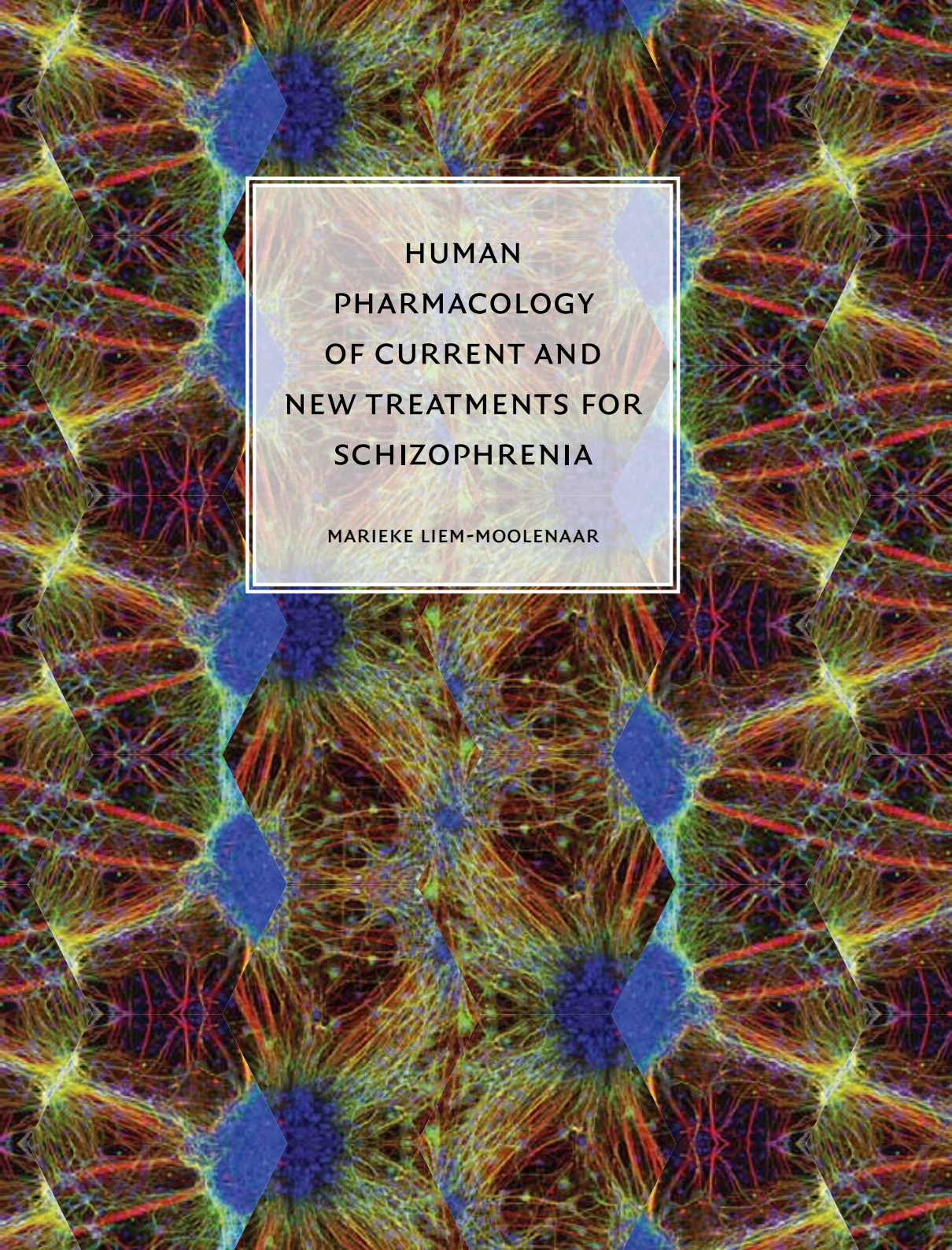


HUMAN PHARMACOLOGY OF CURRENT AND NEW TREATMENTS FOR SCHIZOPHRENIA

MARIEKE LIEM-MOOLENAAR

HUMAN PHARMACOLOGY OF CURRENT AND NEW TREATMENTS FOR SCHIZOPHRENIA

PROEFSCHRIFT

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr.P.F. van der Heijden,
volgens besluit van het College voor Promoties,
te verdedigen op donderdag 8 maart 2012
klokke 16:15 uur

DOOR

Marieke Liem-Moolenaar,
geboren te Nijmegen
in 1974

PROMOTIECOMMISIE

PROMOTORES

Prof. dr. J.M.A. Van Gerven *(promotor)*
Prof. dr. A.F. Cohen *(promotor)*
Dr. K.L. Franson *(co-promotor), Associate Dean for Professional Education, Department of Clinical Pharmacy University of Colorado School of Pharmacy, Denver, United States*

OVERIGE LEDEN:

Prof. dr. A.C.G. Egberts, *hoogleraar klinische farmacie Universiteit Utrecht, Universitair Medisch Centrum Utrecht*
Prof. dr. R.S. Kahn, *hoogleraar klinische en biologische psychiatrie Universiteit Utrecht, Universitair Medisch Centrum Utrecht*
Prof. dr. F.G. Zitman

DESIGN

C de Lint, Voorburg (caro@delint.nl)

ACKNOWLEDGEMENTS

The publication of this thesis was financially supported by the foundation Centre for Human Drug Research (CHDR), Leiden, the Netherlands

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine; serotonin
AD	Alzheimer's disease
AE	Adverse event
AMPA	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
APA	American Psychiatric Association
AUC	Area under the plasma concentration–time curve
BMI	Body Mass Index
C_{MAX}	Maximal mean plasma concentration
CATIE	Clinical Antipsychotic Trials of Intervention Effectiveness
CB	Cannabinoid
CBD	Cannabidiol
CI	Confidence Interval
CL	Total clearance of drug from plasma
CNS	Central Nervous System
COMT	Catechol-O-methyl transferase
CYP	Cytochrome P450
D_1	Dopamine-1 receptor
D_2	Dopamine-2 receptor
DA	Dopamine
DSM	Disease State Manual
ECG	Electrocardiogram
EEG	Electroencephalogram
EPS	Extrapyramidal side effects
FGA	First-generation antipsychotics
FSH	Follicle-Stimulating Hormone
GABA	Gamma-aminobutyric acid
$GlyT_1$	Glycine transporter 1
H_1	Histamine-1 receptor
HPLC/MS/MS	High performance chromatography/mass spectrometry
Kd	Affinity
LH	Luteinizing Hormone
LLQ	Lower Limit of Quantification
LSM	Least Square Mean
LSD	Lysergic acid diethylamide
NA	Nucleus Accumbens
NK3	Neurokinin-3 receptor
NMDA	N-methyl-D-aspartate
PANSS	Positive and Negative Syndrome Scale
PK	Pharmacokinetics
PD	Pharmacodynamics
SAE	Serious Adverse Event
SGA	Second-generation antipsychotics
SPV	Saccadic Peak Velocity
SSRI	Selective Serotonin Reuptake Inhibitors
$T_{1/2}$	Elimination half life
THC	Δ9-tetrahydrobannabinol
T_{MAX}	Time to maximal plasma drug concentration
VAS	Visual Analogue Scales
VTA	Ventral Tegmental Area
VVLT	Visual Verbal Learning Test

CHAPTER 1

INTRODUCTION

Background schizophrenia

Schizophrenia, which is in the top ten of most debilitating psychiatric disorders [1], affects up to 1-1.5% of the world population. Usually the symptoms of schizophrenia occur in young adulthood and persist for the entire lifetime [2]. Schizophrenia means a shorter life expectancy, an almost life-long loss of economic productivity and higher health care costs for many patients [3-5]. There is no schizophrenia therapy available today that addresses these issues.

This thesis describes a number of studies related to early drug development for schizophrenia and related disorders. The reader will note the pharmacological diversity of drugs and systems that are dealt with in this thesis. In a way, this reflects the clinical complexity of schizophrenia. According to the Disease State Manual DSM-IV of the American Psychiatric Association [6], the diagnosis of schizophrenia requires the presence for a significant part of one month of at least two of the following diverse symptoms: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour and negative symptoms. People diagnosed with schizophrenia usually experience a combination of positive symptoms (i.e. hallucinations, delusions, incoherent thoughts), negative symptoms (i.e. apathy, lack of emotion, poor or nonexistent social functioning) and cognitive dysfunction (memory problems, disorganized behaviour, and difficulties planning, concentrating, following instructions or completing tasks) [7-9]. Many researchers include the cognitive deficits with the negative symptoms in the articles they write. Furthermore, many patients suffer from comorbid disorders like depression and anxiety [7]. The most frequently used instrument to measure the signs and symptoms of schizophrenia has been the Positive and Negative Syndrome Scale (PANSS), which in its common form addresses three factors: positive and negative symptoms and general psychopathology [10]. Due to growing knowledge of neurobiology and neuropharmacology of schizophrenia and the increasing recognition of comorbid symptomatology, new ways have been proposed to assess signs and symptoms associated with schizophrenia more accurately. Many publications now identify five symptom categories, which can be roughly divided into positive and negative symptoms, plus disorganization (impaired cognition), excitement, and emotional distress [11-15].

Pathophysiology and etiology

Very little is known about the pathophysiology and etiology of schizophrenia. The situation is complicated by the clinical variability of schizophrenia. It is increasingly suggested that schizophrenia is not a single disease, but a clinical syndrome, perhaps comprising several disease entities [16], and certainly associated with different syndromes like depression and anxiety, in addition to positive, nega-tive and cognitive symptoms. Many hypotheses have been generated in an attempt to explain the pathogenesis and phenomenology of schizophrenia. These can be roughly divided into a neurodevelopmen-tal and neuropharmacological hypothesis [17]. As the neuropharmacological hypothesis will be more relevant for this thesis, this will be discussed in more detail than the neurodevelopmental hypothesis.

Neurodevelopmental hypothesis of schizophrenia

The neurodevelopmental hypothesis of schizophrenia postulates that an environmental insult (e.g. obstetric complications, maternal viral infection, nutritional deficits, psychological experiences) disrupts normal brain maturation, resulting in the emergence of psychosis at puberty or young adulthood [18]. However, a purely environmental origin of schizophrenia is refuted by the strong genetic predisposition, which accounts for 24-80% of the risk of developing the disease (depending on the diagnostic criteria or the use of endophenotypes [17]). The neurodevelopmental hypothesis was therefore later extended to the 'two hit hypothesis', which postulates that an interaction between an early life insult and multiple susceptibility genes is required to cause schizophrenia [19,20]. This hypothesis could be broadened, as Henquet *et al* showed that an environmental factor in a later stage in life (cannabis use), synergistically with genetic predisposition, can also increase the risk of developing psychosis [21-23].

A neurodevelopmental pathogenesis is compatible with the numerous molecular and structural changes that have been found in the brains of schizophrenic patients [24]. Many of the observed changes in gray matter would seem to result from reduced neuropil density (network of dendrites, dendritic spines, axons, and pre- and postsynaptic terminals) and support the suggestion that schizophrenia is a disorder of synaptic connectivity [25,26]. These changes are part of widespread neuroanatomical derangements, which are also reflected by the progressive cerebral (temporal) volume reductions that have repeatedly been found with neuroimaging [27]. Several compounds that target brain growth factors are in early stages of development [28-30]. The currently used drugs are not able to reduce or reverse the structural and neurodegenerative aspects of schizophrenia. However, in some studies indications for secondary structural effects of symptomatic antipsychotics have been found [31,32]. For example, Brennand *et al* showed that loxapine (a registered atypical antipsychotic in the US, structurally related to clozapine) resulted in a statistically significant improvement in neuronal connectivity [26]. If corroborated, these findings illustrate the intricate and reciprocate - though still poorly understood - relationships between psychological, social and biological development of the nervous system in childhood and adolescence.

Neuropharmacological hypotheses of schizophrenia

The pathogenesis of schizophrenia and the pathophysiology of its clinical manifestations have also been related to abnormalities of neurotransmitter systems, which are thought to be the consequences of the underlying neurodevelopmental derangements [33,34]. The most important neuropharmacological hypotheses are based on observations that key symptoms of schizophrenia such as psychotic-like or disorganized states could be either suppressed with or caused by certain drug classes that affect the actions of for example dopamine, serotonine, glutamate, endocannabinoids, GABA (gamma-aminobutyric acid) and acetylcholine.

DOPAMINE

Many different pharmacological systems have been suggested to be implemented in schizophrenia, but the most widely accepted theory regarding the origin of psychotic symptoms is the dopamine hypothesis [35,36]. This hypothesis is based on two observations. First, substances that increase dopamine neurotransmission, such as amphetamine and cocaine, have psychomimetic properties in non-schizophrenics and enhance symptoms in schizophrenia patients [37-39]. And second, the central mechanism of antipsychotic action of even the newer antipsychotics is directed at dopamine D_2 receptors. The first publication of what was later called 'the dopamine receptor hypothesis', reported on the antipsychotic actions of chlorpromazine (introduced in the 50s as the first antipsychotic drug) in a trial with 38 schizophrenic patients [40]. Carlsson and Lindqvist introduced the mechanism of action of antipsychotics by showing that they increased the turnover of monoamines as reflected by increased levels of their metabolites in animals [41]. The dopamine receptor was only identified and firmly linked to antipsychotic response in the 70s [42-45]. It was not until the mid-1990s that imaging studies provided supporting evidence [46-48]. A systematic literature review by De Visser *et al* showed a relationship between D_2 affinity and therapeutic starting dose across a large number of classic and atypical antipsychotic drugs [49].

Changes in dopamine regulation play a major role in both the symptoms and treatment of schizophrenia. In this theory several dopamine tracts are involved (figure 1), which account for the majority of clinical symptoms of schizophrenia and adverse effects of antipsychotic drugs.

Emotional tracts:

1. Mesolimbic pathway (dopamine 2 receptors): begins in the ventral tegmental area (VTA) and connects to the limbic system via the nucleus accumbens (NA), the amygdala, and the hippocampus as well as to the medial prefrontal cortex. Dopamine in the hippocampus is thought to

play a role in new episodic memories and in spatial coding (orientation in space) [50]. Dopamine in the VTA - NA pathway plays a role in the reward system and eating behaviour [51]. The exact role of the mesolimbic dopamine system in the reward system is widely discussed [52]. Three hypotheses have been proposed as explanations for dopamine's function in the reward system: hedonic, learning and motivational salience hypothesis [52]. This hypothesis is discussed in more detail in the next chapter of this introduction.

2. Bulbus olfactorius (not depicted in figure 1 as it is not thought to play an important role in schizophrenia or antipsychotic drug action): involved in olfaction, the perception of odours.

Cognitive tracts:

3. Mesocortical pathway (dopamine 1 receptors): begins in the VTA and connects to the prefrontal cortex. It is thought to be involved in working memory, time orientation, analysis and arguing, planning and mental organisation, and initiative and motivation.

Motor tracts:

4. Nigrostriatal pathway (dopamine 1 receptors): begins in the substantia nigra and projects to the basal ganglia. It is part of a system called the basal ganglia motor loop, and involved in psychomotor and supportive locomotion and facial expression.

Neuroendocrine tracts:

5. Tuberoinfundibular pathway (both dopamine 1 and 2 receptor): begins in the hypothalamus and projects to the posterior pituitary. It is involved in regulation of prolactin secretion from the anterior pituitary gland and metabolic activity.

6. Area postrema (not depicted in figure 1 as it is not thought to play an important role in schizophrenia): controls vomiting through dopamine 2 receptors. Its privileged location in the brain also allows the area postrema to play a vital role in the control of autonomic functions by the central nervous system

MESOLIMBIC DOPAMINE TRACTS At first, it was hypothesized that the mesolimbic dopamine tracts would be hyperactive causing excessive stimulation of D_2 receptors, resulting in positive symptoms. While there is general acceptance of a role for a dopaminergic abnormality in psychosis, what the exact underlying mechanism is, is still a matter of debate. It has been postulated that the mesolimbic dopamine abnormality may be a secondary consequence of another deficit, for example hypo-functionality of the frontal cortex (associated with a reduced glucose utilization and blood flow in the prefrontal cortex, also called cerebral hypofrontality) [53,54], a glutamate deficit [55,56], or a primary neurodevelopmental disorder [34,57]. All these models include a final mesolimbic dopamine dysregulation, but do not specify how this leads to symptoms. The so called aberrant salience hypothesis of Kapur may provide a link between the biological dysfunction of the dopamine system and the symptomatic expression of psychosis [58]. To understand this hypothesis, it is necessary to know one of the roles of dopamine on the mesolimbic pathway (earlier described as 'initiative and motivation' as functions of the mesolimbic tract), called the motivational salience hypothesis [51,59-61]. According to this hypothesis, dopamine mediates the conversion of the neural representation of an external stimulus from a neutral bit of information into an attractive or aversive entity [51]. Thus, dopamine, which under normal conditions is a mediator of contextually relevant saliences, in the psychotic state becomes a creator of aberrant saliences. According to the idea of salience attenuation, antipsychotics do not primarily change thoughts or ideas; instead, they provide a neurochemical milieu in which new aberrant saliences are less likely to form and previously aberrant saliences are more likely to extinguish [62]. They do not remove the core content of the symptom, but rather the degree to which the symptoms occupy the mind, distress the patient, and drive action [63]. In neurochemical terms this means that antipsychotics are seen as blocking the expression of abnormal dopaminergic transmission, but they do not fundamentally alter the dopaminergic dysregulation [64,65]. This explains why delusions and hallucinations do not immediately resolve when treatment is introduced, but do lose their emotional significance and pervasive

character. It is only after some weeks of treatment that the fundamental content of the delusions and hallucinations is deconstructed and recedes (entirely for some) from awareness[66,67]. The detailed mechanism behind this concept is unknown, but it fits well for the phenomenon of relapse, exacerbation and recurrence of psychosis in schizophrenia[68].

MESOCORTICAL DOPAMINE TRACTS The classical dopamine hypothesis of schizophrenia thus postulates that an excess of dopamine subcortically (as a consequence of hyperactivity of the mesolimbic pathway) is associated with positive symptoms of schizophrenia. About 20 years ago, it was proposed that the negative symptoms and cognitive impairment, commonly found in schizophrenia, could be associated with a lack of dopamine in the prefrontal cortex [69]. This idea was raised, because dopamine depletion in the prefrontal cortex produced cognitive impairment in animals [70]. Functional imaging studies [71,72] and postmortem studies of the brains of schizophrenic patients [73-75] demonstrated a range of abnormalities in cortical areas within the working memory network, including (most consistently) the dorsolateral prefrontal cortex, cingulate, and temporal cortices. The overall function of a cortical network presumably relies on the competence of both local information processing within specific local circuits and axonal connections between local circuits and distant cortical areas. Therefore, a deficit of a single region (for example in the dorsolateral prefrontal cortex) could conceivably have functional consequences in the working memory network [72].

MESOLIMBIC AND MESOCORTICAL IMBALANCE Combining the mesolimbic and mesocortical theories, the current dopamine hypothesis suggests an imbalance between mesocortical and mesolimbic dopaminergic systems [53,76]. Several hypotheses have been published on how the overactivity and underactivity in these different regions can co-exist at the same time and whether these are linked. There is an ongoing debate whether subcortical dopamine dysregulation (mesolimbic) is a primary phenomenon, or is rather associated with prefrontal cortical dysfunction (mesocortical) [77-80].

Neuroimaging studies have contributed to a better understanding of the pathophysiology of schizophrenia and give strong evidence for the 'combined mesolimbic and mesocortical dopamine hypothesis' of [76,81,81-83]. Davis *et al* postulates that a deficit of dopamine in the prefrontal cortex results in hypostimulation of D_1 receptors (leading to cognitive deficit symptoms [84,85]), the predominant dopamine receptor subtype in this area [53]. In addition, these alterations feed into each other as it has been shown that cortical dopamine has an inhibitory effect on subcortical dopamine. This deficit in cortical dopamine may itself contribute to excess in subcortical dopamine activity in mesolimbic dopamine neurons (inducing positive symptoms) [86]. The hypothesis of Abi-Dargham is similar to that of Davis *et al*, but slightly more detailed. It makes use of an excitatory (glutamatergic) and inhibitory (GABAergic) system, which both control the dopamine neurons in the VTA to explain a simultaneously-induced imbalance of the two systems [76].

GENETIC VULNERABILITY AND DOPAMINE Although susceptibility to schizophrenia is largely explained by genetic variation, demonstrated by family, twin and adoption studies [87], schizophrenia is a complex disorder, not simply defined by several major genes, but rather evolving from addition or potentiation of a specific cluster of genes, which subsequently determines the genetic vulnerability of an individual. Linkage and association studies suggest that genetic factors increase the vulnerability to the disease, but that penetration is modulated by different triggering factors and environmental influences. Over the past years, a large number of genes or polymorphisms have been evaluated, which could in some way be related to pharmacological systems involved in schizophrenia. Several studies and meta-analyses point at the potential involvement of the gene for dopamine D_2 receptors (DRD2) [88,89]. However, the relationship between polymorphisms and schizophrenia is complex and makes it impossible to draw strong, direct conclusions as dopamine availability and brain functioning are not linearly related [90]. Catechol-O-methyl transferase (COMT), a catabolic enzyme involved in the degradation of particularly dopamine [91,92], has been shown

to be critical for prefrontal dopamine flux as well as prefrontal cortex-dependent cognition and activation [93]. Several COMT polymorphisms substantially influence the activity of the enzyme [90,93]. Except for COMT, studies looking for links between polymorphisms and schizophrenia have mostly been negative or ambiguous [81,94-97].

In summary, the dopamine hypothesis has been and still is the currently most widely accepted theory of schizophrenia. Even though it has been accepted that changes in dopamine are playing a role in schizophrenia, it still remains a matter of debate whether dopamine is the primary or only cause of these derangements.

SEROTONIN

Serotonin was discovered in the late 1940s, and the first serotonin (5-HT) receptors were identified shortly thereafter [98]. In the 1950' and 60's, the partial 5-HT_{2A} agonist lysergic acid diethylamide (LSD) was extensively used and even registered for some time (as Delysid¨), for its hallucinatory effects and as a tool to investigate or experience psychotic-like states. Interest in the role of serotonin for the treatment of schizophrenia was further enhanced by the discovery of the efficacy of clozapine in treatment resistant schizophrenia [99]. The use of this drug is thwarted by the occurrence of rare but serious adverse events like bone marrow suppression, but it is an effective antipsychotic medication that causes fewer extrapyramidal and cognitive side effects than other antipsychotics [100]. Clozapine has a relatively low affinity for the D_2 receptor, but it affects a range of other targets, including 5-HT_2 receptors [101]. Clozapine's specific properties have contributed to the serotonin-dopamine hypothesis, which states that a certain ratio of serotonin 5-HT_2 to dopamine D_2 affinity is the most critical mechanism behind the atypical antipsychotic action. For a long period, this has been the most widely accepted basis for atypicality [102,103]. The thought behind this hypothesis is that by blocking the presynaptic 5-HT_{2A} receptors in the nigrostriatal and mesocortical tracts, the net effect of a decrease in dopamine neurotransmission (and therefore the associated side effects seen with chronic antipsychotic therapy in these tracts) would be reduced.

However, this hypothesis has frequently been criticized as 'pure' 5-HT_{2A} antagonists have never shown antipsychotic properties.

Although the interest in the 5-HT_{2A} antagonists has decreased, there is increasing evidence that 5-HT_6 receptors are involved in cognition and learning, and that they might play a role in convulsive disorders and appetite control [98]. It has been hypothesized that clozapine's relatively high affinity for 5-HT_6 receptors might explain some of its beneficial cognitive effects in schizophrenia [104,105]. In animals 5-HT_6 receptor antagonists showed improvements in several cognition models [106-109], although contradictive results were reported as well [110,111]. Several reports suggest a potential therapeutic role of 5-HT_6 receptor antagonists in the treatment of cognitive dysfunction. Interestingly, these agents seem to be effective in syndromes associated with cholinergic hypofunction [112].

Summarizing, 5-HT_{2A} antagonism by itself has never been persuasively shown to have antipsychotic properties, despite clear evidence for psychomimetic effects of 5-HT_{2A} activation. However, 5-HT_6 antagonism might be promising in the treatment of cognitive impairment in schizophrenia.

GLUTAMATE

In the late 50s it was hypothesized that hypofunction of the glutamatergic system, and specifically of the N-methyl-D-aspartate (NMDA) receptor, contributed to the pathophysiology of schizophrenia [113,114]. This was based on effects of non-competitive antagonists of the N-methyl-D-aspartate (NMDA) glutamate receptor (e.g. ketamine and phencyclidine), which exacerbated symptoms in schizophrenic patients and induce psychotic symptoms in healthy men [115]. The effects were considered to better resemble schizophrenia than those induced by dopamine activation or partial 5-HT_2 agonism: not only positive, but also negative symptoms and cognitive deficits were shown after administering these compounds [115,116]. Furthermore, reciprocal synaptic relationships between forebrain dopaminergic projections and glutamatergic systems have been described and therefore dysfunction of glutamatergic neuronal

systems would not be inconsistent with the dopamine hypothesis of schizophrenia [113]. Olney and Farber conjectured that dopamine receptors inhibit glutamate release [117,118]. A primary defect in the dopamine system that causes dopamine hyperactivity could result in excessive suppression of glutamate release at NMDA receptors, with consequent hypofunction of the NMDA receptor system, which could be the basis for schizophrenic symptoms [113]. It was stated that this theory would fill some of the gaps of the dopamine hypothesis.

Receptors for glutamate are divided into two broad families: 1) ionotropic receptors, which are differentiated based upon sensitivity to the synthetic glutamate derivatives NMDA, AMPA and kainate and 2) metabotropic receptors, which are G protein coupled and mediate longer-term neuromodulatory effects of glutamate [119]. In addition to the recognition site for glutamate, NMDA receptors contain allosteric modulatory sites for glycine, which affects channel open time and desensitization rate in the presence of glutamate. AMPA and kainate receptors mediate the majority of glutamate in the brain. AMPA receptors work heavily in concert with NMDA receptors. Metabotropic receptors, which regulate glutamatergic neurotransmission both pre- and post-synaptically, may serve as an alternative pharmacological target for the treatment of schizophrenia [119].

Summarizing, hypofunction of the glutamatergic system is thought to contribute to the development of schizophrenia. Furthermore, it would not be inconsistent with the dopamine hypothesis.

ENDOCANNABINOIDS

Many articles have been published about the relationship between the use of marijuana and schizophrenia and there is little doubt that cannabis can lead to clinical psychosis, particularly in susceptible individuals and/or after heavy use [21,22]. It is believed that many of the pharmacological effects of marijuana are mediated through the specific endogenous receptors CB_1 and CB_2, and it has been proposed that dysfunction of the endogenous cannabinoid system may play a role in the production of at least some of the symptoms of schizophrenia [120].The finding that levels of two endogenous cannabinoids were increased in the cerebrospinal fluid of schizophrenic patients seems consistent with this hypothesis. However, the direct contribution of endocannabinoid systems in the pathophysiology of the psychotic symptoms of schizophrenia are still unclear [121].

The evidence for pharmacological relationships between endocannabinoid and dopamine systems is increasing [122,123] and psychotic symptoms have consistently been related to increased dopamine function in the striatum [43]. In a study of Fernandez-Ruiz, the literature addressing the cannabinoid-dopamine interactions was reviewed [124]. The CB_1 receptor in particular functions as a retrograde signalling system in many synapses within the CNS, particularly in GABAergic and glutamatergic synapses. They also play a modulatory function on dopamine (DA) transmission, although CB_1 receptors do not appear to be located in dopaminergic terminals in the major brain regions receiving dopaminergic innervations (e.g. the caudate-putamen and the nucleus accumbens or prefrontal cortex). Therefore, the effects of cannabinoids on DA transmission and DA-related behaviours seem generally indirect and exerted through the modulation of GABA and glutamate inputs received by dopaminergic neurons. Recent evidence however suggests a direct interaction between cannabinoid and dopaminergic pathways [125,126]. Cannabinoids have an important influence on various DA-related neurobiological processes (e.g. control of movement, motivation/reward) and pathologies (schizophrenia, basal ganglia disorders, and drug addiction) [124]. Bossong *et al* have shown that striatal dopamine release is increased by an acute dose of Δ9-tetrahydrobannabinol (THC), the most important $CB_{1/2}$-partial agonist from marijuana, which may explain how cannabis contributes to the development and pathophysiology of schizophrenia [127]. In chapter 8 a study is presented which supports the notion that psychotic-like effects induced by THC are mediated by dopaminergic systems, but that other systems also seem to be involved in the 'feeling high' effects. Additionally, the clear reductions of psychotic-like symptoms by a clinically relevant dose of haloperidol suggest that THC administration may be a useful pharmacological cannabinoid model for psychotic effects in healthy

volunteers. A similar study was recently performed by Kleinloog *et al* using olanzapine instead of haloperidol. They found similar results, confirming the use of this practical psychosis model for the currently used antipsychotics. It would be interesting to investigate whether this model can also be used for compounds with other mechanisms investigated for the treatment of schizophrenia.

In summary, relationships between cannabis and psychosis are increasingly clear. There is increasing evidence for a pharmacological relation between (endo)cannabinoid and dopamine systems. However, it is uncertain whether endocannabinoids contribute to the pathophysiology of schizophrenia.

GAMMA-AMINOBUTYRIC ACID (GABA)

The GABA$_{(A)}$ergic (gamma-aminobutyric acid) system has been implicated in the pathophysiology of schizophrenia, both for positive, negative [128,129] and cognitive symptoms [130]. GABA$_{(A)}$ergic interneurons are a core component of the corticolimbic circuitry and modulate the mesolimbic and mesocortical dopaminergic system in a complex way. A growing body of evidence suggests that a malfunction in cortical GABAergic transmission resulting in a disturbance in cortical network activity is a critical factor underlying schizophrenia [131]. The exact role of GABAergic systems in the pathophysiology of schizophrenia is still unclear and in part controversial.

GABA$_{(A)}$ agonists have shown beneficial effects on positive, and to a lesser degree, negative symptoms in some schizophrenic patients [129]. On the other hand, some GABA$_{(A)}$ agonists like zolpidem can induce florid hallucinogenic effects [132,133]. Moreover, GABA$_{(A)}$ agonists such as benzodiazepines, although widely used as sleep aids in schizophrenia, can impair, rather than correct, sleep architecture and cognition [134].

GABA$_{(B)}$ agonists, may be a better option for sleep problems in schizophrenia [134]. Whether GABA$_{(B)}$ agonists have a good or deleterious effect on the positive and negative symptoms has not been sorted out yet. These apparently contradictory reports are at least partly due to two important factors. Firstly, many schizophrenic patients suffer from co-morbid anxiety and disordered sleep, which can have profound effects on the intensity of hallucinations, delusions, and on cognitive organization. Benzodiazepines and other GABA$_{(A)}$ agonists are well-known for their anxiolytic and sleep inducing effects, which during short term treatment may secondarily improve sleep and decrease paranoia. At the same time however, GABA$_{(A)}$ agonists are equally well-known for their detrimental effects on memory and attention, particularly at higher doses and in cognitively vulnerable subjects, and there is no reason to suspect that this would be different in schizophrenia. These two aspects of generalized GABA$_{(A)}$-activation thwart the non-discriminant use of benzodiazepines in schizophrenia, although these treatments can be quite useful in case of pronounced anxiety, restlessness or insomnia. Non-selective GABA$_{(A)}$ receptor inverse agonists (with a negative efficacy at all a subunits) have been shown to improve alertness and memory in experimental animals, but such drugs can also cause anxiety and seizures, and have therefore not been studied extensively in humans [130]. Secondly, GABA$_{(A)}$-receptors are composed of different subunits, which have increasingly been associated with different CNS-functions. GABA$_{(A)}$ agonists containing alpha$_1$ subunits are associated with alertness and sedation. GABA$_{(A)}$ agonists with a high affinity for these subtypes have been developed as potent hypnotics. Particularly these so-called z-hypnotics (including zolpidem, mentioned earlier) can sometimes cause hallucinations, which has been attributed to a dissociated wake-sleep transition [132]. GABA$_{(A)}$ receptors containing alpha$_2$ and alpha$_3$ subunits are linked to anxiety and anxiolysis. Subtype selective alpha$_{2,3}$ agonists are in development for anxiety disorders with an improved side effect profile. These drugs have not been studied in schizophrenia [128] but could be useful as add-on treatments for hallucinations or paranoid delusions or other co-morbid anxieties. Alpha$_4$ containing GABA$_{(A)}$ receptors are located extrasynaptically throughout the brain. Their physiological role is uncertain. GABA$_{(A)}$ receptors containing alpha$_5$ subunits have been associated with memory. Consequently, alpha$_5$-selective inverse agonists have been in development as memory-enhancers for various cognitive disorders, but they have not been examined in schizophrenia.

In conclusion, specific GABA(A)ergic systems (or specific receptor subtypes of the GABA system) may be relevant for various functional abnormalities in schizophrenia, which opens possibilities for potential novel treatments for specific clinical features of this disease. However, schizophrenia is a complex disorder with a multitude of pharmacological derangements, and the role of GABAergic systems is still largely unclear. At present, the development of GABA-ergic drugs is mainly directed at more restricted clinical syndromes, with better established links to the different GABA-systems and their functional characteristics, such as anxiety and memory impairment.

ACETYLCHOLINE

The cholinergic system (and acetylcholine) is thought to play a role in the symptoms of cognitive dysfunction in schizophrenia [129,135,136]. Cognitive impairments observed in schizophrenia in many aspects resemble those that occur in healthy subjects following administration of scopolamine [137,138]. Scopolamine has been applied as a disease model for dementia [139] and for memory impairment in schizophrenia [137]. Although the scopolamine model does not capture the complexity of cognitive decline in schizophrenia (or Alzheimer's disease for that matter), it has become the most frequently used model for studies of cognitive impairment in experimental animals and healthy volunteers [139-142]. Scopolamine-induced memory impairments in animals show similarities to those in humans [143,144]. It is thought that there is an interaction between cholinergic and glutamatergic systems on cognitive function although the exact mechanism behind this interaction has not been resolved [145-147]. In chapter 5 and 6 of this thesis, the scopolamine model was used in healthy volunteers to study the effects of two new glycine reuptake inhibitors. In chapter 7 the pharmacokinetic-pharmacodynamic relationships were investigated.

The evidence that abnormalities in the cholinergic system may play a role in other symptoms of schizophrenia is still marginal and will therefore not be discussed further in this introduction.

NEUROPHARMACOLOGICAL COMBINATION THEORY

The different neuropharmacological theories are not mutually exclusive and can be combined to a 'neuropharmacological combination model' (partially adapted from Stahl [11]). The relationship between dopaminergic, serotonergic, glutamatergic and GABAergic tracts in people without schizophrenia could be simplified as shown in figure 2. The endocannabinoid system has not been included in the picture, since the position of endocannabinoid systems is still unclear and could in fact overlap with several of the other neurotransmitter pathways, by direct or indirect interactions with dopamine and GABA or glutamate [124]. This is also the case for acetylcholine.

In the VTA the cortical brainstem glutamate projection is linked to the mesolimbic dopamine pathway through a GABA interneuron. Stimulation of interneuron NMDA receptors by glutamate induces GABA release. This inhibits dopamine release from the mesolimbic dopamine pathway, which communicates with the parahippocampal gyrus, linked with the sensory associative cortex in both ways, resulting in 'recognition' of sensory information. The mesolimbic pathway also communicates with the amygdala, which also links to the sensory associative cortex, resulting in the realization whether something is 'safe' or whether it is a 'threat'. This can be explained by the motivational salience hypothesis. According to this hypothesis, dopamine can be a mediator of contextually aberrant saliences as was explained before in this introduction [51].

In this relatively simple integrated neuropharmacological model of schizophrenia, NMDA receptors in the cortical brainstem glutamate projection would be hypoactive, resulting in disinhibition of the mesolimbic dopamine pathway, leading to hyperactivity of this pathway and eventually to positive symptoms (figure 3).

The cortical brainstem glutamate projection also communicates directly with the mesocortical dopamine pathway in the VTA, normally causing tonic excitation. In schizophrenia, hypoactivity of NMDA receptors in cortical brainstem glutamate projections leads to a loss of tonic excitation, and mesocortical dopamine pathways become hypoactive, which could contribute to cognitive, negative and affective symptoms (figure 3).

Pharmacological treatment
Currently used antipsychotics

Almost all currently available antipsychotic drugs are based on the dopamine hypothesis, and although many drugs have been designed to affect or avoid certain other specific pharmacological targets, all registered antipsychotics antagonize the dopamine D_2 receptor [49]. Several publications have clearly reviewed these drugs (see for example [148,149]) and therefore only a brief summary will be given.

The current pharmacological treatments can be divided in two groups: 1) first-generation antipsychotics (FGAs), also called classic or conventional antipsychotics or neuroleptics; and 2) second-generation antipsychotics (SGAs), also called atypical or nonconventional antipsychotics. The FGAs (phenothiazines, butyrophenones, thioxanthines) have been the main therapy until the introduction of SGAs in the early 1990s. Although it is not completely clear what differentiates FGAs from SGAs (as discussed in the next chapter of this introduction), the term 'atypicality' refers to drugs that have at least equal antipsychotic efficacy compared to conventional drugs, with less extrapyramidal side effects (EPS) (acute dystonic reactions, akathisia, parkinsonism, tardive dyskinesia) and/or prolactin elevation [148]. The extrapyramidal syndromes such as medication-induced parkinsonism and dystonias are caused by dopamine antagonism in the nigrostriatal tract. Low dopamine in the tuberoinfundibular tract leads to prolactin release, which in some patients leads to gynecomastia.

The high risk of the possibly irreversible movement disorder tardive dyskinesia, and the high rates of acute EPS were the major reason for psychiatrists to switch patients to SGAs when these became available. Many studies have been performed that compare FGAs to SGAs or SGAs to each other. These studies, however, were often performed in patients with acute exacerbation, did not include treatment-resistant patients, were relatively short and did not take into account sufficient efficacy and safety parameters. Moreover, these studies were usually sponsored by the manufacturer of the new antipsychotic and often included a higher than necessary dose of the comparator FGA [149,149]. In the CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) trial, in which discontinuation of treatment was the main endpoint, a difference between the studied SGAs and FGA was found [150]. Olanzapine was the most effective in terms of lower rates of discontinuation, but the efficacy of the FGA perphenazine appeared similar to that of the other SGAs (quetiapine, risperidone, and ziprasidone). However, olanzapine was associated with greater weight gain and increases in measures of glucose and lipid metabolism. Several other meta-analyses did not lead to a definitive answer to the question whether FGAs and SGAs differ in efficacy [150-158].

The only exception seems to be clozapine, which has repeatedly been effective for treating both positive and negative symptoms in treatment resistant schizophrenia [100], but may cause severe side effects such as agranulocytosis that limit its use [159-161]. The beneficial pharmacological properties of clozapine are unknown [162], and many different hypotheses for the superior effectiveness in treatment resistant schizophrenia have been published.

Many have claimed that the SGAs are more efficacious than FGAs in reducing negative symptoms (e.g., lack of emotion, interest, and expression), possibly due to the absence of extrapyramidal symptoms [163] or other secondary causes of negative symptoms (e.g. depression) rather than to direct therapeutic effects [164]. However, it should be noted that this is difficult to assess, because it is hard to distinguish secondary negative symptoms (due to some features of EPSs caused by antipsychotics [163] or exacerbation of mesocortical hypodopaminergic function by D_2 antagonists) from primary negative symptoms of the disease. Lehman *et al* conclude in the second edition of the American Psychiatric Association (APA) Practice Guideline for the treatment of patients with schizophrenia that at this moment there is no effective treatment for primary negative symptoms [165].

Comparable to negative symptoms, it is unknown whether there is a difference in effects on cognitive impairment and mood disturbance [166,167]. The ability of atypical agents to prevent relapse and their effects on social and vocational functioning, quality of life, long-term

outcome, and the caregivers' burden have been incompletely explored [156,163,168]. Similar to negative symptoms, cognitive dysfunction may be secondary to other factors such as EPS, anticholinergic effects and sedation or the underlying disease.

The superior tolerability of the SGAs with regard to EPS seems proven [151,152,152,158,169]. They do, however produce other side effects, of which weight gain [170,171] in combination with an altered glucose, cholesterol and lipid metabolism [172-174] seem to be the most worrisome. Besides the fact that this can adversely affect quality of life and medication adherence [175], weight gain and metabolic abnormalities are important risk factors for premature cardiovascular morbidity and mortality [176]. It is unclear whether the glucose effects are secondary, independent or causative to weight gain. Also, to date, research has been inconclusive whether antipsychotics increase weight via increased appetite and food intake, decreased activity or decreased metabolism. Which pharmacodynamic receptor is responsible for this weight gain and metabolic changes, has yet to be determined [177]. Taken together, the available preclinical and human data indicate that no single neurotransmitter system is responsible for antipsychotic-related weight gain. While rodent and indirect human evidence links the weight gain potential of antipsychotics to histamine H_1 blockade [178], studies also implement other neurotransmitter systems, such as dopamine D_2 blockade, 5-HT_{2C} blockade and interactions with central or peripheral hormones and peptides involved in energy homeostasis [177]. These results are further supported by evidence of an interaction between histamine H_1 and dopamine D_2 blockade [179], genetic data [180] and by the fact that antipsychotics without relevant antihistaminergic activity, such as aripiprazole, amisulpride and haloperidol, have clearly documented weight gain potential, especially in antipsychotic-naive and first episode patients [181].

There are differences in weight gain between the atypical antipsychotics [177]. The risk is highest after the use of clozapine or olanzapine and lower (but still present) after risperidone, quetiapine and aripiprazole.

Another known adverse effect of antipsychotics is cognitive dysfunction. A growing body of evidence from such animal studies indicates that several antipsychotics, including both SGAs and FGAs (if administered for sufficient periods of time), can be associated with impairments in memory-related task performance, as well as alterations in central cholinergic function [182]. It is not completely clarified how this can be explained pharmacologically, but the importance of cholinergic receptor to information processing and cognitive function is thought to play a role [182].

Pharmacological theories for differences between FGAs and SGAs

Many hypotheses have been generated for the pharmacological difference between FGAs and SGAs. According to Kapur and Seeman all efforts to produce antipsychotic action without D_2 blockade have been unsuccessful [183]. Kapur and Seeman proposed that a low affinity at the D_2 receptor in and of itself, is sufficient for producing atypical antipsychotic activity. Affinity (Kd) is defined as the ratio of k_{OFF}/k_{ON} (the rate at which the drug moves off and binds to the receptor). It has been found that koff is the most important determinant of how drugs and dopamine compete [183]. The faster koff, the more quickly the drug will release from the receptor after an endogenous dopamine surge. The slower koff, the slower the drug responds to changes in endogenous dopamine. Antipsychotics with a fast koff under clinical conditions give rise to a fast-on, fast-turnover and fast-off blockade of D_2 receptors. The fast dissociation hypothesis suggests that the combination of a fast koff at the molecular level and transient D_2 occupancy at the system level is sufficient to provide an atypical antipsychotic effect. Kapur and Seeman propose that drugs with fast dissociation when used in doses that lead to appropriately high D_2 blockade modulate the dopamine system in a manner that allows for a better accommodation to changes in physiological dopamine transmission, permitting an antipsychotic effect without motor side effects, prolactin elevation, or anhedonia and other

secondary negative symptoms, and that this leads to what is currently called the atypical antipsychotic effect. Traditional antipsychotics (e.g. haloperidol and chlorpromazine) bind tightly to the dopamine D_2 receptor and slowly dissociate from the D_2 receptor in vitro or in vivo [184]. The atypical antipsychotic drugs (e.g. quetiapine, clozapine, paliperidone, and amisulpride) show rapid dissociation times from the cloned D_2 receptor [185,186], and clinical dissociation times of hours, thus minimizing clinical side effects.

Aripiprazole is a novel antipsychotic agent that is also considered atypical, but with a slightly different mechanism of action. It is a partial agonist for the dopamine and 5-HT_{1A} receptor and an antagonist for the 5-HT_{2A} receptor.

Another hypothesis for differences in FGAs and SGAs is the '5-HT_2 hypothesis'. Since all antipsychotics block D_2 receptors, it has often been thought that atypical antipsychotics must differ due to a separate receptor mechanism. However, high 5-HT_2 occupancy (or a high 5-HT_2/D_2 ratio) seems neither necessary nor sufficient for the atypical antipsychotic effect [183]. Although this hypothesis could be a relatively easy way to explain the differences between atypical and typical antipsychotics, there are several arguments against this theory. One argument is the absence of an atypical clinical profile of antipsychotic effects, after addition of a selective 5-HT_2 antagonist to treatment with an ongoing D_2 antagonist [36]. Many 'atypical' antipsychotics have also been developed because of their 5-HT_2-inhibiting properties, but selective 5-HT_2 antagonists that lack antidopaminergic activity (such as ritanserin) have failed to show a reliable antipsychotic effect [36]. Second, several typical antipsychotics have a high affinity for 5-HT_2 receptors, and some lose 'atypicality' at high doses, which does not support this theory [187]. Furthermore, the relative freedom from extrapyramidal symptoms of the atypical antipsychotics is not related to their 5-HT_2/D_2 ratio's [188] . Also, this ratio is not associated with the therapeutic doses of different classic and atypical antipsychotic drugs, contrary to D_2-affinity [49,189]. Other less well accepted hypotheses for the difference between typical and atypical antipsychotics will not be discussed in this introduction [65].

Past drug development

The majority of currently approved pharmacological agents target psychotic symptoms as their primary effects, and are largely similar in efficacy and effectiveness (except for clozapine in drug-resistant schizophrenia). Each new atypical drug has its own individual side-effect profile and should therefore be individually evaluated for safety. Although currently used antipsychotics can reduce core psychotic symptoms and delay symptom exacerbations very effectively in many patients, schizophrenia has remained a chronic illness with substantial functional impairments, with limited therapeutic developments. The introduction of the atypical antipsychotics has provided a larger and more diverse armamentarium of treatment options, but companies have mainly focussed on alterations of existing medications and on gaining approval on new indications for already marketed drugs [190]. Although many new mechanisms of action have been proposed, several of which have already been examined for a number of decades, these have not led to concrete new treatment strategies or novel drug registrations for schizophrenia. Therapeutic innovations since the discovery of chlorpromazine were mainly limited to pharmacological modifications of the receptor specificity, affinity and intrinsic activity. In general, efforts to discover and develop new drugs have been relatively unsuccessful compared with other disease areas [191-193], and a sceptic could argue that drug development for schizophrenia has not progressed appreciably since the serendipitous discovery of 'chemical lobotomy' with chlorpromazine in the early '50s [194]. Agid *et al* mentioned several reasons that could underlie this lack of success [191]. Perhaps the most important reason is that psychiatry has a diagnostic and classification system that is not based on etiology, neurobiology, epidemiology, genetics or response to medications, but rather on a constellation of signs and symptoms [193]. As with many other neuropsychiatric conditions, schizophrenia research and drug development are also thwarted by the complexity of the CNS, lack of a defined pathology, problematic tissue accessibility, co-morbidity, absence of good animal models, and the daunting fact that

the complexity of behaviour is not simply the sum of its constituents [191].

Another important reason for the lack of progress in development of schizophrenia drug therapy is the absence of adequate animal models to allow determination of clinical efficacy, which therefore often only gets fully established after the drug is widely prescribed on the market. Current preclinical models for schizophrenia are quite effective at predicting whether a candidate molecule will have 'atypical' properties. However, they are less able to make a prognosis for overall efficacy, and completely unable to predict greater efficacy compared to currently available antipsychotics [192]. Moreover, in terms of the negative and cognitive symptom domains in schizophrenia, none of the commonly used animal models are highly predictive, and although it is expected that preclinical memory models will be useful for forecasting the ability to enhance cognition [190], this has not yet been validated in practice. As validity of most currently used animal models is essentially limited to the dopamine hypothesis, these models favour development of (even more) antidopaminergic antipsychotics, but their predictive value for treatments with entirely different mechanisms of action remains to be established. Another disadvantage of using small laboratory animals as a schizophrenia model is that they lack the complex pre-frontal cortical networks that are responsible for many of the clinical manifestations of schizophrenia and psychosis. Additionally (or consequently), the most relevant (clinical) symptoms such as hallucinations and delusions are difficult to assess or measure objectively in animals. Although these limitations are generally acknowledged, current animal models continue to be used because there are no alternative models available. A more rational approach would be to employ human models for the pharmacological, cognitive and antipsychotic effects of putative novel drugs for schizophrenia. Investigating (new) compounds in healthy subjects in an earlier stage of development will increase the predictive value for (new) compounds and will provide reliable information to the development program at the earliest possible time and reduce exposure to vulnerable populations (i.e. schizophrenia patients) [195]. This topic is dealt with in chapters 7 and 8.

New drug development

Although most new pharmacological approaches to schizophrenia are merely symptomatic, they may still cause relevant improvements in patients. Considering the complexity and the multidimensional characteristics of schizophrenia, it is likely that newly developed medications will increasingly be introduced as parts of a polytherapeutic strategy. Each patient will be treated with an individualized drug combination, aimed at his or her personal multidimensional disease profile [190]. This is also called the 'magic shotguns' approach [196]. This competes with the strategy of the 'magic bullet', that still seems to be favoured by the pharmaceutical industry and to be expected by the society. In this strategy a single perfect drug will cure the disease without side effects. Progressions made with polypharmacy are slower than with 'magic bullets', because of the many complexities of dose determinations of combined treatments and interactions, patient selection, and problems associated with patents and ownership. On the other hand, polypharmacy shows close resemblance to clinical practice, where most patients with chronic psychiatric conditions ultimately receive drug combinations that have been individually selected and optimized, targeted at specific clinical problems, and guided by gradual dose escalations and regular assessments of therapeutic and adverse effects.

Gray gives a good impression of the many pharmacological targets which are under investigation for the development of new drugs for schizophrenia [190] and for cognitive deficits specifically in schizophrenia [197]. The increasing awareness that schizophrenia affects different neuropsychological domains such as cognition, mood and social functioning, has fuelled the notion that other systems may also be involved. Thus, drugs that are primarily in development for dementia (e.g. cholinergic or serotonergic enhancers) or depression (like monoaminergic or vasopressinergic agents) are often also explored as potential treatments for abnormal affection, cognition deficits and social functioning in schizophrenia. In more recent years, psychotic symptoms with other drugs or diseases, animal models and other research findings

have also implicated other neuropharmacological systems, such as GABA, endocannabinoids and glucocorticoids [192]. So far, to our knowledge, many of these strategies have been abandoned.

Since 2008, about around 2600 compounds are in development for the treatment of schizophrenia (source: Prous Science Integrity, http://integrity.prous.com/integrity/servlet/xmlxsl/). These experimental compounds show a remarkable pharmacological diversity, with 210 more or less distinct mechanisms of action. Only roughly one third of the compounds is based on known mechanisms of action or on derivatives of currently existing antipsychotics. Frequently reported mechanisms are glycine-1 reuptake inhibition (203 times), dopamine D_2 antagonism or partial agonism (91), 5-HT_{2A} antagonism (70), tachykinin NK_3 antagonism (62) and 5-HT_6 antagonists (60). In the following paragraphs, several active drug development programs targeted at different pharmacological receptors are discussed, which are also dealt with in other chapters of this thesis.

DOPAMINERGIC STRATEGIES

Virtually all currently available antipsychotic drugs are mainly aimed at positive symptoms, by reducing the excessive mesolimbic parahippocampal dopaminergic tone that seems to be an essential element of psychotic manifestations. At the same time, such treatments also reduce other dopaminergic pathways that do not function excessively and are sometimes even less active in patients. Such pathways are involved in important physiological functions like extrapyramidal regulations, mesolimbic reward system, mesocortical cognitive processes, and tuberoinfundibular metabolic and neuroendocrine activity. These mechanisms are responsible for significant adverse effects of antidopaminergic drugs that contribute to poor compliance and cognitive blunting. In recent years, due to new insights in pathogenesis and pathophysiology of schizophrenia, new pharmacological targets and corresponding compounds have been developed, as for example reviewed by Gray *et al* [190,197]. Some new dopaminergic treatments are designed to modulate rather than simply antagonize the activity of dopamine in the brain. Aripiprazole for instance is a partial D_2 agonist, designed to reduce hyperactive dopaminergic functions (involved in positive symptoms) and to activate reduced dopamine tone in mesocortical (cognitive) areas. Other more indirect strategies are currently investigated. Based on the fast dissociation theory of atypicality described earlier, new agents are now in development that dissociate faster from the dopamine receptor [183]. An example is the centrally acting dopamine D_2 receptor antagonist JNJ37822681, which combines specificity for the dopamine D_2 receptor and a fast rate of dissociation from this receptor [198]. Another indirect strategy is targeted at neuropeptides that modify the activity of monoaminergic neurotransmitters. Tachykinins for instance activate neurokinin NK3 receptors that facilitate the release of the biogenic amines dopamine, serotonin and norepinephrine. This opens the possibility that antagonism of NK-receptor reduces the excitatory activation (or hyperactivity) of some or all of these principal systems, without affecting normal dopaminergic baseline activity so much. Various NK antagonists are in development for different psychiatric disorders[199]. In chapter 3, the effects of the neurokinin $(NK)_3$ antagonist talnetant (SB223412) were investigated in healthy volunteers.

SEROTONERGIC STRATEGIES

Although there is no definite proof for the earlier mentioned serotonin-dopamine hypothesis (in which the 5-HT_2 receptor is involved), there are theoretical grounds for investigating other subtypes of the serotonin receptor. Several serotonergic strategies are outlined by Gray *et al* [190,197]. Glennon *et al* have reviewed three possible new receptors which may be implicated in schizophrenia: 5-HT_5, 5-HT_6 and 5-HT_7 [98]. In contrast to the 5-HT_1 to 5-HT_4 receptors, the 5-HT_5, 5-HT_6 and 5-HT_7 receptors have been less extensively investigated and much less is known about their functional properties.

As no selective ligands are available, little is known about the 5-HT_5 receptor [200]. 5-HT_5 receptors have not yet been demonstrated to 'function' in native systems [200]. It is likely that interest in 5-HT_5 pharmacology will follow once selective ligands are available.

Human 5-HT$_7$ receptors are positively coupled to an adenylate cyclase second messenger system. Several reasonably selective antagonists and agonists have been identified, and a number of leads for other structure types have been discovered. 5-HT$_7$ receptors have been implicated in a wide variety of pharmacological functions, although the functions and possible clinical relevance of this receptor are not yet fully understood. Indirect evidence suggests that 5-HT$_7$ receptor antagonism may be clinically useful in the treatment of depression and possibly involved in anxiety, epilepsy, pain, and schizophrenia [201]. Since the involvement of this receptor in schizophrenia is under investigation and no selective 5-HT7 antagonists have been developed for humans, this will not be further discussed in this introduction.

After rat and mouse 5-HT$_6$ receptors were described in 1993 and 1994 [202,203] and human 5-HT$_6$ receptors in 1996 [204], research in this area increased immensely. The human 5-HT$_6$ receptor is positively coupled to adenylate cyclase, has 89% overall sequence homology with the rat receptor and is nearly exclusively localized in the central nervous system [204]. It is predominantly found in the caudate nucleus, with lower concentrations in hippocampus and amygdala [204]. The first 5-HT$_6$-selective antagonist was described in 1998 [205]. Since then, various useful selective 5-HT$_6$ antagonists have been identified, some selective 5-HT$_6$ agonists have been reported and newer agents are developed with improved pharmacokinetic and pharmacodynamic properties [98,206].

Several recent lines of evidence have suggested a role of 5-HT$_6$ receptors in cognitive and memory processes, found in healthy animals and in animal models of cognitive impairment [98,109,207] and in patients with Alzheimer's disease (AD) [206,208]. Maher-Edwards *et al* performed an exploratory clinical study showing that the 5-HT$_6$ antagonist SB742457 and the acetylcholinesterase inhibitor donepezil improved global functioning of AD patients [209]. A combined 5-HT$_6$ receptor antagonist and cholinesterase inhibitor strategy could be a possible treatment of cognitive disorders such as those seen in Alzheimer's disease or schizophrenia. However, this class of drugs needs to be investigated more thoroughly and the role of this receptor in schizophrenia (even more than in AD) still needs to be defined.

The 5-HT$_6$ antagonist SB742457 is developed as a possible 'add-on' therapy for schizophrenia, to improve the negative symptoms or to decrease the cognitive side effects of currently used treatments. Since such compounds are designed to treat only part of the schizophrenic spectrum, they will usually be combined with other therapies. Therefore, interactions with other antipsychotic drugs are relevant, and in chapter 2 SB742457 was investigated in combination with risperidone. Risperidone is a D$_2$/5-HT$_{2A}$ antagonist with low affinity for 5-HT$_6$ receptors, which seems to produce a limited improvement on neurocognitive parameters in schizophrenic patients [210].

GLUTAMATERGIC STRATEGIES

Hypofunction of the glutamatergic system is one of the most promising non-dopaminergic theories for the pathophysiology of schizophrenia. Several targets have been explored to stimulate glutamateric activity [190,197], while avoiding potential adverse reactions like seizures or cell death [211]. Activation of metabotropic receptors indirectly induce ion channel formation and other postreceptor cascades [212]. The selective metabotropic glutamate 2/3 (mGlu2/3) receptor agonist LY404039 [213] has anxiolytic and antipsychotic effects in animal studies, without causing sedation [214] and is thought to work by reducing the presynaptic release of glutamate in brain regions where mGlu2/3 receptors are expressed [215,216]. In a first exploratory clinical study, it seemed effective in patients with schizophrenia and did not show major side effects [217].

Others have tried to stimulate glutamatergic activity through AMPA or kainate activation. AMPAkines, which potentiate AMPA transmission without binding directly to the agonist binding site [218], stimulate memory-dependent processing in animals [219] and could be useful to treat various aspects of schizophrenia. These compounds are currently under development for treatment of cognitive dysfunction in various neuropsychiatric disorders [119,220]. Although far less advanced, the AMPA/kainate receptor antagonist LY293558, showed effect in an animal model for cognitive deficits (ketamine), suggesting a possible utility of AMPA antagonists in the treatment of the cognitive deficits in schizophrenia [221].

The most developed way to augment NMDA receptor function indirectly is through facilitation of glycine, an obligatory co-agonist for glutamate at the NMDA receptor. As direct agonists of NMDA receptors are neurotoxic [114], indirect ways to enhance receptor function have been investigated [222]. After facilitation of glycine, an obligatory co-agonist for glutamate at the NMDA receptor, modest improvements in positive and negative symptoms and cognitive function in schizophrenic patients have been described in exploratory studies [55,211,211,223,224]. However, high doses of glycine are needed to significantly elevate CNS concentrations. Therefore, inhibition of presynaptic glycine reuptake seems a more efficient way to increase pharmacological glycine activity in the brain [225]. Of the two described glycine transporters (glycine transporter 1 or $GlyT_1$ and $GlyT_2$) [226], $GlyT_1$ seems to directly affect NMDA activity [226,227]. Glycine reuptake inhibitors have shown effects in several animal models of schizophrenia [228-230]. In a clinical trial, the glycine reuptake inhibitor sarcosine showed effects on positive and negative symptoms in schizophrenic patients [224,231]. As sarcosine is a low potency antagonist requiring gram-level dosing, more potent glycine reuptake inhibitors are now investigated, as is for example shown in chapters 4-7. In the study described in chapter 4, the first glycine reuptake inhibitor to be administered to humans was examined in healthy subjects. In the other two studies described in chapters 5 and 6, the glycine reuptake inhibitor, was administered to healthy men with and without scopolamine, to induce transient and reversible cognitive and memory impairments, and to reduce ceiling effects by prevention of performance at maximal capacity.

ENDOCANNABINOID STRATEGIES

As several lines of experimental and clinical evidence point to a dysregulation of the endocannabinoid system in schizophrenia, there is a potential for pharmacological manipulation of the endocannabinoid system as a novel approach for treating schizophrenia. Although experimental findings are still controversial, the CB receptor inverse agonist cannabidiol (CBD) and the CB_1 receptor antagonist rimonabant (SR141716) show the most consistent antipsychotic properties in dopamine- and glutamate-based (animal and human) models of schizophrenia, with profiles similar to atypical antipsychotic drugs [232].

CBD is one of the components of cannabis, which may constitute up to 40% of cannabis extracts and is devoid of the typical psychological effects of cannabis in humans [233]. The first evidence that CBD could have antipsychotic effects was obtained in an interactive study of CBD and deltag-THC in healthy volunteers published in 1982 [234]. The antipsychotic-like properties of CBD have been investigated in animal models using behavioural and neurochemical techniques, which suggested that CBD this compound may act as an atypical antipsychotic drug [233,235,236]. Also, in human models of psychotic symptoms in healthy volunteers, the antipsychotic-like activity of CBD was demonstrated [236]. Although CBD did not show effect in a study in treatment-resistant schizophrenic patients, a preliminary report from a 4-week clinical study suggested that CBD is an effective, safe and well-tolerated treatment for schizophrenic patients [236]. In 2007 Leweke *et al* found that CBD significantly reduced psychotic symptoms in acute schizophrenia with potency similar to amisulpride but with fewer side effects such as EPS, increase in prolactin, and weight gain [237].

Although the findings are variable, the selective CB_1 receptor antagonist rimonabant showed activity in preclinical models of antipsychotic efficacy. It failed, however, to demonstrate antipsychotic activity versus placebo and haloperidol in a clinical trial [238]. The ability of CBD and rimonabant to improve the psychosis-related cognitive impairment has not been sorted out yet and these compounds need further investigation for this indication [232].

Scope of this thesis

The introduction of this thesis gave an outline of some important pharmacological hypotheses of schizophrenia, the pharmacological properties of currently used antipsychotics, and compounds with new pharmacological mechanisms that are in the development pipeline.

This thesis describes how these pharmacological characteristics can be determined in healthy volunteers, at a stage of drug development when novel compounds are not yet tested in patients. The different chapters of this thesis illustrate how these concepts are used in practice, during the early development of new therapeutic strategies for schizophrenia. An important aim of these strategies is to identify treatments with an improved therapeutic window, since adverse events and therapeutic failures in schizophrenia form an important impediment in current medical practice.

One way to try to improve the therapeutic window of a drug therapy is by ameliorating the pharmacological mechanism of action, from direct, full and non-selective (D_2) receptor antagonists to more indirect, partial, or selective modulators. Chapters 2 and 4-6 carefully assessed the pharmacological characteristics of novel compounds, by using a large number of different 'drug biomarkers' to create a 'fingerprint' of the pharmacological effects. This is based on the notion that a drug's mechanism of action underlies both its desired and the undesired effects, and therefore provides an indication of the expected therapeutic window. Chapter 2 used a positive control to provide an indication of (dis) advantages of a potential antipsychotic drug from a new class (the NK_3 antagonist talnetant), compared to a widely used therapeutic congener (the D_2-receptor antagonist haloperidol).

Another way to improve the therapeutic window is by development of combination therapies, each targeted at a different clinical syndrome that characterize schizophrenia in addition to the well-known positive psychotic symptoms, in particular cognitive deficits (Chapter 3, examining a 5-HT6 antagonist). In each of these chapters, we used a multifunctional CNS test battery (the neuroCart) to make comprehensive pharmacological profiles of novel schizophrenia treatments in healthy subjects, which were compared with existing medications to identify potential pharmacological advantages of the new therapeutic strategies.

All studies were performed in healthy volunteers, which has the advantage that patients are not burdened with new drugs, at a stage when the therapeutic potential and adverse effect profile are still uncertain. The obvious disadvantage is that healthy volunteers do not have schizophrenia, making it impossible to demonstrate a therapeutic effect. Therefore, new human pharmacological models were developed that can be used in healthy volunteers to study aspects of the disease. Chapters 5 and 6 used a scopolamine model to induce reversible cholinergically mediated cognitive deficits in healthy volunteers, to examine how these 'negative symptoms' were affected by glycine reuptake inhibitors. Chapter 7 describes a pharmacokinetic/pharmacodynamic model of this scopolamine challenge test. In chapter 8, the cannabinoid Δ9-tetrahydrocannabinol (THC) was employed to temporarily cause a mild psychotic-like state in healthy subjects, and described how this was impacted by haloperidol. Thus, physiological perturbations in healthy subjects were used to mimic psychopathophysiological aspects of schizophrenia, in order to compare the effects of novel and established treatments at an early stage of drug development.

These approaches provide clear examples of how clinical pharmacological experiments in healthy volunteers can be used to characterize the pharmacological properties of novel compounds in development for schizophrenia, which basically underlie the therapeutic innovations that current drug development in this field tries to achieve. The studies also show that the main pharmacological focus in this area has shifted from psychosis to improvement of individual negative or cognitive symptom complexes, from direct receptor inhibition to indirect receptor modulation, and from single drug strategies to combination therapies. The diversity of drug development strategies in schizophrenia and related disorders reflects the increasing complexity of neuropharmacological hypotheses in this field. Despite these difficulties, incremental changes in drug characteristics and treatment strategies may well lead to the introduction of new classes or combinations of drugs in the future.

Figure 1 Dopamine pathways thought to be involved in the pathophysiology and adverse drug reactions of schizophrenia

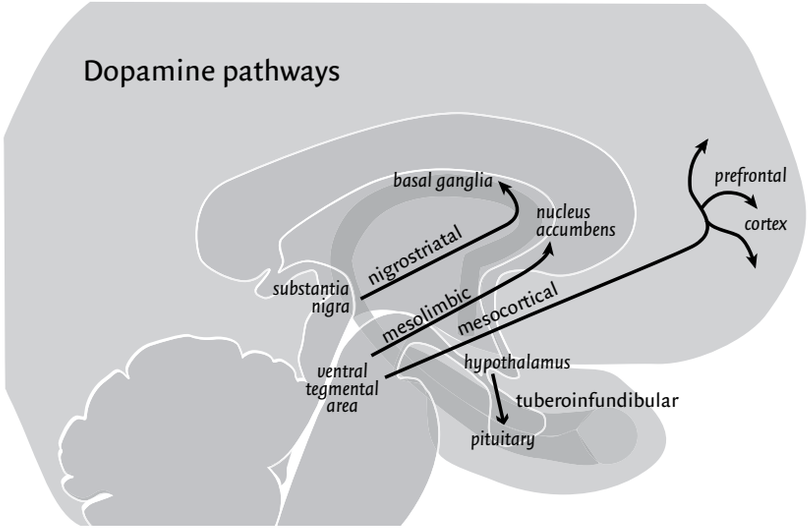


Figure 2 'Neuropharmacological combination model' of non-schizophrenic people (Glu=glutamate; $5HT_{2A}$=serotonin2A; DA=dopamine; GABA= gamma-aminobutyric acid)

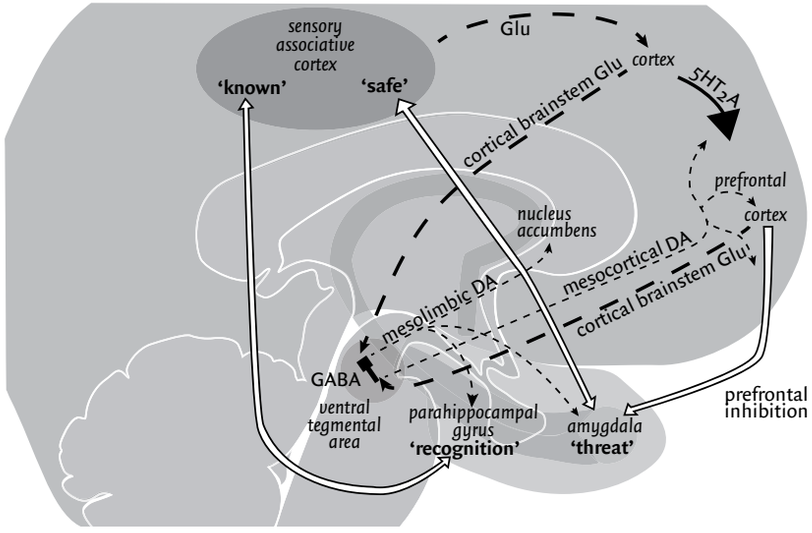
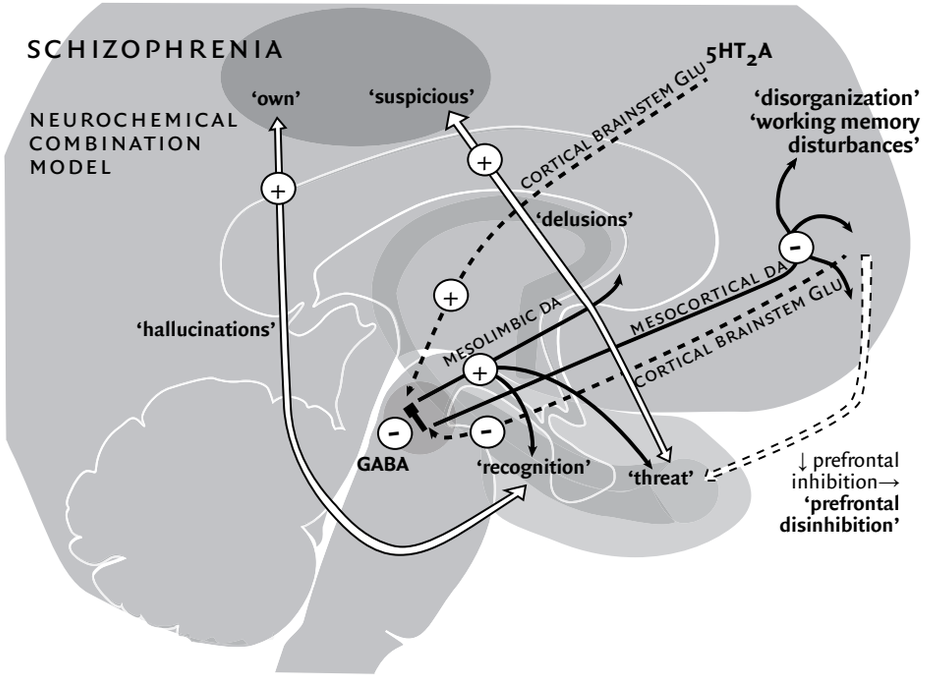


Figure 3 Visualized 'integrated neuropharmacological model' of schizophrenic patients (Glu=glutamate; $5HT_{2A}$=serotonin2A; DA=dopamine; GABA= gamma-aminobutyric acid)



REFERENCES

1 Lopez AD, Murray CC. The global burden of disease, 1990-2020. Nat Med 1998; 4: 1241-3.
2 Sawa A, Snyder SH. Schizophrenia: diverse approaches to a complex disease. Science 2002; 296: 692-5.
3 Salize HJ, McCabe R, Bullenkamp J, Hansson L, Lauber C, Martinez-Leal R, Reinhard I, Rossler W, Svensson B, Torres-Gonzalez F, van den Brink R, Wiersma D, Priebe S. Cost of treatment of schizophrenia in six European countries. Schizophr Res 2009; 111: 70-7.
4 Knapp M, Mangalore R, Simon J. The global costs of schizophrenia. Schizophr Bull 2004; 30: 279-93.
5 Dixon L, Lyles A, Smith C, Hoch JS, Fahey M, Postrado L, Lucksted A, Lehman A. Use and costs of ambulatory care services among Medicare enrollees with schizophrenia. Psychiatr Serv 2001; 52: 786-92.
6 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Washington DC. 2000.
7 Buchanan RW, Gold JM. Negative symptoms: diagnosis, treatment and prognosis. Int Clin Psychopharmacol 1996; 11 Suppl 2: 3-11.
8 Strauss JS, Carpenter WT, Jr., Bartko JJ. The diagnosis and understanding of schizophrenia. Part III. Speculations on the processes that underlie schizophrenic symptoms and signs. Schizophr Bull 1974; 61-9.
9 Meltzer HY, Park S, Kessler R. Cognition, schizophrenia, and the atypical antipsychotic drugs. Proc Natl Acad Sci U S A 1999; 96: 13591-3.
10 Kay SR, Opler LA. The positive-negative dimension in schizophrenia: its validity and significance. Psychiatr Dev 1987; 5: 79-103.
11 Stahl SM. Antipsychotics and Mood Stabilizers. Stahl's Essential Psychopharmacology. 2008; 3rd
12 Bell MD, Lysaker PH, Beam-Goulet JL, Milstein RM, Lindenmayer JP. Five-component model of schizophrenia: assessing the factorial invariance of the positive and negative syndrome scale. Psychiatry Res 1994; 52: 295-303.
13 Kay SR, Sevy S. Pyramidical model of schizophrenia. Schizophr Bull 1990; 16: 537-45.
14 Mass R, Schoemig T, Hitschfeld K, Wall E, Haasen C. Psychopathological syndromes of schizophrenia: evaluation of the dimensional structure of the positive and negative syndrome scale. Schizophr Bull 2000; 26: 167-77.
15 Peralta V, de LJ, Cuesta MJ. Are there more than two syndromes in schizophrenia? A critique of the positive-negative dichotomy. Br J Psychiatry 1992; 161: 335-43.
16 Blom JD. [A hundred years of schizophrenia: from Bleuler to DSM-V]. Tijdschr Psychiatr 2007; 49: 887-95.
17 Jones DNC, Gartlon JE, Minassian A, Perry W, Geyer MA. Developing new drugs for schizophrenia: from animals to the clinic. 2008; 1st: 199-261.
18 Andreasen NC. Schizophrenia: the fundamental questions. Brain Res Brain Res Rev 2000; 31: 106-12.
19 Jarskog LF, Miyamoto S, Lieberman JA. Schizophrenia: new pathological insights and therapies. Annu Rev Med 2007; 58: 49-61.
20 Wong AH, Van Tol HH. Schizophrenia: from phenomenology to neurobiology. Neurosci Biobehav Rev 2003; 27: 269-306.
21 Henquet C, Krabbendam L, Spauwen J, Kaplan C, Lieb R, Wittchen HU, van OJ. Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ 2005; 330: 11.
22 Henquet C, Rosa A, Krabbendam L, Papiol S, Fananas L, Drukker M, Ramaekers JG, van OJ. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology 2006; 31: 2748-57.
23 Henquet C, Di FM, Morrison P, Kuepper R, Murray RM. Gene-environment interplay between cannabis and psychosis. Schizophr Bull 2008; 34: 1111-21.
24 Arango C, Kahn R. Progressive brain changes in schizophrenia. Schizophr Bull 2008; 34: 310-1.
25 Stephan KE, Baldeweg T, Friston KJ. Synaptic plasticity and dysconnection in schizophrenia. Biol Psychiatry 2006; 59: 929-39.
26 Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH. Modelling schizophrenia using human induced pluripotent stem cells. Nature 2011.
27 Siever LJ, Davis KL. The pathophysiology of schizophrenia disorders: perspectives from the spectrum. Am J Psychiatry 2004; 161: 398-413.
28 Buckley PF, Mahadik S, Pillai A, Terry A, Jr. Neurotrophins and schizophrenia. Schizophr Res 2007; 94: 1-11.
29 Pillai A. Brain-derived neurotropic factor/TrkB signaling in the pathogenesis and novel pharmacotherapy of schizophrenia. Neurosignals 2008; 16: 183-93.
30 Stein DJ, Daniels WM, Savitz J, Harvey BH. Brain-derived neurotrophic factor: the neurotrophin hypothesis of psychopathology. CNS Spectr 2008; 13: 945-9.
31 Boonstra G, van Haren NE, Schnack HG, Cahn W, Burger H, Boersma M, de KB, Grobbee DE, Hulshoff Pol HE, Kahn RS. Brain volume changes after withdrawal of atypical antipsychotics in patients with first-episode schizophrenia. J Clin Psychopharmacol 2011; 31: 146-53.
32 Perkins DO, Johnson JL, Hamer RM, Zipursky RB, Keefe RS, Centorrhino F, Green AI, Glick IB, Kahn RS, Sharma T, Tohen M, McEvoy JP, Weiden PJ, Lieberman JA. Predictors of antipsychotic medication adherence in patients recovering from a first psychotic episode. Schizophr Res 2006; 83: 53-63.
33 McCullumsmith RE, Clinton SM, Meador-Woodruff JH. Schizophrenia as a disorder of neuroplasticity. Int Rev Neurobiol 2004; 59: 19-45.
34 Keshavan MS. Development, disease and degeneration in schizophrenia: a unitary pathophysiological model. J Psychiatr Res 1999; 33: 513-21.
35 Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 2009; 35: 549-62.
36 Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D_2 receptors. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27: 1081-90.
37 Angrist BM, Shopsin B, Gershon S. Comparative psychotomimetic effects of stereoisomers of amphetamine. Nature 1971; 234: 152-3.
38 Bell DS. The experimental reproduction of amphetamine psychosis. Arch Gen Psychiatry 1973; 29: 35-40.
39 Griffith JD, Cavanaugh J, Held J, Oates JA. Dextroamphetamine. Evaluation of psychomimetic properties in man. Arch Gen Psychiatry 1972; 26: 97-100.
40 Delay J, Deniker P, Harl JM. [Therapeutic use in psychiatry of phenothiazine of central elective action (4560 RP).]. Ann Med Psychol (Paris 1952; 110: 112-7.
41 Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse braine. Acta Pharmacol Toxicol (Copenh) 1963; 20: 140-4.
42 Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976; 192: 481-3.
43 Seeman P, Lee T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 1975; 188: 1217-9.
44 Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci U S A 1975; 72: 4376-80.
45 Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 1976; 261: 717-9.
46 Farde L, Wiesel FA, Halldin C, Sedvall G. Central D_2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 1988; 45: 71-6.
47 Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2 occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 2000; 157: 514-20.
48 Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G. Central D_2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 1993; 33: 227-35.
49 De Visser S, van der Post J, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 2001; 51: 119-32.
50 Eichenbaum H, Wiener SI, Shapiro ML, Cohen NJ. The organization of spatial coding in the hippocampus: a study of neural ensemble activity. J Neurosci 1989; 9: 2764-75.
51 Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 1998; 28: 309-69.
52 Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl 2007; 191: 391-431.
53 Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 1991; 148: 1474-86.
54 Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system

responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 1991; 41: 1-24.
55 Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001; 158: 1367-77.
56 Javitt DC. Glutamate receptors and schizophrenia: opportunities and caveats. Mol Psychiatry 1996; 1: 16-7.
57 Harrison PJ. Schizophrenia: a disorder of neurodevelopment? Curr Opin Neurobiol 1997; 7: 285-9.
58 Kapur S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. Am J Psychiatry 2003; 160: 13-23.
59 Di CG. A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. J Psychopharmacol 1998; 12: 54-67.
60 Horvitz JC. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 2000; 96: 651-6.
61 Martin-Soelch C, Leenders KL, Chevalley AF, Missimer J, Kunig G, Magyar S, Mino A, Schultz W. Reward mechanisms in the brain and their role in dependence: evidence from neurophysiological and neuroimaging studies. Brain Res Brain Res Rev 2001; 36: 139-49.
62 Clody DE, Carlton PL. Stimulus efficacy, chlorpromazine, and schizophrenia. Psychopharmacology (Berl) 1980; 69: 127-31.
63 Chouinard G, Miller R. A rating scale for psychotic symptoms (RSPS) part I: theoretical principles and subscale 1: perception symptoms (illusions and hallucinations). Schizophr Res 1999; 38: 101-22.
64 Kapur S, Remington G. Atypical antipsychotics: new directions and new challenges in the treatment of schizophrenia. Annu Rev Med 2001; 52: 503-17.
65 Kapur S, Remington G. Dopamine D(2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. Biol Psychiatry 2001; 50: 873-83.
66 Harrow M, Silverstein ML. Psychotic symptoms in schizophrenia after the acute phase. Schizophr Bull 1977; 3: 608-16.
67 Harrow M, MacDonald AW, III, Sands JR, Silverstein ML. Vulnerability to delusions over time in schizophrenia and affective disorders. Schizophr Bull 1995; 21: 95-109.
68 Gitlin M, Nuechterlein K, Subotnik KL, Ventura J, Mintz J, Fogelson DL, Bartzokis G, Aravagiri M. Clinical outcome following neuroleptic discontinuation in patients with remitted recent-onset schizophrenia. Am J Psychiatry 2001; 158: 1835-42.
69 Pratt JA, Winchester C, Egerton A, Cochran SM, Morris BJ. Modelling prefrontal cortex deficits in schizophrenia: implications for treatment. Br J Pharmacol 2008; 153 Suppl 1: S465-S470.
70 Brozoski TJ, Brown RM, Rosvold HE, Goldman PS. Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 1979; 205: 929-32.
71 Ragland JD, Yoon J, Minzenberg MJ, Carter CS. Neuroimaging of cognitive disability in schizophrenia: search for a pathophysiological mechanism. Int Rev Psychiatry 2007; 19: 417-27.
72 Bertolino A, Esposito G, Callicott JH, Mattay VS, Van Horn JD, Frank JA, Berman KF, Weinberger DR. Specific relationship between prefrontal neuronal N-acetylaspartate and activation of the working memory cortical network in schizophrenia. Am J Psychiatry 2000; 157: 26-33.
73 Benes FM, Davidson J, Bird ED. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. Arch Gen Psychiatry 1986; 43: 31-5.
74 Benes FM, McSparren J, Bird ED, SanGiovanni JP, Vincent SL. Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 1991; 48: 996-1001.
75 Glantz LA, Lewis DA. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Regional and diagnostic specificity. Arch Gen Psychiatry 1997; 54: 660-9.
76 Abi-Dargham A. Do we still believe in the dopamine hypothesis? New data bring new evidence. Int J Neuropsychopharmacol 2004; 7 Suppl 1: S1-S5.
77 Bertolino A, Knable MB, Saunders RC, Callicott JH, Kolachana B, Mattay VS, Bachevalier J, Frank JA, Egan M, Weinberger DR. The relationship between dorsolateral prefrontal N-acetylaspartate measures and striatal dopamine activity in schizophrenia. Biol Psychiatry 1999; 45: 660-7.
78 Bertolino A, Breier A, Callicott JH, Adler C, Mattay VS, Shapiro M, Frank JA, Pickar D, Weinberger DR. The relationship between dorsolateral prefrontal neuronal N-acetylaspartate and evoked release of striatal dopamine in schizophrenia. Neuropsychopharmacology 2000; 22: 125-32.
79 Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 1987; 44: 660-9.
80 Abi-Dargham A, Moore H. Prefrontal DA transmission at D_1 receptors and the pathology of schizophrenia. Neuroscientist 2003; 9: 404-16.
81 Seeman P, Kapur S. Schizophrenia: more dopamine, more D_2 receptors. Proc Natl Acad Sci U S A 2000; 97: 7673-5.
82 Abi-Dargham A, van de Giessen E, Slifstein M, Kegeles LS, Laruelle M. Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. Biol Psychiatry 2009; 65: 1091-3.
83 Abi-Dargham A, Kegeles LS, Zea-Ponce Y, Mawlawi O, Martinez D, Mitropoulou V, O'Flynn K, Koenigsberg HW, Van HR, Cooper T, Laruelle M, Siever LJ. Striatal amphetamine-induced dopamine release in patients with schizotypal personality disorder studied with single photon emission computed tomography and [123I] iodobenzamide. Biol Psychiatry 2004; 55: 1001-6.
84 Abi-Dargham A, Jaskiw G, Suddath RL, Weinberger DR. Evidence against progression of in vivo anatomical abnormalities in schizophrenia. Schizophr Res 1991; 5: 210.
85 Goldman-Rakic PS, Muly EC, III, Williams GV. D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 2000; 31: 295-301.
86 Pycock CJ, Kerwin RW, Carter CJ. Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. Nature 1980; 286: 74-6.
87 Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 2005; 10: 40-68.
88 Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, Tanzi RE, Bertram L. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 2008; 40: 827-34.
89 Glatt SJ, Faraone SV, Tsuang MT. Meta-analysis identifies an association between the dopamine D_2 receptor gene and schizophrenia. Mol Psychiatry 2003; 8: 911-5.
90 Williams HJ, Owen MJ, O'Donovan MC. Is COMT a susceptibility gene for schizophrenia? Schizophr Bull 2007; 33: 635-41.
91 Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci U S A 1998; 95: 9991-6.
92 Sesack SR, Hawrylak VA, Guido MA, Levey AI. Cellular and subcellular localization of the dopamine transporter in rat cortex. Adv Pharmacol 1998; 42: 171-4.
93 Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 2001; 98: 6917-22.
94 Barr CL, Kennedy JL, Lichter JB, Van Tol HH, Wetterberg L, Livak KJ, Kidd KK. Alleles at the dopamine D4 receptor locus do not contribute to the genetic susceptibility to schizophrenia in a large Swedish kindred. Am J Med Genet 1993; 48: 218-22.
95 Coon H, Byerley W, Holik J, Hoff M, Myles-Worsley M, Lannfelt L, Sokoloff P, Schwartz JC, Waldo M, Freedman R, . Linkage analysis of schizophrenia with five dopamine receptor genes in nine pedigrees. Am J Hum Genet 1993; 52: 327-34.
96 Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003; 33: 177-82.
97 Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. Cell Physiol Biochem 2007; 20: 687-702.
98 Glennon RA. Higher-end serotonin receptors: 5-HT_5, 5-HT_6, and 5-HT_7. J Med Chem 2003; 46: 2795-812.
99 Meltzer HY. The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 1999; 21: 106S-15S.
100 Kane JM, Freeman HL. Towards more effective antipsychotic treatment. Br J Psychiatry Suppl 1994; 22-31.
101 Cutler A, Ball S, Stahl SM. Dosing atypical antipsychotics. CNS Spectr 2008; 13: 1-16.
102 Meltzer HY, Matsubara S, Lee JC. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther 1989; 251: 238-46.
103 Meltzer HY, Matsubara S, Lee JC. The ratios of serotonin2 and dopamine2 affinities

differentiate atypical and typical antipsychotic drugs. Psychopharmacol Bull 1989; 25: 390-2.
104 Meltzer HY, McGurk SR. The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr Bull 1999; 25: 233-55.
105 Roth BL, Hanizavareh SM, Blum AE. Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. Psychopharmacology (Berl)2004; 174: 17-24.
106 Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 2003; 64: 1295-308.
107 Lieben CK, Blokland A, Sik A, Sung E, van NP, Schreiber R. The selective 5-HT_6 receptor antagonist Ro4368554 restores memory performance in cholinergic and serotonergic models of memory deficiency in the rat. Neuropsychopharmacology 2005; 30: 2169-79.
108 Meneses A, Manuel-Apolinar L, Castillo C, Castillo E. Memory consolidation and amnesia modify 5-HT_6 receptors expression in rat brain: an autoradiographic study. Behav Brain Res 2007; 178: 53-61.
109 Woolley ML, Bentley JC, Sleight AJ, Marsden CA, Fone KC. A role for 5-HT_6 receptors in retention of spatial learning in the Morris water maze. Neuropharmacology 2001; 41: 210-9.
110 Lindner MD, Hodges DB, Jr., Hogan JB, Orie AF, Corsa JA, Barten DM, Polson C, Robertson BJ, Guss VL, Gillman KW, Starrett JE, Jr., Gribkoff VK. An assessment of the effects of serotonin 6 (5-HT_6)receptor antagonists in rodent models of learning. J Pharmacol Exp Ther 2003; 307: 682-91.
111 Russell MG, Dias R. Memories are made of this (perhaps): a review of serotonin 5-HT_6 receptor ligands and their biological functions. Curr Top Med Chem 2002; 2: 643-54.
112 Marcos B, Chuang TT, Gil-Bea FJ, Ramirez MJ. Effects of 5-HT_6 receptor antagonism and cholinesterase inhibition in models of cognitive impairment in the rat. Br J Pharmacol 2008; 155: 434-40.
113 Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995; 52: 998-1007.
114 Stone JM, Morrison PD, Pilowsky LS. Glutamate and dopamine dysregulation in schizophrenia--a synthesis and selective review. J Psychopharmacol 2007; 21: 440-52.
115 Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994; 51: 199-214.
116 Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. Harv Rev Psychiatry 1999; 7: 125-43.
117 Maura G, Giardi A, Raiteri M. Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. J Pharmacol Exp Ther 1988; 247: 680-4.
118 Rowlands GF, Roberts PJ. Activation of dopamine receptors inhibits calcium-dependent glutamate release from corticostriatal terminals in vitro. Eur J Pharmacol 1980; 62: 241-2.
119 Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry 2004; 9: 984-97, 979.
120 Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. Pharmacol Biochem Behav 1997; 56: 803-7.
121 Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. Neuroreport 1999; 10: 1665-9.
122 Gardner EL. Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav 2005; 81: 263-84.
123 Laviolette SR, Grace AA. Cannabinoids Potentiate Emotional Learning Plasticity in Neurons of the Medial Prefrontal Cortex through Basolateral Amygdala Inputs. J Neurosci 2006; 26: 6458-68.
124 Fernandez-Ruiz J, Hernandez M, Ramos JA. Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther 2010; 16: e72-e91.
125 Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A. Distribution of MRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci U S A 2000; 97: 3655-60.
126 Starowicz K, Nigam S, Di M, V. Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 2007; 114: 13-33.
127 Bossong MG, van Berckel BN, Boellaard R, Zuurman L, Schuit RC, Windhorst AD, van Gerven JM, Ramsey NF, Lammertsma AA, Kahn RS. Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 2009; 34: 759-66.
128 Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, Veldic M, Zhang X, Costa E. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berl) 2005; 180: 191-205.
129 Wassef A, Baker J, Kochan LD. GABA and schizophrenia: a review of basic science and clinical studies. J Clin Psychopharmacol 2003; 23: 601-40.
130 Vinkers CH, Mirza NR, Olivier B, Kahn RS. The inhibitory GABA system as a therapeutic target for cognitive symptoms in schizophrenia: investigational agents in the pipeline. Expert Opin Investig Drugs 2010; 19: 1217-33.
131 Charych EI, Liu F, Moss SJ, Brandon NJ. $GABA_{(A)}$ receptors and their associated proteins: implications in the etiology and treatment of schizophrenia and related disorders. Neuropharmacology 2009; 57: 481-95.
132 De Haas S, Dingemanse J, Hoever P, Cohen A, van GJ. Pseudohallucinations after zolpidem intake: a case report. J Clin Psychopharmacol 2007; 27: 728-30.
133 Dolder CR, Nelson MH. Hypnosedative-induced complex behaviours : incidence, mechanisms and management. CNS Drugs 2008; 22: 1021-36.
134 Kantrowitz J, Citrome L, Javitt D. $GABA_{(B)}$ receptors, schizophrenia and sleep dysfunction: a review of the relationship and its potential clinical and therapeutic implications. CNS Drugs 2009; 23: 681-91.
135 Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B. Towards a muscarinic hypothesis of schizophrenia. Mol Psychiatry 2007; 12: 232-46.
136 Scarr E, Dean B. Muscarinic receptors: do they have a role in the pathology and treatment of schizophrenia? J Neurochem 2008; 107: 1188-95.
137 Green A, Ellis KA, Ellis J, Bartholomeusz CF, Ilic S, Croft RJ, Phan KL, Nathan PJ. Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. Pharmacol Biochem Behav 2005; 81: 575-84.
138 Koller G, Satzger W, Adam M, Wagner M, Kathmann N, Soyka M, Engel R. Effects of scopolamine on matching to sample paradigm and related tests in human subjects. Neuropsychobiology 2003; 48: 87-94.
139 Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM. Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 1988; 26: 685-700.
140 Hall ST, Puech A, Schaffler K, Wesnes K, Gamzu ER. Early clinical testing of cognition enhancers: prediction of efficacy. Pharmacopsychiatry 1990; 23 Suppl 2: 57-8.
141 Riedel WJ, Jolles J. Cognition enhancers in age-related cognitive decline. Drugs Aging 1996; 8: 245-74.
142 Vitiello B, Martin A, Hill J, Mack C, Molchan S, Martinez R, Murphy DL, Sunderland T. Cognitive and behavioral effects of cholinergic, dopaminergic, and serotonergic blockade in humans. Neuropsychopharmacology 1997; 16: 15-24.
143 Araujo JA, Chan AD, Winka LL, Seymour PA, Milgram NW. Dose-specific effects of scopolamine on canine cognition: impairment of visuospatial memory, but not visuospatial discrimination. Psychopharmacology (Berl) 2004; 175: 92-8.
144 Araujo JA, Studzinski CM, Milgram NW. Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 411-22.
145 Fishkin RJ, Ince ES, Carlezon WA, Jr., Dunn RW. D-cycloserine attenuates scopolamine-induced learning and memory deficits in rats. Behav Neural Biol 1993; 59: 150-7.
146 Matsuoka N, Aigner TG. Cholinergic-glutamatergic interactions in visual recognition memory of rhesus monkeys. Neuroreport 1996; 7: 565-8.
147 Matsuoka N, Aigner TG. D-cycloserine, a partial agonist at the glycine site coupled to N-methyl-D-aspartate receptors, improves visual recognition memory in rhesus monkeys. J Pharmacol Exp Ther 1996; 278: 891-7.
148 Miyamoto S, Duncan GE, Goff DC, Liebermann JA. Therapeutics of schizophrenia. 2002; 5: 775-807.
149 Sharif Z, Miyamoto S, Lieberman JA. Pharmacotherapy of schizophrenia. 2007; 369-95.
150 Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK.

Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 2005; 353: 1209-23.
151 Leucht S, Pitschel-Walz G, Abraham D, Kissling W. Efficacy and extrapyramidal side-effects of the new antipsychotics olanzapine, quetiapine, risperidone, and sertindole compared to conventional antipsychotics and placebo. A meta-analysis of randomized controlled trials. Schizophr Res 1999; 35: 51-68.
152 Geddes J, Freemantle N, Harrison P, Bebbington P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. BMJ 2000; 321: 1371-6.
153 Wahlbeck K, Tuunainen A, Ahokas A, Leucht S. Dropout rates in randomised antipsychotic drug trials. Psychopharmacology (Berl) 2001; 155: 230-3.
154 Davis JM, Chen N, Glick ID. A meta-analysis of the efficacy of second-generation antipsychotics. Arch Gen Psychiatry 2003; 60: 553-64.
155 Leucht S, Wahlbeck K, Hamann J, Kissling W. New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. Lancet 2003; 361: 1581-9.
156 Leucht S, Barnes TR, Kissling W, Engel RR, Correll C, Kane JM. Relapse prevention in schizophrenia with new-generation antipsychotics: a systematic review and exploratory meta-analysis of randomized, controlled trials. Am J Psychiatry 2003; 160: 1209-22.
157 Leucht S, Komossa K, Rummel-Kluge C, Corves C, Hunger H, Schmid F, Asenjo LC, Schwarz S, Davis JM. A meta-analysis of head-to-head comparisons of second-generation antipsychotics in the treatment of schizophrenia. Am J Psychiatry 2009; 166: 152-63.
158 Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 2009; 373: 31-41.
159 Wahlbeck K, Cheine M, Essali A, Adams C. Evidence of clozapine's effectiveness in schizophrenia: a systematic review and meta-analysis of randomized trials. Am J Psychiatry 1999; 156: 990-9.
160 Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B. Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. Am J Psychiatry 2001; 158: 518-26.
161 Tuunainen A, Wahlbeck K, Gilbody S. Newer atypical antipsychotic medication in comparison to clozapine: a systematic review of randomized trials. Schizophr Res 2002; 56: 1-10.
162 Sawa A, Snyder SH. Schizophrenia: neural mechanisms for novel therapies. Mol Med 2003; 9: 3-9
163 Rosenheck R, Perlick D, Bingham S, Liu-Mares W, Collins J, Warren S, Leslie D, Allan E, Campbell EC, Caroff S, Corwin J, Davis L, Douyon R, Dunn L, Evans D, Frecska E, Grabowski J, Graeber D, Herz L, Kwon K, Lawson W, Mena F, Sheikh J, Smelson D, Smith-Gamble V. Effectiveness and cost of olanzapine and haloperidol in the treatment of schizophrenia: a randomized controlled trial. JAMA 2003; 290: 2693-702.
164 Tollefson GD, Sanger TM. Negative symptoms: a path analytic approach to a double-blind, placebo- and haloperidol-controlled clinical trial with olanzapine. Am J Psychiatry 1997; 154: 466-74.
165 Lehman AF, Lieberman JA, Dixon LB, McGlashan TH, Miller AL, Perkins DO, Kreyenbuhl J. Practice guideline for the treatment of patients with schizophrenia, second edition. Am J Psychiatry 2004; 161: 1-56.
166 Keefe RS, Silva SG, Perkins DO, Lieberman JA. The effects of atypical antipsychotic drugs on neurocognitive impairment in schizophrenia: a review and meta-analysis. Schizophr Bull 1999; 25: 201-22.
167 Tollefson GD, Sanger TM, Lu Y, Thieme ME. Depressive signs and symptoms in schizophrenia: a prospective blinded trial of olanzapine and haloperidol. Arch Gen Psychiatry 1998; 55: 250-8.
168 Csernansky JG, Mahmoud R, Brenner R. A comparison of risperidone and haloperidol for the prevention of relapse in patients with schizophrenia. N Engl J Med 2002; 346: 16-22.
169 Grunder G, Hippius H, Carlsson A. The 'atypicality' of antipsychotics: a concept re-examined and re-defined. Nat Rev Drug Discov 2009; 8: 197-202.
170 Allison DB, Mentore JL, Heo M, Chandler LP, Cappelleri JC, Infante MC, Weiden PJ. Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry 1999; 156-96.
171 Wirshing DA, Wirshing WC, Kysar L, Berisford MA, Goldstein D, Pashdag J, Mintz J, Marder SR. Novel antipsychotics: comparison of weight gain liabilities. J Clin Psychiatry 1999; 60: 358-63.
172 Dawkins K, Lieberman JA, Lebowitz BD, Hsiao JK. Antipsychotics: Past and Future: National Institute of Mental Health Division of Services and Intervention Research Workshop, July 14, 1998. Schizophr Bull 1999; 25: 395-405.
173 Wirshing DA, Spellberg BJ, Erhart SM, Marder SR, Wirshing WC. Novel antipsychotics and new onset diabetes. Biol Psychiatry 1998; 44: 778-83.
174 Henderson DC, Cagliero E, Copeland PM, Borba CP, Evins E, Hayden D, Weber MT, Anderson EJ, Allison DB, Daley TB, Schoenfeld D, Goff DC. Glucose metabolism in patients with schizophrenia treated with atypical antipsychotic agents: a frequently sampled intravenous glucose tolerance test and minimal model analysis. Arch Gen Psychiatry 2005; 62: 19-28.
175 Maayan L, Vakhrusheva J, Correll CU. Effectiveness of medications used to attenuate antipsychotic-related weight gain and metabolic abnormalities: a systematic review and meta-analysis. Neuropsychopharmacology 2010; 35: 1520-30.
176 Newcomer JW. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 2005; 19 Suppl 1: 1-93.
177 Correll CU, Lencz T, Malhotra AK. Antipsychotic drugs and obesity. Trends Mol Med 2011; 17: 97-107.
178 Deng C, Weston-Green K, Huang XF. The role of histaminergic H_1 and H_3 receptors in food intake: a mechanism for atypical antipsychotic-induced weight gain? Prog Neuropsychopharmacol Biol Psychiatry 2010; 34: 1-4.
179 Kirk SL, Glazebrook J, Grayson B, Neill JC, Reynolds GP. Olanzapine-induced weight gain in the rat: role of 5-HT_{2C} and histamine H_1 receptors. Psychopharmacology (Berl) 2009; 207: 119-25.
180 Lencz T, Malhotra AK. Pharmacogenetics of antipsychotic-induced side effects. Dialogues Clin Neurosci 2009; 11: 405-15.
181 Kahn RS, Fleischhacker WW, Boter H, Davidson M, Vergouwe Y, Keet IP, Gheorghe MD, Rybakowski JK, Galderisi S, Libiger J, Hummer M, Dollfus S, Lopez-Ibor JJ, Hranov LG, Gaebel W, Peuskens J, Lindefors N, Riecher-Rossler A, Grobbee DE. Effectiveness of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: an open randomised clinical trial. Lancet 2008; 371: 1085-97.
182 Terry AV, Jr., Mahadik SP. Time-dependent cognitive deficits associated with first and second generation antipsychotics: cholinergic dysregulation as a potential mechanism. J Pharmacol Exp Ther 2007; 320: 961-8.
183 Kapur S, Seeman P. Does fast dissociation from the dopamine d(2)receptor explain the action of atypical antipsychotics?: A new hypothesis. Am J Psychiatry 2001; 158: 360-9.
184 Seeman P, Tallerico T. Rapid release of antipsychotic drugs from dopamine D_2 receptors: an explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. Am J Psychiatry 1999; 156: 876-84.
185 Seeman P, Ko F, Willeit M, McCormick P, Ginovart N. Antiparkinson concentrations of pramipexole and PHNO occupy dopamine D_2(high) and D3(high)receptors. Synapse 2005; 58: 122-8.
186 Seeman P. Atypical antipsychotics: mechanism of action. Can J Psychiatry 2002; 47: 27-38.
187 Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT_2 and D_2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999; 156: 286-93.
188 Schotte A, Janssen PF, Gommeren W, Luyten WH, Van GP, Lesage AS, De LK, Leysen JE. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology (Berl) 1996; 124: 57-73.
189 Jones HM, Pilowsky LS. Dopamine and antipsychotic drug action revisited. Br J Psychiatry 2002; 181: 271-5.
190 Gray JA, Roth BL. The pipeline and future of drug development in schizophrenia. Mol Psychiatry 2007; 12: 904-22.
191 Agid Y, Buzsaki G, Diamond DM, Frackowiak R, Giedd J, Girault JA, Grace A, Lambert JJ, Manji H, Mayberg H, Popoli M, Prochiantz A, Richter-Levin G, Somogyi P, Spedding M, Svenningsson P, Weinberger D. How can drug discovery for psychiatric disorders be improved? Nat Rev Drug Discov 2007; 6: 189-201.
192 Conn PJ, Roth BL. Opportunities and challenges of psychiatric drug discovery: roles for scientists in academic, industry, and government settings. Neuropsychopharmacology 2008; 33: 2048-60.
193 Carpenter WT, Koenig JI. The evolution of drug development in schizophrenia: past issues and future opportunities. Neuropsychopharmacology 2008; 33: 2061-79.

194 Lieberman JA, Phillips M, Gu H, Stroup S, Zhang P, Kong L, Ji Z, Koch G, Hamer RM. Atypical and conventional antipsychotic drugs in treatment-naive first-episode schizophrenia: a 52-week randomized trial of clozapine vs chlorpromazine. Neuropsychopharmacology 2003; 28: 995-1003.
195 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? Nat Rev Drug Discov 2010; 9: 856-65.
196 Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov 2004; 3: 353-9.
197 Gray JA, Roth BL. Molecular targets for treating cognitive dysfunction in schizophrenia. Schizophr Bull 2007; 33: 1100-19.
198 te Beek ET, Moerland M, de BP, van NL, de Kam ML, Burggraaf J, Cohen AF, van Gerven JM. Pharmacokinetics and central nervous system effects of the novel dopamine D_2 receptor antagonist JNJ-37822681. J Psychopharmacol 2011.
199 Quartara L, Altamura M, Evangelista S, Maggi CA. Tachykinin receptor antagonists in clinical trials. Expert Opin Investig Drugs 2009; 18: 1843-64.
200 Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 2002; 71: 533-54.
201 Hedlund PB. The 5-HT_7 receptor and disorders of the nervous system: an overview. Psychopharmacology (Berl) 2009; 206: 345-54.
202 Monsma FJ, Jr., Shen Y, Ward RP, Hamblin MW, Sibley DR. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol 1993; 43: 320-7.
203 Unsworth CD, Molinoff PB. Characterization of a 5-hydroxytryptamine receptor in mouse neuroblastoma N18TG2 cells. J Pharmacol Exp Ther 1994; 269: 246-55.
204 Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY, Sibley DR, Roth BL, Hamblin MW. Cloning, characterization, and chromosomal localization of a human 5-HT_6 serotonin receptor. J Neurochem 1996; 66: 47-56.
205 Sleight AJ, Boess FG, Bos M, Levet-Trafit B, Riemer C, Bourson A. Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT_6 receptors. Br J Pharmacol 1998; 124: 556-62.
206 Upton N, Chuang TT, Hunter AJ, Virley DJ. 5-HT_6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. Neurotherapeutics 2008; 5: 458-69.
207 Pouzet B, Didriksen M, Arnt J. Effects of the 5-HT_6 receptor antagonist, SB-271046, in animal models for schizophrenia. Pharmacol Biochem Behav 2002; 71: 635-43.
208 Geldenhuys WJ, Van der Schyf CJ. The serotonin 5-HT_6 receptor: a viable drug target for treating cognitive deficits in Alzheimer's disease. Expert Rev Neurother 2009; 9: 1073-85.
209 Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P. SB742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 2010; Epub 2010 Sep 24.
210 Keefe RS, Bilder RM, Davis SM, Harvey PD, Palmer BW, Gold JM, Meltzer HY, Green MF, Capuano G, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Davis CE, Hsiao JK, Lieberman JA. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. Arch Gen Psychiatry 2007; 64: 633-47.
211 Javitt DC. Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. Curr Opin Psychiatry 2006; 19: 151-7.
212 Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 2000; 130: 1007S-15S.
213 Rorick-Kehn LM, Johnson BG, Burkey JL, Wright RA, Calligaro DO, Marek GJ, Nisenbaum ES, Catlow JT, Kingston AE, Giera DD, Herin MF, Monn JA, McKinzie DL, Schoepp DD. Pharmacological and pharmacokinetic properties of a structurally novel, potent, and selective metabotropic glutamate 2/3 receptor agonist: in vitro characterization of agonist (-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo [3.1.0]-hexane-4,6-dicarboxylic acid (LY404039). J Pharmacol Exp Ther 2007; 321: 308-17.
214 Rorick-Kehn LM, Johnson BG, Knitowski KM, Salhoff CR, Witkin JM, Perry KW, Griffey KI, Tizzano JP, Monn JA, McKinzie DL, Schoepp DD. In vivo pharmacological characterization of the structurally novel, potent, selective mGlu2/3 receptor agonist LY404039 in animal models of psychiatric disorders. Psychopharmacology (Berl)2007; 193: 121-36.

215 Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Brain Res Rev 2000; 31: 302-12.
216 Swanson CJ, Schoepp DD. The group II metabotropic glutamate receptor agonist (-)-2-oxa-4-aminobicyclo [3.1.0.]hexane-4,6-dicarboxylate (LY379268)and clozapine reverse phencyclidine-induced behaviors in monoamine-depleted rats. J Pharmacol Exp Ther 2002; 303: 919-27.
217 Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev Bv, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 2007; 13: 1102-7.
218 Suppiramaniam V, Bahr BA, Sinnarajah S, Owens K, Rogers G, Yilma S, Vodyanoy V. Member of the Ampakine class of memory enhancers prolongs the single channel open time of reconstituted AMPA receptors. Synapse 2001; 40: 154-8
219 Rivadulla C, Sharma J, Sur M. Specific roles of NMDA and AMPA receptors in direction-selective and spatial phase-selective responses in visual cortex. J Neurosci 2001; 21: 1710-9.
220 Goff DC, Lamberti JS, Leon AC, Green MF, Miller AL, Patel J, Manschreck T, Freudenreich O, Johnson SA. A placebo-controlled add-on trial of the Ampakine, CX516, for cognitive deficits in schizophrenia. Neuropsychopharmacology 2008; 33: 465-72.
221 Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997; 17: 2921-7.
222 Javitt DC. Glycine modulators in schizophrenia. Curr Opin Investig Drugs 2002; 3: 1067-72.
223 Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, Hayden DL, McCarley R, Coyle JT. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry 1999; 56: 21-7.
224 Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 2005; 62: 1196-204.
225 Kemp JA, McKernan RM. NMDA receptor pathways as drug targets. Nat Neurosci 2002; 5 Suppl: 1039-42.
226 Aragon C, Lopez-Corcuera B. Glycine transporters: crucial roles of pharmacological interest revealed by gene deletion. Trends Pharmacol Sci 2005; 26: 283-6.
227 Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 1992; 8: 927-35.
228 Chen L, Muhlhauser M, Yang CR. Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. J Neurophysiol 2003; 89: 691-703.
229 Depoortere R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, Poncelet M, Heaulme M, Santucci V, Decobert M, Cudennec A, Voltz C, Boulay D, Terranova JP, Stemmelin J, Roger P, Marabout B, Sevrin M, Vige X, Biton B, Steinberg R, Francon D, Alonso R, Avenet P, Oury-Donat F, Perrault G, Griebel G, George P, Soubrie P, Scatton B. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology 2005; 30: 1963-85.
230 Harsing LG, Jr., Gacsalyi I, Szabo G, Schmidt E, Sziray N, Sebban C, Tesolin-Decros B, Matyus P, Egyed A, Spedding M, Levay G. The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav 2003; 74: 811-25.
231 Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2004; 55: 452-6.
232 Parolaro D, Realini N, Vigano D, Guidali C, Rubino T. The endocannabinoid system and psychiatric disorders. Exp Neurol 2010; 224: 3-14.
233 Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Braz J Med Biol Res 2006; 39: 421-9.
234 Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology (Berl) 1982; 76: 245-50.

235 Zuardi AW, Rodrigues JA, Cunha JM. Effects of cannabidiol in animal models predictive of antipsychotic activity. Psychopharmacology (Berl) 1991; 104: 260-4.
236 Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Rev Bras Psiquiatr 2008; 30: 271-80.
237 Leweke F, Koethe D, Gerth CW, Nolden BM, Schreiber D, Gross S, Kranaster L, Hoyer C, Schultzelutter F, Hellmich M, Pukrop R, Klosterkotter J. Double blind, controlled clinical trial of cannabidiol monotherapy versus amisulpiride in the treatment of acutely psychotic schizophrenia patients. Schizophr Bull 2007; 33: 310.
238 Meltzer HY, Arvanitis L, Bauer D, Rein W. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. Am J Psychiatry 2004; 161: 975-84.

CHAPTER 2

CENTRAL NERVOUS SYSTEM EFFECTS OF THE INTERACTION BETWEEN RISPERIDONE (SINGLE DOSE) AND THE 5-HT$_6$ ANTAGONIST SB742457 (REPEATED DOSES) IN HEALTHY MEN

M Liem-Moolenaar[1], M Rad[1], S Zamuner[2], AF Cohen[1], F Lemme[3], KL Franson[1], JMA van Gerven[1] & EM Pich[4]

1 Centre for Human Drug Research, Leiden, the Netherlands
2 Clinical Pharmacokinetics, Modelling and Simulation
3 Biometrics Medicine, Psychiatry CEDD, Medicine Centre, Verona, Italy
4 Discovery Medicine, Psychiatry CEDD, Medicine Centre, Verona, Italy

BR J CLIN PHARMACOL 2011; 71: 907-16

Abstract

AIM Several lines of evidence suggest a possible role of 5-HT$_6$ receptor antagonists in cognitive dysfunction of schizophrenia. Atypical antipsychotics, such as risperidone, are currently used in these disorders. Therefore, the pharmacological interactions between the 5-HT$_6$ antagonist SB742457 and risperidone were investigated in the light of possible co-medication.

METHODS A randomized, double-blind, two-way crossover design was used to study the interaction between multiple doses SB742457 50 mg and a single dose risperidone 2 mg in 18 healthy subjects.

RESULTS Treatment was well tolerated. The most common adverse event was somnolence in 83% during the combination *vs.* 50% of subjects after risperidone, 32% after placebo and 11% after SB742457. Combination treatment produced a statistically significant increase in the maximum plasma concentration of risperidone and had no effect on SB742457 pharmacokinetics. Risperidone decreased saccadic peak velocity, finger tapping, adaptive tracking, subjective alertness, delayed word recognition and body sway and increased electroencephalogram (EEG) theta power and prolactin. The only pharmacodynamic interaction of risperidone and SB742457 was an increase of absolute EEG alpha (ratio = 1.25, 95% CI = 1.11, 1.40, $P = 0.0004$) and beta power (ratio = 1.14, 95% CI = 1.03, 1.27, $P = 0.016$). No significant effects of SB742457 alone were found.

CONCLUSION The pharmacokinetic interactions between SB742457 and risperidone detected in this study were not clinically relevant. The increase in EEG alpha and beta power is incompatible with enhanced risperidone activity, but could point to mild arousing effects of the combination. Most pharmacodynamic changes of risperidone are consistent with previously reported data. The potential cognitive effects of SB742457 remain to be established.

Introduction

Several recent lines of evidence have suggested a role of 5-hydroxytryptamine 6 (5-HT$_6$) receptors in cognitive and memory processes. Improvements in memory and other aspects of cognition have been reported using different 5-HT$_6$ antagonists, both in healthy animals and in animal models of cognitive impairment [1-7]. Several research groups have suggested that 5-HT$_6$ blockade may be involved in learning and memory via increased cholinergic transmission [5, 7-10] or modulation of dopaminergic transmission [11], but secondary changes in noradrenergic and glutamatergic neurotransmission may also be involved [12, 13]. Preliminary data from studies in patients with Alzheimer's disease (AD) suggest that the beneficial effects of treatments with 5-HT$_6$ receptor antagonists seen in animal models may translate into humans [14, 15].

In schizophrenia, the role of the 5-HT$_6$ receptor is less well defined. There is post-mortem evidence of reduced expression of the 5-HT$_6$ receptor in the hippocampus of schizophrenic patients [16]. Some of the most effective antipsychotic drugs partially bind to 5-HT$_6$ receptors, and 5-HT$_6$ receptors have been shown to be down-regulated by prolonged clozapine treatment in rats [17]. However, it is unknown if the reduced expression of 5-HT$_6$ receptors is due to the disease or chronic treatment.

At present, over a dozen selective 5-HT$_6$ antagonists are at various stages of development [14]. SB742457 is a potent 5-HT$_6$ antagonist (pKi = 9.6) with high affinity for human 5-HT$_{2A}$ receptors (pKi = 8.0; for structural formula see Figure 1). SB742457 has shown efficacy in different animal models of cognitive impairment [14]. In humans, reports that SB742457 is of clinical benefit in AD patients provided further evidence of the therapeutic potential of this approach [18, 19]. Repeated-dose studies in healthy subjects receiving daily 50 mg showed low occurrence of mild adverse events (AEs), mostly headache. At this dose, the exposure to SB742457 is expected to deliver 5-HT$_6$ receptor occupancy of the central nervous system (CNS) above 90% (unpublished data). Clinical pharmacokinetic assessment showed that SB742457 has a half-life of approximately 30 h, reaching steady state after 7 days with an accumulation ratio of

about fourfold (unpublished data and [14]). Preclinical investigations showed that SB742457 is a moderate inhibitor of CYP450 3A4.

For schizophrenia, SB742457 would be considered for development as an add-on treatment to be used in combination with antipsychotic drugs (e.g. risperidone), known for their lack of clinical effect on cognition [20]. Risperidone is a dopamine 2 (D_2)/5-HT_{2A} antagonist with low affinity for 5-HT_6 receptors and limited effects on cognitive parameters [21] and is commonly used to control agitation and psychotic features. Therefore, the combination of risperidone and SB742457 may constitute a reasonable combination in cognitively impaired patients. Risperidone is known to produce a series of CNS effects (such as sedation, increased theta band power of the electroencephalogram (EEG) spectra and increased prolactin concentrations) at doses of 1-2 mg in healthy volunteers [22]. It is primarily metabolized by CYP2D6, but CYP3A4 is also involved [23]. The main metabolite of risperidone is 9-hydroxyrisperidone, an active compound with a half-life of approximately 20 h [24]. The well-known profile of CNS effects may contribute to the cognitive impairment and the negative syndrome complex in some patients. As 5-HT_6 activity modulates dopaminergic transmission [11], it is hypothesized that some of the CNS effects due to neuroleptic agents are partially reversed by a 5-HT_6 antagonist like SB742457. In this study the pharmacokinetic and pharmacodynamic effects of the interaction between SB742457 and risperidone and of SB742457 and risperidone alone were investigated in healthy volunteers. In this early stage of development of SB742457, the pharmacodynamic effects had not been examined in humans, and its effects on risperidone could not be accurately predicted. Therefore, a multimodal test battery was used repeatedly, consisting of validated neurophysiological and neuropsychological tests. These tests have no direct bearing on schizophrenia and they only partly reflect the negative cognitive and behavioural) effects of this condition (and the positive psychotic effects even less). However the battery accurately covers most drug-responsive CNS-functional domains, and therefore had a large chance of demonstrating pharmacodynamic changes induced by risperidone or SB742457 alone, or the effects of their combination.

Methods

Volunteers

Twenty-four healthy male volunteers aged between 18 and 38 years with a body weight above 50 kg and a body mass index between 18.5-29.9 kg m^{-2} were recruited for the study, with the aim of completing all treatments in at least 18 subjects. Subjects were considered 'healthy' by a responsible study physician, when no clinically significant abnormalities were identified on the medical or laboratory evaluation (haematology, biochemistry, virology, urinalysis and urine drug screen), blood pressure and heart rate or 12-lead ECG before the study starts medical. Exclusion criteria included the use of agents known to affect CNS functions (including drug or alcohol use), smoking more than five cigarettes a day and unable to refrain from smoking during the stay in the research unit. The Ethics Review Board of the Leiden University Medical Centre approved the study protocol. Written informed consent was obtained from all volunteers following a written and oral explanation.

Study design

This was a randomized, double-blind, double-dummy placebo controlled crossover study, consisting of two 11-day multiple dosing periods for either SB742457 50 mg or placebo (see Figure 2). On days 8 and 9 of each period, a single dose of risperidone 2 mg or placebo was administered in a balanced randomized crossover fashion. In this way, the effects of risperidone (either alone or with SB742457) could be examined after acute administration (on day 8 or on day 9 after placebo treatment on the preceding day). There was at least 1 week washout between the two multiple-dose periods. Subjects received SB742457 from day 1 until day 11. They visited the research centre in the morning on days 1 to 7 and days 11 to 14 and remained in house from the morning of day 8 until the morning of day 10. Both SB742457 (capsule) and risperidone (tablet) or their matching placebos were administered once daily with a glass of water.

Before administration of the study medication all participants were instructed to remain fasted. Smoking was not allowed during the study days, volunteers refrained from alcohol and xanthine-containing foods or beverages from 24 h before each study period until day 12, and grapefruit products were not allowed from 14 days prior to the study until the end of the study.

Pharmacokinetics

Blood samples (approximately 3 ml) were obtained pre-dose on days 6 and 7 (for SB742457 measurements only), and days 10, 11 and 12 (for SB742457, risperidone and 9-hydroxyrisperidone measurements). On days 8 and 9, blood samples were taken for SB742457, risperidone and 9-hydroxyrisperidone at baseline and 45, 80, 140, 190, 235, 345 min and 8 and 12 h after administration of both drugs.

Plasma samples were analysed for SB742457 by Drug Metabolism and Pharmacokinetics,GlaxoSmithKline,Ware, UK, using a validated analytical method based on protein precipitation, followed by high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analysis. The lower limit of quantification (LLQ) for SB742457 was 1 ng ml^{-1}, using a 50 ml aliquot of EDTA plasma with a higher limit of quantification (HLQ) of 5000 ng ml^{-1}. Plasma samples were analysed for risperidone and 9-hydroxyrisperidone by York Bio-analytical Solutions, York, UK, using a validated analytical method based on protein precipitation, followed by HPLC-MS/MS analysis.The LLQ for both risperidone and 9-hydroxyrisperidone was 0.1 ng ml^{-1},using a 50 ml aliquot of human plasma with a HLQ of 100 ng ml^{-1} [25].

Quality control (QC) samples, prepared at three different analyte concentrations and stored with study samples, were analysed with each batch of samples against separately prepared calibration standards. For the analysis to be considered acceptable, no more than one-third of the QC results deviated from the nominal concentration by more than 15%, and at least 50% of the results from each QC concentration were within 15% of nominal. The applicable analytical runs m*et a*ll predefined run acceptance criteria.

Pharmacodynamics

On days 8 and 9, a pharmacodynamic test battery was performed twice at baseline and 45, 80, 140, 190, 235, 295, 345 min and 8 and 12 h after administration of both drugs. The battery takes about 20 min and consists of the pharmacodynamic assessments described below, which were performed in a quiet room with subdued light with one volunteer per room. No more than 1 week before the start of the study, the volunteers were familiarized with the test procedures during a training session.

PHARMACO-ELECTROENCEPHALOGRAPHY

Pharmaco-EEG was performed as a general measure of CNS activity [26]. The literature suggests that antipsychotics produce distinct profiles of EEG changes [27, 28]. EEG recordings were made using silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz (international 10/20 system), as previously described [29]. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta-(0.5-3.5 Hz), theta-(3.5-7.5 Hz), alpha-(7.5 -11.5 Hz) and beta-(11.5-30 Hz) frequency ranges. The square root of the total power (μV) was analyzed. The duration of one EEG recording was 64 s (i.e. eight blocks of 8 s).

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS

Eye movements were recorded as previously described [30, 31] and have shown effects with many different CNS active compounds, including GABA-ergic [22, 32, 33], serotonergic [34], noradrenergic [35-37] and dopaminergic agents [22, 29]. For saccadic eye movements, the average values of latency (reaction time), saccadic peak velocity and inaccuracy of all artifact free saccades were calculated. The average percentage of smooth pursuit for all stimulus frequencies was used.

ADAPTIVE TRACKING

The adaptive tracking test has proved to be useful for measurement of the CNS effects of alcohol [38], various psychoactive drugs [26, 39] and sleep deprivation [30, 38]. It measures visuo-motor coordination and vigilance and was performed as originally described by Borland [39],

using customized equipment and software (Hobbs, 2000, Hertfordshire, UK). The average performance scores over a 3-min period were used for analysis, as described previously [29].

BODY SWAY

Changes in body sway have been seen for many different CNS active drugs, including GABA-ergic compounds [32, 33], dopaminergic agents [29] and tetrahydrocannabinol (THC) [40]. The body sway allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxiameter [28]. With a string attached to the waist, all body movements over a period of 2 min were integrated and expressed as millimetre sway on a digital display. Measurements of body sway were made in the sagittal planes. All assessments were made with the eyes closed, standing with feet 10 cm apart wearing comfortable low-heeled shoes. The total amount of movement was used for statistical analysis.

SUBJECTIVE ASSESSMENTS

Visual analogue scales (VAS) consist of 100 mm line segments. Subjects put a mark on a point on the line that best represents their subjective state corresponding to the condition tested. The result is a distance (mm) calculated from the mark on the line.

Subjective effects were quantified using a Dutch translation of the 16 VAS originally described by Norris [41].They have been used previously to quantify subjective effects of a variety of agents, including sedative [32, 33], dopaminergic drugs [22, 29], scopolamine (Centre for Human Drug Research data on file) and THC [40]. From these measurements, three factors are derived as described by Bond and Lader [42], corresponding to alertness, mood and calmness [32, 33, 43]. A lower score on these scales indicates sedation, excitation and decrease in mood (or contentedness) respectively.

A translated version of the Bowdle psychotomimetic VAS [44] showed effects of THC [40], zolpidem [43] and scopolamine (Centre for Human Drug Research data on file). The lowest extreme is '0', signifying complete absence of the state (which is the case under normal circumstances), the highest the 'most extreme state imaginable'. The VAS scores were performed electronically using custom-written and validated E-prime scripts (http:// www.pstnet.com/eprime.cfm).

FINGER TAPPING

The finger tapping test was adapted from the Halstead ReitanTest Battery [45].The test evaluates motor activation and fluency. Speed of finger tapping was measured for the index finger of the dominant hand; a session contained five performances of 10 s. The volunteer was instructed to tap as quickly as possible on the space bar of a keyboard. The mean tapping rate and the standard deviations are used for statistical analysis.

VISUAL VERBAL LEARNING TEST

Memory includes many different components of learning behaviour, such as acquisition, consolidation, storage and retrieval. The Visual Verbal Learning Test (VVLT) contains three different subtests that cover most of the scope of learning behaviour, i.e. immediate and delayed word recall and a delayed word recognition [46].This test is a modified version of the auditory verbal learning test [47] in which 30 words are shown. This test is known to be sensitive to the CNS effects of various compounds such as benzodiazepines [32], cannabinoids [40] and scopolamine [48]. The outcome measures for the immediate and delayed word recall were the average and the maximum number of correct responses. For the delayed word recognition, the number of correct items and mean response time for correct responses were recorded.

PLASMA PROLACTIN CONCENTRATIONS

Prolactin increase induced by antipsychotics is closely related to D_2-receptor antagonism [22]. Prolactin concentrations were measured predose and at 30, 45, 60, 80, 140, 190, 235, 345 min and 8 h after administration of both drugs as described previously. For this purpose, blood samples were collected in plain 3-ml tubes and kept at room temperature for 30 to 45 min. Serum was separated by refrigerated centrifugation

(2000 *g* at 4°C for 10 min) within 1 h of collection and transferred to polypropylene tubes. Serum specimens were stored at approximately -20°C until analysis. The hormone assays were performed by the Central Clinical Chemistry Laboratory of the Leiden University Medical Center and were performed by electrochemiluminescence immunoassay on a Modular Analytics E170 (Elecsys module) immunoassay analyser. The assay had a LLQ of 0.047 mgl^{-1}, an intra-assay precision (expressed as coefficient of variation) of 1.81-1.90% and inter-assay precision of 2.39-2.64%.

STATISTICAL ANALYSIS

PHARMACOKINETIC ANALYSIS Pharmacokinetic analyses of plasma SB742457, risperidone, 9-hydroxyrisperidone and total risperidone active moiety concentration-time data were conducted using non-compartmental methods.

Main pharmacokinetic endpoints were maximum observed concentration (C_{MAX}) and area under the plasma concentration-time curve up to last time point [$AUC_{(0,T)}$] and extrapolated to infinity [$AUC_{(0,\infty)}$] of risperidone and 9-hydroxy-risperidone, while C_{MAX} and AUC over the dosing interval were evaluated for SB742457. Plasma concentration-time data were evaluated by standard non-compartmental analysis using WinNonlin Professional Version.

The study sample size was based on feasibility. A variability estimate of 0.32 for $AUC_{(0,\infty)}$ of risperidone was taken from published results for a risperidone/venlafaxine interaction study [49]. With such variability, it was estimated that with 16 evaluable subjects (of the 20 recruited) completing the study, the lower and upper limits of the 90% confidence interval (CI) for the ratio of geometric means (ratio of SB742457 + risperidone and placebo + risperidone) would have been within 20.9% of the point estimate. In practice, assuming a true ratio of 1, this precision would have lead to a 90% CI of 0.83 to 1.21.

Following log transformation, total risperidone (active moiety) $AUC_{(0,T)}$, $AUC_{(0,\infty)}$ and C_{MAX} and SB742457 $AUC_{(0,T)}$ and C_{MAX} were analysed using a mixed effects model with session, day, regimen and regimen X day as fixed effects and subject as a random effect. Point estimates and corresponding 90% CIs for the differences between risperidone in presence of SB742457 compared with risperidone in presence of placebo were obtained using the residual variance from ANOVA. These data were then exponentially back-transformed to give estimates of the ratios of geometric means and 90% CIs. Lack of drug interaction between SB742457 and risperidone would have been demonstrated if the 90% CI was completely contained within 0.80, 1.25.

PHARMACODYNAMIC ANALYSIS This analysis was exploratory and the formal power estimate was performed according to pharmacokinetic criteria (see above). However, past experience at the study site and published information on EEGs indicated that pharmacodynamic signals could be seen using 8-12 subjects. All endpoints were analysed using an ANOVA model, which was fitted using PROC MIXED in SAS. For VVLT and prolactin endpoints, the model included session, day, regimen, day x regimen, and, when available, baseline as fixed effect terms and subjects as a random effect term. For all other endpoints, the model included session, day, regimen, time, day x regimen, time x regimen and day x time x regimen as fixed effect terms and subjects and subject x session x day as random effect terms.

The following Least Square Means differences were computed to investigate the related treatment effect:
Risperidone effects: placebo SB742457 + risperidone (day 9) vs. placebo SB742457 + placebo risperidone (day 8).
SB-742457 effects: SB742457 + placebo risperidone (day 8) vs. placebo SB742457 + placebo risperidone (day 8).
Effects of SB742457 co-administration on risperidone effects: SB742457 + risperidone (days 8 and 9) vs. placebo SB742457 + risperidone (days 8 and 9).

No correction for multiple comparisons among the various endpoints was performed as this analysis was considered exploratory.

Results

Study population

Twenty-four volunteers were included in the study and six volunteers were withdrawn from the study, resulting in 18 completers. Three subjects withdrew for non drug-related AEs, one for protocol violation, one for personal reasons and one because of a rash (during placebo, see below). Volunteers had a mean (min-max) age of 24.8 (18-38) years, were healthy and took no relevant concomitant medications.

Tolerability

No clinically significant changes were observed for vital signs, respiratory functions, physical examination or laboratory parameters. There were no serious AEs in this trial.The reported AEsare shown in Table 1.The AEs coded as'possibly related to the study medication'were of mild to moderate intensity and resolved spontaneously. The most frequently reported AE, irrespective of causality, was somnolence. More subjects experienced somnolence following SB742457 in combination with risperidone (83%) compared with risperidone alone (50%). On days 8 and 9, somnolence was reported by three subjects (16%) receiving placebo and by two (11%) receiving SB742457 alone. One subject, after exposure to placebo SB742457 for 5 days, was withdrawn from the study because of the occurrence of a papular rash on chest, back, hands and arms. It was not associated with any out-of-range liver enzyme or other laboratory values and resolved without treatment after 11 days. Overall, SB742457 50 mg was generally well tolerated when administered orally once daily for 11 days, and also when administered at steady state in combination with a single 2-mg oral dose of risperidone.

Pharmacokinetic results

The peak plasma concentration of risperidone was 15 ng ml^{-1} at 2.3-2.7 h with an elimination half-life of approximately 4h. Following oral co-administration of SB742457 (50 mg) at steady state with a single dose of risperidone, mean increases in C_{MAX} were estimated for total risperidone active moiety (15%), risperidone (19%) and 9-hydroxyrisperidone (6%) compared with placebo (see Figure 3). The ratio (and 90% CIs) for C_{MAX} were 1.19 (1.04, 1.35) for risperidone, 1.15 (1.02, 1.28) for the total risperidone active moiety and 1.06 (0.96, 1.17) for 9-hydroxyrisperidone. No substantial increases in AUC were found. Overall, co-administration of risperidone with steady state SB742457 did not alter the pharmacokinetics of SB742457 compared with placebo.

Pharmacodynamic results

RISPERIDONE EFFECTS

Risperidone caused a considerable number of effects on subjective, neurophysiological and performance parameters. Compared with placebo, risperidone substantially decreased saccadic peak velocity, finger tapping, adaptive tracking, delayed word recognition and body sway, while subjective alertness and contentedness deteriorated (as assessed by the VAS Bond & Lader) and increased EEG theta power and prolactin concentrations (differences, 95% CI, and P values are shown in Table 2).

SB-742457 EFFECTS

When measured after single doses of risperidone or placebo on both days 8 and 9, no statistically significant differences were detected on any of the pharmacodynamic parameters between subjects daily exposed to 2 weeks of placebo and SB742457 (data not shown).

EFFECTS OF SB742457 CO-ADMINISTRATION ON RISPERIDONE EFFECTS

A single dose of risperidone in subjects during multiple daily doses of SB742457 produced significant increases of absolute alpha Pz-Oz and

beta Pz-Oz power compared with placebo SB742457, while no difference was observed in other endpoints (i.e. saccadic peak velocity,smooth pursuit eye movement,finger tapping, adaptive tracking, VAS Bond & Lader, VVLT, body sway, prolactin serum concentrations and other EEG measures; all values are shown in Table 3).

Discussion

The 5-HT_6 antagonist SB742457 is under development as a possible treatment for the cognitive symptoms in AD and possibly in schizophrenia. This study was set up to evaluate the pharmacokinetic and CNS interactions between SB742457 and risperidone, as this 5-HT_6 antagonist may be used as an add-on therapy in combination with atypical antipsychotics, which may contribute to reduce cognitive impairment in some schizophrenic patients.

The results indicate that co-administration of SB742457 with risperidone did not alter AUC (all AUC 90% CIs were contained within the 0.80, 1.25 equivalence interval). There was a minor increase in peak exposure (C_{MAX})of the total risperidone active moiety (15%), which was caused by an elevation of risperidone concentrations (19%) without a change of the active metabolite 9-hydroxyrisperidone. This could be related to an inhibition by SB742457 of CYP450 3A4, which is one of the cytochrome P450 systems involved in the metabolism of risperidone [23]. Although the differences in C_{MAX} were statistically significant for risperidone and the active moiety, the extent of the increase was very modest and, taking into consideration the inter-subject variability, does not appear to be of any clinical relevance.

Risperidone produced its expected AE profile [50], while SB742457 50 mg was well tolerated when administered orally once daily for 11 days either alone or in combination with a single 2-mg oral dose of risperidone. The most frequently reported AE, irrespective of causality, was somnolence, which occurred in a greater proportion of subjects in the presence of risperidone (with or without active SB742457) than with placebo. However, the numbers of events were too small to draw strong conclusions.

A battery of quantitative CNS tests was used to assess the pharmacodynamic interaction between risperidone and SB742457 in healthy volunteers. These tests were chosen for their sensitivity to classic antipsychotic agents as well as to a wide range of other CNS active drugs. Repeated daily exposure to 50-mg SB742457 did not produce any detectable effects on any of the pharmacodynamic CNS tests when compared with placebo. Additionally, this study yielded an extensive multidimensional pharmacodynamic profile of risperidone in healthy volunteers, showing that this antipsychotic suppresses motor performance (eye-hand coordination, finger tapping and postural stability), alertness, memory and neurophysiological functions (saccadic eye movements and EEG power spectrum). Several of these effects confirm the effects of risperidone found in previous studies in healthy volunteers: decreased behavioural and cognitive performance, increased theta band power of the EEG spectra, decreased saccadic peak velocity and increased prolactin concentrations [50-53]. Although the observed increase in EEG theta power agrees with the effects described by De Visser *et al* [22], a decrease in EEG alpha and an increase in delta power could also have been expected. When risperidone was administered to subjects exposed to daily SB742457, the effects were generally similar to those exposed to daily placebo, except that SB742457 combined with risperidone caused a significant increase of EEG alpha and beta power, compared with risperidone alone. Although it is difficult to assign functional significance to EEG changes, increases in EEG alpha or beta power are not typically because of sedation and have been associated with internally directed attention and increased mental load [54]. This result could be interpreted as mild subclinical arousing activity of SB742457 in the presence of risperidone. Although this finding clearly does not constitute definitive proof, this could be considered as an indication for modulation of dopaminergic hypofunctionality by 5-HT_6 antagonism.

The effects were too limited to be certain of pharmacological interactions between SB742457 and risperidone. This could be related to the unknown sensitivity of this study to detect subtle pharmacodynamic effects, because the study was powered on the pharmacokinetic

outcomes. However, in other studies with similar population sizes, a similar CNS battery detected mild enhancing effects of other serotonergic agents with diverse pharmacological characteristics and for various receptor subtypes [34, 55, 56]. It is also possible that the administered dose of SB742457 did not have any beneficial pharmacodynamic effects by itself, because of ceiling effects in healthy subjects, or functional compensation of 5-HT_6 receptor inhibition in this population.

In conclusion, there was no clinically relevant pharmacokinetic drug-drug interaction between SB742457 50 mg and risperidone 2 mg. Repeated dosing with SB742457 did not increase AEs or cause any pharmacodynamic effects in healthy young males, whereas a single dose of risperidone produced the expected profile of (side) effects. In general, the combination of SB742457 and risperidone did not affect CNS function more than risperidone alone.The only statistically significant pharmacodynamic interaction was an increase of EEG alpha and beta bands, suggesting a mild arousing activity of SB742457 on some CNS-depressive effects caused by risperidone, possibly mediated by 5-HT_6 receptors. The pharmacological or functional significance of these findings remains to be determined, although these interactions might indicate that SB742457 penetrates the blood-brain barrier and modifies some effects of an antipsychotic drug.

Table 1 Adverse events reported on days 8 and 9 after SB742457, risperidone, the combination of SB742457 and risperidone, and placebo

most frequent AEs (at least 2 events)	SB742457	risperidone	SB742457 + risperidone	placebo
	N = 19	N = 20	N = 18	N = 19
	n (%)	n (%)	n (%)	n (%)
somnolence	2 (11)	10 (50)	15 (83)	3 (16)
headache	1 (5)	2 (10)	2 (11)	3 (16)
dizziness	0 (0)	8 (40)	4 (22)	2 (9)
disturbance in attention	1 (5)	3 (15)	1 (6)	0 (0)
nausea	0 (0)	2 (10)	0 (0)	2 (9)
fatigue	2 (11)	5 (25)	3 (17)	2 (9)

Table 2 Pharmacodynamic cross-over effects of risperidone versus placebo risperidone (single dose) in subjects daily exposed to placebo SB742457 (multiple dose)

parameter	placebo SB742457 + risperidone (day 9)[A]	placebo SB742457 + placebo risperidone (day 8)[A]	difference (95% CI)	P-value[C]
saccadic eye movement				
peak velocity (deg/sec)	405.1	475.8	-70.7 (-91.3, -50.1)	<0.0001
smooth pursuit eye movement (%)	47.5	52.7	-5.2 (-10.5, 0.1)	0.0562
finger tapping				
average (taps/10sec)	58.0	63.2	-5.2 (-7.8, -2.6)	0.0002
adaptive tracking				
average (%)	17.6	22.4	-4.8 (-7.1, -2.5)	0.0001
VAS[B] – Bond and Lader				
alertness (mm)	55.7	44.0	11.8 (2.7, 20.8)	0.0124
contentedness (mm)	43.3	35.5	7.8 (1.4, 14.2)	0.0178
Visual Verbal Learning Test				
average reaction time correct recognitions (msec)	840.12	945.89	-105.77 (-203.95, -7.59)	0.0353
body sway (mm)	372.8	301.0	ratio 1.24 (1.07, 1.44)	0.0058
Electroencephalogramc				
absolute power theta Fz-Cz (μV)	2.56	2.19	ratio 1.17 (1.03, 1.33)	0.0175
absolute power theta Pz-Oz (μV)	2.72	2.34	ratio 1.16 (1.02, 1.33)	0.0299
prolactin serum level				
AUC (0.5-8) (h*ng/mL)	179.88	41.30	4.36 (3.65, 5.19)	<0.0001
C_{MAX} (ng/mL)	53.12	8.19	6.48 (5.25, 8.01)	<0.0001

A LSM = Least Square Means; B VAS = Visual Analogue Scale; C only (almost) statistically significant values are reported

Table 3 Results of statistical analysis of pharmacodynamic effects of SB742457 multiple dose on risperidone single dose (estimated adjusted differences with 95%CI)

parameter	SB742457 + risperidone (days 8 + 9)[A]	placebo + risperidone (days 8 + 9) [A]	difference (95% confidence interval)	P-value
saccadic eye movement				
inaccuracy (%)	6.3	6.6	-0.3 (-1.0, 0.5)	0.4613
peak velocity (deg/sec)	406.4	400.9	5.5(-9.3, 20.3)	0.4616
latency (sec)	0.221	0.22	0.001 (-0.006, 0.008)	0.7733
smooth pursuit eye movement (%)	45.6	43.8	1.8 (-2.0, 5.6)	0.3411
finger tapping				
average (taps/10sec)	59.5	57.8	1.7 (-0.2, 3.5)	0.0759
standard deviation (taps/10sec)	3.2	3.5	-0.4 (-0.9, 0.2)	0.2211
adaptive tracking				
average (%)	16.5	15.7	0.8 (-0.8, 2.5)	0.3204
standard deviation (%)	2.9	3.1	-0.2 (-0.5, 0.1)	0.1823
VAS[B] – Bond and Lader				
alertness (mm)	44.2	45.2	-1.0 (-7.5, 5.5)	0.7591
calmness (mm)	31	29.9	1.1 (-5.2, 7.4)	0.7206
contentedness (mm)	32.8	34.1	-1.3 (-5.9, 3.3)	0.563
Visual Verbal Learning Test				
# correct immediate recall	11.2	11.06	0.13 (-1.41, 1.67)	0.863
# correct delayed recall	10.1	10.39	-0.29 (-2.68, 2.10)	0.8071
# correct delayed recognitions	22.8	23.39	-0.59 (-3.06, 1.87)	0.6301
average reaction time correct recognitions (msec)	909.6	877.8	31.79 (-36.21, 99.79)	0.352
body sway (mm)	353.8	358.4	RATIO 0.987 (0.886, 1.099)	0.8042
prolactin serum level				
AUC (0.5-8) (h*ng/mL)	180.06	177.86	1.011 (0.893, 1.145)	0.8571
C_{MAX} (ng/mL)	52.96	51.06	1.037 (0.894, 1.203)	0.6241
electroencephalogramc				
absolute power alpha Fz-Cz (μV)	3.29	3.05	RATIO 1.077 (0.938, 1.238)	0.2861
absolute power alpha Pz-Oz (μV)	6.55	5.26	RATIO 1.247 (1.111, 1.400)	0.0004
absolute power beta Fz-Cz (μV)	1.95	1.91	RATIO 1.024 (0.933, 1.123)	0.6126
absolute power beta Pz-Oz (μV)	2.76	2.43	RATIO 1.139 (1.025, 1.265)	0.0164
absolute power delta Fz-Cz (μV)	1.84	1.85	RATIO 0.996 (0.907, 1.094)	0.9318
absolute power delta Pz-Oz (μV)	1.97	1.87	RATIO 1.055 (0.955, 1.166)	0.2822
absolute power theta Fz-Cz (μV)	2.37	2.42	RATIO 0.976 (0.890, 1.069)	0.5906
absolute power theta Pz-Oz (μV)	2.75	2.6	RATIO 1.056 (0.959, 1.163)	0.2612

A. Least squares means; B. VAS = Visual Analogue Scale; C. only statistically significant EEG absolute powers reported

Figure 1 Structural formula of SB742457

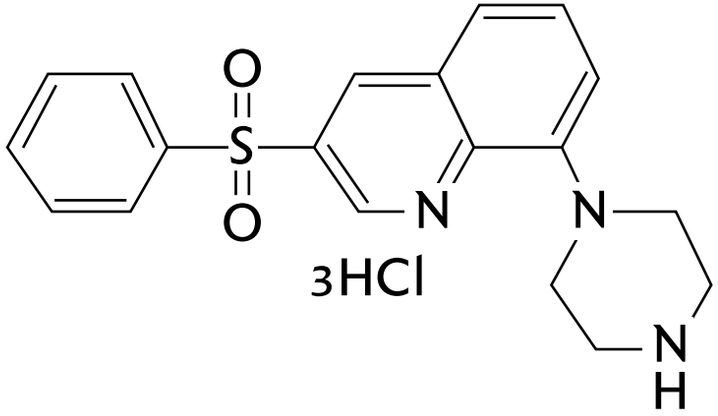


Figure 2 Scheme of study periods 1 and 2, which were similar and separated by at least 1 week washout

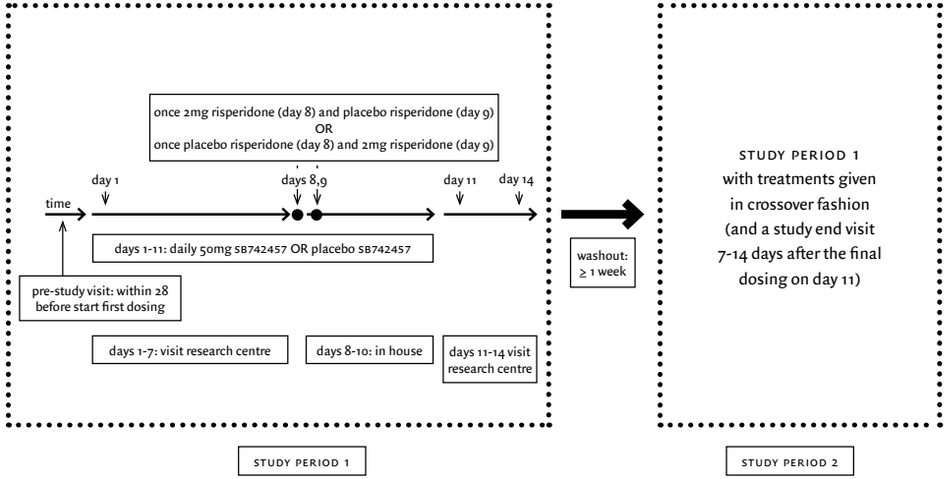
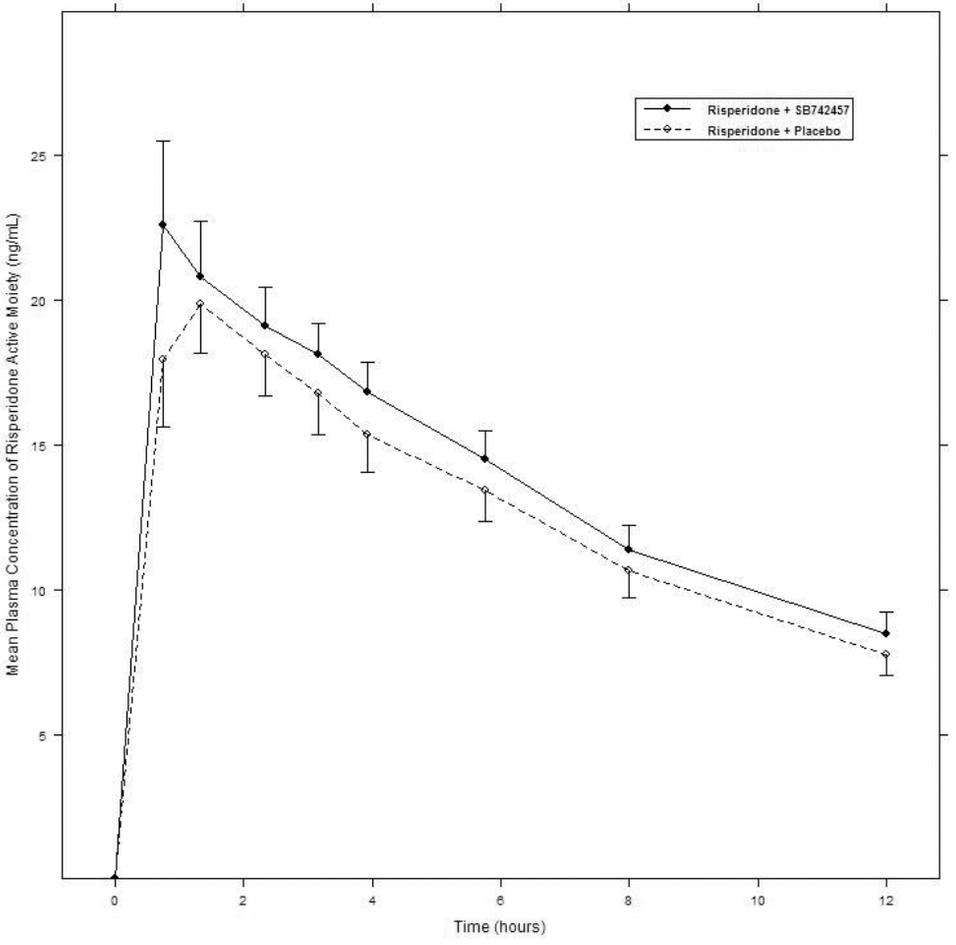


Figure 3 Mean plasma concentrations (standard errors) of risperidone active moiety in the 18 healthy volunteers that completed the study



REFERENCES

1 Glennon RA. Higher-end serotonin receptors: 5-HT$_5$, 5-HT$_6$, and5-HT$_7$. J Med Chem 2003; 46: 2795-812.
2 Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S, Heidbreder CA, Atkins AR, Lacroix LP, Dawson LA, Foley AG, Regan CM, Upton N. SB-399885 is a potent, selective 5-HT$_6$ receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 2006; 553: 109-19.
3 Li Z, Huang M, Prus AJ, Dai J, Meltzer HY. 5-HT$_6$ receptor antagonist SB-399885 potentiates haloperidol and risperidone-induced dopamine efflux in the medial prefrontal cortex or hippocampus. Brain Res 2007; 1134: 70-8.
4 Pouzet B, Didriksen M, Arnt J. Effects of the 5-HT$_6$ receptor antagonist, SB-271046, in animal models for schizophrenia. Pharmacol Biochem Behav 2002; 71: 635-43.
5 Riemer C, Borroni E, Levet-Trafit B, Martin JR, Poli S, Porter RH, Bos M. Influence of the 5-HT$_6$ receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT$_6$ receptor antagonist. J Med Chem 2003; 46: 1273-6.
6 Routledge C, Bromidge SM, Moss SF, Newman H, Riley G, Hagan JJ. Characterisation of SB-271046: a potent and selective 5-HT$_6$ receptor antagonist. Br J Pharmacol 2000; 130: 1606-12.
7 Woolley ML, Marsden CA, Sleight AJ, Fone KC. Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT$_6$ receptor antagonist, Ro 04-6790. Psychopharmacology (Berl) 2003; 170: 358-67.
8 Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 2003; 64: 1295-308.
9 Bourson A, Borroni E, Austin RH, Monsma FJ Jr, Sleight AJ. Determination of the role of the 5-HT$_6$ receptor in the rat brain: a study using antisense oligonucleotides. J Pharmacol Exp Ther 1995; 274: 173-80.
10 Bourson A, Boess FG, Bos M, Sleight AJ. Involvement of 5-HT$_6$ receptors in nigro-striatal function in rodents. Br J Pharmacol 1998; 125: 1562-6.
11 Gerard C, Martres MP, Lefevre K, Miquel MC, Verge D, Lanfumey L, Doucet E, Hamon M, el Mestikawy S. Immuno-localization of serotonin 5-HT$_6$ receptor-like material in the rat central nervous system. Brain Res 1997; 746: 207-19.
12 Mitchell ES, Neumaier JF. 5-HT$_6$ receptors: a novel target for cognitive enhancement. Pharmacol Ther 2005; 108: 320-33.
13 Russell MG, Dias R. Memories are made of this (perhaps): a review of serotonin 5-HT$_6$ receptor ligands and their biological functions. Curr Top Med Chem 2002; 2: 643-54.
14 Upton N, Chuang TT, Hunter AJ, Virley DJ. 5-HT$_6$ receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. Neurotherapeutics 2008; 5: 458-69.
15 Geldenhuys WJ, Van der Schyf CJ. The serotonin 5-HT$_6$ receptor: a viable drug target for treating cognitive deficits in Alzheimer's disease. Expert Rev Neurother 2009; 9: 1073-85.
16 East SZ, Burnet PW, Leslie RA, Roberts JC, Harrison PJ. 5-HT$_6$ receptor binding sites in schizophrenia and following antipsychotic drug administration: autoradiographic studies with [125I]SB-258585. Synapse 2002; 45: 191-9.
17 Frederick JA, Meador-Woodruff JH. Effects of clozapine and haloperidol on 5-HT$_6$ receptor MRNA levels in rat brain. Schizophr Res 1999; 38: 7-12.
18 Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P. SB742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 2010 Sep 24. [Epub ahead of print].
19 Chuang ATT, Foley A, Pugh PL, Sunter D, Tong X, Regan C, Dawson LA, Medhurst AD, Upton N. 5-HT$_6$ receptor antagonist SB742457 as a novel cognitive enhancing agent for Alzheimer's disease. Alzheimers Dement 2006; 2: S631-2.
20 Roth BL, Hanizavareh SM, Blum AE. Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. Psychopharmacology (Berl) 2004; 174: 17-24.
21 Keefe RS, Bilder RM, Davis SM, Harvey PD, Palmer BW, Gold JM, Meltzer HY, Green MF, Capuano G, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Davis CE, Hsiao JK, Lieberman JA. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. Arch Gen Psychiatry 2007; 64: 633-47.
22 de Visser SJ, van der Post JP, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 2001; 51: 119-32.
23 Fang J, Bourin M, Baker GB. Metabolism of risperidone to 9-hydroxyrisperidone by human cytochromes P450 2D6 and 3A4. Naunyn Schmiedebergs Arch Pharmacol 1999; 359: 147-51.
24 Heykants J, Huang ML, Mannens G, Meuldermans W, Snoeck E, Van Beijsterveldt L, Van Peer A, Woestenborghs R. The pharmacokinetics of risperidone in humans: a summary. J Clin Psychiatry 1994; 55 (Suppl.): 13-7.
25 Causon R, Allanson J. Validation of an analytical method for the determination of risperidone and 9-hydroxyrisperidone in human plasma using protein precipitation and liquid chromatography with tandem mass spectrometric detection. York Bioanalytical Solutions Study Number YAY/047. DNG number: WD2005/00549/00. 2008.
26 Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA. Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 1985; 20: 619-29.
27 Sobczak S, Honig A, Schmitt JA, Riedel WJ. Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. Neuropsychopharmacology 2003; 28: 711-9.
28 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-28P.
29 Liem-Moolenaar M, Gray F, de Visser S, Franson K, Schoemaker R, Schmitt J, Cohen A, van Gerven J. Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 2010; 24: 73-82.
30 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-80.
31 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-7.
32 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA$_{(A)}$ alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-83.
33 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.
34 Gijsman HJ, van Gerven JM, Verkes RJ, Schoemaker RC, Pieters MS, Pennings EJ, Hessing TJ, Cohen AF. Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 2002; 17: 83-9.
35 de Visser SJ, van Gerven JM, Schoemaker RC, Cohen AF. Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 2001; 51: 423-8.
36 Kemme MJ, vd Post JP, Schoemaker RC, Straub M, Cohen AF, van Gerven JM. Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 2003; 55: 518-25.
37 van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JM. Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 2004; 18: 221-7.
38 van Steveninck AL. Methods of assessment of central nervous system effects of drugs in man. Thesis. Leiden University, the Netherlands 1993.

39 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 1984; 18 (Suppl. 1): 69S-72S.
40 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, Den Hartigh J, Bender JCME, Verpoorte R, Pinquier J-P, Cohen AF, Van Gerven JMA. Effect of intrapulmonary THC administration in humans. J Psychopharmacol 2008; 22: 707-16.
41 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-91.
42 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-8.
43 de Haas SL, Schoemaker RC, Van Gerven JMA, Hoever P, Cohen AF, Dingemanse J. Pharmacokinetics, pharmacodynamics, and the pharmacokinetic/pharmacodynamic relationship of zolpidem in healthy subjects. J Psychopharmacol 2010; 24: 1619-29.
44 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998; 88: 82-8.
45 Andrew JM. Delinquents and the tapping test. J Clin Psychol 1977; 33: 786-91.
46 Schmitt JAJ, Jorissen BL, Sobczak S, van Boxtel MPJ, Hogervorst E, Deutz NEP, Riedel WJ. Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. J Psychopharmacol 2000; 14: 21-9.
47 Rey A. L'examen Clinique en Psychologie. Paris: Presses Universitaires de France, 1964.
48 Liem-Moolenaar M, Zoethout R, de Boer P, Schmidt M, de Kam ML, Cohen A, Franson K, van Gerven JM. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. J Psychopharmacol 2010; 24: 1681-7.
49 Amchin J, Zarycranski W, Taylor KP, Albano D, Klockowski PM. Effect of venlafaxine on the pharmacokinetics of risperidone. J Clin Pharmacol 1999; 39: 297-309.
50 Hughes AM, Lynch P, Rhodes J, Ervine CM, Yates RA. Electroencephalographic and psychomotor effects of chlorpromazine and risperidone relative to placebo in normal healthy volunteers. Br J Clin Pharmacol 1999; 48: 323-30.
51 Nakagami T, Yasui-Furukori N, Saito M, Tateishi T, Kaneo S. Effect of verapamil on pharmacokinetics and pharmacodynamics of risperidone: in vivo evidence of involvement of P-glycoprotein in risperidone disposition. Clin Pharmacol Ther 2005; 78: 43-51.
52 Artaloytia JF, Arango C, Lahti A, Sanz J, Pascual A, Cubero P, Prieto D, Palomo T. Negative signs and symptoms secondary to antipsychotics: a double-blind, randomized trial of a single dose of placebo, haloperidol, and risperidone in healthy volunteers. Am J Psychiatry 2006; 163: 488-93.
53 Barrett SL, Bell R, Watson D, King DJ. Effects of amisulpride, risperidone and chlorpromazine on auditory and visual latent inhibition, prepulse inhibition, executive function and eye movements in healthy volunteers. J Psychopharmacol 2004; 18: 156-72.
54 Cooper NR, Croft RJ, Dominey SJ, Burgess AP, Gruzelier JH. Paradox lost? Exploring the role of alpha oscillations during externally vs. internally directed attention and the implications for idling and inhibition hypotheses. Int J Psychophysiol 2003; 47: 65-74.
55 van der Post JP, Schram MT, Schoemaker RC, Pieters MS, Fuseau E, Pereira A, Baggen S, Cohen AF, van Gerven JM. CNS effects of sumatriptan and rizatriptan in healthy female volunteers. Cephalalgia 2002; 22: 271-81.
56 Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. Br J Clin Pharmacol 2005; 59: 495-510.

CHAPTER 3

PSYCHOMOTOR AND COGNITIVE EFFECTS OF OF TALNETANT (SB223412) IN HEALTHY VOLUNTEERS COMPARED TO PLACEBO OR HALOPERIDOL

M Liem-Moolenaar[1], FA Gray[2], SJ de Visser[1], KL Franson[1], RC Schoemaker[1], JAJ Schmitt[3], AF Cohen[1], JMA van Gerven[1]

1 Centre for Human Drug Research, Leiden, the Netherlands
2 Clinical Pharmacology & Discovery Medicine, Glaxo SmithKline, Harlow, UK
3 Experimental Psychopharmacology Unit, Maastricht University, Maastricht, the Netherlands

J PSYCHOPHARMACOL 2010; 24: 73-82

Abstract

Central Nervous System (CNS) effects of talnetant, an NK-3 antagonist in development for schizophrenia, were compared to those of haloperidol and placebo. The study was randomised, double-blind, three-way crossover of talnetant 200 mg, haloperidol 3 mg or placebo. Twelve healthy males participated and EEG, saccadic and smooth pursuit eye movements, adaptive tracking, body sway, finger tapping, hormones, visual analogue scales (VAS) for alertness, mood and calmness and psychedelic effects, left/right distraction task, Tower of London and Visual and Verbal Learning Task were assessed. Haloperidol showed (difference to placebo; 95% CI; p-value) decreases in EEG α power (-0.87μV; -1.51/-0.22; p = 0.0110), saccadic inaccuracy (2.0%; 0.5/3.6; p = 0.0133), smooth pursuit eye movements (-7.5%; -12.0/-3.0; p = 0.0026), adaptive tracking (-3.5%; -5.4/-1.7; p = 0.0009), alertness (-6.8 mm; -11.1/-2.4; p = 0.0039), negative mood (-4.6 mm; -8.6/-0.6; p = 0.0266), the ability to control thoughts (1.2 mm; 0.2/2.3; p = 0.0214), and an increase of serum prolactin (ratio 4.1; 3.0/5.6; p < 0.0001). Talnetant showed decreased alpha power (-0.69 μV; -1.34/-0.04; p = 0.0390), improved adaptive tracking (1.9%; 0.1/3.7; p = 0.0370) and reduced calmness on VAS Bond and Lader (-4.5 mm; -8.0/-1.0; p = 0.0151). Haloperidol effects were predominantly CNS-depressant, while those of talnetant were slightly stimulatory.
The results suggest that talnetant penetrates the brain, but it remains to be established whether this dose is sufficient and whether the observed effect profile is class-specific for NK-3 antagonists.

Introduction

Although the atypical antipsychotic drugs are better tolerated than most of the older typical antipsychotics, these drugs still produce less than optimal improvements in quality of life, work and social function and can have serious side effects. It has become increasingly clear that the pathophysiology of schizophrenia probably results from more than dopaminergic dysfunction alone.
The neurokinins (NKs, also called tachykinins) are one of the largest families of peptides and play an important role in neurotransmission and neuromodulation in the central and peripheral nervous system (Tooney *et al*, 2000). The neurokinin receptor consists of three different receptor subtypes: NK1, NK2, and NK3. Recently, NK3 has become of interest with regard to schizophrenia (Spooren *et al*, 2005).

The mRNA encoding these receptors and NK3 agonist binding sites are consistently detected in areas clearly associated with those involved in psychosis and location for therapeutic effects of antipsychotic drugs (Harrison, 1999; Tooney *et al*, 2000; Langlois *et al*, 2001). Other animal studies suggest that NK3 receptors are located on the surface of dopamine cells within the major dopaminergic cell groups (Stoessl, 1994).

Activation of NK3 receptors leads to the release of the biogenic amines dopamine, serotonin, and norepinephrine. Antagonizing these receptors therefore reduces the excitatory activation (or hyperactivity) of some or all of these principal systems without affecting normal baseline activity. This includes a reduction in neurotransmitter release in their target regions, including dopamine in ventral and dorsal striatal regions. This would be expected to have a desirable effect on the positive symptoms of schizophrenia (Spooren *et al*, 2005). The question remains however whether these postulated mechanisms would make NK3-receptors a potential target for novel antipsychotic agents.

Additional support comes from a clinical trial in schizophrenia patients performed by Meltzer *et al* (Meltzer *et al*, 2004). The NK3 receptor antagonist osanetant showed consistent effects on positive symptoms

and Clinical Global Impression Severity of Illness (CGI-S) comparable to haloperidol, without having (worsening or improving) effects on negative or depressive symptoms. Strong effects on hallucinatory symptoms and an excellent tolerability profile were claimed. Side effects noted with osanetant did not differ from those observed with placebo treatment. Although the design of the study did not allow firm conclusions, osanetant showed evidence of efficacy in the treatment of schizophrenia and schizoaffective disorder.

Talnetant (SB223412) is a selective, competitive, non-peptide neurokinin-3 (NK3) receptor antagonist and is in development for the treatment of the positive symptoms of schizophrenia (Dawson L A *et al*, 2008). It was well tolerated in healthy volunteers, but the lack of clear central nervous system (CNS) effects in these early studies also precluded any conclusions about brain penetration and relevant pharmacological activity. The current study was therefore set up to determine the profile and time course of CNS-effects of talnetant 200 mg, and to compare these to the plasma concentrations. Haloperidol has found to be an effective agent in treatment of schizophrenia and has relative specificity for the dopamine2 post-synaptic receptor. Additionally haloperidol has clear CNS-effects in healthy volunteers (Beuzen *et al*, 1999; Legangneux *et al*, 2000; Pretorius *et al*, 2001). NK3 antagonists are hypothesised not to affect baseline dopamine activity, in contrast to haloperidol. Therefore, haloperidol was regarded as a good positive control. In this study the effects of a direct dopamine antagonist are compared to those of talnetant.

Methods and Materials

Subjects

Twelve healthy male and female volunteers aged 18-65 with BMI of 19-30kg/m3 were recruited by the Centre of Human Drug Research (CHDR). Exclusion criteria included the use of agents known to affect CNS performance (including smoking and drug or alcohol abuse) and evidence of relevant clinical abnormalities. The Ethics Review Board of the Leiden University Medical Center approved the study protocol. Written informed consent was obtained from all subjects following a written and oral explanation. The study was performed under Good Clinical Practice quality systems.

Study design

This was a placebo controlled, randomized, double-blind, three-way, cross-over, monocentric study in twelve healthy volunteers, with a two-week wash-out period. All subjects received single oral doses of talnetant 200mg, haloperidol 3mg or their matching double-dummy placebos. Administration sequence was determined using Latin squares balanced for first-order carry-over effects.

For the dosing sessions all participants were instructed to remain fasted from midnight. Smoking, the use of alcohol and quinine- or xanthine-containing foods or beverages was not allowed during the study days. After a standard breakfast at the Centre, patients received the study medication. A standardised lunch and dinner was offered at respectively 4 and 8 hours after drug intake. Water was allowed *ad libitum*. Subjects remained in house until 24 hours after the last study drug administration and returned to the clinic at 36 hours, 48, 72 hours and 7-14 days after dosing for a post-study follow up.

Pharmacokinetics

Blood samples were obtained predose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48 and 72 hours after dosing. Plasma samples were assayed for talnetant using a method based upon protein precipitation with acetonitrile followed by high performance chromatography/mass spectrometry (HPLC/MS/MS) employing positive-ion atmospheric pressure chemical ionization with a lower limit of quantification (LLQ) of 5.00 ng/mL for a 50mL aliquot of human plasma. For haloperidol the method was based upon solid phase extraction followed by LC/MS/MS analysis employing positive ion electrospray (ESI) ionization with LLQ of 0.0250 ng/mL for a 200mL aliquot of human plasma.

Pharmacokinetics (PK) of talnetant were determined using nonlinear mixed effect modelling as implemented in NONMEM Version V (GloboMax LLC, Hanover, MD, USA). A two-compartment model with first order absorption was used and parameters were estimated using first order conditional estimation on log-transformed data. This procedure implements the log-normal residual error model. Inter-individual variability in PK parameters was modelled using a constant CV (coefficient of variation) error model. The model was parameterised in terms of initial ($T_{1/2\alpha}$) and terminal half life ($T_{1/2\beta}$), absorption half life ($T_{1/2\alpha}$), central volume divided by bioavailability ($V_{C/F}$), the rate constant describing transfer from the peripheral to the central compartment (k_{32}), and absorption lag-time (t_{LAG}) (using NONMEM's Advan4 and Trans6).

Pharmacodynamics

All pharmacodynamic measurements were performed at -1, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 hours relative to drug intake. The pharmacodynamic assessments were obtained in a quiet room with subdued light with one subject per room using the 'Neurocart'. This is a transportable CNS measurement battery used for on-site assessment of drug effects and has been used in numerous studies with different kinds of CNS drugs at CHDR (de Haas *et al*, 2007; van Steveninck, 1993; van Steveninck *et al* 1996). It consists of a series of measurements that were chosen for their frequent repeatability, low variability, and their sensitivity to a wide range of drug-induced CNS-effects. Before the study, the subjects were familiarized with the test procedures during a training session, no more than one week before the start of the study.

ADAPTIVE TRACKING

Adaptive tracking measures visuo-motor coordination and vigilance (Borland and Nicholson, 1984) and was performed as originally described by Borland and Nicolson. The test was adapted for use on a personal computer. The adaptive tracking test is more sensitive to impairment of eye-hand coordination by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor.

The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol(van Steveninck), various psychoactive drugs (Borland and Nicholson, 1984; Cohen *et al*, 1985) and sleep deprivation (van Steveninck, 1993). The average performance over a 3-minute period was used for statistical analysis.

VISUAL AND VERBAL LEARNING TASK (VVLT)

Memory (impairment) is a difficult task to study due to the fact that there are so many different components of learning behaviour, i.e. acquisition, consolidation, storage and retrieval. Learning tasks often test only one of these components. However, the Visual Verbal Learning Test (VVLT) (Schmitt *et al*, 2000) contains three different subtests that cover most of the scope of learning behaviour, i.e. immediate and delayed word recall and a delayed word recognition (Schmitt *et al*, 2000). This test is an adapted version of the Auditory Verbal Learning Test (Rey). Thirty words are presented in the same sequence in three trials on a computer screen. Each trial ends with a free recall of the words (immediate recall). Thirty minutes after the first trial, the subject is requested to recall as many words as possible (delayed recall). This is followed by a recognition test, consisting of 15 previously presented words and 15 other but comparable words, in which the subject has to respond 'Yes/No' as quickly as possible to indicate recognition of the word (delayed recognition).

At CHDR this test has shown the CNS effects of various compounds such as benzodiazepines (de Haas *et al*, 2007), cannabis, scopolamine, and risperidone [CHDR data on file]. Outcome variables for the immediate and delayed word recall were the average and the maximum number of correct responses. For the delayed word recognition, the number of correct items and mean response time for correct responses were analysed.

TOWER OF LONDON

Planning capacity can be assessed by a modified version of the One-touch Tower of London (TOL) task (Sobczak *et al*, 2002). This test showed effects for several different CNS active drugs (Beuzen *et al*, 1999; Sobczak *et al* 2002). The test consists of three coloured balls which must be arranged on three sticks to match a picture with the goal positions. The complexity of

the problem is altered by varying the minimum number of moves to reach the goal positions. Each trial consisted of a two- to seven move problem. Prior to each problem, the subjects are informed about the minimum number of moves in which the problem can be solved. Performance was indicated by the slope coefficient of the linear regression of the median response time as a function of the number of steps.

BODY SWAY

An apparatus similar to Wright's ataxiameter was used to measure postural stability, by integrating the amplitude of body movement, transferred through a string attached to the subject's waist (TNO/NIPG, Leiden, The Netherlands) (Wright, 1971). Two-minute measurements were made in the antero-posterior direction with the eyes closed, standing with feet slightly apart wearing comfortable low-heeled shoes. The total amount of movement (in centimeters over 2 min) was used for statistical analysis.

LEFT/RIGHT DISTRACTION TASK

A parametric version of the well-known colour-word response conflict task (Stroop, 1935) was used to measure inhibition by an intervention (Laeng *et al*, 2005). In the past it has been used to measure the effects of several compounds including antipsychotics (Cuesta *et al*, 2001). The words Left and Right are displayed either at the left or the right side of a computer screen. Response instructions were to respond quickly (by pressing a corresponding button) to the location of the word irrespective of its meaning. The output parameters are the response time and accuracy of responding as a function of task difficulty.

FINGER TAPPING

The finger tapping test evaluates motor activation and fluency and was adapted from the Halstead Reitan Test Battery (Yeudall *et al*, 1987). The test Speed of finger tapping was measured for the index finger of the dominant hand; a session contained five performances of ten seconds. The volunteer was instructed to tap as quickly as possible on the space bar of a computer. The mean tapping rate and the standard deviations are used for statistical analysis.

ELECTROENCEPHALOGRAPHY (EEG)

EEG was measured to provide measures of CNS functions (Cohen *et al*1985). In addition, the literature suggests that antipsychotics show distinct profiles of EEG-changes (de Visser *et al*, 2001; Saletu *et al*, 1987). EEG recordings were made using disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark), fixed with collodion at Fz, Cz, Pz and Oz (international 10/20 system). The electrode resistances were kept below 5 kOhm and EEG signals obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customised CED software (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier transform analysis was performed to obtain the absolute power in the delta- (0.5-3.5 Hz), theta- (3.5-7.5 Hz), alpha- (7.5-11.5 Hz) and beta- (11.5-30 Hz) frequency ranges. The duration of EEG measurements was 2 minutes per session and eyes were closed.

EYE MOVEMENT ANALYSIS

Saccadic and smooth pursuit eye movements were recorded as described previously (van Steveninck *et al*, 1996; van Steveninck, 1993) and have shown effects on many different CNS active drugs, including GABA-ergic (de Visser *et al* 2001; de Haas *et al*, 2007), serotonergic (Gijsman *et al*, 2002), noradrenergic (de Visser *et al*, 2001; van der Post *et al* 2004; Kemme *et al*, 2003), and dopaminergic drugs (de Visser *et al*, 2001). The following equipment was used: a micro-computer based system for data recording and analysis (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan) and disposable

surface electrodes (Medicotest N-00-S, Olstykke, Denmark). Average values of latency (= reaction time), peak saccadic velocity and inaccuracy of all artefact-free saccades were used as parameters for saccadic eye movements. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle. The higher the percentage, the worse the performance of the eye movement test. For smooth pursuit, the target moved sinusoidally over 20 degrees of eyeball rotation, at frequencies ranging from 0.3-1.1 Hz. The main parameter was the percentage of time that the eyes were in smooth pursuit of the target.

CORTISOL AND PROLACTIN (PRL)

It is well known that antipsychotics can cause prolactin increase which can lead to clinically undesirable effects(de Visser *et al*, 2001). Blood samples were collected in plain 3mL tubes in order to assess serum levels of cortisol and prolactin (PRL). Serum was separated by refrigerated centrifugation (2000 g at 4°C for ten minutes) within one hour of collection, and transferred to appropriately labelled polypropylene tubes. Serum specimens were stored at -20°C until analysis.

VISUAL ANALOGUE SCALES (VAS)

Visual analogue scales consist of 100 millimeter line segments. Subjects put a mark on a point on the line that best represents their subjective state corresponding to the condition tested. The result is a distance (millimeters) calculated from the mark on the line.

These measures have been shown effects on many different CNS active drugs, including sedative agents (van Steveninck *et al*, 1996; de Haas *et al*, 2007; Norris, 1971), and dopaminergic drugs (de Visser *et al*, 2001).

Subjective effects were quantified using a Dutch translation of the 16 visual analogue scales (VAS) originally described by Norris (Norris, 1971) and applied to drug effect by Bond and Lader. From the set of 16 scales, three factors corresponding to alertness (from nine scores), mood (or contentedness; from five scores), and calmness (from two scores) were derived (Bond and Lader, 1994). A lower score on these scales indicate sedation, excitation and decrease in mood (or contentedness) respectively.

In addition, 13 VAS described by Bowdle (Bowdle *et al*, 1998) assessed the psychedelic effects of the drug. The lowest extreme is usually '0', signifying complete absence of the state (which will be the case under normal circumstances). The highest end of the scale is equivalent with the 'most extreme state imaginable'.

Statistical analysis

All PD endpoints were analysed separately by mixed model analyses of variance (using SAS PROC MIXED) with subject subject-by-treatment and subject-by-time as random effect and treatment, occasion (= period), time and treatment by time as fixed effects, where the baseline value was included as covariate. Graphs of the Least Squares Means estimates over time by treatment were presented with 95% confidence intervals as error bars. Calculation of time and treatment by time effects was for graphical presentation purposes only. Treatment effect was reported as the contrast between placebo and either haloperidol or talnetant where the average of the measurements up to (and including) 10 hours was calculated within the statistical model. All calculations were performed using SAS software (V8.2, SAS Institute, Inc., Cary, NC, USA).

Results

Participant characteristics

Eight males and five females were included in the study. One participant was withdrawn after the first dosing session (talnetant) due to a protocol deviation (undisclosed psychiatric history). Twelve subjects completed the study. Mean (SD) age, height, weight, and body mass index (BMI) of the subjects were 24 (4.6), 178 (7.3), 74.3 (9.1), and 23.6 (3.19) respectively.

Pharmacodynamic results

Since haloperidol was used as a reference dopaminergic agent, the effects of this positive control will be presented before the results of talnetant.

HALOPERIDOL EFFECTS

Haloperidol at a single dose of 3mg was generally well tolerated, with no more than mild adverse events in most subjects. There were no withdrawals due to adverse events and no serious or severe adverse events. Nonetheless, we found a considerable number of effects on neurophysiology and performance. Six subjects reported somnolence and five fatigue. In the results section, differences between treatments were defined as a statistically significant effect at a p-value of 0.05 or lower, without corrections for the number of assessments. The corresponding confidence intervals are shown in the tables 1-4. Haloperidol 3mg caused decreases in alpha power for the summed leads of Pz-Oz and Fz-Cz (table 1). Only absolute power was reported, but similar results were found for total and relative power (data not shown). Haloperidol effects started at one hour after dosing (figure 1).

Haloperidol increased saccadic inaccuracy compared to placebo (table 2). Haloperidol also affected smooth pursuit eye movements. Compared to placebo, treatment with haloperidol caused poorer performance and a clear steady decrease in smooth pursuit eye movements after haloperidol administration was seen.

Adaptive tracking performance deteriorated after haloperidol (difference of -3.5%; 95% CI, -5.4, -1.7%; not shown in table). The time-course of the effects (see figure 2) showed a clear and consistent reduction in tracking performance in the 4-10 hour observation period.

Haloperidol reduced alertness and mood (or contentedness) as indicated by the visual analogue scales (VAS) according to Bond and Lader (table 3). The time-profiles for VAS-alertness and mood showed transient decreases around 4-6 hours after dosing.

Haloperidol affected the VAS of psychedelic effects on item five ('it was difficult to control my thoughts') in comparison to placebo (table 3). The effects mainly occurred six hours post-dose.

Haloperidol caused impairment in the number of correct items after delayed recall (table 4).

Haloperidol caused a prolactin elevation (figure 3), with an increased C_{MAX} (ratio of 4.1; 95% CI, 3.0, 5.6) and a higher Area Under the Curve (AUC) up to 10hr (ratio of 3.1; 95% CI, 2.5, 3.8). Later time-points were not analysed. The time-course showed a clear drug effect from 3-12 hours after dosing, with a maximum around 5-6 hours (figure 3).

Haloperidol did not affect any other PD measures.

TALNETANT EFFECTS

Talnetant was tolerated very well at a dose of 200mg, and it was comparable to placebo in its adverse effects profile. Talnetant was associated with a similar amount of somnolence reports (i.e. three) and headache (i.e. one) as placebo. No other adverse effects were reported for talnetant. There were no withdrawals due to adverse events and no serious or severe adverse events.

The alpha power decreased for talnetant (table 1). The time-course in figure 1 (right panel) showed a decrease for six hours after dosing, which remained detectable for the rest of the ten-hour observation period. Talnetant improved adaptive tracking performance compared to placebo (difference of 1.9%; 95% CI, 0.1, 3.7%; not shown in table). The time-course of the effects suggested a slow increase up to four hours, after which the performance stabilised for the remainder of the observation period (figure 2).

Talnetant reduced feelings of calmness, as indicated by lower scores on the visual analogue scales (VAS) according to Bond and Lader (table 3).

Talnetant did not affect any other PD measures. The data did not allow an analysis of quantitative pharmacokinetic/pharmacodynamic relationships. However, the average time-courses of the effects of talnetant were in agreement with the concentration profile of the compound.

Most effects of talnetant differed from those of haloperidol, both in extent and character (table 6). It was associated with fewer reports of somnolence (i.e. three versus six) and fatigue (no reports versus five) than haloperidol. The alpha power decreased for talnetant and haloperidol to a comparable extent.

Contrary to what was observed for haloperidol, talnetant did not affect saccadic inaccuracy or smooth pursuit eye movements. Also in contrast with haloperidol, talnetant improved adaptive tracking performance compared to placebo. Talnetant reduced calmness of the visual analogue scales (VAS) according to Bond and Lader, for which no change was seen after haloperidol. Contrary to the effects seen with haloperidol, talnetant had no subjective effects on alertness or mood, nor on 'difficulty controlling thoughts' or any of the other Bowdle VAS-scores. Talnetant also did not affect memory, which showed impairments in delayed recall with haloperidol. There were no talnetant effects on serum prolactin level, which also differed from the increase seen with haloperidol.

Pharmacokinetic results

Plasma data are portrayed in figure 4 and NONMEM PK parameters estimates in table 5. The haloperidol plasma profile was similar to that of talnetant, showing a long elimination time with peak plasma concentrations of about 1ng/mL at around 5h and a terminal half life of approximately 30h.

Discussion

This study was performed to evaluate the effects of talnetant, a potential NK3 receptor antagonist, on a battery of quantitative CNS-tests in healthy volunteers, prior to studying its effects in patients. These tests were chosen for their sensitivity to classic neuroleptic agents and other CNS-active drugs (de Visser *et al*, 2001). Haloperidol was chosen as a positive control to prove the sensitivity of the test battery as it was tested before using similar tests (Beuzen *et al*, 1999; Legangneux *et al*, 2000; Pretorius *et al*, 2001). As haloperidol is a relatively selective D_2 antagonist, comparison of the effects of talnetant and haloperidol might give an indication how talnetant would affect the dopaminergic system in healthy subjects.

For this study, a 3 mg dose of haloperidol was selected. While the therapeutic dose range for psychosis in patients is 4 to 10 mg per day, King (King, 1997) recommended a maximum dose of haloperidol 3 mg for healthy volunteer studies. In doses of 1 or 2 mg it is not reliably distinguished from placebo(King ,1997; Legangneux *et al*, 2000). Above 3 mg, there is a dose-dependent rise in reported adverse effects reaching 80 percent at 6 mg (King, 1994).

From this study it was apparent that the pharmacological effects of the two compounds (in the used doses) differ. Haloperidol caused more (predictable) adverse effects than talnetant or placebo. In addition, there were also several differences in the CNS-effect profile. The only comparable effects of haloperidol and talnetant were on EEG alpha power. A reduction in alpha power is shown by many CNS-active drugs, particularly but not exclusively by sedative compounds, and it is not specific for dopaminergic activity (Chavanon *et al*, 2007). Other than this, talnetant effects differed from those of haloperidol. The combination of improved adaptive tracking and reduced calmness could be an indication for slight stimulation. However, these conclusions should be critically evaluated.

First, the talnetant effects were small and it could be argued that these were spurious. The study included many endpoints, and there was no correction for multiple comparisons. The increase in adaptive tracking and decrease in alpha power were marginally significant. Additionally, as the calmness VAS is an individual item of the VAS Bond & Lader, it can be questioned whether this subscale is sufficiently reliable. However, in our experience false-positive statistically significant results are quite rare with this study design, which uses conservative statistical methods based on overall average response, and robust CNS-function tests. So far, we have never observed unexpected statistical significance for stimulatory effects.

The reliability of the design is also confirmed by the effects of haloperidol, which were consistent with earlier reported studies in healthy volunteers (Legangneux *et al*, 2000; Pretorius *et al*, 2001) and with the expected effects of haloperidol in clinical studies (McCue *et al*, 2006).

Second, in the current study the mild stimulant effects of talnetant were only examined at a single dose of 200mg. The effects of higher doses cannot be predicted from these findings. The reason is that some drugs show a dose-related CNS-excitation. For example Selective Serotonin Reuptake Inhibitors (Dumont *et al*, 2005) are mildly stimulatory at low doses, and slightly depressant at higher levels. This study has identified several measurements that can be studied across a wider dose range to investigate the dose-response relationships. Unfortunately, the identification of clear drug-response patterns for talnetant (i.e. a pharmacokinetic-pharmacodynamic (PK/PD) relationship) was precluded by the small effect size and the protracted concentration profile. This was partly due to talnetant's relatively long T_{MAX} and $T_{1/2}$, and the relatively short evaluation period of CNS-effects.

Third, it is still unknown how the effects found in healthy volunteers can be extrapolated to patients. Studies in patients are needed to determine whether talnetant will have the desired effect in schizophrenia and whether the therapeutic window will be improved in comparison with the currently used antipsychotics. The only clinical data available to date demonstrated that osanetant, also an NK3 receptor antagonist, showed antipsychotic activity without any sedative adverse effects(Meltzer *et al*, 2004). The side effect profile of talnetant observed in this study seems consistent with the lack of adverse effects reported for osanetant. Although the effective therapeutic dose is still unknown, the short-term CNS pharmacodynamic effects of talnetant 200 mg in this study, which may provide indications for acute dose-related clinical events in patients, favour talnetant over haloperidol. Most adverse events on these treatments were mild and thought to be unrelated to treatment. Adaptive tracking is a measure of visuo-motor coordination and vigilance and smooth eye pursuit of visual coordination and attention. These parameters show basically opposing effects of haloperidol and talnetant, suggesting that talnetant might not exhibit the typical adverse events of antipsychotic drugs. Possibly, talnetant may be slightly stimulant, but the clinical consequences of this property remain to be established. As expected, haloperidol also showed clear effects on serum prolactin. Talnetant did not cause any hormonal changes, suggesting that this drug might not induce hyperprolactinaemia and its associated problems in patients.

Summarising, it remains to be seen if the identified effects of talnetant are either dose-dependent or class-specific for NK_3 antagonists. The results suggest that talnetant 200 mg will not exhibit the (side) effect profile of antipsychotics, but the clinical relevance of these effects and the therapeutic dose still need to be established. Clearly, other studies with different NK_3 antagonists, are needed to confirm that this drug class may cause slight CNS-stimulation in healthy subjects.

Table 1 Pharmacodynamic EEG effects of haloperidol and talnetant versus placebo

Parameter	Placebo	Haloperidol			Talnetant		
	LS mean[A] placebo	LS mean[A] haloperidol	Difference haloperidol - placebo	95% CI haloperidol - placebo[B]	LS mean[A] talnetant	Difference talnetant - placebo	95% CI talnetant - placebo[B]
Alpha-power summed leads (μV)	8.27	7.40	-0.87	-1.51, -0.22	7.58	-0.69	-1.34, -0.04
Beta-power summed leads (μV)	3.45	3.54	0.09	-0.16, 0.34	3.57	0.13	-0.13, 0.38
Delta-power summed leads (μV)	8.24	8.44	0.20	-0.67, 1.08	8.04	-0.20	-1.09, 0.69
Theta-power summed leads (μV)	5.58	5.62	0.05	-0.32, 0.41	5.70	0.12	-0.25, 0.49

A. LS mean is the Least Squares Means estimate; B. If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level

Table 2 Pharmacodynamic eye movement effects of haloperidol and talnetant versus placebo

Parameter	Placebo	Haloperidol			Talnetant		
	LS mean[A] placebo	LS mean[A] haloperidol	Difference haloperidol - placebo	95% CI haloperidol - placebo[B]	LS mean[A] talnetant	Difference talnetant - placebo	95% CI talnetant - placebo[B]
Saccadic Peak Velocity (deg/sec)	471.3	467.1	-4.2	-21.4, 12.9	475.8	4.5	-12.8, 21.7
Saccadic Latency (sec)	0.204	0.213	0.009	-0.003, 0.021	0.204	-0.000	-0.012, 0.012
Saccadic Inaccuracy (%)	5.6	7.6	2.0	0.5, 3.6	6.3	0.7	-0.8, 2.3
Smooth pursuit (%)	57.9	50.4	-7.5	-12.0, -3.0	58.5	0.6	-4.0, 5.1

A. LS mean is the Least Squares Means estimate; B. If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level

Table 3 Pharmacodynamic VAS effects of haloperidol and talnetant versus placebo

Parameter	Placebo	Haloperidol			Talnetant		
	LS mean[A] placebo	LS mean[A] haloperidol	Difference haloperidol-placebo	95% CI haloperidol-placebo[B]	LS mean[A] talnetant	Difference talnetant - placebo	95% CI talnetant - placebo[B]
VAS Alertness Bond and Lader (mm)	81.1	74.3	-6.8	-11.1, -2.4	80.1	-1.0	-5.2, 3.2
VAS Calmness Bond and Lader (mm)	90.8	88.8	-2.1	-5.6, 1.4	86.3	-4.5	-8.0, -1.0
VAS Mood Bond and Lader (mm)	89.5	84.9	-4.6	-8.6, -0.6	87.3	-2.2	-6.2, 1.7
Psychedelic VAS score 5 (mm)	0.4	1.7	1.2	0.2, 2.3	0.9	0.5	-0.5, 1.5

A. LS mean is the Least Squares Means estimate; B. If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level

Table 4 Pharmacodynamic word recall effects of haloperidol and talnetant versus placebo

Parameter	Placebo	Haloperidol			Talnetant		
	LS mean[A] placebo	LS mean[A] haloperidol	Difference haloperidol-placebo	95% CI haloperidol-placebo[B]	LS mean[A] talnetant	Difference talnetant - placebo	95% CI talnetant - placebo[B]
Immediate word recall number correct -trial 3	17.75	16.08	-1.67	-4.27, 0.94	17.00	-0.75	-3.36, 1.86
Delayed word recall number correct	13.33	10.42	-2.92	-5.30, -0.54	13.50	0.17	-2.21, 2.55
Delayed word recognition number correct	22.50	24.00	1.50	-1.52, 4.52	24.58	2.08	-0.93, 5.10
Delayed word recognition average reaction time correct (msec)	945.36	900.54	-44.82	-148.6, 58.93	878.23	-67.13	-170.9, 36.61

A. LS mean is the Least Squares Means estimate; B. If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level

Table 5 Pharmacokinetic Parameter estimates for talnetant (n = 13)

	Mean[A]	SEM[B]	CV[C]
$T_{1/2\alpha}$ (hr)	2.56	1.20	15.7%
$T_{1/2\beta}$ (hr)	42.1	5.15	34.9%
$t_{1/2A}$ (hr)	1.17	0.504	29.0%
V_C/F (L)	22.8	7.56	18.7%
K_{32} (hr^{-1})	0.105	0.0224	3.9%
t_{LAG} (hr)	0.704	0.118	28.1%
CL (L/hr)[D]	0.957	0.0976	36.1%

A. population average; B. standard error of the population average; C. inter-individual coefficient of variation - residual error (CV) = 10.2%; D. obtained from an alternative parameterization

Table 6 Overview of haloperidol and talnetant effects compared to placebo on different pharmacodynamic tests [A]

Pharmacodynamic test	Haloperidol	Talnetant
Alpha-power summed leads	-	-
Beta/Delta/Theta-power summed leads	0	0
Saccadic Peak Velocity and Latency	0	0
Saccadic Inaccuracy	+	0
Smooth pursuit	--	0
Average adaptive tracking	---	+
VAS Alertness Bond and Lader	--	0
VAS Calmness Bond and Lader	0	-
VAS Mood Bond and Lader	-	0
Psychedelic VAS score 5	+	0
Immediate word recall number correct -trial 3	0	0
Delayed word recall number correct	-	0
Delayed word recognition number correct and average reaction time correct	0	0
AUC up to 10hr and C_{MAX} cortisol serum level	0	0
AUC up to 10hr and C_{MAX} prolactin serum level	++++	0

A. classified as follows: '0' indicates no statistically significant decrease or increase; '-' or '+' indicates a decrease or increase showing a p-value less than 0.05; '--' or '++' indicates a decrease or increase showing a p-value less than 0.01; '---' or '+++' indicates a decrease or increase showing a p-value less than 0.001; '----' or '++++' indicates a decrease or increase showing a p-value less than 0.0001

Figure 1 Adjusted (for baseline) mean time profile of EEG α-power summed leads with 95% CI for placebo and lower 95%CI for talnetant (circle, talnetant; square, haloperidol; dot, placebo).

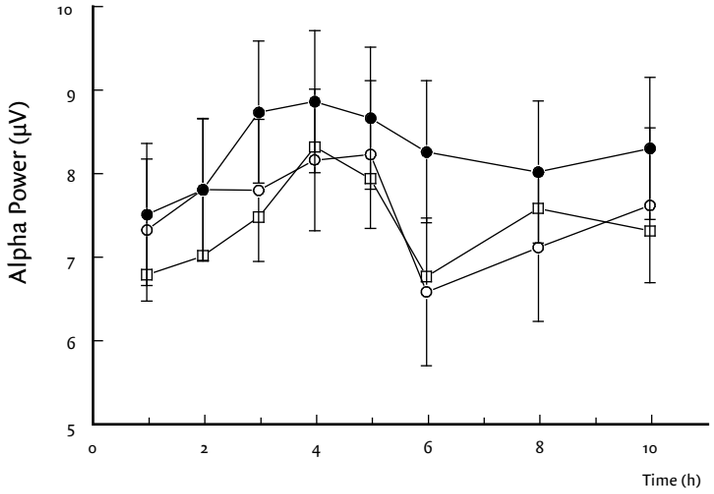


Figure 2 Adjusted (for baseline) mean time profile of average performance on adaptive tracking with 95% CI for talnetant and lower 95% CI for haloperidol (circle, talnetant; square, haloperidol; dot, placebo).

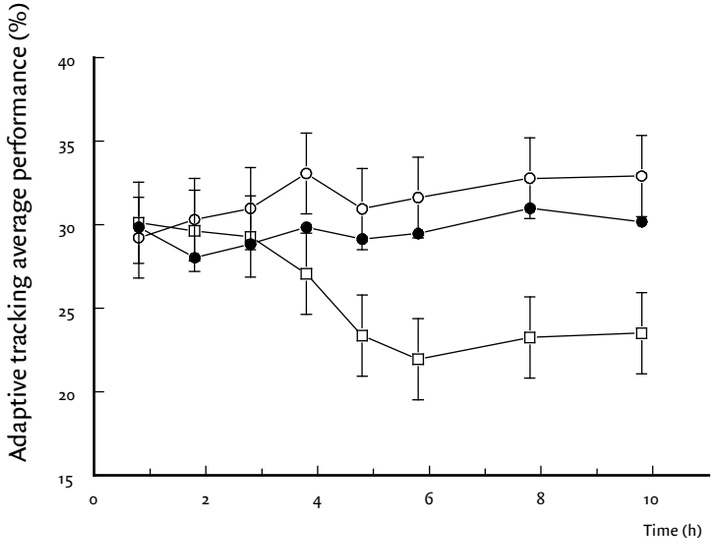


Figure 3 Average (+SD) serum concentration profile of prolactin (circle: talnetant; square: haloperidol; dot: placebo)

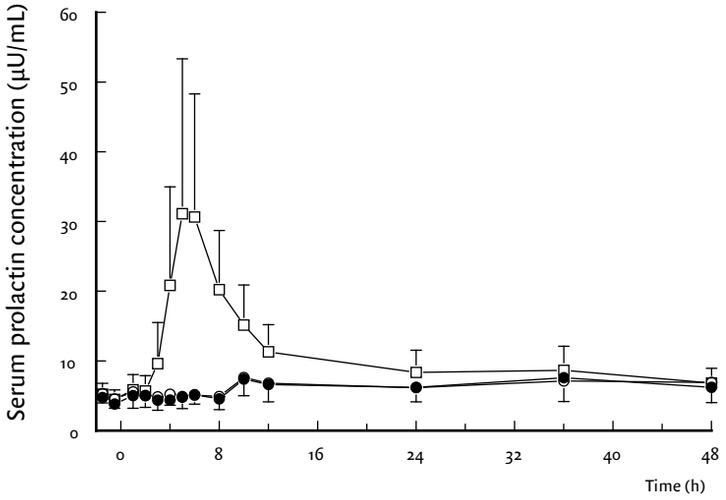
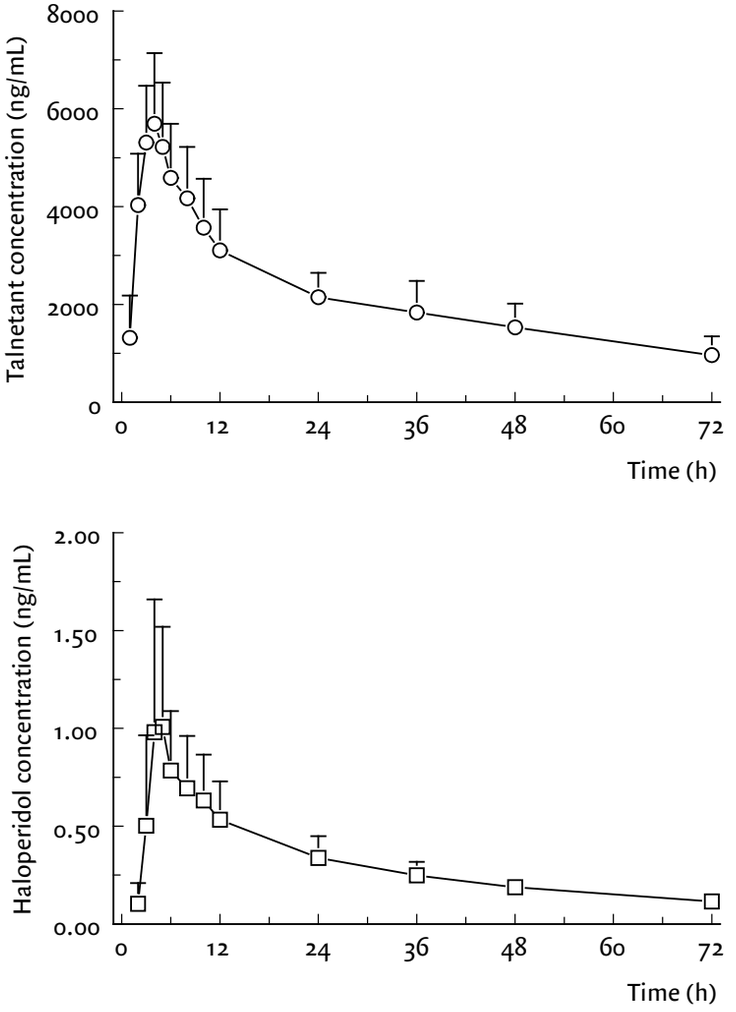


Figure 4 Average (+SD) plasma concentration profile of talnetant and haloperidol



Beuzen J N, Taylor N, Wesnes K, Wood A (1999) A comparison of the effects of olanzapine, haloperidol and placebo on cognitive and psychomotor functions in healthy elderly volunteers. J Psychopharmacol 13: 152-158

Bond A, Lader M (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol 47: 211-218

Borland R G, Nicholson A N (1984) Visual motor coordination and dynamic visual acuity. Br J Clin Pharmacol 18 Suppl 1: 69S-72S

Bowdle T A, Radant A D, Cowley D S, Kharasch E D, Strassman R J, Roy-Byrne P P (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 88: 82-88

Chavanon M L, Wacker J, Leue A, Stemmler G (2007) Evidence for a dopaminergic link between working memory and agentic extraversion: an analysis of load-related changes in EEG alpha 1 activity. Biol Psychol 74: 46-59

Cohen A F, Ashby L, Crowley D, Land G, Peck A W, Miller A A (1985) Lamotrigine (Bw430C), A Potential Anticonvulsant - Effects on the Central Nervous-System in Comparison with Phenytoin and Diazepam. British Journal of Clinical Pharmacology 20: 619-629

Cuesta M J, Peralta V, Zarzuela A (2001) Effects of olanzapine and other antipsychotics on cognitive function in chronic schizophrenia: a longitudinal study. Schizophr Res 48: 17-28

Dawson L A, Cato K J, Scott C, Watson J M, Wood M D, Foxton R, de la F R, Jones G A, Kew J N, Cluderay J E, Southam E, Murkitt G S, Gartlon J, Pemberton D J, Jones D N, Davies C H, Hagan J (2008) In vitro and in vivo characterization of the nonpeptide NK3 receptor antagonist SB-223412 (talnetant): potential therapeutic utility in the treatment of schizophrenia. Neuropsychopharmacology 33: 1642-1652

de Haas S L, de Visser S J, van der Post J P, de Smet M, Schoemaker R C, Rijnbeek B, Cohen A F, Vega J M, Agrawal N G, Goel T V, Simpson R C, Pearson L K, Li S, Hesney M, Murphy M G, van Gerven J M (2007) Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA(A) alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 21: 374-383

de Visser S J, van der Post J P, de Waal P P, Cornet F, Cohen A F, van Gerven J M (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55: 39-50

de Visser S J, van der P J, Pieters M S, Cohen A F, van Gerven J M (2001a) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51: 119-132

de Visser S J, van Gerven J M, Schoemaker R C, Cohen A F (2001b) Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 51: 423-428

Dumont G J, de Visser S J, Cohen A F, van Gerven J M (2005) Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. Br J Clin Pharmacol 59: 495-510

Gijsman H J, van Gerven J M, Verkes R J, Schoemaker R C, Pieters M S, Pennings E J, Hessing T J, Cohen A F (2002) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 17: 83-89

Harrison P J (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122 (Pt 4): 593-624

Kamali F (2001) Osanetant Sanofi-Synthelabo. Curr Opin Investig Drugs 2: 950-956

Kemme M J, vd Post J P, Schoemaker R C, Straub M, Cohen A F, van Gerven J M (2003) Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 55: 518-525

King D J (1994) Psychomotor impairment and cognitive disturbances induced by neuroleptics. Acta Psychiatr Scand Suppl 380: 53-58

King D J (1997) Guidelines for the use of antipsychotic drug studies in healthy volunteers. The BAP Consensus Group. J Psychopharmacol 11: 201-209

Laeng B, Lag T, Brennen T (2005) Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. J Exp Psychol Hum Percept Perform 31: 438-452

Langlois X, Wintmolders C, te Riele P, Leysen J E, Jurzak M (2001) Detailed distribution of neurokinin 3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. Neuropharmacology 40: 242-253

Legangneux E, McEwen J, Wesnes K A, Bergougnan L, Miget N, Canal M, L'Heritier C, Pinquier J L, Rosenzweig P (2000) The acute effects of amisulpride (50 mg and 200 mg) and haloperidol (2 mg) on cognitive function in healthy elderly volunteers. J Psychopharmacol 14: 164-171

McCue R E, Waheed R, Urcuyo L, Orendain G, Joseph M D, Charles R, Hasan S M (2006) Comparative effectiveness of second-generation antipsychotics and haloperidol in acute schizophrenia. Br J Psychiatry 189: 433-440

Meltzer H Y, Arvanitis L, Bauer D, Rein W (2004) Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. Am J Psychiatry 161: 975-984

Norris H (1971) The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10: 181-191

Pretorius J L, Phillips M, Langley R W, Szabadi E, Bradshaw C M (2001) Comparison of clozapine and haloperidol on some autonomic and psychomotor functions, and on serum prolactin concentration, in healthy subjects. Br J Clin Pharmacol 52: 322-326

Rey, A (1964) L'examen Clinique en Psychologie. Paris: Presses Universitaires de France.

Saletu B, Grunberger J, Linzmayer L, Anderer P (1987) Comparative Placebo-Controlled Pharmacodynamic Studies with Zotepine and Clozapine Utilizing Pharmaco-Eeg and Psychometry. Pharmacopsychiatry 20: 12-27

Schmitt J A J, Jorissen B L, Sobczak S, van Boxtel M P J, Hogervorst E, Deutz N E P, Riedel W J (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. Journal of Psychopharmacology 14: 21-29

Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V (2002) The tachykinin peptide family. Pharmacological Reviews 54: 285-322

Shughrue P J, Lane M V, Merchenthaler I (1996) In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. J Comp Neurol 372: 395-414

Sobczak S, Honig A, Nicolson N A, Riedel W J (2002) Effects of acute tryptophan depletion on mood and cortisol release in first-degree relatives of type I and type II bipolar patients and healthy matched controls. Neuropsychopharmacology 27: 834-842

Spooren W, Riemer C, Meltzer H (2005) Opinion: NK3 receptor antagonists: the next generation of antipsychotics? Nat Rev Drug Discov 4: 967-975

Stoessl A J (1994) Localization of striatal and nigral tachykinin receptors in the rat. Brain Res 646: 13-18

Stroop J R (1935) Studies of interference in serial verbal reactions. Journal of Experimental Psychology 18: 643-662

Tooney P A, Au G G, Chahl L A (2000) Localisation of tachykinin NK1 and NK3 receptors in the human prefrontal and visual cortex. Neuroscience Letters 283: 185-188

van der Post J P, de Visser S J, Schoemaker R C, Cohen A F, van Gerven J M (2004) Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 18: 221-227

van Steveninck A L (1993) Methods of assessment of central nervous system effects of drugs in man. Thesis/Dissertation

van Steveninck A L (1994) Methods of assessment of central nervous system effects of drugs in man. Thesis/Dissertation

van Steveninck A L, Gieschke R, Schoemaker R C, Roncari G, Tuk B, Pieters M S, Breimer D D, Cohen A F (1996) Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41: 565-573

van Steveninck A L, Schoemaker H C, Pieters M S, Kroon R, Breimer D D, Cohen A F (1991) A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 50: 172-180

van Steveninck A L, van Berckel B N, Schoemaker R C, Breimer D D, van Gerven J M, Cohen A F (1999) The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 13: 10-17

Wright B M (1971) Simple Mechanical Ataxia-Meter. Journal of Physiology-London 218: 27-&

Yeudall L T, Reddon J R, Gill D M, Stefanyk W O (1987) Normative data for the Halstead-Reitan neuropsychological tests stratified by age and sex. J Clin Psychol 43: 346-367

CHAPTER 4

EARLY STAGE DEVELOPMENT OF THE GLYCINE-1 REUPTAKE INHIBITOR SCH 900435: CENTRAL NERVOUS SYSTEM EFFECTS COMPARED TO PLACEBO IN HEALTHY MEN

M Liem-Moolenaar[1], P Peeters, MSD[2], IMC Kamerling[1], C Hogg[3], G Holder[3], HJ Kleijn, MSD[2], E Spaans, MSD[2], J Udo De Haes, MSD[2], ML de Kam[1], KL Franson[1], AF Cohen[1], JMA van Gerven[1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Early Stage Development, Oss, The Netherlands
3 Moorfields Eye Hospital, London, UK & Institute of Ophthalmology, London, UK

SUBMITTED

Abstract

AIM To report the first three studies with SCH 900435, a highly selective glycine-1 reuptake inhibitor in development for treating schizophrenia, using systematic evaluations of pharmacodynamics to understand the observed (adverse) effects.

METHODS Three double-blind, placebo-controlled studies (single dose, visual effect analysis and multiple dose) were performed. In the single and multiple dose study SCH 900435 (0.5-30mg) was given to healthy males and frequent pharmacokinetic and pharmacodynamic measurements were performed. The visual effects study, which was performed after the single and before the multiple dose study, focussed on the visual system and incorporated visual electrophysiological measures of macular, retinal and intracranial visual pathway function.

RESULTS In the single dose study increases in smooth pursuit eye movements (8, 12, 30mg), pupil/iris ratio (20 and 30mg), VAS colour perception (30mg) and changes in spontaneous reports of short-lasting visual disturbance were found, while FSH (8, 12, 20mg), LH (8-30mg) and EEG alpha$_2$ activity decreased (12, 20, 30mg). A subsequent dedicated visual effects study demonstrated that visual effects were transient without underlying electrophysiological changes. This provided enough safety information for starting a multiple ascending dose study, which showed rapid development of tolerance for visual effects.

CONCLUSIONS Several CNS effects and gonadotropic changes resulted from administration of 8mg and higher, providing evidence for CNS penetration and pharmacological activity of SCH 900435. Transient visual symptoms were reported to which rapid tolerance occurred. It remains to be established whether these effects are specific for this pharmacological class and whether this class of drugs offers any antipsychotic activity in patients.

Introduction

Traditional models of schizophrenia emphasize the importance of dopaminergic (DA) dysregulation, particularly with regard to the positive symptoms [1,2]. An alternative model is based on the effects of non-competitive antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors, which induce psychotic symptoms in healthy subjects and exacerbate symptoms in schizophrenic patients. These effects seem to resemble schizophrenia more closely than those induced by dopamine activation [3-6]. Moreover, dysfunction of glutamatergic neuronal systems would be consistent with the dopamine hypothesis of schizophrenia [7-11]. Due to major concerns over potentially serious adverse events of indiscriminate glutamatergic stimulation, which could affect key functions such as learning, memory and neuronal excitation and cell death, research has focused on alternative strategies to augment NMDA receptor function [12]. One alternative pathway is through the glycine receptor site, an obligatory co-agonist at the NMDA receptor [3]. Direct glycine agonists appear to show some effect in treatment-resistant schizophrenia [3], but this requires gram-level doses. Inhibition of presynaptic reuptake of GlyT$_1$ transporters can also increase local endogenous glycine levels [13,14]. As reviewed by Javitt [3], glycine reuptake inhibitors (e.g. D-cycloserine, glycine and D-serine) have been effective in a variety of rodent schizophrenia models [15-19] and in schizophrenic patients [20-22], but these agents are neither potent nor selective. SCH 900435 is a selective and highly potent GlyT$_1$-reuptake inhibitor (Figure 1 - structural formula), which effectively increases extracellular glycine levels in rat brain regions and spinal cord, but does not affect strychnine-sensitive GlyT$_2$-transporters. Based on its general facilitation of NMDA-receptor activity throughout the nervous system, its effects could be expected to influence a wide range of CNS-effects. Lastly, preclinical studies suggested that SCH 900435 could have a steep dose-effect relationship for adverse events. Therefore, a strategy was chosen for the early development of SCH 900435 that allowed for careful stepwise assessment of the pharmacological and clinical characteristics of the

drug. During the first administration of SCH 900435 in human, each dose escalation step was based on a detailed evaluation of the interim analyses of pharmacokinetics (PK), pharmacodynamics (PD) and clinical effects of the preceding doses. A multimodal test battery was used to frequently measure a wide range of effects, covering most drug sensitive CNS-domains and vital functions. This article describes the three studies in human volunteers performed to determine a dosing regimen of SCH 900435, that were expected to be safe and therapeutically relevant for subsequent patient dose finding studies. Each study was approved by an independent Ethics Review Board and full written informed consent was obtained from all subjects.

Single ascending dose (SD) study

Subjects and methods

Sixteen healthy male volunteers between 18 and 45 years were recruited for the first administration of single ascending doses of SCH 900435 at the Centre for Human Drug Research in Leiden, The Netherlands. Exclusion criteria and study restrictions included the use of agents known to affect CNS performance (including nicotine, alcohol or drugs) and evidence of relevant clinical or psychiatric abnormalities. Subjects remained in house until 72 hours after the last study drug administration. The study was double-blind, placebo controlled and randomized. Each subject was assigned to one of four dosing groups in a four-way crossover study (three active ascending doses and one randomized placebo dose), with a minimum wash-out period of one week (table 1a). Based on preclinical evaluations, the original dosing regimen was planned to cover a range from 0.5 mg (the human starting dose based on animal safety data) to 135 mg (predicted to lead to plasma levels in the anticipated therapeutic range). According to the protocol, doses could be adapted based on investigator-blinded interim assessments.

Adverse events, laboratory safety parameters, ECG, oral body temperature, blood pressure and heart rate measurements were performed regularly throughout the study. Blood samples were obtained pre-dose and frequently up to 72 hours after SCH 900435 administration. Frequent measurements of a multimodal CNS test battery containing a wide range of drug-responsive CNS-domains up to eight hours after dosing were obtained: Visual Analogue Scales (VAS measuring subjective alertness, mood, calmness, psychedelic effects, sleep quality), pharmaco-electroencephalography (pharmaco-EEG), saccadic pursuit eye movements (measure of alertness), smooth pursuit eye movements (measure of motor coordination), adaptive tracking (visuo-motor coordination), body sway (postural stability), pupillometry (pupil-iris ratio) and neuroendocrine effects (serum prolactin, LH, FSH and testosterone levels).

Both a general treatment effect and a simple linear effect was tested for the PD parameters. AUEs (Area Under Effect-time curves) were calculated per subject and divided by the corresponding time span, resulting in a weighted average response used for ANCOVA analysis. If the general treatment effect was significant, contrasts of all treatments versus placebo were calculated. Differences between treatments were defined as statistically significant at a p-value of 0.05 or lower.

If the treatment effect was significant and the linear effect suggested a dose effect relation, an additional regression analysis of the relevant parameter on log dose was performed. The slope of the regression was considered significantly different from zero at a p-value of 0.05 or lower. Conclusions about PK with respect to dose proportionality/ dose independence were primarily based on descriptive statistics of PK parameters and on summary plots. An additional exploratory Analysis of Variance (ANOVA) was performed to test for dose proportionality and dose independence. Detailed methods (pharmacodynamic measurements and statistics) are described in appendix I.

Results and discussion

Sixteen healthy males were included in the single dose study. Two subjects were withdrawn due to visual symptoms (described in more detail below)

and 14 subjects completed the study. The mean (range) age of the subjects was 24 (19-32) years.

After interim assessment of the first dose, it was observed that exposure in terms of plasma AUC was 10-fold higher in humans than that which was allometrically predicted. No pharmacodynamic or adverse effects were noted after the starting dose, but the original anticipated dose range was reduced from 0.5-135 mg to 0.5-30 mg (table 1b).

No statistically significant changes in vital signs, respiratory function, physical examination or laboratory parameters were observed during the study. There were no serious adverse events (SAEs). The most frequently reported adverse events (AEs) during the in-house study period were dizziness, somnolence, headache, fatigue and abnormal vision (table 2). All were mild to moderate and self-limiting. At 3 mg, one subject developed anxiety and other psychological effects in addition to visual changes, which led to his withdrawal. In the subsequent two dosing groups, four of 12 subjects reported visual changes (one each at 3 and 8 and two at 12 mg), often described as spots of enhanced contrast or intensity accompanied by blurred vision and dizziness. These symptoms occurred around 30 minutes after drug administration, and disappeared within minutes to a few hours after the start of the symptoms. They were considered to be drug-related, but were of limited duration and intensity. All four subjects in the fourth dosing group reported spots in the visual fields (starting at 20 or 30 mg) similar to those observed in previous groups. It was decided to withdraw one of these subjects as he reported recurrent symptoms, which, although moderate, increased in the third visit (30 mg) compared to the second visit (20 mg). Ophthalmologic examinations did not reveal any subjective or objective visual system abnormalities, either during the study or at follow-up.

Peak concentrations of SCH 900435 26,7 ng/mL (for 0.5 mg) to 1170 ng/ml (for 30 mg) and were reached between 30 and 50 minute after dosing. Maximal drug plasma concentrations were higher in subjects who reported visual AEs than those who did not (approximately 770 ng/mL versus 140 ng/mL). Peak concentrations of 26.7 ng/mL (for 0.5 mg) to 1170 ng/mL (for 30 mg) SCH 900435 were reached 0.5-0.8 hours after dosing.

Pharmacokinetics (PK) were dose-linear over the tested dose range. The mean concentration-versus time profiles (figure 2) showed that the last parts of the curves for all doses ran essentially parallel and that the terminal elimination phase started approximately 16 hours after dosing. The terminal half-lives varied from 6.6 to 7.8 hours, and clearance from 4.5 to 6.1 L/h.

In agreement with the adverse event reports, significant increases were observed in the 'colours' item of the Bowdle visual analogue scale (table 3). The increases did not exhibit a linear dose-response relationship across all doses (table 4) but were consistent at 30 mg (figure 3). After a maximum at approximately 40 minutes, all effects had disappeared by 3 hours following dosing (figure 4). Pupillometry demonstrated an increase of pupil/iris ratio at 20 mg SCH 900435 (table 3), with the largest effect at 30 mg (table 3, figure 5).

Smooth pursuit (0-4 hours) showed a statistically significant increase at 8, 12, and 30 mg (largest difference) (table 3). The regression analysis showed a statistically significant dose-response relationship for smooth pursuit eye movements (table 4). It should be noted that the smaller number of subjects in the higher dose groups (the 20 mg dose group for smooth pursuit eye movements) may have prevented the detection of a treatment effect from reaching statistical significance (the same could be true for the 30 mg dose group for FSH levels).

Both LH and FSH decreased statistically significantly compared to placebo (table 3, figure 5). LH started to diminish after 12 mg and FSH after 8 mg; the effect was more pronounced with higher doses. Testosterone showed a decrease at 8 and 20 mg (table 3). The LH level decrease followed SCH 900435 administration within one hour and was virtually normalized after ten hours. FSH levels diminished after five to six hours, and remained reduced during the full observation period. Regression analysis showed indications for linear dose response relationships for all three hormones (table 4).

Signs of pharmacological activity in this single ascending dose study were evident from doses starting at 8 mg. Clear increases in pupil size and subjective changes of visual perception were noted after 20 and

30 mg. The effects resolved rapidly and were no more than mild to moderate, but were initially disconcerting and were not anticipated. Therefore, a more specific study on the effect of the drug on visual system function was therefore initiated before a multiple dosing was started.

Visual effects study

Based on the conjecture that the visual symptoms were more likely to reflect a retinal rather than central origin, consultation with ophthalmology experts and a review of the literature revealed that the retina is rich in glycine receptors. It appeared that although CNS-studies of glycinergic or glutamatergic agents did not specifically describe visual disturbances [18,23,24], such effects have been reported with high-dose intravesicular glycine administration in men, following prostate surgery [25,26]. Based on the combination of drug-induced pupil dilation and abnormal vision, direct retinal effects were considered more probable than autonomically induced mydriasis or central visual impairment. Detailed assessments of retinal architecture and function in rats and dogs exposed to SCH 900435 did not demonstrate any electrophysiological or structural abnormalities of the retina or other components of the visual system after repeated dosing (data on file). In the first-in-human study, all symptoms were mild and rapidly reversible without any sequelae, both subjectively and during follow-up. Moreover, the resolution of visual symptoms was faster than the reduction of plasma concentrations, suggesting that the visual effects may not have functional consequences. This was investigated in a dedicated ophthalmologic study with SCH 900435 at Moorfields Eye Hospital in London, UK.

Subjects and methods

The visual effects study was a double-blind, placebo-controlled, four-period crossover study involving the administration of three single oral doses of SCH 900435 (5, 13 and 20 mg) in which the placebo was randomized in an ascending order in 24 healthy male subjects. The exclusion and inclusion criteria and study restrictions were similar to the single ascending dose study. Inclusion criteria for the study included a normal eye examination and good visual acuity (6/9 or better in each eye). Each treatment period lasted one day and was separated by a wash-out of at least 3 days. Subjects were further randomized into one of three groups of eight subjects in which PK, PD and several PD eye assessments were performed. In group 1 (fully dark adapted with dilated pupils; duration: approximately 12 minutes, in cycles of 25 minutes) the following PD tests were performed: dark adaptation - measuring the threshold of light detection without electrophysiology, rod specific single flash, red flash, intermediate, International Society for Clinical Electrophysiology of Vision (ISCEV) standard and ISCEV + 0.6 LV (see appendix II). In group 2 (light conditions with dilated pupils; duration: approximately 20 minutes, in cycles of 25 minutes) the following tests were taken: single flash Cone response - 2 Hz, flicker response - 30 Hz, on-/off- response, S-Cone response and Photopic Negative Response (see appendix II). In group 3 (light conditions with undilated pupils; duration: approximately 45 minutes) the following tests were performed: VEP - pattern reversal/ Flash, pattern ERG, Color Contrast Sensitivity, visual Acuity, pupillometry (see appendix II) and neuroendocrine effects. PK, PD and statistics are described in more detail in appendix II. Endocrinologic investigations (performed in groups 1 and 2 only) included repeated measurements of LH, FSH and testosterone. AEs, laboratory safety parameters, ECG, blood pressure and heart rate measurements were performed regularly during the study. SCH 900435 blood samples were obtained predose and frequently up to 24 h post-dose (for PK methods see single ascending dose study appendix I).

Results and discussion

Twenty-four (three groups of eight) subjects were included. There were no SAEs or clinically relevant changes in laboratory safety parameters, vital signs, and ECG data. The majority of the AEs were considered to be of mild intensity (102 out of 109 AEs), four AEs were of moderate intensity

and three were of severe intensity. One visual AE followed after 13 mg SCH 900435 and two following 20 mg and were similar to the events that were also described in the SD study. The symptoms were transient and resolved without intervention.

Pharmacokinetics and hormone results were also similar to those observed in the SD study (data not shown), with the exception of the FSH profile which showed no decrease in this study. Overall, there was no consistent change in any ophthalmologic test result in any subject, other than those that may be expected from normal variation. The visual symptoms questionnaire was the most sensitive and specific determination of visual disturbance (table 5). Although the results should be treated with caution because of the well documented consequences of multiple hypothesis testing (only unadjusted 95% confidence intervals were calculated) and influence of extraneous factors (e.g. subject tiredness and dizziness, adaptation/intolerance to the testing), the data provided no indication for clinical concerns to perform a multiple (ascending) dose study. This could possibly be an explanation of the reporting of blurred vision and apparent film over the eyes pre-dose and following administration of placebo.

Multiple (ascending) dose (md) study

Subjects and methods

The multiple ascending dose study was performed at Guys Drug Research Unit, London, UK. Selection criteria and study restrictions were similar to the visual effects study. The study design of the multiple dose (MD) study was double-blind, placebo-controlled and parallel group. Multiple (ascending) oral doses between 4 and 30 mg were given once or twice daily to 40 (five groups of eight) healthy male subjects for 13 days (table 6).

Similar to the SD and visual effects study, PK samples were obtained frequently for up to 72 hours after the last SCH 900435 dose, and AEs, laboratory safety parameters, ECG, oral body temperature, blood pressure and heart rate measurements were followed up regularly (see for the study design table 7). PD and safety assessments in the MD study were based on the results of the SD and visual effects study: Visual Analogue Scales (VAS), pharmaco-EEG, pupillometry and neuroendocrine effects (LH, FSH, testosterone) (table 7). In addition, the cognitive effects of multiple dose treatment with SCH 900435 were examined with the following tests from the Cognitive Drug Research (CDR) test battery: simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking. At screening and follow-up, visual examinations were performed at Moorfields Eye Hospital, London, and subjects were given a questionnaire consisting of eight questions to ascertain visual symptoms. The primary aim of this MD study was to examine the clinical course of the events that had been observed during single dosing. Descriptive and summary statistics for the VAS and AEs were used as statistical models were considered to have a chance of obstructing the identification of isolated but relevant clinical observations. The statistical analyses performed on PK, pharmaco-EEG and results of the CDR test battery are described in more detail in appendix III.

Results and discussion

Forty healthy male subjects, with a mean (range) age of 25.9 (18-42) years, were included in the study. There were no clinically relevant abnormal findings in the laboratory safety parameters, vital signs or ECG data. The most common AEs reported by subjects receiving SCH 900435 were eye disorders and CNS disorders (table 8). These symptoms were mostly noted between 0.5 and 1 hours post-dose after both single and multiple doses and did not appear to be prolonged. None of the AEs were severe and no SAEs were reported during the study. One subject was withdrawn from the study on day 12 (before the morning administration of SCH 900435) due to a persistent focussing difficulty; those symptoms disappeared by the evening of day 12. Notably, the number of reported visual symptoms following SCH 900435 12 mg twice daily (after dose

titration) were lower than following 16 mg once daily (table 8). Similarly, the incidence of lethargy, dizziness and somnolence was less during dose titration (table 8).

No clinically relevant deviations in PD effects were shown. Mean VAS data seemed to show increases in dizziness, decreases in alertness and in activeness, sleepiness and nausea and drowsiness on day four, while these effects had diminished or cleared a few days later, suggesting some adaptation to treatment. Although a decrease in pupil diameter was seen (as opposed to the dilation seen in the single dose study), this change was not sustained, not evident under the other conditions or in the other dose groups and it can not exclude that this finding was coincidental in this small group of subjects. There were also no clear results on EEG power and the cognitive data.

Similarly to the SD study, FSH, LH and testosterone significantly decreased during multiple dosing (see figure 7 for LH; the figures for the other hormones were similar and were therefore not shown). There was no difference in hormone reductions between the first and last days of dosing. The effect was generally largest following administration of 16 mg SCH 900435.

The PK parameters of the multiple (ascending) dose study were as expected from the single dose study and the visual effects study (data therefore not shown). Steady state concentrations were reached between 2 and 6 days of daily dosing. Mean accumulation of SCH 900435 exposure (AUC) at steady state after once daily dosing ranged between 5% and 11% and was 22% after 12 mg twice daily treatment. The elimination half-life was increased 20% after 10 days of daily dosing. PK of SCH 900435 was dose-proportional at steady state in the dose range 4 - 16 mg once daily and 12 mg twice daily.

In summary, the incidence of central nervous and visual symptoms measured by AE reporting increased when the dose of SCH 900435 was higher than 8 mg once daily. The other PD measurements did not show relevant or consistent changes in the doses tested in this study, except limited but statistically significant decreases in gonadotrophic hormones. Clinically, the results indicate that dose titration and twice daily (compared to once daily) dosing of SCH 900435 improve tolerance to the subjective visual disturbances that primarily occur shortly after the initiation of treatment.

Discussion

The changes in visual VAS scales, the reported visual symptoms and the pupil dilation that were observed in the SCH 900435 studies are all compatible with a direct retinal effect of SCH 900435. Visual spots can be a manifestation of cortical spreading depression such as that which occurs during a migraine aura, and it cannot be excluded that a similar phenomenon caused the visual symptoms that occurred with SCH 900435. This would be consistent with a potential role of enhanced glutamatergic activity, which among other biochemical derangements has been postulated to play a role in cortical spreading depression [27]. On the other hand, visual auras typically migrate and this was not reported by participants in our studies. Also, no other migrating neurological deficits or headaches occurred. Furthermore, pupillary abnormalities are usually not observed in occipital visual disturbance. A transient change in retinal function may then explain the visual symptoms, even in the absence of any detectable changes in retinal function as measured electrophysiologically.

Glycine is one of the essential neurotransmitters modulating visual signals in the retina [28]. The retinal amacrine cells appear to possess a specific uptake mechanism for glycine [29] and contribute to the generation of the oscillatory potentials (OPs) in the electroretinogram. In different animal models these potentials were found to be blocked by glycine [29,30]. In a recent study with a glycine reuptake inhibitor in healthy humans a high incidence of visual effects was described [31]. Visual effects included visual disturbance, blurred vision, photophobia, diplopia, and photopsia and were only reported in the higher dose ranges. The time course of the effects in the current study was also compatible with an acute pharmacological effect. In the SD study, the reported visual symptoms and pupil dilation were both clearly related to

peak plasma concentrations. Although no formal concentration-effect analyses were performed, it seemed that the subjective visual changes (assessed with the Bowdle VAS colours) resolved more rapidly than the plasma concentrations, perhaps as a result of habituation. In addition, high peak concentrations could be avoided by a slow release formulation which has been developed for this reason. The visual system is highly adaptive, so the question remained whether symptoms faded because of (retinal) adaptation to ongoing functional impairments. Preclinical multiple-dose studies showed no morphological abnormalities in the eyes of experimental animals and the dedicated ophthalmologic study found no electrophysiological or functional indications of significant retinal impairment. In the MD study, the incidence of AEs related to eye disorders increased with ascending doses of SCH 900435. However, reports of visual disturbances were less frequent with dose titration than without. This provided further confidence that the rapid resolution of the visual symptoms after a single dose or during dose titration reflects the development of pharmacological tolerance without further functional consequences. Similarly, in the VAS data there was an indication for tolerance to dizziness, alertness, sleepiness, nausea, activeness and drowsiness.

In all three studies decreases in sex hormones were observed after SCH 900435 (with the exception of FSH in the visual effects study), and the relation between these effects and the dose was linear. Literature on the relationship between NMDA receptor stimulation and FSH, LH or testosterone production in humans is sparse, and most studies show no clear association [32,33] or contradict the present findings [34]. In preclinical experiments, an increase of FSH was found [35,36]. The testosterone reductions found in this study are compatible with the diminished gonadotrophic hormone concentrations, particularly of LH. The differences in the response of LH and FSH after SCH 900435 administration are partly related to the differences in half-life. LH has an elimination half-life of around one to two hours [37], while that of FSH is in the region of five hours [38]. Although a direct effect of SCH 900435 on the expression of any of these hormones individually cannot be excluded, the time-courses of the hormonal effects and the linear relation between dose and effect agree better with a common effect on LH and FSH release, most likely due to reduction of GnRH release [39], and suggesting the site of action of SCH 900435 to be the hypothalamus. These gonadotrophic responses are dose dependent and quite consistent, which makes these hormones (and LH in particular), the most reliable and sensitive pharmacodynamic indicators of a central SCH 900435 effect that were identified in these studies.

In general, animal studies of glycine reuptake inhibitors and glycine agonists showed central nervous system depression (i.e. reduction in motor activity) [7,40,41]. In apparent contrast, SCH 900435 produced slight improvements of smooth pursuit eye movements in the SD study, which seemed to be dose dependent, and hence to be related to the pharmacological activity of the drug. Improved smooth pursuit could point to a mild stimulant effect of SCH 900435. On the other hand, the EEG demonstrated small statistically significant reductions in alpha$_2$ power (10.5 - 12.5 Hz), which are more in line with CNS depression. However, this alpha reduction could be secondary to the visual symptoms caused by SCH 900435, since EEG alpha rhythm is particularly sensitive to visual input (e.g. eye opening or closure) and subjective well being (e.g. suppression by nausea and dizziness). These findings should be replicated before firm conclusions can be drawn.

Since experience with indirect NMDA-receptor activators in humans was limited, a cautious and adjustable approach to development of these drugs in humans is required. The present paper describes how a novel glycine reuptake inhibitor, which had a steep in dose-response curve for adverse events in test animals and showed unanticipated and initially disconcerting visual symptoms in humans, was safely introduced in healthy volunteers using an adaptive research strategy consisting of frequent interim analyses of PK characteristics and of data-intensive PD effects, and a return to dedicated animal studies before the drug was reintroduced in humans. Unanticipated visual effects were detected from adverse event reports, and could be interpreted and pursued more reliably using frequent measurements of visual analogue scales for a broad range

of subjective symptoms, and pharmacodynamic measures of multiple systemic and CNS effects, including pupil size. This strategy is particularly useful for chemical entities with novel mechanisms of action, which have a higher chance of showing unanticipated effects.

Intensive pharmacodynamic measurements can also provide indications for CNS-penetration and dose-related pharmacological activity, especially since the early studies in healthy volunteers almost always cover a large dosage range. The establishment of dose-related changes provides strong evidence for a drug-related effect. This is clearly demonstrated by the consistency of almost all effects that were observed during the first study, even though they were unexpected and may even have initially seemed spurious. It cannot be ascertained whether the indications for CNS penetration and pharmacological activity of SCH 900435 are due to $GlyT_1$ reuptake inhibition, although this is suggested by the high potency and selectivity of SCH 900435 at this site. The retina, which is particularly rich in glycine- and glutamate-containing cells [42], has a structure similar to the blood brain barrier. The observed visual spots in the SD study together with the dose-responsive hormone changes and neurophysiological effects could indicate that SCH 900435 passes the blood retinal barrier and penetrates into the CNS. Long-term studies and investigations with other glycine reuptake inhibitors are needed to confirm whether retinal and gonadotropic changes are pharmacological effects of this novel drug class. Although these studies provided indications that SCH 900435 has a pharmacological effect in the CNS at doses that are well tolerated after titration, the antipsychotic effects and the therapeutically active doses of SCH 900435 and of glycine enhancement strategies in schizophrenia in general [43,44] remain to be established.

Appendix I

Description of pharmacodynamic tests and statistics single ascending dose study

Different measures of the effects of SCH 900435 on the central nervous and visual systems were used in this study. A multimodal test battery was performed regularly, to keep close track of any potential drug-related changes in CNS-functionality.

VISUAL ANALOGUE SCALES (VAS)

Subjective effects were quantified using a Dutch translation of the 16 visual analogue scales (VAS) originally described by Norris [1] and applied to drug effect by Bond and Lader [2]. The Dutch translation showed effects on many different CNS active drugs, including sedative agents [3,4], dopaminergic drugs [5], scopolamine [6], and THC [7]. From the set of 16 scales, three factors corresponding to alertness, mood and calmness were derived.

Bowdle described 13 VAS's to quantify the psychotomimetic effects of ketamine [8]. A translated version of the Bowdle VAS showed concentration-related effects in THC [7], scopolamine [6], and zolpidem [9].

The Leeds Sleep Evaluation Questionnaire (LSEQ) contains 14-item visual analogue scales related to the subjective aspects of sleep and waking [10].

PHARMACO-ELECTROENCEPHALOGRAPHY

Pharmaco-EEG was performed as a general measure of CNS activity [11]. The literature suggests that antipsychotics show distinct profiles of EEG-changes [12,13]. EEG recordings were made using silver-silver chloride electrodes, fixed with collodion according to the international 10/20 system. The electrode resistances were kept below 5 kOhm. The duration of one EEG measurement was 3 minutes. The signals were amplified and stored on a Vitaport-8® system. A/D converted data were subjected to a windowed Fast Fourier transform analysis giving a frequency resolution

of 0.25 Hz. Summary parameters were calculated using the frequency bands as defined by Hermann from a factor analysis of human EEG [14].

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS

Saccadic and smooth pursuit eye movements were recorded as described previously [3,15,15-17] and have shown dose- and concentration-related effects on many different CNS active drugs, including GABA-ergic [4,5,18], serotonergic [19], noradrenergic [20-22], and dopaminergic drugs [23].

ADAPTIVE TRACKING

The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol, various psychoactive drugs and sleep deprivation [3,17]. The adaptive tracking test was performed as described earlier and can be divided into two tasks: the PURSUIT and the FITTS task [24-26]. During the PURSUIT task, the subject was instructed to keep a dot inside a moving circle by operating a joystick. If this effort was successful, the speed of the moving circle was increased. Conversely, the velocity was reduced if the test subject could not maintain the dot inside the circle. The FITTS task was to measure quick motor responses and the subject was asked to aim at targets with a pen as quickly as possible without missing the target.

BODY SWAY

The body sway meter records body movements in a single plane, providing a measure of postural stability. Changes in body sway were seen for many different CNS active drugs, including GABA-ergic drugs [4,18,27], and THC [7] and was performed as previously described [23].

PUPILLOMETRY

Pupil size was included as a measure of autonomic tone and ocular function. In the SD study, exploratory pupillometry was performed using digital photometry. Twa *et al* showed that estimation of pupil size by digital photography was more repeatable and accurate than estimates by common clinical techniques over a wide range of illumination [28].

The subject was instructed to look into the lens of a digital camera after at least five minutes adaptation in ambient lighting. A picture of the eyes was taken using a single flash. All pictures were imported and stored in Adobe Illustrator 9, to determine the diameters of the pupil and the iris in millimetres. For each eye, these values were recorded, and the pupil/iris ratio was calculated as a measure of pupil size.

NEUROENDOCRINE EFFECTS

In humans LH has proven to be a sensitive marker of NMDA activity [29,30]. Prolactin was also measured as an indication of (indirect) dopamine activity [5].

After collection, blood samples for LH, FSH, testosterone, and prolactin were kept at room temperature for about 30 minutes to allow coagulation. Serum was separated by centrifugation (2,000´g at 4°C for 15 minutes) and stored in a deep freezer at - 40 °C. The hormones were performed according to Good Laboratory Practice analysed by Organon Development GmbH using a validated fluoro-immunoassay.

Reference values for men were 2 - 80 U/L for LH, 2-10 U/L for FSH, 10-40 nmol/L for testosterone and < 15 µg/L for prolactin. For cortisol reference values were time dependent: 0,20 - 0,60 µmol/L (8 AM), 0,10 - 0,30 µmol/L (11 PM) and < 0,18 µmol/L (11 PM).

The assays had LLQs of 0.25 mU/mL (FSH), 0.6 mU/mL (LH), 0.14 ng/mL (testosterone), 0.25 ng/mL (prolactin), an intra-assay precision (expressed as coefficient of variation) of 0.8-1.8% (FSH), 1.4-2.2% (LH), 3.3-5.2% (testosterone), 2.8-3.2% (prolactin) and inter-assay precision of 1.4-1.6% (FSH), 1.8-2.6% (LH), 2.7-8.6% (testosterone), and 4.5-6.3% (prolactin).

Pharmacokinetics

Plasma (K-EDTA) was centrifuged within 15 minutes after sampling and stored at -40 degrees Celsius. Plasma samples were assayed for SCH 900435 using a validated liquid chromatographic method with mass spectrometric detection with a lower limit of quantification of 0.1 ng/mL.

The assay had an LLQ of 0.1 ng/mL, an intra-assay precision (expressed as coefficient of variation) of 1.4-6.5% and inter-assay precision of 2.0-24.6%.

Statistical analysis

For the PD parameters both a general treatment effect and a simple linear effect was tested.

To calculate the treatment effect, AUEs (Area Under Effect-time curves) per subject were calculated with the linear trapezoidal rule using protocol times, and divided by the corresponding time span, resulting in a weighted average response. AUEs of the whole time period, of the period 0-4 hours and 0-90 minutes (only for pupil size) of the PD variables were analyzed with an analysis of variance with fixed factors treatment and visit, random factor subject and pre-value as covariate (ANCOVA analysis).

If the treatment effect was significant, contrasts of all treatments versus placebo were calculated. The different time spans of the AUCs were chosen as there were very fast, short lasting effects and slower, more long lasting effects.

If the treatment effect was significant and the linear effect suggested a dose effect relation, an additional regression analysis of the concerning parameter on log dose was performed. This was performed by calculating the regression of relationship between the change from baseline AUEs and the log10 dose with subject, intercept and slope as random factors and visit as fixed factor. Slopes being different from zero were tested with an alpha of 0.05 two sided.

Conclusions with respect to dose proportionality/dose independence were primarily based on descriptive statistics of PK parameters and on summary plots of (dose-normalised-(dn))C_{MAX} and (dn-)AUC versus dose. An additional exploratory Analysis of Variance (ANOVA) was performed on dn-C_{MAX}, dn-AUC, $T_{1/2}$, (weight-normalised-(wn))Cl_{APP}, and (wn-)$V_{Z,APP}$ and an appropriate non-parametric test on T_{MAX} and t_{LAG} to test for dose proportionality/dose independence.

Appendix II

Description of pharmacodynamic tests and statistics visual effects study

VISUAL ELECTROPHYSIOLOGY

A series of electrophysiological tests were performed to obtain a comprehensive overview of various retinal and pupillary functions. Subjects were divided in different groups, to allow investigations under different lighting conditions. All eyes were dilated before full field testing using tropicamide (1%) and/or phenylephrine hydrochloride (2.5%). Measurements were performed according to the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV), as described by Marmor and Zrenner (2004) [31] unless cited otherwise.

One group of subjects was tested under dark conditions, with a number of tests that lasted approximately 12 minutes and was repeated every 25 minutes. Subjects who were examined under dark conditions were adapted to the dark for twenty minutes after pupillary dilation, before the minimum luminance of a test spot required to produce a visual sensation was determined [31]. The full field ('Ganzfeld') ERG protocol incorporated the ISCEV standard and used a commercial system (RETIscan System; Roland Consult, Wiesbaden, Germany). Small disc electrodes were placed on the skin at the side of the subject's eye, and corneal soft gold foil recording electrodes were placed under red light. Rod specific ERG was recorded after a single flash. The rod response was identified as the first signal measured after dark adaptation. Additional recordings included the use of a red flash stimulus [32]. Maximal ERGs were recorded after a brighter maximal flash (Standard + 0.6LU).

Another group was examined under light conditions. The duration of testing was approximately 20 minutes, in cycles of 25 minutes. These subjects also underwent standard ISCEV ERGs after pupillary dilation and a standard period and intensity of light adaptation [31]. Cone responses

were measured after a single white flash preceded by background illumination to suppress rod function. Under the same conditions 30 Hz flicker responses were obtained with repeated single flash stimuli.

Long duration photopic stimulation was used to record ON and OFF ERG responses using previously described techniques [33]. In brief, an amber stimulus of 120 ms or 200 ms duration (luminance 560 cd/m2) was presented upon a bright green background (luminance 160 cd/m2) close to peak rod spectral sensitivity and thus suitable to suppress rod function. A- and b-waves occur at onset of the flash, and a d-wave at light offset. These responses represent different functional pathways of retinal rods and cones. Human S-cones mediate the signals of short wavelength sensitive cones [34]. S-cone responses were measured using a chromatic stimulus ERG as described by Yamamoto *et al* [35].

Under photopic conditions, the ERG shows an a-wave that arises from cones and off-bipolar cells, and a b-wave that originates from on- and off-bipolar cells with a possible contribution from Mueller cells. The late negative component subsequent to the b-wave is called the photopic negative response. This measurement was performed as described by Holder *et al* [36].

A third group of subjects was investigated under light conditions with undilated pupils. These tests lasted approximately 45 minutes. Flash visual evoked potentials (VEPs) were performed according to the ISCEV standard for clinical VEP testing [36]. Pattern ERG was recorded using a black and white checkerboard stimulus, with 98% contrast and a mean luminance of 80 cd/m2. The stimulus frequency was 4.5 reversals/sec. The field size was 15˚ x 11˚, with a check size of 45 min. The amplifier gain was set to 200•103, band pass filter 1-100 Hz, with a sampling rate of 2 kHz. Optimum spectacle correction was used. Fixation was enabled by a red central fixation target and the patient's fixation was monitored using CCTV (closed circuit television). The amplitude and latency of the PERG P50 and N95 components were analysed. The p1 and n1 components of the first order kernel of the mfERG were analysed using the RETIscan software. Trace arrays were divided into 5 concentric rings centred on the fovea (Ring 1, 0-2.1˚; Ring 2, 1.4-6.7˚; Ring 3, 5.7-12.0˚; Ring 4, 9.5-19.8˚; Ring 5, 15.1-28.5˚). The traces within the ring were averaged and the amplitude and latency of p1 and n1 from each ring summation was analysed.

Colour contrast sensitivity was measured psychophysically by determining thresholds along isoluminant protan, deutan, and tritan colour confusion axes using the Arden colour contrast sensitivity system [37]. Pupillometry was performed using a similar method as described in the single ascending dose study (see Appendix I).

NEUROENDOCRINE EFFECTS

See Appendix I single ascending dose study.

VISUAL ANALOGUE SCALES (VAS)

See Appendix I single ascending dose study. This Bond and Lader VAS was extended with a Bastani mood rating scale [1,2].

Additionally, subjects were given a visual questionnaire to ascertain visual symptoms. The questionnaire consisted of eight questions and subjects answered whether they agreed or not and if they agreed then whether the symptoms were mild or severe. The eight questions were as follows:

Your vision is blurred
There appears to be a film over your eyes
You have difficulty assessing how far away objects are
You are seeing flashes of light
You are seeing dark patches
You are having more difficulty than usual focusing
You would not feel safe driving a car with your vision as it is
Do you have any other visual symptoms or disturbance?

The final question had space for a description of other symptoms if present.

Pharmacokinetics

See Appendix I single ascending dose study.

Statistical analysis

Only descriptive and summary statistics were performed for the electrophysiological parameters, hormones, laboratory tests (hematology, serum blood chemistry, urinalysis and any urine microscopy) and pharmacokinetics. An Analysis of Variance (ANOVA) on log-transformed pharmacokinetic parameters (for T_{MAX} after ranking) with factor group; all tests at the 5% level of significance.

Appendix III

Based on the outcomes of the single ascending dose trial, a selection of tests was made for the multiple (ascending) dose study. These measurements were partly targeted at cognitive effects, which were difficult to assess in great detail after single doses and to the visual effects that were observed after the first administration in man.

VISUAL ANALOGUE SCALES (VAS)

See Appendix II visual effects study.

PHARMACO-ELECTROENCEPHALOGRAPHY

During the multiple ascending dose study, more extensive EEG examinations were performed than when the drug was first administered to humans. A Walter Graphtek Polygraph System running validated PL Windsor acquisition and Fourier analysis software was used to collect full 21-lead EEGs, including three minutes eyes closed vigilance control (primary condition), three minute resting, hyperventilation and photic stimulation. Clinical neurophysiologist excluded subjects with clinically significant abnormalities at screening, and recordings with persistent artefacts. Electrodes were attached according to the international 10-20 system using similar materials and methods described for the single ascending dose study. Data from each subject's dominant side was extracted for the frontal (F3 or F4 channel), central (C3 or C4 channel), parietal (P3 or P4 channel) and occipital (O1 or O2 channel) positions at pre-dose, 0.5, 1, 2, 4 and 6 h post-dose on Days 5 and 13 and matching time points on Day -1. The following variables were recorded:total power [μV^2], peak power [μV^2]; peak frequency [Hz]; mean frequency (frequency (1-30 Hz) below which 50 % of total power is located) [Hz]; spectral edge (frequency (1-30 Hz) below which 90 % of total power is located) [Hz]; vigilance index: ($alpha_1$ + $alpha_2$)/(theta + delta) [ASI]; and power [μV^2] and centroid frequency [Hz] for the delta (1-4 Hz), theta (4-8.5 Hz), $alpha_1$ (8.5-10.5 Hz), $alpha_2$ (10.5-12.5 Hz), beta1 (12.5-18.5 Hz) and beta3 (21-30 Hz) frequency bands.

COGNITIVE TESTING

A battery of tasks from the Cognitive Drug Research (CDR, Goring-On-Thames, UK) was administered and consisted of the following tests (described in [38]): simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking. Postural stability measures the ability to stand upright without moving waist assessed using the CDR meter that was modelled on the Wright Ataxiameter [13]. A cord from the meter was attached to the subject who was required to stand as still as possible with feet apart and eyes closed for one minute. During the tracking test the subject used a joystick to track a randomly moving target on the screen for 1 minute. The distance off-target per second was recorded.

PUPILLOMETRY

See Appendix I single ascending dose study. In the multiple (ascending) dose study, the pupil size was measured more extensively than in the single (ascending) dose study in three different conditions (0.04 lux/scotopic, 0.4 lux/low mesopic, 4.0 lux/high mesopic) after the subject was dark adapted to the room for 5 minutes using a Procyon P2000SA Pupillometer (Keeler Instruments, Broomall, Pa.).

Neuroendocrine effects

See Appendix I single ascending dose study (prolactin concentrations were not determined in this study).

Statistical analysis

Only descriptive and summary statistics were performed for the parameters of this study except for pharmacokinetics, pharmaco-EEG parameters (total power, delta, theta, alpha$_1$, alpha$_2$ and vigilance index) and CDR parameters (simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking).

SCH 900435 concentrations in plasma and derived PK parameters were summarized using descriptive statistics. Analysis of variance, to determine time to reach steady state during multiple dosing, was conducted using the Helmert contrast transformation on pre-dose concentrations. Testing on dose proportionality/independence and regimen effects was done using analyses of variance on PK parameters. Food-effect testing was based on C_{MAX} and $AUC_{0\text{-}12}$ of SCH 900435.

Only the primary EEG parameters in both vigilance controlled and rested conditions were statistically analysed. Analysis was performed separately at each electrode location (frontal, central, parietal and occipital). The mixed model described below was used to estimate the effect of each dosing regimen (as ratio of placebo, averaged over time on each day separately) with 95 % CI.

For each log-transformed primary pharmaco-EEG parameter, day and location separately, a repeated measures analysis model was fitted using PROC MIXED in SAS® Version 8.2 with time-matched log-transformed value on Day -1 as baseline covariate and fixed effects terms for time, dose and time x dose interaction term. The repeated nature of the data on each subject was modelled with autoregressive covariance structure [AR(1)]. Data for placebo were pooled across all cohorts (Groups I, II, III and IV). Since only two subjects received placebo in each cohort, no attempt was made to adjust for cohort. Data were included in the model from all available time points (0, 0.5, 1, 2, 4 and 6 h) and the difference in the average value over post dose time points (i.e. excluding 0 time point) of each dosing regimen relative to placebo was estimated with 95 % CI. These estimates and confidence limits were back transformed to give the effect as a ratio of placebo with 95 % CI. No adjustments for multiple comparisons were made since these analyses are considered exploratory. The influence of any outliers was explored on the statistical significance of results, where necessary.

For the CDR parameters repeated measures analysis of variance (ANOVA) was conducted on the 'difference from baseline' scores using SAS® PROC MIXED. A pooled placebo treatment was used. Fixed terms were fitted to the model for treatment (up to 6 levels), day (2 levels), time (4 levels) and the 2-way and 3-way interactions. A random effect of subjects-within-sequence was fitted to the model. Significance of the interactions was tested at the $p<0.05$ level. The interactions were assessed sequentially, highest order first, and non-significant interaction terms were removed from the model. Pairwise comparisons of the active treatments and placebo were performed to clarify any statistically significant interaction effects.

Table 1 Dosing groups with corresponding SCH 900435 (mg) and placebo (P) treatment in single ascending dose study

A. Dosing as originally planned in protocol

Group I				Group II				Group III				Group IV				Group V			
0.5	2.0	8.0	P	8.0	12	18	P	18	27	40	P	40	60	90	P	90	135	P	
0.5	2.0	P	8.0	8.0	12	P	18	18	27	P	40	40	60	P	90	90	P	135	
0.5	P	2.0	8.0	8.0	P	12	18	18	P	27	40	40	P	60	90	P	90	135	
P	0.5	2.0	8.0	P	8.0	12	18	P	18	27	40	P	40	60	90				

B. Actual dosing during study

Group I				Group II				Group III				Group IV			
0.5	1.0	2.0	P	2.0	3.0	5.0	P	5.0	8.0	12	P	12	20	30	P
0.5	1.0	P	2.0	2.0	3.0	P	5.0	5.0	8.0	P	12	12	20	P	30
0.5	P	1.0	2.0	2.0	P	3.0	5.0	5.0	P	8.0	12	12	P	20	30
P	0.5	1.0	2.0	P	2.0	3.0	5.0	P	5.0	8.0	12	P	12	20	30

Table 2 Most frequent Adverse Events single ascending dose study

Reported AE[A]	SCH 900435 dose	Placebo (%)		0.5 mg (%)		30 mg (%)		0.5-30 mg (%)		0.5-30 mg + placebo (%)	
	N of subjects	16		4		4		16		32	
	N of AEs	10		5		15		85		95	
Dizziness		1	(10.0)	0	(0.0)	3	(20.0)	14	(16.5)	15	(15.8)
Somnolence		2	(20.0)	1	(20.0)	3	(20.0)	13	(3.5)	15	(15.8)
Headache		1	(10.0)	0	(0.0)	0	(0.0)	12	(14.1)	13	(13.7)
Fatigue		2	(20.0)	1	(20.0)	1	(6.7)	8	(9.4)	10	(10.5)
Abnormal vision		0	(0.0)	0	(0.0)	3	(20.0)	6	(7.1)	6	(6.3)

A. Not all AEs from each dose are shown for reasons of clarity; there were no relevant differences in these omitted groups

Table 3 Contrasts with placebo on dose groups ANCOVA analysis single ascending dose study (reported for doses SCH 900435 with statistically significant contrasts and higher)

Parameter	Unitc	Dose (mg)	LSM Estimate placebo	LSM Estimate SCH 900435	Estimate of difference	p-value[A]	95% Confidence Interval
Pupil/iris ratio right eye (0-90 minutes)[B]	NA	20	0.41	0.44	-0.030	**0.0145**	-0.05 - -0.01
		30		0.48	-0.065	**<0.0001**	-0.09 - -0.04
VAS colours (0-4 hours)	mm	30	1.45	10.94	-9.48	**<0.0001**	-13.05 - -5.91
VAS dizzy (0-4 hours)	mm	12	43.72	49.57	-5.85	**0.0170**	-10.59 - -1.10
		20		50.34	-6.62	**0.0388**	-12.88 - -0.36
		30		52.14	-8.42	**0.0118**	-14.89 - -1.95
LH (0-final hours)	U/L	8	3.04	2.21	0.82	**0.0025**	0.31 – 1.34
		12		2.69	0.35	0.0864	-0.05 – 0.74
		20		2.10	0.93	**0.0009**	0.41 – 1.46
		30		1.90	1.14	**0.0002**	0.58 – 1.70
LH (0-4 hours)	U/L	8	2.93	2.17	0.76	**0.0335**	0.06 – 1.45
		12		2.15	0.78	**0.0062**	0.24 – 1.32
		20		1.77	1.16	**0.0017**	0.46 – 1.86
		30		1.58	1.35	**0.0003**	0.65 – 2.05
FSH (0-final hours)	U/L	8	2.91	2.50	0.42	**0.0015**	0.18 – 0.66
		12		2.67	0.25	**0.0131**	0.06 – 0.45
		20		2.63	0.28	**0.0301**	0.03 – 0.54
		30		2.68	0.24	0.1068	-0.05 – 0.52
Testosterone (0-final hours)	nmol/L	8	5.88	4.93	0.95	**0.0110**	0.24 – 1.66
		12		5.45	0.43	0.1269	-0.13 – 0.99
		20		4.66	1.22	**0.0024**	0.47 – 1.98
		30		5.26	0.62	0.1440	-0.22 – 1.46
EEG alpha2 (0-4 hours)	µV	12	3.45	3.35	0.11	**0.0449**	0.00– 0.21
		20		3.28	0.17	**0.0099**	0.044 – 0.30
		30		3.18	0.27	**0.0002**	0.14 – 0.41
Smooth pursuit (0-final hours)	%	8	50.94	58.00	-7.06	**0.0172**	-12.80 - -1.32
		12		54.43	-3.49	0.1195	-7.93 – 0.95
		20		51.16	-0.22	0.9397	-6.01 – 5.57
		30		57.26	-6.32	**0.0433**	-12.45 - -0.20
Smooth pursuit (0-4 hours)	%	8	50.281	56.17	-5.89	**0.0289**	-11.14 - -0.64
		12		56.37	-6.09	**0.0042**	-10.14 - -2.04
		20		53.00	-2.71	0.3086	-8.01 – 2.59
		30		57.50	-7.22	**0.0130**	-12.85 - -1.60

A. Significant p-values are indicated in bold (lower than 0.05); B. only results for the right eye were shown as these were similar to the left eye; C. mm = millimetre; U/L = units / liter; µV = microvolt.

Table 4 Regression analysis table of dose-linearity single ascending dose study

Parameter	Unit[B]	p-value slope[A]	Estimate of the slope	95% CI
Log pupil/iris ratio right eye (0-90 minutes)	NA	0.573	0.004	0.017 / -0.010
Log VAS colours (0-4 hours)	NA	0.157	0.081	0.198 / -0.035
VAS dizzy (0-4 hours)	mm	0.062	2.302	4.722 / -0.119
LH (0-final hours)	U/L	**0.032**	-0.356	-0.031 / -0.682
LH (0-4 hours)	U/L	**0.004**	-0.501	-0.164 / -0.838
FSH (0-final hours)	U/L	**0.020**	-0.193	-0.042 / -0.344
Testosterone (0-final hours)	nmol/L	**0.012**	-0.424	-0.101 / -0.747
EEG alpha2 (0-4 hours)	µV	0.142	-0.053	0.020 / -0.126
Smooth pursuit (0-4 hours)	%	**0.001**	3.305	5.125 / 1.485

A. Statistically significant p-values (lower than 0.05) are indicated in bold; B. NA = not applicable; mm = millimetre; U/L = units / liter; µV = microvolt.

Table 5 Results of visual analogue scales in visual effects study - number of subjects responding 'yes' to the question [number of subjects considering the effect as severe]

Question/Study time	Placebo	SCH 900435		
		5 mg	13 mg	20 mg
N	24	24	24	24
Your vision is blurred				
Pre-dose	9 [2]	11 [3]	12 [2]	12 [2]
1.5 hours post-dose	7	10 [1]	16 [1]	14 [1]
8 hours post-dose	0	1	0	1
There appears to be a film over your eyes				
Pre-dose	1	2	2	1
1.5 hours post-dose	1	2	3	3 [1]
8 hours post-dose	0	0	0	0
You have difficulty assessing how far away objects are				
Pre-dose	2	1	2	2
1.5 hours post-dose	2	2	3 [1]	8 [1]
8 hours post-dose	0	0	0	0
You are seeing flashes of light				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	5 [1]	7 [1]
8 hours post-dose	0	0	0	0
You are seeing dark patches				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	2 [1]	3 [1]
8 hours post-dose	0	0	0	0
You are having more difficulty than usual focusing				
Pre-dose	7 [1]	11 [2]	8	7 [1]
1.5 hours post-dose	6	7 [1]	14 [1]	10 [1]
8 hours post-dose	1	2	1	1
You would not feel safe driving a car with your vision as it is				
Pre-dose	5 [1]	4	3	5
1.5 hours post-dose	4	6	11 [1]	12 [1]
8 hours post-dose	3	1	0	1
Do you have any other visual symptoms or disturbance?				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	2 [1]	3 [1]
8 hours post-dose	1	1	0	0

Table 6 Treatment groups multiple (ascending) dose study

Dose group	Treatment [A,C]	Study day [B]												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	4 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
2	8 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
3	16 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
4	12 mg o.d.	x												
	12 mg b.i.d				x	x	x	x	x	x	x	x	X	
	16mg o.d.													x
5 (dose titration)	8 mg b.i.d	x	x	x										
	12 mg b.i.d				x	x	x							
	16 mg b.i.d							x	x	x				
	20 mg b.i.d										x	x	X	
	30 mg o.d.													x

A o.d. = once daily; B.i.d. = twice daily; B x = dose administered; C doses for groups 4 and 5 were determined following blinded review of the data for groups 1 to 3

Table 7 Study design pharmacodynamic measurements multiple (ascending) dose study

	Days										
Assessment	-1	1	2	3	4	5,7,9,11	6,8,10	12	13	14	15
Visual assessments	On medical need only										
SCH 900435 dosing		X	X	X	X	X	X	X	X		
Pharmaco EEG	X [A]					Day 5 [A]			X [D]		
LSEQ					X		X		X		X
VAS, pupillometry [A]				X	X	Day 7		X			X
Visual questionnaire [A]		X		X	X	X	X	X	X		X
LH, FSH, testosterone [B]		X		X			X		X		X
PK sampling [C]		X	X	X	X	X	X	X	X	X	X
Cognitive Test battery [D]	X				X			X			

A Pre-dose and 0.5, 1, 2, 4 and 6 hours post-dose; B Groups I-IV only: Days 1, 3, 6, 8, 10, 13: pre-dose, 2, 6 and 12 h post-dose; Day 15:pre-dose; C PK sampling times were frequent pre-dose and post-dose and were different depending on the treatment group; D Pre-dose and 1, 4 and 6 hours post-dose (in the morning).

Table 8 Number of subjects (%) with AEs possible or probably related to SCH 900435 for 'eye disorders' and 'CNS disorders' in the multiple (ascending) dose study

	Treatment [A]					
	Placebo	4mg q.d.	8mg q.d.	16mg q.d.	12mg b.i.d.	Dose titration
	n=10	n=6	n=6	n=6	n=6	n=6
Eye disorder						
Accommodation disorder	0	0	0	0	1 (17)	0
Eye pain	0	0	0	0	0	2 (33)
Eyelid irritation	0	0	0	1 (17)	0	0
Ocular discomfort	0	0	0	0	1 (17)	0
Photopsia	0	0	0	3 (50)	0	1 (17)
Vision blurred	0	0	1 (17)	5 (83)	2 (33)	4 (67)
Visual disturbance	0	0	1 (17)	3 (50)	2 (33)	1 (17)
Central Nervous System disorders						
Balance disorders	0	0	0	0	1 (17)	0
Coordination abnormal	0	0	0	2 (33)	0	0
Dizziness	1 (10)	0	1 (17)	3 (50)	2 (33)	5 (83)
Dizziness postural	0	0	1 (17)	3 (50)	3 (50)	2 (33)
Headache	2 (20)	0	0	1 (17)	3 (50)	2 (33)
Lethargy	0	0	0	2 (33)	2 (33)	6 (100)
Memory impairment	0	0	0	0	0	1 (17)
Somnolence	1 (10)	0	0	4 (67)	1 (17)	3 (50)
Tremor	1 (10)	0	0	0	0	0
Tunnel vision	0	0	0	1 (17)	0	0

A. q.d. = once daily; b.i.d. = twice daily

Figure 1 Structural formula of SCH 900435

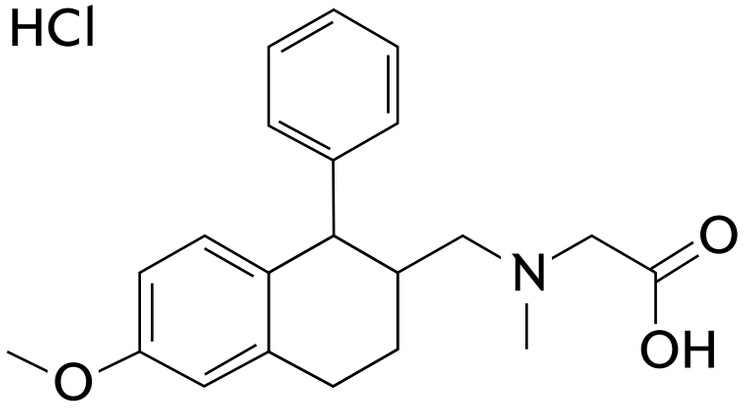


Figure 2 Mean Concentration-versus-Time plot in single ascending dose study

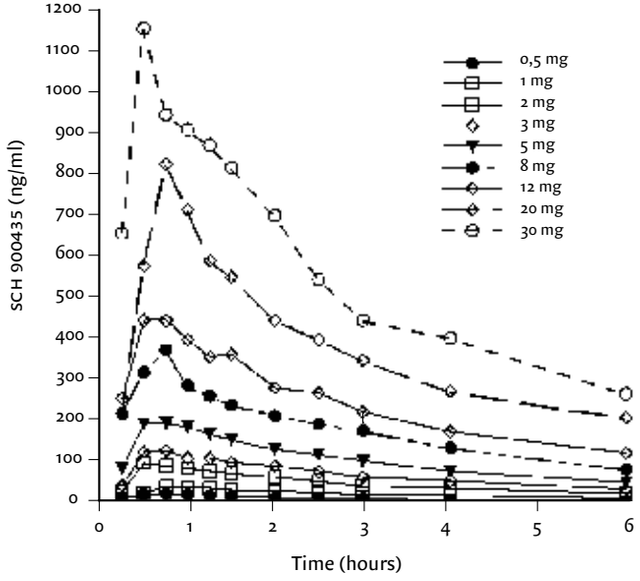


Figure 3 Average graph of AUE change from baseline 0-4 hours VAS Colours by log dose (standard deviations as error bars) in single ascending dose study

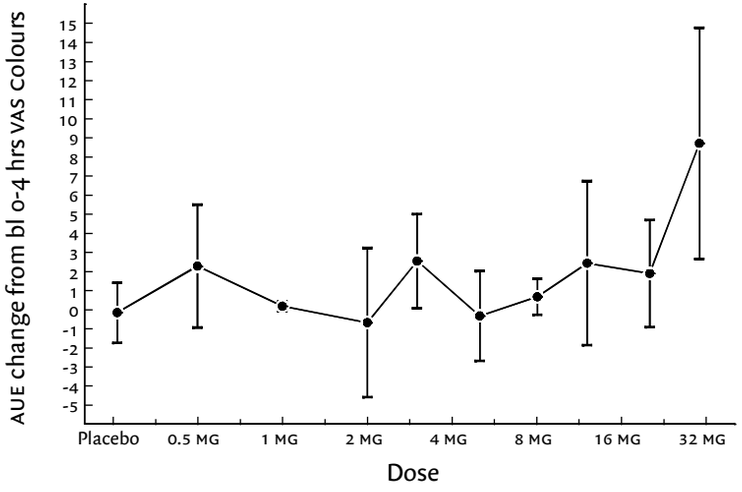


Figure 4 Adjusted (for baseline) mean time profile of different doses of VAS Colours with 95% CI for highest dose and lower 95% CI for lowest dose in single ascending dose study

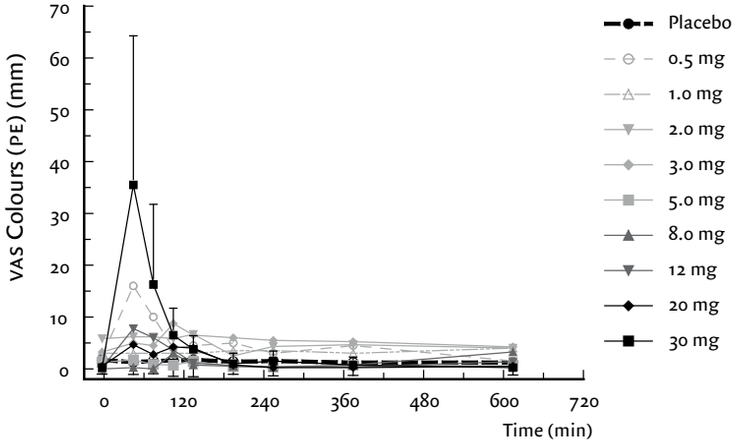


Figure 5 Adjusted (for baseline) mean time profile of different doses of LH with 95% CI for highest dose and lower 95% CI for lowest dose in single ascending dose study.

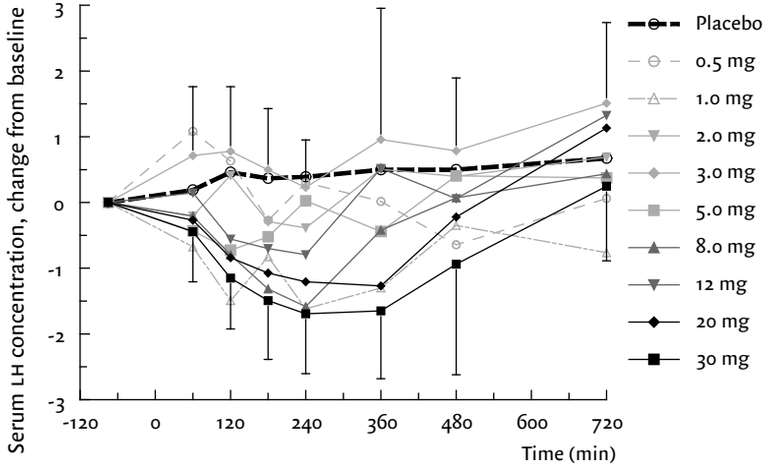


Figure 6 Average graph of AUE change from baseline 0-90 minutes pupil/iris ratio right eye by log dose (standard deviations as error bars) in single ascending dose study

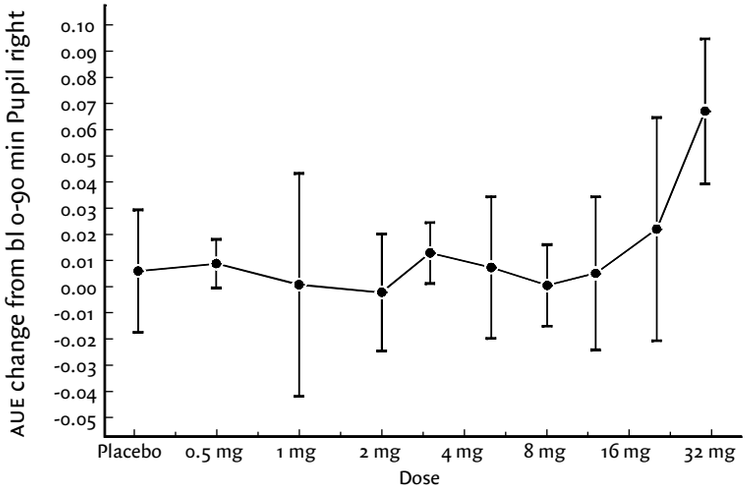
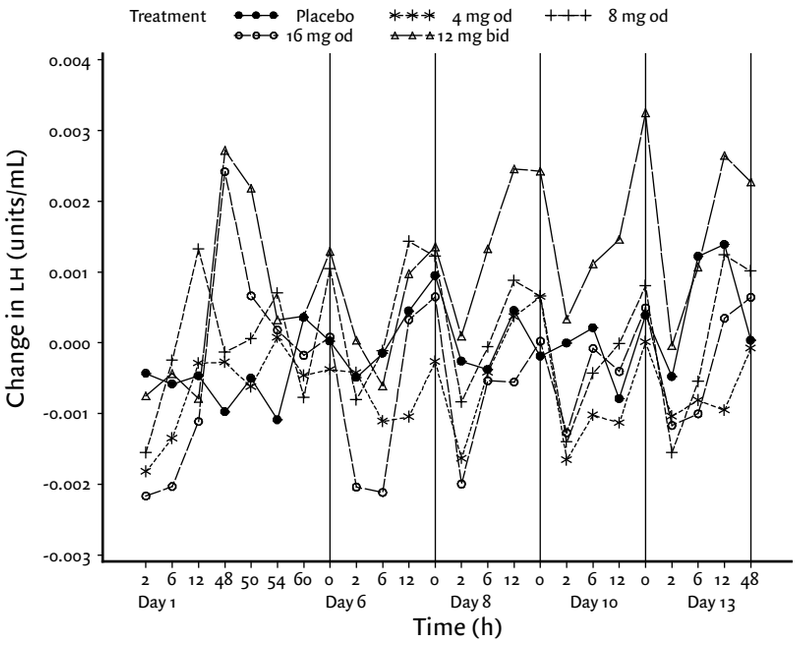


Figure 7 Mean changes from baseline LH values against time following administration of SCH 900435 or placebo in multiple ascending dose study



REFERENCES

1 Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976; 192: 481-3.
2 Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci U S A 1975; 72: 4376-80.
3 Javitt DC. Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. Curr Opin Psychiatry 2006; 19: 151-7.
4 Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994; 51: 199-214.
5 Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991; 148: 1301-8.
6 Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. Harv Rev Psychiatry 1999; 7: 125-43.
7 Millan MJ. N-Methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insights and clinical perspectives. Psychopharmacology (Berl) 2005; 179: 30-53.
8 Schmidt WJ, Kretschmer BD. Behavioural pharmacology of glutamate receptors in the basal ganglia. Neurosci Biobehav Rev 1997; 21: 381-92.
9 Javitt DC, Hashim A, Sershen H. Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacology 2005; 30: 649-56.
10 Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995; 52: 998-1007.
11 Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci 1998; 18: 5545-54.
12 Javitt DC. Glycine modulators in schizophrenia. Curr Opin Investig Drugs 2002; 3: 1067-72.
13 Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 1992; 8: 927-35.
14 Aragon C, Lopez-Corcuera B. Glycine transporters: crucial roles of pharmacological interest revealed by gene deletion. Trends Pharmacol Sci 2005; 26: 283-6.
15 Chen L, Muhlhauser M, Yang CR. Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. J Neurophysiol 2003; 89: 691-703.
16 Depoortere R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, Poncelet M, Heaulme M, Santucci V, Decobert M, Cudennec A, Voltz C, Boulay D, Terranova JP, Stemmelin J, Roger P, Marabout B, Sevrin M, Vige X, Biton B, Steinberg R, Francon D, Alonso R, Avenet P, Oury-Donat F, Perrault G, Griebel G, George P, Soubrie P, Scatton B. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology 2005; 30: 1963-85.
17 Harsing LG, Jr., Gacsalyi I, Szabo G, Schmidt E, Sziray N, Sebban C, Tesolin-Decros B, Matyus P, Egyed A, Spedding M, Levay G. The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav 2003; 74: 811-25.
18 Javitt DC, Balla A, Sershen H, Lajtha A. A.E. Bennett Research Award. Reversal of phencyclidine-induced effects by glycine and glycine transport inhibitors. Biol Psychiatry 1999; 45: 668-79.
19 Le Pen G, Kew J, Alberati D, Borroni E, Heitz MP, Moreau JL. Prepulse inhibition deficits of the startle reflex in neonatal ventral hippocampal-lesioned rats: reversal by glycine and a glycine transporter inhibitor. Biol Psychiatry 2003; 54: 1162-70.
20 Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2004; 55: 452-6.
21 Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 2005; 62: 1196-204.
22 Tsai GE, Yang P, Chang YC, Chong MY. D-alanine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2006; 59: 230-4.
23 Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, Hayden DL, McCarley R, Coyle JT. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry 1999; 56: 21-7.
24 Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001; 158: 1367-77.
25 Mizutani AR, Parker J, Katz J, Schmidt J. Visual disturbances, serum glycine levels and transurethral resection of the prostate. J Urol 1990; 144: 697-9.
26 Hahn RG, Andersson T, Sikk M. Eye symptoms, visual evoked potentials and EEG during intravenous infusion of glycine. Acta Anaesthesiol Scand 1995; 39: 214-9.
27 Dreier JP. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med 2011; 17: 439-47.
28 Shen W, Jiang Z. Characterization of glycinergic synapses in vertebrate retinas. J Biomed Sci 2007; 14: 5-13.
29 Korol S, Leuenberger PM, Englert U, Babel J. In vivo effects of glycine on retinal ultrastructure and averaged electroretinogram. Brain Res 1975; 97: 235-51.
30 Wachtmeister L, Dowling JE. The oscillatory potentials of the mudpuppy retina. Invest Ophthalmol Vis Sci 1978; 17: 1176-88.
31 Ouellet D, Sutherland S, Wang T, Griffini P, Murthy V. First-Time-in-Human Study With GSK1018921, a Selective $GlyT_1$ Inhibitor: Relationship Between Exposure and Dizziness. Clin Pharmacol Ther 2011; 90: 597-604.
32 van Berckel BN, Lipsch C, Timp S, Gispen-de Wied C, Wynne H, van Ree JM, Kahn RS. Behavioral and neuroendocrine effects of the partial NMDA agonist D-cycloserine in healthy subjects. Neuropsychopharmacology 1997; 16: 317-24.
33 van Berckel BN, Oranje B, van Ree JM, Verbaten MN, Kahn RS. The effects of low dose ketamine on sensory gating, neuroendocrine secretion and behavior in healthy human subjects. Psychopharmacology (Berl) 1998; 137: 271-81.
34 van Berckel BN, Lipsch C, Gispen-de Wied C, Wynne HJ, Blankenstein MA, van Ree JM, Kahn RS. The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. Psychopharmacology (Berl) 1998; 138: 190-7.
35 Bonavera JJ, Swerdloff RS, Sinha Hakim AP, Lue YH, Wang C. Aging results in attenuated gonadotropin releasing hormone-luteinizing hormone axis responsiveness to glutamate receptor agonist N-methyl-D-aspartate. J Neuroendocrinol 1998; 10: 93-9.
36 Bettendorf M, de Zegher F, Albers N, Hart CS, Kaplan SL, Grumbach MM. Acute N-methyl-D,L-aspartate administration stimulates the luteinizing hormone releasing hormone pulse generator in the ovine fetus. Horm Res 1999; 51: 25-30.
37 Tornoe CW, Agerso H, Senderovitz T, Nielsen HA, Madsen H, Karlsson MO, Jonsson EN. Population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH analogues. Br J Clin Pharmacol 2007; 63: 648-64.
38 le Cotonnec JY, Loumaye E, Porchet HC, Beltrami V, Munafo A. Pharmacokinetic and pharmacodynamic interactions between recombinant human luteinizing hormone and recombinant human follicle-stimulating hormone. Fertil Steril 1998; 69: 201-9.
39 Brann DW, Mahesh VB. Glutamate: a major neuroendocrine excitatory signal mediating steroid effects on gonadotropin secretion. J Steroid Biochem Mol Biol 1995; 53: 325-9.
40 Lechner SM. Glutamate-based therapeutic approaches: inhibitors of glycine transport. Curr Opin Pharmacol 2006; 6: 75-81.
41 Eulenburg V, Armsen W, Betz H, Gomeza J. Glycine transporters: essential regulators of neurotransmission. Trends Biochem Sci 2005; 30: 325-33.
42 Davanger S, Storm-Mathisen J, Ottersen OP. Colocalization of glutamate and glycine in bipolar cell terminals of the human retina. Exp Brain Res 1994; 98: 342-54.
43 Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The Efficacy of Glutamatergic Agents for Negative Symptoms and Cognitive Impairments. Am J Psychiatry 2007; 164: 1593-602.
44 Duncan EJ, Szilagyi S, Schwartz MP, Bugarski-Kirola D, Kunzova A, Negi S, Stephanides

M, Efferen TR, Angrist B, Peselow E, Corwin J, Gonzenbach S, Rotrosen JP. Effects of D-cycloserine on negative symptoms in schizophrenia. Schizophr Res 2004; 71: 239-48.

REFERENCES APPENDICES

1 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-91.
2 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-8.
3 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-80.
4 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_{(A)}$ alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-83.
5 de Visser SJ, van der PJ, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 2001; 51: 119-32.
6 Liem-Moolenaar M, de BP, Timmers M, Schoemaker RC, van Hasselt JG, Schmidt S, van Gerven JM. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. Br J Clin Pharmacol 2011; 71: 886-98.
7 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, Den Hartigh J, Bender JC, Verpoorte R, Pinquier JL, Cohen AF, van Gerven JM. Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 2008; 22: 707-16.
8 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998; 88: 82-8.
9 de Haas S, Schoemaker R, van GJ, Hoever P, Cohen A, Dingemanse J. Pharmacokinetics, pharmacodynamics and the pharmacokinetic/pharmacodynamic relationship of zolpidem in healthy subjects. J Psychopharmacol 2009.
10 Zisapel N, Laudon M. Subjective assessment of the effects of CNS-active drugs on sleep by the Leeds sleep evaluation questionnaire: a review. Hum Psychopharmacol 2003; 18: 1-20.
11 Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA. Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 1985; 20: 619-29.
12 Sobczak S, Honig A, Schmitt JA, Riedel WJ. Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. Neuropsychopharmacology 2003; 28: 711-9.
13 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-8P.
14 Hermann WM. Development and critical evaluation of an objective procedure for the electroencephalographic classification of psychotropic drugs. 1982; 249-351.
15 van Steveninck A. Methods of assessment of central nervous system effects of drugs in man. Thesis Leiden University 1994.
16 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-7.
17 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, Cohen AF. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 1993; 110: 471-8.
18 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.
19 Gijsman HJ, van Gerven JM, Verkes RJ, Schoemaker RC, Pieters MS, Pennings EJ, Hessing TJ, Cohen AF. Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 2002; 17: 83-9.
20 de Visser SJ, van Gerven JM, Schoemaker RC, Cohen AF. Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 2001; 51: 423-8.
21 Kemme MJ, vd Post JP, Schoemaker RC, Straub M, Cohen AF, van Gerven JM. Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 2003; 55: 518-25.
22 van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JM. Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 2004; 18: 221-7.
23 Liem-Moolenaar M, Gray F, de Visser S, Franson K, Schoemaker R, Schmitt J, Cohen A, van Gerven J. Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 2008.
24 Cutmore TR, Beninger RJ. Do neuroleptics impair learning in schizophrenic patients? Schizophr Res 1990; 3: 173-86.
25 FITTS PM. The information capacity of the human motor system in controlling the amplitude of movement. J Exp Psychol 1954; 47: 381-91.
26 Fitts PM, Peterson JR. Information capacity of discrete motor responses. J Exp Psychol 1964; 67: 103-12.
27 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de Smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a gaba(a) $alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 2008; 22: 24-32.
28 Twa md, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. J Cataract Refract Surg 2004; 30: 381-9.
29 van Berckel BN, Lipsch C, Gispen-de Wied C, Wynne HJ, Blankenstein MA, van Ree JM, Kahn RS. The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. Psychopharmacology (Berl) 1998; 138: 190-7.
30 van Berckel BN, Oranje B, van Ree JM, Verbaten MN, Kahn RS. The effects of low dose ketamine on sensory gating, neuroendocrine secretion and behavior in healthy human subjects. Psychopharmacology (Berl) 1998; 137: 271-81.
31 Marmor MF, Zrenner E. Standard for clinical electroretinography (1999 update). International Society for Clinical Electrophysiology of Vision. Doc Ophthalmol 1998; 97: 143-56.
32 Holder GE. The importance of dark adapted cone function in ERG interpretation. Acta Ophthalmologica 2011; 89.
33 Downes SM, Holder GE, Fitzke FW, Payne AM, Warren MJ, Bhattacharya SS, Bird AC. Autosomal dominant cone and cone-rod dystrophy with mutations in the guanylate cyclase activator 1A gene-encoding guanylate cyclase activating protein-1. Arch Ophthalmol 2001; 119: 96-105.
34 Gouras P. The role of S-cones in human vision. Doc Ophthalmol 2003; 106: 5-11.
35 Yamamoto S, Hayashi M, Tsuruoka M, Ogata K, Tsukahara I, Yamamoto T, Takeuchi S. Selective reduction of S-cone response and on-response in the cone electroretinograms of patients with X-linked retinoschisis. Graefes Arch Clin Exp Ophthalmol 2002; 240: 457-60.
36 Holder GE, Brigell MG, Hawlina M, Meigen T, Vaegan, Bach M. ISCEV standard for clinical pattern electroretinography--2007 update. Doc Ophthalmol 2007; 114: 111-6.
37 Arden G, Gunduz K, Perry S. Color vision testing with a computer graphics system: preliminary results. Doc Ophthalmol 1988; 69: 167-74.
38 Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Wesnes KA, Scholey AB. Positive modulation of mood and cognitive performance following administration of acute doses of Salvia lavandulaefolia essential oil to healthy young volunteers. Physiol Behav 2005; 83: 699-709.

CHAPTER 5

THE EFFECTS OF THE GLYCINE REUPTAKE INHIBITOR R213129 ON THE CENTRAL NERVOUS SYSTEM AND ON SCOPOLAMINE-INDUCED IMPAIRMENTS IN PSYCHOMOTOR AND COGNITIVE FUNCTION IN HEALTHY SUBJECTS

M Liem-Moolenaar[1], RWM Zoethout[1], P de Boer[2], M Schmidt[2],
ML de Kam[1], AF Cohen[1], KL Franson[1] and JMA van Gerven[1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Johnson and Johnson, Pharmaceutical Research and Development, Beerse, Belgium

J PSYCHOPHARMACOL 2010;24:1671-9

Abstract

In this study the effects of R213129, a selective glycine transporter 1 inhibitor, on central nervous system function were investigated in healthy males in the absence and presence of scopolamine. This was a double-blind, placebo-controlled, 4-period crossover ascending dose study evaluating the following endpoints: body sway, saccadic and smooth pursuit eye movements, pupillometry, electroencephalography, visual analogue scales for alertness, mood, calmness and psychedelic effects, adaptive tracking, finger tapping, Visual and Verbal Learning Task, Stroop test, hormone levels and pharmacokinetics. R213129 dose levels were selected based on exposure levels that blocked the $GlyT_1$ sites >50% in preclinical experiments. Forty-three of the 45 included subjects completed the study. Scopolamine significantly affected almost every central nervous system parameter measured in this study. R213129 alone compared with placebo did not elicit pharmacodynamic changes. R213129 had some small effects on scopolamine-induced central nervous system impairments. Scopolamine-induced finger tapping impairment was further enhanced by 3 mg R213129 with 2.0 taps/10 seconds (95% CI -4.0, -0.1), electroencephalography alpha power was increased by 10 mg R213129 with respectively 12.9% (0.7, 26.6%), scopolamine-induced impairment of the Stroop test was partly reversed by 10 mg R213129 with 59 milliseconds (-110, -7). Scopolamine produced robust and consistent effects in psychomotor and cognitive function in healthy volunteers. The most logical reason for the lack of R213129 effects seems to be that the central nervous system concentrations were too low. The effects of higher doses in healthy volunteers and the clinical efficacy in patients remain to be established.

Introduction

It has been proposed that hypofunction of the glutamatergic system, and specifically a hypofunction of N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission, contributes to the pathophysiology of schizophrenia (Javitt, 2006; Stone *et al*, 2007). If schizophrenic symptoms are the result of diminished functioning N-methyl-D-aspartate (NMDA) receptors, stimulation of the NMDA receptors might be a possible mechanism for new schizophrenia medication.

As direct agonists of NMDA receptors are neurotoxic (Stone *et al*, 2007), indirect ways to enhance receptor function have been investigated (Javitt, 2002). One way to indirectly augment NMDA receptor function is through facilitation by glycine, an obligatory co-agonist for glutamate at the NMDA receptor (Javitt, 2006). So far, explorative clinical trials using glycine enhancement strategies have shown (modest) improvements in positive and negative symptoms and cognitive function in schizophrenic patients (Goff *et al*, 1999; Goff and Coyle, 2001; Javitt, 2006; Lane *et al*, 2005). However, gram-level doses of glycine are needed to significantly elevate central nervous system (CNS) concentrations. Inhibition of presynaptic glycine reuptake may be a more efficient way to increase pharmacological glycine activity in the brain (Kemp and McKernan, 2002).

The action of glycine is essentially terminated by rapid reuptake, mediated by at least two glycine transporters, glycine transporter 1 ($GlyT_1$) and glycine transporter 2 ($GlyT_2$) (Aragon and Lopez-Corcuera, 2003). As the distribution of $GlyT_1$ overlaps with that of NMDA receptors (Smith *et al*, 1992) and electrophysiological studies have shown that the responses of NMDA receptors are enhanced after inhibition of $GlyT_1$ (Aragon and Lopez-Corcuera, 2005), it is suggested that $GlyT_1$ is involved in NMDA activity. Glycine reuptake inhibitors have been effective in a variety of schizophrenia animal models (Chen *et al*, 2003; Depoortere *et al*, 2005; Harsing *et al*, 2003; Javitt *et al*, 1999; Le Pen *et al*, 2003) and several have recently entered early phase clinical trials in humans. To date, a few of the clinical trials studying the glycine reuptake inhibitor sarcosine in schizophrenia have been published and show effects on positive and negative symptoms (Lane *et al*, 2005; Tsai *et al*, 2004). The disadvantage of sarcosine is that it is a low potency antagonist that also requires gram-level dosing. Therefore other selective, more potent glycine reuptake inhibitors may be more efficient.

R213129 (see Figure 1 for the structural formula) is a selective inhibitor of the $GlyT_1$ and it is hypothesized to have beneficial effects on positive and negative symptoms and cognitive performance of patients with schizophrenia (Goff *et al*, 1999; Goff and Coyle, 2001; Javitt, 2006; Lane *et al*, 2005).

Preclinical studies have supported the potential therapeutic value of this compound (Johnson & Johnson, data on file). R213129 appeared more potent than sarcosine, evidenced by a lower IC50, and increased extracellular glycine levels in the prefrontal cortex in rats and glycine levels in the cerebrospinal fluid in dogs. R213129 also had efficacy in preclinical models for schizophrenia. It normalized the disturbed prepulse inhibition paradigm in dopamine transporter knockout mice and decreased amphetamine-induced hyperactivity in rats with neonatal lesions of the hippocampus. Furthermore, a reduction of the potentiation of amphetamine-induced dopamine release in the prefrontal cortex of rats receiving phencyclidine was detected. In animal models, effects on cognition were inconsistent.

Three doses of R213129 (3, 10 and 30 mg) were used in this study based on exposure levels that block the $GlyT_1$ site for more than 50% (data on file). In previous clinical studies, R213129 was well tolerated in healthy men in single and multiple doses up to 50 mg. The main focus of these studies was to test the tolerability and pharmacokinetics of the compound, although some CNS effects were evaluated. No clear or consistent pharmacodynamic changes in the electroencephalography and cognitive function tests following single doses of 1, 3, 10, 30, and 50 mg and multiple dose administration of 10, 30 and 50 mg R213129 were measured in these studies.

As Tsai *et al* and Lane *et al* have shown that the glycine reuptake inhibitor sarcosine has effects on positive and negative symptoms and cognitive function in schizophrenia (Lane *et al*, 2005; Tsai *et al*, 2004), the hypothesis is that R213129 may also have effects on these symptoms. Since it is not possible to detect changes in positive and negative symptoms in healthy volunteers and to evaluate the positive effects on cognitive function in subjects already performing at their maximal capacity, CNS biomarkers have been studied in healthy subjects (in the absence and presence of the scopolamine model). By using a battery of quantitative CNS tests sensitive to several CNS-active drugs (De Visser *et al*, 2001b, 2003; Gijsman *et al*, 2002; Kemme *et al*, 2003; Van der Post *et al*, 2004), evidence for CNS brain penetration can be shown, and a global impression of the effects of R213129 could be obtained. In addition, to get an indication of potential pro-cognitive effects of R213129 in healthy men, scopolamine was used to induce a transient and reversible thought and memory disturbance. Although the scopolamine model does not capture the complexity of cognitive decline in human psychopathology, it has become the most frequently used model for studies of cognitive impairment in experimental animals and healthy volunteers (Broks *et al*, 1988; Hall *et al*, 1990; Riedel and Jolles, 1996; Vitiello *et al*, 1997).

A methodological disadvantage of the model is the sedation induced by scopolamine possibly contributing to the impairment of memory and cognition. However, in many studies measures of sedation of scopolamine were unrelated to the adverse effect on memory (Bartus *et al*, 1982; Caine *et al*, 1981; Curran *et al*, 1991; Drachman and Leavitt, 1974; Kopelman and Corn, 1988), and no cognitive impairment was produced by, for example, lorazepam in a dose inducing similar sedation as scopolamine (Sunderland *et al*, 1989). Moreover, stimulant drugs that were added to scopolamine were unable to reverse the scopolamine-induced cognitive impairment (Bartus, 1978; Drachman, 1977; Martinez *et al*, 1997), whereas cholinergic agents were (Ghoneim and Mewaldt, 1977).

In primates, an interaction between cholinergic and glutamatergic systems on cognitive function has been demonstrated (Matsuoka and Aigner, 1996a, b; Sirvio *et al*, 1992). The exact mechanism behind this interaction has not been resolved, but suggestions have been made. One is that glycine would increase acetylcholine release in certain neuronal tissues (Fishkin *et al*, 1993; Matsuoka and Aigner, 1996b). Another is the presence of NMDA receptor sites on cell bodies of cholinergic neurons and a subsequent increased acetylcholine release by glycine (agonists) due to depolarization of the receptor (Fishkin *et al*, 1993; Matsuoka and Aigner, 1996b). Various other preclinical experiments have demonstrated

a reversal of scopolamine-induced impairments using agonists for the glycine site on the NMDA receptor (Andersen *et al*, 2002; Kishi *et al*, 1998; Zajaczkowski and Danysz, 1997). To the best of the authors' knowledge there is only one study in healthy volunteers with the partial glycine site agonist, D-cycloserine, showing significant improvement of scopolamine-induced memory impairment (Jones *et al*, 1991).

Therefore in this study, the objectives were to study the CNS profile of R213129 and its effects on scopolamine-induced impairments in healthy male subjects.

Methods

Subjects

A total of 45 male subjects aged 18-55 with body mass index (BMI) of 18-28.5 kg/m2 were recruited by the Centre of Human Drug Research. After signing an informed consent, subjects were medically screened within three weeks prior to study participation. Exclusion criteria included the use of agents known to affect CNS performance (including nicotine, drugs or alcohol) and evidence of relevant clinical abnormalities. The use of medication and the above-mentioned agents were not allowed during the study period. The Ethics Review Board of the Leiden University Medical Centre approved the study protocol.

Study design

This study was a double-blind, placebo-controlled, four-period crossover ascending dose study. The periods were separated by a washout period of at least one week.

Drugs

Scopolamine 0.5 mg or placebo was given intravenously over a period of 15 minutes starting at T= 0 and 3, 10, or 30 mg of R213129 or placebo was orally administered at T = 0.5 hours. The four treatments were scopolamine+placebo, scopolamine+one dose of the novel compound, placebo+placebo and placebo+one dose of the novel compound, with washout periods of at least one week. Each treatment group consisted of 15 subjects.

Safety

Adverse events, electrocardiogram (ECG), body temperature, blood pressure and heart rate measurements were performed throughout the study. ECGs were assessed using Cardioperfect ECG recorder (Welch Allyn). Blood pressure and heart rate were measured using an automated device (Nihon Kohden, Life Scope EC, Tokyo, Japan).

Pharmacodynamics

Eleven blocks of pharmacodynamic (PD) measurements were performed: pre-dose (twice before scopolamine administration) and 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 6.5 and 8.5 hours post-dose. Average baseline values for each variable were obtained by calculation of the mean of two baseline assessments. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Tests were performed in the following order: body sway, saccadic eye movements, smooth pursuit measurement, pupillometry, pharmacoelectroencephalography (EEG), visual analogue scale (VAS) Bond & Lader, VAS Bowdle, adaptive tracking, finger tapping, Stroop test, and Visual and Verbal Learning Task (VVLT). Blood for hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin) was taken regularly from four minutes until 24 hours after scopolamine administration. Subjects had a standardized breakfast one hour before scopolamine administration. All subjects were thoroughly trained and familiarized with the psychometric tests within 14 days preceding study start to minimize learning effects during the study.

ADAPTIVE TRACKING

The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol, various psychoactive drugs and sleep deprivation (Van Steveninck *et al*, 1991, 1993). The adaptive tracking test was performed as originally described by Borland and Nicholson (Borland and Nicholson, 1984) using customized equipment and software. Adaptive tracking is a pursuit-tracking task with a circle moving randomly on a computer screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance and the standard deviation of scores over a 3.5-minute period were used for analysis, including a 0.5 minute run-in time, during which data were not recorded.

BODY SWAY

Changes in body sway have been seen for many different CNS active drugs, including GABA-ergic drugs (De Haas *et al*, 2007, 2008; De Visser *et al*, 2003) and THC (Zuurman *et al*, 2008). The body sway meter records body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxiameter (Wright, 1971). With a string attached to the waist, all body movements over two minutes were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were instructed to wear the same pair of comfortable, low-heeled shoes on each session. Before starting a measurement, subjects were asked to stand still, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body.

FINGER TAPPING

The finger tapping test was adapted from the Halstead Reitan Test Battery (Andrew, 1977). The test evaluates motor activation and fluency. Speed of finger tapping was measured for the index finger of the dominant hand and a session contained five performances of 10 seconds. The volunteer was instructed to tap as quickly as possible on the space bar of a computer. The mean tapping rate and the standard deviations were used for statistical analysis.

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS

Saccadic and smooth pursuit eye movements have shown dose-and concentration-related effects on many different CNS-active drugs, including GABA-ergic (De Haas *et al*, 2007, 2008; De Visser *et al*, 2003), serotonergic (Gijsman *et al*, 2002), noradrenergic (De Visser *et al*, 2001b; Kemme *et al*, 2003; Van der Post *et al*, 2004), and dopaminergic drugs (CHDR, data on file) which were recorded as described previously (Van Steveninck, 1994; Van Steveninck *et al*, 1993, 1996, 1997, 1999). Average values of saccadic peak velocity (SPV), latency (= reaction time) and inaccuracy were calculated for all artefact-free saccades. The average percentage of smooth pursuit for all stimulus frequencies was used as response parameter.

PHARMACO-ELECTROENCEPHALOGRAPHY

Pharmaco-EEG was performed as a general measure of CNS activity (Cohen *et al*, 1985). The literature suggests that antipsychotics show distinct profiles of EEG changes (Sobczak *et al*, 2003; Wright, 1971). Pharmaco-EEG was measured as previously described (De Haas *et al*, 2008). Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta-(0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha-(7.5-11.5 Hz) and beta-(11.5-30 Hz) frequency ranges. The square root of the total power (in μV) was analysed.

VISUAL AND VERBAL LEARNING TASK

At CHDR the VVLT test has shown the CNS effects of various compounds such as benzodiazepines (De Visser *et al*, 2003), antidopaminergic (De Visser *et al*, 2001a), and cannabinoid drugs (Zuurman *et al*, 2008). Memory function (impairment) includes different aspects of learning behaviour, i.e. acquisition, consolidation, storage and retrieval. The VVLT (Schmitt *et al*, 2000) contains three different subtests that cover most

of these memory components, i.e. immediate and delayed word recall and a delayed word recognition. This test is an adapted version of the Auditory Verbal Learning Test (Rey, 1964) with an increased number of items to reduce ceiling effects. Thirty words are presented in the same sequence in three trials on a computer screen. Each trial ends with a free recall of the words (immediate recall). Thirty minutes after the first trial, the subject is requested to recall as many words as possible (delayed recall). This is followed by a recognition test, consisting of 15 previously presented words and 15 other but comparable words, in which the subject has to respond 'Yes/No' as quickly as possible to indicate recognition of the word (delayed recognition). Outcome variables for the immediate and delayed word recall were the average and the maximum number of correct responses. For the delayed word recognition, the number of correct items and mean response time for correct responses were analysed.

STROOP TEST

The Stroop effect is helpful in understanding attention, perception and reading as well as the cognitive and neural mechanism of mental inhibition, interference and controlled versus automatic processing (Stroop, 1935). Individual differences in the degree of Stroop interference are considered to be among the most reliable or stable measures (Laeng *et al*, 2005). Two single-trial computerized versions of the classic colour-word Stroop tasks are presented to the test subjects. In the first trial, 20 coloured items are presented at random. The subjects are asked to respond as quickly and as accurately as possible by pressing the keys 1, 2 or 3 on the number pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, directly after the first trial, 20 colour and word pairs are presented randomly to the subject, forming either congruent or incongruent matches. The subjects are again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the number pad, corresponding with the correct answer. Three colours are shown, which are green, red and blue. The coloured items are presented in a random order. The words that are used are 'Rood' (red), 'Groen' (green) and 'Blauw' (blue). The outcome parameters are the number of correct answers and the reaction time in both the basic and conflict situation.

VISUAL ANALOGUE SCALES

The Bond and Lader VAS was performed to measure subjective alertness, mood and calmness and the Bowdle VAS of psychedelic effects to evaluate psychedelic effects. These were performed as previously described (De Haas *et al*, 2008). The psychedelic effects measured by the VAS Bowdle cluster into two distinct total sum scores: internal perception (reflects inner feelings that do not correspond with reality, including mistrustful feelings); and external perception (reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings) (Zuurman *et al*, 2008). The Bowdle VAS was expanded with an additional subscore for 'feeling high'.

PUPILLOMETRY

Estimation of pupil size by digital photography is more repeatable and accurate than estimates by common clinical techniques over a wide range of illumination (Twa *et al*, 2004). Pupil diameter was determined using a digital camera (Canon PowerShot A620). After at least five minutes adaptation in ambient lighting, the subject was instructed to look into the lens. A sharp picture of the eyes was taken using a single flash. The program Qpupil (designed by the 'Division of Image Processing (LKEB)', Leiden University Medical Center, the Netherlands) automatically analyses the ratio between the diameter of the iris and the pupil of both eyes.

HORMONES

Blood samples for LH, FSH and prolactin were collected and kept at room temperature for at least 15 and maximally 60 minutes before storage. The hormones were analysed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center) using an electrochemiluminescence-immunoassay (ECLIA) for prolactin, and a fluoroimmunoassay for LH and FSH.

Pharmacokinetics

R213129

Blood samples (5 ml) for plasma concentrations of R213129 were drawn pre-dose, and at 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 8.5, 10 and 24 h post dose. Samples were protected from light at all times to prevent degradation of the compound. Plasma samples were analysed to determine R213129 concentrations using a validated, selective and sensitive liquid LC-MS/MS method (Lower Limit Of Quantification (LLOQ) = 1 ng/ml). R213129 was performed by the Department of Bioanalysis, J&J PRD, Belgium.

SCOPOLAMINE

Blood samples (4 ml) for plasma concentrations of scopolamine were drawn at 0.5, 0.75, 1.0, 2.5, 6.5 h after the scopolamine infusion was stopped. Samples were protected from light at all times. Plasma samples were analysed to determine scopolamine concentrations using a validated, selective and sensitive liquid LC-MS/MS method (LLOQ = 10 pg/ml). Scopolamine bioanalysis was performed by Pharma Bio-Research Group B.V., Zuidlaren, the Netherlands.

Statistical analysis

PHARMACODYNAMICS

PD parameters were analysed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline is defined as the average of the available values obtained prior to dosing. This resulted in LS means estimates that indicate the change from baseline where baseline in the graph is set at 0 for t = 0 min. Treatment effects were reported as the contrasts between placebo and scopolamine, where the average of the measurements up to and including 8.5 hours (22 hours for neuroendocrine parameters) were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals and analyses were two-sided with a significance level of 0.05.

PHARMACOKINETICS

Summary statistics of the plasma concentration data and estimated pharmacokinetic parameters for R213129 and scopolamine were calculated. The following pharmacokinetic parameters for R213129 and scopolamine were determined using Pharsight's WinNonlin pharmacokinetic analysis software (version 4.0.1): C_{MAX},Co.5h (scopolamine), T_{MAX}, AUClast, AUC_{∞}, $t_{1/2}$ term., CL/F (R213129), and CL (scopolamine).

Results

Subject characteristics

Forty-three of the 45 included healthy male subjects completed the study. Two subjects decided to stop participation of the study. For one subject the reason for withdrawal was non-compliance. The other subject experienced a vasodepressive reaction after both scopolamine administrations. Subjects were on average 29.0 years old (range: 18-55).

Clinical effects

Scopolamine induced well-characterized anticholinergic effects (increased pupil size, dry mouth, drowsiness, and impaired eye focusing) in 42 of the 44 subjects. Another reported effect was nausea in six of the 44 subjects. R213129 at all doses of 3, 10, and 30 mg was comparable with placebo in its adverse effects profile.

Pharmacokinetic results

The mean concentration-versus-time profile of R213129 for 3 mg is presented in Figure 2. For all doses of R213129, peak concentrations of around 200, 500, and 1400 ng/ml for the respective doses were reached between two and four hours after dosing, and the terminal elimination half-life was between 12 and 15 hours. Clearance was between 1.2 and 1.5 l/h. Pharmacokinetics were dose-linear for the tested doses. When combined with scopolamine, the pharmacokinetic profile was slightly

altered for some parameters due to an anticholinergic delay of stomach emptying. After combination with scopolamine the area under curve (AUC) of 3 mg R213129 changed from approximately 3400 to 3800, of 10 mg from 9300 to 11000, and of 30 mg from 26500 to 32000 ng h/ml.

The scopolamine concentration 0.5 hours after the end of dosing (with and without R213129) varied between 1200 and 1300 pg/ml and the terminal elimination half-life between 1.4 and 1.6 hours. Clearance was around 190 l/h. Scopolamine was not affected by the addition of R213129.

Pharmacodynamic results

SCOPOLAMINE

Scopolamine resulted in a considerable number of CNS effects and affected almost every parameter measured in this study (Table 1 shows a selection of results, as the complete report is shown in another article describing the PK-PD of scopolamine). Scopolamine deteriorated adaptive tracking, body sway and finger tapping rate performance compared with placebo. Both saccadic peak velocity and smooth pursuit performance decreased after scopolamine compared with placebo.

Alpha and beta power (Fz-Cz and Pz-Oz) decreased after scopolamine compared with placebo. Delta power (Fz-Cz and Pz-Oz) after scopolamine was statistically significantly higher than after placebo. Theta power (Fz-Cz and Pz-Oz) after scopolamine did not differ significantly from placebo.

Scopolamine deteriorated all parameters of the VVLT: delayed word recall (number correct), immediate word recall (number correct), and delayed word recognition (both number correct and reaction time). Similarly, all parameters of the Stroop test were deteriorated compared with placebo (number correct or reaction time and basic situation or conflict situation).

VAS alertness was significantly lower after scopolamine than after placebo, VAS calmness was higher, and VAS mood was not changed by scopolamine. VAS results of internal perception, external perception and feeling high were all higher after scopolamine.

All measured hormone levels increased after scopolamine compared with placebo, as did pupil size.

R213129

R213129 alone compared with placebo did not show pharmacodynamic changes on any of the parameters in this study.

R213129 AND SCOPOLAMINE

R213129 had some small effects on scopolamine-induced CNS impairment (see Table 1). Scopolamine-induced finger tapping impairment was further impaired by 3 mg R213129 with 2.0 taps/ 10 seconds (95% CI -4.0, -0.1). EEG alpha power (Fz-Cz and Pz-Oz) statistically significantly increased with, respectively, 12.9% and 16.0% after the combination of 10 mg R213129 and scopolamine compared with scopolamine alone (respectively, 95% CI 0.7, 26.6% and 0.3, 34.2%). The scopolamine-induced impairment of the number of correct Stroop responses (in the conflict situation) was reversed by 3 mg R213129 with 0.6 items (95% CI 0.0, 1.1). The scopolamine-induced impairment of the reaction time (in the conflict situation) was partly reversed by 10 mg R213129 with 59 milliseconds (95% CI -110, -7). R213129 had no significant impact on the scopolamine effects at a dose of 30 mg.

Discussion

In this study the CNS effects of the new glycine reuptake inhibitor R213129 were investigated using a battery of quantitative CNS tests, sensitive to classic neuroleptic agents and other CNS-active drugs. As this study was performed in healthy volunteers, a scopolamine model was used to examine the potential reversal of cognitive impairments by R213129. Based on experiments with the partial glycine agonist D-cycloserine in animals (Andersen *et al*, 2002; Kishi *et al*, 1998; Zajaczkowski and Danysz, 1997) and healthy subjects (Jones *et al*, 1991), it was hypothesized that scopolamine-induced (cognitive) deficits would be reversed by R213129.

This study found that scopolamine produced very robust and consistent effects in healthy volunteers, across a wide range of CNS functions. Most of the CNS impairments of scopolamine were expected, and are in line

with those observed in previous studies (Ebert and Kirch, 1998; Ebert *et al*, 1998, 2001; Renner *et al*, 2005). However, we are unaware of previous reports of increases in the gonadotrophic hormones LH and FSH after anticholinergic agents. We also found an increase in prolactin, which has previously been found by Benkert *et al* (1981) using the anticholinergic agent biperiden. Most effects disappeared after eight hours, except for pupil size increases, which were still detectable at the end of each scopolamine occasion.

R213129 did not have any CNS effects on its own in this study. Since we have been unable to find reports on other glycine reuptake inhibitors in healthy volunteers, it is difficult to interpret whether this lack of effects is due to insufficient brain penetration, low pharmacological activity, or absence of physiological changes during glycine reuptake inhibition in healthy humans. There may also be methodological reasons that precluded us from detection of small CNS effects, although in our experience almost all CNS-active drugs have some impact on the tests that we used in this study (De Haas *et al*, 2007; De Visser *et al*, 2001b; Kemme *et al*, 2003; Van der Post *et al*, 2004; Van Steveninck *et al*, 1991; Zuurman *et al*, 2008).

Our study showed some modifications of the effects of scopolamine, but these were small and did not seem consistent. Three tests showed statistically significant changes, some of which could be qualified as improvements and others as deteriorations. R213129 slightly reversed some aspects of scopolamine-induced cognitive deterioration on the Stroop test and the scopolamine-induced reduction in alpha EEG power, which might be indicative for improved alertness, but motor impairment (finger tapping rate) was slightly worsened. In addition, the effects were not consistently dose-related in the 3-30 mg dose range.

Several reasons for these marginal effects should be considered. The most likely explanation could be that the doses of R213129 did not adequately cover the dose-response curve. Three different doses of R213129 were investigated in this study. If R213129 had a bell-shaped dose-response curve, which was also suggested by other studies with D-cycloserine (Andersen *et al*, 2002; Jones *et al*, 1991), the selected dose levels could in part have been too high. However, the small and non-dose-related effects that were observed in this study could also suggest an indication of very early CNS changes that were too small and hence too variable to consistently exceed the detection limit. R213129 showed a limited blood-brain barrier penetration, which could increase the variability of its CNS effects in the low 3-30 mg dose range. In this case, (considerably) higher doses may lead to both larger and more consistent effects, provided that the brain penetration increases at a higher dose range.

Another possible explanation for the lack of consistent effects is that the scopolamine model in healthy subjects may have been unsuitable, or the impact of scopolamine may have been too strong to pick up possible cognition-enhancing properties of R213129. Considering the clear effect of scopolamine on all parameters of this study, the 0.5 mg dose could possibly have been too high and may have overshadowed any possible R213129 effects, particularly if these were small. The 0.5 mg dose of scopolamine was selected based on an earlier study of Ebert *et al*, showing that an intravenous dose of 0.5 mg scopolamine was suitable for PK-PD modelling using EEG, and still had an acceptable side effect profile (Ebert *et al*, 2001). Support for reversal of the scopolamine model by glycine reuptake inhibitors primarily comes from several preclinical studies that show reversal of scopolamine-induced impairments by D-cycloserine in various animals (Andersen *et al*, 2002; Fishkin *et al*, 1993; Ohno and Watanabe, 1996; Pitkanen *et al*, 1995; Sirvio *et al*, 1992). The evidence in healthy humans, however, is much more limited. A study similar to ours in scope and objectives showed reversal of scopolamine-induced memory deficits by D-cycloserine (Jones *et al*, 1991). However, this was only a short communication without more comprehensive publication or replication (as far as we could find), and it only showed effects of the 15 mg dose, but not at 5 or 50 mg.

A third factor which might explain the absence of results of R213129 is the composition of the CNS battery. The selected tests might not have been sensitive to the effects of a glycine reuptake inhibitor. In our experience false-negative results are quite rare with these tests, which are chosen for their sensitivity to a wide range of CNS-active compounds

(De Haas *et al*, 2007; De Visser *et al*, 2001b; Kemme *et al*, 2003; Van der Post *et al*, 2004; Van Steveninck *et al*, 1991). This is confirmed by the scopolamine effects in this study, which were very clear and consistent. The previous study with D-cycloserine described reversal of memory effects that were also assessed in our study (immediate and delayed word recall and delayed word recognition) (Jones *et al*, 1991). It cannot be excluded, however, that the effects of R213129 in healthy volunteers would have been detectable with other cognitive tests or impairment models.

In summary, scopolamine proved to be a robust and consistent model of CNS impairment in healthy volunteers. Most of the scopolamine-induced changes were in agreement with the findings of previous studies. R213129 had no CNS effect by itself, and produced marginal changes of scopolamine-induced CNS impairment, without a consistent dose-response relationship. The most logical explanation for this lack of clear effects in this study seems to be that the R213129 brain concentrations were too low and variable to detect any effects in this healthy volunteer study. Studies with higher doses would be required to show pharmacological activity of this compound in healthy volunteers. This could provide support for the dose selection for the patient studies that are required to investigate the clinical efficacy of this glycine reuptake inhibitor, which still remains to be established.

Table 1 Pharmacodynamic effects for placebo, scopolamine, and the combination of scopolamine and R213129[D]

Parameter (unit)[B]	LSM[C] PLAC	LSM[C] SCOP	Scopolamine effect			LSM[C] SCOP + R213129[E]	Interaction effect		
			Difference[A]	95% CI	P value		Difference[A]	95% CI	P value
Adaptive tracking (%)	22.16	12.82	9.33	8.42, 10.25	<0.0001				
Body sway (mm)	288	450	56.3%	44.3, 69.2%	<0.0001				
Finger tapping rate (taps/10 sec)	65.3	62.0	3.3	1.9, 4.6	<0.0001	60.0 (3)	-2.0	-4.0, -0.1	0.0415
Saccadic Peak Velocity (deg/sec)	484.1	454.8	29.2	21.2, 37.3	<0.0001				
Smooth pursuit (%)	46.60	41.52	5.07	2.53, 7.62	0.0001				
EEG alpha Fz-Cz (µV)	2.99	2.04	-31.8%	-36.8, -26.4%	<0.0001	2.31 (10)	112.9%	100.7, 126.6%	0.0383
EEG alpha Pz-Oz (µV)	5.87	3.20	-45.4%	-50.4, -39.8%	<0.0001	3.72 (10)	116.0%	100.3, 134.2%	0.0450
Delayed word recall (# correct)	11.8	6.9	4.9	3.6, 6.2	<0.0001				
Immediate word recall (# correct)	12.0	8.0	4.0	3.1, 4.8	<0.0001				
Delayed word recognition (# correct)	25.4	22.7	2.7	1.0, 4.3	0.0018				
Stroop (conflict, # correct)	19.5	18.7	0.8	0.4, 1.1	0.0002	19.3 (3)	0.6	0.0, 1.1	0.0496
Stroop (conflict, RT, msec)	648	771	-123	-157, -89	<0.0001	713 (10)	-59	-110, -7	0.0259
FSH (U/L)	3.75	3.97	5.8%	3.1, 8.6%	<0.0001				
LH (U/L)	4.65	5.41	16.4%	10.4, 22.7%	<0.0001				
Prolactin (µg/L)	7.56	8.57	13.3%	7.1, 19.8%	<0.0001				

A If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level; B RT = Reaction time; C RSM is the Least Squares Means estimate; D the interaction effects are only indicated in case of a significant effect; E dose (mg) of R213129 inducing a change in scopolamine

Figure 1 Structural formula of R213129

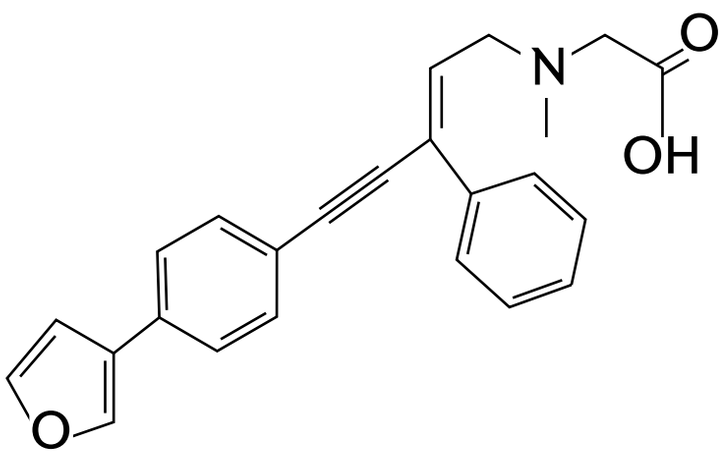
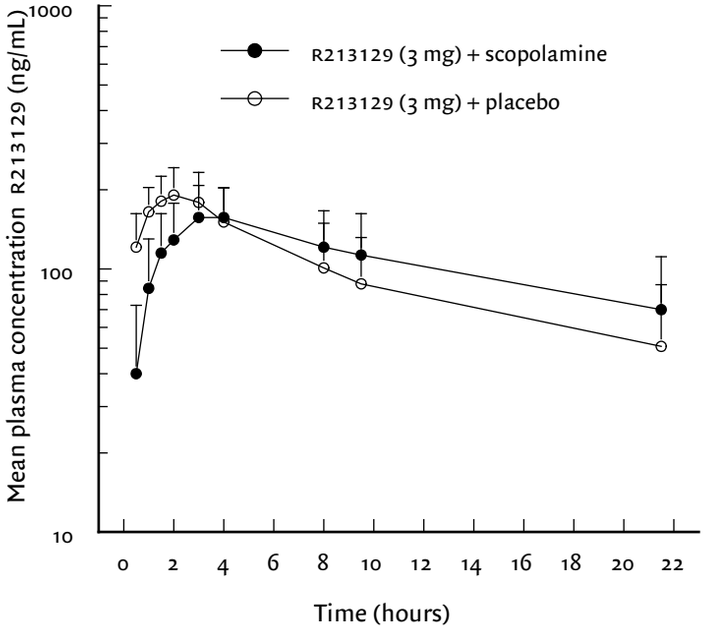


Figure 2 Mean (+SD) plasma concentration profile of 3 mg R213129



REFERENCES

Andersen JM, Lindberg V, Myhrer T (2002) Effects of scopolamine and D-cycloserine on non-spatial reference memory in rats. Behav Brain Res 129: 211-216.

Andrew JM (1977) Delinquents and the Tapping Test. J Clin Psychol
33: 786-791. Aragon C, Lopez-Corcuera B (2003) Structure, function and regulation of glycine neurotransporters. Eur J Pharmacol 479: 249-262.

Aragon C, Lopez-Corcuera B (2005) Glycine transporters: crucial roles of pharmacological interest revealed by gene deletion. Trends Pharmacol Sci 26: 283-286.

Bartus RT (1978) Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys: effects of concurrent administration of physostigmine and methylphenidate with scopolamine. Pharmacol Biochem Behav 9: 833-836.

Bartus RT, Dean III RL, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217: 408-414.

Benkert O, Klein HE, Hofschuster E, Seibold C (1981) Effect of the anticholinergic drug biperiden on pituitary hormones and cortisol. Psychoneuroendocrinology 6: 231-238.

Borland RG, Nicholson AN (1984) Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 18(Suppl 1): 69S-72S.

Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM (1988) Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 26: 685-700.

Caine ED, Weingartner H, Ludlow CL, Cudahy EA, Wehry S (1981) Qualitative analysis of scopolamine-induced amnesia. Psychopharmacology (Berl) 74: 74-80.

Chen L, Muhlhauser M, Yang CR (2003) Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. J Neurophysiol 89: 691-703.

Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA (1985) Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 20: 619-629.

Curran HV, Schifano F, Lader M (1991) Models of memory dysfunction? A comparison of the effects of scopolamine and lorazepam on memory, psychomotor performance and mood. Psychopharmacology (Berl) 103: 83-90.

de Haas SL, de Visser SJ, van der Post JP, *et al* (2007) Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_{(A)}$ alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 21: 374-383.

de Haas SL, de Visser SJ, van der Post JP, *et al* (2008) Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_{(A)}$ alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 22: 24-32.

de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55: 39-50.

de Visser SJ, van der PJ, Pieters MS, Cohen AF, van Gerven JM (2001a) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51: 119-132.

de Visser SJ, van Gerven JM, Schoemaker RC, Cohen AF (2001b) Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 51: 423-428.

Depoortere R, Dargazanli G, Estenne-Bouhtou G, *et al* (2005) Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology 30: 1963-1985.

Drachman DA (1977) Memory and cognitive function in man: does the cholinergic system have a specific role? Neurology 27: 783-790.

Drachman DA, Leavitt J (1974) Human memory and the cholinergic system A relationship to aging?. Arch Neurol 30: 113-121.

Ebert U, Grossmann M, Oertel R, Gramatte T, Kirch W (2001) Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram effects of scopolamine in healthy volunteers. J Clin Pharmacol 41: 51-60.

Ebert U, Kirch W (1998) Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur J Clin Invest 28: 944-949.

Ebert U, Siepmann M, Oertel R, Wesnes KA, Kirch W (1998) Pharmacokinetics and

pharmacodynamics of scopolamine after subcutaneous administration. J Clin Pharmacol 38: 720-726.
Fishkin RJ, Ince ES, Carlezon Jr WA, Dunn RW (1993) D-cycloserine attenuates scopolamine-induced learning and memory deficits in rats. Behav Neural Biol 59: 150-157.
Ghoneim MM, Mewaldt SP (1977) Studies on human memory: the interactions of diazepam, scopolamine, and physostigmine. Psychopharmacology (Berl) 52: 1-6.
Gijsman HJ, van Gerven JM, Verkes RJ, *et al* (2002) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 17: 83-89.
Goff DC, Coyle JT (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 158: 1367-1377.
Goff DC, Tsai G, Levitt J, *et al* (1999) A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry 56: 21-27.
Hall ST, Puech A, Schaffler K, Wesnes K, Gamzu ER (1990) Early clinical testing of cognition enhancers: prediction of efficacy. Pharmacopsychiatry 23(Suppl 2): 57-58.
Harsing Jr LG, Gacsalyi I, Szabo G, *et al* (2003) The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav 74: 811-825.
Javitt DC (2002) Glycine modulators in schizophrenia. Curr Opin Investig Drugs 3: 1067-1072.
Javitt DC (2006) Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. Curr Opin Psychiatry 19: 151-157.
Javitt DC, Balla A, Sershen H, Lajtha A (1999) AE Bennett Research Award. Reversal of phencyclidine-induced effects by glycine and glycine transport inhibitors. Biol Psychiatry 45: 668-679.
Jones RW, Wesnes KA, Kirby J (1991) Effects of NMDA modulation in scopolamine dementia. Ann N Y Acad Sci 640: 241-244.
Kemme MJ, vd Post JP, Schoemaker RC, Straub M, Cohen AF, van Gerven JM (2003) Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 55: 518-525.
Kemp JA, McKernan RM (2002) NMDA receptor pathways as drug targets. Nat Neurosci 5(Suppl): 1039-1042.
Kishi A, Ohno M, Watanabe S (1998) Concurrent activation of hippocampal glycine and polyamine sites of the N-methyl-D-aspartate receptor synergistically reverses working memory deficits in rats. Neurosci Lett 257: 131-134.
Kopelman MD, Corn TH (1988) Cholinergic 'blockade' as a model for cholinergic depletion. A comparison of the memory deficits with those of Alzheimer-type dementia and the alcoholic Korsakoff syndrome. Brain 111 (Pt 5): 1079-1110.
Laeng B, Lag T, Brennen T (2005) Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. J Exp Psychol Hum Percept Perform 31: 438-452.
Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE (2005) Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 62: 1196-1204.
Le Pen G, Kew J, Alberati D, Borroni E, Heitz MP, Moreau JL (2003) Prepulse inhibition deficits of the startle reflex in neonatal ventral hippocampal-lesioned rats: reversal by glycine and a glycine transporter inhibitor. Biol Psychiatry 54: 1162-1170.
Martinez R, Molchan SE, Lawlor BA, *et al* (1997) Minimal effects of dextroamphetamine on scopolamine-induced cognitive impairments in humans. Biol Psychiatry 41: 50-57.
Matsuoka N, Aigner TG (1996a) Cholinergic-glutamatergic interactions in visual recognition memory of rhesus monkeys. Neuroreport 7: 565-568.
Matsuoka N, Aigner TG (1996b) D-cycloserine, a partial agonist at the glycine site coupled to N-methyl-D-aspartate receptors, improves visual recognition memory in rhesus monkeys. J Pharmacol Exp Ther 278: 891-897.
Ohno M, Watanabe S (1996) D-cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. Eur J Pharmacol 318: 267-271.
Pitkanen M, Sirvio J, MacDonald E, Ekonsalo T, Riekkinen Sr P (1995) The effects of d-cycloserine, a partial agonist at the glycine binding site, on spatial learning and working memory in scopolamine-treated rats. J Neural Transm Park Dis Dement Sect 9: 133-144.
Renner UD, Oertel R, Kirch W (2005) Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. Ther Drug Monit 27: 655-665.
Rey A (1964) L'examen Clinique en Psychologie.
Riedel WJ, Jolles J (1996) Cognition enhancers in age-related cognitive decline. Drugs Aging 8: 245-274.
Schmitt JAJ, Jorissen BL, Sobczak S, *et al* (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. Journal of Psychopharmacology 14: 21-29.
Sirvio J, Ekonsalo T, Riekkinen Jr P, Lahtinen H, Riekkinen Sr P (1992) D-cycloserine, a modulator of the N-methyl-D-aspartate receptor, improves spatial learning in rats treated with muscarinic antagonist. Neurosci Lett 146: 215-218.
Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL (1992) Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 8: 927-935.
Sobczak S, Honig A, Schmitt JA, Riedel WJ (2003) Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. Neuropsychopharmacology 28: 711-719.
Stone JM, Morrison PD, Pilowsky LS (2007) Glutamate and dopa-mine dysregulation in schizophrenia-a synthesis and selective review. J Psychopharmacol 21: 440-452.
Stroop JR (1935) Studies of interference in serial verbal reactions. J Exp Psychol 18: 643-662.
Sunderland T, Weingartner H, Cohen RM, *et al* (1989) Low-dose oral lorazepam administration in Alzheimer subjects and age-matched controls. Psychopharmacology (Berl) 99: 129-133.
Tsai G, Lane HY, Yang P, Chong MY, Lange N (2004) Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 55: 452-456.
Twa MD, Bailey MD, Hayes J, Bullimore M (2004) Estimation of pupil size by digital photography. J Cataract Refract Surg 30: 381-389.
van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JM (2004) Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 18: 221-227.
van Steveninck AL, Gieschke R, Schoemaker HC, *et al* (1993) Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 110: 471-478.
van Steveninck AL, Gieschke R, Schoemaker RC, *et al* (1996) Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41: 565-573.
van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF (1991) A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 50: 172-180.
van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF (1999) The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 13: 10-17.
van Steveninck AL, Wallnofer AE, Schoemaker RC, *et al* (1997) A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. Br J Clin Pharmacol 44: 267-275.
van Steveninck A (1994) Methods of assessment of central nervous system effects of drugs in man. Thesis Leiden University.
Vitiello B, Martin A, Hill J, *et al* (1997) Cognitive and behavioral effects of cholinergic, dopaminergic, and serotonergic blockade in humans. Neuropsychopharmacology 16: 15-24.
Wright BM (1971) A simple mechanical ataxia-meter. J Physiol 218: 27P-28P.
Zajaczkowski W, Danysz W (1997) Effects of D-cycloserine and aniracetam on spatial learning in rats with entorhinal cortex lesions. Pharmacol Biochem Behav 56: 21-29.
Zuurman L, Roy C, Schoemaker R, *et al* (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 22: 707-716.

CHAPTER 6

THE EFFECTS OF A GLYCINE REUPTAKE INHIBITOR R231857 ON THE CENTRAL NERVOUS SYSTEM AND ON SCOPOLAMINE INDUCED IMPAIRMENTS IN COGNITIVE AND PSYCHOMOTOR FUNCTION IN HEALTHY SUBJECTS

M Liem-Moolenaar [1], RWM Zoethout [1], P de Boer [2], M Schmidt [2],
ML de Kam [1], AF Cohen [1], KL Franson [1], JMA van Gerven [1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Johnson and Johnson, Pharmaceutical Research and Development, Beerse, Belgium

J PSYCHOPHARMACOL 2010; 24: 1681-7

Abstract

The effects of the selective inhibitor of the glycine transporter 1, might be an indication that R231857 penetrated the CNS, they were not R231857, in development for schizophrenia, on the central nervous system consistent or dose-related. R231857 had some small effects on (CNS) were investigated in healthy males in the absence and presence of scopolamine-induced CNS-impairment, which were also not clearly scopolamine. This was a double-blind, placebo-controlled, four-period dependent on dose. Scopolamine proved to be an accurate, reproducible crossover ascending dose study. Pharmacokinetics, body sway, saccadic and safe model to induce CNS impairment by an anticholinergic and smooth pursuit eye movements, pupillometry, pharmaco-mechanism. R231857 lacked consistent dose-related effects in this study, electroencephalogram (EEG), Visual Analogue Scales (VAS) for alertness, probably because CNS concentrations were too low to produce significant/ mood, calmness and psychedelic effects, adaptive tracking, finger tapping, reproducible CNS-effects or to affect the scopolamine challenge in healthy Stroop test, Visual and Verbal Learning Task (VVLT) and hormone levels volunteers. The effects of higher doses in healthy volunteers and the were assessed. R231857 was administered alone and together with clinical efficacy in patients remain to be established. scopolamine to investigate the potential reversal of anticholinergic CNS impairment by the glycine reuptake inhibitor. Forty-two of the 45 included subjects completed the study. Scopolamine significantly affected almost every CNS parameter measured in this study. R231857 alone showed some pharmacodynamic changes compared with placebo. Although these effects might be an indication that R231857 penetrated the CNS, they were not consistent or dose-related. R231857 had some small effects on scopolamine-induced CNS-impairment, which were also not clearly dependent on dose. Scopolamine proved to be an accurate, reproducible and safe model to induce CNS impairment by an anticholinergic mechanism. R231857 lacked consistent dose-related effects in this study,probably because CNS concentrations were too low to produce significant/reproducible CNS-effects or to affect the scopolamine challenge in healthy volunteers. The effects of higher doses in healthy volunteers and the clinical efficacy in patients remain to be established.

Introduction

As extensively reviewed in previous reports, hypofunction of N-methyl-D-aspartate (NMDA) neurotransmission is hypothesised to play an important role in the pathophysiology of schizophrenia (Javitt, 2006; Stone, *et al*, 2007). R231857 (Figure 1 - structural formula) selectively inhibits the glycine transporter 1 ($GlyT_1$). Tsai and Lane *et al* have shown that the glycine reuptake inhibitor sarcosine causes some improvements of positive and negative symptoms and cognitive function in schizophrenia (Lane, *et al*, 2005; Tsai, *et al*, 2004). Hence, the hypothesis is that R231857 may also have beneficial effects on these symptoms.

Preclinical studies have supported a potential therapeutic potential of this compound (Johnson and Johnson, data on file). R231857 is more potent than sarcosine, evidenced by a lower IC50 (the half maximal inhibitory concentration). It increased extracellular glycine levels in the prefrontal cortex in rats and glycine levels in the cerebrospinal fluid in dogs. It was also effective in some animal models of schizophrenia. Importantly, R231857 normalised the disturbed prepulse inhibition paradigm in dopamine transporter knockout mice. It also decreased amphetamine-induced hyperactivity in rats with neonatal lesions of the hippocampus and reduced the potentiation of amphetamine-induced dopamine release in the prefrontal cortex of rats receiving phencyclidine. R231857 was well tolerated by healthy men in single and multiple doses up to 640 mg but central nervous system (CNS) effects of electroencephalogram (EEG), Cognitive Drug Research (evaluating memory function and attention) and Addiction Research Center Inventory 49-Item Questionnaire (ARCI49) were not observed in these studies (Johnson and Johnson, data on file).

To obtain an indication of CNS penetration and pharmacological activity, a battery of quantitative CNS-tests was used that is sensitive

to a wide range of CNS-active drugs including neuroleptics (de Visser, *et al*, 2001, 2003; Gijsman, *et al*, 2002; Kemme, *et al*, 2003; van der Post, *et al*, 2004). It is difficult to demonstrate cognitive improvements in healthy volunteers, which would be an important therapeutic objective in schizophrenic patients. To induce transient and reversible thought and memory disturbances, which in some ways mimic those observed in patients with negative symptoms and cognitive dysfunction, a scopolamine challenge test was used (Ebert, *et al*, 1998; Green, *et al*, 2005; Koller, *et al*, 2003; Riedel, *et al*, 1995). Various preclinical experiments have demonstrated a reversal of scopolamine-induced impairments using agonists for the glycine site on the NMDA receptor (Andersen, *et al*, 2002; Kishi, *et al*, 1998; Zajaczkowski and Danysz, 1997). Similar findings were obtained in one study in healthy volunteers with the partial glycine site agonist, D-cycloserine (Jones, *et al*, 1991). The neuropharmacological mechanism behind the cholinergic-glutamatergic interaction has not been elucidated. It has been proposed that glycine increases acetylcholine release in the striatum and hippocampus (Nishimura and Boegman, 1990; Ransom and Deschenes, 1989; Scatton and Lehmann, 1982). Another is the presence of NMDA receptor sites on cell bodies of cholinergic neurons and a subsequent increased acetylcholine release by glycine (agonists) due to depolarisation of the receptor (Fishkin, *et al*, 1993; Matsuoka and Aigner, 1996).

In the current study, the objectives were to study the CNS profile of R231857 alone and its effects on scopolamine-induced (cognitive and psychomotor) impairments in healthy male subjects.

Methods

Subjects

A total of 45 male subjects aged 18-55 with body mass index (BMI) of 18-28.5 kg/m2 were recruited by the Centre of Human Drug Research. After signing an informed consent, subjects were medically screened within 3 weeks before study participation. Exclusion criteria included the use of agents known to affect CNS performance (including nicotine, drugs or alcohol), history or presence of psychiatric disease and evidence of relevant clinical abnormalities (checked by a physical examination and haematology, biochemistry and virology). The use of medication was not allowed during the study period. The Ethics Review Board of the Leiden University Medical Center approved the study protocol.

Study design

This study was a double-blind, placebo-controlled, four-period crossover ascending dose study. The periods were separated by a washout period of at least 1 week.

Drugs and study design

Scopolamine 0.5 mg or placebo was given intravenously over a period of 15 min starting at T = 0 and 80, 160 or 320 mg of R231857 or placebo was orally administered at T = 0.5 h. This study was a double-blind, placebo-controlled, four-period crossover ascending dose study. The four treatments given, separated by at least 1 week, were scopolamine + placebo, scopolamine + R231857, placebo + placebo and placebo + R231857. After these four periods, the dose of R231857 was escalated and given to another group of 15 subjects in a four-period crossover fashion. Three dose groups of R231857 were treated, resulting in 45 subjects in total. The three doses of R231857 used in this study (80, 160 and 320 mg) were expected to cause plasma concentrations, corresponding to levels that block the $GlyT_1$ site for more than 50% in preclinical experiments, which was considered sufficient to cause a significant functionally relevant effect. Exploration of higher levels of exposure and inhibition was thwarted by pharmaceutical formulation issues that occurred with the production of higher doses.

The selection of the scopolamine dose was based on an earlier study of Ebert, *et al* showing that an intravenous dose of 0.5 mg scopolamine demonstrated clear concentration-dependent effects using electroencephalography, while still having an acceptable side-effect profile (Ebert, *et al*, 2001).

Safety

Adverse events, electrocardiogram (ECG), body temperature, blood pressure and heart rate measurements were performed throughout the study. ECGs were assessed using Cardioperfect ECG recorder (Welch Allyn, New York, US). Blood pressure and heart rate were measured using an automated device (Nihon Kohden, Life Scope EC, Tokyo, Japan). Haematology, biochemistry and urinalysis were performed.

Pharmacodynamics

Eleven blocks of pharmacodynamic measurements were performed: predose (twice before scopolamine administration) and 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 6.5 and 8.5 h post-dose. Average baseline values for each variable were obtained by calculation of the mean of two baseline assessments. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Tests were performed in the following order: body sway, saccadic eye movements, smooth pursuit measurement, pupillometry, pharmaco-EEG, Visual Analogue Scales (VAS) Bond and Lader, Bowdle, adaptive tracking, finger tapping, Stroop test and Visual and Verbal Learning Task (VVLT). Blood for hormones (follicle-stimulating hormone [FSH], luteinizing hormone [LH] and prolactin) was taken regularly from 4 min until 24 h after scopolamine administration. LH and FSH were measured as animal studies of both R231857 and the related compound R213129 showed an increase in serum prolactin levels and decrease in LH. The prolactin rise was confirmed after single and multiple administration of R231857 in healthy volunteers (Johsnon and Johnson, data on file). Because of the presumed role of excitatory amino acids in the regulation of the release of such hormones, these hormones are potentially interesting endocrine markers for pharmacodynamic activity of $GlyT_1$ inhibitors (Brann and Mahesh, 1995; Mahesh and Brann, 2005). Subjects had a standardised breakfast 1 h before scopolamine administration. All subjects were thoroughly trained and familiarised with the psychometric tests within 14 days before start of the study to minimise learning effects during the study. The pharmacodynamic measurements are described extensively elsewhere (Liem-Moolenaar, 2009).

Pharmacokinetics

R231857

Blood samples (5 mL) for plasma concentrations of R231857 were drawn predose and at 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 8.5, 10 and 24 h post-dose. Samples were protected from light at all times to prevent degradation of the compound. Plasma samples were analysed to determine R231857 concentrations using a validated, selective and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Lower Limit Of Quantification (LLOQ) = 1 ng/mL). R231857 was performed by the Department of Bioanalysis, J&J PRD, Belgium.

SCOPOLAMINE

Blood samples (4 mL) for plasma concentrations of scopolamine were drawn at 0.5, 0.75, 1.0, 2.5 and 6.5 h after the scopolamine infusion was stopped. Samples were protected from light at all times. Plasma samples were analysed to determine scopolamine concentrations using a validated, selective and sensitive liquid LC-MS/MS method (LLOQ = 10 pg/mL). Scopolamine bioanalysis was performed by Pharma Bio-Research Group B.V., Zuidlaren, the Netherlands.

Statistical analysis

PHARMACODYNAMICS (PD)

PD parameters were analysed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects and with the baseline value as covariate, where baseline is defined as the average of the available values obtained before dosing. This resulted in least square means (LSM) estimates that indicate the

change from baseline, where baseline in the graph is set at 0 for t = 0 min. Treatment effects were reported as the contrasts between placebo and scopolamine, where the average of the measurements up to and including 8.5 h (22 h for neuroendocrine parameters) were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals and analyses were two sided with a significance level of 0.05.

PHARMACOKINETICS (PK)

Summary statistics of the plasma concentration data and estimated pharmacokinetic parameters for R231857 and scopolamine were calculated. The following pharmacokinetic parameters for R231857 and scopolamine were determined using Pharsight's WinNonlin pharmacokinetic analysis software (version 4.0.1): C_{MAX}, $C_{0.5h}$ (scopolamine), T_{MAX}, AUC_{last}, AUC_{∞}, $t_{1/2term}$, CL/F (clearance/bioavailability) (R231857) and CL (scopolamine).

Results

Participant characteristics

Forty-two of the 45 included healthy male subjects completed the study. There was no difference in demographics (age and BMI) between the three different dose groups of R231857.

Three subjects decided to stop participation in the study for personal reasons. Subjects were on average 27.5 years old (range: 18-55).

Clinical effects

Scopolamine induced the well-known anticholinergic effects (characterised by increased pupil size, dry mouth, drowsiness and impaired eye focusing) in 43 of the 44 subjects. Other reported effects were dizziness in 4 and headache in 3 of the 44 subjects.

The most frequently reported adverse events for R231857 were headache (12 of 45), somnolence (10 of 45) and nausea (5 of 45).

The incidence of these events seemed slightly higher following dosing with R231857 than with placebo. For headache, this was 6 of 43 after all three doses of R231857 compared with 4 of 44 for placebo, for somnolence 5 of 43 compared with 2 of 44 and for nausea 2 of 43 compared with 0 of 44. However, these numbers are too small to draw any conclusions.

Pharmacokinetic results

The mean concentration-versus-time profile of R231857 is presented in Figure 2. After administration, R231857 was rapidly absorbed with a time of maximum concentration 1 h or less after dose administration and a maximum concentration of 370, 1200 and 2050 ng/mL for the three different doses. The terminal elimination half-life was short, that is, less than 3 h (range: 1.6-2.8 h) and clearance ranged between 125 and 160 L/h. Pharmacokinetics were dose-linear for the tested doses, with the exception of slightly more than dose proportional pharmacokinetics between 80 and 160 mg. When combined with scopolamine, the pharmacokinetic profile of R231857 was slightly altered for some parameters (see Figure 2). For all doses, but mainly at the highest dose, the maximum concentration was reduced. Consequently, exposure was also reduced when 320 mg R231857 was combined with scopolamine (from approximately 2700 to 1900 ng.h/mL).

The average scopolamine concentration 0.5 h after the end of dosing (with and without R231857) was 1335 pg/mL and the terminal elimination half-life was 1.5 h. Clearance was 186 L/h. Scopolamine was not affected by the addition of R231857.

Pharmacodynamic results

SCOPOLAMINE

Scopolamine resulted in a considerable number of CNS-effects and affected almost every parameter measured in this study (Table 1). Scopolamine deteriorated adaptive tracking, body sway and finger tapping rate performance compared with placebo. Both saccadic peak

velocity and smooth pursuit performance decreased after scopolamine compared with placebo administration.

Powers of alpha (Fz-Cz and Pz-Oz) and beta Pz-Oz were statistically significantly lower after scopolamine than after placebo. Delta power (Fz-Cz and Pz-Oz) was statistically significantly higher after scopolamine than after placebo. Beta Fz-Cz and theta power (Fz-Cz and Pz-Oz) after scopolamine was not statistically significantly different from placebo.

Scopolamine deteriorated all parameters of the VVLT (Table 1): delayed word recall (number correct), immediate word recall (number correct) and delayed word recognition (number correct). Similarly, the following parameters of the Stroop test were deteriorated compared with placebo: number correct in both basic and conflict situation and reaction time in conflict situation. The delayed word recognition (reaction time) and the Stroop parameter reaction time in basic situation after placebo were not statistically significantly different than after scopolamine.

The VAS alertness and VAS mood were significantly lower after scopolamine than after placebo and the VAS calmness was not changed by scopolamine. The VAS internal perception, external perception, the VAS feeling high and the VAS colour perception were all higher after scopolamine than after placebo.

All hormone levels increased after scopolamine compared with placebo.

R231857

R231857 80 mg marginally decreased adaptive tracking performance by 1.41% (95% CI -2.78, -0.05). The 160 mg dose marginally decreased the number correct of the immediate word recall test by 2.0 (95% CI -4.0, -0.1) and the VAS alertness scale by 3.4 mm (95% CI -6.2, -0.6).

R231857 AND SCOPOLAMINE

EEG beta power (Fz-Cz), which was not changed by scopolamine alone, was decreased after 160 mg R231857 by 6.7% (95% CI -12.7, -0.4%). The VAS calmness scale, which was also not changed after scopolamine alone, was increased after 320 mg R231857 by 2.5 mm (95% CI 0.2, 4.8). Scopolamine-induced increases in the VAS colour perception scale were further increased after 320 mg R231857 by 1.2 mm (95% CI 0.4, 2.0) and scopolamine-induced hormone increases were reversed after 160 mg R231857 by 6.3% for FSH (95% CI -9.6, -2.8%) and by 17.3% for LH (95% CI -25.9, -7.7%).

Discussion

Scopolamine showed similar effects to our previous study in another cohort of healthy subjects (Liem-Moolenaar, 2009), which demonstrates the accuracy and reproducibility of this model for cognitive and psychomotor impairment. Scopolamine impaired almost every tested CNS-parameter. The concentration effect relationships of the scopolamine challenge will be discussed in more detail elsewhere (data on file).

In this study, the glycine reuptake inhibitor R231857 alone showed some marginal and inconsistent effects compared with placebo. The 80 mg dose caused a small deterioration of adaptive tracking performance and the 160 mg dose decreased the number of correct responses of the immediate word recall test and the subjective alertness. Although these effects might be an indication for limited CNS depression, these were not consistent or dose-related. The study does not allow an interpretation whether this lack of consistent effects is due to insufficient brain penetration, low pharmacological activity, the absence of physiological changes during glycine reuptake inhibition in healthy humans or a methodological reason (i.e., we were not able to detect small CNS-effects with our tests).

The combination of R231857 with scopolamine caused a reduction of C_{MAX} and exposure of the $GlyT_1$-reuptake inhibitor. This could be due to the anticholinergic action of scopolamine, which can lead to delayed gastric emptying and resorption of the highest dose of R231857. Scopolamine also changed the subtle pharmacodynamic effects of R231857, although these changes were small and inconsistent and could not be clearly attributed to either the doses of the $GlyT_1$ reuptake inhibitor or the small scopolamine-related reductions of plasma concentrations.

There were changes in EEG power and subjective calmness, subjective colour perception and hormones, which does not seem to point to alterations of any specific brain network or physiological system. Glycine reuptake inhibition would be expected to cause some glutamatergic effects in the CNS, but also in this respect, we could not identify any trends in the results (such as CNS stimulation). In healthy subjects, the literature has only reported one study on the effects of the glycine reuptake inhibitor D-cycloserine on scopolamine-induced memory impairment (Jones, *et al*, 1991). The only other studies investigating D-cycloserine alone in healthy volunteers on cognitive function report contradictive results (Bailey, *et al*, 2007; D'Souza, *et al*, 2000; Trevisan, *et al*, 2008). Therefore, it is difficult to make useful comparisons to previously published reports.

The lack of clear and consistent R231857 effects on scopolamine-induced CNS-impairment could have several causes. These include non-optimal dosing of the glycine reuptake inhibitor, the unsuitability of the scopolamine model or a dose that was too high to detect effects of glycine reuptake inhibitors and the composition of the CNS battery (which may have consisted of tests that were too insensitive to pick up effects). Because previous studies have shown some reversal of scopolamine effects with D-cycloserine, the most plausible explanation is that the doses of R231857 were too low. If so, the small and non-dose-related effects that were observed in this study could have been an indication of very early CNS-effects, which and may have been too small and/or too variable to consistently surpass the detection limit. In this case, higher doses will cause both larger and more consistent effects. A limited blood-brain-barrier penetration of R231857 may also have increased the variability of its CNS-effects in the low 80- 320 mg dose range. The doses used in this study were based on the in-vitro IC50 for the $GlyT_1$-transporter. Based on pre-clinical experiments, this level was considered to be sufficient to cause pharmacologically relevant and functionally detectable effects in healthy volunteers, but it was actually based on practical limitations with the formulation of higher doses. In hindsight, and particularly if CNS-exposure was low, this exposure level may have been too small.

In summary, the scopolamine challenge test proved to be accurate, reproducible and a safe model for CNS-impairment. Most of the scopolamine-induced changes were in agreement with the findings of previous studies. Both alone and in combination with scopolamine, R231857 only showed marginal, inconsistent effects, which could not be readily attributed to any consistent functional or pharmacological modification. This absence of consistent effects seems most logically explained by R231857 concentrations in the CNS that were too low to cause any reliable changes in healthy volunteers. To show pharmacological activity of this compound in healthy volunteers, studies with higher doses would be required. In addition, clinical trials are needed to investigate which level of glycine reuptake inhibition is needed to show efficacy in patients.

Table 1 Pharmacodynamic effects of placebo, scopolamine and the combination of scopolamine and R231857

Parameter (unit) [B]	LSM[C] PLAC	LSM[C] SCOP	Difference [A]	95% CI	P value	LSM[C] SCOP + R213129 [D, E]	Difference [A]	95% CI	P value
Adaptive tracking (%)	21.45	11.41, 41	10.03	9.10, 10.97	<0.0001				
Body sway (mm)	258	408	58.1%	44.1, 73.9%	<0.0001				
Finger tapping rate (taps/10 sec)	66.2	62.4	3.8	2.5, 5.2	<0.0001				
Saccadic Peak Velocity (deg/sec)	458.8	442.7	16.2	8.3, 24.0	<0.0001				
Smooth pursuit (%)	50.43	43.27	7.16	4.48, 9.83	0.0001				
EEG beta Fz-Cz (μV)	2.03	2.10	3.5%	-1.0, 8.1%	0.125	1.96 (160)	93.3%	87.3, 99.6%	0.0379
Delayed word recall (# correct)	12.9	7.6	5.3	4.0, 6.6	<0.0001				
Immediate word recall (# correct)	13.0	8.5	4.5	3.5, 5.5	<0.0001				
Delayed word recognition (# correct)	24.4	22.6	1.9	0.4, 3.3	0.015				
Delayed word recognition (RT, msec)	903.9	929.3	-25.5	-65.1, 14.1	0.205				
Stroop (conflict, # correct)	19.5	19.1	0.4	0.2, 0.6	0.001				
Stroop (conflict, RT, msec)	663	756	-92	-128, -57	<0.0001				
VAS calmness (mm)	56.8	56.8	-0.1	-1.4, 1.3	0.936	59.3 (320)	2.5	0.2, 4.8	0.0305
VAS feeling high (mm)	0.19	10.23	-10.04	-13.69, -6.39	<0.0001				
VAS colour perception (mm)	0.20	0.92	-0.72	-1.25, -0.20	0.0072	2.13 (320)	1.21	0.40, 2.03	0.0038
FSH (U/L)	3.68	3.87	5.2%	2.6, 7.9%	<0.0001	3.62 (160)	93.7%	90.4, 97.2%	0.0005
LH (U/L)	4.32	5.26	21.8%	13.3, 31.1%	<0.0001	4.35 (160)	82.7	74.1, 92.3%	0.0009
Prolactin (μg/L)	8.41	9.96	18.3%	12.5, 24.7%	<0.0001				

A. If 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level; B. RT = Reaction time; C. LSM is the Least Squares Means estimate; D. The interaction effects are only indicated in case of a significant effect; E. Dose (mg) of R231857 inducing a change in scopolamine

Figure 1 Structural formula of R231857

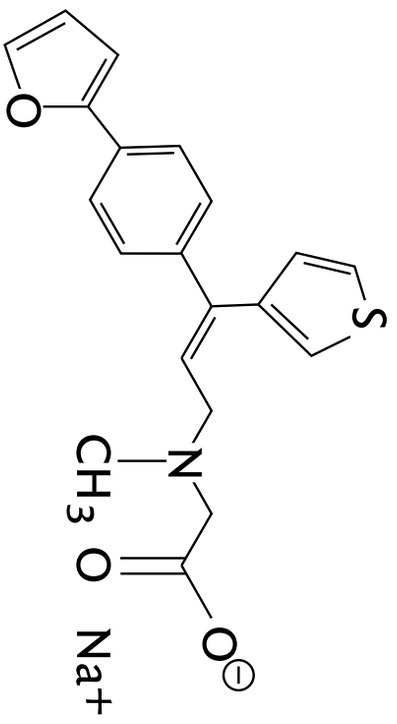
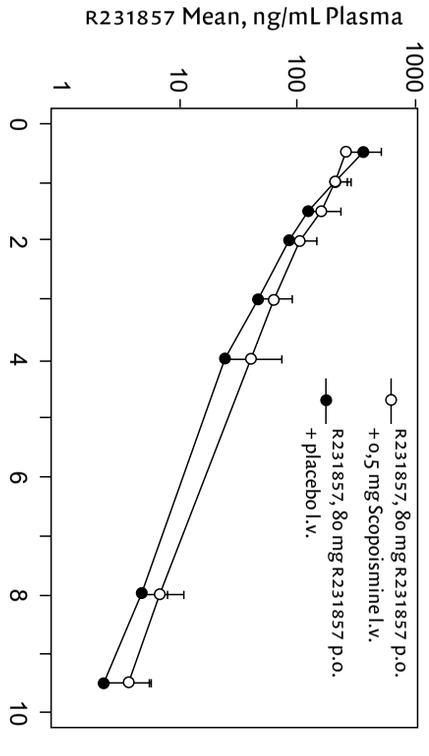


Figure 2 Mean (+SD) plasma concentration profile of 80 mg



REFERENCES

Andersen, JM, Lindberg, V, Myhrer, T (2002) Effects of scopolamine and D-cycloserine on non-spatial reference memory in rats. Behav
Brain Res 129: 211-216.
Bailey, JE, Papadopoulos, A, Lingford-Hughes, A, Nutt, DJ (2007) D-Cycloserine and performance under different states of anxiety in healthy volunteers. Psychopharmacology (Berl) 193: 579-585.
Brann, DW, Mahesh, VB (1995) Glutamate: a major neuroendocrine excitatory signal mediating steroid effects on gonadotropin secretion. J Steroid Biochem Mol Biol 53: 325-329.
D'Souza, DC, Gil, R, Cassello, K, Morrissey, K, Abi-Saab, D, White, J, *et al* (2000) IV glycine and oral D-cycloserine effects on plasma and CSF amino acids in healthy humans. Biol Psychiatry 47: 450-462.
de Visser, SJ, van der Post, JP, de Waal, PP, Cornet, F, Cohen, AF, van Gerven, JM (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55:39-50.
de Visser, SJ, van Gerven, JM, Schoemaker, RC, Cohen, AF (2001) Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 51: 423-428.
Ebert, U, Grossmann, M, Oertel, R, Gramatte, T, Kirch, W (2001) Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram effects of scopolamine in healthy volunteers. J Clin Pharmacol 41:51-60.
Ebert, U, Siepmann, M, Oertel, R, Wesnes, KA, Kirch, W (1998) Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. J Clin Pharmacol 38: 720-726.
Fishkin, RJ, Ince, ES, Carlezon Jr, WA, Dunn, RW (1993) D-cycloserine attenuates scopolamine-induced learning and memory deficits in rats. Behav Neural Biol 59: 150-157.
Gijsman, HJ, van Gerven, JM, Verkes, RJ, Schoemaker, RC, Pieters, MS, Pennings, EJ, *et al* (2002) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 17:83-89.
Green,A,Ellis, KA,Ellis, J,Bartholomeusz,CF,Ilic,S, Croft,RJ, *et al* (2005) Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. Pharmacol Biochem Behav 81:575-584.
Javitt, DC (2006) Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. Curr Opin Psychiatry 19: 151-157.
Jones, RW, Wesnes, KA, Kirby, J (1991) Effects of NMDA modulation in scopolamine dementia. Ann N Y Acad Sci 640: 241-244.
Kemme, MJ, vd Post, JP, Schoemaker, RC, Straub, M, Cohen, AF, van Gerven, JM (2003) Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 55: 518-525.
Kishi, A, Ohno, M, Watanabe, S (1998) Concurrent activation of hippocampal glycine and polyamine sites of the N-methyl-D-aspartate receptor synergistically reverses working memory deficits in rats. Neurosci Lett 257: 131-134.
Koller, G, Satzger, W, Adam, M, Wagner, M, Kathmann, N, Soyka, M, *et al* (2003) Effects of scopolamine on matching to sample paradigm and related tests in human subjects. Neuropsychobiology 48:87-94.
Lane, HY, Chang, YC, Liu, YC, Chiu, CC, Tsai, GE (2005) Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 62: 1196-1204.
Mahesh, VB, Brann, DW (2005) Regulatory role of excitatory amino acids in reproduction. Endocrine 28: 271-280.
Matsuoka, N, Aigner, TG (1996) D-cycloserine, a partial agonist at the glycine site coupled to N-methyl-D-aspartate receptors, improves visual recognition memory in rhesus monkeys. J Pharmacol Exp Ther 278: 891-897.
Nishimura, LM, Boegman, RJ (1990) N-methyl-D-aspartate-evoked release of acetylcholine from the medial septum/diagonal band of rat brain. Neurosci Lett 115: 259-264.
Ransom, RW, Deschenes, NL (1989) Glycine modulation of NMDA-evoked release of [3H] acetylcholine and [3H]dopamine from rat striatal slices. Neurosci Lett 96: 323-328.
Riedel, W, Hogervorst, E, Leboux, R, Verhey, F, van Praag, H, Jolles, J (1995) Caffeine attenuates scopolamine-induced memory impairment in humans. Psychopharmacology (Berl) 122: 158-168.
Scatton, B, Lehmann, J (1982) N-methyl-C-aspartate-type receptors mediate striatal 3H-acetylcholine release evoked by excitatory amino acids. Nature 297: 422-424.
Stone, JM, Morrison, PD, Pilowsky, LS (2007) Glutamate and dopa-mine dysregulation in schizophrenia - a synthesis and selective review. J Psychopharmacol 21: 440-452.
Trevisan, L, Petrakis, IL, Pittman, B, Gueorguieva, R, D'Souza, DC, Perry, E, *et al* (2008) Absence of significant interactive effects of high-dose D-cycloserine and ethanol in healthy human subjects: preliminary insights into ethanol actions at the glycine B site of NMDA glutamate receptors. Alcohol Clin Exp Res 32:36-42.
Tsai, G, Lane, HY, Yang, P, Chong, MY, Lange, N (2004) Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to anti-psychotics for the treatment of schizophrenia. Biol Psychiatry 55: 452-456.
van der Post, JP, de Visser, SJ, Schoemaker, RC, Cohen, AF, van Gerven, JM (2004) Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 18: 221-227.
Zajaczkowski, W, Danysz, W (1997) Effects of D-cycloserine and aniracetam on spatial learning in rats with entorhinal cortex lesions. Pharmacol Biochem Behav 56:21-29.

CHAPTER 7

PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIPS OF CENTRAL NERVOUS SYSTEM EFFECTS OF SCOPOLAMINE IN HEALTHY SUBJECTS

M. Liem-Moolenaar[1], P. de Boer[2], M. Timmers[2], R.C. Schoemaker[1], J.G.C. van Hasselt[1], S. Schmidt[3], J.M.A. van Gerven[1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Johnson and Johnson, Pharmaceutical Research and Development, Beerse, Belgium
3 Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden, The Netherlands

BR J CLIN PHARMACOL 2011; 71: 886-98

Abstract

AIM Although scopolamine is a frequently used memory impairment model, the relationships between exposure and corresponding central nervous system (CNS) effects are mostly unknown. The aim of our study was to characterize these using pharmacokinetic–pharmacodynamic (PK-PD) modelling.

METHODS In two double-blind, placebo-controlled, four-way crossover studies, 0.5-mg scopolamine was administered i.v. to 90 healthy male subjects. PK and PD/safety measures were monitored pre-dose and up to 8.5 h after administration. PK-PD relationships were modelled using non-linear mixed-effect modelling.

RESULTS Most PD responses following scopolamine administration in 85 subjects differed significantly from placebo. As PD measures lagged behind the plasma PK profile, PK-PD relationships were modelled using an effect compartment and arbitrarily categorized according to their equilibration half-lives ($t_{1/2KEO}$; hysteresis measure). $t_{1/2KEO}$ for heart rate was 17 min, saccadic eye movements and adaptive tracking 1–1.5 h, body sway, smooth pursuit, visual analogue scales alertness and psychedelic 2.5–3.5 h, pupil size, finger tapping and visual analogue scales feeling high more than 8 h.

CONCLUSIONS Scopolamine affected different CNS functions in a concentration-dependent manner, which based on their distinct PK-PD characteristics seemed to reflect multiple distinct functional pathways of the cholinergic system. All PD effects showed considerable albeit variable delays compared with plasma concentrations. The $t_{1/2KEO}$ of the central effects was longer than of the peripheral effects on heart rate, which at least partly reflects the long CNS retention of scopolamine, but possibly also the triggering of independent secondary mechanisms. PK-PD analysis can optimize scopolamine administration regimens for future research and give insight into the physiology and pharmacology of human cholinergic systems.

Introduction

Scopolamine, also known as hyoscine, is an antimuscarinic agent approved for use in the prevention of motion sickness. Scopolamine competitively antagonizes the effect of acetylcholine on the muscarinic receptors by occupying postsynaptic receptor sites [1]. Scopolamine has a high affinity for the muscarinic receptor family with little selectivity for any of the five receptor subtypes M1-M5 [2], and it has a negligible affinity for histaminergic and dopaminergic receptors [3]. The peripheral effects of scopolamine include typical anticholinergic effects like a dry mouth, skin and throat, decreased blood pressure, decreased heart rate, difficulty urinating, constipation, pupil dilatation and impaired eye focusing (mydriasis and cycloplegia). The CNS effects consist of drowsiness, reduced attention and memory impairments, and a range of other CNS-effects including changes in several EEG frequency bands [4-6].

At present, scopolamine is the most extensively studied and documented model for cognitive deficits [7-10]. The scopolamine model is frequently used in preclinical studies of cognitive impairment. Studies in animals showed that scopolamine induced memory impairments similar to those seen in humans [11-13]. Scopolamine has been used in humans for several different purposes, for example to study the role of cholinergic systems in memory processing [14]. Since cholinergic systems play an important role in memory processing, scopolamine has been applied as a disease model for dementia [10] and working memory impairment in schizophrenia [15]. The challenge test has been used to show the pharmacological activity of putative cognition enhancers by reversal of scopolamine-induced cognitive and other CNS-deficits in healthy volunteers, including cholinergic drugs [16,17], oxiracetam [18] and D-cycloserine [19].

A methodological disadvantage of the model is the sedation induced by scopolamine. This is not a feature of dementia and may contribute to scopolamine-induced impairment of memory and cognition. However, in many scopolamine studies measures of sedation were unrelated to memory impairment effects [20-24]. Additionally, no cognitive

impairment was produced by lorazepam at a dose which induced similar sedation as scopolamine [25]. Moreover, most stimulant drugs that were added to scopolamine in animals and humans were able to reverse the scopolamine-induced 'fatigue', but not the cognitive impairment [11,26,27], whereas cholinergic agents were [14].

Another source of debate is the dissimilarity between scopolamine effects and the features of memory failure associated with dementia in some studies [28,29]. For example, Alzheimer's disease (AD) is also characterized by other neuropharmacological deficits that are not captured by a single cholinergic deficiency model. These differences are not a reason to completely invalidate the model *per se*, but they would essentially limit the application to cognitive deficits specifically associated with cholinergic dysfunction - of which loss of recent memory might be the most prominent example [11]. With these limitations and caveats, the scopolamine model seems to simulate essential elements of the deficits of AD, and can help studying the physiologic (particularly cholinergic) mechanisms involved in learning and memory [30].

A major part of the uncertainty about the value of the scopolamine challenge is related to the large variability in exposure (due to different administration routes, doses, study populations etc) and/or corresponding effect(s). This limitation is inherent to many pharmacological challenge tests [31], and much of the variability can be better understood by linking exposure and effect(s) in a pharmacokinetic/pharmacodynamic (PK-PD) model [32-34]. Once identified and validated, these PK-PD models can be used to predict the concentration-effect relationship of various dosing regimens and their subsequent optimization.

At present, there is only one study correlating the plasma PK of scopolamine to its corresponding PD effects. However, this study was limited to EEG as a measure of central activity [6]. Therefore, the aim of our study was to evaluate and characterize the PK-PD relationships of scopolamine for a wide range of central nervous system functions in a large group of healthy volunteers using PK-PD modeling.

Methods

Subjects

A total of 90 male subjects aged 18-55 years with a BMI of 18-28.5 kg/m2 were recruited by the Centre of Human Drug Research (Leiden, The Netherlands). After giving written informed consent, subjects were medically screened within 3 weeks prior to study participation. Exclusion criteria included the use of agents known to affect CNS performance (including smoking and drug or alcohol abuse), consuming more than five cups of caffeine-containing drinks per day and evidence of relevant clinical abnormalities. The use of medication was not allowed during the entire study period. Also, subjects were not allowed to consume caffeine-containing drinks from 48 hours prior to dosing until the end of each treatment period. The Ethics Review Board of the Leiden University Medical Centre approved the study protocols.

Study design

Data for this analysis were obtained in two separate studies evaluating the effect of investigational glycinergic compounds on cognitive dysfunction in schizophrenia [35,36]. In these studies, scopolamine was used as a transient cognition impairment model. Both studies had equal double-blind, placebo-controlled, 4-period crossover ascending dose designs. This article is limited to the study visits on which scopolamine or placebo were administered without the experimental glycinergic compounds. Study periods were separated by a washout period of at least 1 week. Scopolamine (0.5 mg) or placebo was administered as a short-term intravenous (i.v.) infusion over 15 minutes starting at T = 0 hours. The sampling and measurement schedules for the scopolamine challenges were identical for both studies.

Clinical observations

Adverse events, electrocardiogram (ECG), body temperature, blood pressure and heart rate (after a supine position for minimally 5 minutes)

were monitored throughout the study. ECGs were assessed using a Cardioperfect ECG recorder (Welch Allyn, Delft, The Netherlands). Blood pressure and heart rate were measured using an automated device (Nihon Kohden, Life Scope EC, Tokyo, Japan).

Pharmacokinetics

Blood samples (4mL) were drawn at 0.5, 0.75, 1.0, 2.5, 6.5 h post dosing of scopolamine. Samples were protected from light at all times. Scopolamine concentrations were determined by using a validated, selective and sensitive liquid LC-MS/MS method (LLOQ = 10 pg/mL). The method consisted of a liquid-liquid extraction sample clean-up with 1-chlorobutane. After evaporation of the organic layer, the residue was reconstituted in a mixture of acetonitrile and 0.1% ammonia (24%) in water (10:90, v/v). 15 µl of this extract was injected onto an X-Bridge shield C18 column (50 x 2.1 mm, 3.5 µm; Waters Corporation,Milford, MA, USA) operated at 30°C. The mobile phase, a mixture of 0.1% ammonia (24%) in water as solvent A and acetonitrile as solvent B, was delivered at 0.4 mL/min. Quantitation was achieved by MS/MS detection in the positive ion mode, using a PE Sciex (Foster City, CA, USA) API 4000 mass spectrometer, equipped with a Turboionspray™ interface. The inter-run precision (expressed as % CV) and accuracy (expressed as % bias) ranged from 1.9% to 5.6% and from -1.0% to 4.3% respectively (n=18). Scopolamine bioanalysis was performed by Pharma Bio-Research Group B.V., Zuidlaren, the Netherlands.

Pharmacodynamics

A battery of quantitative PD measurements was chosen for its repeatability and sensitivity to many different CNS-active drugs in healthy volunteers [35-39] and was incorporated to provide background information on general CNS-performance and functional CNS-domains (neurophysiological, cognitive, neuroendocrine and autonomic changes). Eleven blocks of pharmacodynamic measurements were performed: twice pre-dose (within 1 hour before scopolamine administration) and approximately 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 6.5, and 8.5 hours post-dose. Tests were performed in the following order (relative time difference to the approximate time points): body sway (-10 min), saccadic eye movements (-8 min), smooth pursuit measurement (-7 min), pupil/iris ratio (-5 min), pharmaco-EEG (-4 min), VAS Bond&Lader (-3 min), VAS Bowdle (-2 min), adaptive tracking (+2 min), finger tapping (+6 min), and Stroop test (+8 min). A memory task (i.e. the Visual Verbal Learning Test) was performed once per study period. Blood for hormones (FSH, LH, and prolactin) was taken regularly from 4 minutes up to 24 hours after scopolamine administration. Average baseline values for each variable were obtained by calculation of the mean of the two baseline assessments. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Subjects had a standardized breakfast one hour before scopolamine administration. All subjects were thoroughly trained and familiarized with the psychometric tests within 14 days preceding study start to minimize learning effects during the study.

NEUROPHYSIOLOGICAL

For body sway, the sum of all spontaneous anterior-posterior movements over two minutes (in millimeter) was used for statistical analysis [37]. Saccadic and smooth pursuit eye movements were performed as described before [38]. Average values of saccadic peak velocity (SPV), latency (= reaction time) and inaccuracy were calculated for all artefact free saccades. The average percentage of smooth pursuit for all stimulus frequencies was used as response parameter for smooth pursuit eye movements.

Pharmaco-EEG was measured as previously described [39]. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha- (7.5-11.5 Hz) and beta- (11.5-30 Hz) frequency ranges. The square root of the total power (in µV) was analyzed.

The diameter of the iris and the pupil of both eyes was determined as described previously [35,40].

The adaptive tracking test, as originally described by Borland and Nicholson [41], was performed as previously explained [42]. The average performance and the standard deviation of scores over a 3.5-minute period were used for analysis.

The finger tapping test (adapted from the Halstead Reitan Test Battery [43]) was performed as previously described [35] and evaluates motor activation and fluency. Speed of finger tapping was measured for the index finger of the dominant hand and a session contained five performances of ten seconds. The mean tapping rate and the standard deviations were used for statistical analysis.

COGNITIVE

The Stroop colour-word conflict test is helpful in understanding attention, perception, and reading as well as the cognitive and neural mechanism of mental inhibition, interference and controlled versus automatic processing [44] and was performed as previously described [36]. Outcome parameters are the number of correct answers and the reaction time in basic and conflict situation.

The Visual Verbal Learning Test (VVLT) addresses different components of learning behaviour, such as acquisition, consolidation, storage and retrieval. The VVLT contains three different subtests that cover immediate and delayed word recall and a delayed word recognition [44]. The immediate word recall test was administered 2 hours and 8 minutes after the start of the scopolamine infusion. For the delayed word recall test this was 2 hours and 38 minutes and for the delayed word recognition test 2 hours and 43 minutes. The 30-word learning test avoids ceiling effects in healthy students, and has shown CNS effects for example for benzodiazepines [32,38,46], cannabinoids [33] and antipsychotics [47]. For each study period a different 30-word learning list was used to avoid learning effects over time. All versions of the 30-word learning are validated for differences in complexity. Main parameters for the immediate and delayed word recall were the average and the maximum number of correct responses. For the delayed word recognition, the number of correct items and mean response time for correct responses were analyzed.

SUBJECTIVE

The Bond and Lader Visual Analogue Scale was performed to measure subjective alertness, mood and calmness [38]. The Bowdle VAS evaluates psychedelic effects. These could cluster into two distinct total sum scores (see [33] for further details): internal perception (reflects inner feelings that do not correspond with reality, including mistrustful feelings) and external perception (reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings). The VAS 'feeling high' and 'colour intensity' are subscales of the VAS Bowdle.

NEUROENDOCRINE

Blood samples (3mL) for LH, FSH and prolactin were kept at room temperature for at least 15 and maximally 60 minutes. The hormones were analyzed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center, Leiden, The Netherlands) using an electrochemoluminiscence-immunoassay (ECLIA) for prolactin, and a fluoro-immunoassay for LH and FSH. The assays have a lower limit of quantification (LLOQ) of 0.12 (LH), 0.05 (FSH) U/L and 0.047 µg/L (prolactin). Intra-assay precision (expressed as coefficient of variation) was 3.8 - 5.1% (LH), 3.0 - 14.8% (FSH), and 1.81 - 1.90% (prolactin). Inter-assay precision was 5.5 - 6.8% (LH), 3.8 - 5.3% (FSH), and 2.39 - 2.64% (prolactin).

Statistical analysis

PHARMACODYNAMICS (PD)

PD parameters were analyzed by mixed model analyses of variance using SAS®PROC MIXED (SAS Institute, Inc., Cary, NC, USA). with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline is defined as the average of the available values obtained prior to dosing. Body sway, EEG and neuroendocrine parameters were analyzed after log-transformation and back-transformed after analysis (results may be

interpreted as percentage change). The analysis resulted in Least Square Means (LSM) estimates that indicate the change from baseline where baseline in the graph is set at 0 for t = 0 hours. Treatment effects were reported as the contrasts between placebo and scopolamine, where the average of the measurements up to and including 8.5 hours (22 hours for neuroendocrine parameters) were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals and analyses were two-sided with a significance level of 0.05.

PHARMACOKINETICS (PK) AND PK-PD ANALYSIS

PK and PK-PD modeling and simulations were performed using non-linear mixed effect modeling as implemented in the NONMEM program [45]. Non-linear mixed effect modeling allows the description of a population of individuals using a common structural model while allowing the individuals to vary. Estimates of location and spread between individuals are estimated for the model parameters. Using the population values, individual specific empirical Bayes estimates can be determined that allow description of individual time profiles. Competing models can be compared using the likelihood ratio test, which compares the difference between log-likelihoods for the models (or minimum value of then objective function: MVOF) to a Chi-square distribution with degrees of freedom corresponding to the difference in number of parameters between the two models. First order conditional estimation (FOCE) was used throughout, with additive (on log-scale for concentrations) residual error models.

When analyzing the data, it could be shown that there was no significant placebo response (flat dose-response curve) for most of the parameters. Therefore, it was decided to include a separate placebo model in the analysis for those parameters were a placebo response was observed (tracking performance and smooth pursuit performance), while this was not done for those PD measures where a flat dose-response curve was observed for placebo.

Results

Clinical observations

Eighty-five of the included 90 subjects completed the study. Four subjects withdrew for reasons unrelated to the study. One subject withdrew after what was considered to be a second moderate vasodepressive episode following dosing with scopolamine. The scopolamine treatment was well tolerated by all other subjects. Ninety-eight percent of the subjects reported at least one adverse event. The most commonly reported mild adverse events following dosing with scopolamine were anticholinergic symptoms (97%), nausea (8%), dizziness (7%), and palpitations (6%). The anticholinergic symptoms consisted of variable combinations of drowsiness, dizziness, dry mouth, concentration problems and blurred vision.

Two to 6.5 hours after dosing with scopolamine the mean supine systolic and diastolic blood pressure was lower than with placebo. A similar effect was seen on pulse rate. There was a decrease in mean heart rate (approximately 23%, from 66.8 to 51.2 beats per minute) and consequently a transient increase in mean uncorrected QT interval (approximately 9%, from 381 to 415 milliseconds) without QT_C-changes. There were no other clear consistent changes in vital signs, ECG or laboratory safety parameters.

Pharmacodynamics

NEUROPHYSIOLOGICAL

Except for three EEG parameters (beta Fz-Cz, theta Fz-Cz and Pz-Oz), the changes in the other neurophysiological measures were highly statistically significant after scopolamine compared to placebo treatment (table 1, figure 1). While alpha and beta power increased, delta power decreased. Furthermore, scopolamine decreased saccadic peak velocity (by 22.7 °/sec), adaptive tracking performance (by 9.6%), finger tapping rate (by 3.4 taps/10 seconds) and smooth pursuit (by 6.1%). Body sway and average pupil/iris ratio were both increased by 57.4% and 0.057 respectively.

COGNITIVE

Of the cognitive function tests, the reaction time of the Stroop colour-word conflict test increased after scopolamine with 110.5 msec compared to placebo, while the number of correct responses of this test decreased by 0.6 (table 1). Memory performance deteriorated after scopolamine, since compared to placebo the number of correct responses decreased by 5.8 items for the third immediate recall trial, and by 5.1 items during delayed recall. The average reaction time of the delayed word recognition test (for correct responses) was 48.4 msec longer after scopolamine compared to placebo. The number of correct responses for this same test decreased by 2.3.

SUBJECTIVE MEASUREMENTS

Except for the VAS mood and VAS calmness, all subjective measures were statistically significantly affected by scopolamine (table 1, figure 1). The Bond & Lader VAS alertness scale decreased 9.0 mm compared to placebo. For the Bowdle VAS, an increase in VAS internal perception, external perception, feeling high, and colour perception was seen.

NEUROENDOCRINE MEASUREMENTS

All hormones were increased after scopolamine compared to placebo: FSH by 5.6%, LH by 20.2%, and prolactin by 15.3% (table 1).

Pharmacokinetics

Scopolamine pharmacokinetics were described using a two-compartment model. Individual empirical Bayes estimates were determined that were used to describe the concentration profiles in subsequent PK-PD analyses (see figure 2 for average concentration-time profile with the average NONMEM predicted profile superimposed).

After 30 minutes scopolamine showed a mean maximum concentration of 1287 pg/mL. Plasma concentrations declined rapidly with a mean terminal half-life of 1.5 (range 1.2-2.49) hours (table 2). The $AUC_{0-\infty}$ was 2752 (range 1737-4604) pg.h/mL.

Pharmacokinetic-pharmacodynamic (PK-PD) relationships

When plotting scopolamine plasma concentrations against corresponding effects, PD responses clearly lagged behind the plasma PK profile and showed a much more gradual increase and decline (see figures 3A for SPV and 4A for VAS alertness). In order to overcome this temporal disconnect, data were modeled using an effect compartment, and the relationship between effect compartment concentration and response was assumed to be linear (see figures 3B and 4B). Exploration of the mean predicted and observed time profile for these models did not indicate sufficient model-misspecification to warrant more complex models. With this model average graphs of predicted and observed time-effect curve were made (figure 3C and 4C). In table 3 the PK-PD-parameters intercept, slope, equilibration half-life ($T_{1/2KEO}$) and residual error are presented. $T_{1/2KEO}$ is a measure of hysteresis and is the time in which the effect-compartment concentration reaches half the concentration of the central compartment. In this study, we were not able to measure a maximal effect (E_{MAX}) for any of these outcome parameters. These parameters were categorized according to their equilibration half-lives in four arbitrarily divided groups, *i.e.* shorter than 0.5 hours, 1-1.5 hours, 2.5-3.5 hours and longer than 8 hours. No simple PK-PD model could be fitted adequately for blood pressure, hormones (LH, FSH, prolactin), EEG, pupil/iris ratio, Stroop test, VVLT and VAS scales for calmness, mood and colour (reasons for this are given in the discussion).

Discussion

In this study the PK, PD and PK-PD relationships following the i.v. administration of 0.5 mg scopolamine were assessed using an extensive CNS test battery, frequent measurements and a sizeable study population. The scopolamine challenge proved to be an accurate, reproducible, and safe model for cognitive impairment.

With regard to autonomic changes, the paradoxical reduction in heart rate of 15.6 bpm on average was larger than is typically observed with scopolamine doses of 0.4-0.6 mg, but probably still because of blockade of M1-receptors on postganglionic parasympathetic neurons, as suggested previously [46].

Scopolamine affected most of the measured PD parameters of this study. Most effects were reasonably consistent with the effects described in the literature [5,18,23,26,27,47,48] and disappeared within eight hours. Some of the more noticable PD results will be briefly discussed below.

The very long duration of pupil dilation, in line with the prolonged cycloplegic effects of anticholinergic ocular mydriatics [49], could possibly be explained by an element of retention of the drug in the anterior chamber of the eye and/or a high sensitivity of the iris to cholinergic inhibition. This could not be examined any further, because the effect lasted much longer than the observation period of this study, which refuted PK-PD-analyses.

All tested components of memory showed a significant impairment compared to placebo. In our study, this was particularly due to a reduction of the capacity to immediately actively reproduce memorized words, from 53% (16/30) after placebo to 34% (10.2/30) with scopolamine. This difference was comparable when these words were reproduced 30 minutes later (41% versus 24%). Memory storage (assessed using the delayed word recognition test) was also affected, but to a smaller extent: 75% (22.6/30) of the presented words was still recognized three hours after scopolamine treatment, compared to 83% (24.9/30) with placebo.

Performance of the Stroop test was also significantly impaired, similar to the results of Mintzer and Griffiths [50]. A central mechanism seems the most logical cause for these changes in Stroop performance as the effects were larger in the conflict than in the basic situation, where the colours were the same. Reduced attention could have contributed, since Mintzer [50] also found a strong difference between conflict and basic situation of the Stroop test after benzodiazepine administration.
The observed changes in VAS colour intensity could have been influenced by pupil dilation or cholinergic retinal impairment [51].

The increases in VAS of internal perception, external perception, and feeling high confirms that scopolamine has some psychomimetic properties [2]. When comparing the increase in VAS feeling high with that of tetrahydrocannabinol (THC, the psychoactive component of cannabis), the effects of THC (2, 4, 6 mg intrapulmonary [39]) are twice as large as those of scopolamine. The effects of zolpidem (10 mg orally) on VAS feeling high are close to the values observed with scopolamine [52].

In the PK-PD-analysis of our study, all the effects showed hysteresis and E_{MAX} could not be estimated for any of the effects using a single dose of 0.5 mg. This delay was modeled by assuming a direct relationship between the concentrations in the effect compartment (estimated from the pharmacodynamic effect) and those in the central compartment. Variations in $T_{1/2KEO}$ can be caused by strong retention of the drug within a certain compartment (like for lipophilic compounds in the CNS) or subsequent pharmacological and physiological processes that can lead to a delay or prolongation of a pharmacodynamic effect [34]. It should be kept in mind that the descriptive approach of our model is a limitation of the analysis. As we are evaluating a large variety of effects that certainly can not be characterized by a single mechanism-based model we decided to keep it reasonably simple and consistent for all outcome parameters. We arbitrarily divided the equilibration half-lives in four groups, namely shorter than 0.5 hour, 1-1.5 hours, 2.5-3.5 hours and longer than 8 hours. Although these differences seem large enough to be related to meaningful functional distinctions, a physiological explanation is not immediately apparent and may in fact differ among pharmacodynamic effects even within the same group.

Heart rate showed a short $T_{1/2KEO}$ of 17 minutes. The fact that an effect compartment with a significant equilibration half-life was needed suggests that both central and peripheral effects are involved. In case of purely peripheral effects, we would have expected a more direct link the plasma PK to the corresponding PD effect. However, the physiological situation is more complex. An apparently long equilibration half-life can also ensue from a high affinity of the drug for cardiac tissue leading to prolongation of the (peripheral) effects of scopolamine on

the heart. Moreover, heart rate is determined by complex peripheral cardiovascular regulatory systems, and changes in one part of the system (like muscarinic blockade) will usually cause compensatory changes in the components. Similar $T_{1/2KEO}$ values are found for cannabis-induced tachycardia [34].

Adaptive tracking and saccadic peak velocity (SPV) were both characterized by longer equilibration half-lives of about one hour (see table 3). SPV is one of the most sensitive parameters for alertness [53-55]. Adaptive tracking performance is a visuomotor task that is influenced by attention and vigilance. These measurements usually show fairly direct concentration-effect relationships with other CNS-active drugs [56-59] indicating that an underlying prolonged physiological mechanism is unlikely to be the cause for an extended effect duration. It is more likely that an effect prolongation is mainly due to retention of scopolamine in a certain part of the central nervous system, and not solely to induction of independent physiological processes. This is confirmed by a PET-study performed by Frey *et al* [60], who showed that ^{11}C-scopolamine was retained in different brain regions for many hours. The compound was most rapidly removed from the cerebellum, where receptor binding still had a half-life of several hours. Functionally, this region is most relevant for body sway, which also had an effect half-life of approximately two-and-a-half hours. The tracer showed even longer retention times in other brain regions (pons, thalamus, occipital cortex and caudate nucleus), which cannot be easily related to functional parameters in our study. It is possible therefore that some very long lasting effects like on some of the VAS-scales and finger tapping (see table 3) are still related to the active presence of scopolamine in relevant brain regions. Obviously, it cannot be excluded that functional adaptations play a role in the final effect duration and the length of its equilibration half-life. Although all effects seem closely related to the activity (i.e. inhibition) of cholinergic systems in the brain, the features of these relationships vary considerably. This indicates that different mechanisms could be involved in the effects of scopolamine, and that they are not caused by a single underlying anticholinergic effect like for instance 'CNS-depression'. More complex functional mechanisms may have refuted an adequate PK-PD-analysis, which was not possible for all endpoints. This was the case for blood pressure, possibly because of homeostatic mechanisms interfering between the direct relation between scopolamine concentration and blood pressure. Once LH and FSH are activated (by scopolamine), these hormones have their own complicated and poorly described system dynamics. For EEG parameters, the complex profiles during placebo treatment (which probably not only reflect diurnal rhythms but also unspecified variability) made PK-PD modelling too complicated. The excessive duration of the effects on the pupil/iris ratio and the absence of measurements in this late phase refuted an adequate description of the PK-PD relationships. For many cognitive processes (e.g. Stroop and memory tasks), it is hard - if not impossible - to include enough measurements in a study to have reliable PK-PD, since repeated assessments are complicated by learning effects, different versions and long test durations. PK-PD relationships could also not be described for three other VAS scales (calmness, mood, and colour), mainly because the effects were too small and/or inconsistent.

The results of this study can be used for the design of new studies of the cholinergic system. One example would be when stable CNS-effects (e.g. a certain level of objective alertness) of scopolamine are required for a functional imaging study of longer duration. The dosing regimen predicted to result in reasonably stable reductions of saccadic peak velocity (SPV - an objective measure of sedation) of 50°/sec and of VAS alertness of 10 mm for approximately seven hours (figure 3D and 4D), would result in a 0.45 mg continuous infusion for 15 minutes and 0.15 mg infusions for 15 minutes at t=150, t=250 and t=350 minutes.

The models can also be used for individual dose optimization in patient studies when large inter-individual differences are anticipated, and to study the integrity of the cholinergic system in different individuals, populations or disorders (i.e. disease progression or effects of treatment on the cholinergic system in dementia). However, as this study was done in healthy young men, it is not yet known whether the results are also applicable to older (or demented) men.

In conclusion, the results of our PK-PD-analyses show that scopolamine causes a range of different effects, which are regulated by systems that are functionally and pharmacologically at least partly distinct. The rather extensive data set obtained in this study allowed for accurate analyses of the effects of scopolamine and their concentration-effect relationships across a wide range of different CNS-domains, and provides a useful set of normal reference values for the design optimization of future studies.

Table 1 Pharmacodynamic differences in Least Square Means (LSMs) relative to placebo for all measurements

Variable	Least Square Means		Difference	95%CI		p-value
	Placebo	Scopolamine				
Neurophysiological						
Body sway (mm)	268	422	57.4%	48.3	67.1	<0.0001
Saccadic peak velocity (°/sec)	471.0	448.3	-22.7	-28.5	-16.8	<0.0001
Saccadic latency (sec)	0.200	0.217	0.017	0.013	0.022	<0.0001
Saccadic inaccuracy (%)	5.73	8.14	2.41	2.14	2.68	<0.0001
Smooth pursuit eye movements (%)	48.37	42.32	-6.05	-8.10	-3.99	<0.0001
EEG alpha Fz-Cz (μV)	2.889	2.064	-28.6%	-32.8	-24.1	<0.0001
EEG alpha Pz-Oz (μV)	5.472	3.133	-42.7%	-46.9	-38.3	<0.0001
EEG beta Fz-Cz (μV)	1.998	1.982	-0.8%	-4.3	2.8	0.6492
EEG beta Pz-Oz (μV)	2.508	2.010	-19.8%	-23.1	-16.4	<0.0001
EEG delta Fz-Cz (μV)	1.801	2.064	14.6%	9.6	19.9	<0.0001
EEG delta Pz-Oz (μV)	1.779	1.983	11.5%	6.4	16.8	<0.0001
EEG theta Fz-Cz (μV)	2.112	2.134	1.1%	-3.3	5.6	0.6332
EEG theta Pz-Oz (μV)	2.293	2.310	0.7%	-4.8	6.6	0.8011
Average pupil/iris ratio	0.532	0.590	0.057	0.042	0.072	<0.0001
Adaptive tracking performance (%)	21.70	12.07	-9.62	-10.29	-8.95	<0.0001
Finger tapping rate (taps/10sec)	65.4	62.0	-3.4	-4.4	-2.4	<0.0001
Cognitive						
Stroop test RT conflict situation (msec) [A]	657.0	767.4	110.5	82.2	138.8	<0.0001
Stroop test # correct conflict situation [B]	19.5	18.9	-0.6	-0.9	-0.3	<0.0001
Immediate word recall # correct 3rd trial b	16.0	10.2	-5.8	-6.7	-5.0	<0.0001
Delayed word recall # correct [B]	12.4	7.3	-5.1	-6.0	-4.3	<0.0001
Delayed word recognition average RT correct a answers (msec)	903.1	951.4	48.4	17.4	79.3	0.0025
Delayed word recognition # correct [B]	24.9	22.6	-2.3	-3.3	-1.4	<0.0001
Subjective						
VAS alertness (mm)	54.7	45.7	-9.0	-10.9	-7.2	<0.0001
VAS mood (mm)	57.2	56.6	-0.6	-1.9	0.6	0.3243
VAS calmness (mm)	57.5	58.7	1.2	-0.5	2.9	0.1567
VAS internal perception (log mm)	0.35	0.43	0.08	0.04	0.11	<0.0001
VAS external perception (log mm)	0.34	0.50	0.15	0.11	0.19	<0.0001
VAS feeling high (log mm)	0.36	0.68	0.32	0.24	0.40	<0.0001
VAS colour perception (log mm)	0.35	0.42	0.08	0.04	0.11	<0.0001

Neuroendocrine						
FSH (U/L)	3.70	3.90	5.6%	3.7	7.6	<0.0001
LH (U/L)	4.39	5.28	20.2%	13.6	27.1	<0.0001
Prolactin (U/L)	7.91	9.12	15.3%	10.5	20.3	<0.0001

A RT = reaction time; B # = number

Table 2 Population PK parameters of scopolamine

	Mean [A]	SEM [B]	IIV ar [C]
Clearance (L/min)	2.53	0.0791	15.4%
Central volume (L)	66.3	8.37	36.6%
Intercompartmental Clearance (L/min)	4.78	0.309	8.9%
Steady State Vd (L)	250	12.6	7.2%
Residual error (SD/Mean)	0.102	0.00736	

A. Mean: population average; B. SEM: Standard error population mean; C. IIV ar: Inter-individual variability.

Table 3 Population parameters of PK-PD analysis sorted by equilibration half-life; depicted as population mean (SEM[D] - IICV[F])

Outcome parameter mean (IICV)	Intercept	Slope [C]	Equilibration $t_{1/2}$ (min)	Residual error
mean (SEM - IICV)				
< 0.5 hours				
Heart rate (beats per minute)	55.2 (0.96 – 1.72)	-0.00675[G] (-0.000976 – -14.46)	16.8 (2.73 – 16.25)	0.103 (NA[E])
1- 1.5 hours				
Saccadic peak velocity (°/sec) [A]	485 (NA[E] - 55.05)	-0.0737 (NA[E] - 45.3%)	65.1 (NA[E] - 0.0%)	28.4 (NA[E])
Adaptive tracking (%)	0.0479 (0.457 - 4.336)	-0.0217 (0.000931 - 22.6%)	86.6 (4.14 - 28.4%)	2.98 (0.155)
2.5 - 3.5 hours				
VAS external perception (log mm) [A]	0.34 (0.00931 - 28.6%)	0.000633 (0.000109 - 107%)	161 (25.5 - 86.5%)	0.0935 (0.0062)
Body Sway (log mm) [A]	2.4 (0.0219 - 8.2%)	0.00147 (0.000105 - 32.4%)	181 (12 - 30.6%)	0.102 (0.0053)
VAS alertness (mm) [A]	53.6 (NA[E] - 14.8%)	-0.0622 (NA[E] - 62.9%)	199 (NA[E] - 53.8%)	4.05 (NA[E])
VAS internal perception (log mm) [A]	0.336 (NA[E] - 28.1%)	0.000331 (NA[E] - 130%)	200 (NA[E] - 1.6%)	0.083 (NA[E])
Smooth pursuit (%) [A]	3.1 (0.951 - 12.25)	-0.0264 (0.0029 - 44.4%)	221 (31.5 - 97.8%)	6.28 (0.238)
> 8 hours				
VAS feeling high (log mm) [A]	0.328 (NA[E] - 21.0%)	0.00313 (NA[E] - 93.9%)	483 (NA[E] - 207%)	0.216 (NA[E])
Finger tapping (taps/10 sec) [A,B]	63.4 (0.895 - 12.6%)	-0.0797 (0.0571 - 25.3%)	649 (472 - 62.9%)	3.09 (0.217)

A. Placebo results not subtracted; B. Finger tapping has a time slope of 19.3 (9.187) number/day; C. Unit of the slope = unit of the outcome parameter/pg/mL.dose (mg) of R231857 inducing a change in scopolamine; D. SEM = Standard Error of the Mean; E. NA = not available due to aborted covariance step; for the residual error only the SEM is depicted; F. IICV = inter-individual variability coefficient of variation; G. effect is parameterized: a decrease from baseline is depicted as a positive value.

Figure 1 Time–effect profiles for all pharmacodynamic measurements which resulted in a successful pharmacokinetic–pharmacodynamic analysis.

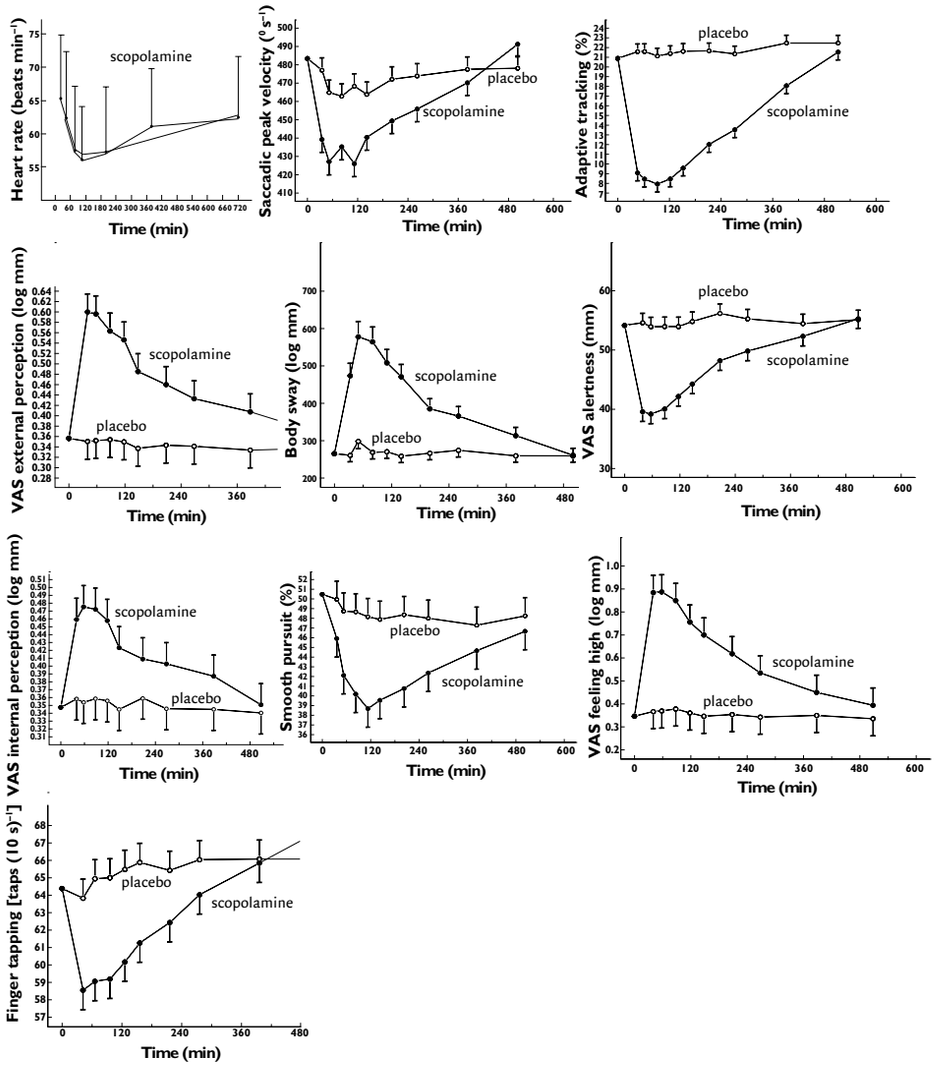


Figure 2 Average observed (with SD error bars) and predicted (two-compartment model) scopolamine plasma concentrations

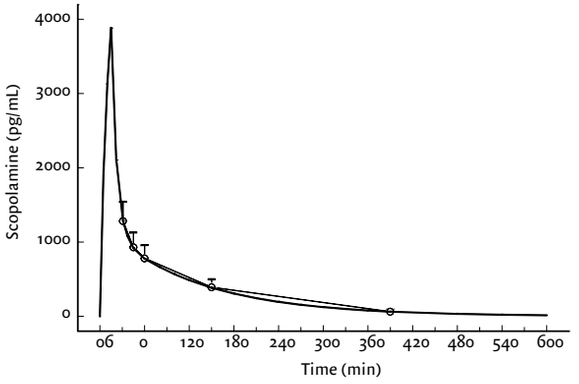


Figure 3 Saccadic peak velocity (SPV)
A. clockwise hysteresis loop formed by the plasma concentration-effect curve; B. effect compartment concentration-effect curve; C. predicted (dark line) and observed (closed circles) time-effect curve with 95% CI error bars; D. mean predicted dosing regimen (0.45 mg i.v. infusion 15 minutes at t=0 and 0.15 mg i.v. infusion over 15 minutes at t=150, t=250 and t=350 minutes) to attain a SPV reduction of 50°/sec

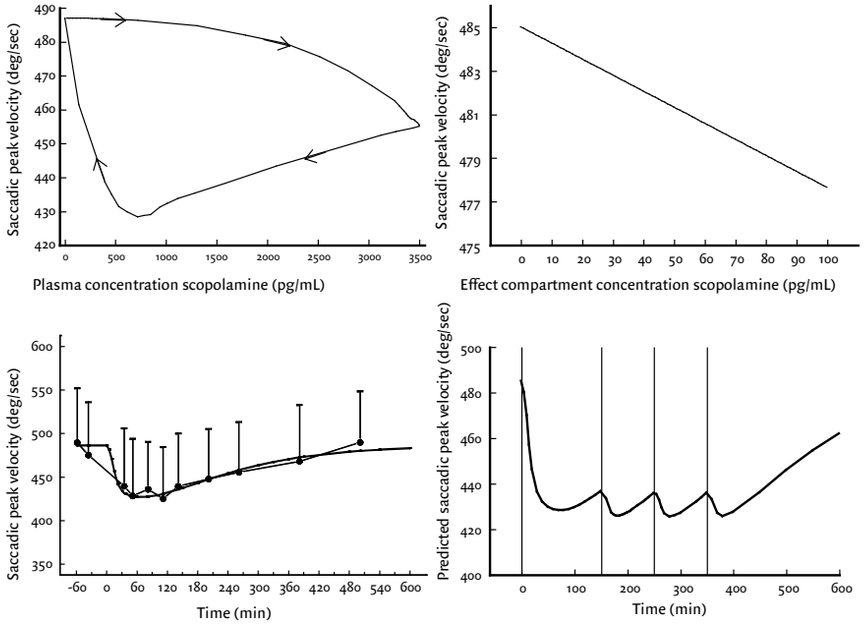
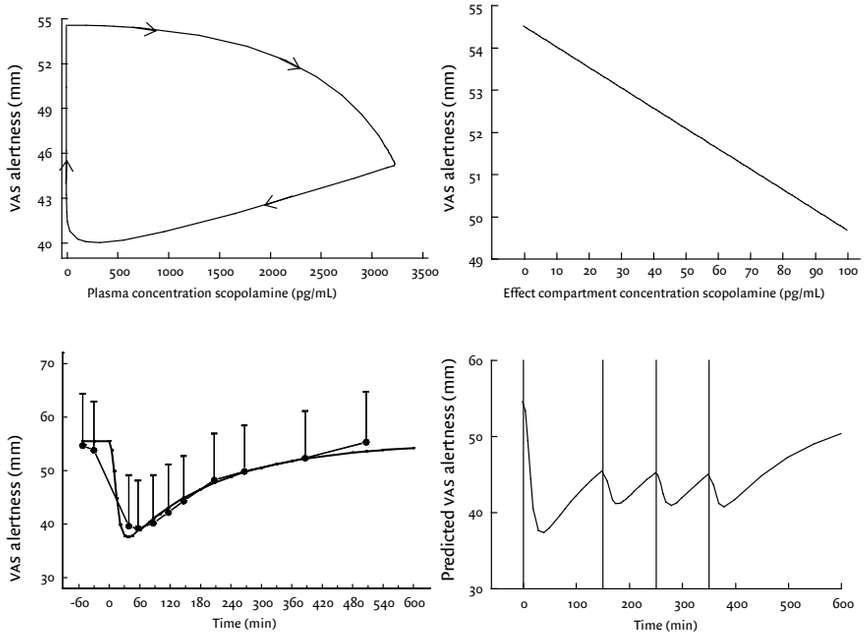


Figure 4 For VAS alertness

A. clockwise hysteresis loop formed by the plasma concentration-effect curve; B. effect compartment concentration-effect curve; C. predicted (dark line) and observed (closed circles) time-effect curve with 95% CI error bars; D. mean predicted dose regimen (0.45 mg i.v. infusion over 15 minutes at t=0 and 0.15 mg i.v. infusion over 15 minutes at t=150, t=250 and t=350 minutes) to attain a VAS alertness reduction of 10 mm



REFERENCES

1 Deutsch JA. The cholinergic synapse and the site of memory. Science 1971; 174: 788-94.

2 Renner UD, Oertel R, Kirch W. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. Ther Drug Monit 2005; 27: 655-65.

3 Peroutka SJ, Snyder SH. Antiemetics: neurotransmitter receptor binding predicts therapeutic actions. Lancet 1982; 1: 658-9.

4 Ebert U, Kirch W. Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur J Clin Invest 1998; 28: 944-9.

5 Ebert U, Siepmann M, Oertel R, Wesnes KA, Kirch W. Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. J Clin Pharmacol 1998; 38: 720-6.

6 Ebert U, Grossmann M, Oertel R, Gramatte T, Kirch W. Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram effects of scopolamine in healthy volunteers. J Clin Pharmacol 2001; 41: 51-60.

7 Vitiello B, Martin A, Hill J, Mack C, Molchan S, Martinez R, Murphy DL, Sunderland T. Cognitive and behavioral effects of cholinergic, dopaminergic, and serotonergic blockade in humans. Neuropsychopharmacology 1997; 16: 15-24.

8 Hall ST, Puech A, Schaffler K, Wesnes K, Gamzu ER. Early clinical testing of cognition enhancers: prediction of efficacy. Pharmacopsychiatry 1990; 23 Suppl 2: 57-8.

9 Riedel WJ, Jolles J. Cognition enhancers in age-related cognitive decline. Drugs Aging 1996; 8: 245-74.

10 Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM. Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 1988; 26: 685-700.

11 Bartus RT. Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys: effects of concurrent administration of physostigmine and methylphenidate with scopolamine. Pharmacol Biochem Behav 1978; 9: 833-6.

12 Araujo JA, Studzinski CM, Milgram NW. Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 411-22.

13 Araujo JA, Chan AD, Winka LL, Seymour PA, Milgram NW. Dose-specific effects of scopolamine on canine cognition: impairment of visuospatial memory, but not visuospatial discrimination. Psychopharmacology (Berl) 2004; 175: 92-8.

14 Ghoneim MM, Mewaldt SP. Studies on human memory: the interactions of diazepam, scopolamine, and physostigmine. Psychopharmacology (Berl) 1977; 52: 1-6.

15 Green A, Ellis KA, Ellis J, Bartholomeusz CF, Ilic S, Croft RJ, Phan KL, Nathan PJ. Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. Pharmacol Biochem Behav 2005; 81: 575-84.

16 Siegfried KR. Pharmacodynamic and early clinical studies with velnacrine. Acta Neurol Scand Suppl 1993; 149: 26-8.

17 Prohovnik I, Arnold SE, Smith G, Lucas LR. Physostigmine reversal of scopolamine-induced hypofrontality. Journal of Cerebral Blood Flow and Metabolism 1997; 17: 220-8.

18 Preda L, Alberoni M, Bressi S, Cattaneo C, Parini J, Canal N, Franceschi M. Effects of acute doses of oxiracetam in the scopolamine model of human amnesia. Psychopharmacology (Berl) 1993; 110: 421-6.

19 Jones RW, Wesnes KA, Kirby J. Effects of NMDA modulation in scopolamine dementia. Ann N Y Acad Sci 1991; 640: 241-4.

20 Caine ED, Weingartner H, Ludlow CL, Cudahy EA, Wehry S. Qualitative analysis of scopolamine-induced amnesia. Psychopharmacology (Berl) 1981; 74: 74-80.

21 Curran HV, Schifano F, Lader M. Models of memory dysfunction? A comparison of the effects of scopolamine and lorazepam on memory, psychomotor performance and mood. Psychopharmacology (Berl) 1991; 103: 83-90.

22 Bartus RT, Dean RL, III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982; 217: 408-14.

23 Drachman DA, Leavitt J. Human memory and the cholinergic system. A relationship to aging? Arch Neurol 1974; 30: 113-21.

24 Kopelman MD, Corn TH. Cholinergic 'blockade' as a model for cholinergic depletion. A comparison of the memory deficits with those of Alzheimer-type dementia and the alcoholic Korsakoff syndrome. Brain 1988; 111 (Pt 5): 1079-110.

25 Sunderland T, Weingartner H, Cohen RM, Tariot PN, Newhouse PA, Thompson KE, Lawlor BA, Mueller EA. Low-dose oral lorazepam

administration in Alzheimer subjects and age-matched controls. Psychopharmacology (Berl) 1989; 99: 129-33.
26 Drachman DA. Memory and cognitive function in man: does the cholinergic system have a specific role? Neurology 1977; 27: 783-90.
27 Martinez R, Molchan SE, Lawlor BA, Thompson K, Martinson H, Latham G, Weingartner H, Sunderland T. Minimal effects of dextroamphetamine on scopolamine-induced cognitive impairments in humans. Biol Psychiatry 1997; 41: 50-7.
28 Beatty WW, Butters N, Janowsky DS. Patterns of memory failure after scopolamine treatment: implications for cholinergic hypotheses of dementia. Behav Neural Biol 1986; 45: 196-211.
29 Christensen H, Maltby N, Jorm AF, Creasey H, Broe GA. Cholinergic 'blockade' as a model of the cognitive deficits in Alzheimer's disease. Brain 1992; 115 (Pt 6): 1681-99.
30 Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, Sunderland T. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. Brain Res Brain Res Rev 1992; 17: 215-26.
31 Gijsman HJ, Cohen AF, van Gerven JM. The application of the principles of clinical drug development to pharmacological challenge tests of the serotonergic system. J Psychopharmacol 2004; 18: 7-13.
32 Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. Int J Clin Pharmacol Ther 1997; 35: 401-13.
33 Cawello W. Connection of pharmacokinetics and pharmacodynamics--how does it work? Int J Clin Pharmacol Ther 1997; 35: 414-7.
34 Strougo A, Zuurman L, Roy C, Pinquier JL, van Gerven JM, Cohen AF, Schoemaker RC. Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol 2008; 22: 717-26.
35 Liem-Moolenaar M, Zoethout R, de BP, Schmidt M, de KM, Cohen A, Franson K, van GJ. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. J Psychopharmacol 2009; 24: 1681-7.
36 Liem-Moolenaar M, Zoethout RW, de BP, Schmidt M, de Kam ML, Cohen AF, Franson KL, van Gerven JM. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. J Psychopharmacol 2010; 24: 1671-9.
37 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_{(A)}$ alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-83.
38 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de Smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_{(A)}$ $alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 2008; 22: 24-32.
39 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, Den Hartigh J, Bender JC, Verpoorte R, Pinquier JL, Cohen AF, van Gerven JM. Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 2008; 22: 707-16.
40 Twa MD, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. J Cataract Refract Surg 2004; 30: 381-9.
41 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 1984; 18 Suppl 1: 69S-72S.
42 Liem-Moolenaar M, Gray F, de Visser S, Franson K, Schoemaker R, Schmitt J, Cohen A, van Gerven J. Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 2008.
43 Andrew JM. Delinquents and the Tapping Test. J Clin Psychol 1977; 33: 786-91.
44 Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol 1935; 18: 643-62.
45 Version VI, NONMEM Users Guides (1989-2006). Beal SL, Sheiner LB, Boeckmann AJ (Eds). Icon Development Solutions, Ellicott City, Maryland, USA 2008.
46 Wellstein A, Pitschner HF. Complex dose-response curves of atropine in man explained by different functions of M1- and M2-cholinoceptors. Naunyn Schmiedebergs Arch Pharmacol 1988; 338: 19-27.
47 Duka T, Ott H, Rohloff A, Voet B. The effects of a benzodiazepine receptor antagonist beta-carboline ZK-93426 on scopolamine-induced impairment on attention, memory and psychomotor skills. Psychopharmacology (Berl) 1996; 123: 361-73.
48 Riedel W, Hogervorst E, Leboux R, Verhey F, van Praag H, Jolles J. Caffeine attenuates scopolamine-induced memory impairment in humans. Psychopharmacology (Berl) 1995; 122: 158-68.
49 Morrison JD, Reilly J. The effects of 0.025% hyoscine hydrobromide eyedrops on visual function in man. Ophthalmic Physiol Opt 1989; 9: 41-5.
50 Mintzer MZ, Griffiths RR. Lorazepam and scopolamine: A single-dose comparison of effects on human memory and attentional processes. Exp Clin Psychopharmacol 2003; 11: 56-72.
51 Hutchins JB, Hollyfield JG. Acetylcholine receptors in the human retina. Invest Ophthalmol Vis Sci 1985; 26: 1550-7.
52 de Haas SL, Schoemaker RC, Van Gerven JMA, Hoever P, Cohen AF, Dingemanse J. Pharmacokinetics, pharmacodynamics, and the pharmacokinetic/pharmacodynamic relationship of zolpidem in healthy subjects. Accepted for publication in Journal of Psychopharmacology. 2008.
53 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-80.
54 van Steveninck AL. Methods of assessment of central nervous system effects of drugs in man. 1993.
55 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-7.
56 van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JM. Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 2004; 18: 221-7.
57 Dingemanse J, van Gerven JM, Schoemaker RC, Roncari G, Oberye JJ, van Oostenbruggen MF, Massarella J, Segala P, Zell M, Cohen AF. Integrated pharmacokinetics and pharmacodynamics of Ro 48-6791, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects. Br J Clin Pharmacol 1997; 44: 477-86.
58 van Gerven JM, Roncari G, Schoemaker RC, Massarella J, Keesmaat P, Kooyman H, Heizmann P, Zell M, Cohen AF, Dingemanse J. Integrated pharmacokinetics and pharmacodynamics of Ro 48-8684, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects. Br J Clin Pharmacol 1997; 44: 487-93.
59 van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, van Gerven JM, Cohen AF. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. Br J Clin Pharmacol 1997; 44: 267-75.
60 Frey KA, Koeppe RA, Mulholland GK, Jewett D, Hichwa R, Ehrenkaufer RL, Carey JE, Wieland DM, Kuhl DE, Agranoff BW. In vivo muscarinic cholinergic receptor imaging in human brain with [11C]scopolamine and positron emission tomography. J Cereb Blood Flow Metab 1992; 12: 147-54.

CHAPTER 8

CENTRAL NERVOUS SYSTEM EFFECTS OF HALOPERIDOL ON THC IN HEALTHY MALE VOLUNTEERS

M Liem-Moolenaar[1], ET te Beek[1], ML de Kam[1], KL Franson[1],
RS Kahn[2], R Hijman[2], D Touw[3] and JMA van Gerven[1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Department of Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands
3 Apotheek Haagse Ziekenhuizen and Haga Teaching Hospital, The Hague, The Netherlands

J PSYCHOPHARMACOL 2010; 24: 1697-708

Abstract

In this study, the hypothesis that haloperidol would lead to an amelioration of Δ9-tetrahydrocannabinol (THC)-induced 'psychotomimetic' effects was investigated. In a double-blind, placebo-controlled, partial three-way crossover ascending dose study the effects of THC, haloperidol and their combination were investigated in 35 healthy, male mild cannabis users, measuring Positive and Negative Syndrome Scale, Visual Analogue Scales for alertness, mood, calmness and psychedelic effects, saccadic and smooth pursuit eye measurements, electroencephalography, Body Sway, Stroop test, Visual and Verbal Learning Task, hormone levels and pharmacokinetics. Compared with placebo, THC significantly decreased smooth pursuit, Visual Analogue Scales alertness, Stroop test performance, immediate and delayed word recall and prolactin concentrations, and significantly increased positive and general Positive and Negative Syndrome Scale score, Visual Analogue Scales feeling high, Body Sway and electroencephalography alpha. Haloperidol reversed the THC-induced positive Positive and Negative Syndrome Scale increase to levels observed with haloperidol alone, but not THC-induced 'high' feelings. Compared with placebo, haloperidol significantly decreased saccadic peak velocity, smooth pursuit, Visual Analogue Scales mood and immediate and delayed word recall and significantly increased Body Sway, electroencephalography theta and prolactin levels. THC-induced increases in positive Positive and Negative Syndrome Scale but not in Visual Analogue Scales feeling high were reversed by haloperidol. This indicates that psychotic-like effects induced by THC are mediated by dopaminergic systems, but that other systems are involved in 'feeling high'. Additionally, the clear reductions of psychotic-like symptoms by a clinically relevant dose of haloperidol suggest that THC administration may be a useful pharmacological cannabinoid model for psychotic effects in healthy volunteers.

Introduction

The underlying mechanisms of the contribution of cannabinoid systems in the pathophysiology of the psychotic symptoms of schizophrenia are still unclear. There is increasing evidence for pharmacological relationships between cannabinoid and dopamine systems (Gardner, 2005; Laviolette and Grace, 2006). Both animal and human studies show contradictive results. Preclinical evidence was recently summarized (D'Souza *et al*, 2008). In general, animal models are reasonably predictive of several characteristics of schizophrenia, such as disorganized behaviour, psychomotor agitation and social withdrawal, but do not adequately represent typical positive features like delusions, hallucinations and disorganized speech. These positive phenomena are reminiscent of the effects of cannabis in humans, and although there is an ongoing debate about the link between cannabis use and the development of schizophrenia (Phillips and Johnson, 2001), there is little doubt that cannabis can lead to clinical psychosis, particularly in susceptible individuals and/or after heavy use (Henquet *et al*, 2005, 2006). Since psychotic symptoms have consistently been related to increased dopamine function in the striatum (Angrist and Van Kammen, 1984; Seeman and Lee, 1975), elevated striatal dopamine release after Δ9-tetrahydrocannabinol (THC) use may explain how cannabis contributes to the development and pathophysiology of schizophrenia (Bossong *et al*, 2009). Dopamine release from the ventral tegmental area into the nucleus accumbens is also a consistent feature of drugs that affect the brain reward system. It is possible therefore that the typical pleasurable 'high feelings' of cannabis are also mediated by this reward system (Lupica *et al*, 2004).

The Centre for Human Drug Research (CHDR) has recently developed an intrapulmonary THC-challenge model (Zuurman *et al*, 2008) and demonstrated clear effects on most of the psychedelic Visual Analogue Scales (VAS). This VAS was previously used by Bowdle *et al* (1998) to quantify psychotomimetic effects of ketamine, which is suggested to induce some schizophrenia-like symptoms in healthy volunteers

(Adler *et al*, 1999). Different VAS subscales and various other central nervous system (CNS) effects showed clear but distinct concentration-effect relationships with THC, suggesting that different pathways mediate the various effects of THC (Strougo *et al*, 2008; Zuurman *et al*, 2008).

THC-induced psychotomimetic effects could be used as a pharmacological challenge test to study the involvement of cannabinoid systems in psychosis, or even as a practical 'psychosis' model to assess therapeutic effects of antipsychotic agents. As haloperidol is a well-known classic antipsychotic that is particularly effective against the positive symptoms of schizophrenia, our hypothesis was that haloperidol would lead to an amelioration of the psychotic-like effects of the THC challenge. At the same time, it was expected that the antidopaminergic effects of haloperidol in the reward system would cause a reduction of the pleasurable subjective effects of THC. These issues were recently also addressed by D'Souza *et al* (2008), who were unable to demonstrate unequivocally that haloperidol reverses psychotic-like symptoms induced by cannabis. In the current study, which started before D'Souza *et al*'s results were published, a novel mode of intrapulmonary THC-administration was employed.

Methods

Subjects

Healthy male subjects aged between 18 and 45 years with a body mass index (BMI) of 18-28.5 kg/m2 were recruited by the CHDR. The subjects had to be cannabis users, but not use more than once a week in the last year. After signing informed consent, subjects were medically screened within three weeks prior to study participation and excluded when relevant clinical abnormalities were found. The use of medication and agents known to affect CNS performance (including nicotine, xanthines, drugs or alcohol) were not allowed and were screened during screening and prior to each study day (i.e. the 24-h period at CHDR). The Ethics Review Board of the Leiden University Medical Center approved the study protocol.

Study design

This was a randomized, double-blind, placebo-controlled, partial three-way crossover interaction study with washout periods of at least two weeks. All 24 subjects received the treatment combinations 'THC + placebo' and 'THC + haloperidol', but half of the subjects received 'haloperidol + placebo' and the other half 'placebo + placebo'.

In order to estimate the sample size for a study that has 80% power of detecting half of the THC effect on the VAS Bowdle 'external perception' subscale (i.e. 50% of 0.437) as observed in a previous THC study, a sample size of 26 was calculated (two-sided test, alpha = 0.05) and rounded to 24 for randomization using Williams Squares.

Drugs

Each study day started with a dose of 3 mg haloperidol (or its matching placebo) orally. After 3 h, three consecutive doses of THC 2, 4 and 6 mg (or matching placebo) were given intrapulmonary (described in more detail elsewhere (Zuurman *et al*, 2009b)) around the expected maximum of plasma concentrations and pharmacodynamic effects of haloperidol, at t = 180, t = 270, and t = 360 min (Chakraborty *et al*, 1989). Subjects remained in the unit for a 24-h observation period (see Table 1).

The dose of haloperidol in healthy volunteers was based on recommendations by King (1997) and was previously used at CHDR (Liem-Moolenaar *et al*, 2008) showing clear effects with only limited, usually mild, and some moderate adverse events. THC doses were based on previous CHDR studies using similar dosages, showing clear effects and concentration-effect relationships on a range of different CNS effects, including VAS Bowdle subscales feeling high and postural instability (Zuurman *et al*, 2008, 2009b, 2010).

Safety

Adverse events, electrocardiography, blood pressure, heart rate, and routine haematology and biochemistry measurements were performed throughout the study (see Table 1).

Pharmacodynamics

The extensive CNS battery was incorporated to provide background information on general CNS performance and functional CNS domains, which could have had a secondary impact on the primary endpoints of the study (e.g. changes in alertness or vigilance), with each drug alone or in combination.

Pharmacodynamic measurements were performed pre-dose (twice before haloperidol administration) and frequently after THC dosing. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the room per session. All subjects were trained within three weeks preceding study start to minimize learning effects during the study. The tests were performed as described below.

POSITIVE AND NEGATIVE SYNDROME SCALE

The Positive And Negative Syndrome Scale (PANSS) includes 30 items on three subscales: seven items covering positive symptoms (e.g. hallucinations and delusions), seven covering negative symptoms (e.g. blunted affect), and 16 covering general psychopathology (e.g. guilt, uncooperativeness). Each item is scored on a seven-point Likert scale ranging from 1 to 7, resulting in a range from 7 to 49 for the positive and negative and 16 to 112 for the general psychopathology scale. Reliability and validity have been shown to be high (Kay and Opler, 1987). For the current study, several PANSS raters were trained to perform the interview, to observe changes in attitude, and inter-rater correlation was assessed. During the study, two interviewers both made their own assessment, and the final score was determined by consensus. PANSS assessments were videotaped during the interview to permit review at a later time point.

VISUAL ANALOGUE SCALES (VAS)

The VAS Bond and Lader, originally described by Norris (1971) and applied to drug effect by Bond and Lader (1974), was performed to measure subjective alertness, mood (or contentedness) and calmness, and the VAS Bowdle (Bowdle *et al*, 1998) to measure psychedelic effects, and were performed as previously described (De Haas *et al*, 2008). The VAS feeling high is a subscale of the VAS Bowdle. After THC administration, the Bowdle sub-scales clustered in two distinct groups, following stepwise discriminant, cluster and factor analysis. The first group could be described as 'internal perception' of changes in the way that the subject perceived his inner self (e.g. body composition, paranoid ideas), and the second group as 'external perception' of changes in the environment (e.g. time, colours) (Zuurman *et al*, 2008).

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS

Saccadic and smooth pursuit eye movements have shown dose-and concentration-related effects of many different CNS active drugs, including GABA-ergic (De Haas *et al*, 2008; De Visser *et al*, 2003), serotonergic (Gijsman *et al*, 2002) and noradrenergic compounds (Kemme *et al*, 2003; Van der Post *et al*, 2004b), and to a limited extent dopaminergic drugs (Liem-Moolenaar *et al*, 2008). Eye movements were recorded as described previously (Van Steveninck *et al*, 1999). Saccadic eye movements are used to measure alertness and smooth pursuit eye movements to measure motor coordination. Average values of saccadic peak velocity (SPV) for all artefact-free saccades and the average percentage of smooth pursuit for all stimulus frequencies were measured.

PHARMACO-ELECTROENCEPHALOGRAPHY (-EEG)

Pharmaco-EEG was performed as a general measure of CNS activity (Cohen *et al*, 1985) and was performed as previously described (De Haas *et al*, 2008). The literature suggests that antipsychotics show distinct profiles of EEG changes (De Visser *et al*, 2001). Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta-(0.5-3.5 Hz), theta-(3.5-7.5 Hz), alpha-(7.5-11.5 Hz) and beta(11.5-30 Hz) frequency ranges. The square root of the total power (in microvolts) was analysed.

BODY SWAY

The Body Sway meter records body movements in a single plane, providing a measure of postural stability.

Changes in Body Sway have been seen for many different CNS active drugs, including GABA-ergic drugs (De Haas *et al*, 2008; De Visser *et al*, 2003) and THC (Zuurman *et al*, 2008) and was performed as described previously (Liem-Moolenaar *et al*, 2008).

STROOP TEST

The Stroop effect is helpful in understanding attention, perception and reading as well as the cognitive and neural mechanism of mental inhibition, interference and controlled versus automatic processing (Laeng *et al*, 2005). Individual differences in Stroop interference are considered to be among the most reliable or stable measures (Laeng *et al*, 2005). The test battery is affected by many different CNS-active compounds (Bohnen *et al*, 2005; Griffiths *et al*, 1986; Rose and Duka, 2007). In the first part of the test (E-prime, http://www.pstnet.com/e-prime/support/samples), 20 coloured items (green, red and blue) are randomly presented (basic situation). In the second part, 20 colour and word pairs are randomly presented, forming either congruent or incongruent matches (conflict situation). Subjects are asked to respond as quickly and accurately as possible by pressing the keys 1, 2 or 3 on the number pad with the index, middle and ring finger of the dominant hand. Outcome parameters are the number of correct answers and the reaction time in basic and conflict situation.

VISUAL AND VERBAL LEARNING TASK (VVLT)

The VVLT test has shown CNS effects of various compounds such as benzodiazepines (De Visser *et al*, 2008), antidopaminergic (Liem-Moolenaar *et al*, 2008), and cannabinoid drugs (Zuurman *et al*, 2008) and was performed as described previously (Liem-Moolenaar *et al*, 2008). The primary outcome parameters for the immediate and delayed word recall tasks were the numbers of correct responses, and for the delayed word recognition the number of correct items and mean response time for correct responses. Learning was assessed from the change in reproduced words with three consecutive memorization trials, decay from the change in reproduced words after a time delay (delayed word recall versus last trial of immediate word recall), and retrieval from the difference between delayed word recognition and delayed word recall.

HORMONES

Prolactin levels were measured as a biomarker for dopaminergic activity (De Visser *et al*, 2001; Liem-Moolenaar *et al*, 2008). Blood samples for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin and cortisol were collected pre-dose, frequently and up to 24 h after haloperidol administration (for frequency see Table 1) and kept at room temperature for between 30 and 45 minutes. Serum was separated by centrifugation (2000g at 4°C for 10 min) within 1 h of collection. The hormones were analysed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center) using an electrochemoluminiscence-immunoassay for prolactin and cortisol, and a fluoro-immunoassay for LH and FSH (Bieglmayer *et al*, 2004).

Pharmacokinetics

THC

Blood samples for plasma concentrations of THC were drawn frequently up to 24 h after haloperidol administration (for frequency see Table 1). Samples were protected from light at all times to prevent degradation of the compound, centrifuged (2000g at 4°C for 10 min) within 1 h and stored at a temperature of at least -20°C.

Pharmacokinetic analysis was performed by the Analytisch Biochemisch Laboratorium BV (ABL), Assen, The Netherlands (http://www.abl.nl/), using a validated Good Laboratory Practices assay The analysis was performed by protein precipitation followed by online solid phase extraction on a Symbiosis Pharma System using an Agilent HPLC Column followed by an API 4000 liquid chromatography/ tandem mass spectrometry (LC-MS/MS). One of the two most prominent metabolites of THC, 11-nor-9-COOH-THC, has been reported to have no psychotropic or cardiologic effects (Barnett *et al*, 1982; Grotenhermen, 2003). The other metabolite, 11-OH-THC, was shown to be equipotent (Grotenhermen,

2003) to twice as potent (Howlett *et al*, 2004; Perez-Reyes *et al*, 1972) as THC, but the contribution to overall activity was assumed insignificant since 11-OH-THC concentrations were approximately 25 times lower than THC concentrations (Zuurman *et al*, 2008). Therefore, only THC concentrations are reported. The assay was linear from 1.00-500 ng/mL, the limit of quantitation (LOQ) 1.0 ng/mL, recovery 99.99% and the variation coefficient (CV) ranged between 3.5 and 5.7%.

HALOPERIDOL

Blood samples for haloperidol were collected pre-dose and frequently, up to 24 h after haloperidol administration (for frequency see Table 1). After collection, tubes were kept at room temperature for between 30 and 60 minutes, and serum was separated by refrigerated centrifugation (2000g at 4°C for 10 min) and stored at approximately -20°C. Pharmacokinetic analysis was performed by the laboratory of the 'Apotheek Haagse Ziekenhuizen', Den Haag, The Netherlands (www.ahz.nl), using a validated high-performance LC/MS/MS assay. The assay was linear from 0.05-2.0 µ/L, the LOQ 0.0517 µ/L, the recovery 101.8% and the CV ranged between 2.4 and 4.6%.

Statistical analysis

PHARMACODYNAMICS

The pharmacodynamic endpoints were analysed by mixed-model analyses of variance (using SAS PROC MIXED) with subject, subject by treatment and subject by time as random effects, with treatment, study day, time and treatment by time as fixed effects, and the average baseline value as covariate. Contrasts were estimated within the overall treatment effect and contrasts between treatments and between treatments over 360 and 420 min were calculated. Body Sway, EEG and neuroendocrine parameters were analysed after log-transformation and back-transformed after analysis (results may be interpreted as percentage change).

In patient studies using the PANSS, a comparison of PANSS scores and an expert assessment was performed to ensure inter-rater reliability. As there is no 'gold standard' for PANSS scores in healthy volunteers using THC, the comparison to this standard was not possible. Instead, we determined the inter-rater variability of PANSS scores by performing a Pearson's correlation after showing all eight raters four PANSS interviews on video. Based on the correlations and means, one rater showed a low correlation (<0.8) and one rater showed a good correlation but with a substantially higher mean value for the negative PANSS subscale. Therefore the negative subscales for these two raters were re-rated from video recordings by two independent raters, and these new values were used for the analysis. To take rater effects into account, this was added as a random factor to the mixed model for the analysis of the PANSS scores. Results are presented as differences with 95% confidence intervals (CI).

PHARMACOKINETICS

THC Pharmacokinetic modelling was performed using non-linear mixed effect modelling as implemented in the NONMEM software package (Version VI, NONMEM Project Group, University of California, San Francisco, CA). Previous assessment of THC pharmacokinetics indicated the requirement of a two-compartment model with a bolus administration in the central compartment (Strougo *et al*, 2008).

The first-order conditional estimation method with interaction was used. Individual empirical Bayesian estimates were generated in a post hoc step and used to plot predicted concentration profiles. To account for variability between different THC administrations within a subject, a relative bioavailability parameter was used.

The terminal half life and area under curve (AUC) were obtained using a non-compartmental analysis which was performed using the software package WinNonLin (version 5.0, Pharsight Corp., Mountain View, CA).

HALOPERIDOL Individual pharmacokinetic parameters of haloperidol were calculated by a non-compartmental analysis using WinNonLin software (version 5, Pharsight Corp., Mountain View, CA, USA). Terminal half life was estimated by log-linear regression of the terminal part of the concentration time curve. $AUC_{0-\infty}$ was calculated using the log-down trapezoidal method in plasma concentration-time curves. Terminal half life, clearance/F (calculated as Dose/$AUC_{0-\infty}$), C_{MAX} and T_{MAX} were reported.

Results

Subjects

A total of 35 subjects aged 18-38 years with a BMI of 18.8- 28.4 kg/m2 were included in 2007 and 2008. Thirteen subjects (37%) stopped the study after participation in at least one study day: six due to non-compliance (three were excluded by the investigator and three stopped for personal reasons) and seven due to adverse events (after THC alone or after the combination with haloperidol). Three subjects considered their subjective high effects too intense, and the study physician decided to not continue dosing of THC. Five other participants dropped out due to physical adverse effects (nausea, drowsiness, dizziness). The resulting loss of power was considered to be adequately compensated by the incorporation of all completed occasions of the 13 dropouts in the final analyses.

Clinical effects

Following THC dosing the most frequently reported adverse events were sleepiness, 'feeling high', dizziness and headache. After haloperidol administration sleepiness was most frequently reported. All adverse events were transient and mild (discomfort, but no reduced normal daily activity) to moderate (normal daily activity reduced) in severity. After the combination of haloperidol and THC, sleepiness (24 versus 18) and headache (9 versus 5) were reported more frequently and dizziness was reported less frequently (4 versus 7) than after THC alone.

Pharmacodynamic results

PANSS AND VAS SCALES (TABLE 2)

Compared with placebo, the positive and general PANSS scale, the VAS external and internal perception and VAS feeling high were increased after THC administration, while the VAS alertness was decreased. THC caused a significant increase of 2.5 points in positive PANSS, which was significantly reduced by 1.1 points after pre-treatment with haloperidol (Figure 1), comparable to those with haloperidol alone (baseline values were 7.4 after placebo, 7.8 after THC, 8.2 after haloperidol and 7.7 after the combination). In contrast, the significant and strong increase in VAS feeling high after THC was not affected by haloperidol (Figure 2). Compared with placebo, haloperidol caused a small reduction of VAS mood.

A visual inspection of the distributions of the PANSS data provided no clear indications for excessive skewness. Therefore, this distribution was considered not to refute further parametric analyses, also because non-parametric methods would not account for the complexity of the study design and the data set. However, since the data were not completely normally distributed and could not be fully normalized using log-transformation, a non-parametric check of the main outcome of the study was performed. The PANSS scores after the second THC inhalation (at t = 306 min) were analysed with a Wilcoxon signed rank test, to compare the THC and THC + haloperidol treatments. The result was highly significant ($p = 0.0091$).

NEUROPHYSIOLOGICAL PARAMETERS (TABLE 3)

THC administration decreased smooth pursuit eye movements, while Body Sway and EEG alpha Fz-Cz power were increased. Body Sway and EEG theta Fz-Cz power were increased after haloperidol administration, but SPV and smooth pursuit eye movements were decreased. Further reductions in these last two parameters were found after the combination of haloperidol and THC compared with THC alone.

COGNITION (TABLE 4)

THC administration deteriorated the number of correct responses on the Stroop test (conflict situation). Both THC and haloperidol caused an impairment of immediate and delayed word recall tasks. The deterioration in correct responses of immediate word recall by THC alone (on average 10.7/30 words compared with 16.2/30 words with placebo) was statistically significantly reversed by haloperidol by 2.2 words, to levels observed with the antipsychotic alone (12.5-12.9/30 words). Delayed word recognition was not affected by any treatment.

HORMONES (TABLE 5)

Compared with placebo, prolactin concentrations were slightly decreased after THC administration (baseline values were 9.7 after placebo, 10.8 after THC, 11.4 after haloperidol and 9.7 after the combination). This effect was completely reversed by the strong increase after haloperidol administration. Cortisol levels were increased after THC compared with placebo, but haloperidol had no effects on cortisol. Neither THC nor haloperidol alone had an effect on LH and FSH concentrations, but the combination caused (near) significant reductions of both these gonadotrophic hormones.

Pharmacokinetic results

Analysis of concentration-time profiles of THC revealed a biphasic clearance from the plasma, confirmed by the successful implementation of a two-compartment model (Figure 3). The NONMEM pharmacokinetic population parameter estimates were very comparable with a previously published PK- PD model of THC (Strougo *et al*, 2008). Haloperidol clearly did not influence THC pharmacokinetics (determined by a paired t-test).

Haloperidol pharmacokinetics were described using a non-compartmental analysis. Haloperidol showed a relatively long absorption phase (T_{MAX} after almost 5 h) and terminal half life (more than 19 h), a maximum concentration around 1 mg/L and clearance/F of 2.9 L/min (Figure 4). Haloperidol administration alone resulted in a higher maximum concentration for haloperidol than after the combination with THC (on average (SD) 1.07 (0.53) versus 0.79 (0.38) mg/L, $p < 0.05$ using a paired t-test, see Figure 4).

Discussion

The main objective of this study was to examine the effects of haloperidol on behavioural effects of THC assessed with PANSS scores and VAS Bowdle scales. The increase in positive PANSS with THC alone was reduced after pre-treatment with haloperidol to a level that was similar to the effects of haloperidol alone. This is consistent with the reported associations between cannabinoid and dopamine systems in the brain (Bossong *et al*, 2009; Gardner, 2005). THC had another dopaminergic effect, consisting of a small decrease in prolactin. This completely disappeared in combination with haloperidol, which produced an overwhelming prolactin increase. This THC-induced decrease in prolactin concentrations was probably mediated by (indirect) cannabinoid 1 receptor activation of tuberoinfundibular dopaminergic neurons (Rodriguez *et al*, 1992). This decrease was comparable to levels observed with the dopamine agonist lisuride (Van der Post *et al*, 2004a). It is much harder to show a statistically significant decrease in prolactin due to a 'floor effect' (i.e. it is more difficult to find a decrease of low prolactin levels than an increase). Although the reduction in prolactin after THC seems small, this finding should not be neglected, since these findings provide functional support for the stimulation of dopaminergic systems by THC. The only other ameliorating effect of haloperidol was a partial reversal of THC-induced working memory impairment (immediate recall). Dopaminergic systems have been implicated in working memory (Mehta and Riedel, 2006), but their role in memory and cognition is complex (Kulisevsky, 2000). Many studies have shown that cannabis causes deteriorations of immediate recall and working memory (Ranganathan and D'Souza, 2006; Zuurman *et al*, 2009a), although the dose- response relationships are complex (Zuurman *et al*, 2009a).

Haloperidol did not interact with other THC-induced CNS effects, such as the general PANSS, VAS 'internal', 'external' perception, and alertness, Body Sway, EEG alpha reductions, number of correct answers of delayed word recall and Stroop test and cortisol concentrations. In most cases, the effects of the combination were similar to those of the individual compound with the largest impact. Since haloperidol is a potent D_2 antagonist, it would seem that the unmodified effects of THC are mediated by other than dopaminergic mechanisms. This also appears to be the case for the 'high feelings' induced by THC, which remained very clear after co-treatment with haloperidol. This was unexpected, since the literature contains many links between striatal dopamine release

and mood improvement for drugs such as amphetamine (Drevets *et al*, 2001), cocaine (Schlaepfer *et al*, 1997), alcohol (Yoder *et al*, 2007) and nicotine (Brody *et al*, 2009; Drevets *et al*, 2001; Yoder *et al*, 2007). Although the reward system has also been implicated in the typical 'high feelings' caused by cannabis (Lupica *et al*, 2004), this was until recently not as clearly demonstrated in humans. However, in line with earlier animal studies (Gardner and Lowinson, 1991; Tanda and Goldberg, 2003), Bossong *et al* showed a 3% decrease in [11C]-raclopride binding after THC in humans, as a measure of dopamine release in the ventral striatum. Bossong *et al* used the same mode of THC administration as in the current study, which caused comparable levels of 'feeling high' (Bossong *et al*, 2009). The amount of dopamine release following cannabinoids, however, seems to be considerably smaller (or even absent after oral dronabinol (Stokes *et al*, 2009)) than with typical addictive drugs such as ethanol (15% (Boileau *et al*, 2003)), nicotine (8.4-37% (Brody *et al*, 2004, 2009)), alfentanil (7.4% (Hagelberg *et al*, 2002)), amphetamine (12-16% (Drevets *et al*, 2001; Martinez *et al*, 2003, 2007)) or cocaine (12% (Schlaepfer *et al*, 1997)). In several studies, statistically significant correlations were found between the level of striatal dopamine release and the intensity of subjective intoxication or mood changes (Brody *et al*, 2009; Drevets *et al*, 2001; Yoder *et al*, 2007), which was not the case for THC (Bossong *et al*, 2009). Thus, the absence of this correlation with THC and the limited striatal dopamine release reported suggest that stimulation of the reward system after THC administration is not mediated (exclusively) by dopamine (Wise, 1988, 2004). Most addictive drugs cause rewarding effects by activation of dopamine release from the ventral tegmentum into the nucleus accumbens, but some drugs, including cannabinoids, are also thought to produce reward partly through direct stimulation of the accumbens (Hyman *et al*, 2006).

The relationships between dopamine release and THC-induced psychosis have not been directly studied with positron emission tomography (PET,) but are likely to involve brain areas other than the striatum and the reward system. Low-affinity dopamine receptors in the cerebral cortex, for instance, are not captured accurately with [11C]-raclopride PET, but require other ligands such as [11C]-fallypride or [11C]-FLB 457. Such experiments have not been performed with THC, but several PET studies using [11C]-FLB 457 (Narendran *et al*, 2009) or [11C]-fallypride (Cropley *et al*, 2008; Riccardi *et al*, 2006a,b) have shown cortical dopamine release after amphetamine administration. Haloperidol is not region specific, and shows comparable binding to both cortical and striatal D_2 receptors (Kessler *et al*, 2005).

The literature about the relationships between antipsychotics and cannabis-induced psychotic symptoms is not conclusive. In clinically stable and medication-responsive schizophrenia patients, antipsychotic treatment did not prevent all THC-induced PANSS-increases (D'Souza *et al*, 2005). The study did not compare the results with unmedicated patients, so it cannot be excluded that the positive symptoms were actually blunted. Recently, another study of D'Souza *et al* in healthy volunteers was published after the start of our experiment. This study was reminiscent of ours, and PANSS effects of THC either alone or combined with haloperidol were studied (D'Souza *et al*, 2008). PANSS effects were similar to those observed in the current study. Haloperidol appeared to reduce the positive PANSS and perceptual alterations induced by THC, but this was not statistically significant. Also, D'Souza *et al* found a worsening of cognitive function after the combination of haloperidol and THC compared with THC alone, while we found improvement. The differences in findings could have been caused by different factors. First, the doses of THC and haloperidol and the administration routes of THC differed. D'Souza *et al* used doses of active oral haloperidol (0.057 mg/kg) or placebo in random order followed 90 and 215 min later by a fixed-order intravenous administration over 20 min of placebo and active (0.0286 mg/kg) THC, respectively. Haloperidol doses are generally lower in our study and those of THC substantially higher. It is hard to exactly compare these as no blood concentrations were measured in D'Souza *et al*'s study. When comparing pharmacokinetics to an earlier study by D'Souza *et al* (2004), which used slightly higher doses than in the THC-haloperidol interaction study (D'Souza *et al*, 2008), the observed maximum concentrations

seemed at least 10 times higher in our study. Another reason for the differences in results could have been the rate of increase of THC brain levels, which would have been higher in our study due to the inhalation method for THC. Stokes *et al* (2009) also hypothesized that this could have contributed to a lack of dopamine release in their study with oral dronabinol, as opposed to Bossong *et al*'s (2009) study that used our method. Also, different relative timing of administration of THC and haloperidol and assessment methods could have caused differences. In our study the THC administration and measurements were performed later than in D'Souza *et al*'s trial, aiming for the T_{MAX} of haloperidol and continuing for longer. In this way, we tried to achieve the maximum effects of THC when D_2 occupancy by haloperidol was also maximal.

In the current study, THC mainly affected the individual positive PANSS items 'delusions', 'conceptual disorganization' and 'hallucinatory behaviour'. 'Excitement', 'grandiosity', 'suspiciousness/persecution' and 'hostility' did not change significantly. Haloperidol completely reversed THC-induced increases in 'delusions' and 'conceptual disorganization' and almost halved the increase in 'hallucinatory behaviour'. Although not statistically significant, haloperidol seemed to increase the items 'conceptual disorganization', 'suspiciousness/persecution' and 'hostility' compared with placebo. This may in part correspond to the reduced contentedness (as shown by the VAS mood), and is reflected by the somewhat higher average positive PANSS values after haloperidol treatment shown in Figure 1.

A limitation of this study is the drop-out rate. Thirty-five subjects started the study, but only 22 completed all three study days according to protocol. It is hard to determine an exact reason for this, but the intensiveness of the study could have been a contributing factor. As subjects who withdrew from the study did not differ clearly from the ones who stayed in and all the effects of THC were similar to those of previous studies, drop-out does not seem to have introduced a bias. Also, all completed study days were included, even if subjects withdrew after one or two occasions.

In this group of mild cannabis users without a personal or family history of psychosis or other relevant psychiatric disorders, the THC effects were small compared with a clinical psychotic episode in schizophrenia. The PANSS has not been designed for short time intervals such as in this study (i.e. four times per study day). Therefore, some modifications were made in conjunction with psychiatric experts in this field, mainly consisting of adaptations in the time periods and the situations addressed in the interview. In addition, all raters were trained by an expert using standard interview samples, and the inter-rater variability was assessed and found to be low. Nonetheless, the effects were very robust; they seemed to cover many aspects of positive psychotic symptoms, and the percentage changes in positive PANSS reduction with haloperidol pre-treatment were similar to clinically relevant reductions. Clearly, this does not validate the THC challenge as a psychosis model. However, the results provide support for a role of cannabinoid systems in some aspects of schizophrenia, and could suggest applications of the THC challenge model in further pathophysiological research in this area.

Table 1 Overview of study day procedures

Time (hours)	Procedures study day
-1.5h00m – 0h00m	Arrival, breakfast, vital signs, ECG, safety lab, drug screen, alcohol breath test, AE assessment, pharmacodynamic measurements (twice)
0h00m	Haloperidol administration
1h00m	Blood sampling hormones, haloperidol
2h00m	Blood sampling hormones, haloperidol
2h55m	Blood sampling hormones, haloperidol, THC
3h00m	1st THC administration
3h00m - 4h10m	Pharmacodynamic blocka, blood sampling hormones & THC (3h05m, 3h20m), haloperidol (4h00m), vital signs, ECG, AE assessment (twice), lunch
3h35m – 3h50m	Positive And Negative Syndrome Scale (PANSS)
4h25m	Blood sampling hormones, THC
4h30m	2nd THC administration
4h30m – 5h35m	Pharmacodynamic blocka, blood sampling hormones & THC (4h35m, 4h50m), haloperidol (5h00m, 6h00m), vital signs, ECG, AE assessment (twice), snack
4h35m – 4h50m	WLT (Visual and Verbal Learning Task) word learning and immediate recall
5h05m – 5h20m	PANSS
5h20m – 5h30m	WLT delayed word recall & delayed word recognition
5h55m	Blood sampling hormones, THC
6h00m	3rd THC administration
6h00m – 7h10m	Pharmacodynamic blocka, blood sampling hormones & THC (6h05m, 6h20m), vital signs, ECG, AE assessment (twice), snack
6h35m – 6h50m	PANSS
7h25m	Blood sampling hormones & THC
8h00m	Blood sampling hormones, THC & haloperidol
8h45m – 9h20m	Pharmacodynamic block[A], AE assessment, dinner
12h00m – 13h00m	Blood sampling hormones & haloperidol, AE assessment, vital signs, bed
24h00m – 24h30m	Blood sampling hormones & haloperidol, AE assessment, ECG, breakfast, home

A. Pharmacodynamic block consists of Body sway, VAS (B&L, Bowdle), eye movements, EEG, VAS (B&L, Bowdle), Stroop test, VAS (B&L, Bowdle), Body Sway respectively (PANSS and VVLT is indicated separately)

Table 2: Least square means, estimates of differences and confidence intervals of PANSS (Positive and Negative Syndrome Scale) and VAS (Visual Analogue Scale) effects of THC, haloperidol and the combination

Parameter[D]	Least square means				Estimate of difference (95% CI[C]) P-value					
	PLA[A]	THC	HAL[B]	THC + HAL[B]	THC vs PLA[A]		HAL[B] vs PLA[A]		THC + HAL[B] vs THC	
Positive PANSS	7.4	9.8	8.4	8.7	**2.5 (1.2 / 3.7)**	**0.0002**	1.0 (-0.5 / 2.6)	0.18	**-1.1 (-2.1 / -0.1)**	**0.03**
Negative PANSS	9.2	10.2	11.4	11.2	1.0 (-0.9 / 3.0)	0.29	2.2 (-0.1 / 4.4)	0.06	0.9 (-0.7 / 2.6)	0.26
General PANSS	18.5	22.5	19.7	21.8	**4.0 (2.0 / 6.0)**	**0.0002**	1.3 (-1.1 / 3.7)	0.30	-0.7 (-2.3 / 0.9)	0.39
VAS external perception log (mm)	0.32	0.52	0.33	0.48	**0.20 (0.11 / 0.29)**	**<0.0001**	0.01 (-0.10 / 0.13)	0.84	-0.04 (-0.11 / 0.03)	0.29
VAS internal perception log (mm)	0.31	0.39	0.34	0.37	**0.08 (0.02 / 0.14)**	**0.01**	0.03 (-0.05 / 0.11)	0.46	-0.01 (-0.06 / 0.04)	0.60
VAS feeling high log (mm)	0.34	1.08	0.37	0.92	**0.74 (0.50 / 0.98)**	**<0.0001**	0.03 (-0.28 / 0.33)	0.87	-0.17 (-0.36 / 0.03)	0.09
VAS Alertness (mm)	52.0	44.9	49.5	44.7	**-7.1 (-11.4 / -2.7)**	**0.002**	-2.5 (-7.6 / 2.6)	0.34	-0.2 (-3.8 / 3.4)	0.89
VAS Calmness (mm)	52.8	55.1	51.0	54.6	2.3 (-0.9 / 5.5)	0.15	-1.8 (-5.5 / 1.9)	0.34	-0.5 (-3.2 / 2.1)	0.69
VAS Mood (mm)	54.6	54.5	51.5	52.7	-0.1 (-2.7 / 2.5)	0.94	**-3.1 (-6.2 / -0.1)**	**0.05**	-1.8 (-4.0 / 0.3)	0.09

A. PLA = placebo; B. HAL = haloperidol; C. 95% CI = 95% confidence interval; D. statistically significant differences (i.e. p<0.05) are indicated in bold

Table 3 Least square means, estimates of differences and confidence intervals of neurophysiological effects of THC, haloperidol and the combination

Parameter[D]	Least square means				Estimate of difference (95% CI[C]) p-value					
	PLA[A]	THC	HAL[B]	THC + HAL[B]	THC vs PLA[A]		HAL[B] vs PLA[A]		THC + HAL[B] vs THC	
Body sway (mm)	274	395	318	405	**44.1% (29.3 / 60.5%)**	**<0.0001**	**16.0% (1.5 / 32.5%)**	**0.03**	2.5% (-5.5 / 11.2%)	0.54
Saccadic peak velocity (deg/sec)	490.6	489.3	465.1	474.6	-1.3 (-15.8 / 13.3)	0.86	**-25.5 (-42.8 / -8.3)**	**0.004**	**-14.7 (-26.8 / -2.7)**	**0.02**
Smooth pursuit (%)	52.2	48.9	46.0	45.7	**-3.3 (-6.3 / -0.3)**	**0.03**	**-6.2 (-9.9 / -2.6)**	**0.001**	**-3.2 (-5.6 / -0.7)**	**0.01**
EEG alpha Fz-Cz (power)	2.77	3.49	3.03	3.37	**26.0% (4.4 / 52.2%)**	**0.02**	9.3% (-12.4 / 36.5%)	0.43	-3.5% (-17.5 / 12.8%)	0.65
EEG theta Fz-Cz (power)	2.03	2.19	2.38	2.30	7.8% (-5.2 / 22.5%)	0.25	**17.0% (1.1 / 35.4%)**	**0.04**	4.9% (-5.8 / 16.9%)	0.38

A. PLA = placebo; B. HAL = haloperidol; C. 95% CI = 95% confidence interval; D. statistically significant differences (i.e. p<0.05) are indicated in bold E. EEG parameters for which there are no significant (i.e. p<0.05) estimates of differences are not included in the table

Table 4 Least square means, estimates of differences and confidence intervals of cognition effects of THC, haloperidol and the combination

Parameter[D,E]	Least square means				Estimate of difference (95% CI[C]) P-values					
	PLA[A]	THC	HAL[B]	THC + HAL[B]	THC vs PLA[A]		HAL[B] vs PLA[A]		THC + HAL[B] vs THC	
Learning (immediate word recall #3 – #1)[E]	6.8	4.7	4.8	6.1	-2.1 (-4.3 / 0.0)	0.05	-2.0 (-4.6 / 0.6)	0.12	1.4 (-0.3 / 3.1)	0.10
Immediate word recall number correct (# 3)[E]	16.2	10.7	12.5	12.9	**-5.5 (-7.8 / -3.2)**	**<0.0001**	**-3.6 (-6.5 / -0.8)**	**0.01**	**2.2 (0.4 / 3.9)**	**0.02**
Decay (immediate word recall #3 – delayed recall)	3.0	2.0	3.6	3.7	-0.9 (-2.6 / 0.7)	0.26	0.6 (-1.3 / 2.5)	0.51	**1.7 (0.3 / 3.1)**	**0.02**
Delayed word recall number correct	12.9	9.2	9.1	9.3	**-3.7 (-6.1 / -1.3)**	**0.003**	**-3.8 (-6.8 / -0.9)**	**0.01**	0.1 (-1.9 / 2.0)	0.95
Retrieval (delayed word recognition – delayed recall)	9.3	10.2	11.6	11.0	0.9 (-4.2 / 5.9)	0.73	2.3 (-3.0 / 7.6)	0.38	0.8 (-3.6 / 5.2)	0.72
Delayed word recognition number correct	23.1	20.2	19.9	20.7	-2.9 (-7.7 / 1.9)	0.23	-3.2 (-8.3 / 1.9)	0.22	0.5 (-3.6 / 4.7)	0.80
Stroop # correct[G] (conflict situation)	19.5	19.2	19.5	19.1	**-0.3 (-0.7 / -0.0)**	**0.04**	-0.0 (-0.4 / 0.4)	0.92	-0.1 (-0.3 / 0.2)	0.58
Stroop RT[F] (conflict situation)	658	663	666	697	5 (-39 / 48)	0.82	8 (-44 / 59)	0.76	34 (-1 / 70)	0.06

A. PLA = placebo; B. HAL = haloperidol; C. 95% CI = 95% confidence interval; D. statistically significant differences (i.e. p<0.05) are indicated in bold; E. # = trial number; F RT = reaction time; G. stroop data for the basic situation are not statistically significant and are not shown

Table 5 Least square means, estimates of differences and confidence intervals of hormone effects of THC, haloperidol and the combination

Parameter[D]	Least square means				Estimate of difference (95% CI[C]) P-values					
	PLA[A]	THC	HAL[B]	THC + HAL[B]	THC vs PLA[A]		HAL[B] vs PLA[A]		THC + HAL[B] vs THC	
Prolactin (ng/mL)	8.71	7.26	20.11	19.53	**-16.6% (-28.3 / -3.0%)**	**0.02**	**130.9% (91.0 / 179.1%)**	**<0.0001**	**168.9% (138.6 / 203.1%)**	**<0.0001**
Cortisol (umol/L)	0.27	0.32	0.26	0.29	**18.4% (4.0 / 34.8%)**	**0.01**	-0.7% (-15.6 / 16.7%)	0.93	-8.0% (-16.9 / 1.8%)	0.11
FSH (U/L)[E]	3.0	2.9	3.1	2.9	-2.5% (-5.7 / 0.9%)	0.15	2.5% (-1.7 / 6.9%)	0.24	-0.6% (-3.3 / 2.1%)	0.64
LH (U/L)[F]	4.21	3.71	4.45	3.64	-12.0% (-23.4 / 1.1%)	0.07	5.7% (-11.4 / 26.3%)	0.53	-1.7% (-12.0 / 9.9%)	0.76

A. PLA = placebo; B. HAL = haloperidol; C. 95% CI = 95% confidence interval; D. statistically significant differences (i.e. p<0.05) are indicated in bold; E. FSH = Follicle-Stimulating Hormone; F. LH = Luteinizing Hormone

Figure 1 Graph of Least Square Means Positive PANSS change from baseline with 95% CI error bars for THC (up) and placebo (down) and drug administration (vertical lines) at T=0 (haloperidol), T=180, 270, 360 (THC)

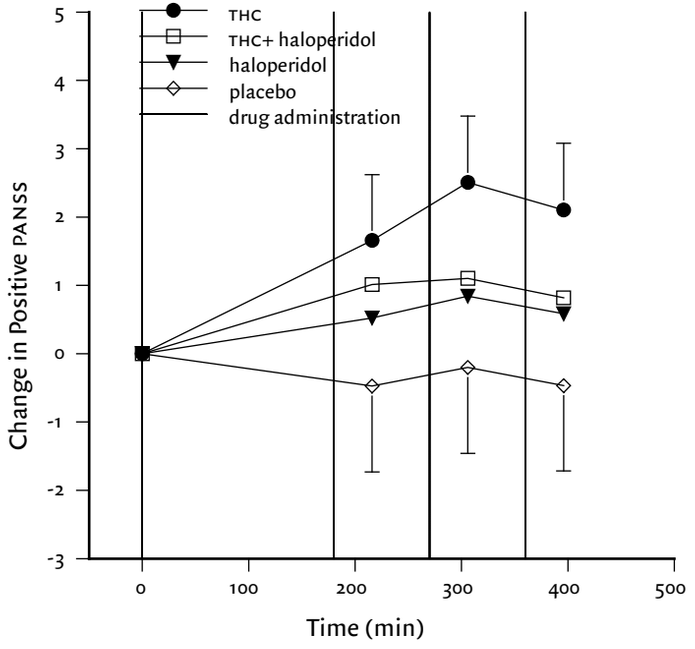


Figure 2 Graph of Least Square Means VAS feeling high (subscale of VAS Bowdle) change from baseline with 95% CI error bars THC (up) and placebo (down) and drug administration (vertical lines) at T=0 (haloperidol), T=180, 270, 360 (THC)

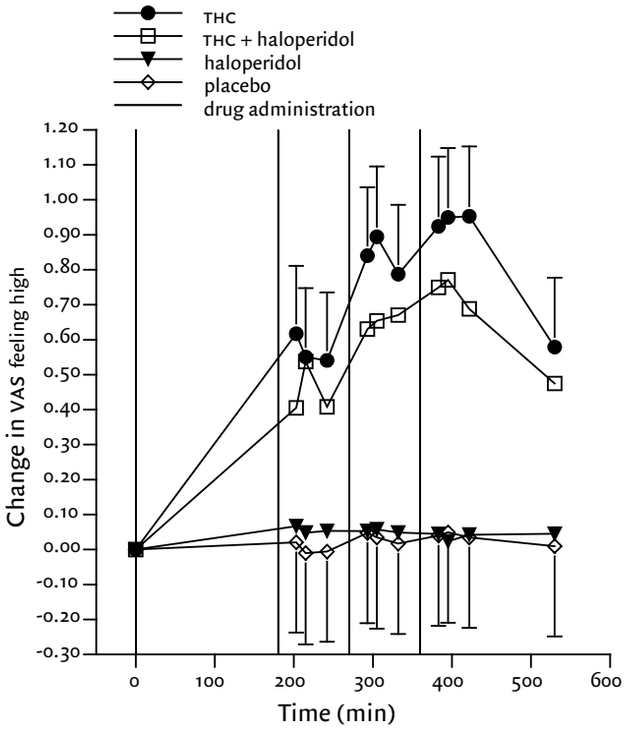


Figure 3 Average graph of predicted (solid black line) and observed concentration-time profiles of THC (open circles) and THC + haloperidol (closed circle) with SD error bars for THC (down) and THC + haloperidol (UP) and drug administration (vertical lines) at T=0 (haloperidol), T=180, 270, 360 (THC)

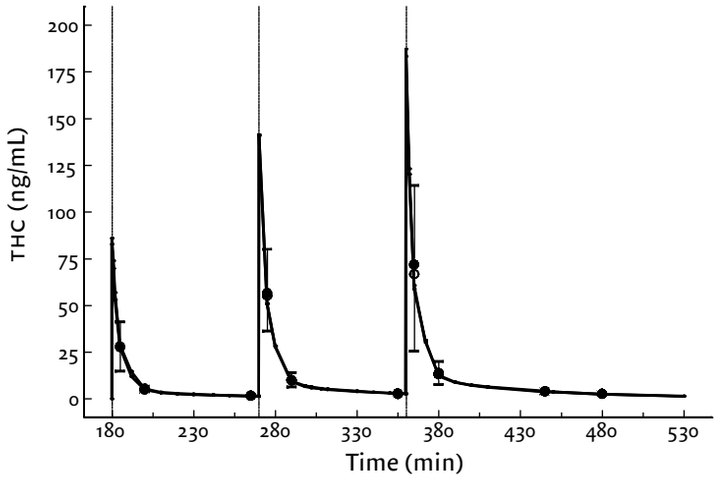
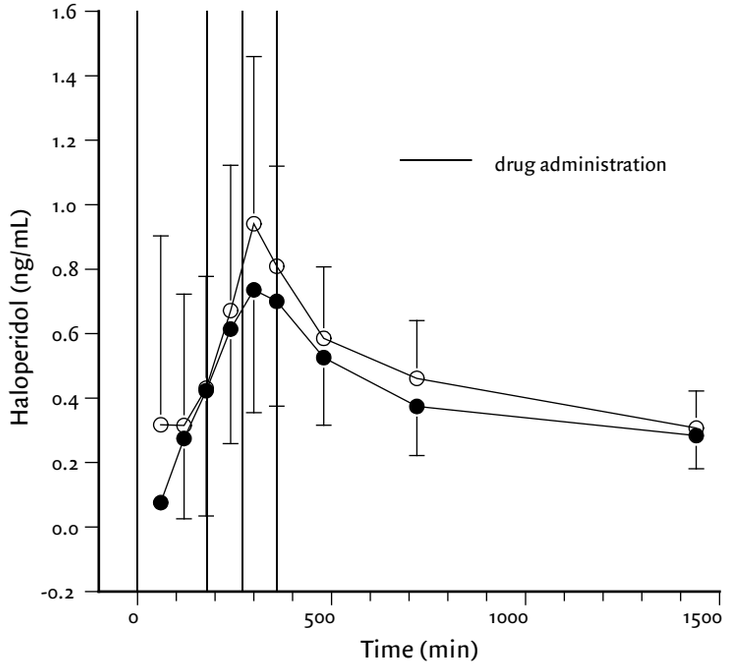


Figure 4 Average concentration-time profile of haloperidol (open circle) and haloperidol + THC (closed circle) with SD error bars for haloperidol + THC (down) and haloperidol (UP) and drug administration (vertical lines) at T=0 (haloperidol), T=180, 270, 360 (THC)



REFERENCES

Adler CM, Malhotra AK, Elman I, *et al* (1999) Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. Am J Psychiatry 156: 1646-1649.

Angrist B, Van Kammen DP (1984) CNS stimulants as tools in the study of schizophrenia. Trends Neurosci 7: 388-390.

Barnett G, Chiang CW, Perez-Reyes M, Owens SM (1982) Kinetic study of smoking marijuana. J Pharmacokinet Biopharm 10: 495-506.

Bieglmayer C, Chan DW, Sokoll L, *et al* (2004) Multicentre performance evaluation of the E170 module for modular analytics. Clin Chem Lab Med 42: 1186-1202.

Bohnen NI, Kaufer DI, Hendrickson R, *et al* (2005) Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. J Neurol Neurosurg Psychiatry 76: 315-319.

Boileau I, Assaad JM, Pihl RO, *et al* (2003) Alcohol promotes dopa-mine release in the human nucleus accumbens. Synapse 49: 226-231.

Bond A, Lader M (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol 47: 211-218.

Bossong MG, van Berckel BN, Boellaard R, *et al* (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology: 34: 759-766.

Bowdle TA, Radant AD, Cowley DS, Kharasch D, Strassman RJ, Roy-Byrne PP (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 88: 82-88.

Brody AL, Mandelkern MA, Olmstead RE, *et al* (2009) Ventral striatal dopamine release in response to smoking a regular vs a denicotinized cigarette. Neuropsychopharmacology 34: 282-289.

Brody AL, Olmstead RE, London ED, *et al* (2004) Smoking-induced ventral striatum dopamine release. Am J Psychiatry 161: 1211-1218.

Chakraborty BS, Hubbard JW, Hawes EM, *et al* (1989) Interconversion between haloperidol and reduced haloperidol in healthy volunteers. Eur J Clin Pharmacol 37: 45-48.

Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA (1985) Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 20: 619-629.

Cropley VL, Innis RB, Nathan PJ, *et al* (2008) Small effect of dopa-mine release and no effect of dopamine depletion on [18F]fallypride binding in healthy humans. Synapse 62: 399-408.

D'Souza DC, Abi-Saab WM, Madonick S, *et al* (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry 57: 594-608.

D'Souza DC, Braley G, Blaise R, *et al* (2008) Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of delta-9-tetrahydrocannabinol in humans. Psychopharmacology (Berl) 198: 587-603.

D'Souza DC, Perry E, MacDougall L, *et al* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 29: 1558-1572.

De Haas SL, De Visser SJ, Van der Post JP, *et al* (2008) Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_{(A)}$ $alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 22: 24-32.

De Visser SJ, Van der Post JP, De Waal PP, Cornet F, Cohen AF, Van Gerven JM (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55: 39-50.

De Visser SJ, Van der Post J, Pieters MS, Cohen AF, Van Gerven JM (2001) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51: 119-132.

Drevets WC, Gautier C, Price JC, *et al* (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry 49: 81-96.

Gardner EL (2005) Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav 81: 263-284.

Gardner EL, Lowinson JH (1991) Marijuana's interaction with brain reward systems: update 1991. Pharmacol Biochem Behav 40: 571-580.

Gijsman HJ, Van Gerven JM, Verkes RJ, *et al* (2002) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 17: 83-89.

Griffiths AN, Jones DM, Richens A (1986) Zopiclone produces effects on human performance similar to flurazepam, lormetazepam and triazolam. Br J Clin Pharmacol 21: 647-653.

Grotenhermen F (2003) Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42: 327-360.

Hagelberg N, Kajander JK, Nagren K, Hinkka S, Hietala J, Scheinin H (2002) Mu-receptor agonism with alfentanil increases striatal dopamine D_2 receptor binding in man. Synapse 45: 25-30.
Henquet C, Krabbendam L, Spauwen J, *et al* (2005) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. Br Med J 330: 11.
Henquet C, Rosa A, Krabbendam L, *et al* (2006) An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology 31: 2748-2757.
Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004) Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 47(Suppl 1): 345-358.
Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29: 565-598.
Kay SR, Opler LA (1987) The positive-negative dimension in schizophrenia: its validity and significance. Psychiatr Dev 5: 79-103.
Kemme MJ, Van der Post JP, Schoemaker RC, Straub M, Cohen AF, Van Gerven JM (2003) Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 55: 518-525.
Kessler RM, Ansari MS, Riccardi P, *et al* (2005) Occupancy of striatal and extrastriatal dopamine D_2/D3 receptors by olanzapine and haloperidol. Neuropsychopharmacology 30: 2283-2289.
King DJ (1997) Guidelines for the use of antipsychotic drug studies in healthy volunteers. The BAP Consensus Group. J Psychopharmacol 11: 201-209.
Kulisevsky J (2000) Role of dopamine in learning and memory: implications for the treatment of cognitive dysfunction in patients with Parkinson's disease. Drugs Aging 16: 365-379.
Laeng B, Lag T, Brennen T (2005) Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. J Exp Psychol Hum Percept Perform 31: 438-452.
Laviolette SR, Grace AA (2006) Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. J Neurosci 26: 6458-6468.
Liem-Moolenaar M, Gray F, de V S, *et al* (2008) Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol (Epub ahead of print).
Lupica CR, Riegel AC, Hoffman AF (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol 143: 227-234.
Martinez D, Narendran R, Foltin RW, *et al* (2007) Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. Am J Psychiatry 164: 622-629.
Martinez D, Slifstein M, Broft A, *et al* (2003) Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab 23: 285-300.
Mehta MA, Riedel WJ (2006) Dopaminergic enhancement of cognitive function. Curr Pharm Des 12: 2487-2500.
Narendran R, Frankle WG, Mason NS, *et al* (2009) Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: a comparative evaluation of the high affinity dopamine $D_{2/3}$ radiotracers [11C]FLB 457 and [11C]fallypride. Synapse 63: 447-461.
Norris H (1971) The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10: 181-191.
Perez-Reyes M, Timmons MC, Lipton MA, Davis KH, Wall ME (1972) Intravenous injection in man of 9-tetrahydrocannabinol and 11-OH-9 -tetrahydrocannabinol. Science 177: 633-635.
Phillips P, Johnson S (2001) How does drug and alcohol misuse develop among people with psychotic illness? A literature review. Soc Psychiatry Psychiatr Epidemiol 36: 269-276.
Ranganathan M, D'Souza DC (2006) The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl) 188: 425-444.
Riccardi P, Li R, Ansari MS, *et al* (2006a) Amphetamine-induced displacement of [18F] fallypride in striatum and extrastriatal regions in humans. Neuropsychopharmacology 31: 1016-1026.
Riccardi P, Zald D, Li R, *et al* (2006b) Sex differences in amphetamine-induced displacement of [(18)F] fallypride in striatal and extrastriatal regions: a PET study. Am J Psychiatry 163: 1639-1641.
Rodriguez DF, Fernandez-Ruiz JJ, Murphy LL, *et al* (1992) Acute effects of delta-9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. Pharmacol Biochem Behav 42: 269-275.
Rose AK, Duka T (2007) The influence of alcohol on basic motoric and cognitive disinhibition. Alcohol Alcohol 42: 544-551.
Schlaepfer TE, Pearlson GD, Wong DF, Marenco S, Dannals RF (1997) PET study of competition between intravenous cocaine and [11C] raclopride at dopamine receptors in human subjects. Am J Psychiatry 154: 1209-1213.
Seeman P, Lee T (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 188: 1217-1219.
Stokes PR, Mehta MA, Curran HV, Breen G, Grasby PM (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage 48: 186-190.
Strougo A, Zuurman L, Roy C, *et al* (2008) Modelling of the concentration-effect relationship of THC on central nervous system parameters and heart rate - insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol 22: 717-726.
Tanda G, Goldberg SR (2003) Cannabinoids: reward, dependence, and underlying neurochemical mechanisms - a review of recent preclinical data. Psychopharmacology (Berl) 169: 115-134.
Van der Post J, De Waal PP, De Kam ML, Cohen AF, Van Gerven JM (2004a) No evidence of the usefulness of eye blinking as a marker for central dopaminergic activity. J Psychopharmacol 18: 109-114.
Van der Post JP, De Visser SJ, Schoemaker RC, Cohen AF, Van Gerven JM (2004b) Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 18: 221-227.
Van Steveninck AL, Van Berckel BN, Schoemaker RC, Breimer DD, Van Gerven JM, Cohen AF (1999) The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 13: 10-17.
Wise RA (1988) The neurobiology of craving: implications for the understanding and treatment of addiction. J Abnorm Psychol 97: 118-132.
Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5: 483-494.
Yoder KK, Constantinescu CC, Kareken DA, *et al* (2007) Heterogeneous effects of alcohol on dopamine release in the striatum: a PET study. Alcohol Clin Exp Res 31: 965-973.
Zuurman L, Roy C, Schoemaker R, *et al* (2010) Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB_1 receptor antagonist AVE1625. J Psychopharmacol 24: 374-382.
Zuurman L, Ippel AE, Moin E, Van Gerven JM (2009a) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 67: 5-21.
Zuurman L, Passier PC, de K M, Kleijn HJ, Cohen AF, van Gerven JM (2009b) Pharmacodynamic and pharmacokinetic effects of the intravenously administered CB_1 receptor agonist Org 28611 in healthy male volunteers. J Psychopharmacol 23: 633-644.
Zuurman L, Roy C, Schoemaker RC, *et al* (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 22: 707-716.

CHAPTER 9

DISCUSSION

In the introduction (**chapter 1**) of this thesis we discussed the pharmacology of the currently existing treatments for schizophrenia, the lack of concrete treatment options with new pharmacological mechanisms of action, and the relatively unsuccessful drug development for schizophrenia compared to other disease areas in the last 50 years. At this moment, however, there are several new compounds in development involving new pharmacological mechanisms of action. Most if not all of these drugs are aimed at treating one or more symptoms of schizophrenia, more than its underlying processes. Despite their limited therapeutic scope, these drugs could still be used to improve the patient's quality of life, particularly in individually targeted polytherapeutic strategies, if the symptomatic benefits outweigh the adverse effects. It is important to determine the potential added value of a new schizophrenia therapy and its therapeutic position as well as to identify the potential (dis)advantages of new compounds as early as possible during development. There are several ways to predict the clinical effects of a drug, since these largely depend on the compound's pharmacological properties. In some cases, it is possible to examine the functional effects of a new drug, and to obtain an impression of the potentially beneficial and less desirable effects of a drug in patients. In this thesis we have also described different ways to characterize the human pharmacology of drugs in development for the various symptomatic domains of schizophrenia. To this end, we have used a range of different functional and pharmacological tests that were combined in a CNS test battery, the NeuroCart.

The central nervous system (CNS) can be divided into different functional domains, for example in neurophysiological, neuropsychological and neuroendocrine functions. Drugs acting on the CNS will usually influence more than one of these functions, which offers the possibility to frequently measure drug-induced changes across a range of different CNS-activities. For the studies in this thesis, the NeuroCart test battery was used in healthy volunteers, with the aim of covering a broad range of CNS-functional domains that could be responsive to drug effects. Healthy volunteers function optimally and are therefore quite sensitive to drug effects that interfere with normal CNS activity. However, in most cases, it is impossible to measure therapeutic effects of a new schizophrenia drug in healthy volunteers, because they do not suffer from the disease. The main aim of early human studies with such drugs is to characterize the (new) compound, using a range of different tests. Integration of this information can help to provide support for the compound's distinctive pharmacological properties, to predict interactions or adverse events in an early stage of development, and to identify dosing regimens with an optimal balance between potentially beneficial and undesirable pharmacological effects. Many of the tests that are described in this thesis provide direct or indirect indications for drug-related adverse effects. The broad composition of the NeuroCart test battery provides some protection against unnoticed adverse drug effects. This thesis has used a large number of 'drug biomarkers', and developed and validated several others, which provide different types of information about the effects of a new drug. These so called drug biomarkers can be divided in functional biomarkers, pharmacological biomarkers and other biomarkers.

For each functional domain of the central nervous system, several different tests or measurement instruments were employed in this thesis (see table 1).

Psychotic-like effects were determined with the Positive and Negative Syndrome Scale (PANSS) and the Bowdle visual analogue scales for psychomimetic effects. Cognitive impairment was evaluated using memory tests (visual and verbal immediate & delayed recall and recognition), the Tower of London (TOL), the Stroop Test, the Simple Reaction Time Test, the Left/Right Distraction Task, the Digit Vigilance Choice Reaction Test, the Spatial Working Memory Task and the Numeric Working Memory Task. Depression and anxiety were assessed with the Visual Analogue Scales assessing mood and calmness. Extrapyramidal adverse effects were indirectly measured with tests of motor function (Body Sway) or eye-hand-coordination (Adaptive Tracking, Reaction Time Tests). Metabolic and autonomic adverse effects were determined by measurement of several hormones (cortisol, LH, FSH, testosterone and prolactin). In most studies, several of these tests were combined to generate functional effect profiles that provide important information about the new drug's expected side

effects -or anticipated lack thereof. This information is quite useful during early drug development, but it is restricted to functional outcomes and may miss more subtle CNS-effects that can be used to answer several specific questions about the drug's pharmacological characteristics in more detail. To this end, pharmacological biomarkers can be used.

Some tests are validated reasonably well for a certain specific pharmacological process. Prolactin release for instance is closely regulated by D_2-receptor activity and hence strongly induced by drugs that directly or indirectly affect dopaminergic activity, such as neuroleptics. Cortisol release is under serotonergic 5-HT_2-control, and stimulated by many serotonergic compounds. Subjective 'high feelings' are strongly increased after activation of the cannabinoid system. Anticholinergic activity causes pupil dilation, which is a nonspecific effect of many psychopharmacological compounds. In this way, specific physiological or even subjective effects can be interpreted as biomarkers for a particular pharmacological activity of a new CNS-active drug. Most tests however are neither validated as surrogate parameters for clinical endpoints, nor as primarily receptor-mediated pharmacological biomarkers. This holds true for the majority of tests used in early drug development. In principle, tests that do not allow a clear functional or pharmacological interpretation are less useful in drug development, but such tests cannot always be avoided in exploratory studies of compounds with novel mechanisms of action. In such cases, the interpretation of the test results can be strongly facilitated by the design or the analysis of the study. This thesis explored several ways of providing indications for a drug's pharmacological characteristics, using biomarkers that were not unequivocally validated for proof-of-pharmacological activity. In chapters 2 and 3, the effect profiles of a novel compound were compared with a carefully selected *positive control*. Chapter 4 used the demonstration of *dose- effect relationships* to provide support for the pharmacological activity of a new drug. The last four chapters of this thesis employed a *pharmacological challenge test* to study new drugs, which by themselves were expected to have limited direct effects in healthy volunteers. A challenge test consists of stimulation of a central neurotransmitter system by a single dose of a relevant drug, which leads to known quantitative effects in healthy volunteers or patients [5]. Although a challenge test in healthy volunteers is not an attempt to imitate a disease, it can often lead to a better understanding of both the pharmacological and the functional aspects of a treatment. A scopolamine challenge test was used in chapters 5 and 6 as a cognitive impairment model and a model of cholinergic activity. The scopolamine model was explored further in chapter 7, where *pharmacokinetic-pharmacodynamic* (PK-PD) *modelling* was employed as a sophisticated technique to examine the drug's pharmacological characteristics in greater detail. Chapter 8 used delta-9-tetrahydrocannabinol (THC) to produce functional signs and symptoms in healthy volunteers that are reminiscent of psychosis. The model was used to examine whether it is possible to demonstrate the acute antipsychotic effects of haloperidol (and hence possibly also of other antipsychotics). At the same time, the clear demonstration of psychotic-like phenomena in healthy volunteers during the THC challenge supported the involvement of endocannabinoid systems in psychosis.

Most of these tests would not be immediately considered as validated pharmacological biomarkers, because they are not unequivocally linked to the pathophysiology of schizophrenia. Nonetheless, many of the tests provided indirect but important support for the pharmacological activity of novel compounds that are in development for aspects of schizophrenia and related conditions or other disorders.

In **chapter 2**, a basic pharmacology interaction effects study was performed between the 5-HT_6 receptor antagonist SB742457 and risperidone. Several lines of evidence suggest a possible role of 5-HT_6 receptor antagonists in cognitive dysfunction of schizophrenia. Risperidone, commonly used to control agitation and psychotic features in schizophrenia, is a D_2/5-HT_{2A} antagonist with low affinity for 5-HT_6 receptors. As the combination of risperidone and SB742457 may constitute a reasonable combination in cognitively impaired patients, pharmacologic interaction effects were investigated in this study in healthy volunteers. The chance of showing improvement in cognitive dysfunction is low in healthy volunteers. However risperidone causes

significant CNS-depression in healthy subjects, and it is also a significant side effect in psychiatric patients, albeit less than with many older neuroleptics. This notion was used to investigate the effects of SB742457 alone and in combination with risperidone.

As expected, risperidone caused mild depression of many CNS tests, and we did not detect clear effects of SB742457 by itself in this group of optimally functioning healthy young males. However, SB742457 did cause a small increase of EEG alpha and beta bands in combination with risperidone. This suggests a mild arousing activity of SB742457 on the CNS-depressant effects of risperidone, and provides indications for a pharmacological effect of this compound in the CNS. It cannot be certified that this isolated finding represents a true pharmacological reversal of risperidone-induced CNS-depression. Also there is no indication on the potential clinical relevance for patients.

In **chapter 3**, the dopamine receptor antagonist haloperidol was used as a positive control for the new neurokinin 3 (NK_3) antagonist talnetant. By adding a positive control the effects of a new compound can be compared to the effects of a currently used treatment. While haloperidol effects were predominantly CNS-depressant (affecting almost all pharmacodynamic measures in the study), those of talnetant seemed slightly stimulatory (decreased EEG alpha power, improved visuo-motor control, and reduced calmness on visual analogue scales). As an NK_3 antagonist is thought to decrease dopamine by indirect modulation of dopamine release rather than by dopamine receptor inhibition, a lower effect on prolactin was expected than following haloperidol. There appeared to be no prolactin release after the NK_3 antagonist compared to the anticipated high release after haloperidol. The results suggest that talnetant penetrates the blood-brain-barrier, but it remains to be established whether this dose and the effect is sufficient in patients, and whether the observed effect profile is class-specific for NK_3 antagonists.

In **chapter 4** three studies of the development of the new glycine reuptake inhibitor SCH 900435, given to healthy males were described. In the first study, a single ascending dose study, this compound was unexpectedly found to induce dose-related visual disturbance (increases in pupil/iris ratio, changes in VAS colour perception and spontaneous reports of short-lasting visual disturbance). Dose-related effects were also seen in the neuroendocrine system (decreases in FSH and LH). As dose escalation was based on frequent interim analyses, this resulted in a far smaller dose range (0.5-30mg) than predicted before the study (0.5-135mg). Instead of discontinuing drug development for this compound or expose subjects or patients unnecessarily to the visual effects, a side step was taken by further examining the compound in dedicated visual effects studies in animals and humans. These studies demonstrated that the visual effects were transient without underlying electrophysiological changes. This provided enough safety information for starting a multiple ascending dose study, which showed rapid development of tolerance for visual effects without detrimental long-term consequences.

The development strategy that was chosen allowed for careful stepwise assessment of the pharmacological and clinical characteristics of the drug. The visual adverse effects were not foreseen, but preclinical and biological data about a new drug can be used to predict the anticipated effects of this new drug, even if it has a new mechanism of action. As extensively discussed in the introduction of this thesis, many such new compounds are currently in development. Frequent measurement of multiple pharmacodynamics can also provide indications for CNS-penetration and dose-related pharmacological activity, especially since the early studies in healthy volunteers cover a large dose range. This is clearly demonstrated by the consistency of almost all effects that were observed during the first study, which may have seemed spurious at first. Many of the biomarkers showed dose-related effects. The changes in visual VAS scales, pupil dilation, and reported visual symptoms are most compatible with a direct retinal effect, although central causes cannot be excluded. The neuroendocrine and CNS measurements following doses of 8 mg and higher provide indications for CNS penetration and pharmacological activity of SCH 900435. The antipsychotic activity still needs to be investigated in a patient study. It also remains to be established whether the observed effects are pharmacological effects of this drug class, or specific for this compound.

In **chapter 5 and 6** the effects of two other glycine reuptake inhibitors R213129 and R231857 on CNS function were investigated in healthy males. In these cases, the drugs were not administered to humans for the first time, and the dose range was limited by systemic adverse effects that had been observed earlier. This refuted the use of dose response relationships to demonstrate pharmacological activity, and required an alternative approach. Based on preclinical experiments, it was decided to use a pharmacological challenge test to show that the glycine reuptake inhibitors penetrated into the brain, and indirectly affected the cholinergic system. Scopolamine produces robust and consistent effects in psychomotor and cognitive function in healthy volunteers and is used both as a cholinergic pharmacological model, and as a cognitive dysfunction model because of the importance of acetylcholine in memory function. R231857 alone showed some pharmacodynamic changes compared with placebo, whereas R213129 did not. Both R213129 and R231857 had some small effects on scopolamine-induced central nervous system impairments, which were not clearly related to the limited dose range. There were no visual effects, like had been observed with high doses of SCH 900435. Although these effects might be an indication that these glycine reuptake inhibitors penetrated the CNS to some extent, it seemed likely that the CNS concentrations were too low to cause reproducible CNS-effects or to affect the scopolamine challenge in healthy volunteers. It is also possible that the tests were not sensitive enough to detect the pharmacological actions of the two compounds. It cannot be excluded that other techniques (like PET-imaging or cerebrospinal fluid sampling) or higher doses in healthy volunteers would have been able to demonstrate effects. At any rate, clinical trials are needed to provide feedback on the predictive value of the pharmacological biomarkers that we used in our studies, for therapeutic effects in patients.

In **chapter 7** a pharmacokinetic-pharmacodynamic (PK-PD) analysis of scopolamine was performed to improve the scopolamine challenge test. The cholinergic system is important for different central nervous system functions, including memory, learning and attention. Scopolamine is a centrally active muscarinic antagonist, which is frequently used as a memory impairment model. Nevertheless, the relationships between exposure and corresponding central nervous system (CNS) effects are mostly unknown. We determined the PK-PD relationships of scopolamine using a multidimensional CNS test battery in a large group of healthy volunteers. The results suggested there are various functional cholinergic systems with different pharmacological characteristics, which can be used to study the effects of drugs that directly or indirectly modify cholinergic systems. The design of such studies should take the different concentration-effect relationships into account. Other reasons to perform PK-PD modelling are the use of the model for individual dose optimization in studies when large inter-individual differences are anticipated, or to give insight into the physiology and pharmacology of human cholinergic systems and to find proof-of-pharmacological-activity.

In **chapter 8** a human cannabinoid model was used in healthy volunteers, by administration of tetrahydrocannabinol (THC), the most active ingredient of cannabis. Animal models for schizophrenia or psychosis typically do not capture the complex and unravelled pathophysiology of this disease. In general, preclinical models are reasonably predictive of several characteristics of schizophrenia, such as disorganized behaviour, psychomotor agitation and social withdrawal, but do not adequately represent typical positive features like delusions, hallucinations and disorganized speech. Therefore, human models for (the symptoms) of the disease could be useful.

The hypothesis that haloperidol would lead to an amelioration of THC-induced 'psychotomimetic' effects was investigated in healthy volunteers. THC-induced increases in positive Positive and Negative Syndrome Scale (PANSS) but not in Visual Analogue Scales feeling high were reversed by haloperidol. This indicates that psychotic-like effects induced by THC are mediated by dopaminergic systems, but that other systems are primarily involved in 'feeling high'. The clear reductions of psychotic-like symptoms by a clinically relevant dose of haloperidol suggest that THC administration seems a useful pharmacological cannabinoid model for psychotic effects in healthy volunteers. This could be of value for the assessment of therapeutic effects of antipsychotic agents.

Short summary and conclusions

All studies in this thesis make use of pharmacological monitoring in an early stage of drug development in healthy volunteers. An intensive CNS test battery consisting of multiple tests was used to measure effects in different functional domains of the brain. In this way we have tried to create a pharmacological fingerprint of the investigated compounds. Besides this test battery, we have made use of additional methods and 'tools' (such as positive controls, dose escalation, PK-PD modelling and challenge tests) to improve the reliability of tests, which each on its own has a limited functional of pharmacological validation.

We have studied the effects of the currently used antipsychotics risperidone (chapter 2) and haloperidol (chapter 3 and 8) and of compounds in development for schizophrenia: the 5-HT_6 antagonist SB742457 (chapter 2), the NK_3 antagonist talnetant (chapter 3) and the three glycine reuptake inhibitors SCH 900435, R213129 and R231857 (chapters 4-6). We have used two functional models by administration of scopolamine (chapter 5-7) and THC (chapter 8). These compounds give a good impression of the trends in drug development for schizophrenia. They affect a range of neurotransmitters (or systems) such as dopamine, serotonin, glutamate, acetylcholine and the cannabinoid system, and are often targeted at negative rather than (or in addition to) positive symptoms of schizophrenia.

Table 2 gives a summary of the main neurotransmitters that are currently thought to be involved in the pathophysiology of schizophrenia. Additionally, the table shows several functional relationships between neurotransmitters and symptoms, and where these are addressed in this thesis.

We investigated both pure agonists and antagonists, but also drugs that rather modulate or indirectly influence neurotransmitters. Newer compounds are increasingly being developed to treat different symptoms of schizophrenia (other than psychotic symptoms), rather than for the cause of the disease.

The framework of this thesis is drugs in development for schizophrenia. However, this disorder is characterized by a range of different functional abnormalities that also occur in other neuropsychiatric diseases, not being one disease but a spectrum of symptoms that partly overlap. Consequently, the pharmacological results and especially the way of investigating the human pharmacology and the models can be applied to other related CNS disorders, such as for Alzheimer's disease (5-HT6 antagonist) or other related psychiatric disorders (schizoaffective disorder, psychotic disorder). In fact many of the novel compounds described in this thesis are also considered for other indications than schizophrenia. This tendency is quite prevalent in drug development, where compounds are designed to have specific pharmacological characteristics, rather than to correct a certain pathophysiological deficit (which in case of neuropsychiatric disorders is often unknown).

There are many reasons why frequent and multiple human pharmacology measurements in an early stage of drug development can be helpful. One of the reasons is the possibility to create a pharmacological fingerprint of a new compound in a much earlier stage. This makes it possible to earlier predict effects and to select doses (or compounds) that avoid side-effects in study subjects and subsequently in patients. Consequently, vulnerable patients are not unnecessarily burdened and development costs can be reduced. Careful stepwise flexible decisions can be taken based on actual findings. In addition, a human pharmacological model as a practical 'psychosis' model could be an alternative to assess therapeutic effects of new antipsychotic agents as there are no suitable animal models for psychosis.

Summarizing, the studies in this thesis together show different ways of studying human pharmacology, give an impression of the current drug development in schizophrenia, and provide examples how human pharmacology can be applied in an early stage of drug development.

Table 1: Organization of all tests used in this thesis, into their corresponding clusters, domains (in grey) according to Zoethout *et al* [1] and reference to the chapter(s) of this thesis

Language		
Cluster	Test	Chapter(s)
Semantics	Verbal Learning Test*	2, 3, 5-8
Attention		
Cluster	Test	Chapter(s)
Divided attention	Left/Right Distraction Task Digit Vigilance Choice Reaction*	3 4
Reaction time	Stroop Test* Simple Reaction Time Task Digit Vigilance Choice Reaction*	5-8 4 4
'DSST-like'	Digit Vigilance Choice Reaction*	4
Time/distance estimation	Digit Vigilance Choice Reaction*	4
Working memory	Digit Vigilance Choice Reaction*	4
Executive		
Cluster	Test	Chapter(s)
Inhibition	Stroop Test*	5-8
Planning	Tower of London	3
Working memory	Spatial Working Memory Task Numeric Working Memory Task	4 4
Memory		
Cluster	Test	Chapter(s)
Auditory/verbal memory: immediate recall	Immediate Word Recall (VVLT)*	2-8
Visual/spatial memory: immediate recall	Immediate Word Recall (VVLT)*	2-8
Learning	Verbal Memory Task (VVLT)*	2-8
Auditory/verbal memory: delayed recognition	Delayed Word Recognition (VVLT)*	2-8
Visual/spatial memory: delayed recognition	Delayed Word Recognition (VVLT)*	2-8
Visual/spatial memory: delayed recall	Delayed Word Recall (VVLT)*	2-8
Auditory/verbal memory: delayed recall	Delayed Word Recall (VVLT)*	2-8
Motor		
Cluster	Test	Chapter(s)
Motor control	Finger Tapping Task	2,3, 5-7
Postural stability	Body Sway	2-8
Visuo-motor control	Adaptive Tracking Test	2-7
Neurophysiologic		
Cluster	Test	Chapter(s)
EEG – frequency spectrum	Pharmaco-EEG	2-8
Eye movements - pursuit	Smooth Pursuit Eye Movements Task	2-8
Eye movements - saccadic	Saccadic Eye Movements Task	2-8
Pupil-iris ratio	Pupillometry	4-7
Perception		
Cluster	Test	Chapter(s)
Scale perception	VAS colour perception (Bowdle)	4
Subjective experience		
Cluster	Test	Chapter(s)
Scale alertness	Visual Analogue Scale (VAS) alertness[2] Leeds Sleep Evaluation Questionnaire (LSEQ)[3]	2-8 4
Scale mood	Visual Analogue Scale (VAS) mood[2]	2-8
Scale calmness	Visual Analogue Scale (VAS) calmness[2]	2-8
Scale psychomimetic	Visual Analogue Scale (VAS) psychomimetic[4] – high – external perception – internal perception Positive and Negative Syndrome Scale (PANSS)	2-8 8
(Neuro)endocrine		
Cluster	Test	Chapter(s)
Cortisol	Cortisol determination	3, 8
FSH	FSH determination	4-8
LH	LH determination	4, 5, 6, 7, 8
Prolactin	Prolactin determination	2, 3, 4, 5, 6, 7, 8
Testosterone	Testosterone determination	4

Whenever tests provided different parameters with information on more than one functional domain, de tests were marked (*); VVTL = Visual and Verbal Learning Task (consists of immediate & delayed word recall and delayed word recognition)

Table 2: Summary of neurotransmitters which are thought to be involved in the pathophysiology of schizophrenia with expected symptoms after activation (↑) or inhibition (↓) of the neurotransmitter and chapter number (in superscript) in which this is addressed

Neurotransmitter	Subtype	Positive symptoms	Negative symptoms	Cognitive impairment	Extrapyramidal adverse effects	Metabolic adverse effects (e.g. hyperprolactinaemia)
Dopamine	D2	↓8		↑2,3	↑	↑2,3,8
Serotonin	5-HT_6		↑/↓	↓2		
Glutamate	NMDA/AMPA	↑	↑	↑4–6		
Endocannabinoids	CB_1	↓8				↓8
	CB_2	↑				
Acetylcholine	N			↑5–7		
GABA (gamma-aminobutyric acid)	A	↑/↓		↑		

REFERENCES

1 Zoethout RW, Delgado WL, Ippel AE, Dahan A, van Gerven JM. Functional biomarkers for the acute effects of alcohol on the central nervous system in healthy volunteers. Br J Clin Pharmacol 2011; 71: 331-50.

2 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-8.

3 Zisapel N, Laudon M. Subjective assessment of the effects of CNS-active drugs on sleep by the Leeds sleep evaluation questionnaire: a review. Hum Psychopharmacol 2003; 18: 1-20.

4 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998; 88: 82-8.

5 Gijsman HJ. Pharmacological aspects of challenge tests of the serotonergic system of the brain. 2002.

NEDERLANDSE SAMENVATTING

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SUMMARY IN DUTCH

In dit proefschrift wordt de humane farmacologie (de wisselwerking tussen een geneesmiddel en de mens) van bestaande en nieuwe geneesmiddelen voor schizofrenie besproken. Schizofrenie is een van de meest slopende psychiatrische aandoeningen en komt bij 1-1,5% van de wereldbevolking voor. Het ontstaat meestal bij jong volwassenen en blijft gedurende het hele leven aanwezig. Bij de diagnose van schizofrenie wordt gekeken naar bepaalde karakteristieke symptomen, die kunnen worden onderverdeeld in zogenaamde positieve symptomen (hallucinaties, wanen, incoherente gedachten), negatieve symptomen (apathie, gebrek aan emotie, matig sociaal functioneren) en cognitief disfunctioneren (geheugen- en concentratieproblemen, ongeorganiseerd gedrag). Er is nog weinig bekend over hoe schizofrenie ontstaat en welke 'afwijkingen' in de hersenen precies bijdragen aan het ontstaan van deze aandoening. Dit heeft te maken met de ondoorgrondelijkheid van de hersenen en de variabiliteit in de klinische verschijningsvormen van schizofrenie. Er zijn al zeer veel hypothesen geopperd voor de oorsprong van schizofrenie. Ditzelfde geldt voor de geneesmiddelen die deze aandoening zouden moeten tegengaan. Deze onbekendheid met de aandoening heeft zijn weerslag gehad en heeft nog steeds effect op de ontwikkeling van nieuwe geneesmiddelen voor schizofrenie.

HOOFDSTUK 1: INTRODUCTION

In de introductie wordt de farmacologie van de huidige behandelingen en de relatief trage geneesmiddelenontwikkeling voor schizofrenie besproken. Ook het gebrek aan behandelingsmogelijkheden komt aan het licht. Op dit moment is er een aantal middelen in ontwikkeling die nieuwe farmacologische werkingsmechanismen hebben. Het is opvallend dat een groot deel van de nieuwe middelen zich meer richt op bepaalde symptomen van schizofrenie dan op de onderliggende oorzaak van de aandoening. Deze middelen kunnen worden gebruikt om de kwaliteit van leven van een pati'nt te verbeteren, maar dan moeten de voordelen wel opwegen tegen de bijwerkingen. Om dit te bepalen is het daarom belangrijk dat

effecten en mogelijke bijwerkingen in een zo vroeg mogelijk stadium van de ontwikkeling van een geneesmiddel worden herkend.

In dit proefschrift beschrijven we een verzameling van onderzoeken, waarin in een vroege fase van onderzoek bij gezonde vrijwilligers wordt gekeken naar effecten van nieuwe middelen. Aangezien de klinische effecten van een geneesmiddel voor een groot deel afhangen van de farmacologische eigenschappen van een geneesmiddel, hebben wij farmacologische testen uitgevoerd met bestaande en nieuwe middelen. Deze testen werden uitgevoerd met behulp van een testbatterij voor het centraal zenuwstelsel (genaamd de NeuroCart), waarmee verschillende testen kunnen worden afgenomen, die leiden tot veranderingen in zogenaamde 'biomarkers' (parameters die gebruikt kunnen worden om het beloop van een aandoening of het effect van een behandeling te meten). Daarnaast werd de concentratie van alle geneesmiddelen regelmatig in het bloed bepaald. Door het frequent meten van zeer veel verschillende 'biomarkers' hebben we geprobeerd een farmacologisch profiel te verkrijgen van alle middelen in dit proefschrift. Gezonde vrijwilligers zijn over h*et a*lgemeen gevoelig voor geneesmiddeleffecten die het centraal zenuwstelsel beïnvloeden. Het is echter vaak niet mogelijk om bij gezonde vrijwilligers therapeutische effecten te meten, omdat de ziekte niet aanwezig is. Daarom hebben we gebruik gemaakt van farmacologische modellen om dit toch mogelijk te maken (bijvoorbeeld door het geven van een middel waardoor iemand cognitief verslechtert).

HOOFDSTUK 2: CENTRAL NERVOUS SYSTEM EFFECTS OF THE INTERACTION BETWEEN RISPERIDONE (SINGLE DOSE) AND THE 5-HT6 ANTAGONIST SB742457 (REPEATED DOSES) IN HEALTHY MEN

In dit hoofdstuk wordt een nieuw middel, bedoeld voor het verbeteren van de cognitieve disfunctie bij schizofrenie, onderzocht bij gezonde vrijwilligers. Het gaat om het middel SB742457, een 5-HT_6 (hydroxytryptamine 6) antagonist. Dit middel zou effectief zijn bij cognitieve verslechtering,

zoals is aangetoond in dieronderzoeken en in kleinschalige onderzoeken bij patiënten met de ziekte van Alzheimer. Aangezien gezonde vrijwilligers geen cognitieve verslechtering hebben, is het therapeutisch effect moeilijk aan te tonen. Het gaat er in dit onderzoek dan ook vooral om te kijken of het middel veilig lijkt voor mensen en of het veilig te combineren is met een ander bestaand geneesmiddel voor schizofrenie, namelijk risperidon. Dit middel, dat veel wordt gebruikt, lijkt niet in staat de cognitieve problemen van schizofrenie patiënten (voldoende) tegen te gaan en het antagoneert de 5-HT_6 receptor vrijwel niet. De combinatie van deze middelen lijkt logisch en we hebben daarom onderzocht of er een interactie optreedt tussen deze twee middelen. Dit wordt gedaan door bij gezonde vrijwilligers op frequente basis verschillende testen af te nemen.

Zoals verwacht, werd er bij gezonde vrijwilligers geen cognitieve verbetering waargenomen na toediening van SB742457. Ook liet SB742457 geen andere effecten zien. Risperidon daarentegen liet op de meeste testen wel een duidelijke depressie van het centraal zenuwstelsel zien. De combinatie (t.o.v. risperidon alleen) vertoont in bepaalde frequenties een kleine stijging van het elektro-encefalogram (EEG), wat een mild opwekkend effect van SB742457 zou kunnen betekenen. Aangezien het slechts een alleenstaand effect is, dient dit nog verder onderzocht te worden in patiënten.

HOOFDSTUK 3: PSYCHOMOTOR AND COGNITIVE EFFECTS OF A SINGLE ORAL DOSE OF TALNETANT (SB223412) IN HEALTHY VOLUNTEERS COMPARED WITH PLACEBO OR HALOPERIDOL

In dit onderzoek werd het nieuwe middel talnetant (een neurokinine 3 antagonist) vergeleken met het bestaande middel haloperidol. Haloperidol werd in dit onderzoek gebruikt als positieve controle. We weten goed wat voor effecten er optreden bij haloperidol en die effecten kunnen we vergelijken met de onbekende effecten van talnetant. Haloperidol is een dopamine2 antagonist en deze gaat dus de werking van dopamine tegen. Daarentegen is talnetant een middel dat de dopamine meer moduleert dan tegengaat. De verwachting van dit onderzoek was daarom dat de bijwerkingen van talnetant veel milder zullen zijn dan die van haloperidol.

Zoals verwacht, had haloperidol een 'dempend' effect op bijna alle testen van het centraal zenuwstelsel, terwijl de effecten van talnetant (op verschillende testen) juist wat stimulerend leken. Een bekend resultaat van het antagoneren van dopamine is prolactinestijging in het bloed. Bij haloperidol was er de verwachte prolactinestijging, terwijl dit voor talnetant niet het geval was.

De resultaten lijken aan te geven dat talnetant in de gegeven dosering veilig is bij gezonde vrijwilligers en dat het door de bloed-hersen-barrière heen lijkt te gaan. Wat echter de effecten zijn voor patiënten moet nog verder worden onderzocht.

HOOFDSTUK 4: EARLY STAGE DEVELOPMENT OF THE GLYCINE-1 REUPTAKE INHIBITOR SCH 900435: CENTRAL NERVOUS SYSTEM EFFECTS COMPARED TO PLACEBO IN HEALTHY MEN

Dit hoofdstuk beschrijft de drie eerste onderzoeken van de glycine heropnameremmer SCH 900435, in ontwikkeling voor schizofrenie. Glycine heropname-remmers zouden een nieuwe effectieve behandeling voor schizofrenie kunnen zijn. De ervaring met dit type middelen is schaars, maar dieronderzoeken en beperkt onderzoek bij patiënten laten zien dat het mogelijk effectief zou zijn. De theoretische verklaring van de werking is dat het een indirecte manier is om het glutamaterge-systeem in het lichaam te stimuleren. En dit systeem zou nu bij schizofreniepatiënten net verlaagd zijn.

SCH 900435 laat in het eerste onderzoek (enkelvoudige, oplopende doseringen) veel sterkere effecten zien dan vooraf te verwachten was op basis van onderzoeken bij dieren. Daarnaast werden onverwachte effecten waargenomen, bijvoorbeeld veranderingen in een aantal hormonen en op

het eerste gezicht verontrustende visuele verstoringen (o.a. het zien van vlekken, veranderde kleurperceptie). Ophoging van de dosis werd gedaan op basis van een frequente interim-analyse, die resulteerde in een dosis range van 0.5-30mg (waarbij steeds kleine stappen werden genomen) i.p.v. 0.5-135mg. In plaats van staken van het middel, werd er een specialistisch oog-onderzoek bij dieren en bij gezonde vrijwilligers uitgevoerd (tweede onderzoek dat in dit hoofdstuk wordt beschreven). Deze onderzoeken toonden aan dat de visuele effecten van voorbijgaande aard leken zonder elektrofysiologische veranderingen. Hierdoor kon het nieuwe middel veilig aan gezonde vrijwilligers worden gegeven (in meervoudige dosering, derde onderzoek). Dit onderzoek liet zien dat er tolerantie leek op te treden voor de visuele effecten.

Op basis van deze onderzoeken is aangetoond dat de door ons gekozen onderzoeksstrategie een veilige introductie van middelen met een nieuw werkingsmechanisme mogelijk maakt, ondanks het optreden van onverwachte effecten. Dit werd gedaan door het frequent uitvoeren van verschillende farmacologische metingen. Deze metingen toonden bovendien aan dat een middel door de bloed-hersen-barrière heen gaat en dat er een dosis-gerelateerde farmacologische activiteit is. Dit was in dit onderzoek bijvoorbeeld het geval voor de resultaten van de hormonen, omdat zij een duidelijke dosis-effect relatie lieten zien. Of de effecten specifiek zijn voor deze groep middelen en of de antipsychotische activiteit aanwezig is bij patiënten moet nog worden onderzocht.

HOOFDSTUK 5&6: THE EFFECTS OF THE GLYCINE REUPTAKE INHIBITOR R213129 OR R231857 ON THE CENTRAL NERVOUS SYSTEM AND ON SCOPOLAMINE-INDUCED IMPAIRMENTS IN PSYCHOMOTOR AND COGNITIVE FUNCTION IN HEALTHY SUBJECTS

In de hoofdstukken 5 en 6 worden de effecten van twee verschillende glycineheropname-remmers, R213129 en R231857, op het centraal zenuwstelsel bij gezonde mannelijke vrijwilligers onderzocht. Aangezien

positieve effecten op cognitie bij gezonde vrijwilligers lastig zijn aan te tonen, werd in deze onderzoeken gebruik gemaakt van het farmacologisch model scopolamine. In diermodellen waren effecten waargenomen na combinatie met de glycineheropname-remmer t.o.v. scopolamine alleen. Scopolamine is het meest gebruikte model voor het onderzoeken van de cognitieve disfunctie bij dieren en mensen. Het laat immers robuuste en consistente verslechtering zien van de psychomotorische en de cognitieve functie.

Na toediening van R231857 waren een aantal effecten te zien (vergeleken met placebo), terwijl dit voor R213129 niet het geval was. Er werden geen visuele effecten waargenomen zoals bij SCH 900435 (hoofdstuk 4). Zowel R213129 als R231857 leken in staat om kleine veranderingen in scopolamine-geinduceerde effecten te bewerkstelligen. Het zou een indicatie kunnen zijn dat deze glycineheropname-remmers de bloed-hersen-barrière passeren. Deze effecten waren echter niet gerelateerd aan de dosis. De reden hiervoor zou kunnen zijn dat de concentraties in het centraal zenuwstelsel te laag zijn of de effecten van de scopolamine-test te groot voor deze nieuwe middelen om hierin een duidelijke verandering te brengen. Ook zou het kunnen zijn dat de testen niet gevoelig genoeg waren om de farmacologische effecten te detecteren. Helaas konden we door omstandigheden de doseringen niet meer verder verhogen om de effecten hiervan te onderzoeken. Daarom zullen de effecten in klinische onderzoeken nader moeten worden onderzocht bij patiënten.

HOOFDSTUK 7: PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIPS OF CENTRAL NERVOUS SYSTEM EFFECTS OF SCOPOLAMINE IN HEALTHY SUBJECTS

In hoofdstuk 7 wordt een farmacokinetische-farmacodynamische (PK-PD) analyse van scopolamine uitgevoerd om het scopolamine model te optimaliseren. Farmacokinetiek (PK) kan worden omschreven als de invloed van het lichaam op het geneesmiddel en farmacodynamiek (PD) als de invloed van een geneesmiddel op het lichaam. Farmacokinetiek kan

bepaald worden door bloedspiegels van het geneesmiddel in het lichaam te meten. Hoe deze concentraties veranderen in het lichaam is voor ieder geneesmiddel weer anders. Farmacodynamiek kan bepaald worden door bepaalde functies van het lichaam te meten en de verandering hierin door een geneesmiddel, bijvoorbeeld hartslagfrequentie, vergroting van de pupil, cognitieve prestaties, reactiesnelheid, etcetera. De resultante van de farmacokinetische en farmacodynamische processen, die zich uiteraard gelijktijdig in het lichaam afspelen, bepalen uiteindelijk het farmacologisch profiel van een middel.

Zoals in de hoofdstukken 5 en 6 is besproken, is scopolamine het meest gebruikte model voor het onderzoeken van cognitief disfunctioneren. Het antagoneert het cholinerge systeem (d.w.z. het antagoneert de neurotransmitter acetylcholine). Dit systeem is belangrijk voor verschillende functies van het centraal zenuwstelsel zoals bijvoorbeeld geheugen, leren en aandacht. De exacte relaties tussen de PK van scopolamine en PD zijn nog vrijwel onbekend. Daarom hebben we in dit onderzoek de PK bepaald door bloedspiegels te meten (wat doet het lichaam met het geneesmiddel) en de PD door het afnemen van diverse testen van het centraal zenuwstelsel (wat doet het geneesmiddel met het lichaam). Vervolgens hebben wij in dit onderzoek de PK-PD relaties bepaald in een grote groep gezonde vrijwilligers.

Het onderzoek liet zien dat veel farmacologische systemen werden beïnvloed door scopolamine. De verandering in deze systemen kan worden gebruikt om de effecten van (genees)middelen, die het cholinerge systeem (direct of indirect) beïnvloeden, te onderzoeken. Bij het ontwerpen van nieuwe onderzoeken met scopolamine kan gebruik worden gemaakt van de PK-PD relatie die beschreven in dit hoofdstuk is. Dit is een van de redenen voor het bepalen van PK-PD relatie. Andere redenen zijn de toepassing van deze relaties voor individuele dosis optimalisatie wanneer grote inter-individuele verschillen worden voorzien, het vergroten van inzicht in de fysiologie en farmacologie van humane cholinerge systemen en het bewijzen van de farmacologische activiteit van een stof.

HOOFDSTUK 8: CENTRAL NERVOUS SYSTEM EFFECTS OF HALOPERIDOL ON THC IN HEALTHY MALE VOLUNTEERS

In hoofdstuk 8 is gebruik gemaakt van een cannabinoid model in gezonde vrijwilligers door het toedienen van Δ9-tetrahydrocannabinol (THC), het meest actieve ingrediënt van cannabis. Het is bekend dat er een relatie is tussen THC en het ontstaan van psychosen en schizofrenie, maar wat de onderliggende mechanismen zijn van het cannabinoid systeem (geactiveerd door THC), waardoor psychotische symptomen en schizofrenie ontstaan, is nog steeds onduidelijk. Er is meer en meer bewijs voor een farmacologische relatie tussen het cannabinoid en het dopaminerge systeem.

Diermodellen voor schizofrenie of psychose zijn redelijk goed voorspellend voor bepaalde symptomen van schizofrenie, zoals desorganisatie in gedrag, psychomotorische opwinding en sociale teruggetrokkenheid, maar ze zijn helaas geen goede voorspeller voor de typische positieve symptomen, zoals waanvoorstellingen, hallucinaties en spraakverwarring. Daarom zouden *humane* modellen voor deze symptomen van de aandoening nuttig kunnen zijn.

In dit onderzoek hebben we de hypothese onderzocht dat haloperidol THC-geïnduceerde 'psychomimetische' effecten zou verminderen bij gezonde vrijwilligers. Haloperidol is een geregistreerd antipsychoticum dat dopamine antagoneert (zie ook hoofdstuk 3). Psychomimetische effecten werden gemeten met behulp van de Positive and Negative Syndrome Scale (PANSS), een internationaal geaccepteerd, gestandaardiseerd en semi-gestructureerd interview bij schizofrenie.

We zagen in dit onderzoek dat de toename in positieve symptomen na THC weer werd teruggedrongen door toevoegen van haloperidol. Dit was niet het geval voor de 'feeling high', gemeten met de Visual Analogue Scales. Dit geeft aan dat de 'psychomimetische' effecten, die worden opgewekt door THC, waarschijnlijk worden gemedieerd door dopaminerge systemen, maar dat andere systemen betrokken zijn bij het ontstaan van 'feeling high'. De resultaten van dit onderzoek wijzen er op

dat de toediening van THC een nuttig farmacologisch cannabinoid model voor 'psychomimetische effecten' lijkt in gezonde vrijwilligers. Mogelijk kan dit model voor de bepaling van therapeutische effecten van nieuwe antipsychotica van nut zijn.

HOOFDSTUK 9: DISCUSSION AND CONCLUSIONS

Alle onderzoeken in dit proefschrift maken gebruik van farmacologische monitoring in een vroege fase van geneesmiddelenontwikkeling bij gezonde vrijwilligers. Er werd gebruik gemaakt van een testbatterij voor het centraal zenuwstelsel die uit diverse testen bestond. Op deze manier hebben we geprobeerd om een farmacologisch profiel van de onderzochte middelen te bepalen. Dat dit in een zo vroeg stadium van de geneesmiddelenontwikkeling gebeurt, is momenteel nog vrij ongewoon.

Daarnaast hebben we gebruik gemaakt van andere methoden om de betrouwbaarheid van de testen te verbeteren, bijvoorbeeld van een positieve controle, van PK-PD modelling (het bepalen van PK-PD relaties) en van farmacologische modellen. We hebben effecten bestudeerd van geneesmiddelen die al lang op de markt zijn, zoals de antipsychotica risperidon (hoofdstuk 2) en haloperidol (hoofdstukken 3 en 8) en van nieuwe middelen in ontwikkeling voor schizofrenie, zoals de 5-HT_6 antagonist SB742457 (hoofdstuk 2), de NK_3 antagonist talnetant (hoofdstuk 3) en drie glycine heropname-remmers SCH 900435, R213129 en R231857 (hoofdstukken 4-6). Deze nieuwe middelen geven een goede indruk van de huidige trends op het gebied van geneesmiddelenontwikkeling voor schizofrenie. Ze beïnvloeden een aantal neurotransmitters (of systemen) zoals dopamine, serotonine, glutamaat, acetylcholine en het cannabinoid systeem, en ze zijn vaak meer (of met name) gericht op de negatieve dan op de positieve symptomen van schizofrenie. We hebben gebruik gemaakt van twee farmacologische modellen door het toedienen van scopolamine (hoofdstukken 5-7) en THC (hoofdstuk 8). We onderzochten zowel pure agonisten en antagonisten als middelen die neurotransmitters meer moduleren of indirect beïnvloeden (een trend van de laatste jaren).

Alle geneesmiddelen in dit proefschrift werden ontwikkeld voor schizofrenie. Deze aandoening wordt gekarakteriseerd door een aantal afwijkingen, die ook bij andere neuropsychiatrische aandoeningen optreden. Daarom kunnen de farmacologische resultaten, de methode van onderzoek van deze humane farmacologie en het gebruik van modellen worden toegepast op andere aandoeningen van het centraal zenuwstelsel, zoals bijvoorbeeld de ziekte van Alzheimer (5-HT_6 antagonist) of andere gerelateerde psychiatrische aandoeningen (bijvoorbeeld schizo-affectieve aandoening). De middelen die werden onderzocht in dit proefschrift, werden daarom ook vaak - behalve voor de toepassing schizofrenie - voor andere indicaties ontwikkeld.

Er zijn vele redenen waarom frequent meten d.m.v. veel verschillende testen in een vroeg stadium van de geneesmiddelenontwikkeling nuttig kan zijn. Een van de redenen is de mogelijkheid om een farmacologisch profiel van een nieuw middel te verkrijgen in een vroeger stadium dan dat nu meestal het geval is. Dit maakt het mogelijk om eerder effecten en bijwerkingen te voorspellen, doseringen te selecteren en aldus deze gewilde en ongewilde effecten in volgende onderzoeken te voorkomen. Hierdoor worden kwetsbare patiënten niet onnodig belast en kunnen ontwikkelingskosten worden teruggedrongen. Aangezien er geen geschikte diermodellen zijn voor psychose, kan een humaan farmacologisch model als praktisch model voor psychose of cognitief disfunctioneren worden gebruikt om therapeutische effecten van nieuwe antipsychotische middelen te onderzoeken.

Heel kort samengevat bevatten de onderzoeken in dit proefschrift de volgende onderwerpen: er wordt getoond op welke verschillende manieren humane farmacologie van bestaande en nieuwe middelen bij schizofrenie kan worden onderzocht, ze geven een indruk van de huidige ontwikkeling van middelen tegen schizofrenie en ze laten voorbeelden zien van hoe humane farmacologie van nut is en kan worden toegepast in vroege stadia van geneesmiddelenontwikkeling.

CURRICULUM VITAE

Marieke Liem-Moolenaar was born on December 5th 1974 in Nijmegen, The Netherlands. She grew up together with her older brother and parents in Wijchen and completed high school at the 'Dominicus College' (gymnasium) in Nijmegen in 1993. In the same year she started her pharmacy study at the University of Utrecht. She performed a research project in psychopharmacology (The Effects of Ephedrine on the Development of Fatigue in a Prolonged Driving-Related Task) at 'The Department of Pharmacology, University of Sydney, Australia' (1997-1998). During her two hospital pharmacy internships in the 'Ziekenhuisapotheek Midden-Brabant' in Tilburg (supervisor: prof. A.C.G. Egberts) and 'Canisius-Wilhelmina Ziekenhuis' in Nijmegen (supervisor: drs J.C.A. Smit/dr E.J. Vollaard), she got interested in working in a hospital pharmacy. After finishing her pharmacist's degree at the University of Utrecht in 2000, she went back to Sydney for an internship in the hospital pharmacy of the 'North Shore Hospital', Sydney, Australia. Subsequently, she worked at the 'Canisius-Wilhelmina Ziekenhuis' and started the degree as hospital pharmacist in the 'Ziekenhuisapotheek Midden-Brabant', which she finished in 2003. In 2004 she started working at the Centre for Human Drug Research in Leiden (2004), the former pharmacology department of the Leiden University Medical Centre (promotores: prof. dr. J.M.A. Van Gerven and prof. dr. A.F. Cohen). She worked as a project leader, finished a degree in clinical pharmacology in 2007 and started the work presented in this thesis. Since September 2009 she has worked as a hospital pharmacist at the Erasmus Medical Centre in Rotterdam and currently works in 'Ziekenhuis Rivierenland Tiel', The Netherlands. Marieke married Yves in 2006 and they have two sons, Ward (2007) and Krijn (2009).

NAWOORD

Eigenlijk zou ik het liefst iedereen in één zin bedanken, maar dan doe ik toch geen recht aan de energie die anderen in dit boekje hebben gestoken. Allereerst mijn promotores: Joop en Adam, heel erg bedankt voor de leerzame en leuke tijd bij CHDR met veel goede herinneringen. Het was voor mij als apotheker weer een kijkje in de keuken van een geheel ander bedrijf dan ik gewend was. De kennis en vaardigheden die ik bij het CHDR heb opgedaan komen mij nu en ook in de toekomst steeds weer van pas. Dan mijn co-promotor: Kari, er was nog één apotheker bij het CHDR en ook nog één waarvan ik veel kon leren en die ook nog eens erg aardig was (en is). Ik ben daarom erg blij dat je je als co-promotor met mijn onderzoek wilde "bemoeien". Uiteraard ben ik iedereen van het CHDR veel dank verschuldigd (dank!), maar wil ik toch even een paar personen even expliciet noemen: Marieke en Rik, dank dat jullie mij de statistiek op een goede manier helder hebben weten te maken, zonder dat ik afdwaalde. Esther, Monique, Eva, Herbert, Ria, Margreet, Eleonora, Carmen, Ron en dames van het secretariaat bedankt voor de zeer waardevolle hulp bij het vloeiend laten verlopen van alle onderzoeken. sSanne, sGabriel en sRemco, door jullie werd het werken op CHDR heel gemakkelijk en gezellig, hopelijk kunnen we nog vele Oxford en Scroppino momenten beleven.

Buiten het CHDR/werk zijn er natuurlijk nog een aantal zeer belangrijke personen die ik niet mag vergeten. Allereerst alle vrienden die het steeds weer durfden te vragen hoe het er voor stond met mijn promotie. Mijn paranimfen Kris en Emmeke (en Kirsten en Camiel niet te vergeten!), jullie stonden natuurlijk aan het begin van dit project en weten als geen ander hoe lastig dit te combineren is met een druk leven. Vorige keer beviel zo goed in de senaatszaal in Utrecht, dat ik het daarom weer over wil doen. Liever sta ik toch eigenlijk weer aan de zijkant en niet in het midden, maar ja dat is even niet anders.

Fong en Lany, bedankt voor jullie interesse en grote hulp altijd in huis en voor de kinderen. Zonder deze hulp had de promotie zeker nu nog niet plaats kunnen vinden. Roger, jij ook bedankt voor je interesse. Constant, jij ook bedankt voor je steun in een voor jou hele zware periode, die nu

hopelijk steeds verder weg gaat lijken. En Yvonne, wat ben ik blij dat jij er ineens was. Ik hoop dat we je nog lang mogen blijven zien. Pap en mam, bedankt voor heel veel dingen: het altijd klaar staan voor ons en Ward en Krijn (en binnenkort weer voor ???), het begrip en het medeleven. Ik vind het fijn dat ons contact nog hechter is geworden doordat jullie zo vaak bij ons zijn.

Lieve Yves, ik kan het helaas niet zo mooi verwoorden als dat jij dat deed in je nawoord. Jij hebt mijn laatste zware loodjes denk ik het meest gevoeld van iedereen en ik denk dat jij ook blij bent dat we dit hoofdstuk kunnen afsluiten. Een periode met drie zwangerschappen viel vrij ongelukkig samen met het afronden van de promotie. Zeker nu we binnenkort weer aan een zeer welkom, nieuw, druk "project" zullen beginnen. Door de lol met elkaar en met Ward en Krijn en door het meedenken zijn de zware loodjes gelukkig steeds weer stukken lichter geworden en dat heeft mede geholpen om mijn promotie dan toch echt af te ronden. Ik hoop dat we altijd veel lol blijven houden met z'n vijven.

Marieke Liem-Moolenaar
Januari 2012

Liem-Moolenaar M, Gray FA, de Visser SJ, Franson KL, Schoemaker RC, Schmitt JA, Cohen AF, van Gerven JM. Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 2010; 24: 73-82.

Liem-Moolenaar M, Zoethout RW, de BP, Schmidt M, de Kam ML, Cohen AF, Franson KL, van Gerven JM. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. J Psychopharmacol 2010; 24: 1671-9.

Liem-Moolenaar M, te Beek ET, de Kam ML, Franson KL, Kahn RS, Hijman R, Touw D, van Gerven JM. Central nervous system effects of haloperidol on THC in healthy male volunteers. J Psychopharmacol 2010; 24: 1697-708.

Liem-Moolenaar M, Zoethout RW, de BP, Schmidt M, de Kam ML, Cohen AF, Franson KL, van Gerven JM. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. J Psychopharmacol 2010; 24: 1681-7.

Liem-Moolenaar M, de BP, Timmers M, Schoemaker RC, van Hasselt JG, Schmidt S, van Gerven JM. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. Br J Clin Pharmacol 2011; 71: 886-98.

Liem-Moolenaar M, Rad M, Zamuner S, Cohen AF, Lemme F, Franson KL, van Gerven JM, Pich EM. Central nervous system effects of the interaction between risperidone (single dose) and the 5-HT6 antagonist SB742457 (repeated doses) in healthy men. Br J Clin Pharmacol 2011; 71: 907-16.