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THE ROLE OF DOPAMINE, 5-HYDROXYTRYPTAMINE, SIGMA AND NMDA RECEPTORS IN THE ACTION OF ANTIPSYCHOTIC DRUGS

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present study is based on the following original publications, which are referred to in the text by Roman numerals (I–III):

I Lang A., Vasar E., Soosaar A. and Harro J. The involvement of sigma and phencyclidine receptors in the action of antipsychotic drugs. *Pharmacology & Toxicology* 1992, 71: 132–138.

II Lang A., Soosaar A., Kõks S., Volke V., Bourin M., Bradwejn J. and Vasar E. Pharmacological comparison of antipsychotic drugs and σ-antagonists in rodents. *Pharmacology & Toxicology* 1994, 75: 222–227.

III Lang A., Harro J., Soosaar A., Kõks S., Volke V., Oreland L., Bourin M., Vasar E., Bradwejn and Männistö P. T. Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behaviour in rats. *Naunyn-Schmiedeberg's Archive of Pharmacology* 1995, 351: 363–370.

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ABBREVIATIONS

B_{MAX}	apparent number of binding sites
DTG	1,3-di(2-tolyl)guanidine
ED_{50}	effective $dose_{50}$, the dose which causes the desired effect in 50% compared to drug-induced effect in control animals
EPS	extrapyramidal side effects
5-HT	5-hydroxytryptamine
IC_{50}	50% inhibitory concentration
i. p.	intraperitoneal(ly)
K_D	dissociation constant
NMDA	N-methyl-D-aspartic acid
PCP	phencyclidine
3-PPP	3-(3-hydroxyphenyl)-N-n-propyl-piperidine
s. c.	subcutaneous(ly)
(±)-SKF 10,047	(±)N-allyl-normetazocine
TCP	1-[1-(2-thienyl)cyclohexyl]piperidine

1. INTRODUCTION

Compounds having antipsychotic activity belong to a chemically heterogenous group of drugs. The blockade of dopamine D_2 receptors is a common feature of these drugs and a necessary requisite to treat positive symptoms of schizophrenia (Seeman, 1980; Richelson, 1984). Long-term administration of antipsychotic drugs such as chlorpromazine and haloperidol is accompanied with the development of extrapyramidal side effects (EPS) ranging from acute dystonia and drug-induced parkinsonism to tardive dyskinesia. Therefore, a substantial effort has been directed to develop new antipsychotic drugs lacking EPS. Recently Seeman and Van Tol (1994) have proposed the hypothesis that effective antipsychotic medication without disabling EPS requires blockade of more than one receptor in the brain. Indeed, risperidone and clozapine, blocking dopamine D_2, 5-hydroxytryptamine 5-HT_{2A}, and 5-HT_{2C} receptors do not cause significant EPS but potently ameliorate positive and negative symptoms of schizophrenia (Leysen *et al.*, 1993).

In 1976, Martin developed a new functional classification of opioid receptors according to action of various drugs in spinal dogs (Martin *et al.*, 1976). Psychotomimetic action of SKF 10,047 in dogs was attributed to sigma opioid receptors (Martin *et al.*, 1976). However, the subsequent studies did not confirm belonging of these receptors to opioid receptors since unselective opioid antagonist naloxone was ineffective against SKF 10,047 (Iwamoto, 1981; Brady *et al.*, 1982; Slifer & Balster, 1983; Katz *et al.*, 1985). Moreover, interaction of SKF 10,047 with two distinct binding sites was established in the radioligand binding studies. SKF 10,047 was fully active at NMDA-gated channels and haloperidol-sensitive sigma receptors (Zukin & Zukin, 1979; Tam, 1983). Therefore, the psychotomimetic action of SKF 10,047 can be mediated by both of these binding sites in the brain (Itzhak & Alerhand, 1989; Itzhak & Stein, 1990).

Sigma receptors are characterized by insensitivity to opioid antagonist naloxone and also by extremely high affinity for haloperidol, a potent antipsychotic drug (Pasternak *et al.*, 1981; Brady *et al.*, 1982; Slifer & Balster, 1983; Tam & Cook, 1984; Katz *et al.*, 1985; Itzhak, 1988). Neurotransmitters dopamine and 5-hydroxytryptamine are lacking major activity at sigma receptors (Tam & Cook, 1984; Weber *et al.*, 1986). Although little is known about the physiological functions of sigma receptors, several lines of evidence suggest the involvement of these receptors in the action of antipsychotic drugs (Deutsch *et al.*, 1988). It is now clear that several antipsychotic drugs have a significant affinity for sigma receptors, often greater than their affinity for dopamine receptors (Tam, 1983; Tam & Cook, 1984; Largent *et al.*, 1988). There is a growing body of evidence about anatomical and functional interaction between sigma receptors and dopaminergic systems (Graybiel *et al.*, 1989; Taylor *et al.*, 1990; Walker *et al.*, 1990). Sigma receptors are involved in the control of the firing rate of dopamine neurons and release of dopamine from neuronal terminals (Berkowitz, 1974; Wachtel & White, 1988; Steinfels & Tam, 1989;

Steinfels *et al.*, 1989; Taylor *et al.*, 1990). Stimulation of sigma receptors increases release of dopamine, whereas blockade of these receptors decreases activity of the dopaminergic system.

Recently, involvement of glutamate in the action of antipsychotic drugs has been suggested. Glutamate is also shown to contribute to the control of dopamine release in various brain structures (Whitton *et al.*, 1994). Glutamate released from the corticostriatal terminals seems to have a strong influence on the dopaminergic neurotransmission in the striatum (Martinez-Fong *et al.*, 1992; Whitton *et al.*, 1994). One subtype of glutamate-gated channels, N-methyl-D-aspartate (NMDA) channel, is believed to be involved in psychotomimetic action of phencyclidine-like compounds (Javitt & Zukin, 1991). Phencyclidine and related compounds block these channels in non-competitive manner (Wong *et al.*, 1986). The antipsychotic compound clozapine also interacts with NMDA-gated channels both in radioligand binding and behavioural experiments (Janowsky & Berger, 1989; Tiedtke *et al.*, 1990). It has been even claimed that effectiveness of clozapine against negative symptoms of schizophrenia might be linked to its interaction with NMDA-gated channels (Tiedtke *et al.*, 1990; Schmidt *et al.*, 1991).

The present study aimed to extend our understanding of the mechanism of action of antipsychotic drugs. We attempted to find out to what extent the various brain receptors (dopamine, 5-hydroxytryptamine, NMDA-gated channels and sigma receptors) are involved in the action of antipsychotic drugs. Since mainly acute experiments have been employed to date in the study of the effects of antipsychotic drugs, we made an attempt to use a long-term model of psychotic behaviour in laboratory animals. In this model, repeated administration of apomorphine, an unselective dopamine agonist, was used to mimic psychotic state in the rat.

2. REVIEW OF LITERATURE

2.1. Properties of typical and atypical antipsychotic drugs

Antipsychotic drugs are classified as typical or atypical according to their clinical and pharmacological effects. The similarities and differences of these two groups of drugs are summarized in Table 1.

Antipsychotic effect of typical antipsychotic drugs is accompanied with a high probability of occurrence of extrapyramidal side effects (EPS) in man (Table 1). Catalepsy is believed to be the rodent equivalent of EPS. Atypical antipsychotic drugs, on the contrary, are unlikely to cause these motor disturbances. It has been believed that EPS is a prerequisite for antipsychotic action (Mattke, 1968). However, recent studies have shown that several effective antipsychotic drugs do not induce EPS.

Many animal tests to reveal antipsychotic drugs are based on the inhibition of dopamine agonist-induced behaviour. For example, apomorphine-induced climbing test in mice is inhibited by low doses of all classes of antipsychotic drugs, including: phenothiazines, butyrophenones, thioxanthenes, butaclamol, clozapine, and benzamides. Higher doses of typical antipsychotic drugs are needed to block the apomorphine- and amphetamine-induced hypermotility in rodents and stereotyped behaviour in rats. Atypical antipsychotic drugs, in contrast, are almost ineffective against dopamine agonist induced stereotyped behaviour but they efficiently block the hyperlocomotion induced by amphetamine or apomorphine (Table 1).

Besides behavioural tests reflecting the increased stimulation of dopamine receptors, the conditioned avoidance paradigm has been used to study antipsychotic drugs. Typical antipsychotic drugs disrupt the development of conditioned avoidance. By contrast, atypical antipsychotic drugs are completely ineffective in this study design (Table 1).

Acute treatment with the classical antipsychotic drugs increases spontaneously active dopamine neurons in the substantia nigra (A_9) and ventral tegmental area (A_{10}). Their repeated administration causes opposite action — decreases the number of spontaneously active dopamine neurons in these two brain regions. Acute and long-term treatment with atypical antipsychotic drugs affects only the electrical activity of A_{10} neurons (Table 1). The selective action on the dopamine neurons in the ventral tegmental area is a possible reason why atypical drugs do not induce EPS.

All typical antipsychotic drugs have significant affinity for dopamine D_2 receptors, whereas the activity of atypical drugs on dopamine D_2 receptors is variable.

Table 1

Comparative effects of typical and atypical antipsychotic drugs

Effect	Typical antipsychotic drugs (e.g., haloperidol, chlorpromazine)	Atypical antipsychotic drugs (e.g., clozapine, sulpiride, thioridazine, remoxipride)
Extrapyramidal side effects in man	high probability	low probability
	(Stille *et al.*, 1971; Costall & Naylor, 1975; Janssen & Van Bever, 1978; Niemegeers & Janssen, 1979)	
Catalepsy in laboratory animals	high	low
	(Stille *et al.*, 1971; Costall & Naylor, 1975; Janssen & Van Bever, 1978; Niemegeers & Janssen, 1979; Worms & Lloyd, 1979)	
Apomorphine- and amphetamine-induced hyperlocomotion	yes	yes
	(Puech *et al.*, 1976; Simon & Puech, 1979; Bischoff *et al.*, 1988)	
Blockade of amphetamine- and apomorphine-induced stereotypy	yes	no
	(Puech *et al.*, 1976; Simon & Puech, 1979; Robertson & MacDonald, 1984)	
Suppression of conditioned avoidance response	yes	no
	(Worms *et al.*, 1983; Blackburn & Phillips, 1989; Bruhwyler *et al.*, 1990; Britton *et al.*, 1992)	
Regional selectivity for dopamine neurons	both A_9 and A_{10}	A_{10}
	(White & Wang, 1983; Hand *et al.*, 1987) (White & Wang, 1983; Freeman & Bunney, 1987; Hand *et al.*, 1987)	
Affinity for dopamine D_2 receptors	high	variable
	(Seeman, 1980; Richelson, 1984)	

2.2. Interactions of antipsychotic drugs with neurotransmitter systems and their receptors

Dopaminergic system and dopamine receptors are proposed to be the primary targets for the action of antipsychotic drugs (Mortimer, 1994). However, other neurotransmitters are also affected by antipsychotic drugs. Below a short review is given about the role of some of these neurotransmitters in the action of antipsychotic compounds.

2.2.1. Dopamine

Dopaminergic neurons innervating all major parts of brain arise from cell bodies located in the substantia nigra (area A_9), ventral tegmental area (area A_{10}), and hypothalamus (area A_{12} and A_{13}). These neurons form major dopaminergic pathways: nigrostriatal (A_9), mesolimbic (A_{10}), mesocortical (A_{10}), and tuberoinfundibular (A_{12} and A_{13}) tracts (Dahlström & Fuxe, 1964). The mesolimbic and mesocortical dopamine systems are considered as the main sites for antipsychotic action of drugs, whereas hormonal and motor impairments are mediated by the tuberoinfundibular and nigrostriatal tracts respectively.

The original pharmacological classification divides dopamine receptors into two major groups and five currently cloned dopamine receptors fall into these classes (Seeman & Van Tol, 1994). The D_1-like receptors include D_1 and D_5, while the D_2-like receptors include D_2, D_3 and D_4 (Seeman & Van Tol, 1994).

Dopamine D_2 receptor blockade, a common feature of all known antipsychotic drugs, is believed to be a basic mechanism to treat positive symptoms in schizophrenia (Seeman, 1980; Richelson, 1984). As mentioned above, the antipsychotic activity of drugs is presumably the result of blockade of the dopamine D_2 receptors in the mesolimbic system, while the blockade of dopamine D_2 receptors in the striatum is related to EPS (Carlsson, 1978). Therefore, drugs with higher selectivity for dopamine D_2 receptors in the mesolimbic area are less likely to induce EPS and catalepsy. They display significantly higher selectivity against apomorphine-induced hyperlocomotion compared to stereotypies elicited by dopamine agonists. Raclopride and remoxipride, new antipsychotic drugs with selectivity for dopamine D_2 receptors, differ from haloperidol and chlorpromazine by their markedly higher potency against apomorphine-induced hyperlocomotion (Ögren *et al.*, 1986). Relatively higher doses of raclopride and remoxipride were needed to produce catalepsy, indicating lower potential to produce EPS (Hillegaart & Ahlenius, 1987; Farde *et al.*, 1989).

The role of dopamine D_1 receptors in the action of antipsychotic drugs should also be considered. Several studies suggest that the blockade of dopamine D_1 receptors is important in the mechanism of action of atypical antipsychotic drugs

(Chipkin & Latranyi, 1987; Alter *et al.*, 1988; Imperato & Angelucci, 1989). The repeated administration of SCH 23390, a selective dopamine D_1 receptor antagonist, caused depolarization inactivation of A_{10} dopamine cells in the same way as atypical antipsychotic drugs (Goldstein & Litwin, 1988).

Seeman and Van Tol (1994) suggested that pharmacotherapy of schizophrenia may be improved by the selective blockade of new subtypes of dopamine receptors (D_3, D_4). Clozapine is most active at dopamine D_4 receptor subtype (Van Tol *et al.*, 1991, 1992; Meltzer & Gudelsky, 1992). Moreover, the sixfold elevation of the density of dopamine D_4 receptors was described in patients suffering from schizophrenia (Seeman *et al.*, 1993). Dopamine D_3 receptors are mainly located in the mesolimbic structures, which are important in the control of motivations (Bouthenet *et al.*, 1991; Van Tol *et al.*, 1991; Sokoloff *et al.*, 1992).

2.2.2. 5-Hydroxytryptamine

5-Hydroxytryptamine (5-HT) is widely distributed throughout the central nervous system. The neurons located in the brainstem raphe nuclei innervate both forebrain and spinal cord. The recent classification divides 5-HT receptors into at least seven groups with several subtypes (Humphrey & et al., 1993). Abnormalities in 5-HT-ergic transmission may be implicated in the pathogenesis of several mental disorders including major depression and schizophrenia (Ohuoha *et al.*, 1993).

Several clinically potent antipsychotic drugs have significant activity both at dopamine D_2 and 5-HT_2 receptors. Some of them display even higher selectivity for 5-HT_2 than for D_2 receptors (Meltzer *et al.*, 1989). For example, clozapine had nearly 50 times and risperidone 20 times higher affinity for 5-HT_2 than they do to dopamine D_2 receptors (Leysen *et al.*, 1993). A hypothesis has been presented that high affinity of antipsychotic drugs for 5-HT_2 receptor reduces the risk of development of EPS, and it might have a role in alleviation of negative symptoms of schizophrenia resistant to other antipsychotic drugs (Leysen *et al.*, 1988; Gelders *et al.*, 1990). Moreover, 5-HT_2 antagonists reduce the antipsychotic-induced catalepsy in rats (Balsara *et al.*, 1979; Hicks, 1990). Differently from typical antipsychotic drugs, the administration of atypical compounds changes 5-HT turnover in the brain structures (Csernansky *et al.*, 1993).

2.2.3. Sigma receptors

Despite significant efforts there is no consensus about the endogenous ligands for sigma receptors (Su *et al.*, 1986; Contreras *et al.*, 1987; Su & Vaupel, 1988; Zhang *et al.*, 1988). Sigma receptors are localized in the brain, as well as, in many peripheral tissues including liver, spleen, and blood cells (Samoilova *et al.*, 1988; Su *et al.*,

1988; Wolfe, S. A. *et al.*, 1988; Wolfe, S. E. *et al.*, 1989). In the brain, sigma receptors are mostly concentrated in the brainstem areas subserving motor functions (the red nucleus, cerebellum, substantia nigra pars compacta, and various cranial nerve nuclei: the facial, hypoglossal, and motor trigeminal nuclei) (Gundlach *et al.*, 1986; McLean & Weber, 1988). However, the high densities of sigma receptors are also found in certain limbic structures, in the brain areas related to the regulation of sensory and endocrine function (McLean & Weber, 1988). Such location of sigma receptors may reflect their role in the regulation of a variety of physiological functions. However, it has been strongly established that sigma receptors are involved in the coordination of motor functions (Walker *et al.*, 1990). Indeed, the injection of several sigma agonists caused significant dystonia in rats (Matsumoto *et al.*, 1990).

Several antipsychotic drugs (haloperidol, chlorpromazine, remoxipride) interact with sigma receptors whereas the others (clozapine, raclopride, sulpiride) lack any activity on these receptors (Su, 1982; Largent *et al.*, 1984; Tam & Cook, 1984). Haloperidol is the most potent drug interacting with sigma receptors (Tam & Cook, 1984; Itzhak, 1988). Recent behavioural, electrophysiological and receptor binding experiments provide evidence that haloperidol may act as a mixed agonist-antagonist of sigma receptors (Largent *et al.*, 1984; Bowen *et al.*, 1988; Tam *et al.*, 1988; Walker *et al.*, 1988; Beart *et al.*, 1989). The chronic administration of haloperidol considerably reduced the density of sigma receptors, but not their affinity in the rat brain (Itzhak & Alerhand, 1989; Kizu *et al.*, 1991; Jansen *et al.*, 1992).

2.2.4. Glutamate and NMDA-gated channels

Glutamate released from the fibers originating from the frontal cortex appears to be a major factor in the control of dopaminergic neurotransmission in the striatum and nucleus accumbens (Fonnum *et al.*, 1981; Christie *et al.*, 1985). Moreover, dopamine receptors are located on the glutamatergic corticostriatal terminals, showing reciprocal interactions between dopamine and glutamate (Schwarcz, R. *et al.*, 1978; Roberts & Anderson, 1979; Nishikawa *et al.*, 1983). Therefore, antipsychotic drugs seem to affect the activity of the glutamatergic system. Moreover, clozapine displaces [^{3}H]-MK-801, a non-competitive antagonist of N-methyl-D-aspartate gated channels, from striatal homogenate (Lidsky *et al.*, 1993). Chlorpromazine also inhibits the binding of NMDA channel ligand [^{3}H]-TCP to the rat brain membranes, but chronic administration of chlorpromazine does not change the density of NMDA channels in the rat cerebral cortex (Rehavi & Schnitzer, 1991). Clozapine efficiently blocks the stereotypies induced by dizocilpine. In the electrophysiological studies, clozapine suppresses electrical responses in the striatum evoked by the stimulation of glutamatergic corticostriatal pathways (Lidsky *et al.*, 1993). These data are apparently in favour of interaction of clozapine with glutamatergic neurotransmission.

2.3. Sigma receptor antagonists as potential antipsychotic drugs

Interaction of several clinically potent antipsychotic drugs with sigma receptors led to the hypothesis that selective sigma receptor antagonists may possess antipsychotic activity. Limited clinical trials with rimcazole, a sigma receptor antagonist, have strengthened this idea. Rimcazole exhibited moderate antipsychotic activity in patients suffering from acute schizophrenic psychosis (Davidson *et al.*, 1982, 1985; Guy *et al.*, 1983; Chouinard & Annable, 1984; Schwarcz, G. A. *et al.*, 1985). Furthermore, the density of sigma receptors was markedly reduced in the temporal lobe of schizophrenic patients compared to age-matched controls (Weissman *et al.*, 1988, 1991).

2.3.1. Biochemical effects

Several compounds display relatively higher affinity for sigma receptors than for any other known receptor types in the mammalian brain. The putative sigma receptor antagonists (haloperidol, cinuperone, BMY 14802, and rimcazole) inhibit [^{3}H]-SKF 10,047, an agonist of sigma receptors, binding to sigma receptors at lower concentrations than they inhibit its binding to NMDA-gated channels (Su *et al.*, 1988). Nevertheless, the interaction with other receptors should not be neglected if the action of these compounds is under the scope. Su (1986) established that cinuperone affected binding of radioligands to dopamine D_2 and sigma receptors in the rat brain (Su, 1986). The enantiomers of BMY 14802 bind with approximately equal potency to 5-HT_{1A} receptor (Taylor *et al.*, 1990).

Subchronic treatment with rimcazole increased the number of sigma binding sites in the rat brain (Manallack & Beart, 1988; Beart *et al.*, 1989), while haloperidol had the opposite action; reducing the density of sigma receptors (Itzhak & Alerhand, 1989).

Data of several studies suggest the modulation of dopaminergic activity by sigma receptors. First, using autoradiographic methods, sigma binding sites were detected on dopamine neurons of the rat (Gundlach *et al.*, 1986) and cat (Graybiel *et al.*, 1989) brain. Second, in electrophysiological and biochemical studies, sigma agonists and antagonists either increase or decrease the activity of dopamine neurons (Berkowitz, 1974; Freeman & Bunney, 1984; Matthews *et al.*, 1986; Steinfels *et al.*, 1989; Iyengar *et al.*, 1990). The administration of sigma antagonists (BMY 14802, rimcazole and remoxipride) markedly accelerated dopamine turnover in the brain (Matthews *et al.*, 1986; Beart *et al.*, 1989; Iyengar *et al.*, 1990; Köhler *et al.*, 1990; Rao *et al.*, 1990; Gudelsky & Nash, 1992).

2.3.2. Behavioural effects

The close interaction between sigma receptors and dopaminergic system determines the effectiveness of sigma antagonists in the behavioural tests used to reveal the potential antipsychotic activity of drugs. Rimcazole was identified as a potential antipsychotic drug in the behavioural experiments potently antagonizing apomorphine-induced behaviours in rodents (Ferris *et al.*, 1982). Rimcazole was effective against apomorphine-induced aggressiveness in rats, and apomorphine-induced climbing behaviour in mice, but did not affect apomorphine-induced stereotyped gnawing (Ferris *et al.*, 1982). Furthermore, rimcazole displayed very limited activity at dopamine receptors in the radioligand binding studies whereas it efficiently blocked sigma receptors (Ferris *et al.*, 1986a, b). Cinuperone displayed a very similar behavioural profile to rimcazole (Hock *et al.*, 1985)). The subsequent radioligand binding studies also excluded the role of dopamine in the action of cinuperone (Su, 1986). Remoxipride was more potent to inhibit apomorphine-induced stereotyped behaviour than to induce catalepsy in the rat (Florvall & Ögren, 1982). Moreover, remoxipride displayed higher selectivity against apomorphine-induced hyperlocomotion compared to stereotypies elicited by dopamine agonists (Ögren *et al.*, 1984). The sigma antagonist BMY 14802 did not cause catalepsy at any dose administered and it reversed catalepsy produced by haloperidol (Matthews *et al.*, 1986). Accordingly, the above described sigma antagonists resemble, in many respects, the atypical antipsychotic drugs.

3. AIMS OF THE PRESENT STUDY

As discussed above, not only dopamine D_2 receptors, but also several other receptors, are involved in the action of antipsychotic drugs and the pathophysiology of schizophrenia. According to current understanding the most concerned receptors are 5-HT_{2A} and 5-HT_{2C}, dopamine D_1 and sigma receptors, and also NMDA-gated channels. Moreover, it has been hypothesized that effective antipsychotic treatment with the reduced liability of EPS requires blockade of two or more receptor systems in the brain (Kahn & Davidson, 1993; Seeman & Van Tol, 1994). Taking into account the complicated nature of the antipsychotic action of various drugs, the main aims of the present study were as follows:

1) To compare the typical and atypical antipsychotic drugs by means of receptor binding studies and animal models reflecting the possible antipsychotic potency of drugs. Special attention was paid to the interaction of these drugs with dopamine, sigma, 5-HT_{2A} receptors, and NMDA-gated channels.

2) To study the role of sigma receptors in the action of antipsychotic drugs. Selective sigma antagonists were compared with the antipsychotic drugs in acute and long-term experiments using radioligand binding and behavioural studies.

3) The current practice to reveal antipsychotic drugs utilizes a disease-like state induced by acute drug treatments. However, schizophrenia and other psychotic states are chronic and highly devastating diseases. Therefore, the use of subchronic animal models was considered to be important. For that purpose we used the aggressive behaviour induced by repeated apomorphine treatment. Two major questions were addressed. First, to examine the selectivity of this model to reveal the antipsychotic activity of drugs. Second, special attention was paid to the role of NMDA-gated channels in the development of aggressive behaviour.

4. MATERIALS AND METHODS

4.1. Animals

Male Wistar rats (Kuo:WIST) and (Rap:WIST) were supplied by the National Animal Center, Kuopio, Finland and Rappolovo Farm of Laboratory Animals, Russia, respectively. The source of male albino mice was the Rappolovo Farm of Laboratory Animals, Russia. Rats weighing 180–300 g were housed in groups of five. Mice weighing 25–30 g were housed in groups of 15–25. Animals were kept under standard laboratory conditions (temperature 20±3°C) with free access to tap water and pelleted rat/mouse feed (STS, UK). An artificial 12 h light-dark cycle was used (lights on at 8:00).

4.2. Drugs

The commercial solution of *haloperidol* (Gedeon Richter, Hungary) was diluted in saline immediately before the experiment. *Raclopride:* (-)(S)-3,5-dichloro-N[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide tartrate (Astra AB, Sweden), *chlorpromazine* (Sigma, USA), *remoxipride:* (S)(-)-3-bromo-2,6-dimethoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide (Astra AB, Sweden), *BMY 14802:* α-(4-fluorophenyl)-4-(-flouro-2-pyrimidinyl)-1-piperazine butanol (Bristol-Myers & Squibb, USA), *apomorphine* hydrochloride (Sigma, USA), *d-amphetamine* (Sigma, USA), *quipazine* dimaleate (Research Biochemicals International, USA), *dizocilpine* maleate: (+)-5-methyl-10,11-dihydro-5-H-dibenzo[a,d]cycloheptan-5,10-imine maleate (Merck Sharp & Dohme, UK) and *rimcazole:* (cis-9-[3-3,5-dimethyl-1-piperazinyl)propyl]carbazole dihydrochloride (Burroughs-Wellcome, USA) were dissolved in saline. *Clozapine* (Sandoz, Switzerland) and *cinuperone:* 3-(4-(3-(4-fluorobenzoyl)-propyl-piperazino-l-yl)-isoquinolino-hydrochloride (Hoechst, Germany) were suspended in the saline with the help of 1-2 drops of Tween-85. *SCH 23390:* R(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (Research Biochemicals International, USA) was dissolved in 0.1% ascorbic acid.

4.3. Radioligand binding studies

The radioligand binding studies included two sets of experiments. In *in vitro* studies the IC_{50} values of respective drugs were determined. In the case of subchronic treatment of drugs the affinity and the capacity of binding sites were detected.

4.3.1. Preparation of brain samples for radioligand binding studies

For *in vitro* binding studies the membranes prepared from various structures of the rat brain were used. Animals were killed by decapitation. The brains were rapidly removed from the skull and the brain structures were dissected on ice. The brain structures were stored at -20°C until the following procedures. Brain tissues were thawed on the day of experiment and were homogenized with a Potter-S homogenizer (1000 rpm, 12 passes) in 20 vol ice-cold 50 mM Tris-HCl buffer (pH 7.4 or 7.7 in the case of [^{3}H]-spiperone, [^{3}H]-haloperidol, [^{3}H]-SCH 23390, [^{3}H]-ketanserin, [^{3}H]-MK-801 or [^{3}H]-TCP, respectively). Membranes were washed twice by centrifugation at 48000 × g for 15 min. After the last centrifugation the tissues were suspended in incubation buffer for the appropriate binding assay.

4.3.2. [^{3}H]-Spiperone binding (I, II, III; dopamine D_2 receptors)

[^{3}H]-Spiperone (specific activity 109 Ci/mmol, Amersham, UK, final concentrations 0.06–2 nM to create saturation curves or 0.1 nM to measure IC_{50} values of drugs) was incubated 30 min at 37°C with the membrane preparation (1 mg wet weight/tube) in 0.5 ml of incubation buffer consisting of Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM (pH 7.4). Nonspecific binding was determined in the presence of raclopride. The reaction was stopped by rapid centrifugation at 11000 × g for 4 min.

4.3.3. [^{3}H]-Haloperidol binding (I, II; sigma receptors)

The rat brain homogenates (12 mg wet weight/tube) were incubated with increasing concentrations (2.5–80 nM for saturation studies) or 1.7 nM (to detect IC_{50} values for different drugs) of [^{3}H]-haloperidol (specific activity 8.9 Ci/mmol, Dupont-NEN, USA) in the absence and presence of 10 μM haloperidol to determine specific binding. Spiperone (50 nM) was added to each tube to block [^{3}H]-haloperidol binding to dopamine D_2, 5-HT_2, α_1 adrenergic receptors. This approach was used since spiperone was inactive at sigma binding up to low micromolar concentrations. The incubation was carried out in the total volume of 1 ml 50 mM Tris-HCl buffer (pH 7.7). After a 90-min incubation at room temperature the membrane-bound [^{3}H]-haloperidol was separated from free radioligand by rapid filtration through Whatman GF/B glass fibre filters (presoaked with 0.05% polyethyleneimine) using a Brandel Cell Harvester (MS-24S, USA). After filtration the filters were washed twice (4.5 ml each) with incubation buffer.

4.3.4. [^{3}H]-TCP binding (I; NMDA-gated channels)

NMDA-gated channels were measured by means of using 7.5 or 2 nM [^{3}H]-TCP (specific activity 60 Ci/mmol, Dupont-NEN, USA) in the presence of 2–100 nM of dizocilpine (IC_{50} studies). The incubation of brain membranes (12 mg wet weight/tube) was carried out in the total volume of 0.5 ml 5 mM Tris-HCl buffer (pH 8.1 at 20 °C) for 45 min at room temperature. The incubation was terminated by rapid filtration as described above.

4.3.5. [^{3}H]-Ketanserin binding (II; 5-HT_2 receptors)

Binding of [^{3}H]-ketanserin (specific activity 72.3 Ci/mmol, Dupont-NEN, USA, 1 nM) to the frontal cortex membranes (2 mg wet weight/tube) was performed in the total volume of 1 ml 50 mM Tris-HCl buffer (pH 7.4) at room temperature. To detect nonspecific binding unlabelled ketanserin (10 µM) was added. After 30 min the incubation was stopped as described earlier with [^{3}H]-haloperidol binding.

4.3.6. [^{3}H]-SCH-23390 binding (II; dopamine D_1 receptors)

The incubation of the rat striatal membranes (1 mg wet weight/tube) was carried out in 0.5 ml of incubation buffer (Tris-HCl, 50 mM, pH 7.4) during 45 min with [^{3}H]-SCH-23390 (specific activity 85 Ci/mmol, Amersham, UK, 2 nM) at room temperature. Nonspecific binding was detected in the presence of 10 µM SCH-23390. The reaction was stopped by rapid centrifugation at 11000 × g for 4 min.

4.3.7. [^{3}H]-MK-801 binding (III; NMDA-gated channels)

[^{3}H]-MK-801 (specific activity 25 Ci/mmol, Dupont-NEN, USA, 1–80 nM) was incubated with the homogenized membranes in Tris-HCl buffer (50 mM) at room temperature in total incubation volume of 0.5 ml. In order to detect nonspecific binding 100 µM of unlabelled MK-801 (dizocilpine) was used. The incubation was terminated after 60 min by rapid filtration over Whatman GF/B filters. The filters were washed with 10 ml cold incubation buffer.

The radioactivity of samples was measured by means of liquid scintillation spectrometry at 50 per cent efficiency level. Saturation curves were processed by means of non-linear least squares regression analysis (Leatherbarrow, 1987). The protein content (III) was measured according to a modification of the Lowry procedure (Markwell *et al.*, 1978).

The IC_{50} values for antipsychotic drugs and sigma antagonists were detected using the methods described above. Always 10–12 concentrations of test compounds were used to inhibit [^{3}H]-ligand binding. These experiments were repeated at least 4 times.

4.4. Behavioural experiments

4.4.1. Apomorphine-induced yawning in rats (I, II)

The behavioural testing was performed as described by Morelli *et al.* (1986). The number of yawns was counted during 1 h after subcutaneous (s.c.) treatment with apomorphine (0.1 mg/kg). Sigma antagonists and antipsychotic compounds were injected intraperitoneally (i.p.) 30 min before the administration of apomorphine.

4.4.2. Apomorphine-induced climbing in mice (I, II)

The climbing behaviour was studied according to the method of Moore and Axton (1988): apomorphine (3 mg/kg, s.c.) and test compounds were injected 5 min and 30 min respectively before the placement of animals into individual wire net cages where the climbing activity was observed over 30 min.

4.4.3. Amphetamine- and dizocilpine-induced motor excitation in mice (I, II)

Locomotor activity was measured in individual cylindrical cages (diameter 40 cm), with 2 photocells located in the wall (Vasar *et al.*, 1990). Locomotor activity was counted between 15 and 45 min after administration of d-amphetamine (5 mg/kg, s.c.) or dizocilpine (0.25 mg/kg, i.p.). The test compounds were injected 30 min before the measurement of motor activity.

4.4.4. Apomorphine-induced stereotyped behaviour in rats (I, II)

Apomorphine (0.5 mg/kg, s.c.) was injected 30 min and the test drugs 60 min prior to the registration of stereotyped behaviour according to the scale of Costall and Naylor (Costall & Naylor, 1974). Stereotyped behaviour was measured simultaneously with the aggressive behaviour.

4.4.5. Apomorphine-induced aggressiveness in rats (I, II, III)

The animals were sensitized to apomorphine aggressiveness by 10 days of repeated treatment with apomorphine (0.5 mg/kg twice daily, s.c.) (Allikmets & Vasar, 1982). Aggressiveness was studied in the grouped rats (8–10 rats together in the test cage). Aggressive behaviour was measured in a cage (the walls of the cage were made of glass) 55×40×40 cm. Apomorphine-induced (0.5 mg/kg, s.c.) aggressiveness was assessed in a 5-minute observation period at the end of each 15-minute monitoring period. Rating of aggressive behaviour was performed on a 0–4 point scale described by Allikmets *et al.* (1979). The number of rats showing apomorphine-induced aggressive behaviour (aggressive posturing, boxing, biting, vocalization etc.) was registered. All the drugs under the study were injected i.p. 30 min before treatment with apomorphine.

4.4.6. Quipazine-induced head twitches (II)

Head twitches were induced by quipazine, an agonist of 5-HT receptors (Vetulani *et al.*, 1980). The number of head twitches was observed during 30 min after the administration of quipazine (2.5 mg/kg, i.p.). The studied compounds were injected i.p. 30 min before treatment with quipazine.

4.4.7. Behavioural studies after chronic treatment (I)

Haloperidol (0.5 mg/kg daily, i.p.), clozapine (10 mg/kg daily, i.p.) and BMY 14802 (10 mg/kg daily, i.p.) were administered to the rats for 15 days. Dizocilpine-induced behaviour was investigated 72 h after the last injection of test drugs. Dizocilpine (0.2 mg/kg) was administered s.c. 30 min prior to the registration of stereotyped behaviour according to the scale of Costall and Naylor (Costall & Naylor, 1974). After that the animals were placed into an open field (1×1×0.4 m). The numbers of line crossings and rearings during 5 min was counted. The intensity of ataxia was measured according to the method of Contreras *et al.* (1986).

Apomorphine-induced behavioural effects were also measured 72 h after the last injection of haloperidol, clozapine and BMY 14802. Apomorphine (0.15 mg/kg) was injected s.c. 15 min prior to the experiment. The intensity of stereotyped gnawing (Costall & Naylor, 1974), the numbers of line crossings, rearings, and head-dippings in the open field were registered.

4.5 Statistical analysis

In binding studies the mean apparent equilibrium dissociation constants (K_D) and maximum number of binding sites (B_{MAX}) were calculated using the nonlinear iterative curve-fitting program (Enzfitter) of Leatherbarrow (Leatherbarrow, 1987). The remainder of statistical data analyses were calculated by using Statistica for Windows (Statsoft Inc., USA) and Pharmacological Calculation System (Tallarida & Murray, 1986) statistical software. Log-plot analysis was applied to determine IC_{50} values for test compounds.

In behavioural studies the ED_{50} values for all drugs were calculated from log dose-response curves. However, in the case of apomorphine-induced stereotypy and aggressiveness, the dose of drug was detected completely blocking the behavioural effects of apomorphine.

The mean values ± S.E.M. are presented in tables and figures. Mann-Whitney U-test was used to evaluate the significance of behavioural effects of drugs. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test or Student's t-test (if two mean values were compared) were used for evaluation of the radioligand binding data.

The Pearson correlation analysis was employed to reveal the relations between the behavioural and radioligand binding data.

5. RESULTS

5.1. *In vitro* receptor binding studies

In the radioligand binding studies the antipsychotic compounds and sigma antagonists were tested to inhibit specific radioligand binding to different receptor types to find the IC_{50} values for them. These results are presented in Table 2.

Table 2

Inhibition of *in vitro* radioligand binding by test compounds

Radioligand Receptor type Structure Drug	[^{3}H]-Spiperone Dopamine D_2 Striatum	[^{3}H]-SCH 23390 Dopamine D_1 Striatum and mesolimbic area	[^{3}H]-Haloperidol Sigma Cerebellum	[^{3}H]-Ketanserin 5-$HT_{2A/2C}$ Frontal cortex
Haloperidol	5.5±0.8	350±59	1.2±0.4	200±18
Raclopride	8.9±1.1	>100000	>10000	9800±910
Chlorpromazine	16±2.9	400±60	180±11	67±4.2
Clozapine	300±25	210±42	>10000	28±1.3
Remoxipride	1400±110	>100000	110±11	15000±1400
Cinuperone	76±13	6200±550	32±3.3	240±28
BMY 14802	5100±420	44000±5600	83±9.1	15000±2200
Rimcazole	5200±530	15000±2500	280±24	2300±290
SCH 23390	1700±150	0.93±0.1	>10000	19±1.2

Results are presented as IC_{50} values in nM.

All tested antipsychotic drugs exerted notable affinity for dopamine D_2 receptors labeled with [^{3}H]-spiperone. Haloperidol and raclopride were the most potent inhibitors of [^{3}H]-spiperone binding to dopamine D_2 receptors, reducing it at a low nanomolar level. Other antipsychotic drugs such as chlorpromazine and especially clozapine were less effective inhibitors of [^{3}H]-spiperone binding in the striatum. By variance from antipsychotic compounds the IC_{50} values of sigma antagonists

(remoxipride, BMY 14802 and rimcazole) against [^{3}H]-spiperone binding were in micromolar range. Cinuperone was an exception among sigma antagonists, being a potent inhibitor of [^{3}H]-spiperone binding (IC_{50}=76 nM) to dopamine D_2 receptors.

Antipsychotic drugs like haloperidol, chlorpromazine, and clozapine displayed moderate affinity for dopamine D_1 receptors, whereas raclopride was unable to interact with this type of receptor (Table 2). High concentrations of cinuperone, BMY 14802, remoxipride and rimcazole (>10 μM) were needed to inhibit [^{3}H]-SCH 23390 binding to dopamine D_1 receptors. The most effective blocker of [^{3}H]-SCH 23390 binding was the selective dopamine D_1 antagonist SCH 23390. Majority of tested compounds (haloperidol, chlorpromazine, remoxipride, cinuperone, BMY 14802, rimcazole) were more effective at dopamine D_2 than at dopamine D_1 receptors. The only exception was an atypical antipsychotic drug clozapine that had nearly similar affinity for both dopamine receptors.

Studying drug potencies at sigma receptors showed the inability of clozapine, raclopride and SCH 23390 to inhibit specific binding of [^{3}H]-haloperidol to sigma receptors in the cerebellum up to the concentration of 10 μM, while all the other tested compounds had IC_{50} values less than 300 nM (Table 2). Again, haloperidol was by far the most potent antagonist of [^{3}H]-haloperidol binding to sigma receptors in the rat cerebellum. Remoxipride, BMY 14802, rimcazole and cinuperone were apparently more potent at sigma receptors compared to the other receptors studied.

Chlorpromazine, clozapine and SCH 23390 displayed significant affinity for 5-HT_2 receptors labeled with [^{3}H]-ketanserin (Table 2). Haloperidol and cinuperone exerted only moderate activity at these 5-HT-receptors. Very high concentrations of other compounds were needed to inhibit [^{3}H]-ketanserin binding.

All studied compounds (up to 100 μM) were ineffective against [^{3}H]-TCP and [^{3}H]-MK-801 binding in the rat frontal cortex.

5.2. Results of acute behavioural experiments (I, II)

5.2.1. Antipsychotic drugs (I, II)

In acute behavioural studies the antipsychotic compounds and sigma antagonists were tested to inhibit different drug-induced behaviours to find the ED_{50} values for them. These results are presented in Table 3.

The studied antipsychotic drugs inhibited drug-induced behaviour in the following order of potency: haloperidol > raclopride > chlorpromazine ≥ clozapine. It is worthy to note that clozapine was a relatively more effective antagonist of dizocilpine-induced motor excitation as compared to its action against amphetamine-induced locomotor stimulation. This behavioural profile differed from the other studied antipsychotics.

Table 3

Inhibition of drug-induced behaviour

Drug	Apomorphine-induced stereotypy	Apomorphine-induced aggressiveness	Apomorphine-induced climbing	Apomorphine-induced yawning	Amphetamine-induced hypermotility	Dizocilpine-induced hypermotility	Quipazine-induced head twitches
Haloperidol	0.67	0.67	0.35 (0.22–1.3)	0.13 (0.08–0.36)	0.37 (0.26–0.70)	0.43 (0.27–0.56)	0.27 (0.15–0.67)
Raclopride	2	1	1 (0.80–1.5)	0.34 (0.18–0.67)	1.6 (0.61–3.0)	1.6 (0.32–3.0)	2.1 (0.11–32)
Chlorpromazine	28	28	5.6 (2.5–14)	3.9 (0.56–6.5)	3.7 (1.9–5.9)	3.4 (0.70–7.3)	3.8 (0.98–15.0)
Clozapine	>31	31	24 (8.3–46)	3.4 (1.1–8.0)	17 (10–29)	6.4 (3.4–12)	1.9 (0.24–15)
Remoxipride	24	12	4.7 (1.4–16)	1.7 (0.29–3.3)	4.2 (1.4–8.0)	5.2 (2.1–12)	15 (3.8–57)
Cinuperone	>51	>51	10 (2.1–63)	15 (1.8–32)	4 (0.60–6.5)	2.2 (1.4–5.6)	0.97 (0.18–5.3)
BMY 14802	>115	>115	45 (20–67)	7.5 (1.5–14)	30 (16–50)	27 (7.8–68)	15 (9.7–23)
Rimcazole	>127	>127	127 (34–150)	60 (35–61)	48 (18-71)	70 (32–120)	>102
SCH 23390	2.5	0.62	0.052 (0.019–0.15)	>2.5	0.097 (0.06–0.16)	0.66 (0.10–4.2)	0.04 (0.0057–0.27)

Results are ED_{50} values and confidence limits (in brackets) for them in μmol/kg. In case of apomorphine-induced stereotypy and aggressiveness doses completely blocking these behavioural effects of apomorphine are given.

Both typical and atypical antipsychotic drugs effectively antagonized the manifestation of aggressive behaviour (Table 3). The atypical antipsychotic drug clozapine did not block the apomorphine-induced stereotypies. At high doses (>31 μmol/kg) clozapine even increased the intensity of apomorphine-induced gnawing. Raclopride also displayed higher effectiveness against apomorphine-induced aggressiveness and amphetamine-induced hyperlocomotion compared to its action against apomorphine-induced stereotyped behaviour.

The selective dopamine D_1 receptor antagonist SCH 23390 was effective in most behavioural tests at very low doses. However, much higher doses, inducing very significant catalepsy, were required to block apomorphine-induced aggressiveness and stereotyped behaviour.

5.2.2. Sigma receptor antagonists (I, II)

Overall, markedly higher doses of sigma antagonists (cinuperone, BMY 14802, and rimcazole) were needed to antagonize the behavioural effects of apomorphine, amphetamine, and quipazine, compared to antipsychotic drugs (Table 3). Furthermore, the preferential sigma antagonists (cinuperone, BMY 14802 and rimcazole) did not antagonize apomorphine-induced stereotypy and aggressiveness in the rat at all. Rimcazole did not block quipazine-induced head twitches either. In a majority of behavioural tests remoxipride was the most potent among the sigma antagonists, but in case of dizocilpine-induced hyperlocomotion and quipazine-induced head twitches cinuperone was more active compared to remoxipride.

5.2.3. Correlations between radioligand binding data and behavioural effects

Pearson correlation coefficients, calculated between the potencies of all studied compounds in behavioural tests, revealed very good correlation between different behavioural tests ($r=0.7286-0.9969$). The ED_{50} values of tested antipsychotics and sigma antagonists in the behavioural experiments correlated extremely well with their IC_{50} values at dopamine D_2 receptors ($r=0.6526-0.8732$), but not with their affinities at dopamine D_1, 5-$HT_{2A/2C}$ and sigma receptors ($r<0.48$).

5.3. Subchronic administration of antipsychotic drugs and sigma antagonists (I)

5.3.1. Results of radioligand binding (I)

Results of effects of subchronic treatment with haloperidol, clozapine and BMY 14802 on brain receptor binding parameters are presented in Table 4.

Table 4

Effect of subchronic treatment with haloperidol, clozapine and BMY 14802 on dopamine D_2, NMDA-gated channels and sigma receptors

Receptor type	Structure		Subchronic treatment			
			Saline	Haloperidol	Clozapine	BMY 14802
Dopamine D_2 receptors	Striatum	B_{MAX}	22.8±1.2	35.4±3.8*	27.4±2.0	25.4±2.1
		K_D	0.17±0.001	0.45±0.1*	0.23±0.06	0.19±0.05
	Mesolimbic area	B_{MAX}	7.5±0.8	13.1±1.7*	9.6±0.6	9.6±1.0
		K_D	0.08±0.01	0.38±0.11*	0.14±0.02*	0.12±0.04
NMDA-gated channels	Frontal cortex	B_{MAX}	115±8	120±10	152±12*	110±12
		K_D	7.0±1.5	5.2±1.2	6.5±0.6	10.1±1.6
	Hippocampus	B_{MAX}	142±13	143±14	165±15	153±14
		K_D	10.1±1.4	9.8±1.6	10.8±1.2	12.7±1
Sigma receptors	Frontal cortex	B_{MAX}	336±20	367±18	345±30	450±30*
		K_D	7.8±0.8	16.4±2.0*	10.4±1.0	13.4±1.0*
	Cerebellum	B_{MAX}	415±34	206±22*	344±30	456±32
		K_D	16.4±2.0	10±1.2	12.8±1.0	16.2±1.6

B_{MAX} values are presented in fmol/mg of wet weight tissue; K_D values are in nM. * $P < 0.05$ compared to saline treated animals.

Repeated treatment with haloperidol significantly increased the number and reduced the affinity of dopamine D_2 receptors in the striatum and mesolimbic area. The affinity of dopamine D_2 receptors labelled by [^{3}H]-spiperone in the mesolimbic structures was also decreased to some extent after long-term treatment with clozapine. The elevation of density of dopamine D_2 receptors in the subcortical structures,

induced by repeated administration of clozapine and BMY 14802, was not statistically significant.

Only the long-term administration of clozapine, an atypical antipsychotic drug, increased the density of [^{3}H]-TCP binding sites in the rat frontal cortex, whereas BMY 14802 and haloperidol were ineffective.

Repeated treatment with BMY 14802, a selective sigma receptor antagonist, increased the density and reduced the affinity of [^{3}H]-haloperidol-labelled sigma receptors in the frontal cortex. By contrast, subchronic treatment with haloperidol, as with BMY 14802, decreased the affinity of sigma sites in the frontal cortex, but unlike the sigma antagonist, it decreased nearly 50% the density of sigma receptors in the cerebellum. Repeated treatment with clozapine did not cause any noticeable changes in [^{3}H]-haloperidol binding at sigma receptors.

5.3.2. Results of behavioural experiments (I)

The administration of dizocilpine at a dose of 0.2 mg/kg induced the stereotyped behaviour like sniffing, head movements, circling behaviour and ataxia, but never gnawing stereotypies in the rat. Simultaneously, dizocilpine increased the number of line crossings but decreased the number of rearings in the open field test. Repeated treatment (15 days) with haloperidol (0.5 mg/kg daily), clozapine (10 mg/kg daily) and BMY 14802 (10 mg/kg daily) did not change stereotyped sniffing and ataxia induced by dizocilpine. Repeated treatment with clozapine significantly enhanced the effect of dizocilpine on line crossings and nearly restored the number of rearings suppressed by NMDA-gated channel non-competitive antagonist. Long-term treatment with haloperidol and BMY 14802 also increased to some extent the locomotor effect of MK-801 but this increase was statistically insignificant.

The administration of unselective dopamine agonist apomorphine (0.15 mg/kg) induced syndrome of stereotyped gnawing and significantly inhibited motor activity in rats treated with saline. Apomorphine decreased the numbers of line crossings, rearings and head-dips in the open field test. Chronic pretreatment with clozapine and BMY 14802 antagonized completely motor depressant effect of low dose of apomorphine. Long-term treatment with clozapine differently from that of haloperidol and BMY 14802 reduced intensity of stereotyped behaviour in the rat. The subchronic treatment with haloperidol also attenuated, to some extent, the motor depressant effect of apomorphine but only antagonism with the decreased number of head-dips was statistically significant.

5.4. Apomorphine-induced aggressiveness (III)

The first injection of apomorphine (0.5 mg/kg) caused a set of behavioural effects characterized by repeated sniffing, licking, and gnawing. Repeated administration of apomorphine made animals irritable on the third day of treatment: rats presented sudden bursts of locomotor activity in response to noise or the approach of another rat. Some rats displayed also the upright threatening posture, sham boxing and vocalization. The intensity of aggressive behaviour became gradually stronger during the course of repeated injections with apomorphine. All rats became aggressive by the seventh day of treatment. Increasingly vigorous tail-vibration and short bursts of locomotion always preceded this behavioural syndrome of aggressive behaviour. These behavioural manifestations occurred later after each injection of apomorphine. Aggressive behaviour became longer (it lasted about 45 min on the 10th day of treatment) during the course of repeated injections with apomorphine. Syndrome of apomorphine-induced aggressive behaviour was very steady. Once induced, any subsequent injection of apomorphine resulted in a similar behaviour. Administration of apomorphine even 3 months after the last injection of 10-day medication induced aggressive behaviour.

Neither density nor affinity of dopamine D_2 receptors labelled by [^{3}H]-spiperone was changed in the striatum after a single or a repeated treatment of rats with apomorphine (0.5 mg/kg). Ten-day apomorphine treatment significantly increased the number of [^{3}H]-MK-801 binding sites in the frontal cortex and hippocampus, but not in the striatum. The challenging of previously sensitized rats to apomorphine (0.5 mg/kg) almost normalized the density of [^{3}H]-MK-801 binding sites in the frontal cortex. However, in the hippocampus, parameters of [^{3}H]-MK-801 binding sites remained unchanged after the challenge dose of apomorphine.

Effects of antipsychotic drugs and sigma antagonists against apomorphine-induced aggressiveness were described in earlier parts (5.2.1. and 5.2.2).

A single injection of dizocilpine, a non-competitive NMDA-gated channel antagonist, did not affect stereotyped behaviour in rats sensitized to apomorphine. Administration of dizocilpine at doses above 0.25 mg/kg blocked signs of aggressive behaviour caused by apomorphine. However, this effect of dizocilpine was accompanied by a marked impairment of muscular coordination, starting at 0.25 mg/kg and worsening at 0.5 mg/kg.

The coadministration (twice daily for 10 days) of dizocilpine (0.25 mg/kg) with apomorphine (0.5 mg/kg) completely blocked the development of aggressive behaviour. However, the lowest dose of dizocilpine (0.025 mg/kg) even accelerated the onset of aggressive behaviour — all rats became aggressive already on the third day of treatments.

After completing the experiments where the aggression-blocking dose of dizocilpine (0.25 mg/kg) and apomorphine (0.5 mg/kg) were injected together the action of apomorphine was studied. The first injection of apomorphine did not induce

any signs of aggressive behaviour in these rats. The response of these rats to repeated treatment with apomorphine did not differ from that of saline-treated animals. Only on the third day of apomorphine administration were the first signs of aggressiveness evident. The administration of dizocilpine (0.25–1 mg/kg daily for 7 days) to rats sensitized to apomorphine did not affect the increased sensitivity of rats to apomorphine-induced aggressiveness.

6. DISCUSSION

6.1. Profile of tested compounds in radioligand binding studies

In radioligand binding studies performed *in vitro*, the tested compounds had rather different profiles. Haloperidol, chlorpromazine and raclopride displayed high affinity for dopamine D_2 receptors. Haloperidol was the most potent inhibitor of [^{3}H]-spiperone binding to dopamine D_2 receptors, whereas raclopride displayed the highest selectivity for these receptors. Differently from the other dopamine antagonists haloperidol had very high affinity for sigma receptors. The second important target for the action of chlorpromazine was 5-$HT_{2A/2C}$ receptor. The interaction with 5-$HT_{2A/2C}$ receptors was dominating in the action of clozapine. SCH 23390 was by far the most potent compound to inhibit radioligand binding to dopamine D_1 receptors. None of the tested antipsychotic drugs interacted with NMDA-gated channels labelled by [^{3}H]-TCP or [^{3}H]-MK-801. Therefore, the interaction with dopamine and 5-hydroxytryptamine receptors should be considered when the antipsychotic action of drugs is under the scope. This statement is in good agreement with the recent studies of Leysen *et al.* (1993).

The sigma antagonists (remoxipride, cinuperone, BMY 14802, rimcazole) displayed the highest affinity for sigma receptors compared to other receptors studied. The affinity of these compounds for dopamine and 5-HT receptors was very variable but it was always lower than their efficacy at sigma receptors. In conclusion, the radioligand binding studies revealed the interaction of antipsychotic drugs with dopamine D_1, dopamine D_2, 5-$HT_{2A/2C}$ and sigma receptors.

6.2. Comparison of typical and atypical antipsychotic drugs in acute and subchronic studies

A substantial amount of data are in favour of significant difference in the behavioural action of atypical and typical antipsychotic drugs (Costall & Naylor, 1975; Simon & Puech, 1979; Worms *et al.*, 1983). As a rule, the atypical drugs do not block apomorphine-induced stereotyped behaviour or significantly higher doses are required compared to their action in the other behavioural tests. The cataleptogenic potency of atypical compounds is very weak or missing (Costall & Naylor, 1975; Janssen & Van Bever, 1978; Niemegeers & Janssen, 1979; Worms & Lloyd, 1979).

Haloperidol, a widely used, typical antipsychotic drug, was not only a potent inhibitor of dopamine D_2 receptor binding, but it also had the most prominent action in behavioural studies. Haloperidol displayed nearly equal efficacy in all behavioural tests performed. It is worth noting that haloperidol antagonized apomorphine-induced aggressiveness only in doses blocking the stereotyped behaviour. As a matter of fact, identical doses of haloperidol induced marked catalepsy in rats. However, the

relevance of sigma receptors in the action of haloperidol should also be taken into account due to its high affinity for these binding sites.

Another typical antipsychotic drug, chlorpromazine, exerted a behavioural pattern similar to haloperidol but at substantially higher doses. The action of chlorpromazine was particularly weak against apomorphine-induced aggressiveness. The antiaggressive action of chlorpromazine was accompanied, like in case of haloperidol, with significant catalepsy and a blockade of stereotyped behaviour. The different effectiveness of haloperidol and chlorpromazine against apomorphine-induced aggressiveness is in good correlation with their clinical potency (Seeman *et al.*, 1976).

The behavioural profile of raclopride differed from that of haloperidol and chlorpromazine. Raclopride was weak in inducing catalepsy (Hillegaart & Ahlenius, 1987) and it was less effective to inhibit quipazine-induced head-twitches and apomorphine-induced stereotyped behaviour. The reduced effectiveness of raclopride against quipazine-induced head-twitches could be explained in light of the negligible affinity of raclopride for 5-HT receptors mediating this particular behaviour (Yocca *et al.*, 1991). The weaker potency of raclopride against apomorphine-induced stereotyped behaviour compared to the antagonism of raclopride with apomorphine-induced aggressiveness is linked to the regional selectivity of drug toward the mesolimbic dopaminergic system (Hillegaart & Ahlenius, 1987). Consequently, our data are in favour of the proposed idea that raclopride belongs to the atypical antipsychotic drugs (Hillegaart & Ahlenius, 1987).

Clozapine, another antipsychotic drug with atypical action, was the most effective drug against quipazine-induced head-twitches. This is in good accordance with the radioligand binding data since in the present study clozapine displayed the highest effectiveness against the binding of [^{3}H]-ketanserin to 5-HT$_{2A/2C}$ receptors. Clozapine did not prevent the stereotyped behaviour induced by apomorphine. Moreover, it even tended to increase the intensity of gnawing syndrome. The potentiation of stereotyped behaviour by clozapine differentiates it from other antipsychotic drugs. However, this effect of clozapine occurred at doses remarkably higher than required to inhibit other apomorphine-induced behaviours. Clozapine rather selectively antagonized dizocilpine, a non-competitive antagonist of NMDA-gated channels, induced hyperlocomotion in mice. This is in line with the existing data that clozapine is a potent antagonist of dizocilpine-induced behaviours in rodents (Tiedtke *et al.*, 1990). In some studies the interaction of clozapine with NMDA-gated channels has been established even in the radioligand binding studies (Janowsky & Berger, 1989). However, in the present study clozapine did not affect binding of [^{3}H]-TCP to NMDA-gated channels in *in vitro* studies at all. However, it could be possible that clozapine affects NMDA-gated channels indirectly via the noradrenergic mechanisms in the brain (Rao *et al.*, 1991). Therefore, two major conclusions can be drawn about the action of clozapine. First, clozapine obviously discriminates the dopaminergic systems in the distinct brain structures. Clozapine increased the dopaminergic activity in the striatum (increase of stereotyped behaviour), but blocked it in the mesolimbic

structures (antagonism with aggressive behaviour and amphetamine-induced hyperlocomotion). Also selective interaction of clozapine with dopamine D_4 receptors should be taken into account (Van Tol *et al.*, 1991). Second, the interaction of clozapine with more than one receptor is apparently related to the action of drug. This is in agreement with the recent hypothesis that blockade of more than one receptor is necessary to achieve antipsychotic action with limited EPS (Kahn & Davidson, 1993; Seeman & Van Tol, 1994).

Selective dopamine D_1 antagonist SCH 23390 potently antagonized most behavioural effects of apomorphine, amphetamine and quipazine. SCH 23390 was rather effective against quipazine-induced head-twitches. This is not surprising since SCH 23390 had a significant affinity for 5-$HT_{2A/2C}$ receptors (IC_{50}=19 nM). However, substantially higher doses of SCH 23390 were needed to block apomorphine-induced aggressiveness and stereotyped behaviour. This effect of SCH 23390 was accompanied by strong catalepsy. In fact the rats were unable to move at all. Therefore, the role of selective blockade of dopamine D_1 in the mechanism of action of antipsychotic drugs is rather doubtful. The correlation analysis revealed a relationship between behavioural effects of antipsychotic drugs and their affinity for dopamine D_2 receptors, but not to other receptors. Consequently, the blockade of dopamine D_2 receptors is essential for the action of antipsychotic drugs.

In subchronic studies marked differences between haloperidol and clozapine became evident. Repeated treatment with haloperidol reduced the affinity but increased the density of dopamine D_2 receptors both in the striatum and mesolimbic area. The former effect could be explained by the presence of haloperidol in the brain even 72 hours after the discontinuation of treatment. However, the increased density of dopamine D_2 receptors is probably the cause of hypersensitivity to the behavioural effects of dopamine agonists (stereotyped behaviour, hyperlocomotion) after the withdrawal of long-term haloperidol treatment (Jenner *et al.*, 1985; Gordon *et al.*, 1987). By contrast, clozapine did not increase the number of dopamine D_2 receptors in the brain. This may explain why withdrawal of long-term clozapine treatment did not increase the intensity of apomorphine-induced stereotypy. Moreover, repeated clozapine administration even tended to reduce this behaviour. Established lack of hypersensitivity at dopamine receptors is a likely reason of low incidence of EPS after long-term clozapine treatment. Furthermore, clozapine has been used to treat tardive dyskinesia caused by long-term antipsychotic treatment (Littrell & Magill, 1993; Tamminga *et al.*, 1994). Clozapine, unlike haloperidol, increased the number of NMDA-gated channels in the open state and intensity of dizocilpine induced hyperlocomotion. This finding is obviously in favour of above described action of clozapine on the neurotransmission at NMDA-gated channels. In conclusion, interaction with several receptors in the brain, including dopamine D_2, 5-HT_2 and NMDA, seems to be important in the action of clozapine.

6.3. Comparison of antipsychotic drugs and sigma antagonists

Several antipsychotic drugs (haloperidol, chlorpromazine) have a considerable affinity for sigma receptors (Su, 1982; Largent *et al.*, 1984; Tam & Cook, 1984). This was the first reason why sigma receptors were proposed to be the possible targets of antipsychotic action (Meltzer, 1991; Su, 1991). In various behavioural experiments sigma antagonists exerted a profile resembling in many ways that of atypical antipsychotic drugs. Rimcazole blocked apomorphine-induced climbing and aggressiveness but not stereotyped behaviour induced by dopamine agonist (Ferris *et al.*, 1982). Rimcazole did not disrupt the conditioned avoidance behaviour and did not produce catalepsy either (Ferris *et al.*, 1982). Cinuperone inhibited apomorphine-induced climbing, but was too weak to block amphetamine-induced stereotyped behaviour (Hock *et al.*, 1985). Remoxipride antagonized apomorphine-induced hyperlocomotion and caused modest catalepsy only at high doses (Ögren *et al.*, 1984). BMY 14802 blocked apomorphine-induced hyperlocomotion and the conditioned avoidance behaviour (Taylor *et al.*, 1985). Moreover, BMY 14802 efficiently reversed catalepsy induced by haloperidol (Taylor *et al.*, 1985). However, it should be noted that the selectivity of these compounds is not high enough and other receptors are also involved into the action of sigma antagonists (Ferris *et al.*, 1986b; Su, 1986; Largent *et al.*, 1988; Taylor & Dekleva, 1988). Therefore, to distinguish the role of sigma receptors in the action of antipsychotic drugs the sigma antagonists were compared with the typical and atypical antipsychotic compounds.

In the current study the sigma antagonists rather efficiently blocked several effects of apomorphine, amphetamine, and quipazine in acute experiments. Namely, they inhibited apomorphine-induced climbing and yawning, amphetamine- and dizocilpine-induced hyperlocomotion, and quipazine-induced head-twitches. Differently from haloperidol and chlorpromazine sigma antagonists (cinuperone, BMY 14802, rimcazole) did not block apomorphine-induced stereotypy at all or did it only at high doses (remoxipride). In this respect their pattern of action resembled that of atypical antipsychotic drugs. However, much higher doses of sigma antagonists were required compared to antipsychotic drugs and this does not support the idea that the existing sigma antagonists might be the strong antipsychotic drugs. Furthermore, sigma antagonists (except remoxipride), unlike antipsychotic drugs, were ineffective against apomorphine-induced aggressiveness even at very high doses. This is contradictory to the study of Ferris *et al.* (1982), where rimcazole was a potent antagonist of apomorphine-induced aggressiveness (Ferris *et al.*, 1982). However, in that study, an acute high challenge dose of apomorphine (5 mg/kg) was given to induce apomorphine-induced aggressiveness. This is entirely different from our approach in the present study. We employed the repeated apomorphine treatment at moderate doses, not inducing aggressive behaviour after the acute injection. In our study, doses of apomorphine as low as 0.25 mg/kg were able to evoke aggressive behaviour after the repeated treatment. Therefore, it is likely that subchronic

administration of apomorphine causes the sensitization of dopamine receptors (to be discussed in the next chapter). Consequently, rimcazole was effective against apomorphine-induced aggressiveness when sensitivity of dopamine receptors linked to aggressive behaviour was low. It is likely that the antiaggressive effect of rimcazole established by Ferris *et al.* (1982) is not due to its interaction with sigma, but with dopamine D_2 receptors. Indeed, rimcazole interacts with dopamine D_2 receptors at low micromolar range.

The only preferential sigma antagonist showing marked effectiveness against apomorphine-induced aggressiveness was remoxipride. It also inhibited most behavioural effects induced by apomorphine, amphetamine and quipazine, and even at lower doses than clozapine did. It is important to note that remoxipride was described at first as a selective dopamine D_2 antagonist (Ögren *et al.*, 1984). Only later, was the high activity of remoxipride to sigma receptors established (Largent *et al.*, 1988). In our studies remoxipride had ten-fold higher affinity for sigma receptors than for dopamine D_2 receptors. Despite that the role of dopamine D_2 receptors in the action of remoxipride should not be underestimated. Köhler *et al.* (1990) have demonstrated that in *in vivo* experiments the affinity of remoxipride for dopamine D_2 receptors was markedly higher than in *in vitro* studies.

Subchronic experiments with BMY 14802 are in favour of the idea that sigma receptors affect dopaminergic neurotransmission via presynaptic mechanisms. The motor depressant action of apomorphine, shown to be linked to stimulation of dopamine autoreceptors (Wilmot *et al.*, 1989; Reith & Selmeci, 1992), was reversed after long-term administration of BMY 14802 and haloperidol. Haloperidol induced a significant upregulation of dopamine receptors in the striatum and mesolimbic area, whereas BMY 14802 did not induce any changes at dopamine receptors. By contrast, BMY 14802 increased the density of sigma receptors in the forebrain. Therefore, the different mechanisms in the action of haloperidol and BMY 14802 must be considered. Haloperidol, by increasing the density of dopamine D_2 receptors, probably sensitized the postsynaptic dopamine receptors to the action of apomorphine. The increased sensitivity of postsynaptic dopamine receptors is a likely reason why dopamine agonist was not able to cause motor depression after long-term treatment with haloperidol. The action of BMY 14802, differently from haloperidol, seems to be related to the inhibition of presynaptic dopaminergic mechanisms. The repeated treatment with BMY 14802 is shown to inhibit electrical activity of dopamine neurons in the ventral tegmental area (Wachtel & White, 1988).

In chronic experiments, haloperidol is shown to decrease (Bremer *et al.*, 1989; Itzhak & Alerhand, 1989; Matsumoto *et al.*, 1989) and rimcazole to increase the number of sigma binding sites in the rat brain (Mariallack & Beart, 1988; Beart *et al.*, 1989). These findings were confirmed by the present study. Repeated treatment with haloperidol markedly reduced the density of sigma receptors in the cerebellum, whereas sigma antagonist BMY 14802 increased the number of these receptors in the frontal cortex. The down-regulation of a particular receptor is usually related to the

repeated action of a drug having the agonistic action at this receptor. Thus, the decrease of sigma receptors by haloperidol can be taken as a sign of agonistic, but not antagonistic interaction with sigma receptors. This opinion is in accordance with the studies of Walker *et al.* (1988) where the microinjections of haloperidol, like sigma agonists (+)-SKF 10,047, (+)-pentazocine, dextrallorphan and DTG, into the red nucleus caused significant dystonia in rats. Consequently, the possiblity exists that sigma receptors may mediate one of the side effects of antipsychotic treatment — dystonia.

Two possible mechanisms should be taken into account to explain the behavioural effects of sigma antagonists against dopamine agonists. First, sigma receptors located at dopamine neurons and nerve terminals are shown to stimulate the release of dopamine (Matthews *et al.*, 1986). Therefore, sigma antagonists reduce the dopaminergic activity by blocking these sigma receptors. Second, they interact directly with dopamine receptors. The data of correlation analysis are rather in favour of this statement since it revealed a strong positive relation between the behavioural effects of studied drugs and their affinity for dopamine D_2 receptors. Comparison of action of sigma antagonists at sigma receptors and in behavioural studies established only weak and negative correlation. Therefore, it is likely that at least some effects of sigma antagonists are linked to their weak activity at dopamine D_2 receptors, but not to their interaction with sigma receptors. There are two more arguments in favour of this statement. Clozapine and raclopride, the drugs having significant antipsychotic action, did not influence the activity of sigma receptors at all. Remoxipride, despite considerable affinity for sigma receptors, exerted the majority of its effects via blocking of dopamine D_2 receptors.

In conclusion, it is very unlikely that the existing sigma receptor antagonists could be used as antipsychotic drugs with strong action. The moderate antipsychotic-like action of sigma antagonists in the behavioural studies is probably related to their weak interaction with dopamine D_2 receptors.

6.4. Apomorphine-induced aggressiveness: a model to reveal and study new antipsychotic drugs

Psychotic disorders are mainly chronic diseases. Therefore, acute animal models can hardly be reliable to reveal new antipsychotic drugs and study their mechanism of action. Previous parts of current investigation are in favour of that. Sigma antagonists that effectively work in acute behavioural models were completely ineffective against apomorphine-induced aggressiveness. Accordingly, subchronical models should be used to reveal and study antipsychotic drugs.

Aggressiveness induced by a single high dose of apomorphine has been found to be a sensitive test to reveal antipsychotic activity of drugs. Both typical and atypical antipsychotic drugs blocked this aggressiveness. However, aggressive behaviour can

be induced also by repeated injections of moderate doses of apomorphine for 10–15 days (Allikmets & Vasar, 1982; Porreca *et al.*, 1982). Repeated treatment with dopamine agonists induces sensitization of dopaminergic system, which appears as increased motor activity and aggressiveness (Rowlett *et al.*, 1991; Druhan *et al.*, 1993; McNamara *et al.*, 1993).

Our data agree with the results of earlier studies (Allikmets & Vasar, 1982; Porreca *et al.*, 1982) which showed that repeated injections of apomorphine at moderate doses (0.5–1 mg/kg) induced aggressive behaviour in the rats. On the 7th day of repeated apomorphine treatment all rats became aggressive. Apomorphine-induced aggressiveness was very steady. Once induced, every subsequent injection of apomorphine caused a similar behaviour. Even a single treatment with apomorphine 3 months after the last injection of the 10-day apomorphine regimen induced aggressive behaviour.

Haloperidol and chlorpromazine did not show any selectivity for apomorphine-induced aggressiveness over other behaviours. They blocked apomorphine-induced stereotyped behaviour and aggressiveness at equal dose. However, several drugs belonging to atypical antipsychotic drugs (raclopride, clozapine, remoxipride, SCH 23390) antagonized apomorphine-induced aggressiveness at much lower doses as compared to stereotyped behaviour. Consequently, the target of antipsychotic action of atypical compounds seems to be the mesolimbic dopaminergic system related to aggressive behaviour, but not to the induction of stereotyped behaviour. Differently from the experiments of Levy *et al.* (1988) and Mattingly *et al.* (1991) the role of dopamine D_1 receptors in the development of aggressive behaviour is less evident as compared to that of dopamine D_2 receptors. Indeed, the data of present study are strongly in favour of dopamine D_2 receptor to be a major target of apomorphine-induced aggressiveness. There is a good correlation between antiaggressive action of antipsychotic drugs and their affinity for dopamine D_2 receptors ($r=0.86$). However, we were not able to detect any changes at dopamine D_2 receptors after a long-term treatment with apomorphine. This is in accordance with earlier studies showing that repeated administration of dopamine agonists did not change the parameters of striatal dopamine receptors in mice and rats (Riffee *et al.*, 1982; Jenner *et al.*, 1988). Therefore, one can state that the other mechanisms must be involved.

Dizocilpine, a non-competitive NMDA antagonist, has been shown to block the sensitization of rats to apomorphine-induced hyperlocomotion (Druhan *et al.*, 1993). Therefore, we used dizocilpine to reveal the involvement of NMDA-gated channels in the development of apomorphine-induced stereotypy. Dizocilpine (0.01–0.5 mg/kg) blocked aggressive behaviour at doses 0.25 mg/kg or higher. This effect was accompanied by marked impairment of motor coordination in rats. Thus, the specificity of antiaggressive effect of dizocilpine remains questionable at this point. Nevertheless, coadministration of dizocilpine (0.25 mg/kg) with apomorphine blocked, whereas a low dose of dizocilpine (0.025 mg/kg) even accelerated the development of aggressive behaviour. The blockade of apomorphine-induced

behavioural sensitization by dizocilpine is in good correlation with the studies of Druhan *et al.* (1993). Accordingly, NMDA-gated channels are involved in the changes of emotional behaviour induced by apomorphine, as they are implicated in the development of increased motor activity. The acceleration of development of apomorphine-induced aggressive behaviour by 0.025 mg/kg of dizocilpine might be related to the NMDA agonist-like effect of low doses of dizocilpine. This proaggressive action of dizocilpine seems to support the specific nature of anti-aggressive effect of NMDA antagonist at high doses. The background of such action of dizocilpine is not clear, but could be related to the accelerated development of increased sensitivity at NMDA-gated channels and subsequently at dopamine D_2 receptors.

Repeated administration of apomorphine increased the density of dizocilpine labelled NMDA-gated channels in the two brain regions studied (the frontal cortex and hippocampus). Acute exposure of these rats to apomorphine induced a marked down-regulation (to the level of control animals) of NMDA channels in the frontal cortex and striatum. Therefore, one could speculate that apomorphine induced a significant release of endogenous ligands for NMDA-gated channels (glutamate, aspartate) which desensitized their receptors.

It is very likely that NMDA-gated channels play one of the key roles in the development of apomorphine-induced aggressive behaviour. Repeated treatment with apomorphine increased the density of NMDA-gated channels in the open state since dizocilpine is the antagonist of open NMDA channels (Sills & Loo, 1989). These changes at NMDA-gated channels somehow affect the functioning of dopamine D_2 receptors without altering their number or affinity for [^{3}H]-spiperone. This poorly characterized phenomenon seems to be the main reason for the development of apomorphine-induced aggressive behaviour.

In conclusion, apomorphine-induced aggressiveness is a more valid model of psychotic behaviour than the behavioural effects induced by acute administration of apomorphine, amphetamine, and quipazine. There is a correlation between antipsychotic activity of drugs and their antiaggressive action in the described model. The sensitization of dopamine D_2 receptors seems to play the central role in the development of this behaviour. The increase of NMDA-gated channels in the open state is a possible reason of increased sensitivity at dopamine D_2 receptors.

7. CONCLUSIONS

1. The results of radioligand binding studies are in favour of involvement of dopamine D_2, 5-$HT_{2A/2C}$ and sigma receptors in the action of antipsychotic drugs and sigma antagonists. Haloperidol displayed high affinity for dopamine D_2 and sigma receptors, whereas chlorpromazine preferentially interacted with dopamine D_2 and 5-$HT_{2A/2C}$ receptors. Raclopride was the selective ligand for dopamine D_2 receptors and clozapine displayed significant affinity for 5-$HT_{2A/2C}$ receptors. SCH 23390 had very high affinity for dopamine D_1 receptors, but it also interacted with 5-$HT_{2A/2C}$ receptors. Sigma antagonists (cinuperone, remoxipride, BMY 14802 and rimcazole) had a preferential action on sigma receptors, but they also had very variable affinity for dopamine D_2 and 5-$HT_{2A/2C}$ receptors.

2. Atypical antipsychotic drugs (clozapine, raclopride, remoxipride), unlike typical ones (haloperidol, chlorpromazine), possess the selectivity for dopamine D_2 receptors in the mesolimbic area. These drugs were much stronger antagonists of apomorphine-induced aggressiveness and amphetamine-induced hyperlocomotion compared to apomorphine-induced stereotyped behaviour. Atypical antipsychotic drugs clozapine and SCH 23390 are also potent antagonists of 5-HT_2 receptors in the behavioural studies. They blocked in low doses quipazine-induced head-twitches in rats. Clozapine, unlike the other antipsychotic drugs, interacted with NMDA-gated channels. Repeated treatment with clozapine increased the density of NMDA-gated channels in the frontal cortex. This effect of clozapine was accompanied by the augmented response to dizocilpine, a non-competitive antagonist of NMDA, induced hyperlocomotion.

3. The behavioural pattern of sigma antagonists resembled that of atypical antipsychotic drugs. However, sigma antagonists did not antagonize apomorphine-induced aggressiveness. The weaker behavioural activity of sigma antagonists is probably related to their modest antagonistic activity at dopamine D_2 receptors. Repeated treatment with haloperidol, differently from BMY 14802, induced the down-regulation of sigma receptors in the brain. Thus, it is very unlikely that the blockade of sigma receptors plays a major role in the action of antipsychotic drugs.

4. Apomorphine-induced aggressiveness is a reliable model to mimic psychotic behaviour in rats. This model clearly distinguishes the action of antipsychotic drugs and sigma antagonists. There was positive correlation between the antipsychotic activity of drugs and their antiaggressive action in the described model. Repeated administration of moderate doses of apomorphine caused persistent emotional and behavioural changes in rats. The sensitization of dopamine D_2 receptors is probably a key mechanism in the development of this behaviour. The increased number of

NMDA-gated channels in the open state is a likely reason for enhanced sensitivity of dopamine D_2 receptors.

5. In general, the results of *in vitro* binding and behavioural studies confirm that the action of tested antipsychotic compounds is related mainly to their activity at dopamine D_2 receptors. The correlation analysis revealed a good correlation between the potency of tested compounds in the behavioural experiments and their affinity for dopamine D_2 receptors. However, clozapine had also a significant interaction with 5-$HT_{2A/2C}$ receptors and NMDA-gated channels. The interaction of clozapine with several neurotransmitter systems may explain its unusual antipsychotic action.

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DOPAMIINI, 5-HÜDROKSÜTRÜPTAMIINI, SIGMA JA NMDA RETSEPTORITE ROLL ANTIPSÜHHOOTILISTE AINETE TOIMES

Kokkuvõte

Antipsühhootilised ravimid on keemiliselt heterogeense struktuuriga ained, kuid enamik antipsühhootikume on tugevad dopamiini D_2 retseptorite antagonistid (Seeman, 1980; Richelson, 1984). Paraku on tüüpiliste antipsühhootiliste ainete pikaajaline kasutamine seotud ekstarpüramidaalhäirete tekkega. Seeman ja Van Tol (1994) oletavad, et edukaks kõrvaltoimetevabaks antipsühhootiliseks raviks on lisaks dopamiini D_2 retseptorite blokaadile vaja ravimi toimet ka teistel retseptoritel. Tõepoolest, risperidoon ja klosapiin, blokeerides dopamiini D_2, 5-hüdroksütrüptamiini 5-HT_{2A} ja 5-HT_{2C} retseptoreid, kõrvaldavad skisofreenia positiivse ja negatiivse sümptomaatika, põhjustamata sealjuures olulisi ekstrapüramidaalhäireid (Leysen *et al.*, 1993).

Viimastel aastatel on antipsühhootikumide ühe võimaliku toimepunktina käsitletud sigma retseptoreid. Nimelt omavad mitmed antipsühhootilised ravimid sigma retseptorite suhtes isegi suuremat afiinsust kui dopamiini retseptorite suhtes (Tam, 1983; Tam & Cook, 1984; Largent *et al.*, 1988). Samuti on kindlaks tehtud anatoomiline ning funktsionaalne seos sigma retseptorite ja dopamiinergilise süsteemi vahel ajus (Graybiel *et al.*, 1989; Taylor *et al.*, 1990; Walker *et al.*, 1990). Antipsühhootiliste ainete toimes peetakse oluliseks ka glutamaati ja NMDA-retseptorkompleksi, sest glutamaat reguleerib NMDA-retseptorkompleksi kaudu dopamiini vabanemist aju mitmetes piirkondades (Whitton *et al.*, 1994) ja mõjutab tugevasti dopamiinergilist närviülekannet striatumis (Martinez-Fong *et al.*, 1992; Whitton *et al.*, 1994). NMDA-retseptorkompleksi kuuluv mittespetsiifiline katioonkanal on oluline fentsüklidiini ja sarnaste ainete psühhootomimeetilises toimes (Javitt & Zukin, 1991), sest kõik need ained blokeerivad nimetatud kanali (Wong *et al.*, 1986). Retseptorisidumis- ja käitumiskatsete tulemused lubavad väita, et atüüpiline antipsühhootikum, klosapiin, interakteerub nimetatud sidumiskohaga (Janowsky & Berger, 1989; Tiedtke *et al.*, 1990). On isegi väidetud, et klosapiini toime skisofreenia negatiivse sümptomaatika leevendamisel võib olla seotud tema toimega NMDA-kanalitele (Tiedtke *et al.*, 1990; Schmidt *et al.*, 1991).

Eelnevast lähtudes oli antud töö eesmärgiks täpsustada arusaamu antipsühhootiliste ainete võimalikest toimemehhanismidest. Selle probleemi lahendamiseks püstitati kolm konkreetsemat ülesannet:

1) Võrrelda tüüpilisi ja atüüpilisi antipsühhootilisi aineid retseptorisidumiskatsetes ja eksperimentaalsetes käitumuslikes mudelites, mis peegeldavad

ainete antipsühhootilist aktiivsust. Erilist tähelepanu pöörati nende ainete interaktsioonile dopamiini, sigma, 5-$HT_{2A/2C}$ retseptoritega ja NMDA-retseptorkompleksiga.

2) Selgitada sigma retseptorite tähtsust antipsühhootiliste ainete toimes. Sel eesmärgil võrreldi selektiivsete sigma retseptorite antagonistide ja antipsühhootiliste ainete ühekordse ja kestva manustamise efekte käitumuslikes ja radioligand-uuringutes.

3) Senises praktikas on ainete antipsühhootiliste toime selgitamiseks rakendatud peamiselt akuutsete psühhoosilaadsete seisundite modelleerimist katseloomadel, kasutades psühhootomimeetiliste ainete ühekordset manustamist. Skisofreenia ja mitmed teised psühhootilised seisundid on aga kroonilise iseloomuga haigused. Eeltoodut silmas pidades oli otstarbekas rakendada meetodit, mis modelleeriks kestvat psühhootilist seisundit. Selleks manustati rottidele korduvalt apomorfiini, mis kutsus neil esile agressiivse käitumise. Eksperimentide käigus püüti selgitada mudeli adekvaatsust ainete antipsühhootilise toime uurimisel, samaaegselt uuriti täpsemalt NMDA-kanalite osa agressiivse käitumise kujunemisel.

Käesoleva töö *in vitro* retseptorisidumiskatsete tulemused on kooskõlas hüpoteesiga, et dopamiini D_2, 5-$HT_{2A/2C}$ ja sigma retseptorid osalevad antipsühhootiliste ainete ja sigma antagonistide toimes. Haloperidool oli väga afiinne dopamiini D_2 ja sigma retseptorite suhtes, samal ajal kui kloorpromasiin interakteerus eelistatult dopamiini D_2 ja 5-$HT_{2A/2C}$ retseptoritega. Klosapiin omas aga olulist afiinsust 5-$HT_{2A/2C}$ retseptoritel. SCH 23390 oli väga afiinne dopamiini D_1 retseptorite suhtes, kuid ta interakteerus ka 5-$HT_{2A/2C}$ retseptoritega. Sigma antagonistid (tsinuperoon, remoksipriid, BMY 14802 ja rimkasool) toimisid eelistatult sigma retseptoritel, kuid nende afiinsus dopamiini D_2 ja 5-$HT_{2A/2C}$ retseptorite suhtes oli varieeruv. Tuginedes käitumiskatsete ja *in vitro* retseptorisidumiskatsete tulemustele, võib kinnitada, et uuritud ainete antipsühhootiline efekt on eelkõige seotud nende aktiivsusega dopamiini D_2 retseptoritel. Korrelatsioonianalüüs tõi ilmsiks olulise seose ainete käitumusliku toime ja nende afiinsuse vahel dopamiini D_2 retseptorite suhtes.

Erinevalt tüüpilistest (haloperidool, kloorpromasiin) antipsühhootilistest ainetest toimisid atüüpilised (klosapiin, raklopriid ja remoksipriid) eelistatult mesolimbiliste dopamiini D_2 retseptorite kaudu. Atüüpilised antipsühhootikumid antagoniseerisid apomorfiini agressiivsust ja amfetamiini hüperlokomototsiooni märksa väiksemates annustes kui apomorfiini poolt põhjustatud stereotüüpset käitumist. Atüüpilised antipsühhootikumid, klosapiin ja SCH 23390, blokeerisid efektiivselt kvipasiinist tingitud pearaputusi rottidel, demonstreerides sellega oma tugevat toimet 5-$HT_{2A/2C}$ retseptoritel. Erinevalt teistest antipsühhootilistest ainetest toimis klosapiin ka NMDA-retseptorkompleksile. Klosapiini korduv manustamine suurendas avatud NMDA-kanalite hulka frontaalses koores. Klosapiini sellise retseptoorse efektiga kaasnes NMDA mittekonkureeriva antagonisti ditsotsilpiini motoorikat stimuleeriva toime tugevnemine. Klosapiini omapärane antipsühhootiline toimespekter on seega

põhjendatav aine toimimisega mitmetele erinevatele virgatsainesüsteemidele (dopamiin, 5-HT ja glutamaat).

Sigma antagonistide aktiivsus käitumiskatsetes meenutas atüüpiliste antipsühhootikumide toimespekterit. Ainsa olulise erinevusena sigma antagonistid ei blokeerinud apomorfiini agressiivsust. Sigma antagonistide suhteliselt nõrk aktiivsus käitumiskatsetes on ilmselt seotud nende nõrga antagonistliku toimega dopamiini D_2 retseptoritel. Oluline on ka mainida, et haloperidooli korduv manustamine erinevalt sigma antagonisti BMY 14802 manustamisest põhjustas sigma retseptorite hulga vähenemise ajus. Seetõttu on ebatõenäoline, et sigma retseptorite blokaad omaks olulist rolli antipsühhootiliste ainete toimes.

Apomorfiini mõõdukate annuste korduv manustamine kutsus esile püsiva agressiivse käitumise rottidel. Selles mudelis eristus selgelt antipsühhootiliste ainete ja sigma antagonistide toime. Eksisteeris tugev positiivne korrelatsioon ainete antipsühhootilise aktiivsuse ja antiagressiivse toime vahel antud mudelis. Püsivate käitumuslike muutuste tekkimisel omab ilmselt peamist tähtsust dopamiini D_2 retseptorite sensitiseeerumine. Dopamiini D_2 retseptorite suurenenud tundlikkuse kujunemise üheks põhjuseks on arvatavasti avatud olekus NMDA-kanalite hulga suurenemine. Läbiviidud katsed lubavad väita, et apomorfiini agressiivsus on adekvaatseks mudeliks psühhootilise käitumise uurimisel rottidel.

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PUBLICATIONS

I

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The Involvement of Sigma and Phencyclidine Receptors in the Action of Antipsychotic Drugs

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Abstract: An atypical antipsychotic drug clozapine and a selective sigma antagonist BMY 14802 were significantly less effective in the behavioural experiments (against apomorphine, d-amphetamine and MK-801), as well in the radioligand binding studies against ^{3}H-spiperone (dopamine$_2$-receptors) and ^{3}H-haloperidol (sigma receptors) in the rat brain, as compared to a typical antipsychotic compound haloperidol. Contrary to haloperidol and BMY 14802, clozapine was a relatively selective antagonist of MK-801-induced motor excitation in the mouse. A nearly 3-fold lower dose of clozapine was needed to block the effect of MK-801 (6.4 μmol/kg) as compared to the action of amphetamine (17 μmol/kg). Haloperidol and clozapine, but not BMY 14802, antagonized apomorphine-induced aggressiveness in the rat. After long-term treatment (for 15 days) with BMY 14802 (10 mg/kg daily), haloperidol (0.5 mg/kg daily) and clozapine (10 mg/kg daily) the motor depressant effect of apomorphine (0.15 mg/kg) was reversed. Chronic haloperidol treatment, but not administration of BMY 14802 and clozapine, increased the number of dopamine$_2$-receptors in the rat brain. BMY 14802 caused upregulation of sigma receptors in frontal cortex, whereas haloperidol induced the opposite change in cerebellum. Repeated treatment with clozapine significantly augmented the motor stimulating effect of MK-801 in rats. Simultaneously with a behavioural change the density of ^{3}H-TCP binding sites in the rat forebrain was elevated after long-term treatment with clozapine, probably indicating the involvement of PCP binding sites at NMDA channel in the action of clozapine.

Martin *et al.* (1976) have identified sigma opiate receptors as the sites accounting for the "mania" induced by N-allylnormetazocine (SKF 10,047) and related benzomorphans in spinal dogs. The psychotomimetic action of benzomorphans have since been attributed to non-opioid binding sites that are not sensitive to naloxone and etorphine (Su 1982). In radioligand binding studies an important separation has been made between two distinct binding sites for SKF 10,047: 1) the phencyclidine (PCP) site with low affinity for SKF 10,047, is known to be related to the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor; 2) a site with high affinity for SKF 10,047, which is now known as the sigma receptor (Largent *et al.* 1986; Tam 1985). This sigma receptor exhibits high affinity for some neuroleptic drugs, e.g. haloperidol, chlorpromazine, remoxipride (Largent *et al.* 1986 & 1988; Taylor & Dekleva 1987). Rimcazole and BMY 14802 were shown to be relatively selective ligands at sigma binding sites in CNS tissues with no appreciable affinity for dopamine receptors (Ferris *et al.* 1986; Taylor & Dekleva 1988). A putative sigma antagonist rimcazole has been shown to cause the upregulation of rat cortical sigma receptors after subchronic administration (Manallack & Beart 1988).

Several studies suggest a significant functional connection of PCP and sigma receptors with the mesolimbic and cortical dopaminergic neurones. According to Deutch *et al.* (1987) PCP increases dopamine release in the mesolimbic/cortical region and decreases it in nigrostriatal structures. The sigma receptor agonist (+)SKF 10,047 is shown to stimulate the activity of dopamine neurones in the ventral tegmental area (A_{10}) of the rat brain (Freeman & Bunney 1984) innervating the nucleus accumbens, lateral septum, prefrontal cortex and olfactory tubercle (Dahlström & Fuxe 1964). Rimcazole, an antagonist at sigma sites, effectively antagonizes (+)SKF 10,047-induced excitation of dopamine neurones in the ventral tegmental area, while having no effect on spontaneous firing of A_{10} neurones (Ceci *et al.* 1988). Wachtel & White (1988) have demonstrated that the repeated administration of the selective sigma antagonist BMY 14802, like that of the atypical neuroleptic drug clozapine, reduces the number of spontaneously active dopamine cells in ventral tegmental area without affecting the activity of dopamine cells in the substantia nigra. The observation that benzomorphans with high affinity at the sigma receptor are psychotomimetics in humans has prompted the suggestion that the selective antagonists at the sigma receptor may represent a class of novel antipsychotic compounds without extrapyramidal side effects (Tam *et al.* 1988). Janowsky & Berger (1989) have demonstrated that clozapine, moderately active compound at dopamine$_2$ receptors, is a rather potent inhibitor of ^{3}H-MK-801 binding in the rat brain, suggesting the interaction of clozapine with PCP binding sites. Byrd *et al.* (1987) have shown that long-term treatment with haloperidol increases significantly the density of PCP binding sites in the rat brain. Thus, to establish the role of dopamine$_2$-, PCP and sigma receptors in the action of antipsychotic drugs we have compared the effects of acute and chronic treatment with the classical neuroleptic drug haloperidol, the atypical antipsychotic compound clozapine and the selective antagonist at sigma receptors BMY 14802 in behavioural and radioligand binding experiments.

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Table 1.

Antagonism of haloperidol, clozapine and BMY 14802 to the behavioural effects of apomorphine, amphetamine and MK-801 in rodents and the inhibition of *in vitro* ^{3}H-radioligand binding by haloperidol, clozapine and BMY 14802 in rat brain.

Drug-induced behaviour	Haloperidol	Clozapine	Ratio CLZ vs. HAL	BMY 14802	Ratio BMY vs. HAL
Apomorphine-induced yawning (rat)	0.13 (0.08–0.36)	3.4 (1.1–8.0)	26	7.5 (1.5–14.4)	58
Apomorphine-induced climbing (mouse)	0.35 (0.22–1.3)	24.0 (8.3–46.2)	69	45.0 (20.1–67.1)	129
Amphetamine-induced motor excitation (mouse)	0.37 (0.26–0.7)	17.0 (10.1–29.3)	46	30.0 (16.4–50.0)	81
MK-801-induced motor excitation (mouse)	0.43 (0.27–0.56)	6.4 (3.4–11.9)	15	27.0 (7.8–68.0)	63
Apomorphine-induced stereotypy (rat)	0.67	>31	>46	>115	>172
Apomorphine-induced aggressiveness (rat)	0.67	31	46	>115	>172
Radioligand binding					
^{3}H-spiperone binding in striatum	5.5±0.8	300±25	55	5100±420	927
^{3}H-haloperidol binding in frontal cortex	1.8±0.4	>10000	>5555	92±10	51

The table presents ED_{50} values (μmol/kg) of compounds and confidence limits for them. The doses of compunds, completely blocking the behaviour are shown only in the case of apomorphine-induced stereotyped gnawing and aggressiveness. The results of radioligand binding studies are presented as IC_{50} values±S.E.M. in nM.

Materials and Methods

Animals. Male albino rats, weighing 250–300 g, and male albino mice, weighing 25-30 g, were used in the experiments. Animals were kept under standard laboratory conditions (temperature 20±3°), with free access to food and water.

Acute behavioural studies.

Apomorphine-induced yawning in rats. The test was performed as described by Morelli *et al.* (1986). Haloperidol, clozapine and BMY 14802 were injected intraperitoneally 30 min. before the administration of apomorphine. The number of yawns was counted during 1 hr after treatment with apomorphine (0.1 mg/kg, subcutaneously). The commercial solution of haloperidol (Gedeon Richter, Hungary) was diluted in saline, BMY 14802 (Bristol-Myers, U.S.A.) was dissolved in saline and clozapine (Sandoz, Switzerland) was suspended in saline with the help of 1–2 drops of Tween-85 (Ferak, Germany).

Apomorphine-induced climbing in mice was studied according to the method of Moore & Axton (1988): apomorphine (3 mg/kg, subcutaneously) and test compounds were injected respectively 5 min. and 30 min. prior to the placement of animals into individual wire net cages, where the climbing activity was registered during 30 min.

Amphetamine- and MK-801-induced motor excitation in mice was measured in individual cages (Vasar *et al.* 90). The cage was a cylinder (diameter 40 cm) with 2 photoce. located in the wall. Locomotor activity was counted between 15 and 45 min. after administration of d-amphetamine (7.5 mg/kg, subcutaneously) or MK-801 ((+)-5-methyl-10,11-dihydro-5-H-dibenzo[a,d]cyclohept-an-5,10-imine maleate) (0.25 mg/kg, intraperitoneally). The test compounds were injected 30 min. before the measurement of motor activity.

The ED_{50} values for all drugs were calculated from the dose-response curves.

Apomorphine-induced stereotyped behaviour in rats. Apomorphine (0.5 mg/kg, subcutaneously) was injected 30 min. and the test drugs 60 min. prior to the registration of stereotyped behaviour according to the scale of Costall & Naylor (1974). Stereotyped behaviour was measured simultaneously with aggressive behaviour.

Apomorphine-induced aggressiveness was studied in the grouped animals (8 rats in the test cage). The animals were sensitized previous to apomorphine aggressiveness by 3-weeks chronic treatment with apomorphine (0.5 mg/kg twice daily, subcutaneously) (Allikmets & Vasar 1982). The number of rats showing apomorphine-induced (0.5 mg/kg, subcutaneously) aggressive behaviour was registered. Haloperidol, clozapine and BMY 14802 were administered 30 min. before the treatment with apomorphine. In the case of apomorphine-induced stereotyped behaviour and aggressiveness the dose of drug which produced a complete blockade of the behavioural effects of apomorphine was registered.

Table 2.

The effect of repeated administration (for 15 days) of haloperidol (0.5 mg/kg daily), clozapine (10 mg/kg) and BMY 14802 (10 mg/kg) on behavioural effects of MK-801 (0.2 mg/kg) in the rat

Treatment	Intensity of stereotyped behaviour	Intensity of ataxia	No. of lines crossing	No. of rearings
Saline+saline	–	–	48±4	8.4±1.4
Saline+MK-801	1.3±0.3	1.3±0.3	97±14[a]	1.4±0.3[a]
Haloperidol+MK-801	1.6±0.3	1.8±0.3	118±12	2.3±0.8
Clozapine+MK-801	1.3±0.3	1.8±0.3	139±19[b]	6.0±2.7[b]
BMY 14802+MK-801	1.3±0.2	1.1±0.2	120±23	2.0±0.9

MK-801 was administered 72 hr after the administration of haloperidol, clozapine or BMY 14802. Saline or MK-801 were injected 30 min. before the experiment. [a]-$P<0.05$ (as compared to saline+saline-treated animals); [b]-$P<0.05$ (as compared to saline+MK-801-treated animals), Mann-Whitney U-test.

Table 3.

The effect of repeated administration (for 15 days) of haloperidol (0.5 mg/kg daily), clozapine (10 mg/kg daily) and BMY 14802 (10 mg/kg daily) on behavioural effects of apomorphine (0.15 mg/kg) in the rat.

Treatment	Intensity of stereotyped behaviour	No. of line crossings	No. of rearings	No. of head-dips
Saline + saline	–	27.3 ± 4.4	13.1 ± 2.8	3.0 ± 0.8
Saline + apomorphine	3.0 ± 0.1	14.3 ± 2.5[a]	4.1 ± 2.0[a]	1.3 ± 0.4[a]
Haloperidol + apomorphine	2.8 ± 0.3	21.6 ± 3.0	4.8 ± 3.5[a]	4.4 ± 0.6[b]
Clozapine + apomorphine	2.3 ± 0.3[b]	25.0 ± 4.7[b]	8.0 ± 2.6[b]	2.8 ± 0.9
BMY 14802 + apomorphine	2.8 ± 0.4	29.0 ± 4.5[b]	8.8 ± 3.4[b]	4.3 ± 1.3[b]

Apomorphine was injected 72 hr after the last injection of saline, haloperidol, clozapine or BMY 14802. The behaviour of rats was registered 15 min. after the administration of saline or apomorphine. [a]-$P < 0.05$ (as compared to saline + saline-treated animals); [b]-$P < 0.05$ (as compared to saline + apomorphine-treated animals), Mann-Whitney U-test.

Behavioural studies after chronic treatment. Haloperidol (0.5 mg/kg daily, intraperitoneally), clozapine (10.0 mg/kg daily, intraperitoneally) and BMY 14802 (10.0 mg/kg daily, intraperitoneally) were administered for 15 days. Seventy-two hr after the last injection of test drugs, MK-801-induced behaviour was investigated. MK-801 (0.2 mg/kg) was administered subcutaneously 30 min. prior to the registration of stereotyped behaviour according to the scale of Costall & Naylor (1974). Then the animals were placed in the open field (1 × 1 × 0.4 m). The number of line crossings and rearings during 5 min. was counted. The intensity of ataxia was measured according to the method of Contreras *et al.* (1986). Apomorphine-induced behaviour was also investigated 72 hr after the last injection of haloperidol, clozapine and BMY 14802. Apomorphine (0.15 mg/kg) was injected subcutaneously 15 min. prior to the experiment. The intensity of stereotyped gnawing (Costall & Naylor 1974), the number of line crossings, rearings and head-dippings in the open field were registered.

Binding studies. For binding studies the animals were killed by decapitation 72 hr after the last injection of drugs. The brains were rapidly removed from the skull and the brain structures were dissected on the ice. The brain structures were stored at −20° until the following procedures. Brain tissues were thawed on the day of the experiment. Pooled tissues from 4 animals were used in all radioligand experiments. Tissues were homogenized with a Potter-S homogenizer in 20 vol. ice-cold 50 mM Tris-HCl buffer (pH 7.4 or 7.7 in the case of ^{3}H-spiperone, ^{3}H-haloperidol or ^{3}H-TCP, respectively). Membranes were washed twice by centrifugation at 48000 × g for 15 min. After the last centrifugation the tissues were suspended in the incubation buffer for the appropriate binding assay. The radioligand binding studies were repeated at least three times.

^{3}H-Spiperone (109 Ci/mmol, Amersham, final concentrations 0.06–2 nM) was incubated for 30 min. at 37° with the membrane preparation (1 mg wet weight/tube) in 0.5 ml of incubation buffer consisting of Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM (pH 7.4). The non-specific binding was determined in the presence of 500 nM raclopride. The reaction was stopped by rapid centrifugation at 11,000 × g for 4 min.

^{3}H-Haloperidol binding. To define specific binding, homogenates (12 mg wet weight/tube) were incubated with increasing concentrations (2.5–80 nM) of [^{3}H]haloperidol (8.9 Ci/mmol, NEN) in the absence and presence of 10 μM haloperidol. Raclopride (500 nM) was added to each tube to block ^{3}H-haloperidol binding to dopamine$_2$ receptors. Incubation was carried out at room temperature in a total volume of 1 ml 50 mM Tris-HCl buffer (pH 7.7). After a 90 min. incubation at room temperature membrane-bound ^{3}H-haloperidol was separated from free radioligand by rapid filtration through Whatman GF/B glass fibre filters that were presoaked with 0.05% polyethyleneimine. After filtration, the filters were washed twice (4.5 ml each) with incubation buffer.

PCP binding sites were detected on membranes using 7.5 nM ^{3}H-TCP (60 Ci/mmol, NEN) in the presence of 2–100 nM of MK-801. The incubation of brain membranes (12 mg wet weight/tube) was carried out in the total volume of 0.5 ml 5 mM Tris-HCl buffer (pH 8.1 at 20°) for 45 min. at room temperature. The incubation was terminated by rapid filtration as described above.

The mean apparent equilibrium dissociation constants (K_d) and

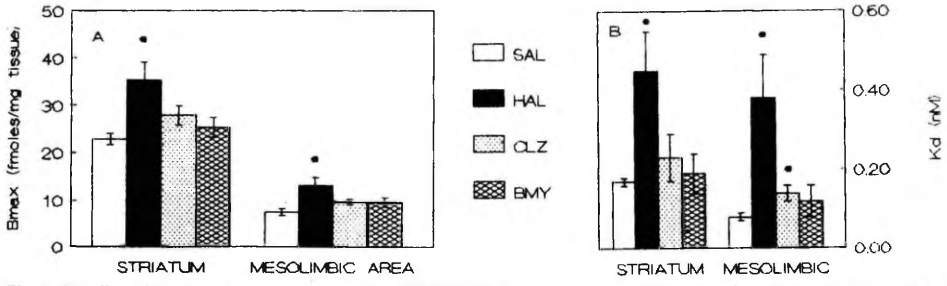

Fig. 1. The effect of long-term treatment (for 15 days) with haloperidol, clozapine and BMY 14802 on ^{3}H-spiperone binding in the striatum and mesolimbic region.
Part A – the apparent density and part B – the apparent affinity of dopamine$_2$ receptors. B_{max} – density of binding sites; K_d – dissociation constant. * – $P < 0.05$ compared to saline treated animals (Student's t-test). SAL = saline, HAL = haloperidol, CLZ = clozapine, BMY = BMY 14802.

maximum number of binding sites (B_{max}) were calculated from binding studies performed 72 hr after the last injection of test drugs using a non-linear iterative computer curve-fitting program (Enzfitter) of Leatherbarrow (1987).

The IC_{50} values for haloperidol, clozapine and BMY 14802 were detected using the methods described above. The concentrations of ^{3}H-spiperone, ^{3}H-haloperidol and ^{3}H-TCP used in displacement experiments were respectively 0.1 nM, 1.7 nM and 2 nM. Ten–12 concentrations of test compounds were used to inhibit ^{3}H-ligand binding. The experiments were repeated at least 4 times. The IC_{50} values were determined by log-plot analysis.

Results

As shown in the table 1, the studied drugs inhibited drug-induced behaviour in the following order of potency: haloperidol ≫ clozapine > BMY 14802. All three compounds had the lowest ED_{50} value against yawning induced by a low dose (0.1 mg/kg) of apomorphine (table 1). It is worth noting that clozapine was a relatively more effective antagonist of MK-801-induced motor excitation as compared to its action against amphetamine-induced locomotor stimulation. A nearly 3-fold lower dose of clozapine was needed to antagonize the effect of MK-801 (ED_{50} value 6.4 μmol/kg) compared with the action of amphetamine (ED_{50} value 17 μmol/kg). By contrast, haloperidol and BMY 14802 did not differentiate between the locomotor effects of amphetamine and MK-801. Very similar doses of haloperidol, as well as BMY 14802, were effective against amphetamine and MK-801 (table 1). Clozapine and BMY 14802, differently from haloperidol, were unable to inhibit apomorphine-induced stereotyped behaviour in the rat. Clozapine at higher doses (> 31 μmol/kg) even increased the intensity of apomorphine-induced gnawing stereotypies (data not shown). BMY 14802 did not block apomorphine-induced aggressiveness. In the radioligand binding studies haloperidol was the most potent inhibitor of ^{3}H-spiperone binding at dopamine$_2$ receptors in the rat striatum, while clozapine was only a moderately potent compound and BMY 14802 had only a very weak interaction with dopamine$_2$ receptors (table 1). In ^{3}H-haloperidol binding studies at sigma sites (the binding was performed in the presence of a selective antagonist to dopamine$_2$ receptors – raclopride) in the rat frontal cortex haloperidol had significantly higher affinity in comparison with BMY 14802. Clozapine was ineffective in antagonizing ^{3}H-haloperidol binding at sigma sites (table 1). All three compounds studied (up to 100 μM) were completely ineffective against ^{3}H-TCP binding in rat frontal cortex (data are not shown). The ratio between IC_{50} values of clozapine and haloperidol against ^{3}H-spiperone in the rat striatum was very similar to the ratio between ED_{50} values of clozapine and haloperidol against apomorphine-induced climbing, amphetamine-induced motor excitation and apomorphine-induced aggressiveness (table 1). A very similar relation was found between the IC_{50} values of haloperidol and BMY 14802 against ^{3}H-haloperidol binding in the rat frontal cortex and their ED_{50} values against apomorphine-induced yawning, amphetamine-induced motor excitation and MK-801-induced motor excitation (table 1).

MK-801 (0.2 mg/kg) caused stereotyped behaviour (sniffing, head movements, circling behaviour, but never gnawing stereotypies) and ataxia in the rat. Simultaneously MK-801 increased the number of line crossings, but decreased the number of rearings in the open field (table 2). Long-term treatment (15 days) with haloperidol (0.5 mg/kg daily), clozapine (10 mg/kg daily) and BMY 14802 (10 mg/kg daily) did not change the stereotyped sniffing and ataxia induced by MK-801. Repeated treatment with clozapine significantly enhanced the effect of MK-801 on line crossings and nearly restored the number of rearings suppressed by MK-801 (table 2). Long-term treatment with haloperidol and BMY 14802 also increased to some extent the locomotor effect of MK-801, but this increase was statistically insignificant. The administration of apomorphine (0.15 mg/kg) induced stereotyped gnawing behaviour and significantly decreased the motor activity in saline treated animals (table 3). Apomorphine decreased the number of line crossings, rearings and head-dips in the open field test. The chronic pretreatment with clozapine and BMY 14802 antagonized completely the motor depressant effect of low dose of apomorphine. Long-term treatment with clozapine, differently from haloperidol and BMY 14802, reduced the intensity of stereotyped gnawing behaviour in the rat. The chronic treatment with haloperidol also attenuated to some

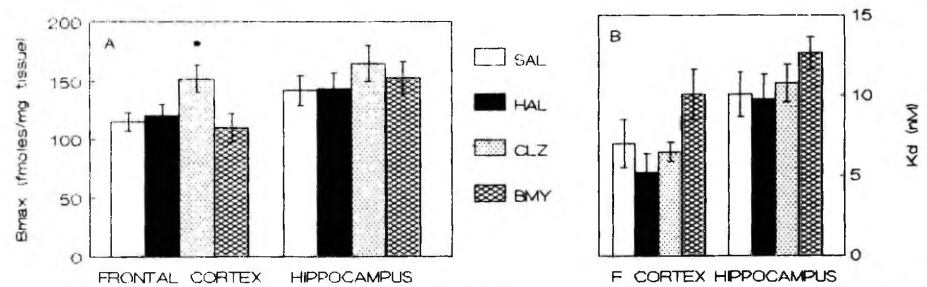

Fig. 2. The effect of long-term treatment (for 15 days) with haloperidol, clozapine and BMY 14802 on ^{3}H-TCP binding in the rat frontal cortex and hippocampus (see also explanation to fig. 1).

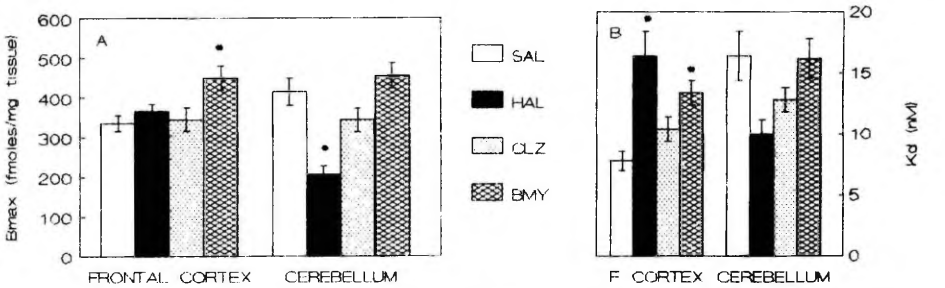

Fig. 3. The effect of long-term treatment (for 15 days) with haloperidol, clozapine and BMY 14802 on ^{3}H-haloperidol binding in the rat frontal cortex and cerebellum (see also text to fig. 1).

extent the motor depressant effect of apomorphine, but only the antagonism to the decreased number of head-dips was statistically significant (table 3).

The density of apparent binding sites for ^{3}H-spiperone in the striatum and mesolimbic area (nucleus accumbens and tuberculum olfactorium) was 22.8 ± 1.2 and 7.5 ± 0.8 fmol/mg wet weight tissue, respectively. The dissociation constant for ^{3}H-spiperone was 0.17 ± 0.01 nM in the striatum and 0.08 ± 0.01 nM in the mesolimbic structures. Repeated treatment with haloperidol significantly increased the apparent number and reduced the affinity of ^{3}H-spiperone binding sites in the striatum and mesolimbic area (fig. 1). The affinity of ^{3}H-spiperone binding sites in mesolimbic structures also decreased to some extent after long-term treatment with clozapine. The elevation of ^{3}H-spiperone binding sites' density induced by repeated administration of clozapine and BMY 14802 was not statistically significant. The apparent number of binding sites for ^{3}H-TCP in the frontal cortex and hippocampus was 115 ± 8 and 142 ± 13 fmol/mg original tissue wet weight, respectively. The dissociation constant for ^{3}H-TCP was 7.0 ± 1.5 nM in the frontal cortex and 10.1 ± 1.4 nM in the hippocampus. Only the long-term administration of clozapine induced the increase of ^{3}H-TCP binding sites' density in the rat frontal cortex, whereas BMY 14802 and haloperidol were ineffective (fig. 2). The apparent number of binding sites for ^{3}H-haloperidol (in the presence of 500 nM raclopride) in the frontal cortex and cerebellum was 336 ± 20 and 410 ± 32 fmol/mg original tissue wet weight, respectively. The dissociation constant for ^{3}H-haloperidol was 7.8 ± 0.8 nM in the frontal cortex and 15 ± 2.5 nM in the cerebellum. The long-term treatment with BMY 14802 increased the density and decreased the affinity of ^{3}H-haloperidol-labelled sigma receptors in the frontal cortex (fig. 3). By contrast, chronic treatment with haloperidol decreased like BMY 14802 the affinity of sigma sites in the frontal cortex (fig. 3), but differently from the selective sigma antagonist, haloperidol reduced the density of sigma sites in the cerebellum nearly 50% (from 410 ± 32 to 215 ± 21 fmol/mg original tissue wet weight). Long-term treatment with clozapine did not cause any statistically significant changes in ^{3}H-haloperidol binding at sigma receptors (fig. 3).

Discussion

According to the present study, haloperidol is significantly more potent than clozapine and BMY 14802 in blocking the behavioural effects of amphetamine, apomorphine and MK-801, and in inhibiting ^{3}H-radioligand binding at dopamine$_2$ and sigma receptors. Despite the significant interaction of haloperidol with sigma receptors in *in vitro* binding studies, it is rather doubtful that these receptors are playing the main role in the action of haloperidol. Haloperidol evidently antagonizes the behavioural effects of dopamine agonists (apomorphine and amphetamine) and after long-term treatment induces the up-regulation of dopamine$_2$-receptors in the striatum and mesolimbic structures. Therefore, it is most likely that the dopamine$_2$ receptor is the main target for the action of haloperidol. Nevertheless, evidence exists that sigma receptors modulate the activity of dopaminergic neurones (Steinfels & Tam 1989). The results of the present study seem to support this opinion to some extent. Indeed, the selective sigma antagonist BMY 14802 antagonized apomorphine-induced yawning and amphetamine-induced motor excitation – the behavioural effects related to the presynaptic dopaminergic mechanisms (Andén 1970; Stahle & Ungerstedt 1984; Stoessel *et al.* 1987). By contrast, BMY 14802 was completely ineffective against apomorphine-induced stereotyped behaviour and aggressiveness, where probably the postsynaptic dopamine receptors are involved (Seeman 1980; our unpublished data). The results of long-term treatment with BMY 14802 and haloperidol also seem to support the idea about the functional interaction between dopamine neurons and sigma receptors. Repeated treatment with haloperidol and BMY 14802 caused different changes in the binding of ^{3}H-spiperone and ^{3}H-haloperidol in the rat brain, but very similar changes at the behavioural level. In behavioural experiments long-term treatment with haloperidol and BMY 14802 similarly reversed the motor depressant effect of apomorphine (0.15 mg/kg), but enhanced moderately the motor stimulant effect of MK-801. Different from haloperidol, the long-term administration of BMY 14802 failed to change the parameters of ^{3}H-spiperone binding, but increased the den-

sity of sigma sites in the rat frontal cortex. Manallack & Beart (1988) have shown similar up-regulation of rat cortical sigma receptors after subchronic administrations (for 5 or 7 days) of putative sigma antagonists, rimcazole and 1,3-di(2-tolyl)guanidine. By contrast, repeated administration of haloperidol reduced significantly the density of sigma sites in the cerebellum. This finding is in good agreement with the study of Itzhak & Alerhand (1989) where also the inhibition of (+)-^{3}H-3-PPP labelled sigma sites was established in the mouse brain after repeated treatment by haloperidol. In electrophysiological studies the chronic administration of BMY 14802 suppressed the activity of mesolimbic, but not of nigrostriatal dopaminergic neurons (Wachtel & White 1988). This effect of BMY 14802 resembles very much the action of atypical neuroleptic drugs (i.e. clozapine) (Chiodo & Bunney 1983).

Clozapine, differently from haloperidol and BMY 14802, antagonized at relatively low dose the MK-801-induced hyperlocomotion. Furthermore, clozapine potently reversed MK-801-induced stereotypy in rats whereas haloperidol only delays its onset and duration (Tiedtke *et al.* 1990). Taking into consideration the finding of Janowsky & Berger (1989) that clozapine is an effective inhibitor of ^{3}H-MK-801 binding at PCP receptors, it is possible that this anti-MK-801 effect is explainable by the direct interaction of clozapine with PCP binding sites at NMDA receptors. However, our experiments do not support this opinion, clozapine even in concentrations of up to 100 μM was not able to inhibit ^{3}H-TCP binding. Nonetheless, repeated treatment with clozapine, differently from the action of haloperidol and BMY 14802, increased significantly MK-801-induced hyperlocomotion. This behavioural phenomenon was parallel to the up-regulation of PCP binding sites in the rat frontal cortex after chronic administration of clozapine, i.e. repeated treatment with clozapine induced hypersensitivity at PCP binding sites inside the NMDA receptor channel. Long-term treatment with clozapine did not change significantly the density of dopamine$_2$ receptors, however, the involvement of dopamine$_2$ receptors in the action of clozapine also seems to be obvious. A good relation exists between the ratio of IC_{50} values of clozapine and haloperidol at dopamine$_2$ receptors in the rat striatum and their ED_{50} values in the behavioural experiments (apomorphine-induced climbing and aggressiveness, amphetamine-induced motor excitation). Clozapine is a relatively active and selective antagonist of apomorphine-induced yawning and aggressiveness. Apomorphine-induced yawning and aggressiveness are probably related to the stimulation of dopamine$_2$ receptors (Yamada *et al.* 1986; our unpublished data).

In conclusion, it is probable that not only dopamine$_2$ receptors, but also sigma and PCP receptors may be involved in the action of antipsychotic drugs. Evidence exists that the selective sigma antagonist BMY 14802 decreases activity of dopamine neurones by blocking the sigma receptors (Wachtel & White 1988). Nevertheless, according to the present study the action of BMY 14802 was significantly weaker as compared to haloperidol and clozapine, the clinically effective antipsychotic drugs. Therefore, it is rather doubtful that BMY 14802 will be a strong antipsychotic drug. Kane *et al.* (1988) have shown that clozapine is an effective drug in the medication of schizophrenic patients resistant to conventional antipsychotic treatment. Thus, one could speculate that the indirect interaction with PCP binding sites has some significance in the beneficial clinical action of clozapine.

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Pharmacological Comparison of Antipsychotic Drugs and σ-Antagonists in Rodents

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Abstract: We compared antipsychotic drugs (haloperidol, chlorpromazine and clozapine) and σ antagonists (remoxipride, cinuperone, α-(4-fluorophenyl)-4-(-fluoro-2-pyrimidinyl)-1-piperazine butanol (BMY 14802) and rimcazole) in the radioligand binding and behavioural experiments in rodents. A good correlation was established between the affinity of compounds at dopamine$_2$-receptors in the striatum and their ability to block apomorphine-, amphetamine- and quipazine-induced behavioural effects in rodents. By contrast, no correlation was found between the behavioural effects of these drugs and their affinity at dopamine$_1$-, 5-HT$_2$- and σ receptors. The rank order of potency among the studied antipsychotic drugs in the behavioural tests and at dopamine$_2$-receptors was following: haloperidol≥chlorpromazine≥clozapine. The effectiveness of chlorpromazine and clozapine was nearly similar against apomorphine-induced aggressiveness and yawning, whereas at 5-HT$_2$-receptors clozapine was more active than chlorpromazine. The weak activity of σ antagonists at dopamine$_2$ receptors could be a possible reason why these compounds were less effective in the behavioural studies compared to antipsychotic drugs. However, the antagonism of remoxipride against apomorphine-induced stereotypy and aggressiveness is not related to its activity at σ receptors, because the other σ antagonists did not block these effects of apomorphine. It is probable that remoxipride exerts its action through blocking of dopamine$_2$ receptors. In conclusion, the present study revealed only weak activity of σ antagonists in the behavioural models widely used to study the antipsychotic drugs. Therefore, the antipsychotic activity of σ antagonists is doubtful.

It is generally accepted that the clinical potency of antipsychotic drugs is related to their affinity at dopamine$_2$-receptors in the striatum (Seeman *et al.* 1976). However, the antipsychotic drugs do not interact only with dopamine$_2$-receptors, but they also share high affinity for the other receptors occurring in the mammalian brain (for example dopamine$_1$, 5-HT$_2$, muscarinic, α-adrenergic, σ etc.). Nevertheless, the role of these receptors in the action of antipsychotic drugs remains still to be established.

Recently, a new group of purported antipsychotic drugs, displaying significantly higher affinity for σ than dopamine$_2$-receptors, has been developed (Largent *et al.* 1988). Several σ antagonists, like rimcazole (Ferris *et al.* 1986), cinuperone (Su 1986) and α-(4-fluorophenyl)-4-(-fluoro-2-pyrimidinyl)-1-piperazine butanol (BMY 14802) (Taylor & Dekleva 1988), has been characterized in the behavioural experiments as the 'atypical' antipsychotic compounds. Moreover, the significant relationship bwteen σ receptors and dopaminergic neurones has been established in the brain (Steinfels & Tam 1989). The localization of σ receptors on the mesencephalic dopamine neurones was shown in autoradiographic binding studies (Graybiel *et al.* 1989). The administration of the σ agonist (+)SKF 10,047 (N-allylnormetazocine) stimulates the activity of dopamine cells in the ventral tegmental area (Freeman & Bunney 1984). Rimcazole, an antagonist of σ receptors, effectively antagonizes (+)SKF 10,047-induced excitation of dopamine neurones (Ceci *et al.* 1988). In addition, long-term treatment with the selective σ antagonist BMY 14802, like that of the 'atypical' antipsychotic drug clozapine, reduces the number of spontaneously active dopamine cells in the ventral tegmental area without affecting the activity of dopamine cells in the substantia nigra (Wachtel & White 1988).

Considering the possible antipsychotic activity of σ antagonists, in the present study, an attempt was done to compare the effects of various clinically effective antipsychotic drugs (haloperidol, chlorpromazine, clozapine) with that of the preferential σ antagonists (rimcazole, remoxipride, cinuperone, BMY 14802). The first part of experiments was dedicated to reveal the interaction of all these drugs with dopamine$_1$-, dopamine$_2$-, 5-HT$_2$- and σ receptors, because all these receptors were shown to be involved to some extent in the action of various antipsychotic drugs. Simultaneously with the radioligand experiments, the behavioural studies were employed. The action of antipsychotic drugs and σ antagonists was studied upon the behavioural effects of drugs interacting with dopamine- and 5-HT-receptors (apomorphine-induced climbing and amphetamine-induced hypermotility in mice, apomorphine-induced aggressiveness, stereotypy and yawning behaviour, and quipazine-induced head-twitches in rats). The selection of these behavioural models was motivated by the wide use of these behavi-

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oural models in the preclinical screening of drugs with possible antipsychotic activity.

Materials and Methods

Animals. Male albino rats, weighing 250-300 g, and male albino mice, weighing 25-30 g, were used in the experiments. The animals were kept under standard laboratory conditions (temperature 20±3°), with free access to food and water. Mice were used in apomorphine-induced climbing and amphetamine-induced hyperlocomotion experiments. Rats were employed in the following tests: apomorphine-induced aggressiveness, stereotypy and yawning behaviour, and quipazine-induced head twitches.

Drugs. The commercial solution of haloperidol (Gedeon Richter, Hungary) was diluted in saline immediately before of the experiment. Remoxipride (Astra AB, Sweden), rimcazole (Burroughs-Wellcome, U.S.A.), BMY 14802 (α-(4-fluorophenyl)-4-(-flouro-2-pyrimidinyl)-1-piperazine butanol) (Bristol-Myers & Squibb, U.S.A.) and chlorpromazine (Sigma, U.S.A.) were dissolved in saline, whereas clozapine (Sandoz, Switzerland) and cinuperone (Hoechst, Germany) were suspended in the saline with the help of 1-2 drops of Tween-85.

Behavioural studies.

Amphetamine-induced motor excitation was measured in individual cylindrical cages (diameter 40 cm), with 2 photocells located in the wall (Vasar *et al.* 1990). Locomotor activity was counted between 15 and 45 min. after the administration of d-amphetamine (Sigma, U.S.A., 5 mg/kg, subcutaneously). The test compounds were injected 30 min. before the measurement of motor activity.

Apomorphine-induced climbing was studied according to the method of Moore & Axton (1988): apomorphine (Sigma, U.S.A., 3 mg/kg, subcutaneously) and test compounds were given respectively 5 min. and 30 min. before the placement of animals into individual wire net cages, where the climbing activity was registered during 30 min.

Apomorphine-induced stereotyped behaviour. Apomorphine (0.5 mg/kg, subcutaneously) was injected 30 min. and the test drugs 60 min. before the registration of stereotyped behaviour according to the scale of Costall & Naylor (1974). Stereotyped behaviour was detected simultaneously with aggressive behaviour.

Apomorphine-induced aggressiveness was studied in grouped animals (8 rats in the test cage). The animals were sensitized previously to apomorphine aggressiveness by 2-weeks repeated treatment with apomorphine (0.5 mg/kg, twice daily, subcutaneously) (Allikmets & Vasar 1982). The number of rats exhibiting the aggressive behaviour (aggressive posturing, boxing, biting, vocalization etc.) after the administration of apomorphine (0.5 mg/kg, subcutaneously) was registered. All the drugs in the study were injected 30 min. before treatment with apomorphine.

Apomorphine-induced yawning. The rats were tested according to method of Morelli *et al.* (1986). The number of yawns was counted during 1 hr after treatment with apomorphine (0.1 mg/kg, subcutaneously). σ Antagonists and antipsychotic compounds were injected 30 min. before the administration of apomorphine.

Quipazine-induced head twitches. Head twitches were induced by quipazine, an agonist at 5-HT-receptors (Vetulani *et al.* 1980). The number of head twitches was registered during 30 min. after the administration of quipazine (2.5 mg/kg, intraperitoneally). The compounds in the study were injected intraperitoneally 30 min. before treatment with quipazine.

The ED_{50} values for all drugs were calculated from the log dose-response curves. However, in the case of apomorphine-induced stereotypy and aggressiveness the dose of drug was detected completely blocking these behavioural effects of apomorphine.

Radioligand binding studies.

For *in vitro* binding studies the membranes prepared from the various brain structures of the rat were used. Animals were killed by decapitation. The brains were rapidly removed from the skull and the brain structures were dissected on ice. The striatum was used for [^{3}H]-spiperone and [^{3}H]-SCH 23390, the cerebellum for [^{3}H]-haloperidol, and the frontal cortex for [^{3}H]-ketanserin binding studies. Pooled brain structures from 6 rats were homogenized with a Potter-S homogenizer in 20 vol. ice-cold 50 mM Tris-HCl buffer (pH 7.4, at 4°). Membranes were washed twice by the centrifugation at 48000×g for 15 min. After the last centrifugation the crude membranes were suspended in the incubation buffer for the appropriate binding assay.

[^{3}H]-Spiperone binding. [^{3}H]-Spiperone (109 Ci/mmol, Amersham, final concentration 0.1 nM) was incubated 30 min. at 37° with the striatal membrane preparation (1 mg wet weight/tube) in 0.5 ml of incubation buffer, consisting of Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM (pH 7.4). The non-specific binding was detected in the presence of 10 μM raclopride. The reaction was stopped by rapid centrifugation at 11,000×g for 4 min.

[^{3}H]-SCH 23390 binding. Incubation of the rat striatal membranes (1 mg wet weight/tube) was carried out in 0.5 ml of incubation buffer (Tris-HCl, 50 mM, pH 7.4) during 45 min. with [^{3}H]-SCH 23390 (Amersham, 85 Ci/mmol, 2 nM) at room temperature. The non-specific binding was detected in the presence of 10 μM SCH 23390. The reaction was stopped by rapid centrifugation at 11,000 ×g for 4 min.

[^{3}H]-Haloperidol binding. The rat cerebellar membranes (12 mg wet weight/tube) were incubated with 1.7 nM of [^{3}H]-haloperidol (8.9 Ci/mmol, Dupont-NEN) in the absence and presence of 10 μM haloperidol. Spiperone (50 nM) was added to each tube to block [^{3}H]-haloperidol binding to dopamine$_2$-, 5-HT_2-, α_1 adrenergic receptors. It should be noted that the affinity of spiperone at σ receptors is only in low micromolar range (Tam & Cook 1984). The incubation was carried out at the room temperature in the total volume of 1 ml 50 mM Tris-HCl buffer (pH 7.7). After a 90 min. incubation at room temperature membrane-bound [^{3}H]-haloperidol was separated from free radioligand by rapid filtration through Whatman GF/B glass fibre filters presoaked with 0.05% polyethyleneimine. After filtration, the filters were washed twice (4.5 ml each) with incubation buffer.

[^{3}H]-Ketanserin binding. Binding of [^{3}H]-ketanserin (Dupont-NEN, 72.3 Ci/mmol, 1 nM) to frontal cortex membranes (2 mg wet weight/tube) was performed in the total volume of 1 ml 50 mM Tris-HCl buffer (pH 7.4) at room temperature. To detect non-specific binding ketanserin (10 μM) was used. After 30 min. incubation the reaction was stopped as described earlier with [^{3}H]-haloperidol binding.

Ten to twelve concentrations of test compounds were used to inhibit [^{3}H]-ligand binding. The radioactivity of samples was measured by means of liquid scintillation spectrometry, at 50 per cent efficiency level. The IC_{50} values for test compounds were determined by log-plot analysis. The experiments were repeated at least 4 times. The Spearman rank correlation test was employed to reveal the relation between the affinity of drugs at various receptors and their behavioral effects.

Results

The results of the binding studies are presented in table 1. All tested compounds exerted certain affinity at dopamine$_2$-receptors labeled with [^{3}H]-spiperone. Haloperidol was the most potent antagonist of [^{3}H]-spiperone binding to dopamine$_2$-receptors, inhibiting it at a low nanomolar level. Chlorpromazine and especially clozapine were less effective inhibitors of [^{3}H]-spiperone binding in the striatum. By

Table 1.

Inhibition of *in vitro* radioligand binding by antipsychotic drugs and σ antagonists.

Radioligand Binding site	Spiperone Dopamine$_2$	SCH 23390 Dopamine$_1$	Haloperidol σ	Ketanserin 5-HT$_2$
Drug				
Haloperidol	5.5±0.8	350±59	1.1±0.4	200±18
Chlorpromazine	16±2.9	400±60	200±13	67±4.2
Clozapine	300±25	210±42	>10000	28±1.3
Remoxipride	1400±110	>10000	120±13	>10000
Cinuperone	76±13	6200±550	54±3.9	240±28
BMY 14802	5100±420	>10000	90±9.7	>10000
Rimcazole	5200±530	>10000	260±29	2300±290

Results are presented as IC_{50} values±S.E.M. in nM.

variance from antipsychotic compounds the IC_{50} values of σ antagonists (remoxipride, BMY 14802 and rimcazole) against [^{3}H]-spiperone binding were in micromolar range. An exception among σ antagonists was cinuperone, which was a potent inhibitor of [^{3}H]-spiperone binding (IC_{50}=76 nM) to dopamine$_2$-receptors in the present experiments.

The antipsychotic drugs haloperidol, chlorpromazine and clozapine displayed only moderate affinity at dopamine$_1$ receptors (IC_{50}=210-400 nM) labeled with [^{3}H]-SCH 23390 (table 1). High concentrations of cinuperone, BMY 14802, remoxipride and rimcazole (>10 μM) were needed to block [^{3}H]-SCH 23390 binding at dopamine$_1$- receptors. Most of tested compounds (haloperidol, chlorpromazine, remoxipride, cinuperone, BMY 14802, rimcazole) were more effective at dopamine$_2$- than at dopamine$_1$-receptors, the only exception was clozapine having nearly similar affinity at both dopamine receptors.

The studying of drug potencies at σ receptors showed the inability of clozapine to inhibit the specific binding of [^{3}H]-haloperidol to σ receptors in the cerebellum up to the concentration of 10 μM, whereas all the other tested compounds had IC_{50} values less than 300 nM (table 1). Again, haloperidol was by far the most potent antagonist (IC_{50}= 1.1 nM) of [^{3}H]-haloperidol binding to σ receptors in the rat cerebellum. Remoxipride, BMY 14802, rimcazole and cinuperone were apparently more potent at σ receptors compared to the other receptors studied.

Chlorpromazine and clozapine displayed a significant affinity at 5-HT$_2$-receptors labeled with [^{3}H]-ketanserin (table 1). Haloperidol and cinuperone exerted moderate activity at these 5-HT-receptors (IC_{50} values 200 and 240 nM, respectively), whereas the remaining compounds had IC_{50} values higher than 10 μM.

The ED_{50} values of drugs against the behavioural effects of apomorphine, amphetamine and quipazine are presented in table 2. Generally, a typical antipsychotic drug haloperidol was the most effective antagonist in all behavioural experiments done. Chlorpromazine, clozapine and remoxipride were less effective if compared to haloperidol. The rank order of potency of these drugs in the behavioural studies was following: haloperidol>remoxipride>chlorpromazine ≥clozapine. Remoxipride was more effective in the behavioural tests where dopamine agonists apomorphine and amphetamine were used. Differently from haloperidol and chlorpromazine it was more selective antagonist of apomorphine-induced aggressiveness compared to stereotyped behaviour. The comparison of chlorpromazine and clozapine revealed that chlorpromazine was stronger antagonist of apomorphine-induced climbing and sterotypy, and amphetamine-induced hypermotility. Clozapine did not block apomorphine-induced stereotypy, but by variance from the other antipsychotic drugs even increased the intensity of this behaviour. Chlorpromazine and clozapine had nearly similar effectiveness against apomorphine-induced aggressiveness and yawning behaviour, whereas in the case of quipazine-induced head twitches clozapine was two times more potent than chlorpromazine. The later finding was in correlation with their affinity for 5-HT$_2$-receptors labeled by [^{3}H]-ketanserin (table 1).

The comparison of σ antagonists and antipsychotic compounds revealed a big difference between these compounds. In general, much higher doses of σ antagonists (cinuperone, BMY 14802 and rimcazole) were needed to block the above described behavioural effects of apomorphine, amphetamine and quipazine. Furthermore, the preferential σ antagonists (cinuperone, BMY 14802 and rimcazole) did not antagonize apomorphine-induced sterotypy and aggressiveness in the rat.

The correlation was established between the affinity of drugs at dopamine$_2$-receptors and their effectiveness in the various behavioural studies (the correlation coefficients 0.65-0.93, $P<0.05$). No correlation was found between the behavioural effects and affinity of drugs at the other receptors (the correlation coefficients less than 0.30, $P>0.1$).

Discussion

The present study revealed clear evidence that the potency of antipsychotic drugs and σ antagonists in various behavioural tests correlated with their affinity at dopamine$_2$-receptors. No correlation has been established between the affinity of drugs at dopamine$_1$-, 5HT$_2$- and σ receptors and their action against apomorphine-, amphetamine- and quipazine-induced behaviours. Fritz *et al.* (1993) were also unable to establish the correlation between ability of drugs to block

Table 2.

Antagonism of antipsychotic drugs and σ antagonists to the behavioural effects of apomorphine, amphetamine and quipazine in rodents.

Drug-induced behaviour	Apomorphine stereotypy	Apomorphine aggressiveness	Apomorphine climbing	Apomorphine yawning	Amphetamine hypermotility	Quipazine head-twitches
Drug						
Haloperidol	0.67	0.67	0.35	0.13	0.37	0.27
			0.22–1.3	0.08–0.36	0.26–0.70	0.15–0.67
Chlorpromazine	28	28	5.6	3.9	3.7	3.8
			2.5–14	0.56–6.5	1.9–5.9	0.98–15.0
Clozapine	>31	31	24	3.4	17	1.9
			8.3–46	1.1–8.0	10–29	0.24–15
Remoxipride	24	12	4.7	1.7	4.2	15
			1.4–16	0.29–3.3	1.4–8.0	3.8–57
Cinuperone	>51	>51	10	15	4.0	0.97
			2.1–63	1.8–32	0.60–6.5	0.18–5.3
BMY 14802	>115	>115	45	7.5	30	15
			20–67	1.5–14	16–50	9.7–23
Rimcazole	>127	>127	127	60	48	>102
			76–163	34–150	35–61	

Results are presented as ED_{50} values (μmol/kg) of compounds and confidence limits for them. The doses of compounds completely blocking the behaviour are shown in the case of apomorphine-induced stereotyped gnawing and aggressiveness.

apomorphine-induced climbing and their potency at σ receptors. Haloperidol, a widely used antipsychotic drug, was the most potent compound to inhibit [^{3}H]-spiperone binding at dopamine$_2$-receptors in the rat striatum and it also displayed the highest effectiveness in the behavioural studies. Indeed, haloperidol was very potent at σ receptors in the cerebellum, but haloperidol appears to be an agonist of σ receptors. It was established that long-term treatment with haloperidol induced the down-regulation of σ receptors in the mouse and rat brain (Itzhak & Alerhand 1989; Kizu *et al.*1991), whereas several σ antagonists (rimcazole, BMY 14802) caused the opposite effect (Manallack & Beart 1988; Lang *et a.* 1992). Thus, it is very unlikely that the current behavioural effects of haloperidol are related to the blockade of σ receptors.

Chlorpromazine and clozapine were less active at dopamine$_2$-receptors and they were less effective against apomorphine-, amphetamine- and quipazine-induced behavioural effects compared to haloperidol. The rank order of potency of antipsychotic drugs at dopamine$_2$-receptors and in the behavioural studies was as follows: haloperidol≫ chlorpromazine≥clozapine. However, it should be noted that clozapine instead of blocking of apomorphine-induced stereotypy even potentiated the intensity of this behaviour. Nevertheless, clozapine and chlorpromazine had nearly similar effectiveness against apomorphine-induced aggressiveness and yawning in rats. However, it is worthy to stress that clozapine is clinically more effective antipsychotic compound compared to chlorpromazine (Kane *et al.* 1988). Recently, very high affinity of clozapine at one subpopulation of dopamine$_2$-receptor family was established. Namely, clozapine displayed a significant activity for dopamine$_4$-receptors (Van Tol *et al.* 1991). The marked potency of clozapine and chlorpromazine at 5-HT$_2$-receptors should be also mentioned. However, clozapine was more potent than chlorpromazine to inhibit [^{3}H]-ketanserin binding to 5HT$_2$-receptors in the rat frontal cortex and to block quipazine-induced head twitches. By variance from haloperidol and chlorpromazine, clozapine did not interact with σ receptors. However, there is some evidence that clozapine can affect in some indirect way the function of N-methyl-D-aspartate receptor channels (Tiedtke *et al.* 1990; Lang *et al.* 1992).

In general, the preferential σ antagonists were apparently less effective than antipsychotic drugs to antagonize the behavioural effects of apomorphine, amphetamine and quipazine. Nevertheless, there is a growing body of evidence about the functional interaction of σ receptors and dopaminergic neurones. Indeed, the high density of σ receptors has been described on the mesencephalic dopaminergic cells (Graybiel *et al.* 1989). In addition, the administration of σ agonists increased the electrical activity of dopaminergic neurones (Freeman & Bunney 1984; Ceci *et al.* 1988; French & Ceci 1990) and facilitated the release of dopamine (Patrick *et al.* 1993). The σ agonist-induced increase of electrical activity of dopamine neurones was potently antagonized by the selective σ antagonist rimcazole (Ceci *et al.* 1988). The preferential σ antagonist BMY 14802 is shown to inhibit dose-dependently the neostriatal motor-dependent neurones in freely moving rats (Wang *et al.* 1992). According to the present study the preferential σ antagonists were effective against apomorphine-induced yawning and climbing, amphetamine-induced hyperlocomotion and quipazine-induced head twitches. However, only remoxipride blocked apomorphine-induced stereotyped behaviour and aggressiveness, whereas the other σ antagonists were completely ineffective. In the radioligand binding studies remoxipride had nearly tenfold preference for σ receptors compared to dopamine$_2$-receptors. Recent evidence suggests that remoxipride is an apparently more potent drug at dopamine$_2$-receptors than it was established in the present study. Indeed, the affinity of remoxipride against [^{3}H]-raclopride, a highly selective antagonist of dopamine$_2$-receptors,

was 10 times higher than in our present study, because K_i value for remoxipride in this experiment was 113 nM (Mohell *et al.* 1993). The presence of metabolites of remoxipride having moderate affinity for dopamine$_2$-receptors could be also a possible explanation for the marked activity of remoxipride in the behavioural experiments (Mohell *et al.* 1993). The statement about the significant activity of remoxipride at dopamine$_2$-receptors is in good accordance with results of Köhler *et al.* (1990). In contrast to classical antipsychotic drugs remoxipride is more potent at preventing of [^{3}H]-spiperone *in vivo* binding to dopamine$_2$-receptors in certain mesolimbic structures and other extrastriatal brain areas compared to its effects on striatal binding (Köhler *et al.* 1990). The higher potency of remoxipride against apomorphine-induced aggressiveness compared to stereotyped behaviour seems also to be related to its higher selectivity for limbic dopamine$_2$-receptors. The site of action of the other σ antagonists upon the behavioural effects of apomorphine, amphetamine and quipazine is less clear. However, at least two mechanisms should be considered. First, by blocking σ receptors at dopamine cells they could decrease the activity of these neurones. Second, the direct interaction of σ antagonists with dopamine$_2$-receptors should not be neglected either, because all these drugs (cinuperone, BMY 14802 and rimcazole) possessed some activity at dopamine$_2$-receptors in the radioligand binding experiments.

It is difficult to explain why cinuperone, having a considerable affinity at dopamine$_2$-receptors in *in vitro* binding studies, displayed weak activity in the behavioural experiments. However, one could speculate that the poor penetration of cinuperone through the blood-brain barrier would be a possible reason. Rimcazole was an effective antagonist of apomorphine-induced aggressiveness in earlier studies (Ferris *et al.* 1982), but we were unable to confirm this finding in the present study. This discrepancy could be explained in the light of different use of apomorphine, namely that Ferris *et al.* (1982) induced aggressiveness by acute injection of high dose of apomorphine, whereas in the present work the animals were sensitized to aggressive behaviour by two weeks pretreatment with apomorphine. Probably, the repeated dosing of apomorphine induced the hypersensitivity of dopamine receptors (Allikmets & Vasar 1982), and after that rimcazole, as weak antagonist of dopamine$_2$-receptors, was not able to block apomorphine-induced aggressiveness.

In conclusion, the present study revealed only weak activity of σ antagonists in behavioural models widely used for the study of antipsychotic drugs in rodents. Therefore, the antipsychotic activity of σ antagonists is doubtful. This pessimistic view is in accordance with the recent clinical studies where rimcazole, by contrast from remoxipride (Den Boer *et al.* 1990), did not show any considerable antipsychotic action in schizophrenic patients (Borison *et al.* 1991). However, the data of our experiments favour the existing view that the blockade of dopamine$_2$-receptors is a common feature in the action of antipsychotic drugs.

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ORIGINAL ARTICLE

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Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behaviour in rats

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Abstract We studied the aggressive behaviour induced by repeated treatment with apomorphine, a dopamine agonist (0.5 mg/kg s.c. twice daily, 10 days), in rats. The first signs of defensive aggressiveness appeared on the third day of apomorphine treatment and were generally seen on the 7th day. Aggressiveness induced by a challenge dose of apomorphine (0.5 mg/kg s.c.) on the 11th day was antagonized by haloperidol (0.05 and 0.1 mg/kg i.p.) and clozapine (10 mg/kg i.p.). An antagonist of N-methyl-D-aspartate (NMDA)-gated channels, dizocilpine (MK-801), also blocked the aggressive behaviour at 0.25 and 0.5 mg/kg i.p. but caused ataxia. When dizocilpine (0.25 mg/kg i.p.) and apomorphine were coadministered for 10 days, aggressive behaviour did not develop. At 0.025 mg/kg i.p., dizocilpine even accelerated the appearance of apomorphine-induced aggressive behaviour, which manifested on the 3rd day in all rats. In a separate study, a 7-day treatment with dizocilpine (0.25–1 mg/kg i.p.) of rats, sensitized by a prior 10-day apomorphine treatment, did not reverse the established aggressive behaviour. The coadministration of apomorphine and cholecystokinin (CCK) -A or -B antagonists, devazepide or L-365,260 (0.01–2.5 mg/kg i.p.) respectively, neither affected development of apomorphine-induced aggressive behaviour nor intensity of aggressiveness in the sensitized rats.

In binding studies neither density nor affinity of striatal dopamine D_2 receptors was changed by acute or chronic apomorphine treatment. The number of [^{3}H]pCCK-8 binding sites in the frontal cortex increased already after a single injection of apomorphine. After 10-day administration of apomorphine, a significant upregulation of [^{3}H]pCCK-8 binding sites occurred in the frontal cortex and striatum, but a downregulation was observed in the hippocampus. A challenge dose of apomorphine (0.5 mg/kg s.c.) on the 11th day of experiment, normalized the upregulated CCK receptors in the frontal cortex and striatum. Acute apomorphine did not change [^{3}H]-MK-801 binding in the rat brain. However, in rats treated for 10 days with apomorphine, the number of NMDA-gated channels in open state was increased in the frontal cortex and hippocampus. In these rats, a challenge dose of apomorphine (0.5 mg/kg s.c.) normalized also the increased number of [^{3}H]-MK-801 binding sites in the frontal cortex.

In conclusion, repeated treatment with apomorphine seems to modify the function of dopamine D_2 receptors without affecting their number or affinity. The increased number of NMDA-gated channels in open state appears to be related to this alteration of dopamine D_2 receptors. The increased density of [^{3}H]pCCK-8 binding sites in the frontal cortex may reflect anxiety and fear due to chronic exposure of rats to apomorphine.

Key words Apomorphine · Dopamine receptors · NMDA-gated channels · CCK receptors · Dizocilpine · Aggressive behaviour · CCK antagonists

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Introduction

Long-term treatment with various agonists at dopamine receptors (apomorphine, amphetamine and cocaine) induces increased expression of motor behaviour in rodents (Karler et al. 1989; Vaughn et al. 1990; Druhan et al. 1993). This sensitization appears as increased motor activity in rats and stereotyped behaviour in mice (Karler et al. 1990; Druhan et al. 1993). Even the emotional behaviour of rats can be altered by repeated administration of dopamine agonists. The acute administration of high doses (above 5 mg/kg) of apomorphine, an unselective agonist at dopamine receptors, induces aggressive behaviour in rats (Lapin and Samsonova 1968; Schneider 1968; Senault 1970). However, after prior repeated treatments with apomorphine, low doses (0.15–0.2 mg/kg) of the dopamine agonist cause defensive aggressiveness in rats (Allikmets and Vasar 1982; Porreca et al. 1982). Consequently, in the course of long-term administration of apomorphine, an apparent sensitization develops to apomorphine-induced hyperlocomotion and aggressive behaviour. Induction of aggressive behaviour by apomorphine is a well reproducible phenomenon in both male and female rats (Porreca et al. 1982).

Development of increased sensitivity to apomorphine-induced hyperlocomotion is mediated through dopamine D_1 receptors (Mattingly et al. 1991). However, there is some evidence that long-term administration of amphetamine induced hypersensitivity of dopamine D_2 instead of dopamine D_1 receptors (Levy et al. 1988). In addition, the development of increased sensitivity to amphetamine-induced hyperlocomotion seems to be related to presynaptic, but not to postsynaptic dopaminergic mechanisms in the mesolimbic structures (Wise and Leeb 1993). It is worth stressing that repeated treatment with methamphetamine induced a long-lasting decrease of dopamine uptake sites in the rat striatum (Nakayama 1993). In contrast to apomorphine-induced hyperlocomotion dopamine D_2 receptors are involved in the mediation of defensive aggressiveness induced by apomorphine (Lang et al. 1992). The clinically effective antipsychotic drugs (haloperidol, clozapine, remoxipride, chlorpromazine etc.) potently block this behavioural effect of apomorphine (Lang et al. 1992, 1994). The antiaggressive effect of antipsychotic drugs is correlated with their affinity at dopamine D_2, but not at dopamine D_1, sigma, 5-HT_2 or NMDA-receptors (Lang et al. 1992). Consequently, apomorphine-induced aggressive behaviour may serve as an animal model to reveal new potential antipsychotic drugs.

There is a growing body of evidence demonstrating a functional interaction between dopamine receptors and NMDA-gated channels. NMDA-gated channels seem to regulate the activity of the dopaminergic system not only at the level of presynaptic mechanisms, but also by affecting postsynaptic dopamine receptors. Indeed, NMDA-gated channels are involved in the regulation of dopamine release and their stimulation evoked a substantial release of dopamine in the striatum (Wang 1991; Cai et al. 1991). Furthermore, long-term administration of dizocilpine (MK-801), a noncompetitive antagonist of NMDA-gated channels, significantly increased the expression of D_2 dopamine receptor mRNA and the density of D_2 dopamine receptors in the striatum without changing the presynaptic dopaminergic activity (Micheletti et al. 1992). On the other hand, the lesion of nigrostriatal dopaminergic neurons by means of intranigral 6-hydroxydopamine injections resulted in an elevated number of NMDA-gated channels in the rat striatum (Samuel et al. 1990). Recently, it was established that the coadministration of amphetamine, cocaine and apomorphine with dizocilpine antagonized the development of hypersensitivity to dopamine agonist induced motor excitation (Karler et al. 1989; Druhan et al. 1993). These findings probably reflect an indirect involvement of NMDA-gated channels in the action of dopamine agonists, and it might explain the development of hypersensitivity of dopamine receptors. Therefore, studies on the effect of dizocilpine on apomorphine-induced aggressive behaviour were needed.

Cholecystokinin octapeptide (CCK-8), the most widely distributed neuropeptide in the brain, has been shown to regulate the activity of the dopaminergic system in the limbic structures (Crawley 1991). Two subtypes of CCK receptors (CCK_A [peripheral subtype] and CCK_B[brain subtype] receptors) mediate opposite effects of CCK-8 on K^+-stimulated dopamine release from the nucleus accumbens (Marshall et al. 1991). Moreover, the application of CCK-8 into the posterior part of nucleus accumbens (the area where CCK_A receptors dominate) enhances the stimulatory action of dopamine upon the adenylate cyclase activity, while CCK infusions into the anterior part of nucleus accumbens (the area of CCK_B receptor domination) apparently inhibit this effect of dopamine (Studler et al. 1986; Marshall et al. 1991). However, little is known about the role of CCK in the development of hypersensitivity to dopamine agonists. A high dose of caerulein (100 μg/kg) attenuated the development of apomorphine-induced aggressive behaviour (Allikmets and Vasar 1990). Therefore, in the present work an attempt was made using selective CCK receptor antagonists to clarify the role of CCK receptors in the aggressiveness induced by repeated apomorphine injections.

We first examined the actions of the noncompetitive NMDA antagonist, dizocilpine, and selective CCK antagonists (CCK_B: L-365,260 and CCK_A: devazepide), on the intensity of apomorphine-induced aggressiveness in rats already sensitized to apomorphine. Haloperidol and clozapine were used as positive controls. Then we studied the effect of dizocilpine and

CCK antagonists on the development of apomorphine-induced aggressive behaviour. In addition, changes in the density of dopamine D_2, MK-801 labeled NMDA-gated channels and CCK receptors were studied in the brains of rats treated chronically with apomorphine.

Methods

Animals. Male Wistar (Han/Kuo) rats (National Animal Center, Kuopio, Finland), weighing 180–200 g at the beginning of the experiments were kept in home cage in groups of five. The animals were maintained on food (animal pellets) and tap water ad libitum. Rats were kept in a temperature-controlled room (22 ± 1 °C) with a 12 h light-dark cycle (lights on: 8:00). Behavioural testing, as well as collecting of brain samples for radioligand binding studies were carried out between 14:00–19:00.

Behavioural testing. Apomorphine-induced aggressive behaviour was studied in grouped rats (8–10 rats together in test cage). The animals were labelled before the beginning of experiment. The highest score of aggressive behaviour displayed by each rat during the observation time was registered. Aggressive behaviour was measured in a cage with glasswalls, size 55 × 40 × 40 cm. The observation was performed 30 min after the administration of apomorphine (0.5 mg/kg) during a 5-min observation period on the 1st, 3rd, 7th and 10th day of repeated treatment with dopamine agonist. Rating of aggressive behaviour (Allikmets et al. 1979) was performed on a 0–4 point scale:

0, no aggressive manifestations; 1, intermittent upright attack posturing and contact with other rat, no vocalizations; 2, intermittent upright attack posturing and boxing, mild vocalizations; 3, continuous upright attack posture, boxing and vocalization; 4, continuous fighting and vocalization, attempts to bite an opponent rat.

When the development of aggressive behaviour with the highest score (attempts to bite an opponent animal) occurred, the rats were immediately isolated to avoid injuries.

Treatments. Apomorphine (0.5 mg/kg twice daily, at 9:00 and 17:00) was given subcutaneously for ten days. Dizocilpine (0.01–0.5 mg/kg), haloperidol (0.01–0.1 mg/kg), clozapine (1–10 mg/kg), L-365,260 (0.01–2.5 mg/kg) and devazepide (0.01–2.5 mg/kg) were injected intraperitoneally 30 min before apomorphine. To investigate the effect of dizocilpine (0.025–0.25 mg/kg) and CCK antagonists (0.01–1 mg/kg) on the development of apomorphine-induced aggressiveness, these drugs were injected 30 min before apomorphine for 10 days.

In the radioligand binding studies half of the rats (16 rats) received injections of saline and the other half injections of apomorphine (0.5 mg/kg twice daily) for 10 days. The rats were kept in the individual cages during their exposure to apomorphine in order to avoid a possible influence of fighting on the parameters of radioligand binding. When the behavioural manifestations of apomorphine's action ceased the animals were again transferred into their home cage. The same approach was used in the case of saline-treated control animals. Thus, twice a day during the exposure to apomorphine and saline (between 9:00–10:00, and between 17:00–18:00), rats were kept isolated. On the 11th day of the experiment, half the animals (8 rats) from both groups (saline and apomorphine-treated, respectively) received either saline or apomorphine (0.5 mg/kg) injections. Thirty min after the administration of saline and apomorphine, rats were killed by decapitation.

Radioligand binding experiments. Rats were decapitated and the brains were quickly dissected. The binding studies were generally performed on brain structures of individual animals. Only in the case of striatum, tissue from two rats was pooled. Brain tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 4° C) using a Potter-S glass-teflon homogenizer (1000 rpm, 12 passes). The membranes were washed twice in the same buffer by centrifugation and resuspension. For CCK-8 binding experiments the pellets were homogenized in HEPES buffer (10 mM HEPES; 130 mM NaCl; 5 mM KCl; 1 mM EDTA; pH 6.5 adjusted with 5 N NaOH) containing bovine serum albumin (0.5 mg/ml). CCK-8 receptor labelling was carried out in the presence of 0.05–2.4 nM [propionyl-^{3}H]propionylated-CCK-8sulphated ([^{3}H]pCCK-8, specific activity 79 Ci/mmol, Amersham, UK) at room temperature in a total incubation volume of 0.5 ml. Caerulein (100 nM) was added to determine nonspecific binding. For [^{3}H]-MK-801 (1–80 nM, specific activity 25 Ci/mmol, Dupont-NEN, USA) binding, the homogenized membranes were incubated in Tris-HCl buffer (50 mM) at room temperature in a total incubation volume of 0.5 ml. To detect nonspecific binding, 100 µM of unlabelled dizocilpine was used. Incubation was terminated after 120 min ([^{3}H]pCCK-8 binding) or 60 min ([^{3}H]-MK-801 binding) by rapid filtration over Whatman GF/B filters using a Brandel Cell Harvester (M-24S, USA). The filters were washed with 10 ml cold incubation buffer, dried and assayed for radioactivity by liquid scintillation spectrometry. For dopamine D_2 receptor assay [^{3}H]-spiperone (0.03–2 nM, specific activity 109 Ci/mmol, Amersham, UK) was incubated 30 min at 37° C with the striatal membrane preparation (1 mg wet weight/tube) in 0.5 ml of incubation buffer, consisting of Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM (pH 7.4). Nonspecific binding was determined in the presence of 1 µM raclopride (Astra, Sweden). The reaction was stopped by rapid centrifugation at 11000 × g for 4 min. The protein content was measured according to a modification of the Lowry procedure (Markwell et al. 1978). Saturation curves were analyzed using non-linear least squares regression (Leatherbarrow 1987).

Drugs. Apomorphine hydrochloride (Sigma, USA), dizocilpine maleate (formerly MK-801, (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate, Merck Sharp and Dohme, UK), and haloperidol (Gedeon Richter, Hungary) were dissolved in saline. Clozapine (Sandoz, Switzerland), CCK antagonists L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-3-methyl-phenyl urea) and devazepide (1-methyl-3-(2-indoloyl)amino-5-phenyl-3H-1,4-benzodiazepin-2-one) (Merck Sharp and Dohme, UK) were made soluble with the help of 1–2 drops of Tween-85 (Ferak, Germany).

Statistics. Mean values ± SEM are presented in table and figures. Mann-Whitney U-test was used for evaluation of the behavioural data. One-way analysis of variance followed by Duncan's multiple range test was used for evaluation of the radioligand binding data.

Results

Development of aggressive behaviour during repeated apomorphine treatment

The well-documented acute effects of apomorphine such as sniffing, licking and gnawing were seen following the first injection of apomorphine (0.5 mg/kg). On the third day of treatment the animals became irritable and showed sudden bursts of locomotor activity in response to noise or the approach of another rat. Some rats also displayed upright threatening posture, sham boxing and vocalization. The rats established also dominant-subordinate relationships which did not

change throughout the duration of the observation period. Further administrations of apomorphine rendered all grouped rats aggressive by the 7th day of treatment. Increasingly vigorous tail-vibration and short bursts of locomotion always preceded this syndrome of aggressive behaviour. These behavioural manifestations occurred after each injection of apomorphine. The intensity of aggressive behaviour became gradually stronger and lasted longer (about 45 min on the 10th day of treatment) during the course of repeated injections with apomorphine. The syndrome of apomorphine-induced aggressive behaviour was very steady. Once induced, any subsequent injection of apomorphine resulted in a similar behaviour. Even administration of apomorphine 3 months after the last injection of the 10-day medication induced aggressive behaviour.

Effects of single doses of dizocilpine, haloperidol and clozapine with a challenge dose of apomorphine in rats sensitized to apomorphine

Haloperidol (0.05 and 0.1 mg/kg) and clozapine (10 mg/kg) effectively antagonized the manifestation of aggressive behaviour (Fig. 1). Unlike dizocilpine, haloperidol also dose-dependently decreased the stereotyped behaviour, whereas clozapine (10 mg/kg) even potentiated this phenomenon (data not shown).

The administration of dizocilpine (0.01–0.5 mg/kg), a noncompetitive NMDA antagonist, at doses above 0.25 mg/kg blocked the signs of aggressive behaviour caused by apomorphine (Fig. 1). However, this effect of dizocilpine was accompanied by a marked failure of muscular coordination, starting at 0.25 mg/kg and worsening at 0.5 mg/kg.

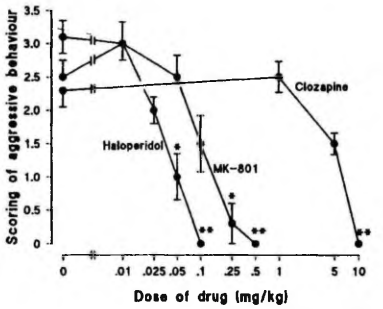

Fig. 1 Effect of dizocilpine (0.01–0.5 mg/kg i.p.), haloperidol (0.01–0.1 mg/kg i.p.) and clozapine (1–10 mg/kg i.p.) on apomorphine-induced aggressiveness in rats. The intensity of aggressive behaviour was measured 30 min after the administration of apomorphine (0.5 mg/kg s.c.). Dizocilpine, haloperidol and clozapine were given 30 min before administration of the dopamine agonist. $^{*}P < 0.05$;** $P < 0.01$ (Mann-Whitney U-test, compared to saline + apomorphine treated rats)

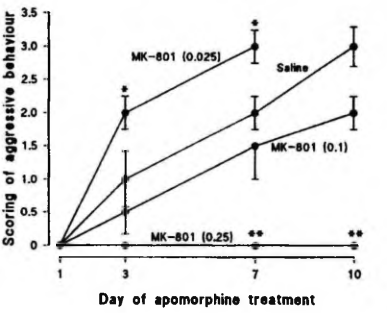

Fig. 2 Effect of repeated treatment with dizocilpine (0.025–0.25 mg/kg i.p.) and apomorphine upon the development of aggressive behaviour. The intensity of aggressive behaviour was assessed 30 min after the administration of apomorphine. $^{*}P < 0.05$; $^{**}P < 0.01$ (Mann-Whitney U-test, compared to saline + apomorphine treated rats)

Effect of coadministration of dizocilpine and apomorphine on the development of aggressive behaviour

Coadministration (twice daily for 10 days) of dizocilpine (0.25 mg/kg) with apomorphine (0.5 mg/kg) completely blocked the development of aggressive behaviour (Fig. 2). However, the lowest dose of dizocilpine (0.025 mg/kg) even accelerated the onset of aggressive behaviour. Already on the third day of treatment all rats became aggressive if treated with both apomorphine and dizocilpine (0.025 mg/kg; Fig. 2). After completing the experiment in which dizocilpine (0.25 mg/kg) and apomorphine (0.5 mg/kg) were injected together, the action of apomorphine was studied. The first injection of apomorphine did not induce any signs of aggressive behaviour in these rats. Moreover, the response of these rats to repeated treatment with apomorphine did not differ from that of saline-treated animals. This means that only on the third day of apomorphine administration the first signs of aggressiveness became evident.

Effect of repeated administration of dizocilpine on aggressive behaviour induced by the prior 10-day apomorphine treatment

The administration of dizocilpine (0.25–1 mg/kg daily for 7 days) to rats sensitized to apomorphine did not affect the increased sensitivity to apomorphine in terms of aggressiveness (data no shown).

Effect of single doses of CCK antagonists on a challenge dose of apomorphine in rats sensitized to apomorphine

Single doses of the preferential CCK_B receptor antagonist L-365,260 (0.01–2.5 mg/kg) or the preferential CCK_A receptor antagonist devazepide (0.01–2.5 mg/kg) were completely ineffective against apomorphine-induced aggressiveness (data not shown).

Effect of coadministration of CCK antagonists and apomorphine on development of aggressive behaviour

Neither L-365,260 (0.01–1 mg/kg) nor devazepide (0.01–1 mg/kg) changed the development of apomorphine-induced aggressive behaviour when injected together with apomorphine twice daily for 10 days (data not shown).

Radioligand binding experiments

Neither density ($B_{max} = 56.8 \pm 5.2$ fmol/mg protein) nor affinity ($K_d = 0.48 \pm 0.08$ nM) of dopamine D_2 receptors labelled by [^{3}H]-spiperone was changed in the striatum (the number of samples in each group was 4) after single ($B_{max} = 53.2 \pm 6.8$ fmol/mg protein; $K_d = 0.60 \pm 0.09$ nM) or repeated treatment ($B_{max} = 61.2 \pm 4.8$ fmol/mg protein; $K_d = 0.52 \pm 0.10$ nM) of rats with apomorphine (0.5 mg/kg).

Addition of apomorphine to the incubation mixture (up to 100 μM) did not affect [^{3}H]-MK-801 and [^{3}H]pCCK-8 binding in the cerebral cortex of rats in 'in vitro' experiments (data not shown).

Acute administration of apomorphine (0.5 mg/kg) to saline-treated control rats induced a significant increase of [^{3}H]pCCK-8 binding sites in the frontal cortex, but not in the other brain regions studied (Table 1).

Apomorphine treatment for 10 days increased the apparent density of [^{3}H]pCCK-8 binding sites in the frontal cortex ($F_{3,28} = 6.38$, $P < 0.01$) and striatum ($F_{3,12} = 3.65$, $P < 0.05$), but decreased it in the hippocampus ($F_{3,28} = 4.12$, $P < 0.05$) (Table 1). After the same treatment, the number of [^{3}H]-MK-801 binding sites was also significantly increased in the frontal cortex ($F_{3,28} = 3.80$, $P < 0.05$) and hippocampus ($F_{3,28} = 3.56$, $P < 0.05$), but not in the striatum ($F_{3,12} = 1.68$, $P > 0.05$) (Table 1).

Table 1 Effect of repeated apomorphine treatment on [^{3}H]-MK-801 and [^{3}H]pCCK-8 binding in the rat forebrain

Treatment Repeated: Acute:	Saline + saline	Saline + apomorphine	Apomorphine + saline	Apomorphine + apomorphine
[^{3}H]-MK-801				
Frontal cortex	$n = 8$			
K_d	14.5 ± 0.9	16.6 ± 1.1	20.8 ± 3.0	15.7 ± 0.3
B_{max}	2408 ± 136	2578 ± 142	3284 ± 216[a]	2638 ± 204[a]
Striatum	$n = 4$			
K_d	17.2 ± 0.9	15.9 ± 2.2	21.8 ± 2.2	18.5 ± 1.9
B_{max}	1088 ± 98	1028 ± 102	1356 ± 86	1158 ± 98
Hippocampus	$n = 8$			
K_d	15.9 ± 1.2	16.7 ± 1.6	15.4 ± 1.4	17.4 ± 1.7
B_{max}	1776 ± 178	2048 ± 220	2524 ± 190[a]	2714 ± 245[a]
[^{3}H]pCCK-8 binding				
Frontal cortex	$n = 8$			
K_d	0.55 ± 0.14	0.72 ± 0.19	0.79 ± 0.15	0.58 ± 0.04
B_{max}	12.5 ± 1.0	17.1 ± 1.6[a]	19.2 ± 2.0[b]	12.6 ± 0.6[a]
Striatum	$n = 4$			
K_d	0.71 ± 0.08	0.58 ± 0.05	0.76 ± 0.06	0.58 ± 0.06
B_{max}	14.5 ± 1.0	14.2 ± 1.0	17.9 ± 0.8[a]	11.2 ± 0.8[a]
Hippocampus	$n = 8$			
K_d	0.54 ± 0.08	0.53 ± 0.06	0.56 ± 0.06	0.58 ± 0.08
B_{max}	8.5 ± 1.0	8.7 ± 1.0	5.6 ± 0.9[a]	5.2 ± 0.7[a]

The experiment was performed 30 min after the administration of apomorphine or saline. The rats were pretreated with saline or apomorphine for 10 days. Results are expressed as mean values ± SEM (K_d in nM, B_{max} in fmol/mg protein). [a] $P < 0.05$ (compared to saline + saline), [b] $P < 0.01$ (compared to saline + saline); [x] $P < 0.05$ (compared to apomorphine + saline). *n* number of samples in each group

A challenge injection of apomorphine (0.5 mg/kg) to rats previously sensitized to apomorphine reduced the density of [^{3}H]pCCK-8 binding sites in the frontal cortex and striatum to the level of saline-treated control rats. The same was true for [3H]-MK-801 binding in these rats: the administration of apomorphine normalized the density of [^{3}H]-MK-801 binding sites in the frontal cortex. However, in the hippocampus, the parameters of [^{3}H]pCCK-8 and [^{3}H]-MK-801 binding sites remained unchanged after a challenge dose of apomorphine if compared to saline + apomorphine treated rats (Table 1).

Discussion

From a methodological point of view the present results are in good agreement with the study of Porreca et al. (1982) and our previous studies (Allikmets and Vasar 1982) which showed that repeated injections of apomorphine, at moderate doses (0.5–1 mg/kg), induced aggressive behaviour in the rats. The intensity of aggressive behaviour became gradually stronger during the course of repeated injections with apomorphine. The first signs of defensive aggressiveness became evident on the third day of treatment. The rats became irritable and showed sudden bursts of locomotor activity in response to noise or the approach of another rat. Some rats also displayed an upright threatening posture, sham boxing and vocalization. On the 7th day of apomorphine treatment all rats became aggressive. It is worth stressing that the apomorphine-induced aggressiveness is very steady. Once induced, every subsequent injection of apomorphine caused a similar behaviour. Even an acute administration of apomorphine 3 months after the last injection of the 10-day apomorphine regimen induced the aggressive behaviour.

Apomorphine-induced aggressiveness might serve as a model of psychotic behaviour since several antipsychotic drugs reverse this behaviour (Lang et al. 1992 1994). In the present study, a typical antipsychotic drug, haloperidol, and an atypical antipsychotic compound, clozapine, both blocked the aggressive behaviour, confirming that the model was working. There is a correlation between the antiaggressive action of antipsychotic drugs and their affinity at dopamine D_2 receptors (Lang et al. 1994). Nevertheless, we were not able to detect any changes at dopamine D_2 receptors after repeated treatment with apomorphine. This in accordance with earlier studies showing that the repeated administration of dopamine agonists did not change the parameters of striatal dopamine receptors in mice and rats (Riffee et al. 1982; Jenner et al. 1988). Therefore, other factors must be involved.

Dizocilpine (0.01–0.5 mg/kg), a noncompetitive NMDA antagonist, blocked the aggressive behaviour at doses of 0.25 mg/kg or higher. However, the effect was accompanied by marked impairment of motor coordination. Therefore, the specificity of the antiaggressive effect of dizocilpine remains questionable at this point. Nonetheless, coadministration of dizocilpine (0.25 mg/kg) with apomorphine blocked, whereas a low dose of dizocilpine (0.025 mg/kg) even accelerated, the development of aggressiveness. The acute challenge of rats with apomorphine, after repeated coadministration of apomorphine and dizocilpine (0.25 mg/kg), did not cause any signs of aggressive behaviour. Moreover, the response of these rats to subsequent repeated administration of apomorphine did not differ from that of saline-treated rats. The antagonistic interaction of dizocilpine with apomorphine-induced aggressiveness is in good accordance with recent studies which showed that an NMDA antagonist blocked the sensitization of rats to apomorphine-induced hyperlocomotion (Druhan et al. 1993). Accordingly, NMDA-gated channels are involved in the changes of emotional behaviour induced by apomorphine as they are in the development of increased motor activity. The acceleration of appearance of apomorphine-induced aggressiveness by 0.025 mg/kg of dizocilpine is difficult to explain in the light of existing data. The background of such an action of dizocilpine is ambiguous and remains to be established.

Repeated administration of apomorphine increased the density of [^{3}H]-MK-801 binding sites in two brain regions studied (frontal cortex and hippocampus). Dizocilpine was shown to be an open-channel blocker at NMDA-gated channels (Sills and Loo 1989). Therefore, it is likely that apomorphine treatment induced an increase in the proportion of NMDA channels in the open state rather than a true increase in channel numbers. Acute exposure of these rats to apomorphine induced a marked down-regulation (to the level of control animals) of [^{3}H]-MK-801 binding sites in the frontal cortex and striatum. Therefore, one could speculate that apomorphine induced a significant release of endogenous ligands for NMDA-gated channels (glutamate, aspartate), which may close these channels.

Abundant evidence supports the hypothesis that CCK-8 affects the activity of the dopaminergic system, especially in the mesolimbic region (Vaccarino and Rankin 1989; Crawley 1991). The coadministration of the unselective CCK agonist caerulein (100 μg/kg) with apomorphine attenuated the development of apomorphine-induced aggressiveness in rats (Allikmets and Vasar 1990). However, caerulein was unable to modify the already existing hypersensitivity to apomorphine-induced aggressive behaviour (Allikmets and Vasar 1990). Based on electrophysiological findings, Rasmussen et al. (1991, 1993) recently proposed that CCK_B antagonists could display an antipsychotic activity. In our study, blocking of CCK receptors by the CCK_B receptor antagonist L-365,260 (0.01–2.5 mg/kg) and the CCK_A receptor antagonist devazepide (0.01–2.5 mg/kg) neither affected the development nor

the intensity of apomorphine-induced aggressiveness. Taking into account the potent interaction of antipsychotic drugs with apomorphine-induced aggressive behaviour, it is unlikely that the currently existing CCK antagonists possess a strong antipsychotic action.

Acute administration of apomorphine (0.5 mg/kg) to saline-treated control rats induced a significant increase of [^{3}H]pCCK-8 binding sites in the frontal cortex, but not in the other structures. Moran et al. (1986) have established that CCK-8 binding sites in the cerebral cortex belong to the CCK_B receptor subtype. It is worth noting that the acute administration of apomorphine (1 mg/kg) also increased the amount of CCK-8-like immunoreactivity in the medial prefrontal cortex (Fukamauchi and Takahashi 1988). Different anxiogenic manipulations of rats (administration of beta-carbolines, exposure of rats to plus-maze, benzodiazepine withdrawal, social isolation of rats, etc.) have similarly been shown to induce an increase in cortical [^{3}H]pCCK-8 binding (Harro et al. 1993; Vasar et al. 1993). Therefore, we suggest that the acute treatment with apomorphine caused an anxiogenic-like action in rats. Further administrations of apomorphine induced even more pronounced elevation of [^{3}H]pCCK-8 binding sites in the frontal cortex and striatum, whereas in the hippocampus a decrease of these binding sites was evident. It is likely that these alterations are a reflection of anxiety and fear due to repeated exposure of rats to apomorphine.

In conclusion, the present results suggest that NMDA-gated channels play a role in the development of apomorphine-induced aggressive behaviour. Repeated treatment with apomorphine increased the number of NMDA-gated channels in the open state in the frontal cortex and hippocampus. These changes affect the functioning of dopamine D_2 receptors without altering their number on affinity to [^{3}H]-spiperone. This poorly characterized phenomenon seems to be the main reason for the development of apomorphine-induced aggressive behaviour. The role of CCK in the development of apomorphine-induced aggressiveness, if any, is probably indirect and the increased density of CCK_B receptors in the frontal cortex is likely reflecting anxiety and fear due to chronic exposure of rats to apomorphine.

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CURRICULUM VITAE

Aavo Lang was born June 15, 1964, in Tartu, Estonia. In 1988, he graduated from the Medical faculty of the University of Tartu as an M.D. After that he worked at the Laboratory of Hormonal Regulation in the Department of Science, University of Tartu (1988–1992). Between 1992 and 1993, he worked as a Researcher at the Laboratory of Experimental and Clinical Psychopharmacology, in the University's Department of General and Molecular Pathology. Since 1992, he has been an Assistant in the Department of Physiology, University of Tartu.

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He has passed several study courses: Drug Research Course, Espoo, Finland, 1991; Laboratory Animals in Biomedical Research, Tartu, 1994.

Aavo Lang is a member of the Estonian and the European Physiological Societies.

His main scientific interest is pharmacology of antipsychotic drugs and pharmacology of cholecyctokininergic system. He has published 33 scientific papers.

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