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SPECT imaging in young patients with schizophrenia

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Jules Lavalaye

Chapter 3 Figure 3.1 123I-IBZM SPECT images of a healthy control and two schizophrenic patients treated with olanzapine (15 mg) and risperidone (4 mg). Transverse slices from the brain at the level of the striatum. The images of the patients clearly show much lower striatal binding of 123I-IBZM than in the control subject. Levels of SPECT activity are colour encoded from low (black) to high (white).
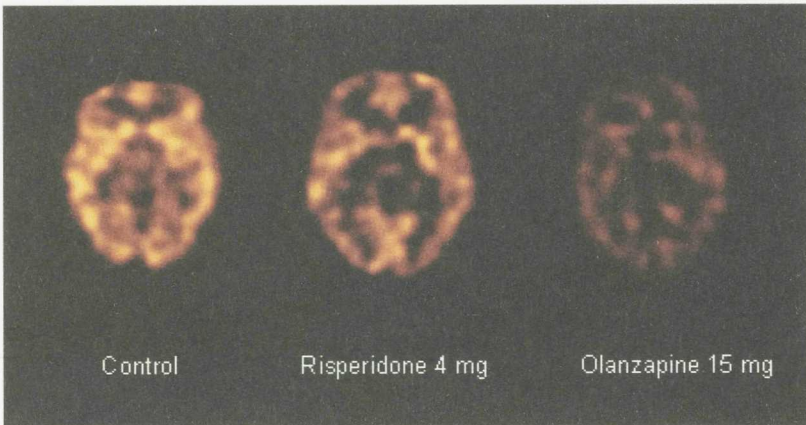
Chapter 10 Figure 3. [123I]-IDEX SPECT transversal slices at the level of the striatum of a patient treated with olanzapine 15 mg, risperidone 4 mg and a control subject. [123I]-IDEX binding in the striatum and cortex of the patient with olanzapine is lower than in the control, reflecting higher level of muscarinic receptor occupancy.

Stellingen behorende bij het proefschrift:

SPECT imaging in young patients with schizophrenia

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- Olanzapine en risperidone verschillen niet in dopamine D_2 receptorbezetting in vivo

- Hogere D_2 receptorbezetting door antipsychotica gaat gepaard met een slechter subjectief welbevinden van patiënten met schizofrenie

- Antipsychotica en dopaminomimetica hebben geen effect op de binding van $[^{123}I]$-FP-CIT aan dopamine transporters in het striatum van de rat

- De gepostuleerde overactiviteit van het dopamine systeem bij patiënten met schizofrenie berust waarschijnlijk niet op een verhoogd aantal nigrostriatale dopaminerge neuronen

- Vrouwen hebben meer dopamine en serotonine transporters dan mannen

- Tardieve dyskinesie wordt niet verklaard door dopaminerge neurotoxiciteit van antipsychotica

- Olanzapine veroorzaakt een hogere bezetting van muscarine receptoren dan risperidone

- Hoger doseren van antipsychotica leidt helaas niet tot een beter klinisch effect, maar wel tot meer bijwerkingen

- Voor een eerlijke vergelijking dienen nieuwe antipsychotica in medicatieonderzoeken vergeleken te worden met klassieke antipsychotica in een klinisch relevante dosering

- Het samenvoegen van de afdeling psychiatrie met het hoofdgebouw van het AMC getuigt van inzicht in de inhoudelijke toenadering tussen somatiek en psychiatrie, en daarbij is het ook een stuk minder ver lopen

- De hersenen als biologisch orgaan zijn een tijd lang over het hoofd gezien

- De biologische verklaringen van de hersenen doen niets af aan het unieke karakter ervan

- The noise is not the real activity - (Dessislava Karoushkova)

SPECT imaging

in young patients with schizophrenia

Cover design by Robin Uleman

SPECT imaging

in young patients with schizophrenia

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. J.J.M. Franse

ten overstaan van een door het college voor promoties ingestelde

commissie, in het openbaar te verdedigen in de Aula der Universiteit

op woensdag 25 april 2001, te 10.00 uur

door Jules Lavalaye

geboren te Sint Michielsgestel

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Faculteit der Geneeskunde

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General introduction

The research project "SPECT imaging in young patients with schizophrenia" was started in 1997 as a co-operation between the Adolescent Clinic (department of Psychiatry) and the department of Nuclear Medicine of the Academic Medical Center in Amsterdam. This project was preceded by a large study funded by the Prevention Fund (Preventiefondsonderzoek I) which took place in the Adolescent Clinic from 1986 to 1992. The focus of that study was also on young patients with recent-onset schizophrenia and related psychotic disorders, studying personal and environmental risk as well as protective factors in relation with psychotic relapse. The study used the vulnerability-stress model as developed by Zubin and Spring (Zubin and Spring, 1977) and Nuechterlein an co-workers (Nuechterlein et al., 1992) as a framework. The model had as a primary focus the clinical course of schizophrenia, rather than aetiology, and included components that were supposed to play a role in psychotic relapse. Four classes of components were distinguished in the model: 1) enduring personal vulnerability factors, 2) personal protective factors, 3) environmental potentiators and stressors and 4) environmental protective factors.

Three dissertations of the first project covered the relations between symptom aetiology, cognitive and social dysfunctioning.
One covered the personal vulnerability domain (Van der Does, 1994) and one covered the family affective style and communication deviance (Nugter, 1997) in the environmental domain, both from a longitudinal perspective. The third dissertation examined the effects of psychosocial interventions including pharmacotherapy in a randomised clinical trial and other risk and protective factors in a population, that appeared to have an usual short duration of untreated psychosis (Linszen, 1993). Family support, education and problem-solving plus individual supportive treatment turned out to be effective: the relapse rate was low (15%) and the compliance rate to antipsychotic medication was high (Linszen et al., 1996). Cannabis abuse turned out to be a risk factor for psychotic relapse (Linszen et al., 1994).

A sequel of the study was planned to examine some findings in detail, i.e., in the biological domain, in a larger population and with a longer follow-up with regular ratings of psychopathology, subjective experience and social functioning: "Risk and protective factors in the early course of recent-onset schizophrenia". The project was sponsored by a grant of the Dutch Health Research and Development Council (ZON).

This second study was expanded with biological markers in the fields of neurotransmission, neurophysiology, neuropsychology, and psychopharmacology. The expansion served a comprehensive biopsycho-social model of schizophrenia as a brain disease, including etiological and pathophysiological aspects. The dopamine hypothesis stood at the cradle of crucial questions, including the role of dopamine antagonists ameliorating symptoms of schizophrenia and subjective experience.

The research line of the department of Nuclear Medicine was focussed on neuroimaging, covering both presynaptic and postsynaptic dopamine imaging (thesis by Booij (Booij, 1998) and by Verhoeff (Verhoeff, 1993), respectively). A co-operation between the two departments resulted in the plans for integrating dopamine neuroimaging in the new Prevention Fund II project. Moreover, the new project was completed with an antipsychotic drug trial, comparing two new atypical antipsychotics, olanzapine, and risperidone. A grant was obtained from the Dutch Health Research and Development Council (ZON) to carry out the multi-modality Prevention Fund II project. Moreover, this project was part of a co-operation between the universities of Groningen (AZG) and Utrecht (UMCU) through a grant of NWO. The new project was completed with an antipsychotic drug trial, comparing two new atypical antipsychotics, olanzapine, and risperidone, partly sponsored by a grant of Eli Lilly.

The dopamine hypothesis of schizophrenia
The dopamine hypothesis of schizophrenia, as originally formulated by Van Rossum (Van Rossum, 1967) is the most enduring explanation for

the pathophysiology of schizophrenia. Revised versions hypothesise that schizophrenia is characterised by abnormally low prefrontal dopamine activity (causing deficit symptoms) leading to excessive dopamine activity in mesolimbic dopamine neurons (causing positive symptoms) (Davis et al., 1991; Carlsson, 1995). Furthermore, several studies point at the difference between a tonic low level of dopamine in schizophrenia, combined with a phasic higher dopamine release, replacing the model of an overall higher dopaminergic activity (Grace, 1991). The specific significance of these findings for first or second episode patients with schizophrenia is unclear.

In the last decades of the 20th century the technique of neuro-imaging in vivo, using single photon emission computed tomography (SPECT) and positron emission tomography (PET), was evolved far enough to test the dopamine hypothesis in vivo. Three main findings in this field will be mentioned shortly because they were the basis of our aims and hypotheses that were tested in this SPECT project.

Part 1

Dopamine receptor occupancy by antipsychotic medication

According to the dopamine hypothesis, antipsychotic medication acts by blocking the D_2 dopamine receptors in the brain. This is predominantly in the striatum due to the abundance of dopamine receptors in that area. Early PET studies made this blockade visible and measurable, using radioligands with a high affinity for the D_2 receptor. In patients treated with antipsychotic medication the striatal binding of the radioligand was found to be much lower compared to control values, reflecting a high D_2 receptor occupancy (Farde et al., 1986). This landmark study set off a world-wide interest in dopamine receptor imaging in schizophrenia, and this imaging

paradigm was used in a large number of studies to test different aspects of the dopamine hypothesis. An overview of dopamine receptor studies is presented in Chapter 2.

A major finding, derived from these PET and SPECT studies, is that there is a threshold of D_2 receptor occupancy of about 60% required to induce clinical effect of antipsychotic medication (Nyberg et al., 1995; Kapur et al., 1996). Furthermore the risk of extrapyramidal side effects seems particularly high at D_2 receptor occupancy levels over 80% (Farde et al., 1992).

Side effects of antipsychotics are relevant because they are a major reason for treatment non-compliance, while discontinuation of antipsychotic therapy is probably the most important risk factor for developing a psychotic relapse (Prevention Fund I project) (Robinson et al., 1999). Therefore, side effects are always an essential item in the introduction of new antipsychotics. Since the classic antipsychotics were found to occupy a high percentage of D_2 receptor, a search was started of new antipsychotic medication with good clinical effect and low extrapyramidal side effects. Both olanzapine and risperidone were developed according to these criteria. In our first study, we compared side effects and D_2 receptor occupancy and of these two new antipsychotics. Specific aim was to correlate to D_2 receptor occupancy of both antipsychotics (Chapter 3).

Apart from the traditional side effects rating scales, including specific extrapyramidal rating scales, we also assessed the subjective experience of patients treated with antipsychotics. Our hypothesis was that a higher occupancy of D_2 receptors by antipsychotics was correlated with a worse subjective experience. This is a different approach to evaluate the overall effect of well-being under antipsychotic treatment (Naber, 1995), which may be more useful in the understanding of treatment compliance than traditional side effect ratings.

The findings of neuroimaging studies that relatively low doses of antipsychotic already induce high receptor occupancy slowly influenced

clinical practice, as 80% occupancy of the D_2 receptors is already achieved at lower doses than routinely used. One conclusion is that antipsychotic medication is often prescribed in doses that induce more side effects than necessary, with no further reduction of psychotic symptoms (de Haan and Maksmovic, 1999). In line with this argumentation, a study was set up to compare the D_2 receptor occupancy and efficacy of a classic antipsychotic (haloperidol) and an atypical antipsychotic (olanzapine) in a low dose. This low dose study is carried out at the Adolescent Clinic at the moment of writing and no data are yet available. Meanwhile, a Canadian group recently reported favourable results with very low doses of haloperidol (starting at 1 or 2.5 mg per day), which were related with D_2 receptor occupancies of 38 to 87% (Kapur et al., 2000).

A more chronic and lasting side effect of antipsychotic medication that may occur months or years after the start of treatment is tardive dyskinesia. Symptoms of tardive dyskinesia are mostly found in the face. Involuntary movements of the tongue and facial muscles are prominent features. For patients these symptoms can be strongly invalidating and are often a major hindrance in social life. One hypothesis for the occurrence of tardive dyskinesia is a decrease in dopaminergic neurons (Lohr et al., 1988). The chronic increase of synaptic dopamine, caused by the blocking of dopamine receptors, is thought to induce free radicals that are toxic to the dopaminergic neuron. To test the free radical hypothesis we assessed the dopamine transporter density (as a measure of intact dopaminergic neurons) in a group of patients with tardive dyskinesia (Chapter 9) in comparison with controls.

Apart from understanding the mechanism of antipsychotic medication and its side effects, another aim of in vivo imaging of the dopamine system is to evaluate the density of dopamine receptors in patients with schizophrenia. It was predicted that the hyperactivity of the dopamine

system was reflected in a higher density of dopamine receptor. After elaborate imaging studies with conflicting results, consensus was that no large difference in dopamine receptor density between patients with schizophrenia and controls exists (overview by Laruelle, 1998).

However, using a different paradigm, in which endogenous dopamine is depleted before SPECT imaging with [^{123}I]-IBZM, a very recent study claims a higher D_2 receptor occupancy by endogenous dopamine in patients with schizophrenia, stating that endogenous dopamine obscures proper measurement of D_2 receptors (Abi-Dargham et al., 2000). The findings in their study, after depletion of endogenous dopamine, reflect a higher density of striatal D_2 receptors in patients with schizophrenia (Seeman and Kapur, 2000).

Part 2
The presynaptic Dopamine transporter

The second major finding in schizophrenia imaging research involves the presynaptic part of the dopamine system. The focus of dopaminergic imaging was diverted from the effect of a possible over-activity of the system, as measured in the postsynaptic receptor, to the system that could be responsible for this over-activity: the synthesis and release of dopamine. Therefore, presynaptic aspects of the dopamine system were studied. This resulted in the finding of a higher uptake of the dopamine precursor ^{18}F-DOPA in the striatum of patients with schizophrenia compared with controls, as was measured with PET imaging (Reith et al., 1994; Hietala et al., 1995). A recent study, did not replicate these findings (Hietala et al., 1995; Elkashef et al., 2000). However, in this last study patients were not antipsychotic-naive. The higher uptake of this dopamine precursor could reflect either a more active decarboxylase activity, or more nigrostriatal dopaminergic nerve terminals. We tested the hypothesis of an

increased number of dopaminergic nerve terminals by comparing the dopamine transporter density in the striatum, reflecting the density of functional nigrostriatal dopamine neurons, in patients with schizophrenia with healthy controls (Chapter 6).

First, to determine a possible medication effect on the SPECT imaging, we studied the effect of antipsychotic medication on dopamine transporter binding of the radioligand $[^{123}I]$-FP-CIT in rat brain (Chapter 5). To avoid any possible medication effect, the SPECT study also included a subgroup of patients who were never before exposed to antipsychotic medication.

The third and related milestone study in support of the dopamine hypothesis was the finding of a higher dopamine release in patients with schizophrenia (Laruelle et al., 1996). In this neuro-imaging studies, amphetamine was used to provoke dopamine release, resulting in displacement of the radioligand. This study was later replicated (Breier et al., 1997; Abi-Dargham et al., 1998) and it was found that the hyper-dopaminergic state of patients with schizophrenia is present in the initial episode and subsequent relapses, but not in periods of remission (Laruelle et al., 1999). Our initial plan to replicate this important finding and expand it to first episode patients was abolished. Although the testing of the challenge paradigm was already performed at our department (Booij et al., 1997), the set-up of the Prevention Fund II project, was not suitable to perform this study.

Gender differences in the presynaptic dopamine system

Gender differences in neuropsychiatric disorders are well described, and especially play a role in schizophrenia. The male to female ratio is striking when entering the Adolescent Clinic for young patients with a first psychotic episode. More evidence-based, a later age of onset and a initially better outcome has been described in female patients with schizophrenia (Wanderling, 1994; Faraone et al., 1994; Häfner et al., 1994). The relevance of studying these gender differences in

schizophrenia has been put forward (Seeman and Lang, 1990), since it may ultimately have consequences for a more gender-specific treatment (Szymanski et al., 1995).

Previous imaging studies of the postsynaptic dopamine D_2 receptor already revealed that women had a lower D_2 receptor affinity in the striatum (Pohjalainen et al., 1998), suggesting an increased endogenous striatal dopamine concentration in women. This may be related to a higher number of dopaminergic nerve terminals in females. In rats it was shown already that females had a higher dopamine and transporter density, putting estrogen forward as a crucial factor. (Rivest et al., 1995). However, gender differences in the presynaptic dopamine system had not been studied before. Therefore, we explored the effect of gender on dopamine transporter density in patients with schizophrenia (Chapter 6) and in healthy controls (Chapters 7 and 8). Furthermore, the effect of gender on serotonin transporter density was studied (Chapter 8).

The gender effect in schizophrenia has even been expanded into the therapeutic field, by adding estrogen therapy to the standard anti-psychotic medication regime, a project which is, however, still in the experimental phase (Kulkarni et al., 2000).

Part 3
Schizophrenia and the cholinergic system

Although dopamine and schizophrenia have long been inextricably linked, several other neurotransmitters may be involved in the pathophysiology of schizophrenia, e.g. serotonin and glutamate. Although the cholinergic system is mostly mentioned in relation to side effects of antipsychotics, also the cholinergic aspects of schizophrenia itself are subject of discussion (Tandon, 1999).

Some antipsychotics are known to induce cholinergic side effects such as hyposalivation, constipation, or cognitive disturbances. These effects have been described in a low incidence in olanzapine treated patients, but are seen hardly in patients treated with risperidone. On the other hand, anticholinergic medication like biperiden is used to counteract the extrapyramidal side effects of antipsychotics. Therefore, an anti-psychotic drug with anticholinergic effect may well be responsible for the low incidence of extrapyramidal side effects, apart from the D_2 receptor occupancy.

With $[^{123}I]$-Dexetimide as a radioligand, it is possible to study the density of muscarine receptor in the brain in vivo. We used $[^{123}I]$-Dexetimide SPECT to compare the muscarine receptor occupancy of olanzapine and risperidone in patients with schizophrenia and looked for a correlation with extrapyramidal and anticholinergic side effects (Chapter 10).

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2

Dopamine receptor occupancy by antipsychotic medication, an overview of SPECT and PET research receptor

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Summary

By means of literature search, an overview of neuroimaging research on dopamine D_2 receptor occupancy by antipsychotic medication has been framed. Single-photon emission computed tomography (SPECT) and positron emission tomography (PET) techniques are useful to determine the occupancy of dopamine receptors by antipsychotic drugs in vivo. Clozapine and possibly quetiapine appear to result in low D_2 receptor occupancy though being effective. These techniques have led to a better insight in the relation between dosages of antipsychotic drugs, therapeutic effect, and the occurrence of extrapyramidal side effects.

Introduction

Although the efficacy of antipsychotic medication has been established, there is no conclusive explanation for their pharmacological effects. Occupancy of dopamine receptors in the brain is a property of all anti-psychotic drugs, and is supposed to be the primary mechanism of action.

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are research techniques from nuclear medicine, in which (patho-) physiological processes can be visualised by means of radio-labelled substances (radioligands). Specific radioligands bind to specific receptors. The administered dose is very low and does not induce any clinical effect. The gamma-camera or PET camera detects gamma radiation which is then transformed into an image. The radiation load a subject receives with these techniques is comparable to that of a CT scan.

PET offers higher resolution than SPECT and the possibility of absolute quantification, however PET is expensive and limited available.

A clear overview of differences between SPECT and PET was previously published in this journal (Louwerens and Korf, 1994). Dopamine receptors are divided into subgroups. Most prevalent and most studied is the D_2 receptor, to which antipsychotic medication binds most. Verhoeff (Verhoeff, 1999) gives an overview of studies concerning other dopamine receptor subtypes.

In this article we give a concise overview of the imaging research that has been performed on dopamine D_2 receptor occupancy by antipsychotic medication.

Methods

Literature concerning SPECT and PET research on dopamine receptor occupancy by antipsychotic medication was collected via Ovid Medline from 1966 to 2000 (keywords: antipsychotics, SPECT, PET, dopamine and antipsychotic brand names) and by means of references in articles.

Results

The D_2 receptor occupancy by antipsychotic medication as found in SPECT and PET studies is presented in Table 1.

Antipsychotic	Dose	Dopamine D_2 receptor occupancy	Reference
Classical	variable	85 to 90%	(Farde et al., 1986)
		Over 65%	Various studies
Clozapine	300-600 mg	40 to 65%	(Farde et al., 1989)
	300-600 mg	38-63%	(Farde et al., 1992)
	125-600 mg	20-67%	(Nordström et al., 1995)
	175-900 mg	18 to 80%	(Pickar et al., 1996)
	75-900 mg	16-68%	(Kapur et al., 1999)
	300-600 mg	20-49%	(Tauscher et al., 1999)
Olanzapine	10-20 mg	Lower than classical AP	(Pilowsky et al., 1996)
	10-20 mg	68-84% comparable with	(Nordström et al., 1998)
	20 mg	risperidone	(Dresel et al., 1999)
	5-30 mg	68%	(Lavalaye et al., 1999)
	10-25 mg	63-85%	(Tauscher et al., 1999)
		43-84%, comparable with risperidone, higher than	
	5-60 mg	clozapine	(Kapur et al., 1999)
Risperidone	6 mg	75-80%	(Farde et al., 1995),
	2, 4 and 6mg	66, 73 and 79% comparable with classical	(Kapur et al., 1995)
	4-14 mg	AP	(Busatto et al., 1995)
	3 and 8 mg	64 and 74%, (8 mg lower than 20 mg haloperidol)	(Küfferle et al., 1996)
	1,5-10 mg	60-90%, equal to 4-20 mg haloperidol	(Knable et al., 1997)
	2-8 mg	76%	(Lavalaye et al., 1999)
	2-12 mg	63-89%	(Kapur et al., 1999)
	3 and 6 mg	72 and 82%	(Nyberg et al., 1999)
Quetiapine	300-700mg	Lower than 30%	(Küfferle et al., 1997)
	450 mg	44%	(Gefvert et al., 1998)
Ziprasidone	40 mg	comparable with risperidone and olanzapine	(Bench et al., 1996)

Table 1. Overview of dopamine D_2 receptor occupancy by antipsychotic medication determined by SPECT and PET imaging

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Dosages and D_2 receptor occupancy

The relation between dosage and D_2 receptor occupancy of both classical and atypical antipsychotic medication is described as a saturation hyperbole. Increasing low doses resulted in a high rise in D_2 receptor occupancy, while high doses showed no further rise (Nordström et al., 1993; Kapur et al., 1999).

Clinical effects and D_2 receptor occupancy

With classical antipsychotic medication it was determined whether the absence of a clinical effect was caused by lower dopamine receptor occupancy in non-responders. In multiple studies it was found that the occupancy is equal in both responders and non-responders, and therefore non-response is not likely to be explained by a lack of D_2 receptor occupancy (Wolkin et al., 1989; Geaney et al., 1992; Pilowsky et al., 1993). Nordström and co-workers (Nordström et al., 1993) found no clinical effect at very low D_2 receptor occupancy (as low as 35%).

Extrapyramidal side effects, subjective experience and D_2 receptor occupancy

Extrapyramidal side effects (EPS) occur at higher doses of classical antipsychotics. A PET study showed an average D_2 receptor occupancy of 74% in patients with no EPS, and of 82% in patients with EPS (Farde et al., 1992). This difference in receptor occupancy between patients with and without EPS was later confirmed (Scherer et al., 1994). It is suggested that D_2 receptor occupancy of classical antipsychotics has to stay below 80% to prevent EPS.

Furthermore, a higher D_2 receptor occupancy in patients with olanzapine or risperidone is correlated with a worse subjective experience (de Haan et al., 2000).

Low dosage of antipsychotic medication

Dosages in recent studies are often lower in recent studies than in the beginning of the 1980's. One study found no clinical difference in patients that were treated with both 5 and 20 mg olanzapine, with a high D_2 receptor occupancy at 20 mg (60 vs. 83%, respectively) (Raedler et al., 1999). In a study with 2 mg of haloperidol, a D_2 receptor occupancy of 53-74% was found, at which most patients showed clinical improvement. (Kapur et al., 1996). Partly because of these high D_2 receptor occupancies, lower dosages are now recommended (Heinz et al., 1996; de Haan and Maksmovic, 1999).

Discussion

SPECT and PET imaging can be used to determine the occupancy of dopamine receptors by antipsychotic medication in vivo. These techniques improved the insight into the correlation between dosages of antipsychotics, clinical effects, and the occurrence of extrapyramidal side effects. These imaging techniques are therefore important for the introduction of treatment guidelines and the development and evaluation of new antipsychotic medication.

It is sometimes hard to compare dopamine receptor occupancy studies due to the differences in radioligand and in method of analysis. However, an important finding is that results from different groups are more or less comparable.

A clinically relevant finding is that an increase in dose at low doses induces a high increase in D_2 receptor occupancy, whereas an already high D_2 receptor occupancy hardly increases when doses are further increased. A direct application of PET studies is an advice to administer risperidone in a 4 mg dose, to induce a D_2 receptor occupancy of 70-80% (Nyberg et al., 1999). Moreover, on account of neuro-imaging studies

there is a recent plead for equivalent dosages when switching from haloperidol to risperidone (Remington et al., 1998). The working mechanism of antipsychotics remains unclear. Because the D_2 receptor occupancy is not different in responders and non-responders, this occupancy may not be the only reason for the effect of antipsychotics in all schizophrenic patients.

Clozapine, and possibly quetiapine, appeared to induce a lower D_2 receptor occupancy, and are effective antipsychotic drugs. New radio-ligands for the determination of binding to other receptors such as the serotonin receptor, the dopamine D_1 receptor, the muscarinic and GABA receptors can be used in future to unravel the complex working mechanism of antipsychotic drugs.

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3

Dopamine D$_2$ receptor occupancy by olanzapine or risperidone in young patients with schizophrenia

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Abstract

A crucial characteristic of antipsychotic medication is the occupancy of the dopamine D_2 receptor. We assessed striatal dopamine D_2 receptor occupancy by olanzapine and risperidone in 36 young patients (31 Male, 5 Female; mean age 21.1 years, (16-28)) with first episode schizophrenia, using ^{123}I-IBZM SPECT. The occupancy of dopamine D_2 receptors was not significantly different between olanzapine and risperidone. However, in subgroups of most prescribed doses, dopamine D_2 occupancy was higher in the risperidone 4 mg group (79%) compared to the olanzapine 15 mg group (62%). ^{123}I-IBZM binding ratios decreased with olanzapine dose ($r = -0.551$; $p<0.01$), indicating higher dopamine D_2 receptor occupancy with higher olanzapine dose. Akathisia and positive symptoms were correlated with ^{123}I-IBZM binding ratio ($r = -0.442$; $p < 0.01$; and $r = -0.360$; $p < 0.05$, respectively). Prolactin levels were elevated in the risperidone, but not in the olanzapine group, at comparable D_2 receptor occupancy levels. In the olanzapine group, prolactin levels were correlated with ^{123}I-IBZM binding ratio ($r = -0.551$; $p<0.01$).

In conclusion, both olanzapine and risperidone induce a high striatal D_2 receptor occupancy, dependent on dose and group formation. The lower incidence of prolactin elevation with olanzapine, compared to risperidone, may not be attributed to a lower D_2 receptor occupancy.

Introduction

Schizophrenia is a severe and disabling psychiatric disorder with a heterogeneous symptomatology. Various theories have been developed to explain the pathophysiology of these various symptoms. The dopamine hypothesis (Carlsson, 1959) has generated strong arguments for the involvement of dopamine in the pathophysiology of psychotic symptoms.

More recent studies refined this theory by stressing the enhanced sensitivity of the dopaminergic neurotransmission system (Lieberman et al., 1997). An enhanced responsiveness of the nigrostriatal dopaminergic pathway has been shown in vivo with a pharmacological stress model and SPECT (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998). The proposed major role for dopamine in the pathophysiology of schizophrenia underlines the importance of studying intensively the in vivo influence of different antipsychotic drugs on the dopaminergic neurotransmission system.

Olanzapine and risperidone have been introduced as atypical anti-psychotics with a good effect on positive and negative symptoms of schizophrenia (Peuskens, 1995; Beasley et al., 1996). The main benefit of these antipsychotics their relatively low incidence of extrapyramidal symptoms compared with classic antipsychotics (Borison et al., 1992; Tran et al., 1997). However, extrapyramidal symptoms have also been observed in patients treated with risperidone or olanzapine in high dosages (Marder and Meibach, 1994; Kapur et al., 1998). Several studies have been performed to explain the low incidence of extrapyramidal symptoms by examining the striatal D_2 receptor occupancy by these two antipsychotics. However, the results of these studies are not consistent in the percentage of striatal D_2 receptor occupancy by olanzapine (Pilowsky et al., 1996; Nordström et al., 1998; Kapur et al., 1998; Tauscher et al., 1999).

The aim of the present study was to evaluate the occupancy of striatal D_2 receptors by maintenance doses of olanzapine and risperidone, using iodine-123 iodobenzamide (^{123}I-IBZM) SPECT in a large patient cohort. All patients were adolescents or young adults, administered after a first or second psychotic episode. The small age range in this group is an advantage over other studies because of the decrease of D_2 receptors with age (Volkow et al., 1998), which can be of influence on the total radioligand binding. ^{123}I-IBZM is a suitable SPECT radioligand for visualisation of striatal D_2 receptors in the human brain (Verhoeff et al.,

1991). Moreover, we studied the correlation between occupancy of D_2 receptors, clinical symptoms, and prolactin levels.

Methods

Subjects

A consecutive series of thirty-nine young patients with schizophrenia were initially included in this study. Excluded were patients with prominent or recent alcohol or drugs dependency.

All patients were admitted to the Adolescent Clinic of the AMC and attended a special program for adolescents with a first psychotic episode. Clinical diagnosis was confirmed in 37 out of 39 patients after discharge. Two patients were excluded from the study because of a different diagnosis (mood disorder with psychotic features), and one was excluded because of discontinuation of medication therapy during the study. Analysis of SPECT data was performed on the resulting 36 patients with schizophrenia (31 males, 5 females) according to DSM IV (American Psychiatric Association, 1994), ranging in age from 16-28 (mean = 21.1) years. All patients gave their written informed consent. This study is part of an ongoing trial in which the clinical response to two new atypical anti-psychotic drugs (olanzapine (Zyprexa®) and risperidone (Risperdal®)) is compared. The two drugs under study were randomly allocated to patients who used classic neuroleptic drugs at intake. However, patients who were well responding to either one of the two drugs under study at intake continued their original medication. Drug dosing was flexible, according to psychotic symptoms. Eventually, 23 patients were treated with olanzapine in an average dose of 15.4 mg (range 5-30 mg), and 13 patients were treated with risperidone (average dose 4.2 mg (range 2-8 mg)).

SPECT imaging was always performed after a stable dose period of at least 6 weeks of study medication to ensure stabilisation of psychotic

symptoms. At the moment of imaging, co-medication was kept as low as clinically achievable. A few patients used selective serotonin re-uptake inhibitors (SSRI) (n = 7) or benzodiazepines (n = 6) in the week of SPECT imaging. Three patients (no 11, 12, and 35) received a depot AP more than 3 months before SPECT imaging. Last intake of antipsychotic medication was the evening before scintigraphy in most patients. This study was approved by the medical ethical committee of the Academic Medical Centre in Amsterdam.

^{123}I-IBZM SPECT procedure

SPECT imaging was performed with a brain-dedicated SPECT multi-detector system (SME 810, Strichman Medical Equipment Inc., USA), linked to a Macintosh II computer. The Strichman camera consists of twelve individual crystals, each equipped with a focussing collimator. The transaxial resolution of this camera is 7.6 mm full width half maximum (FWHM) of a line source in air, and the axial resolution is 13.5 mm FWHM. All subjects received potassium iodide orally to block thyroid uptake of free radioactive iodide. ^{123}I-IBZM (specific activity of >185 MBq/nmol; radio-chemical purity of > 95%) was injected intravenously at an approximate dose of 110 MBq. ^{123}I labelling of IBZM was performed by Amersham Cygne (Technical University Eindhoven, The Netherlands). SPECT image acquisition was always performed at 2 h p.i. (Verhoeff et al., 1991a). Slices were acquired during 220 s periods from the orbitomeatal line to the vertex using an interslice distance of 5 mm. Attenuation correction and reconstruction of the images was performed as described earlier (Verhoeff et al., 1993).

Data processing

For analysis of striatal ^{123}I-IBZM binding, the ratio of striatal to non-specific binding was calculated by summing up two transversal slices, representing the most intense striatal binding. Analyses were performed by one observer (J.L.), blinded to clinical data. A standard region of interest (ROI)

template (constructed according to a stereotactic atlas and including regions for striatum and occipital cortex) was placed bilaterally on the acquired image. The ratio of specific striatal binding was calculated by dividing the striatal binding by occipital binding. This method is used as a reliable measure for estimation of ^{123}I-IBZM binding (Verhoeff et al., 1993). Data obtained in normal controls (average age 24 years (range 19-31), (n = 9) were used to calculate the percentage of occupancy by antipsychotics in the striatum. ^{123}I-IBZM binding in controls was symmetrical in the striatum with an average binding ratio of 1.92 (SD 0.08). Percentage of occupancy of antipsychotics in patients was calculated as (ratio str/occ-1) / (1.92-1) * (-100) + 100, as described by Küfferle and co-workers (1996).

Clinical interviews

Psychotic symptoms were rated, in the same week of SPECT imaging, by one of the researchers (J.L.) using the Structured Clinical Interview for the Positive and Negative syndrome Scale (SCI-PANSS; (Kay et al., 1987). Depression was rated with the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979). Extrapyramidal symptoms were rated with three different rating scales. The Barnes Akathisia rating scale (Barnes, 1989), the Simpson Angus rating scale, (Simpson and Angus, 1970), and the AIMS (Abnormal Involuntary Movement Scale) (National Institute of Mental Health, 1974). All tests were performed at the time of the PANSS interview. The rater of the tests was blind to the results of the SPECT imaging. The Simpson Angus and AIMS rating scales were performed in 15 patients.

Prolactin

Blood samples for prolactin levels were taken in 27 patients, between 10.00 and 12.00 AM, at the time of injection of ^{123}I-IBZM. Samples were measured with fluoroimmunoassay. Prolactin elevation was defined as higher than 15 µg/l for men and higher than 22 µg/l for women. Thyroid

function was screened at intake at the clinic and was normal in all
subjects.

Statistics

Correlations were calculated using a one-tailed Spearman's rho non-parametric test. Analysis of variance (ANOVA) was used to compare the regional SPECT data in the patients and healthy controls. A Mann-Whitney U test was used for comparison between two groups. Data analysis was carried out with statistical software (SPSS version 7.5).

Results

^{123}I-IBZM SPECT

Both patient groups showed a low striatal binding of ^{123}I-IBZM on visual inspection (Fig. 1). ^{123}I-IBZM binding in the striatum was symmetric (left/right ratio = 1.01), with no correlation of handedness for the patients, so average binding in left and right striatum was used for further analysis. Gender and age were not significantly correlated with ^{123}I-IBZM binding in both patient groups. Ratios in patient groups were significantly lower than in controls ($p < 0.01$; data not shown). The ratios of striatal to non-specific ^{123}I-IBZM binding for olanzapine and risperidone are shown in Table 1 and Fig. 2. The occupancy of striatal D_2 receptors by olanzapine was not significantly different from that in patients treated with risperidone (Mann Whitney U=107,0; p=0.16). To exclude a possible confounding effect of co-medication, we also analyzed our data restricted to patients without SSRI and benzodiazepines. In these subgroups, the mean binding ratio was 1.31 (SD 0.15, n=13) and 1.20 (SD 0.10, n=11) for the olanzapine and the risperidone group, respectively. This difference between groups was also not significant (Mann Whitney U=41.5, p=0.082).
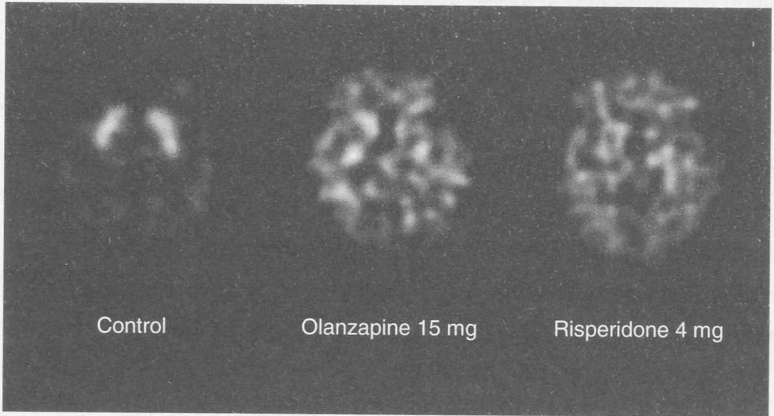
Figure 1. ^{123}I-IBZM SPECT images of a healthy control and two schizophrenic patients treated with olanzapine (15 mg) and risperidone (4 mg). Transverse slices from the brain at the level of the striatum. The images of the patients clearly show much lower striatal binding of ^{123}I-IBZM than in the control subject. Levels of SPECT activity are colour encoded from low (black) to high (white). Colour version on the inside of the cover.

Table 1 Demographic data and ^{123}I-IBZM binding ratios of 36 patients with schizophrenia

Patient no.	Age (years)	Sex	Medication	Dose (mg/day)	Dose (mg/kg)	Co-medication
1	17	M	olanzapine	5	0.100	
2	17	F	olanzapine	5	0.077	
3	22	M	olanzapine	10	0.133	paroxetin 20 mg
4	19	M	olanzapine	10	0.154	
5	20	F	olanzapine	10	0.128	
6	25	M	olanzapine	10	0.120	
7	27	M	olanzapine	10	0.141	
8	20	M	olanzapine	15	0.231	oxazepam 30 mg
9	25	F	olanzapine	15	0.188	fluvoxamine 150 mg
10	22	M	olanzapine	15	0.231	
11	19	M	olanzapine	15	0.176	
12	23	M	olanzapine	15	0.250	
13	16	F	olanzapine	15	0.208	
14	20	M	olanzapine	15	0.224	paroxetin 30 mg
15	22	M	olanzapine	15	0.163	oxazepam 75 mg
16	20	M	olanzapine	15	0.172	oxazepam 10 mg
17	21	M	olanzapine	20	0.267	
18	18	F	olanzapine	20	0.408	paroxetin 20 mg, alprazolam 1 mg
19	23	M	olanzapine	20	0.222	oxazepam 75 mg
20	21	M	olanzapine	20	0.253	paroxetin 20 mg
21	16	M	olanzapine	20	0.263	
22	18	M	olanzapine	30	0.300	
23	22	M	olanzapine	30	0,242	amitriptyline 200 mg
Mean				15.4	0.202	
(SD)				(6.4)	(0.074)	

Table 1 Demographic data and ^{123}I-IBZM binding ratios of 36 patients with schizophrenia (continued)

Patient no.	Age (years)	Sex	Medication	Dose (mg/ day)	Dose (mg/kg)	Co-medication
24	19	M	risperidone	2	0.027	
25	25	M	risperidone	3	0.030	
26	22	M	risperidone	3	0.038	
27	27	M	risperidone	4	0.057	
28	17	M	risperidone	4	0.044	biperiden 2 mg, lithium 1000 mg
29	21	M	risperidone	4	0.040	biperiden 4 mg
30	19	M	risperidone	4	0.075	biperiden 2mg
31	28	M	risperidone	4	0.066	biperiden 2 mg
32	28	M	risperidone	4	0.057	biperiden 2 mg, fluoxetin 10 mg
33	17	M	risperidone	4	0.054	
34	21	M	risperidone	4	0.054	
35	24	M	risperidone	6	0.073	biperiden 2mg
36	18	M	risperidone	8	0.100	biperiden 2mg, clorazepate 50 mg
Mean (SD)				4.2 (1.5)	0.055 (0.020)	

Table 1 Demographic data and ^{123}I-IBZM binding ratios of 36 patients with schizophrenia (continued)

Patient no.	IBZM ratio	Occupancy (%)	PRL (µg/l)	Akathisia	PANSS pos.	PANSS neg.	PANSS general	MADRS
1	1.15	83.96		0	11	12	21	2
2	1.41	55.45	9.5	0	10	11	21	2
3	1.53	42.36		0	13	17	34	18
4	1.24	74.16	10	1	7	7	22	3
5	1.41	55.07	12.5	0	7	12	19	2
6	1.45	51.22	8.5	0	7	9	22	4
7	1.29	68.94	9.5	0	7	17	16	0
8	1.40	56.00		0	7	9	26	2
9	1.20	78.26		0	7	24	28	20
10	1.38	59.02	13	0	14	7	20	1
11	1.57	38.35	14.5	0	7	26	28	19
12	1.44	51.69	8.5	1	8	8	24	3
13	1.13	86.41	23*	0	9	11	18	9
14	1.40	57.03			10	13	33	
15	1.41	55.63	13	0	17	23	43	18
16	1.23	74.64	21*	2	9	13	22	6
17	1.24	73.91		0	9	15	25	11
18	1.10	89.65		3	20	22		25
19	1.28	69.57	10	2	7	17	25	5
20	1.15	84,02	19.5*	1	15	17	27	4
21	1.16	83.13	14	1		11	19	2
22	1.12	86.82		3	13	9	24	5
23	1.11	87.83	5	2	19	17	44	18
Mean	1.29	68			10.6	14.2	25.5	8.1
(SD)	(.14)	(16)			(4.2)	(4.2)	(7.4)	(7.8)

IBZM ratio is striatum/occipital ^{123}I-IBZM binding
*Prolactin (PRL) elevated measure

Table 1 Demographic data and ^{123}I-IBZM binding ratios of 36 patients with schizophrenia (continued)

Patient no.	IBZM ratio	Occupancy (%)	PRL (µg/l)	Aka-thisia	PANSS pos.7	PANSS neg.	PANSS general	MADRS
24	1.29	68.94		0				2
25	1.20	77.74	26*	0	11	22	34	4
26	1.28	69.12	41*	0	8	15	20	7
27	1.07	92.91	39*	1	10	16	30	33
28	1.13	85.59	29*	3	9	12	20	4
29	1.36	60.98	48*	0	9	8	19	0
30	1.26	71.34	27*	2	14	15	31	5
31	1.21	77.70	23*	0	12	21	35	17
32	1.33	63.77	30*	1	9	19	35	17
33	1.02	98.02	42*	2	13	21	33	10
34	1.15	84.07	56*	0	10	9	17	4
35	1.40	56.93	51*	2	16	14	46	25
36	1.18	80.49	48*	1	14	16	29	10
Mean	1.22	76			11.3	15.7	29.1	10.6
(SD)	(.11)	(12)			(2.5)	(4.6)	(8.6)	(9.8)

IBZM ratio is striatum/occipital ^{123}I-IBZM binding

*Prolactin (PRL) elevated measure
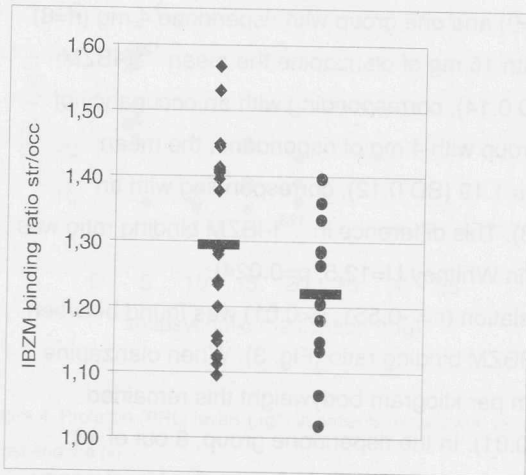
Figure 2. Individual ¹²³I-IBZM binding ratios (striatum/occipital binding) in patients treated with olanzapine (♦) or risperidone (•).
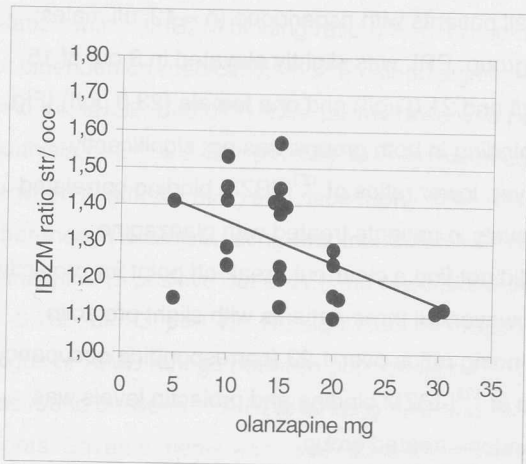
Figure 3. Individual ¹²³I-IBZM binding ratio (striatum/occipital binding) in patients treated with different doses of olanzapine.

To enable direct comparison with other studies, we also specified two subgroups of patients with the clinically most prescribed doses, one group with olanzapine 15 mg (n=9) and one group with risperidone 4 mg (n=8). In the subgroup treated with 15 mg of olanzapine the mean ^{123}I-IBZM binding ratio was 1.35 (SD 0.14), corresponding with an occupancy of 62% (SD 15). In the subgroup with 4 mg of risperidone, the mean ^{123}I-IBZM binding ratio was 1.19 (SD 0.12), corresponding with an occupancy of 79% (SD 13). This difference in ^{123}I-IBZM binding ratio was significantly different (Mann Whitney U=12,5; p=0.024).

A negative linear correlation (r = -0.551; p<0.01) was found between olanzapine dose and ^{123}I-IBZM binding ratio (Fig. 3). When olanzapine was converted to milligram per kilogram bodyweight this remained significant (r = -0.509; p<0.01). In the risperidone group, 8 out of 13 patients were treated with 4 mg (table 1). Therefore, a dose-occupancy correlation was not calculated.

Prolactin

Prolactin was elevated in all patients with risperidone (n = 12, all males; Fig. 4). In the olanzapine group, PRL was slightly elevated in 3 out of 15 patients, 2 males (19.5 μg/l and 21.0 μg/l) and one female (23.0 μg/l) (Fig. 4). The striatal ^{123}I-IBZM binding in both groups was not significantly different (p = 0.07). However, lower ratios of ^{123}I-IBZM binding correlated negatively with prolactin levels in patients treated with olanzapine (r = -0.551; p<0.01). We did not find a clear-cut break-off point for prolactin elevation in this group. However, all three patients with slight prolactin elevation had ^{123}I-IBZM binding ratios over 1.23 (corresponding occupancy of 74.6%). The correlation of ^{123}I-IBZM binding and prolactin levels was not significant in the risperidone treated group.
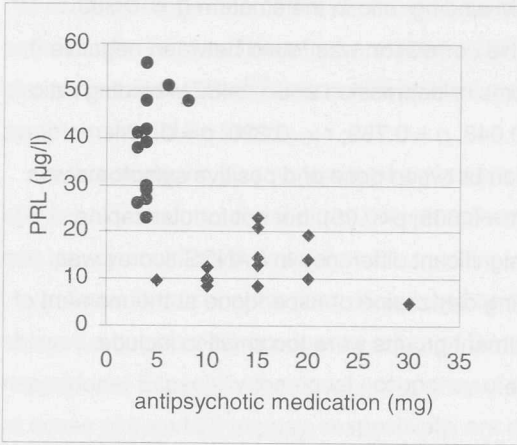
Figure 4. Prolactin (PRL) levels (μg/l) in patients treated with various dosages of olanzapine (♦) or risperidone (•).

Extrapyramidal Symptoms

Mild to moderate akathisia was scored in 25% of the patients (9 out of 35). The Barnes akathisia rating scores showed a significant negative correlation with ^{123}I-IBZM binding ratio (r = -0.417; p<0.01). When corrected for biperiden co-medication this remained significant (multiple regression β=-0.44; df=32; p=0.011). Olanzapine dose was positively correlated with akathisia rate (r = 0.496; p<0.05). No significant correlation was found between akathisia rating and risperidone dose. No statistically significant difference in akathisia rating was found between patients treated with olanzapine (5 positive out of 22) and risperidone (4 out of 13) (t-test t= -0.785; df=33; p=0.43). No significant difference was found in Simpson Angus or AIMS ratings between both groups, and no significant correlation was found between ^{123}I-IBZM binding ratio and Simpson Angus or AIMS scores. Seven patients with risperidone were also treated with biperiden (Akineton).

PANSS

Positive symptoms of schizophrenia were significantly negatively

correlated with the ^{123}I-IBZM binding ratio in the striatum (r = -0.360; p<0.05) in the total group. No correlation was found between negative symptoms, general symptoms or depression and ^{123}I-IBZM binding ratio (r = -0.134, p = 0.443; r = 0.048, p = 0.789; r = -0.220, p = 0.204, respectively). The correlation between dose and positive symptoms was significant for risperidone (r = 0.605; p<0.05), but not for olanzapine (r = 0.375; p = 0.086). No significant difference in PANSS scores was found between patients using olanzapine or risperidone at the moment of SPECT imaging. Both treatment groups were too small to include medication as a co-variable.

Discussion

In this clinical study, we found no significant difference in occupancy of striatal D_2 receptors between the total olanzapine and risperidone group, at a comparable clinical efficacy. However, when we studied subgroups of patients with the most prescribed doses we found a significant lower occupancy in patients treated with 15 mg olanzapine (62%) compared to patients treated with 4 mg risperidone (79%). This clearly demonstrates the influence of the composition of the groups that are under study. The high D_2 occupancy in the olanzapine treated group is mainly caused by doses of 20 mg or more. In this dose range, the D_2 occupancy is comparable to 4 mg of risperidone. Nevertheless, a large overlap in occupancy was found between olanzapine and risperidone.

We tried to explain the wide spread in D_2 receptor occupancy, especially in patients treated with equal dosages. Our hypothesis was that patients with a more "sensitive dopamine system" have a higher release of endogenous dopamine. This may explain the lower binding of ^{123}I-IBZM on comparable antipsychotic doses. However, with multiple regression analysis we found no significant influence of psychotic symptoms on

^{123}I-IBZM binding when corrected for antipsychotic dose. In individual patients who were outliners in the scatterplot of ^{123}I-IBZM binding and olanzapine dose, no correlation with positive, negative nor depressive symptoms could be found.

In this study, no plasma levels of antipsychotic medication were obtained. Therefore, correlations between plasma levels and D$_2$ receptor occupancy could not be made.

In line with our observations, Kapur and co-workers (1998;1999) showed a comparable occupancy range of D$_2$ receptors by olanzapine and risperidone. Especially the equal occupancy of risperidone and olanzapine at doses of 5 and 20 mg/day, respectively, are comparable to our findings in patients with 4 mg risperidone and 20 mg of olanzapine. The high occupancy of D$_2$ receptors in patients treated with olanzapine was confirmed in a PET study by Nordström and co-workers (1998). In contrast to our findings, a SPECT study by Pilowsky and co-workers (1996) showed a relatively low occupancy for olanzapine. This was more in the range of clozapine, as opposed to risperidone and classic antipsychotics. Nyberg and co-workers (1997) also found a relatively low occupancy (61%). The low occupancy of D$_2$ receptors reported by the last two mentioned studies may be explained by the low dosage of olanzapine.

Risperidone has been found to have a high D$_2$ receptor occupancy. Occupancy rates range in different studies from 66% with 2 mg (Kapur et al., 1995), to 99% with 10 mg (Knable et al., 1997). The 73% occupancy found by Kapur and co-workers (1995), at 4 mg is comparable to our finding of 79% at the same dose. A recent ^{123}I-IBZM SPECT study indicates a D$_2$ receptor occupancy of risperidone between haloperidol and clozapine (Dresel et al., 1998). Differences in occupancy rates are probably most dependent on dosage, imaging technique, and calculations.

To determine preclinical and clinical profiles of antipsychotics, Kapur (1998) suggests dividing antipsychotic medication in 4 groups, high and low D$_2$ and serotonin (5HT$_2$) receptor occupancy groups. We found a high occupancy of the D$_2$ receptor by olanzapine and risperidone, placing both

antipsychotics in the same group of high D_2 antagonists. The high $5HT_2$ in vivo receptor occupancy by olanzapine and risperidone has already been shown in different studies (Nyberg et al., 1997; Kapur et al., 1998; Travis et al., 1998).

Placing olanzapine and risperidone in a high D_2 receptor occupancy group, as opposed to clozapine, is in accordance with in vitro data (Bymaster et al., 1996).

The high occupancy of olanzapine and risperidone supports important aspects of the dopamine hypothesis of schizophrenia. The low D_2 receptor occupancy of clozapine is still one of the disturbing factors in this theory. However, interestingly, Seeman and Kapur (1997) shed a new light on the supposed low binding of clozapine to the D_2 receptor. They show that clozapine has also a high in vivo occupancy of the D_2 receptor. This is a strong support to the dopamine hypothesis, according to which all anti-psychotics mainly function through D_2 receptor antagonism.

To calculate the percentage of occupancy we compared our patient ^{123}I-IBZM data to data obtained in normal controls. It might be more optimal to calculate the occupancy by comparing ^{123}I-IBZM binding in a drug-free and medicated state. However, several studies showed that the D_2 receptor density is not significantly different between drug-naive patients and normal controls (Farde et al., 1990).

A small number of patients were treated with benzodiazepines and SSRIs as concomitant medication at the moment of imaging. In a PET study, lorazepam has been shown to have no influence on striatal ^{11}C-raclopride binding (Hietala et al., 1997). In contrast to this, the SSRI citalopram has been shown to induce a slight decrease in striatal ^{11}C-raclopride binding (Tiihonen et al., 1996). However, in this study, statistical analysis showed no significant effect of the concomitant medication on ^{123}I-IBZM binding in the striatum. Moreover, after analysis of our data restricted to patients without SSRI and benzodiazepine, the conclusion that olanzapine and risperidone induced no significant

difference in D_2 receptor occupancy remained intact. Finally, although a slight influence of SSRIs on striatal ^{123}I-IBZM binding could not be completely excluded, it was, from a clinical point of view, not eligible to stop this medication at the moment of SPECT imaging.

Prolactin

We evaluated prolactin levels after a stable dose period of six weeks or longer to exclude initial fluctuations. No baseline values for prolactin were available, so it can not be excluded that elevations in prolactin were persistent from before treatment. Also, the phase of the menstrual cycle of the 5 participating female patients, was not recorded. In this study, we found a significant difference of prolactin levels in patients with olanzapine and risperidone. Atypical antipsychotics, like olanzapine, have been shown to induce smaller prolactin elevations, which are often reversible, compared to typical antipsychotics (Beasley et al., 1996). In this study we found, in the olanzapine treated group, higher prolactin levels in patients with a higher D_2 receptor occupancy. Risperidone is considered an atypical antipsychotic drug, but increases prolactin levels significantly more than olanzapine, and prolactin remains more often elevated (Tran et al., 1997). There are several explanations for the difference in prolactin levels in patients treated with olanzapine and risperidone. One explanation is a lower binding of olanzapine to the D_2 receptor compared to risperidone. This explanation is not supported by the results of this study. Secondly, the occupancy of D_2 receptors by olanzapine and risperidone might be different in the striatum and the pituitary. However, there are no studies to date that showed different D_2 receptors in these two regions. Nevertheless, it would be of interest to examine the D_2 receptor occupancy of antipsychotic medication in both striatum and pituitary with a radioligand with a higher affinity for extrastriatal D_2 receptors, e.g. ^{123}I-epidepride. Thirdly, Leysen and co workers showed a different binding profile of olanzapine and risperidone for a serotonin receptor subtype (1998).

Olanzapine, as opposed to risperidone, showed a strong in vitro antagonist for the $5HT_{2C}$ receptor. Blocking of the $5HT_{2C}$ receptor results in a suppression of prolactin release (Coccaro et al., 1996). Thus, a difference in occupancy of the $5HT_{2C}$ receptor by olanzapine and risperidone may be the most likely explanation for the presently observed difference in prolactin levels.

The postulated importance of a non-dopaminergic effect of antipsychotic medication in the hypothalamic/pituitary pathway may also play a major role in the mesolimbic/mesocortical dopamine pathway. To clarify differences in clinical response of antipsychotics it may be essential to characterise also the in vivo binding to other neurotransmission systems than the dopaminergic.

Extrapyramidal symptoms

Although we found low to moderate scores of extrapyramidal symptoms in both patient groups, with no clear difference between groups, the akathisia rating was higher than expected. This rate of akathisia correlated positively with the D_2 receptor occupancy and with the dosage of olanzapine. This finding might be clinically relevant for dosing as low as possible to optimise compliance.

There are different explanations for the low incidence of extrapyramidal symptoms with olanzapine and risperidone. First, the D_2 receptor binding for most patients is just below the proposed crucial level of D_2 receptor occupancy above which extrapyramidal symptoms are thought to occur (Farde et al., 1992). Second, olanzapine is a strong muscarinic antagonist in vitro (Bymaster et al., 1996). Olanzapine may therefore function in vivo as a built-in anticholinergic agent. Third, mild extrapyramidal symptoms can also be a symptom of schizophrenia, as the rate of abnormal involuntary movements is not negligible in drug-naive first episode schizophrenic patients (Gervin et al., 1998). Finally, treatment with anticholinergic medication can mask extrapyramidal symptoms.

Interestingly, in this study we found a high correlation between different

side effects. In line with this finding, patients who experienced side effects often had positive scores on all extrapyramidal symptom scales. Patients with higher scores on the positive items of the PANSS had a significantly lower ^{123}I-IBZM binding. This is likely to be an effect of the fact that this was not a fixed dose study, resulting in a significantly higher dose of antipsychotic medication in patients with more psychotic symptoms. An overall improvement in symptoms was registered in the period between intake and the moment of SPECT imaging. Because the patients used a variety of antipsychotic drugs at intake, it was not possible to assess optimally the response to olanzapine or risperidone treatment.

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4

Subjective experience and striatal dopamine D_2 receptor occupancy in patients with schizophrenia stabilised on olanzapine or risperidone

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Abstract

Objective: The authors' goal was to study the relationship between subjective experience during treatment with olanzapine or risperidone and dopamine D_2 receptor occupancy in stabilised patients with schizophrenia. Method: Subjective experience, psychopathology, and extrapyramidal symptoms were assessed, and D_2 receptor occupancy was determined with [^{123}I]iodobenzamide single photon emission computed tomography, in 22 patients whose schizophrenia was stabilised by olanzapine or risperidone. Results: Subjective experience, depression, and negative symptoms were related to dopamine D_2 receptor occupancy, but extrapyramidal symptoms were not. Conclusions: These results provide preliminary evidence that negative subjective experience is related to high D_2 receptor occupancy. Longitudinal study is required since this relationship may have implications for dosing strategies.

Introduction

Subjective experience of patients treated with antipsychotic medication is related to quality of life (Naber, 1995) and predicts medication compliance (Hogan et al., 1983; Naber, 1995; Van Putten et al., 1981). Subjectively experienced side effects are more distressing than other side effects (Buis, 1992).

Occupancy of dopamine D_2 receptors in the striatum by antipsychotic medication is thought to influence patients' subjective experiences. The influence of cocaine on the dopamine system has a profound effect on subjective experience (Volkow et al., 1997). The striatum is involved in the

control of motivation and reward. Extrapyramidal symptoms (EPS) are related to D$_2$ receptor occupancy (Farde et al., 1992) and, in particular, akathisia has an important subjective component.

Olanzapine and risperidone may be associated with a better subjective experience than typical antipsychotic drugs (Franz et al., 1997; Tollefson et al., 1998). However, if the severity of negative subjective experience is related to D$_2$ receptor occupancy of olanzapine or risperidone, then these agents might not demonstrate benefits for subjective experience than typical anti-psychotic drugs in doses that lead to the same range of D$_2$ receptor occu-pancy (Kapur et al., 1999).

In this study, we evaluated the relationship between subjective experience and striatal D$_2$ receptor occupancy in patients with schizophrenia stabilised on olanzapine or risperidone.

Method

Subjects

Twenty-one patients with schizophrenia and one patient with schizo-affective disorder, diagnosed according to DSM-IV criteria, were included in the study. Four of the patients were female and 18 were male; their mean age was 22 years (SD = 4, range =16-28). Exclusion criteria were neurological or endocrine disease and mental retardation. Nine healthy controls were also included; their mean age was 24. After complete description of the study to the subjects, written informed consent was obtained from all.

Instruments

Patients' subjective experience during the previous 7 days was measured with the Subjective Well-being under Neuroleptic Treatment Scale (Naber,

1995) and the Subjective Deficit Syndrome Scale (Jaeger et al., 1990). Patients' psychopathology was assessed with the Positive and Negative Syndrome Scale and the Montgomery Åsberg Depression Rating Scale. Extrapyramidal symptoms and akathisia were assessed with the Simpson Angus Rating scale (range = 0-40) and the Barnes Rating Scale for Drug Induced Akathisia scale, respectively.

Single photon emission Computed Tomography imaging (SPECT) imaging was performed with a brain-dedicated SPECT camera 2 hours after intravenous injection of 110 MBq of iodobenzamide ($[^{123}I]$-IBZM). Specifications and imaging procedures have been described elsewhere (11). SPECT imaging was performed after a stable dose period of at least 6 weeks of olanzapine (N=15, mean dose=14.7 mg, SD=5.8 mg, range= 5-30) or risperidone (N=7, mean dose=4.1 mg SD=0.9 mg, range=3-6).

Subjective experience, psychopathology, and extrapyramidal symptoms were assessed within 2 days after SPECT imaging. None of the patients used alcohol, cannabis, or other nonprescribed drugs. Patients were blind to the main goal of this study: i.e., assessment of the relationship between medication and subjective experience.

Data-analysis

Semiquantification of $[^{123}I]$IBZM binding was performed by placing a template of fixed regions of interest over the striatum and occipital cortex (Verhoeff et al., 1993). Specific striatal binding was defined as striatal binding divided by occipital binding (Verhoeff et al., 1993). All analyses were performed blind to clinical data.

The nine healthy comparison subjects had a mean $[^{123}I]$IBZM binding ratio of 1.92 (SD=0.08), which was used to calculate the percentage of occu-pancy by medication as (ratio striatum/occipital region)-1/(1.92-1)*(-100)+100 (Küfferle et al., 1996).

We conducted one-tailed analyses because we hypothesised that high

D_2 receptor occupancy was related to worse subjective experience. Analyses were performed for the total group of patients (N=22) and for the group receiving olanzapine (N=15). Analyses for the subgroup using risperidone were not performed, because there was minimal variation of doses in this group.

Results

Both self-control and emotional regulation items on the Subjective Well-Being Under Neuroleptic Treatment Scale correlated with the percentage of D_2 receptor occupancy (Table 1). Positive and Negative Syndrome Scale negative symptoms ratings and the Montgomery Åsberg Depression Rating Scale scores also correlated with D_2 receptor occupancy (Table1). Other Positive and Negative Syndrome Scale sub-scale ratings were not significantly correlated with D_2 receptor occupancy.

Simpson-Angus ratings were zero in most patients, except in two patients each had a total score of 3 and in two patients each had a total score of 4. Six patients had a score of 2 on the Barnes Akathisia scale. Extra-pyramidal symptoms were not correlated with percentage D_2 receptor occupancy.

In the subgroup of patients using olanzapine, a significant correlation was found between D_2 receptor occupancy percentages and the Subjective Wellbeing Under Neuroleptic Treatment Scale self-control total score (r_s=-0.53, N=15, p=0.02) and the Subjective Deficit Syndrome Scale total score (r_s=0.45, N=15, p=0.05). Olanzapine dosage correlated with percentage D_2 receptor occupancy (r_s=0.60, N=15, p=0.01).

The average D_2 receptor occupancy was 67% in the olanzapine group and 77% in the risperidone group.

The correlations found would not have reached statistical significance if they

had been corrected for multiple comparisons by the Bonferroni test.

Measure of Subjective Experience of Psychopathology	Spearman Correlation of Score With Percentage of D_2 receptor occupancy (N=22)	
	r_s	p
Subjective Well-Being under Neuroleptic treatment Scale		
Total score	-0.33	0.06
Emotional regulation	-0.36	0.05
Self-control	-0.41	0.03
Subjective Deficit Syndrome Scale total	0.30	0.09
Montgomery Åsberg Depression Rating Scale	0.46	0.02
Positive and Negative Syndrome Scale		
Negative	0.45	0.02
Positive	0.26	0.12
General	0.22	0.16

Table 1. Correlations between D_2 receptor occupancy and subjective experience and psychopathology in 22 patients with schizophrenia stabilised on olanzapine or risperidone

Discussion

We found a correlation between striatal D_2 receptor occupancy by olanzapine and risperidone and subjective experience, negative symptoms, and de-pression, in the absence of extrapyramidal symptoms. Higher doses of olanzapine were correlated with higher D_2 receptor occupancy and worse subjective experience. Negative subjective experience might be more sensitive to D_2 receptor occupancy than extrapyramidal symptoms. The substantial D_2 receptor occupancy of olanzapine and risperidone we found is in agreement with recent findings of others (Kapur et al., 1999).

Our study design permitted assessment only of correlation between

subjective experience and D_2 receptor occupancy. Therefore, a longitudinal study is required to confirm these results, since a relationship between higher D_2 receptor occupancy and worse subjective experience may have important implications for dosing strategies and compliance with antipsychotic medication.

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[^{123}I]FP-CIT binding in rat brain after acute and sub-chronic administration of dopaminergic medication

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Abstract

The recently developed radioligand [^{123}I]FP-CIT is suitable for clinical single photon emission tomography (SPECT) imaging of the dopamine transporter in vivo. To date, it has remained unclear whether dopaminergic medication influences the striatal [^{123}I]FP-CIT binding. The purpose of this study was to investigate the influence of this medication on [^{123}I]FP-CIT binding in the brain. We used an animal model in which we administered dopaminomimetics, antipsychotics and an antidepressant. In vivo [^{123}I]FP-CIT binding to the dopamine and serotonin transporters was evaluated after sub-chronic and acute administration of the drugs. The administered medication induced small changes in striatal [^{123}I]FP-CIT binding which were not statistically significant. As expected, the dopamine reuptake blocker GBR 12,909 induced a significant decrease in [^{123}I]FP-CIT binding. [^{123}I]FP-CIT binding in the serotonin-rich hypothalamus was decreased only after acute administration of fluvoxamine. The results of this study suggest that dopaminergic medication will not affect the results of dopamine transporter SPECT imaging with [^{123}I]FP-CIT.

Introduction

The development of positron emission tomography (PET) and single photon emission tomography (SPECT) radiotracers for quantification of the dopaminergic nigrostriatal pathway has been successful. Especially quantification of dopamine transporters, situated in the membrane of presynaptic neurons, is now possible. One of the tracers for SPECT imaging of this transporter is [^{123}I]FP-CIT (N-ω-fluoropropyl-2β-carbomethoxy-3β-[4-iodophenyl]nortropane). Dopamine transporter imaging has been mainly focussed on studying parkinsonian syndromes. These studies showed loss of striatal dopamine transporters in

Parkinson's disease, an observation in line with post-mortem findings (Booij et al., 1997a). Imaging of dopamine transporters has also been applied to investigate other neuropsychiatric diseases such as schizophrenia. Due to the practical applicability of [^{123}I]FP-CIT SPECT, this technique will be available in the near future as a routine diagnostic tool to investigate the integrity of the nigrostriatal pathway in vivo. However, in clinical practice, patients referred for SPECT imaging will often be medicated, especially using dopaminomimetic, antipsychotic, or antidepressant medication. To date, it has remained unclear whether this medication influences in vivo binding of [^{123}I]FP-CIT to striatal dopamine transporters. Therefore, we studied acute and sub-chronic administration of various drugs in rats.

Materials and Methods

Chemicals and radiolabelling of FP-CIT

Medication under study consisted of the following compounds (Table 1). Sinemet® (100 mg levodopa combined with 25 mg of the decarboxylase inhibitor carbidopa) was obtained from Merck Sharp & Dome. The dopamine receptor agonist pergolide (Permax®) was a generous gift from Eli Lilly Corp. Antipsychotic medication consisted of two atypical antipsychotics (olanzapine and risperidone, obtained from Eli Lilly Corp. and Janssen Pharm., respectively) and one classical antipsychotic (haloperidol, obtained from Janssen Pharm.). The selective serotonin reuptake inhibitor fluvoxamine was a generous gift from Solvay Pharmaceuticals. The potent selective dopamine uptake blocker GBR 12,909 and MAO-B inhibitor selegiline (L-deprenyl®) were obtained from Research Biochemicals International (RBI, Natrick, Mass.). All compounds were

administered in a quantity higher than the therapeutic human doses; however administration of excessive doses was avoided.

[^{123}I]FP-CIT was provided by Amersham Cygne (Eindhoven, The Netherlands) at a specific activity of >185 MBq/nmol and a radiochemical purity >95% in a sodium acetate buffer (pH 4.75) with 5% ethanol. The radioligand was further diluted with 5% ethanolic acetate buffer to the appropriate concentration for intravenous (i.v.) injection into rats.

Sub-chronic administration		Acute administration	
Pergolide	1 mg/kg	Pergolide	5 mg/kg
Olanzapine	2 mg/kg	Olanzapine	10 mg/kg
Sinemet®	125 mg/kg	Sinemet®	125 mg/kg
Selegiline	1 mg/kg	Selegiline	5 mg/kg
Risperidone	1 mg/kg	Risperidone	5 mg/kg
Haloperidol	1 mg/kg	Haloperidol	5 mg/kg
Fluvoxamine	2 mg/kg	Fluvoxamine	10 mg/kg
		GBR12,909	5 mg/kg

Table 1. Medication administered to rats for two weeks (sub-chronic administration) or on the day of imaging (acute administration)

In vivo distribution in rats

Drug administration

Male Wistar rats (Broekman Institute B.V., Someren, The Netherlands; bodyweight 250-350 g) were used. Sub-chronic administration was performed in groups of 4-5 rats per compound, for 2 weeks with a daily i.v. administration of a dose dissolved in 0.5 ml 5% glucose solution. A control group of rats received an i.v. dose of 0.5 ml 5% glucose solution. Olanzapine, Sinemet® and pergolide were administered orally because of their low solubility. Radioligand distribution studies (*vide infra*) were done at 4 h after the last drug administration. Doses of chemicals are listed in Table 1. This sub-chronic study was split in two sessions because of logistic reasons, with use of a separate control group to exclude confounding factors.

In a second series of experiments, acute administration of compounds was performed i.v. 5 min prior to injection of [^{123}I]FP-CIT, whereas a group of controls received an i.v. injection of 0.5 ml 5% glucose solution. Olanzapine, Sinemet® and pergolide were administered orally 2 h prior to [^{123}I]FP-CIT injection because of their low solubility.

To validate our animal model, a third series of experiments was added in which a group of rats received an i.v. injection with an excess of GBR 12,909, 5 min prior to injection with [^{123}I]FP-CIT, whereas a control group received an i.v. injection of 0.5 ml 5% glucose solution.

Biodistribution

Rats were injected with approximately 1.85 MBq [^{123}I]FP-CIT/0.4 ml buffer into the tail vein under ether anaesthesia. Groups of rats were sacrificed 2 h after injection of [^{123}I]FP-CIT. Rats were killed via heart puncture under ether anaesthesia. Several brain areas were rapidly excised and weighed. Radioactivity in each region was assayed in a gamma counter. All data were corrected for radioactive decay. Radioactivity concentrations were

expressed as percent injected dose, multiplied by the body weight per gram tissue (%ID * g/g tissue), which is a slight modification from the procedure as described previously (Rijks et al., 1996).

The striatum was chosen as an area of binding to dopamine transporters due to its high concentration of dopamine reuptake sites. The hypothalamus was chosen as an area representative of binding to serotonin transporters because it contains many serotonin, but few dopamine transporters (Kuhar et al., 1972). The cerebellum was used as the reference region for the estimation of free and non-specifically bound radioligand.

The study was approved by the Animal Ethical Commission of the Academic Medical Center, according to international laws on protection of animals.

Statistical analysis

The difference in radioligand binding in individual brain regions was analysed by ANOVA using SPSS 7.5 software. In the case of multiple comparisons, the Tukey post hoc test was used. In all statistical analyses, probability values < 0.05 were considered significant.

Results

Striatal and cerebellar binding

In control rats, injection of [^{123}I]FP-CIT resulted in higher striatal binding than cerebellar binding. Sub-chronic administration of none of the compounds showed a significant difference in [^{123}I]FP-CIT binding compared with controls (Table 2). This was true for both absolute striatal and cerebellar binding, and for striatum-to-cerebellum binding ratios.

After acute administration of all drugs under study, the absolute striatal binding of [^{123}I]FP-CIT was not significantly different from controls

(Table 3). The striatum-to-cerebellum binding ratios were also not affected by acute administration of medication.

After pre-treatment with GBR 12,909, the absolute binding in the striatum as well as the striatum-to-cerebellum binding ratio was significantly decreased (Table 4). The cerebellum proved to be a suitable reference region, since GBR 12,909 did not significantly influence [¹²³I]FP-CIT binding in this region.

	Controls 1[b]	Pergolide	Olanzapine	Sinemet
Striatum	223.48±22.83	256.30±37.29	274.63±45.06	223.85±46.17
Hypothalamus	115.73±6.27	125.68±19.43	143.15±33.96	121.35±15.39
Cerebellum	56.30±0.88	65.57±11.13	70.77±12.99	57.80±11.06
Striatum/ Cerebellum	3.97±0.42	3.95±0.49	3.90±0.28	3.74±0.31
Hypothalamus/ Cerebellum	2.06±0.14	1.93±0.21	2.01±0.12	2.05±0.18

	Controls 2[b]	Selegiline	Risperidone	Haloperidol	Fluvoxamine
Striatum	208.08±25.21	184.00±24.97	188.32±45.32	206.61±29.85	207.12±12.81
Hypothalamus	107.01±21.67	94.42±12.53	88.01±14.25	102.11±22.91	115.57±23.13
Cerebellum	52.31±11.65	53.63±8.45	43.13±4.44	43.76±6.63	52.30±4.56
Striatum/ Cerebellum	4.07±0.65	3.45±0.30	4.42±1.32	4.54±1.11	3.97±0.28
Hypothalamus/ Cerebellum	2.08±0.42	1.77±0.09	2.04±0.31	2.15±0.56	2.20±0.28

[a] Data are given as %ID*g/g and represent the mean ± SD of 4 or 5 rats. Drugs were administered daily for 2 weeks. Radioactivity was measured 2 h after injection of the radiotracer
[b] For logistic reasons, this study was split in two sessions with separate control groups.

Table 2. [^{123}I]FP-CIT binding in groups of 4 or 5 rats after sub-chronic administration of pergolide, olanzapine, Sinemet, selegiline, risperidone, haloperidol and fluvoxamine [a]

	Controls	Pergolide	Olanzapine	Sinemet
Striatum	272.37±73.14	296.38±8.85	292.39±17.32	319.47±36.63
Hypothalamus	132.30±8.78	120.59±3.33	164.29±7.42	149.22±24.64
Cerebellum	70.98±5.56	67.31±1.60	74.14±3.95	75.62±10.27
Striatum/Cerebellum	3.83±0.91	4.40±0.05	3.96±0.43	4.24±0.24
Hypothalamus/Cerebellum	1.88±0.14	1.79±0.04	2.22±0.03	1.97±0.14

	Risperidone	Haloperidol	Fluvoxamine	Selegiline
Striatum	342.32±93.37	297.85±24.49	258.24±15.97	212.39±55.98
Hypothalamus	130.22±30.07	131.18±9.79	79.88*±6.93	114.07±29.24
Cerebellum	68.83±13.27	67.28±4.16	68.90±7.85	59.22±12.08
Striatum/ Cerebellum	4.94±0.59	4.43±0.26	3.76±0.21	3.57±0.48
Hypothalamus/Cerebellum	1.88±0.14	1.95±0.16	1.16*±0.09	1.92±0.14

$*P<0.01$; statistically significant different from controls;

[a] Data are given as %ID * g/g and represent the mean ± SD of 4 or 5 rats. Drugs were administered orally 2 h before injection of [¹²³I]FP-CIT and radioactivity was measured 2 h p.i.

Table 3. [¹²³I]FP-CIT binding in groups of 4 or 5 rats after acute administration of pergolide, olanzapine, Sinemet, risperidone, haloperidol, fluvoxamine and selegiline [a].

	Controls	GBR12,909
Striatum	194.22±40.49	104.35*±3.91
Hypothalamus	102.82±22.23	82.89±13.97
Cerebellum	49.46±7.64	43.38±9.80
Striatum/Cerebellum	3.90±0.24	2.51*±0.66
Hypothalamus/Cerebellum	2.07±0.15	1.93±0.13

$*P<0.01$ statistically significant different from controls

[a] Data are given as %ID * g/g and represent the mean ± SD of 4-5 rats. Drugs were administered i.v. 5 min before injection of [¹²³I]FP-CIT and radioactivity was measured 2 h p.i.

Table 4. [¹²³I]FP-CIT binding in groups of 4 rats after acute administration of GBR12,909 [a]

Hypothalamus binding

Sub-chronic administration of all compounds showed no statistical significant effect on absolute [^{123}I]FP-CIT binding in the hypothalamus and in hypothalamus-to-cerebellum binding ratios (Table 2). Acute administration of fluvoxamine showed a significant decrease in absolute [^{123}I]FP-CIT binding in the hypothalamus, as well as in hypothalamus-to-cerebellum binding ratios (Table 3). None of the other compounds induced a significant change in absolute [^{123}I]FP-CIT binding in the hypothalamus, or in hypothalamus-to-cerebellum uptake ratio.

Discussion

Sub-chronic administration

In this study, only small changes in absolute [^{123}I]FP-CIT binding in rat striatum and hypothalamus were found after sub-chronic administration of dopaminergic medication or fluvoxamine. None of these changes was statistically significant. Ratios of striatum- and hypothalamus-to-cerebellum binding were also not significant influenced by sub-chronic administration of this medication.

Our findings are in line with recent studies, in which no effect of levodopa on in vivo radioligand binding to striatal dopamine transporters was found in humans (Innis et al., 1999) and animals (Vander Borght T. et al., 1995). While no effect of selegiline administration on in vivo radioligand binding to striatal dopamine transporters was found in humans (Innis et al., 1999), animal studies showed inconsistent results (Scheffel et al., 1996; Vander Borght T. et al., 1995).

We found no significant influence of pergolide on [^{123}I]FP-CIT binding to dopamine transporters. In agreement with this, no effect of treatment with the dopamine agonist apomorphine on striatal [^{3}H]WIN 35,428 binding was recently reported (Vander Borght T. et al., 1995). However, the finding

in the latter study of decreased striatal [^{3}H]WIN 35,428 binding after halo-peridol administration was not confirmed in our study. In addition, our study showed no significant influence of sub-chronic administration of olanzapine and risperidone on striatal [^{123}I]FP-CIT binding. Nevertheless, significant influences of administration of chronic administration of these drugs cannot be excluded conclusively by means of the employed experimental paradigm.

Acute administration

Absolute striatal [^{123}I]FP-CIT binding and the striatum-to-cerebellum uptake ratio were not affected by acute administration of dopaminergic drugs and fluvoxamine. In agreement with our findings, striatal [^{3}H]WIN 35,428 binding has been shown to be insensitive to acute haloperidol administration (Scheffel et al., 1996). Our present data also show that acute administration of the atypical antipsychotics olanzapine and risperidone had no influence on striatal [^{123}I]FP-CIT binding.

A previous animal study showed that acute administration of 50 mg/kg of levodopa in baboons had no influence on specific striatal [^{123}I]β-CIT binding (Laruelle et al., 1993). Moreover, using [^{99m}Tc]TRODAT-1, Dresel et al. (Dresel et al., 1998) found no acute effect of dopaminergic drugs on striatal binding. In contrast to this, intraperitoneal administration of 50 mg/kg of levodopa or more resulted in a decrease of striatal [^{3}H]WIN 35,428 binding in mice (Scheffel et al., 1996). However, this decrease was not found when levodopa was administrated more than 2.5 h before injection of [^{3}H]WIN 35,428. In our study, 100 mg/kg levodopa was administered orally 2 h before injection of [^{123}I]FP-CIT, without inducing a decrease in striatal binding. This difference may be explained by the fact that oral administration, which resembles clinical practice, induces slow delivery of the compound to the brain. Accordingly, the peak brain concentration of the compound is lower than following intraperitoneal injection.

In the present as well as in previous studies from our laboratory, GBR 12,909 and fluvoxamine significantly blocked absolute [^{123}I]FP-CIT binding in the striatum and the hypothalamus, respectively (Booij et al., 1997b). These findings suggest that this animal model can be used to detect possible effects of medication on [^{123}I]FP-CIT binding to dopamine or serotonin transporters.

The finding of a decrease of [^{123}I]FP-CIT binding in the hypothalamus after acute administration of fluvoxamine is in line with a study that reports that clomipramine decreased [^{123}I]β-CIT binding in the hypothalamus (Fujita et al., 1997). By contrast, the finding in that study of enhanced striatal [^{123}I]β-CIT binding after clomipramine administration was not matched in our study with fluvoxamine and [^{123}I]FP-CIT. However, this enhancement was found with relatively high doses of clomipramine. It would be of interest to see whether or not this phenomenon might also be found with higher doses of fluvoxamine than were used in the present study.

A limitation of this study is the relatively small number of animals per group. This might explain the high variance in some treatment groups. Therefore, our results are quite vulnerable to false-negative errors.

Conclusion

The results of this study suggest that it is most likely that dopaminergic drugs, in therapeutic dosages, do not affect the results of dopamine transporter imaging with [^{123}I]FP-CIT in humans. Therefore it seems unnecessary to interrupt dopaminergic therapy for [^{123}I]FP-CIT SPECT imaging.

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6

Dopamine transporter density in young patients with schizophrenia assessed with [123I]FP-CIT SPECT

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Abstract

Disturbances in the dopamine system are thought to play a major role in schizophrenia. Amphetamine-induced release of endogenous dopamine is shown to be enhanced in schizophrenia, as is striatal [^{18}F]FDOPA uptake in the striatum. It is not clear if the density of dopamine neurons is altered in schizophrenia. By studying the dopamine transporter with [^{123}I]FP-CIT Single Photon Emission Computed Tomography (SPECT), the density of nigrostriatal dopaminergic cells can be studied.

Dopamine transporter density in the striatum, was studied in 36 young patients with schizophrenia using [^{123}I]FP-CIT SPECT. Ten patients were antipsychotic-naive, 15 were treated with olanzapine, 8 with risperidone and 3 were antipsychotic-free. A control group of 10 age-matched volunteers was included.

Striatal [^{123}I]FP-CIT binding was not significant different between antipsychotic-naive patients (2.87), patients treated with olanzapine (2.76), patients treated with risperidone (2.76), antipsychotic-free patients (2.68) and controls (2.82) (F = 0.07, p = 0.98). Unexpectedly, striatal [^{123}I]FP-CIT binding in females was significantly higher than in males (3.29 and 2.70, respectively) (t = -2.56, p = 0.014).

Concluding, functional changes in the dopaminergic system in schizophrenia are not likely to be reflected in a change in dopamine transporter density. Moreover, dopamine transporter density does not seem to be altered by antipsychotic medication.

Introduction

A dysregulation of the dopaminergic neurotransmission system has been thought to contribute to the pathophysiology of schizophrenia (Davis et al., 1991). Pharmacological evidence shows that dopamine antagonists have

beneficial effects in patients suffering from schizophrenia, whereas dopamimetic agents exacerbate symptoms (Lieberman et al., 1987). Recent neuro-imaging studies found significant changes in the presynaptic dopamine system in schizophrenic patients. First, the endogenous dopamine release, following an amphetamine challenge, is increased in patients with schizophrenia (Laruelle et al., 1996a; Breier et al., 1997; Abi-Dargham et al., 1998). Second, in two [^{18}F]FDOPA Positron Emission Tomography (PET) studies, an increased [^{18}F]FDOPA uptake in the putamen was found in schizophrenia (Reith et al., 1994; Hietala et al., 1995). In a more recent PET study, an increased [^{18}F]FDOPA uptake was confirmed. Moreover, depressive symptoms were found to correlate negatively with [^{18}F]FDOPA uptake (Hietala et al., 1999). These studies suggest a disturbed presynaptic dopamine neurotransmission in schizophrenia, which can be detected in the striatum.

The increased striatal [^{18}F]FDOPA uptake and increased drug-induced endogenous dopamine release could be explained by a higher density of dopamine nerve terminals and, consequently, dopamine transporters in the striatum. The dopamine transporters (or reuptake sites) are located on nerve terminals and play a role in the reuptake of dopamine from the synaptic cleft.

Dopamine transporter density in the human brain can be assessed in vivo with Single Photon Emission Computed Tomography (SPECT) imaging, using N-ω-fluoropropyl-2β-carbomethoxy-3β[4-iodophenyl]-tropane (FP-CIT), labeled with ^{123}I, as a radioligand. [^{123}I]FP-CIT SPECT is a highly reproducible technique, which is used in clinical studies to detect presynaptic degeneration of dopaminergic neurons in patients with Parkinson (Booij et al., 1997; 1998).

To examine possible changes in striatal dopamine transporter density in schizophrenia, we compared dopamine transporter density in young patients with schizophrenia with age-matched healthy volunteers by using [^{123}I]FP-CIT SPECT.

Antipsychotic medication is the cornerstone of therapy in patients with schizophrenia. However, the influence of antipsychotic medication on striatal [^{123}I]FP-CIT binding has not been elucidated in humans. Therefore, in our study, patients who were never before treated with antipsychotics (antipsychotic-naive) were compared with patients treated with the atypical antipsychotic olanzapine or risperidone, and with patients previously treated with antipsychotics who were now antipsychotic-free. Finally, we studied correlations between clinical symptoms of schizophrenia and dopamine transporter density.

Methods

Subjects

SPECT imaging was performed in 38 first-admitted patients at the Adolescent clinic of the Academic Medical Center. All patients suffered from a first or second psychotic episode and had a diagnosis of schizo-phrenia according to DSM IV (American Psychiatric Association, 1994), which was confirmed during outpatient follow-up. Two patients were left out of the analysis because of a different diagnosis during follow-up (i.e. substance-induced psychotic disorder and obsessive compulsive dis-order). Therefore, 36 patients were studied, divided into 4 subgroups (characteristics are listed in Table 1). The first group consisted of 10 anti-psychotic-naive patients. Moreover, at the moment of SPECT imaging no other medication was taken. A second group of 15 patients was treated with 5 to 30 mg of olanzapine (mean 16.3 mg, SD 7.2). A third group of 8 patients was treated with 2 to 6 mg of risperidone (mean 3.5 mg, SD 1.3). Antipsychotic type and dose were stable from at least 6 weeks before SPECT imaging in both antipsychotic-treated groups. A fourth group consisted of 3 patients who did not take antipsychotic medication for more than 4 weeks before SPECT imaging. Six patients of the medicated group

were co-medicated with selective serotonin reuptake inhibitor (SSRI) medication at the moment of SPECT imaging, (4 with paroxetine 20-30 mg, one with fluvoxamine 150 mg, one with venlafaxine 300 mg), one was co-medicated with amitriptyline 225 mg and one was co-medicated with lithium 1000 mg.

A group of 10 healthy volunteers, with a mean age comparable to that of the patient groups, was included in this study. Volunteers were free from any neurological or psychiatric disease and were not taking any drugs.

All patients and controls participated after informed consent. The research protocol was approved by the medical ethical committee of the Academic Medical Center in Amsterdam.

Groups	n	Age in years (S.D.)	Sex	Illness duration[a]	Subtype[b]	Medication in mg (S.D.)
AP-naive	10	22.1 (3.7)	9M 1F	33.5 (8.5)	5 Par, 1 Dis, 3 Und, 1 Sch-aff	
Olanzapine	15	21.0 (2.5)	12M 3F	16.5 (17.0)	6 Par, 8 Und, 1 Sch-aff	16.3 (7.2)
Risperidone	8	22.8 (4.1)	8M	18.5 (20.5)	4 Par, 2 Und, 2 Sch-aff	3.5 (1.3)
AP-free	3	19.0 (2.0)	3M	13.7 (7.0)	1 Par, 1 Und, 1 Sch-aff	
Control	10	20.3 (0.5)	7M 3F			

[a]Illness duration in months at the time of SPECT imaging (median)
[b]Subtype: Paranoid (Par), Disorganized (Dis) and Undifferentiated (Und) subtype of schizophrenia, and Schizoaffective disorder (Sch-aff)

Table 1 Composition of groups of schizophrenic patients and controls

SPECT procedure

For SPECT imaging a brain-dedicated camera was used (Strichman Medical Equipment Inc, Medfield, Mass., USA). This camera consists of twelve individual crystals each equipped with a focussing collimator. The transaxial resolution of this camera is 7.6 mm full width half maximum (FWHM) of a line source in air, and the axial resolution is 13.5 mm FWHM. The energy window was set at 135-190 keV.

All subjects received potassium iodide orally to block thyroid uptake of free radioactive iodide. [^{123}I]FP-CIT (specific activity of > 185 MBq/nmol; radiochemical purity of > 95 %) was injected intravenously at an approximate dose of 110 MBq. ^{123}I labeling of FP-CIT was performed by Amersham Cygne (Eindhoven, The Netherlands) with the trimethylstannyl precursor of FP-CIT obtained from Research Biochemicals International (Natick, MA, USA). SPECT acquisition was performed at 3 h p.i. (Booij et al., 1999). Images were acquired during periods of 210 s from the orbitomeatal line to the vertex with an interslice distance of 5 mm. Data acquisition took place in a 128x128 matrix.

Attenuation correction and reconstruction of the images were performed as described earlier (Booij et al., 1997). The measured concentration of radioactivity was expressed as Strichman Medical Units (SMUs; 1 SMU = 100 Bq/ml as specified by the Strichman Medical Equipment Inc).

Data processing

Assessment of [^{123}I]FP-CIT binding in the entire striatum, caudate nucleus, putamen and occipital cortex (non-specific binding) was performed with a recently developed fully automated three-dimensional technique. This technique has been described in detail by Habraken and co-workers (1999). Briefly, this method automatically places volumes of interest (VOI) over the brain areas instead of manually placing predefined two-dimensional regions of interest (ROI), as in traditional SPECT data analysis. Binding activity is compared on a voxel-by-voxel base to achieve

the best fit. This automated arranging of volumes is operator independent and repeatable. Caudate nucleus and putamen were defined as sub-regions of the striatum and occipital cortex (OCC) was used as a reference region. Specific to non-specific [^{123}I]FP-CIT binding was calculated as: [^{123}I]FP-CIT binding = (VOI - OCC) / OCC, in which VOI represents the mean radioactivity (in SMU) in the VOI (striatum, caudate nucleus or putamen).

Asymmetry of striatal [^{123}I]FP-CIT binding ratios was calculated with the Asymmetry Index (AI): (right - left) / (right + left). If the index is positive, the binding ratio is higher at the right than left side.

Clinical Measurements

Psychotic symptoms were assessed in all patients in the week of imaging by one of the investigators (J.L.) who was blinded to the results of SPECT imaging. All antipsychotic-naive patients were interviewed on the day of imaging. Psychotic symptoms were rated with the structured clinical interview of the PANSS (Positive And Negative Symptoms Scale for schizophrenia; (Kay et al., 1987). Depression was rated with the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979). Akathisia was assessed with the Barnes akathisia rating scale (Barnes, 1989).

Statistics

Differences between groups were calculated with one-way ANOVA. Linear regression was performed in the total group of all subjects under study, and separately in one group with all patients, and in the group of medicated patients. Striatal [^{123}I]FP-CIT binding was used as dependent variable. Correlations between variables were measured using a two-tailed Spearman's rho (ρ). A significance level of $p < 0.05$ was used if not otherwise indicated. All statistical analyses were carried out with SPSS 9.0 for windows.

Results

[^{123}I]FP-CIT SPECT imaging

Visually, striatal [^{123}I]FP-CIT binding was not different among the 5 groups
(Figures 1 and 2). No significant differences in specific to nonspecific
[^{123}I]FP-CIT binding ratios were found between patient groups and controls
(Table 2). This was true for binding ratios in the entire striatum (F = 0.07,
p = 0.98), caudate nucleus (F = 0.04, p = 0.99) and putamen (F = 0.15,
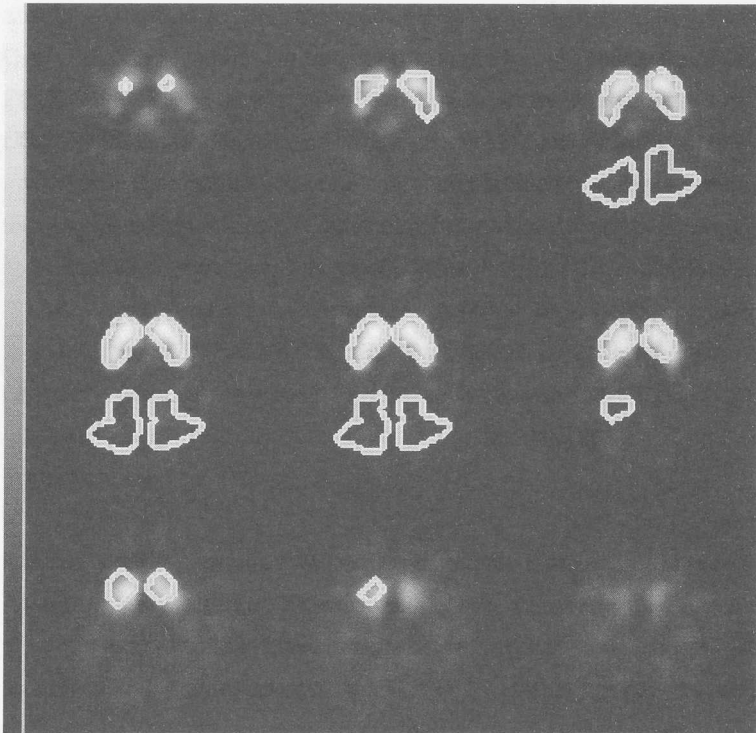p = 0.93).

Figure 1 [^{123}I]FP-CIT SPECT images of an antipsychotic-naive patient with schizophrenia.
Transverse slices of the striatum with overlaid Volumes of Interest for striatum and occipital
cortex. The level of radioactivity is color encoded from low (black) to high (white).
Colour version on the inside of the cover.
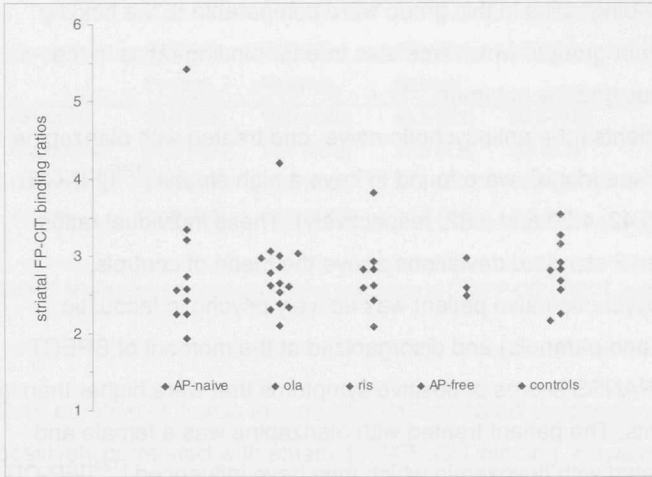
Figure 2 Specific striatal to non-specific Striatal [^{123}I]FP-CIT binding ratios (mean left and right side) in schizophrenic patients and normal controls. At the moment of imaging patients were antipsychotic-naive, treated with olanzapine (ola), treated with risperidone (ris), or antipsychotic-free.

Group	Striatum (S.D.)	Putamen (S.D.)	Caudate Nucleus (S.D.)	Asymmetry Index (S.D.)[b]
AP-naive	2.87 (0.99)	2.97 (1.09)	2.81 (0.94)	-0.001 (0.018)
Olanzapine	2.76 (0.49)	2.76 (0.51)	2.76 (0.49)	-0.013 (0.014)
Risperidone	2.76 (0.50)	2.83 (0.52)	2.73 (0.50)	-0.009 (0.031)
AP-free	2.68 (0.26)	2.71 (0.30)	2.66 (0.25)	-0.016 (0.006)
Control	2.82 (0.43)	2.86 (0.45)	2.79 (0.44)	0.000 (0.017)

[a][^{123}I]FP-CIT binding ratio is determined as (region of interest-occipital binding) / occipital binding

[b]Asymmetry Index is (right - left) / (right + left) of the striatal binding ratios

Table 2 Mean [^{123}I]FP-CIT SPECT binding ratios in patients with schizophrenia and controls[a]

The Asymmetry Index was low in all groups (Table 2). No significant difference in asymmetry index was found between groups in the entire striatum, caudate nucleus, or putamen.

The antipsychotic-free group was left out of the analysis because of the small number of subjects. However, as shown in figure 2, the striatal

[^{123}I]FP-CIT binding ratios in this group were comparable to the binding ratios of the other groups, which was also true for binding ratios in the caudate nucleus and the putamen.

Three patients (one antipsychotic-naive, one treated with olanzapine and one with risperidone), were found to have a high striatal [^{123}I]FP-CIT binding ratio (5.42, 4.20 and 3.82, respectively). These individual ratios were more than 2 standard deviations above the mean of controls.

The antipsychotic-naive patient was actively psychotic (acoustic hallucinations and paranoia) and disorganized at the moment of SPECT imaging, with PANSS scores of positive symptoms that were higher than in all other patients. The patient treated with olanzapine was a female and was co-medicated with fluvoxamin which may have influenced [^{123}I]FP-CIT SPECT imaging.

Symptoms and [^{123}I]FP-CIT SPECT imaging

In the antipsychotic-naive group, PANSS rates of positive and negative symptoms of schizophrenia and general psychopathology (Table 3), did not correlate with [^{123}I]FP-CIT binding ratios in the entire striatum ($\rho = 0.40$, $p = 0.26$; $\rho = 0.13$, $p = 0.71$; $\rho = 0.49$, $p = 0.16$, respectively). These PANSS rates were also not significantly correlated with [^{123}I]FP-CIT binding ratios in the caudate nucleus or putamen.

Also, these symptoms did not correlate with [^{123}I]FP-CIT binding ratios in both medicated patient groups. In all patient groups, depression rates did not correlate with [^{123}I]FP-CIT binding ratios in all 3 regions.

Group	PANSS Positive	PANSS Negative	PANSS General	MADRS
AP-naive	22.8 (3.8)	18.9 (6.66)	43.5 (8.1)	22.5 (9.5)
Olanzapine	11.2 (4.4)	14.7 (6.08)	27.0 (4.8)	9.3 (8.3)
Risperidone	11.7 (4.9)	14.0 (4.20)	27.7 (7.7)	10.6 (10.4)
AP-free	11.0 (2.0)	15.0 (8.89)	24.3 (7.6)	2.0 (2.0)

Table 3 Mean psychotic and depressive symptoms at [^{123}I]FP-CIT SPECT imaging (S.D.)

Both duration of illness and duration of untreated psychosis were positively correlated with striatal [^{123}I]FP-CIT binding. However, this correlation was caused by outliers. Specifically, the three patients with exceptionally high striatal [^{123}I]FP-CIT binding also had longer duration of illness (60, 35 and 98 months) and duration of untreated psychosis (60, 29 and 85 months), compared with the median of all 4 patient groups (16 and 6.5 months for duration of illness and duration of untreated psychosis, respectively).

Age of onset of psychotic symptoms and subtype of schizophrenia were not significantly correlated with [^{123}I]FP-CIT binding ratios.

Medication and [^{123}I]FP-CIT SPECT imaging

In the group of patients with present or previous antipsychotic medication ($n = 26$), linear regression showed no significant influence of duration of antipsychotic treatment and of SSRI co-medication on striatal [^{123}I]FP-CIT binding ratios. Furthermore, no correlation was found between daily dose of olanzapine or risperidone and [^{123}I]FP-CIT binding ratios ($n = 15$, $\rho = -0.099$, $p = 0.725$; $n = 8$, $\rho = -0.210$, $p = 0.618$, respectively).

Six out of 15 patients treated with olanzapine experienced questionable to moderate akathisia and 4 out of 8 patients treated with risperidone. Akathisia rates were not significantly correlated with striatal [^{123}I]FP-CIT binding in patients treated with olanzapine or risperidone.

Gender and [^{123}I]FP-CIT binding

In females, the striatal [^{123}I]FP-CIT binding ratios were significantly higher
than in males, 3.29 and 2.70, respectively (t = -2.56, p = 0.014). This
difference was found both in the 3 female controls compared with male
controls, as well as in the 4 female patients compared with the male
patients, though the latter only at a trend level (p = 0.07). Moreover, in a
linear regression analysis including all subjects (n = 45), gender was the
only variable with a significant effect on striatal [^{123}I]FP-CIT binding ratios
(R^2 = 0.341, β = -0.565, p < 0.001). Group, handedness and age were of
no significant influence on [^{123}I]FP-CIT binding ratios. One AP-naive
patient was left out of the regression analysis because the [^{123}I]FP-CIT
binding ratio was more than three standard deviations above the mean.

Discussion

Dopamine transporter density in schizophrenia

In this study we found no significant difference in specific to nonspecific
striatal [^{123}I]FP-CIT binding ratios between young antipsychotic-naive
patients with schizophrenia, medicated patients with schizophrenia and
controls. This was also true for [^{123}I]FP-CIT binding ratios in the two
subdivisions of the striatum, the caudate nucleus and the putamen. These
findings, in a large group of schizophrenic patients, may indicate that there
are no significant changes in striatal dopamine transporter density in
schizophrenia.

Our findings are in line with a study by Laruelle and co-workers,
which showed no change in striatal dopamine transporter density in
schizophrenia assessed by [^{123}I]β-CIT SPECT (1996b). Our findings are
also in line with post mortem studies, in which no change in dopamine
transporter density in schizophrenia was found (Hirai et al., 1988 Joyce et

al., 1988; Czudek and Reynolds, 1989; Pearce et al., 1990; Chinaglia et al., 1992; Knable et al., 1994).

In contrast with our findings, Tatsch and co-workers recently found a lower density of striatal dopamine transporters in patients with schizophrenia than in controls, assessed by [^{123}I]IPT SPECT (1999). However, their preliminary results were obtained in a relatively small group of patients, and it is not clear whether data were analyzed with an operator independent analysis technique. SPECT imaging with [^{123}I]FP-CIT, using an operator independent three-dimensional analysis technique, has been shown to be a sensitive and reproducible technique to visualize and quantify the dopamine transporter density in vivo (Booij et al., 1998; Habraken et al., 1999). Therefore, the lack of changes we found in striatal dopamine transporter density in schizophrenia is a reliable observation.

Earlier studies showed a higher striatal [^{18}F]DOPA uptake in schizophrenic patients (Hietala et al., 1999). Taking into account the results of the present study, the increased [^{18}F]DOPA uptake may be explained by an increased decarboxylase activity instead of an increased number of dopamine terminals.

Antipsychotic medication and the dopamine transporter
In patients treated with olanzapine or risperidone, the [^{123}I]FP-CIT binding ratios in all measured striatal regions were not significantly different from those in antipsychotic-naive patients and those in controls. Therefore, it can be presumed that treatment with atypical AP medication does not change dopamine transporter density. Chronic administration of the classic antipsychotic haloperidol showed different effects on dopamine transporter density in two animal studies. In one study a reduced pre-synaptic dopamine reuptake was found (Vander Borght et al., 1995) in rats with chronic administration of antipsychotic medication, whereas no difference with controls was found in another study (Rivest et al., 1995). Chronic administration of olanzapine and risperidone induced no significant effect on striatal [^{123}I]FP-CIT binding in rats (Lavalaye et al.,

2000). Unexpectedly, we found a higher striatal [^{123}I]FP-CIT binding in women than in men. This was found both in patients and in controls. Higher dopamine transporter density in females than in males has been reported in animal studies (Rivest et al., 1995), but no effect of gender on dopamine transporter density was found in a human study (van Dyck et al., 1995). In addition, an [^{18}F]DOPA PET study performed in patients with attention deficit hyperactivity disorder (ADHD) and controls, showed higher striatal uptake ratios in women compared to men (Ernst et al., 1998). This was found both in patients and controls. The higher striatal [^{18}F]DOPA uptake may imply a higher number of dopaminergic terminals and, consequently, of dopamine transporters in females, which is in line with our findings.

In general, dopamine transporter imaging studies are performed in groups of a wide age range, mostly including subjects who are older than the subjects in this study. Therefore, gender- related differences might be overlooked easily. Nevertheless, gender differences in the dopamine system play an important role in schizophrenia (Szymanski et al., 1995). This is closely related to hormonal influence, which is of influence in schizophrenia, and which has been extensively discussed (Seeman, 1997). However, it has to be kept in mind that the number of females in this study was limited. Therefore, it would be of interest to confirm our finding of a higher dopamine transporter density in women in a larger study, or by using a meta-analytical approach.

The small age range of the subjects in the present study is an important feature, since aging is associated with a clear loss of dopamine transporters, according to several PET and SPECT studies (van Dyck et al., 1995; Volkow et al., 1998). Therefore, a wide age range is a relevant confounding factor in many imaging studies.

Three patients in this study were found to have a very high striatal [^{123}I]FP-CIT binding, compared with controls. In these 3 patients the duration of untreated psychosis was more than two years. It is not clear whether this could explain the high [^{123}I]FP-CIT binding, because a large

variability in the number of striatal dopamine transporters has been observed in controls (van Dyck et al., 1995). In addition, a high density of dopamine D_2 receptors was found in a subgroup of antipsychotic-naive schizophrenics (Hietala et al., 1994).

Conclusion

Striatal dopamine transporter density in young patients with schizophrenia is not different than in controls, as assessed with [^{123}I]FP-CIT SPECT. Moreover, dopamine transporter density is not significantly different in drug-naive compared to medicated patients. In controls, as well as in patients, we observed a higher striatal density of dopamine transporters in women than in men. Further exploring the dopamine system in schizophrenia, and the possible involvement of presynaptic changes, may lead to a better understanding of this devastating disease, and finally to a more causal therapy.

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7

Effect of age and gender on dopamine transporter imaging with [123I]FP-CIT SPECT in healthy volunteers

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Abstract

Dopamine transporter imaging is a valuable tool to investigate the integrity of the dopaminergic neurons. To date, several reports have shown an age-associated decline of dopamine transporters in healthy volunteers. Although animal studies suggest an effect of gender on dopamine transporter density, this gender effect has not yet been confirmed in human studies.

To study the influence of age and gender on dopamine transporter imaging in healthy volunteers, we performed single photon emission computed tomography (SPECT) imaging with [123I]FP-CIT to quantify dopamine transporters. Forty-five healthy volunteers (23 males and 22 females) were included, ranging in age from 18 to 83 years. SPECT imaging was performed 3 h after injection of ± 110 MBq [123I]FP-CIT. An operator independent volume of interest analysis was used for quantification of [123I]FP-CIT binding in the striatum.

The ratio of specific striatal to non-specific [123I]FP-CIT binding was found to decrease significantly with age. Moreover, we found a high variance in [123I]FP-CIT binding in young adults. Finally, females were found to have significantly higher [123I]FP-CIT binding ratios than males. This effect of gender on [123I]FP-CIT binding ratios was not related to age.

The results of this study are consistent with findings from previous studies, which showed that dopamine transporter density declines with age. The intriguing finding of a higher dopamine transporter density in females than in males is in line with findings from animal studies.

Introduction

Dopamine transporter imaging with [123I]FP-CIT single photon emission computed tomography SPECT is a valuable tool in the diagnostic process

for patients with parkinsonian symptoms. However, confounding factors have to be taken into account when using dopamine transporter imaging as a diagnostic tool. For example, an age-related decline in dopamine transporter density in healthy volunteers has been reported (Kuikka et al., 1999; Mozley et al., 1999; Tissingh et al., 1998; van Dyck et al., 1995; Volkow et al., 1996). Most of these studies described a linear pattern of decline, although one study proposed a broken stick-model with a faster decrease in young adults than in old age (Mozley et al., 1999).

A gender effect on dopamine transporter heterogeneity has been reported in humans, with females having a higher heterogeneity in the striatum than males (Kuikka et al., 1997). Furthermore, a gender effect on dopamine transporter density has been reported in animals (Rivest et al., 1995). However, a gender effect has not been described in humans so far.

We used N-ω-fluoropropyl-2β-carbomethoxy-3β-[4-iodophenyl]tropane (FP-CIT), labeled with iodine-123, to evaluate the effect of both age and gender on [^{123}I]FP-CIT binding to dopamine transporters in healthy volunteers, including a relatively large group of young adults. To exclude operator-dependant variability, we used automated data analysis of [^{123}I]FP-CIT SPECT images.

Materials and Methods

[^{123}I]FP-CIT SPECT imaging was performed in 45 healthy volunteers, 23 males and 22 females, aged 18-83 years (mean 47.7 years, SD 21.4). All volunteers were free from any neurological or psychiatric disease and were not on medication or using drugs of abuse. Seven were left-handed and 38 were right-handed. All subjects gave their written informed consent for the study, which was approved by the medical ethical committee of the Academic Medical Center.

SPECT procedure

For SPECT imaging a brain-dedicated camera was used (Strichman Medical Equipment Inc, Medfield, Mass., USA). This camera consists of twelve individual crystals each equipped with a focussing collimator. The transaxial resolution is 7.6 mm full width half maximum of a line source in air. The energy window was set at 135-190 keV. All subjects received potassium iodide to block thyroid uptake of free radioactive iodide. [^{123}I]FP-CIT (specific activity of > 185 MBq/nmol; radiochemical purity of > 95 %) was injected intravenously at an approximate dose of 110 MBq. ^{123}I labeling of FP-CIT was performed by Amersham Cygne (Eindhoven, The Netherlands) with the trimethylstannyl precursor of FP-CIT. SPECT acquisition was performed at 3 h p.i. (Booij et al., 1997). Images were acquired during periods of 150 s from the orbitomeatal line to the vertex with an interslice distance of 5 mm. Data acquisition took place in a 128×128 matrix.

Attenuation correction and reconstruction of the images were performed as described earlier (Booij et al., 1997). The measured concentration of radioactivity was expressed as Strichman Medical Units (SMUs; 1 SMU = 100 Bq/ml as specified by the Strichman Medical Equipment Inc).

Data analysis

Assessment of [^{123}I]FP-CIT binding in the whole striatum, caudate nucleus and putamen was performed with a recently developed fully automated three-dimensional technique (Habraken et al., 1999). Briefly, this method automatically places volumes of interest (VOIs) over the brain areas, instead of manually placing predefined two-dimensional regions of interest, as in traditional SPECT data analysis. Binding activity is compared on a voxel-by-voxel base to achieve the best fit. This automated arranging of volumes is operator independent and repeatable. Caudate nucleus and putamen were defined as sub-regions of the striatum. Occipital cortex (OCC) was used as a reference region for non-specific

binding. Ratios of specific to non-specific [^{123}I]FP-CIT binding were calculated as: [^{123}I]FP-CIT binding = (VOI - OCC) / OCC, in which VOI represents the mean radioactivity (in SMU) in the VOI (striatum, caudate nucleus or putamen).

Statistics

Stepwise linear regression analyses were performed in the total group of all 45 subjects, with [^{123}I]FP-CIT binding in the striatum, caudate nucleus and putamen as dependent variables. Age, gender, and the product of age and gender were used as independent variables. A significance level of p < 0.05 was used. All statistical analyses were carried out with SPSS 9.0 for Windows.

Results

No significant differences between left and right striatum, caudate nucleus or putamen were found (Table 1). A decrease in specific to non-specific striatal [^{123}I]FP-CIT binding ratios with age was found (Fig. 1). In young adults, aged 18-30 years, the variance in [^{123}I]FP-CIT binding ratios was 0.25, compared with 0.08 and 0.18, respectively, in the age groups 30-60 and 60-90 years of ages. Linear regression analysis demonstrated a significant effect of both age and gender on striatal [^{123}I]FP-CIT binding ratios (β=-0.62, t=-4.96, p<0.001 and β=-0.33, t=-2.62, p=0.012, respectively), but the interaction between age and gender was not found to have a significant effect (β=0.20, t=0.64, p=0.53). Because of the number of included subjects and visual assessment of our data (Fig. 1), a linear model was used to investigate the correlation between [^{123}I]FP-CIT binding and age.

	Left (SD)	Right (SD)	Mean (SD)
Whole striatum	2.56 (0.48)	2.55 (0.47)	2.55 (0.47)
Caudate nucleus	2.62 (0.55)	2.60 (0.54)	2.61 (0.54)
Putamen	2.53 (0.45)	2.51 (0.44)	2.52 (0.44)

Table 1. Specific to non-specific [123I]FP-CIT binding ratios in 45 healthy volunteers
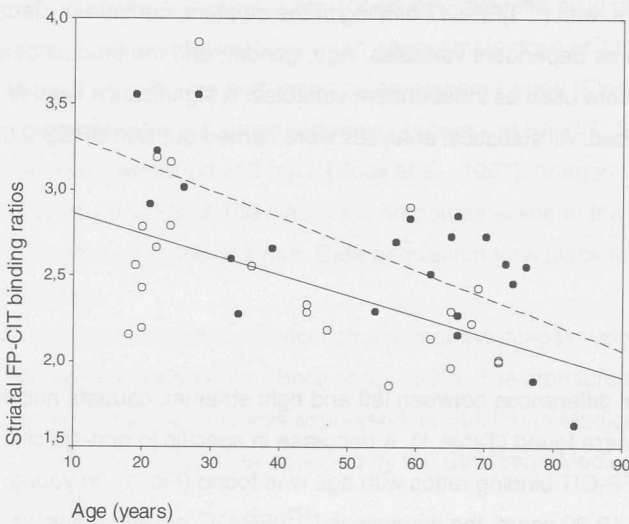
Figure 1. Specific to non-specific striatal [123I]FP-CIT binding ratios versus age in 45 healthy volunteers. Closed circles represent females (dotted line), open circles represent males (unbroken line)

Striatal [123I]FP-CIT binding ratios were significantly higher in females than males. Linear regression showed a decrease of 4.1% per decade.

[123I]FP-CIT binding ratios in both the caudate nucleus and in the putamen decreased significantly with age (β=-0.61, t=-4.77, p<0.001 and β=-0.62, t=-4.99, p<0.001, respectively), with a significant gender effect (β=-0.27, t=-2.14, p=0.039 and β=-3.66, t=-2.95, p=0.005, respectively).

The interaction between age and gender was not a significant variable in both caudate nucleus and putamen. No significant increase in the ratio of caudate over putamen was found with age. [^{123}I]FP-CIT binding ratios in the putamen were not significantly lower than in the caudate nucleus.

Discussion

The results of this study show that [^{123}I]FP-CIT binding in the striatum in healthy volunteers decreases significantly with age. The decline in [^{123}I]FP-CIT binding with age (4.1% per decade) is consistent with published studies (Mozley et al., 1999; Tissingh et al., 1998; van Dyck et al., 1995; Volkow et al., 1996). To describe the influence of age on dopa-mine transporter density, a linear model gave the best fit for our data, which is in agreement with earlier studies.

Furthermore, the high variance in [^{123}I]FP-CIT binding in a relatively large group of young volunteers is in line with a previous study (Mozley et al., 1999). However, the pattern of a relatively rapid rate of decline during young adulthood followed by a less rapid decline during middle age is not confirmed in our study.

It has to be kept in mind that there are no large prospective studies to date on decline in dopamine transporter density with age. The findings from cross-sectional studies of volunteers in various age groups may differ from individual longitudinal findings.

All subjects were imaged at 3 h p.i. Although peak time of [^{123}I]FP-CIT binding may be earlier with higher age, specific striatal to non-specific binding ratios are stable between 3 and 6 h p.i., independent of the density of striatal dopamine transporters.

The higher density of dopamine transporters in females is in line with studies in rats (Rivest et al., 1995), but not with a previous [^{123}I]β-CIT

SPECT study (van Dyck et al., 1995). In female rats, the oestrogen hormone was found to be a crucial factor in the expression of dopamine transporters, which might explain the gender difference in dopamine transporter density in humans as well. Studying the postsynaptic side of the synapse, gender differences in dopamine D_2 receptor affinity have been reported (Pohjalainen et al., 1998). In their study, females were found to have a lower affinity, suggesting an increased endogenous striatal dopamine concentration in women. This may be related to the higher number of dopaminergic nerve terminals in females, as found in our study.

In conclusion, both age and gender should be taken into account in dopamine transporter imaging studies, especially in neuropsychiatric disorders with involvement of the dopaminergic system and established gender differences. For example, female patients with schizophrenia have a higher age of onset than males and in general a less invalidating course of disease. Interestingly, changes in dopamine transporter density have also been described in alcoholism. It may be of interest to study whether the lower occurrence of alcoholism in females is related to gender differences in dopamine transporter density.

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Gender differences in serotonin and dopamine transporter densities in healthy volunteers: a [123I]β-CIT SPET study

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Abstract

Several neuropsychiatric disorders are linked to disturbances of monoaminergic transmission and exhibit gender differences. However, little is known about the basis of gender differences in monoaminergic transmission in humans.

In 15 young, healthy human volunteers (8 females and 7 males) midbrain serotonin transporter (5-HTT) and striatal dopamine transporter (DAT) densities were examined with $[^{123}I]\beta$-CIT SPET. Data for a functional polymorphism of the 5-HTT gene were also assessed. 5-HTT and DAT densities were significantly higher in females than males (p=0.01 and p=0.03, respectively). A strong positive correlation was observed between 5-HTT and DAT densities ($\rho = 0.65$, $p = < 0.01$). 5-HTT density was not significantly associated with 5-HTT genotype. Distinct liability for females and males to suffer from neuropsychiatric disorders responding to monoaminergic agents may be related to differences in brain 5-HTT and DAT densities. This study supports the view that there is a need to examine gender-based treatment and prevention approaches.

Introduction

Evidence is accumulating that several neuropsychiatric disorders, such as schizophrenia and drug abuse, are linked to disturbances of mono-aminergic transmission and exhibit gender differences. For instance, it has been hypothesized that gender differences in monoaminergic neuro-transmission play a role in lower vulnerability to alcohol dependence in females than males. However, little is known about the basis of gender differences in monoaminergic transmission in humans.

Therefore, we assessed the effect of gender on serotonin transporter (5-HTT) and dopamine transporter (DAT) densities in healthy volunteers

imaged with [^{123}I]β-CIT SPET, and analyzed the data controlling for the potential confounding effects of age and 5-HTT genotype. In addition, previous studies have repeatedly demonstrated correlations between cerebrospinal fluid (CSF) measures of the 5-HT and DA metabolites 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) (Geracioti, Jr. et al., 1998). These data suggest that 5-HT and DA systems are coupled in the CNS. Therefore, we hypothesized that 5-HTT and DAT densities would be associated with each other.

Materials and Methods

Subjects and genetic analysis

Fifteen healthy, drug-free volunteers, were enrolled (eight females aged 23.3 ± 1.3 years, range 22-26 years and 7 males aged 29.3 ± 6.9, range 23-42 years). Subjects were free from any neuropsychiatric diagnosis, and underwent urine drug screening. Women were scanned randomly relative to their menstrual cycle. Since a genetic contribution to the expression of 5-HTT was recently described, in which the activity of the long allele of the 5-HTT promoter region has been shown to be twice that of the short allele, polymerase chain reaction based genotype analysis of the 5-HTT gene regulatory region was performed, as described elsewhere (Lesch et al., 1996). Exclusion criteria were: a positive urine test for psychoactive drugs, pregnancy, severe medical or neuropsychiatric illness. Written informed consent was obtained from all participants. The institutional Medical Ethics Committee approved the study.

Imaging

Subjects underwent SPET imaging with the Strichmann Medical Equipment 810X tomographic system. This 12-detector single-slice scanner has a full-width at half-maximum resolution of approximately

7.5 mm. Each acquisition consisted of at least 15 slices (acquired in a 64 x 64 matrix), 3 min per slice (interslice distance 5 mm). The energy window was set at 135-190 keV. Subjects lay supine with the head parallel to the orbitomeatal line. Acquisition was started 4 and 20 h after intravenous injection of approximately 140 MBq [^{123}I]β-CIT (specific activity > 185 MBq/nmol; radiochemical purity > 98%), a time when specific binding to 5-HTT and DAT in the midbrain and striatum, respectively, is stable. Attenuation and reconstruction correction were performed as described elsewhere (Lavalaye et al., 2000).

For binding analysis, a standard template with regions of interest (ROIs) was constructed manually from MR images. For positioning we used these images as a guide. Analysis was performed blinded for gender. The template for the striatum (representing binding to DAT) was placed on three consecutive SPECT slices demonstrating best visualization of the striatum. An additional template was constructed with an ROI for the midbrain (representing binding to 5-HTT) and cerebellum. The cerebellar binding was used as a reference for background activity (non-specific binding + free radioligand). The ratios of specific to non-specific binding were calculated by dividing specific binding by cerebellar binding.

Statistics

Differences in regional [^{123}I]β-CIT binding ratios between females and males were assessed by ANCOVA with age as confounding variable. Using the same statistical analysis, we explored the potentially confounding influence of the 5-HTT genotype on midbrain [^{123}I]β-CIT binding. Correlations were assessed with Spearman's correlation coefficient. The chance of a type I error ($\forall$) was set at 0.05 using two-tailed tests of significance. All data are presented as mean ± SD. Results were considered significant at $p < .05$. Data were analyzed using SPSS version 9.0.

Results

[^{123}I]β-CIT binding ratios in the midbrain and striatum were significantly higher in females than males (p = 0.01 and 0.03, respectively; Figure 1). The covariance effect of age was not significant (p = 0.92 and 0.19 for the midbrain and striatum, respectively).

Figure 1 Specific to non-specific $[^{123}I]\beta$-CIT binding ratios in the midbrain and striatum of 15 healthy volunteers. In females (n=8; mean = 1.43, SD = 0.12) the binding ratios were significantly higher in the midbrain than in males (n=7; mean = 1.25 ; SD = 0.13). This was also true for binding ratios in the striatum (ratios in females; mean = 7.27; SD = 1.10 versus mean = 5.81; SD = 1.21 in males).

Six females and 5 males were heterozygote carriers of the long and short alleles of the 5-HTT, while 2 females and 1 male were homozygote for the long allele. One male was homozygote for the short allele. The genotype did not predict midbrain [^{123}I]β-CIT binding ratios ($p = 0.60$). A strong positive correlation was observed between midbrain and striatal [^{123}I]β-CIT binding ratios ($\rho = 0.65$, $p = < 0.01$; Figure 2).
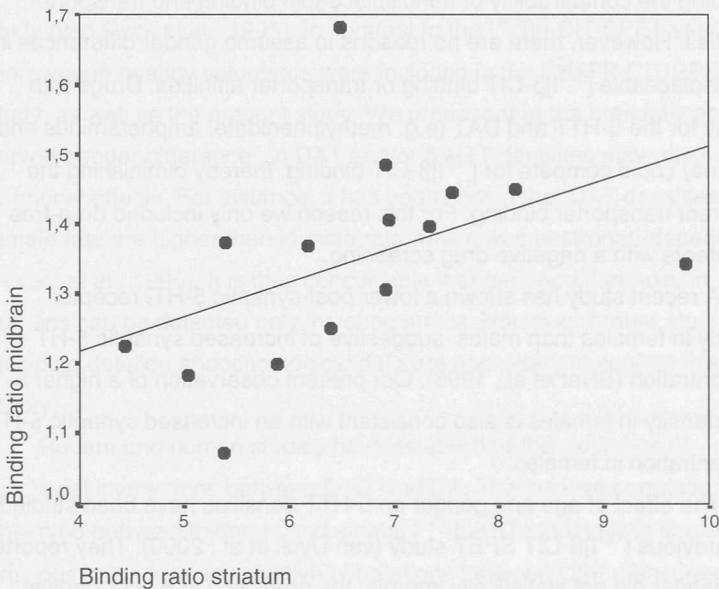
Figure 2 Correlation between specific to non-specific [^{123}I]β-CIT binding ratios in the midbrain (vertical axis) and striatum (horizontal axis) for individual subjects.

Discussion

In the present study, we observed higher 5-HTT and DAT densities in healthy females compared to males. Furthermore, we showed a significant correlation between 5-HTT and DAT densities.

While the higher specific binding of [^{123}I]β-CIT in the midbrain and striatum of women is most likely explained by a higher density of 5-HTT and DAT, respectively, this assertion rests on several assumptions, including the comparability of nondisplaceable binding and transporter affinities. However, there are no reasons to assume gender differences in nondisplaceable [^{123}I]β-CIT binding or transporter affinities. Drugs with affinity for the 5-HTT and DAT (e.g. methylphenidate, amphetamines and cocaine) could compete for [^{123}I]β-CIT binding, thereby diminishing the apparent transporter binding. For this reason we only included drug-free volunteers with a negative drug screening.

A recent study has shown a lower post-synaptic 5-HT$_2$ receptor density in females than males, suggestive of increased synaptic 5-HT concentration (Biver et al., 1996). Our present observation of a higher 5-HTT density in females is also consistent with an increased synaptic 5-HT concentration in females.

The effect of age and gender on 5-HTT densities have been studied in a previous [^{123}I]β-CIT SPET study (van Dyck et al., 2000). They reported that gender did not statistically improve the prediction of 5-HTT density after controlling for ageing effects. Although the sample size was large in their study, the divergence between their and our study likely accrues from several important methodological differences: 1) They used the occipital cortex, instead of the cerebellum, as a reference region. The occipital cortex, however, contains a higher 5-HTT density than the cerebellum. Consequently, smaller differences in 5-HTT densities may be detected easier when using the cerebellum instead of the occipital cortex. 2) Their age range of controls was larger (from 18 to 88 years; present study 22 to

42 years). However, we recently have shown that the variation in DAT density is higher in young than old adults (Lavalaye et al., 2000). Assuming that such a strong variation on 5-HTT density also exists in young adults, one may detect gender differences in 5-HTT densities when including young healthy volunteers only. 3) Furthermore, they investigated gender effects statistically. However, can one adequately investigate gender effects by simply "covarying" for gender ?

The higher density of DAT in females is in line with a recent [^{123}I]FP-CIT SPET study (Lavalaye et al., 2000), but not with a [^{123}I]β-CIT SPET study (van Dyck et al., 1995). In contrast to the [^{123}I]β-CIT SPET study, more young healthy volunteers were included in the [^{123}I]FP-CIT SPET study, as well as the present study. We propose that the presently observed gender differences in DAT and/or 5-HTT densities may relate to hormonal effects. For instance, it has been shown that DAT densities in female rats are higher than in male rats, which was oestrogen-dependent (Rivest et al., 1995). It is thus conceivable that gender differences in humans can be detected only in young adults. However, further studies including detailed endocrinological data are necessary to confirm this theory.

Rodent and human studies have established the existence of functional interactions between 5-HT and DA. The positive correlation we observed between midbrain and striatal [^{123}I]β-CIT binding is in line with a previous study in which positive correlations between CSF measures of 5-HIAA and HVA were observed (Geracioti, Jr. et al., 1998). It was shown that 5-HIAA to HVA ratio for an individual remains relatively stable over time, with little variability. It has been hypothesized that the balance between of 5-HT and DA function in the CNS is of physiologic importance in the human. The imbalance of activity of 5-HT and DA systems is already an important consideration in the pathophysiology of psychoses and may become relevant to the treatment of depression.

It could be argued that the presently observed association between 5-HTT and DAT may be attributable in part to the fact that midbrain [^{123}I]β-

CIT binding may include some binding to DATs. Displacement studies in primates have shown that the uptake of $[^{123}I]\beta$-CIT in the brainstem is primarily associated with 5-HTT (i.e., displaceable by citalopram but not by GBR-12909) (Laruelle et al., 1993). However, since the substantia nigra is part of brainstem and contains relatively high DAT densities, $[^{123}I]\beta$-CIT binding in the brainstem cannot be completely ascribed to 5-HTT alone.

In the present study, we explored the potentially confounding influence of heritable effects. We did not observe an association between the 5-HTT genotype and density of midbrain 5-HTT, consistent with other reports. Even though it has been suggested by Lesch and co-workers (Lesch et al., 1996) that the long allele of the genotype is associated with an increased 5-HTT density, it does not seem to explain our present findings. However, because of our small sample size, this remains to be proven.

The data may bear relevance to a number of fields. For instance, numerous studies have noted differences in the age at onset, treatment response, course, and outcome between females and males suffering from schizophrenia. Furthermore, it has been indicated that the causes and consequences of drug abuse may be different for females and males. For instance it may be hypothesized that the observed higher 5-HTT densities in females may play a role in lower vulnerability to alcohol dependence in females. Our findings may also have implications for the action of neurotoxic derivatives such as 3,4-methylenedioxymet-amphetamine (MDMA). It is thought that blocking the 5-HTT prevents neurotoxicity of MDMA, since administration of 5-HT re-uptake inhibitors prevent the 5-HT neurotoxic effects of MDMA. It could be speculated that the presently observed higher 5-HTT densities in women renders them more susceptible to neurotoxic actions of MDMA. In line with this, females have been found to experience stronger adverse effects of MDMA than males and greater depletions in 5-HIAA (McCann et al., 1994; Liechti et al., 2001).

Conclusion

Our preliminary data show gender differences in 5-HTT and DAT densities, and suggest a close relationship between these two systems. Our results indicate the importance of taking gender into account in future 5-HTT and DAT imaging studies. Further studies including detailed endocrinological and genotypic data obtained in large samples of healthy volunteers are required to delineate the basis of variation in 5-HTT and DAT densities between males and females.

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9

Dopamine transporter density
in patients with tardive dyskinesia:
a single photon emission computed
tomography study

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Abstract

Tardive dyskinesia occurs frequently in schizophrenic patients chronically treated with classical antipsychotic medication. It may be caused by loss of dopaminergic cells, due to free radicals as a product of high synaptic dopamine levels. Our objective: was to evaluate dopamine transporter density in the striatum in patients with tardive dyskinesia. Striatal [^{123}I]FP-CIT binding was measured with SPECT in 7 schizophrenic patients with tardive dyskinesia and 8 healthy controls. o significant difference was found between striatal [^{123}I]FP-CIT binding ratios in patients with tardive dyskinesia and controls. his preliminary study indicates no change in striatal dopamine transporter density in schizophrenic patients with tardive dyskinesia. This finding does not support the hypothesis that tardive dyskinesia is caused by dopaminergic cell loss.

Introduction

Chronic treatment with classical antipsychotic medication can induce tardive dyskinesia, a chronic choreoathetoid movement disorder. One theory on the pathophysiology of tardive dyskinesia supposes a super-sensitivity of dopamine receptors from prolonged receptor blockade or upregulation of these receptors.

An alternative hypothesis is the free radical hypothesis. According to this theory tardive dyskinesia is a neurodegenerative process, with neuronal damage in the basal ganglia. Prolonged treatment with anti-psychotic medication increases the dopamine metabolism and turnover. In this process free radicals are generated. This excessive production of free radicals could lead to neuronal membrane instability and dopaminergic cell death (Lohr et al., 1988). Treatment with the free radical scavenger vitamin E is based on this theory (Soares and McGrath, 1999). However,

to date it has to be established whether there is loss of dopaminergic cells in patients with tardive dyskinesia. Furthermore, the role of atypical antipsychotic in inducing tardive dyskinesia is still unclear. Clozapine hardly induces tardive dyskinesia, and both olanzapine and risperidone seem to induce less tardive dyskinesia than classical antipsychotics. Dopamine transporter density can be used as a marker for the integrity of the dopaminergic neurons. Single photon emission computed tomography (SPECT) with N-ω-fluoropropyl-2β-carbomethoxy-3β[4-iodophenyl]tropane ([^{123}I]FP-CIT, ioflupane) makes it possible to visualize dopamine transporters in the striatum (Booij et al., 1999) and has proved to be a sensitive tool to demonstrate even a small loss of nigrostriatal dopaminergic neurons.

Method

Imaging was performed in 7 patients (3 males and 4 females) with schizophrenia according to DSM IV, with a mean age of 50.6 years (range 42 to 60 years, SD 5.94). Mean duration of illness was 24 (14 to 36) years, with a mean duration of antipsychotic medication of 20 (14 to 24) years. Five patients were treated with classic antipsychotic medication at the time of imaging. Two were treated with olanzapine, with a history of chronic treatment with classic antipsychotics of 14 and 20 years. Five patients were co-medicated with benzodiazepines and with paroxetine, which were withheld at the day of assessment.

Eight healthy volunteers, 4 males and 4 females (mean age 48.5 years, range 39 to 59 years, SD 7.8) were included. The difference in age between patients and controls was not significant. Volunteers were free from any neurological or psychiatric disease and were not taking any drugs, as assessed by a clinical interview. After complete description of the study to the subjects, written informed consent was obtained. The

research protocol was approved by the medical ethical committee of the Academic Medical Center in Amsterdam.

For SPECT imaging a brain-dedicated camera was used (Strichman Medical Equipment Inc, Medfield, Mass., USA). All subjects received potassium iodide orally to block thyroid uptake of free radioactive iodide. An approximate dose of 110 MBq [^{123}I]FP-CIT was injected intravenously and SPECT acquisition was performed 3 hours later, as previously described (Booij et al., 1999). Assessment of [^{123}I]FP-CIT binding in the whole striatum, caudate nucleus, putamen and occipital cortex (as a reference region) was performed with a recently developed fully automated three-dimensional technique (Habraken et al., 1999). This method automatically places volumes of interest (VOI) over the brain areas. Specific to non-specific [^{123}I]FP-CIT binding was calculated as (VOI - OCC) / OCC, in which VOI represents the mean radioactivity in the VOI (striatum, caudate nucleus or putamen) and OCC the occipital binding. Image analysis was performed blind to clinical data.

Tardive dyskinesia was rated with the Abnormal Involuntary Movement Scale (AIMS). All assessments were made in the week of imaging by two of the investigators (J.L. and A.S.). Interviews were recorded on video and movement disorders were rated by a senior psychiatrist. All patients were diagnosed as moderate to severe tardive dyskinesia by all three raters. While AIMS scores were not significantly different between raters a consensus score was used in the analysis.

Differences in [^{123}I]FP-CIT binding ratios between groups were calculated with a Student's t-test. A significance level of $p < 0.05$ was used.

Results

No significant difference in specific to non-specific striatal [^{123}I]FP-CIT binding ratios in patients with tardive dyskinesia (mean ± S.D.: 2.62 ± 0.27) and controls (2.48 ± 0.26) was found (t=1.05, df=13, p=0.31, Figure 1).
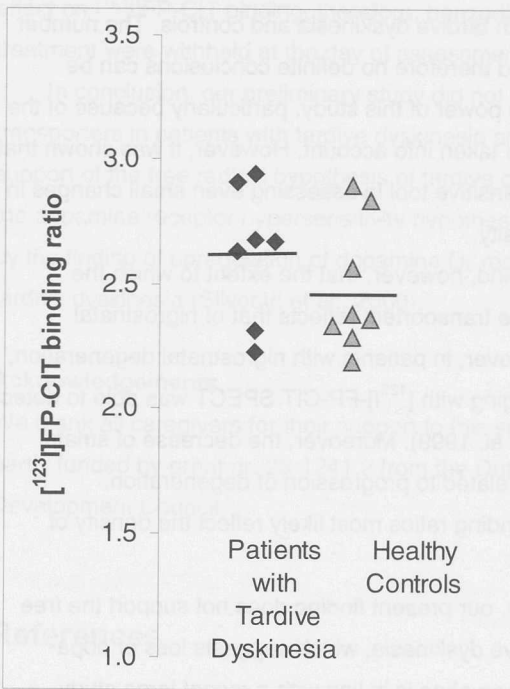

Figure 1. Individual ratios of specific striatal to non-specific [^{123}I]FP-CIT binding in patients with tardive dyskinesia (N=7) and controls (N=8).

This was also true for binding ratios in the caudate nucleus (2.60 ± 0.34 in patients, and 2.51 ± 0.32 in controls; t=0.54, df=13, p=0.60) and in the putamen (2.64 ± 0.25 in patients, and 2.46 ± 0.23 in controls; t=1.43, df=13, p=0.18).

The mean total AIMS score was 8.4 (range 4-13, SD 3.1). [123I]FP-CIT binding ratios were not significantly correlated with AIMS ratings.

Discussion

This study showed no significant difference in striatal [123I]FP-CIT binding ratios between patients with tardive dyskinesia and controls. The number of subjects was limited, and therefore no definite conclusions can be drawn from this study. The power of this study, particularly because of the negative finding, should be taken into account. However, it was shown that [123I]FP-CIT SPECT is a sensitive tool in assessing even small changes in dopamine transporter density.

It should be kept in mind, however, that the extent to which the density of striatal dopamine transporters reflects that of nigrostriatal neurons is uncertain. However, in patients with nigrostriatal degeneration, dopamine transporter imaging with [123I]-FP-CIT SPECT was able to detect this degeneration (Booij et al. 1999). Moreover, the decrease of striatal [123I]-FP-CIT binding was related to progression of degeneration. Therefore, [123I]-FP-CIT binding ratios most likely reflect the density of nigrostriatal neurons.

If replicated, however, our present finding does not support the free radical hypothesis of tardive dyskinesia, which suggests loss of dopaminergic neurons. Our observation is in line with a recent large study, which reported no evidence for efficacy of vitamin E in the treatment of tardive dyskinesia (Adler et al., 1999).

In SPECT (Lavalaye et al., 2000a) and PET studies (Laakso et al., 2000), patients with schizophrenia, but without tardive dyskinesia, were shown to have no change in dopamine transporter density. Therefore, in this study, patients were compared with healthy controls. This study did match patients and controls for age and gender, since both age and

gender have shown a clear effect on dopamine transporter density (Lavalaye et al., 2000b). All patients were on antipsychotic medication at the moment of imaging. However, this medication was shown not to influence striatal [^{123}I]FP-CIT binding (Lavalaye et al., 2000a; Lavalaye et al., 2000c). No co-medication was used with a known influence on [^{123}I]FP-CIT binding. However, it can not be completely excluded that this medication has an effect on [^{123}I]FP-CIT binding, therefore, benzodiazepine and paroxetine treatment were withheld at the day of assessment.

In conclusion, our preliminary study did not show loss of dopamine transporters in patients with tardive dyskinesia and is therefore not in support of the free radical hypothesis of tardive dyskinesia. Interestingly, the dopamine receptor hypersensitivity hypothesis received recent support by the finding of upregulation of dopamine D$_2$ receptor in patients with tardive dyskinesia (Silvestri et al., 2000).

Acknowledgements

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Higher occupancy of muscarinic receptors by olanzapine than risperidone in patients with schizophrenia, a [123I]-IDEX SPECT study

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Abstract

In vitro data have shown strong anticholinergic properties of the atypical antipsychotic drug olanzapine. Substantial occupancy of muscarinic receptors may be an explanation for the low incidence of extrapyramidal side effects induced by olanzapine.

Our objective was to obtain an in vivo measurement of muscarinic receptor occupancy by olanzapine compared with risperidone in patients with schizophrenia stabilised on medication.

Five patients with schizophrenia treated with olanzapine and five patients treated with risperidone were studied. Muscarinic receptor occupancy in the striatum and cortex was studied in vivo with SPECT using [^{123}I]-IDEX as a radioligand. SPECT data were compared with those of six healthy subjects.

Patients stabilised on olanzapine showed significantly lower mean (±SD) striatal and cortical (1.50±0.21 and 1.51±0.22, respectively) muscarinic receptor binding of [^{123}I]-IDEX (reflecting higher levels of muscarinic receptor occupancy) than controls (3.91±0.61 and 3.65±0.70, respectively). Furthermore, [^{123}I]-IDEX binding ratios in patients treated with risperidone were slightly lower than controls, reaching significance only in the striatum (2.99±0.27 versus 3.91±0.61, for risperidone and controls).

The substantial occupancy of muscarinic receptors in the striatum and cortex by olanzapine may be an explanation for the low incidence and severity of extrapyramidal side effects of this antipsychotic drug. Furthermore, it may also explain the anticholinergic side effects of olanzapine.

Introduction

Antipsychotic medication is effective in decreasing psychotic symptoms of schizophrenia and in preventing psychotic relapse. However, antipsychotic medication, such as olanzapine or risperidone, also induces a variety of side effects. Moreover, side effects are a main reason to withdraw from medication after the acute psychotic phase. Furthermore, it has been shown that discontinuing treatment with antipsychotic drugs is the most important predictor of relapse (Robinson et al., 1999). Therefore, the aetiology of side effects of antipsychotic drugs is important to study.

High occupancy of dopamine D_2 receptors in the brain is thought to induce extrapyramidal symptoms. Recently, a Cochrane systematic review of 20 studies (Duggan et al., 2000) has shown a higher incidence and severity of extrapyramidal side effects in patients treated with risperidone than with olanzapine. Interestingly, positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies showed no difference in dopamine D_2 receptor occupancy between olanzapine and risperidone at therapeutic dosages (Dresel et al., 1999; Kapur et al., 1999; Lavalaye et al., 1999). This finding suggests that dopamine D_2 receptor occupancy may be necessary for inducing side effects, although this does not completely explain the pathophysiology of extrapyramidal side effects. However, neurotransmitter systems other than the dopaminergic system may also be involved in the occurrence of extrapyramidal side effects induced by antipsychotics.

In addition, in the Cochrane review (Duggan et al., 2000), anti-cholinergic symptoms such as dizziness and dry mouth were found to be more common in the olanzapine-treated group than in the risperidone group. This may be explained by a higher in vivo occupancy of muscarinic receptors by olanzapine than risperidone. This is supported by in vitro studies using rat striatum which showed that olanzapine has a relatively high affinity for muscarinic receptors (K_i 26 nM), whereas risperidone has

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a low affinity (K_i >5000 nM; Schotte et al., 1996). Another in vitro study has shown that the affinity of olanzapine is relatively high for subtypes of the muscarinic receptor (K_i for the m_1, m_2, m_3 and m_4 receptors were 1.9 nM, 18 nM, 25 nM and 13 nM, respectively), comparable with clozapine. The receptor affinity of risperidone was low for these muscarinic receptor subtypes (inhibition of binding <50% at 10,000 nM concentration) (Bymaster et al., 1996). A recent study also found a clear affinity of olanzapine for the muscarinic receptor (K_i ranging from 32 nM to 132 nM; Bymaster and Falcone, 2000).

[123]I-Dexetimide ((S)-(+)-3-phenyl-3-(4-piperidinyl)-2,6-piperidinedione ((S)-nordexetimide) ([^{123}I]-IDEX) is a high-affinity, non-selective muscarinic receptor antagonist which was developed and evaluated as a radioligand for imaging muscarinic receptors (Boundy et al., 1995) with SPECT (Müller-Gärtner et al., 1992). In order to determine whether higher in vivo occupancy of muscarinic receptors by olanzapine than risperidone account for higher occurrence of anticholinergic side effects in olanzapine-treated patients, the following study was designed. We used [^{123}I]-IDEX SPECT to determine the in vivo occupancy of the muscarinic receptor in patients with schizophrenia treated with olanzapine or risperidone. We hypothesised that the in vivo occupancy of muscarinic receptors by olanzapine is higher than the in vivo occupancy of muscarinic receptors by risperidone. In addition, we hypothesised that lower in vivo occupancy of muscarinic receptors is associated with lower anticholinergic side effects.

Materials and Methods

Subjects

We studied ten young patients with schizophrenia according to DSM IV (for details see Table 1). All patients were admitted to the Adolescent

Clinic of the Academic Medical Centre and were following a program for first episode schizophrenia.

	Controls (n=6)	Olanzapine (n=5)	Risperidone (n=5)
Age in years	27.3 (22-36)	20.0 (18-22)	22.8 (19-27)
Sex (male/female)	4/2	4/1	5/0
Dose in mg	-	15.0 (10 – 20)	4.0 (3-5)

Data are presented as mean (range)

Table 1. Subject characteristics

Five patients were treated with olanzapine, and five patients were treated with risperidone (Table 1). Patients were stabilised on a fixed dose for at least 4 weeks and no concomitant medication was used, except for low-dose benzodiazepines that were withheld at the day of imaging. A group of six healthy subjects (Table 1) with no history of neurological or mental disorders was included to obtain control data. All patients and healthy controls gave their written informed consent to the research protocol, which was approved by the medical ethics committee of the Academic Medical Centre.

Clinical Measurements

Psychotic symptoms were rated in all patients on the day of imaging by one of the investigators (J.L.). The structured clinical interview of the Positive and Negative Symptoms Scale of schizophrenia (PANSS; Kay et al., 1987) was used to rate psychotic symptoms. Akathisia was assessed with the Barnes akathisia rating scale (Barnes, 1989). Cholinergic side effects were measured using the AMDP-5 rating scale (Association for Methodology and Documentation in Psychiatry, 1981). The interviewer was blind to the results of SPECT imaging.

SPECT procedure

Subjects received potassium iodide orally to block thyroid uptake of free radioactive iodide the day before the injection and on the day of injection (total amount of approximately 230 mg). [^{123}I]-IDEX (specific activity of > 200 MBq/nmol; radiochemical purity of > 95%) was injected intravenously at an approximate dose of 185 MBq. ^{123}I labelling was performed by Amersham Cygne (Eindhoven, The Netherlands) with the precursor dexetimide obtained from Janssen Pharmaceuticals (Beerse, Belgium).

SPECT acquisition was performed at 8 h post injection using a brain-dedicated camera (Strichman Medical Equipment Inc, Medfield, Mass.). This camera consisted of 12 individual crystals each equipped with a focussing collimator. The transaxial resolution was 7.6 mm full width at half maximum (FWHM) of a line source in air. Images were acquired in periods of 150 s from the orbitomeatal line to the vertex with an interslice distance of 5 mm. The energy window was set at 135-190 keV. Data acquisition took place in a 64x64 matrix. The measured concentration of radioactivity was expressed as Strichman Medical Units (SMUs; 1 SMU = 100 Bq/ml as specified by the Strichman Medical Equipment Inc).

Data analysis

[^{123}I]-IDEX binding was assessed in the total cortex, striatum, frontal, temporal and occipital cortices using a template with predefined regions of interest (ROIs) constructed according to a stereotactic atlas. ROIs were placed manually over two slices with the highest striatal and cortical [^{123}I]-IDEX binding and in a separate slice over the cerebellum.

At plateau (or pseudoequilibrium), it is assumed that striatal and cortical uptake represents total radioligand binding [(specific + non-specific binding) + free ligand]. Cerebellar activity provides a reference region for background activity (non-specific binding + free ligand). The cerebellum was used as a reference region for non-specific binding (Müller-Gärtner et al., 1992; Boundy et al., 1995) since the cerebellum is devoid of muscarinic receptors (Lin et al., 1986).

Occupancy of cortical and striatal muscarinic receptors by cold antipsychotic drugs decreases the amount of receptors available for specific binding to the radioligand, [^{123}I]-IDEX. The cortical/cerebellar ratio (or muscarinic binding index) is reduced in proportion to the degree of occupancy by the antipsychotic drugs. Thus, a high muscarinic binding implies low muscarinic receptor occupancy by the antipsychotic drug.

Statistical analysis

Differences between groups were analysed by analysis of variance (ANOVA) with a Tukey post-hoc test for multiple comparisons. A significance level of $P < 0.05$ was considered significant. All statistical analyses were carried out with SPSS 9.0 for windows.

Results

Patients treated with olanzapine exhibited [^{123}I]-IDEX binding ratios that were, overall, significantly lower than in controls and than in patients treated with risperidone in all studied brain regions (Table 2). Figure 1 shows a transversal [^{123}I]-IDEX SPECT slice at the level of the striatum of a control subject, a patient treated with risperidone and a patient treated with olanzapine.
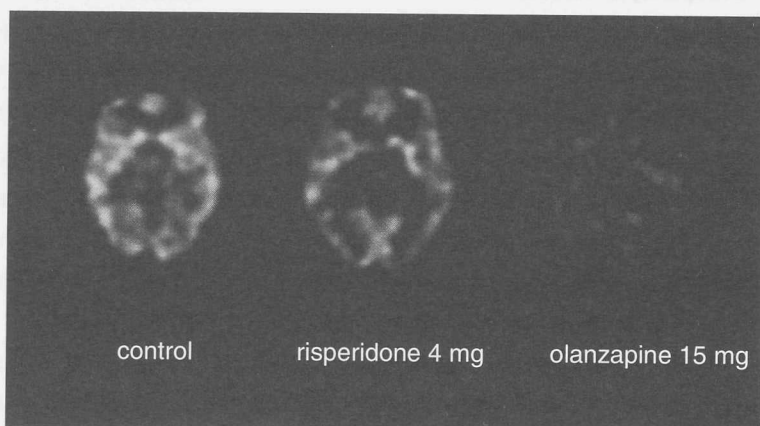
Figure 1. [^{123}I]-IDEX SPECT transversal slices at the level of the striatum of one patient treated with olanzapine 15 mg, one with risperidone 4 mg and a control subject. [^{123}I]-IDEX binding in the striatum and cortex of the patient with olanzapine is lower than in the control, reflecting a higher level of muscarinic receptor occupancy. Colour version on the inside of the cover.

	cortex	striatum	frontal	temporal	occipital
Controls	3.65	3.91	3.51	3.01	3.37
	(0.70)	(0.61)	(1.18)	(0.30)	(0.79)
Olanzapine	1.51	1.50	1.49	1.47	1.34
	(0.22)*	(0.21)*	(0.21)*	(0.13)*	(0.13)*
Risperidone	2.90	2.99	2.80	2.59	2.73
	(0.21)**	(0.27)*/**	(0.30)**	(0.37)**	(0.32)**

Data are binding ratios of region/cerebellum, presented as mean values (SD)
*Significantly different from controls, P<0.01
**Significantly different from olanzapine, P<0.001

Table 2. [^{123}I]-IDEX binding ratios in patients treated with olanzapine and risperidone and in controls

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Figure 2A. [^{123}I]-IDEX binding ratios of total cortex divided by cerebellum in controls and patients treated with risperidone or olanzapine

Figure 2B. [^{123}I]-IDEX binding ratios of striatum divided by cerebellum in controls and patients treated with risperidone or olanzapine

As expected, total [^{123}I]-IDEX binding in the cerebellum was low and not significantly different between groups (data not shown). Therefore, the cerebellum was considered as a region of non-specific binding, and all regions were divided by cerebellum values to obtain [^{123}I]-IDEX binding ratios.

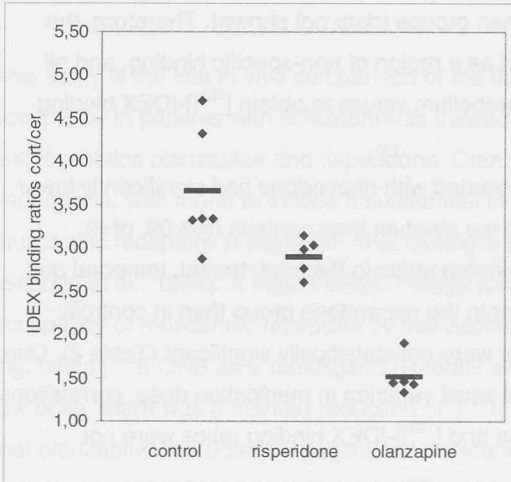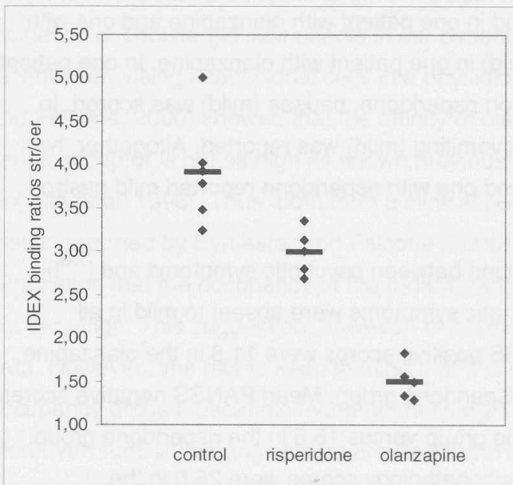Surprisingly, patients treated with risperidone had significantly lower [^{123}I]-IDEX binding ratios in the striatum than controls (t=3.09, df=9, P< 0.05). The [^{123}I]-IDEX binding ratios in the total, frontal, temporal and occipital cortices were lower in the risperidone group than in controls. However, these differences were not statistically significant (Table 2). Due to the small group size and small variation in medication dose, correlations between antipsychotic dose and [^{123}I]-IDEX binding ratios were not thought to be reliable.

Side effects and psychotic symptoms
No correlations between side effects and [^{123}I]-IDEX binding ratios were found. Akathisia was absent or questionable in all studied patients. Hyper-salivation (mild) was scored in one patient with olanzapine and one with risperidone, dry mouth (mild) in one patient with olanzapine. In one patient on olanzapine and in one on risperidone, nausea (mild) was scored. In one patient on risperidone vomiting (mild) was reported. Altogether, two patients with olanzapine and one with risperidone reported mild gastro-intestinal disturbances.

No correlation was found between psychotic symptoms and [^{123}I]-IDEX binding ratios. Psychotic symptoms were absent to mild in all patients, mean total PANSS positive scores were 11.8 in the olanzapine group versus 14.6 in the risperidone group. Mean PANSS negative scores were 14.6 in the olanzapine group versus 16.6 in the risperidone group. Mean PANSS general psychopathology scores were 25.0 in the olanzapine group versus 35.4 in the risperidone group.

Discussion

This study is the first in vivo comparison of the muscarinic receptor occupancy in patients with schizophrenia treated with the atypical antipsychotics olanzapine and risperidone. Olanzapine, in contrast to risperidone, was found to induce a substantial in vivo occupancy of muscarinic receptors in the brain. This finding is in line with in vitro studies (Schotte et al., 1996). A recent SPECT study also reported a significant occupancy of muscarinic receptors by olanzapine at doses of 5 mg and 20 mg, using [^{123}I]-QNB as a radioligand (Raedler et al., 2000). Even at the low dose, there was a marked reduction of [^{123}I]-QNB binding, suggesting that olanzapine is a potent antagonist of muscarinic receptors.

The presently observed very low [^{123}I]-IDEX binding ratios in patients with olanzapine compared with controls suggests a high in vivo occupancy of muscarinic receptor by olanzapine. A high occupancy of these receptors would, however, likely induce anticholinergic side effects. In contrast to this, and in line with the results of other studies, we did not find a high incidence of cholinergic side effects in our patient sample. Interestingly, a recent study using intact clonal cells in a physiological medium (Bymaster and Falcone, 2000) showed that the affinity of olanzapine for the muscarinic receptor is not as high as shown previously (Bymaster et al. 1996; Schotte et al. 1996). Thus, both from a clinical point of view and from the results reported by Bymaster and Falcone (2000), it may be a reasonable suggestion that the occupancy of the muscarinic receptor by olanzapine is not very high. This suggestion, however, is not in line with our present data. However, one has to keep in mind that it is still not clear at which occupancy of the muscarinic receptor cholinergic side effects occur. Moreover, further imaging studies are needed to examine the exact relationship between in vitro affinity of muscarinic receptor and in vivo [^{123}I]-IDEX binding ratios. Finally, since SPECT imaging cannot produce absolute quantitative measurements, we cannot exclude completely that

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our binding ratios overestimate muscarinic receptor occupancy. Nevertheless, our data at least suggest substantial occupancy of muscarinic receptor by olanzapine.

The higher occupancy of muscarinic receptors by olanzapine than risperidone might be an intriguing explanation for the lower incidence of extrapyramidal side effects by olanzapine than risperidone, even at high doses and high dopamine D_2 receptor occupancy. The anticholinergic property of olanzapine may be described as an intrinsic anticholinergic compound.

The finding of a significantly lower [^{123}I]-IDEX binding in the striatum in patients with risperidone than in controls was unexpected, as in vitro studies showed that risperidone had a low affinity for muscarinic receptors in the striatum (Bymaster et al., 1996; Schotte et al., 1996). This lower [^{123}I]-IDEX binding in patients with risperidone could reflect low occupancy of muscarinic receptors. However, as expected, the occupancy of muscarinic receptors by risperidone was significantly lower than that by olanzapine.

Another explanation for the lower [^{123}I]-IDEX binding is that muscarinic receptor density in patients with schizophrenia may be lower than in controls. This was also suggested in a recent SPECT study (Raedler et al., 2000) and was a finding of recent post-mortem studies (Crook et al., 1999, 2000). Therefore, schizophrenia per se may also explain the lower [^{123}I]-IDEX binding in patients with risperidone than in healthy controls. The cholinergic aspects of schizophrenia are an interesting field, with studies suggesting an increased muscarinic cholinergic activity in schizophrenia, and the various anticholinergic aspects of antipsychotic drugs (Tandon, 1999). For instance, an agonist effect of both clozapine and olanzapine on the muscarinic m_4 receptor has been described (Zeng et al., 1997), which may also be a relevant mechanism for the therapeutic efficacy and side effects of these anti-psychotic drugs. Nevertheless, more work will be needed to achieve a complete understanding of the relationship between schizophrenia and

muscarinic receptor binding availability, and the anticholinergic properties of antipsychotics.

A limitation of the present study is that patients treated with antipsychotics were compared with healthy volunteers, instead of with antipsychotic-free patients with schizophrenia. However, due to practical reasons, it was not feasible to include a large enough number of anti-psychotic-free patients. Nevertheless, definite conclusions both on the occupancy of muscarinic receptors by antipsychotics, and on possible differences in density of the muscarinic receptor between schizophrenic patients and controls can only be drawn after the inclusion of anti-psychotic-naive patients. Future studies with a wide dosing range of antipsychotic medication and antipsychotic-free patients (to make a better estimation of occupancy of muscarinic receptors) should be performed to clarify the effect of the antipsychotic dose on the occupancy of the muscarinic receptor.

Patients with olanzapine or risperidone in our study experienced only mild anticholinergic and extrapyramidal side effects of medication. Therefore it was not possible to determine a correlation between side effects and [^{123}I]-IDEX binding. It would be of interest to determine [^{123}I]-IDEX binding in patients with severe anticholinergic side effects to determine whether this is related to the degree of occupancy of muscarinic receptors. Moreover, psychotic effects might have an influence on either muscarinic receptor density or [^{123}I]-IDEX binding. Nevertheless, in this study we found no correlation between [^{123}I]-IDEX binding ratios and psychotic symptoms.

In conclusion, in this study we showed that the in vivo occupancy of muscarinic receptors is higher in olanzapine-treated patients than in risperidone-treated patients with schizophrenia. This finding might be an explanation for the lower incidence of extrapyramidal side effects by olanzapine than risperidone.

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11

Summary and conclusions

The project "SPECT imaging in young patients with schizophrenia" was started to investigate different aspects of the central dopaminergic neurotransmission system in schizophrenic patients with a first or second psychotic episode. SPECT (single photon emission computed tomography) is a nuclear medicine technique, which is used to image functional processes in the body after administration of a radioligand. In this project, both postsynaptic and presynaptic aspects of the dopaminergic neurotransmission system were studied, as well as specific side effects of antipsychotic medication. The postsynaptic dopamine system was studied by focusing on the dopamine D_2 receptor occupancy by two recently introduced antipsychotic drugs (Part 1). The presynaptic dopamine system was studied by imaging dopamine transporter densities in patients with schizophrenia and in controls, and by focusing on tardive dyskinesia, a specific side effect of antipsychotic medication (Part 2). In addition, with respect to side effects, the muscarinic receptor occupancy of two recently developed antipsychotics was determined (Part 3).

Part 1

Dopamine receptor occupancy by antipsychotic medication

Part one focuses on the postsynaptic dopamine D_2 receptor. A literature overview of the large number of SPECT and PET (Positron Emission Tomography) imaging studies concerning dopamine D_2 receptor occupancy by antipsychotic medication is presented in Chapter 2. This overview shows that most antipsychotics induce a high occupancy of the D_2 receptors in the striatum, with the exception of clozapine and possibly quetiapine.

A minimum level of 60% D_2 receptor occupancy was found to be required to induce an antipsychotic effect, and above 80% D_2 receptor

occupancy the incidence of extrapyramidal side effects increases (Farde et al., 1992; Nyberg et al., 1995; Kapur et al., 1996).

Several previous reports have shown that olanzapine and risperidone, two new antipsychotic drugs, both show a low incidence of extrapyramidal side effects.

In order to find a possible explanation for the low extrapyramidal side effects, and to determine the difference in D_2 receptor occupancy between both antipsychotics, we assessed the D_2 receptor occupancy with [^{123}I]-IBZM SPECT and rated the side effects of young patients treated with olanzapine or risperidone. In agreement with these previous studies, we also found that both recently developed antipsychotics showed a low incidence of extrapyramidal side effects. Moreover, we showed that there was no significant difference in D_2 receptor occupancy between the two groups under study (Chapter 3). In addition, we observed that risperidone treatment was related to high levels of prolactin, in contrast to olanzapine. The most likely explanation for this finding would be that this is due to a higher occupancy of dopamine receptors. However, as we found no difference in dopamine receptor occupancy between olanzapine and risperidone, an alternative hypothesis was suggested, stressing the different affinities of olanzapine and risperidone for the $5HT_{2c}$ receptor (Leysen et al., 1998). Olanzapine, as opposed to risperidone, is a strong $5HT_{2c}$ receptor antagonist, and blocking this specific receptor results in suppression of prolactin release. This may explain why the high prolactin levels are found in risperidone, and not in olanzapine.

Apart from extrapyramidal side effects, we studied the subjective experience of patients with schizophrenia treated with antipsychotics. We found a positive correlation between subjective experience and D_2 receptor occupancy in patients treated with moderate doses of olanzapine or risperidone (Chapter 4). This means that patients with a higher D_2 receptor occupancy, although with low rates on classical side effect rating scales, showed worse subjective experience. Therefore, subjective

experience may be more sensitive to D_2 receptor occupancy than extrapyramidal symptoms. Further analysis of these data, specifically looking at the sub-scales of the subjective experience rating scales, showed a strong correlation between negative subjective experience and D_2 receptor occupancy. These results look promising and resulted in the planning of a forthcoming larger study.

General considerations regarding dopamine D_2 receptor imaging with [^{123}I]-IBZM SPECT

A number of limitations should be taken into account when discussing dopamine receptor SPECT imaging studies. The foremost critique on these studies is the brain region that is studied. As the highest density of dopamine receptors is situated in the striatum, almost all dopamine receptor imaging studies, both PET and SPECT, focus on this region. However, it is thought that in schizophrenia, dopamine abnormalities in the limbic cortical areas are of more importance for understanding sympto-matology than abnormalities in the striatum. This general consideration has been under discussion since the introduction of dopamine neuro-imaging. However, the finding of a lower threshold for antipsychotic action of antipsychotic medication, and studies suggesting a higher striatal dopamine release in patients, and especially the relationship to illness phase (Laruelle et al., 1999), show that imaging studies in the striatum are representative of the limbic dopaminergic system.

Recently, a new radioligand with higher affinity for the D_2 receptor than [^{123}I]-IBZM was introduced, [^{123}I]-epidepride, enabling visualisation of also extrastriatal dopamine receptors (Bigliani et al., 1999, 2000; Stephenson et al., 2000;). These studies showed a substantial occupancy of receptors in the temporal cortex by antipsychotics, and suggest limbic selectivity in atypical antipsychotics. The availability of this and other radioligands, with higher affinity for the dopamine receptor than

[^{123}I]-IBZM, enables imaging of extrastriatal dopamine receptors. This may shed a new light on the basic question of changes in dopamine receptors in schizophrenia.

A second limitation is the selectivity of the radioligand. Apart from affinity for the dopamine D_2 receptor, [^{123}I]-IBZM also has affinity for the dopamine D_3 receptor. However, the density of D_3 receptors in the striatum is much lower than that of D_2 receptors, so that the contribution of D_3 receptor binding to the striatal [^{123}I]-IBZM SPECT measurement is probably negligible (Murray et al., 1994).

A third limitation of the methods of imaging dopamine receptor occupancy, is the comparison of data from patients treated with anti-psychotics with data from normal controls. Ideally, one would compare the patient before and during antipsychotic treatment. This would minimise the error from the natural variance in dopamine receptor density. However, since no large difference was found in dopamine receptor density between patients with schizophrenia and healthy controls, and because of the practical and ethical problem of withdrawing antipsychotics for a number of weeks to make a baseline scan, nearly all dopamine receptor occupancy imaging studies are carried out by comparing patient data with data from controls.

A very recent discovery with potential impact on dopamine receptor imaging is the finding of distinct function of two isoforms of the dopamine D_2 receptor, namely D2S (D2 short) and D2L (D2 long) (Usiello et al., 2000). It was found that D2L acts mainly at postsynaptic sites, and D2S serves presynaptic autoreceptor functions. It was suggested that haloperidol has its extrapyramidal side effects through the D2L receptor, and, therefore, that drugs which could discriminate between D2L and D2S in vivo could lead to more effective treatment with fewer extrapyramidal side effects. In addition, the development of new radioligands to analyse these isoforms of the dopamine receptor would be of value to study the role of these isoforms in the pathophysiology of schizophrenia.

In conclusion, the large and growing number of studies on the occupancy of dopamine receptors by antipsychotic medication shows that dopamine receptor SPECT imaging is a valuable tool. This imaging technique improved the insight into the psychopharmacological effect of antipsychotic medication and is therefore important for the introduction of treatment guidelines, the development and evaluation of new antipsychotic medication and for further research concerning the subjective experience of individual patients with schizophrenia.

Part 2

The presynaptic Dopamine transporter

Part two focuses on the presynaptic dopamine system, covering the dopaminergic nerve terminal from which dopamine is released and where reuptake of dopamine is performed by the dopamine transporter. Two types of dopamine imaging studies found indications for changes in the presynaptic dopamine system in patients with schizophrenia. Using the amphetamine-challenge paradigm, it was found that patients with schizophrenia have a higher release of endogenous dopamine after stimulation with amphetamine (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998). Secondly, it was found that the uptake of ^{18}F-DOPA, was increased in patients with schizophrenia (Reith et al., 1994; Hietala et al., 1995, 1999). These findings could be explained by a higher number of dopamine nerve terminals in schizophrenia. Our aim was to elucidate whether the number of dopaminergic nerve terminals was increased in schizophrenic patients, to explain both the higher amphe-tamine-induced dopamine release and the higher uptake of ^{18}F-DOPA.

However, to properly study the density of dopamine transporters, it first had to be determined whether antipsychotic medication had an effect on the binding of the SPECT radioligand [^{123}I]-FP-CIT to dopamine trans-

porters. In our study, in which we used a rat model, it was shown that neither antipsychotic drugs nor dopaminomimetic medication had an effect on [^{123}I]-FP-CIT binding in the rat striatum (Chapter 5). Therefore, antipsychotic medication was not withdrawn in our study comparing young patients with schizophrenia with controls. However, to exclude all possible effects of medication, also a subgroup was included with patients who had never been treated with antipsychotic medication at the moment of SPECT imaging. Using [^{123}I]-FP-CIT SPECT, we found no significant difference in dopamine transporter density between patients and controls (Chapter 6). At the time of that investigation, others published a study with a similar conclusion: the over-activity of the dopamine system is not likely to be explained by an increased number of dopamine transporters (Laakso et al., 2000; Laruelle et al., 2000). It appears that the increased ^{18}F-DOPA uptake and higher dopamine release in patients with schizophrenia may better be explained by an over-activity of the dopamine system, than by an increased number of dopaminergic terminals.

Dopamine transporters and gender

In two studies, we investigated the influence of gender on dopamine transporter density in healthy controls. This work was done in addition to the [^{123}I]-FP-CIT SPECT study in patients with schizophrenia, in which an effect of gender on dopamine transporter density was found. In combination with the intriguing difference between men and women in the occurrence and course of schizophrenia, this finding was studied in a larger group. In one study, we assessed the effect of age and gender on dopamine transporter density (Chapter 7). In this [^{123}I]-FP-CIT SPECT study, we found that healthy females have a slightly but significantly higher striatal dopamine transporter density than males. A second finding was that the density of dopamine transporters decreases with age in healthy controls, a finding confirming earlier studies.

In a second study on the effect of gender on dopamine transporter density, we used a different SPECT radioligand, [^{123}I]-β-CIT, to image both

the dopamine and serotonin transporter in healthy controls. We found a significantly higher density of both dopamine and serotonin transporters in women than in men and observed a relationship between the serotonin and dopamine system in healthy controls (Chapter 8). The observed gender differences may have implications for dopamine imaging studies and for diseases with an established gender difference, such as schizo-phrenia, but also for dopamine-related disorders, such as addiction. Since an imbalance between the dopamine and serotonin system may play a role in the pathophysiology of schizophrenia, it would be of interest to further investigate this relationship (Kapur and Remington, 1996).

Dopamine transporters and tardive dyskinesia

Tardive dyskinesia is a severe extrapyramidal side effect of antipsychotic medication. One hypothesis on the pathophysiology of tardive dyskinesia is the free-radical hypothesis (Lohr et al., 1988). According to this theory, tardive dyskinesia is a neurodegenerative process, with neuronal damage in the basal ganglia. Prolonged treatment with antipsychotic medication increases the dopamine metabolism and turnover. In this process free radicals are generated. This excessive production of free radicals could lead to neuronal membrane instability and dopaminergic cell death. To test the free-radical hypothesis of tardive dyskinesia in vivo, we investigated the density of the dopaminergic nerve terminal in patients with tardive dyskinesia using [^{123}I]FP-CIT SPECT (Chapter 9). We found no change in [^{123}I]FP-CIT binding in the striatum in patients with tardive dyskinesia compared to normal controls. This finding is in line with a large randomised clinical trial on the efficacy of the free radical scavenger vitamin E. That study found no evidence for efficacy of vitamin E in the treatment of tardive dyskinesia (Adler et al., 1999). A recently more dominating theory on the pathophysiology of tardive dyskinesia is the dopaminergic hypersensitivity theory, which explains tardive dyskinesia by an upregulation of dopamine D_2 receptors as a result of the chronic blockade by antipsychotic medication. Interestingly, this theory was

supported by a very recent study that showed an upregulation of striatal dopamine receptors in patients treated with antipsychotics, with the highest upregulation in patients with tardive dyskinesia (Silvestri et al., 2000).

General considerations regarding dopamine transporter imaging

Dopamine transporter imaging has recently been made widely available by the registration of a new radioligand, DaTSCAN, formerly called [^{123}I]-FP-CIT. This radioligand is designed for clinical studies in patients with dopaminergic degeneration such as Parkinson's disease. However, there is one limitation that can be relevant in fundamental research. [^{123}I]-FP-CIT is not strictly selective for the dopamine transporter, as also binds to the serotonin transporter. Because serotonin transporter density in the striatum is only a fraction of the dopamine transporter density, this will not be of great influence clinically. However, in fundamental studies a small serotonergic effect cannot be completely ruled out when studying striatal [^{123}I]-FP-CIT binding.

Part 3

Muscarinic receptor occupancy

Apart from the dopaminergic system, we evaluated side effects related to the occupancy of muscarinic receptors by the antipsychotic drugs olanzapine and risperidone (Chapter 10). In vitro studies showed that olanzapine induces a high occupancy of muscarinic receptors, in contrast with risperidone, which shows hardly any affinity for muscarinic receptors. In our study, we used [^{123}I]-IDEX, a radioligand derived from dexetimide, which is an anticholinergic drug used e.g. in patients with Parkinson's disease. SPECT imaging 8 hours after injection of [^{123}I]-IDEX resulted in clear binding patterns with high activity in the striatum and cortex in

healthy volunteers. In schizophrenic patients treated with olanzapine the [^{123}I]-IDEX binding in all brain regions was dramatically decreased, compared to controls. This is thought to reflect a substantial occupancy of muscarinic receptor by olanzapine, and is in line with in vitro data.

Unexpectedly, patients treated with risperidone showed a significantly lower [^{123}I]-IDEX binding in the striatum than controls. However, in vitro studies showed that risperidone had a very low affinity for muscarinic receptors in the striatum (Schotte et al., 1996). Therefore, it is possible that the lower [^{123}I]-IDEX binding is not an effect of risperidone. Another explanation for the lower [^{123}I]-IDEX binding is that muscarinic receptor density in patients with schizophrenia is lower than in controls. This has also been suggested in another recent SPECT study (Raedler et al., 2000) and in recent post-mortem studies (Crook et al., 1999, 2000). Therefore, schizophrenia per se may also explain the lower [^{123}I]-IDEX binding in schizophrenic patients with risperidone. This interesting finding requires further study, especially with antipsychotic-naive patients, to exclude any medication effect.

General Conclusion

Several studies were performed in this project "SPECT imaging in young patients with schizophrenia", mostly concerning the central dopaminergic system. SPECT imaging was found to be a non-invasive, reliable tool to investigate the central dopamine system in young patients with schizophrenia. The presynaptic and postsynaptic properties of this system can be adequately studied with various radioligands.

The dopamine hypothesis of schizophrenia, since its development in the middle of the 20th century, has, surprisingly, remained of interest (Davis et al., 1991). That this is remarkable is due to the many attempts to falsify this hypothesis. An overwhelming number of studies, stressing the

interaction of neurotransmitter systems (e.g. the serotonin system (Lieberman et al., 1998), and the cholinergic system (Tandon, 1999)) have put the hypothesis under fire, but have not falsified it.

The beneficial effect of antipsychotic drugs, which are all dopamine receptor antagonists, is a major basis for the dopamine hypothesis. Furthermore, the psychotogenic effect of dopamine agonists is still a relevant argument in favour of the dopamine hypothesis. The dopamine hypothesis therefore remains the most important hypothesis in schizophrenia.

In this project, we focused on side effects of antipsychotics and on the presynaptic dopamine system. Our presynaptic study in patients with schizophrenia, however, showed no changes in the presynaptic dopamine transporter density, and seems not to support the dopamine hypothesis. However, when we place our finding alongside those from other recent studies on the presynaptic dopamine system, it is well in line with the latest theory of a hypersensitive dopamine system, that supports an increased dopamine release, despite a normal number of dopaminergic nerve terminals (Duncan et al., 1999).

A fundamental aspect of schizophrenia is the variety of symptoms and course of the disease. Therefore, a specification of symptoms, or degree of illness, of patients under study would be a clarifying contribution to imaging studies. A larger number of patients in a study would make it possible to make analyses of sub-groups, for example of patients with predominantly negative symptoms.

Another characteristic aspect of schizophrenia is the age of onset, which is in most patients during adolescence. The majority of SPECT and PET studies in schizophrenia is performed in young patients. This group of patients is studied with the emphasis on the short lifetime period of treatment with antipsychotic medication, or the antipsychotic-naive state of these patients, but not on specific first episode aspects. As in these studies, and since both dopamine receptors and transporters decline with

age, we decided to include only young patients in a small age range. In addition, in we also included a subgroup of antipsychotic-naive patients. Assessment of typical clinical aspects of this young patient group was not the aim of our project. However, in the debate as to whether the disease process in schizophrenia involves only neurodevelopmental pathology, or also a progressive neurodegenerative component, the comparison of aspects of the dopamine system in first episode and in chronic patients with schizophrenia would be highly interesting.

A general remark on all schizophrenia imaging studies is that even studies that show significant differences between patients with schizo-phrenia and healthy controls always have a large overlap in group outcome, therefore, to date, no diagnosis can be made by scanning the individual patient (fig. 1).
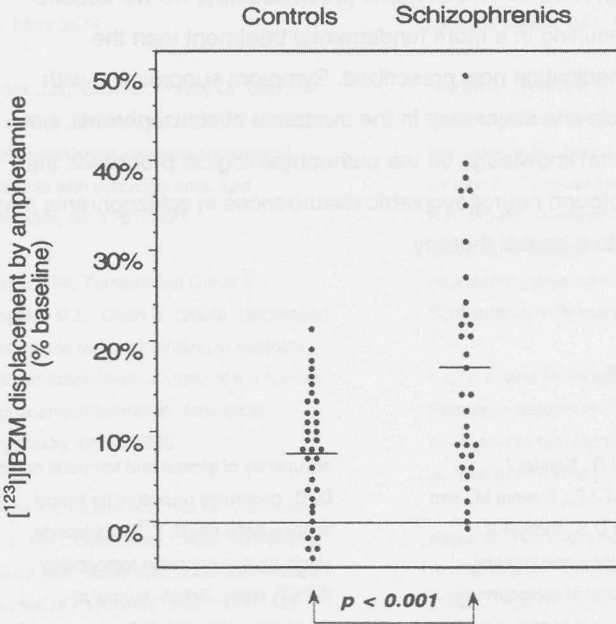
Figure 1. The effect of amphetamine on [^{123}I]-IBZM binding in healthy controls and in untreated patients with schizophrenia. The y-axis shows the percentage decrease in [^{123}I]-IBZM binding potential induced by amphetamine, which is a measure of the increased occupancy of D_2 receptors by dopamine following the challenge. (M. Laruelle et al. Biological Psychiatry 1999; 46:56-72, reprinted with permission).

The combination of dopamine SPECT with other imaging modalities, such as Magnetic Resonance Spectroscopy (MRS) will probably have greater success in providing additional insight into the pathophysiology of this invalidating disease. For example, the finding of a correlation between D_2 receptor density in the striatum and prefrontal neuronal pathology, a combined [^{123}I]IBZM SPECT and MRS study (Bertolino et al., 1999) may well set a trend for imaging strategies.

Finally, no total picture of schizophrenia will be possible with neuroimaging alone. Only in co-operation with other fields, e.g.

neuropsychology, neurochemistry, and pharmacology, we will acquire more insight, resulting in a more fundamental treatment than the antipsychotic medication now prescribed. Symptom suppression with fewer side effects is a major step in the treatment of schizophrenia, but more fundamental knowledge on the pathophysiological processes that underlie the profound neuropsychiatric disturbances in schizophrenia may ultimately lead to a causal therapy.

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Samenvatting en conclusies

Het project "SPECT imaging bij jonge patiënten met schizofrenie" is opgezet om verschillende aspecten van het centrale dopamine systeem bij patiënten met schizofrenie met een eerste of tweede psychotische episode te onderzoeken. SPECT (single photon emission computed tomography) is een nucleair geneeskundige techniek waarbij na toedienen van een radioligand afbeeldingen gemaakt kunnen worden van functionele processen in het lichaam. In dit project werden zowel postsynaptische als presynaptische aspecten van het dopamine systeem bestudeerd, evenals specifieke bijwerkingen van antipsychotische medicatie. Het postsynaptische dopamine systeem werd bestudeerd in twee studies die zich richtten op de dopamine receptorbezetting door twee recent geïntroduceerde antipsychotica (Deel 1). Het presynaptische systeem werd bestudeerd door het vergelijken van de dopamine transporterdichtheid bij patiënten met schizofrenie met controlepersonen. Tardieve dyskinesie werd bestudeerd als specifieke bijwerking van antipsychotica (Deel 2). Verder werd, in relatie met bijwerkingen, de bezetting van de muscarine receptor door twee recent ontwikkelde antipsychotica bepaald (Deel 3).

Deel 1
Dopamine receptorbezetting door antipsychotica

In Deel 1 ligt de nadruk op de postsynaptische dopamine D_2 receptor. Een literatuuroverzicht van het grote aantal SPECT en PET (Positron Emission Tomography) imaging studies dat betrekking heeft op de dopamine D_2 receptorbezetting door antipsychotica wordt gepresenteerd in Hoofdstuk 2. Dit overzicht laat zien dat de meeste antipsychotica een hoge bezetting van de dopamine D_2 receptor in het striatum veroorzaken, met de uit-

zondering van clozapine en mogelijk quetiapine. Een minimum van 60% D_2 receptorbezetting bleek nodig om een antipsychotisch effect te induceren, met een maximum van 80% D_2 receptorbezetting waarboven de incidentie van extrapiramidale bijwerkingen toeneemt (Farde et al., 1992; Nyberg et al., 1995; Kapur et al., 1996).

Verschillende eerdere onderzoeken toonden aan dat olanzapine en risperidone, twee nieuwe antipsychotische medicijnen, beide een lage incidentie van extrapiramidale bijwerkingen laten zien. Om een mogelijke verklaring voor de lage extrapiramidale bijwerkingen te vinden, en om het verschil in D_2 receptorbezetting tussen beide antipsychotica te bepalen, bestudeerden wij de D_2 receptorbezetting met [^{123}I]-IBZM SPECT en beoordeelden we de bijwerkingen bij jonge patiënten die behandeld werden met olanzapine of risperidone. In overeenstemming met deze eerdere studies vonden wij ook dat beide recent ontwikkelde antipsychotica weinig extrapiramidale bijwerkingen veroorzaakten. Bovendien toonden we aan dat er geen significant verschil in D_2 receptorbezetting bestond tussen de twee bestudeerde groepen (Hoofdstuk 3). Daarbij werd gevonden dat behandeling met risperidone gepaard ging met hoge prolactine spiegels in het bloed, in tegenstelling tot behandeling met olanzapine. De meest voor de hand liggende verklaring zou zijn dat dit komt door een hogere bezetting van de D_2 receptoren. Aangezien wij echter geen verschil in dopamine receptorbezetting vonden tussen olanzapine en risperidone, werd een alternatieve hypothese voorgesteld, die de nadruk legt op de verschillen in affiniteit van olanzapine en risperidone voor de $5HT_{2C}$ receptor (Leysen et al., 1998). Olanzapine, in tegenstelling tot risperidone, is een sterke $5HT_{2C}$ receptor-antagonist, en het blokkeren van deze specifieke receptor resulteert in een onderdrukking van het vrijkomen van prolactine. Dit kan een verklaring zijn voor de hoge prolactine spiegels die gevonden werden bij risperidone en niet bij olanzapine.

Naast extrapiramidale bijwerkingen bestudeerden we de subjectieve ervaringen van patiënten met schizofrenie die behandeld werden met antipsychotica. We vonden een positieve correlatie tussen subjectieve ervaring en D_2 receptorbezetting bij patiënten die behandeld werden met gemiddelde doseringen olanzapine of risperidone (Hoofdstuk 4). Dat wil zeggen, patiënten met een hogere D_2 receptorbezetting, met slechts lage scores op de standaard extrapiramidale bijwerkingen onderzoeken, meldden een slechtere subjectieve ervaring. Subjectieve ervaring is daarom misschien meer gevoelig voor D_2 receptorbezetting dan extrapiramidale symptomen. Aanvullende analyses van deze data, waarbij specifiek naar bepaalde sub-schalen van de subjectieve ervaring beoordelingslijsten werd gekeken, toonden een sterke correlatie tussen negatieve subjectieve ervaring en D_2 receptorbezetting. Deze resultaten zijn veelbelovend en maken deel uit van een uitbreiding van deze studie.

Algemene overwegingen over dopamine D_2 receptor imaging met [^{123}I]-IBZM SPECT

Een aantal beperkingen moet in acht worden genomen bij de discussie over dopamine receptor SPECT imaging studies. Een van de meest gehoorde kritiekpunten op deze studies is het gebied in de hersenen dat wordt bestudeerd. Aangezien de hoogste dichtheid van dopamine receptoren zich in het striatum bevindt, richten vrijwel alle dopamine receptor imaging studies, zowel SPECT als PET, zich op dit gebied. Er wordt echter gedacht dat bij schizofrenie dopamine afwijkingen in limbische corticale gebieden meer van belang zijn voor het begrijpen van de symptomatologie dan veranderingen in het striatum. Deze algemene overweging is bediscussieerd vanaf het begin van de dopamine neuro-imaging. Toch laat de bevinding van een ondergrens van D_2 receptor-bezetting voor antipsychotische activiteit van antipsychotische medicatie zien dat imaging studies van het striatum representatief zijn voor het limbische dopaminerge systeem. Evenals studies die een verhoogde

vrijlating van dopamine aantonen bij schizofrenie patiënten, met name het verband met de fase van de ziekte (Laruelle et al., 1999).

Recent werd een nieuw radioligand geïntroduceerd met een hogere affiniteit voor de D_2 receptor dan [^{123}I]-IBZM; namelijk [^{123}I]-epidepride, dat het mogelijk maakt om ook extrastriatale dopamine receptoren te visualiseren (Bigliani et al., 1999, 2000; Stephenson et al., 2000). Deze studies toonden een substantiële bezetting van dopamine receptoren in de temporale cortex door antipsychotica, en suggereren limbische selectiviteit van atypische antipsychotica. De beschikbaarheid van deze en andere radioliganden, met een hogere affiniteit voor de dopamine receptor dan [^{123}I]-IBZM, maken het afbeelden van extrastriatale dopaminereceptoren mogelijk. Dit kan nieuwe inzichten geven in de basale vraag of er veranderingen zijn aan de dopamine receptoren in schizofrenie.

Een tweede beperking is de selectiviteit van het radioligand. Naast affiniteit voor de dopamine D_2 receptor, heeft [^{123}I]-IBZM ook affiniteit voor de dopamine D_3 receptor in de hersenen. Echter, de dichtheid van D_3 receptoren in het striatum is veel lager dan die van D_2 receptoren, zodat de bijdrage van D_3 receptor binding aan de striatale [^{123}I]-IBZM SPECT bepaling waarschijnlijk te verwaarlozen is (Murray et al., 1994).

Een derde beperking in de methode van dopamine receptor imaging is de vergelijking van patiënten behandeld met antipsychotica met gezonde controles. Idealiter zou men de patiënt voor en tijdens antipsychotica behandeling willen vergelijken. Dit zou de fout die geïnduceerd wordt door de natuurlijke variatie in dopamine receptor-dichtheid minimaliseren. Aangezien geen groot verschil in receptor-dichtheid werd gevonden tussen patiënten met schizofrenie en controles, en het praktische en ethische probleem van het stoppen met anti-psychotica voor een aantal weken om een uitgangsscan te maken, worden bijna alle dopamine receptorbezettingsstudies uitgevoerd door patiëntendata te vergelijken met data van controlepersonen.

Een zeer recente ontdekking met potentiële impact op dopamine receptor imaging is de ontdekking van verschillen in functie tussen twee

iso-vormen van de dopamine D_2 receptor, namelijk D2S (D2 short) en D2L, (D2 lang) (Usiello et al., 2000). Er werd gevonden dat D2L zich vooral op postsynaptische plaatsen bevindt, en D2S een presynaptische auto-receptor functie heeft. Er werd hierbij gesuggereerd dat haloperidol zijn extrapiramidale bijwerking uitoefent door de D2L receptor, en dat medicatie die het onderscheid kan maken tussen D2L en D2S, in vivo zou kunnen leiden tot een effectievere behandeling met minder extra-piramidale bijwerkingen. Daarbij zou de ontwikkeling van nieuwe radioliganden met de mogelijkheid tot specifieke analyse van deze twee isovormen waardevol zijn bij de bestudering van de rol van isovormen van dopamine receptoren bij de pathofysiologie van schizofrenie.

Concluderend, het grote en toenemende aantal studies naar de bezetting van dopamine receptoren door antipsychotica laat zien dat dopamine receptor SPECT imaging een waardevolle techniek is. Deze imaging techniek verschaft inzicht in het psychofarmacalogische effect van antipsychotica en is daarom belangrijk voor de introductie van behande-lingsrichtlijnen, voor de ontwikkeling en evaluatie van nieuwe antipsychotica, en voor verder onderzoek naar de subjectieve ervaring van de patiënt met schizofrenie.

Deel 2

Presynaptische dopamine transporters

Deel twee richt zich op het presynaptische dopamine systeem, en omvat de dopaminerge zenuw-terminal waar dopamine vrijkomt en waar de heropname van dopamine plaatsvindt door de dopamine transporter. Twee verschillende types van dopamine imaging studies vonden eerder aanwijzingen voor veranderingen in het presynaptische dopamine systeem bij patiënten met schizofrenie. Met het paradigma van de amfetamine-stimulatie studie werd gevonden dat bij patiënten meer dopamine vrijkomt na stimulatie met amfetamine (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998). De tweede serie studies vond dat de opname van ^{18}F-DOPA toegenomen was bij patiënten met schizofrenie (Reith et al., 1994; Hietala et al., 1995, 1999). Deze bevindingen zouden verklaard kunnen worden door een hoger aantal dopaminerge zenuw-terminals. Ons doel was om op te helderen of de dichtheid van dopamine transporters in het striatum verhoogd is bij patiënten met schizofrenie, om zo een verklaring te geven voor de hogere amfetamine geïnduceerde dopamine vrijlating en de hogere opname van ^{18}F-DOPA.

Echter, om de dichtheid van de dopamine transporters eenduidig te kunnen bepalen, moest eerst duidelijk zijn of antipsychotica effect had op de binding van het SPECT radioligand [^{123}I]-FP-CIT aan de dopamine transporters. In onze studie, waarbij we gebruik maakten van een rattenmodel, toonden we aan dat noch antipsychotica, noch dopaminomimetica een effect hadden op de binding van [^{123}I]-FP-CIT in het striatum van de rat (Hoofdstuk 5). Daarom werd antipsychotische medicatie niet gestaakt in onze studie waarbij we jonge patiënten met schizofrenie vergeleken met controles. Toch werd, om alle mogelijke medicatie invloeden uit te sluiten, een subgroep met patiënten geïncludeerd die nooit eerder met antipsychotica behandeld was op h et moment van SPECT

imaging. Met ons [^{123}I]-FP-CIT SPECT onderzoek vonden we geen significant verschil in dopamine transporterdichtheid tussen patiënten en controles (Hoofdstuk 6). Op het moment dat deze studie uitgevoerd werd, verschenen studies met een vergelijkbare conclusie: de overactiviteit van het dopamine systeem wordt waarschijnlijk niet verklaard door een verhoogd aantal dopamine transporters (Laakso et al., 2000; Laruelle et al., 2000). De verhoogde opname van ^{18}F-DOPA en de hogere vrijlating van dopamine bij patiënten met schizofrenie worden dus waarschijnlijk beter verklaard door overactiviteit van het dopamine systeem dan door een hoger aantal dopaminerge terminals.

Dopamine transporters en geslacht

In twee studies werd gekeken naar de invloed van het geslacht op de dopamine transporter. Dit werk werd gedaan in aanvulling op de [^{123}I]-FP-CIT SPECT studie, waarin een effect van geslacht op de dopamine transporter gevonden was. In combinatie met het intrigerende verschil in voorkomen en beloop van schizofrenie bij mannen en vrouwen, werd deze bevinding nader onderzocht in een grotere groep. In de eerste [^{123}I]-FP-CIT SPECT studie werd gekeken naar het effect van leeftijd en geslacht op de dopamine transporterdichtheid (Hoofdstuk 7). In deze [^{123}I]-FP-CIT SPECT studie vonden we dat gezonde vrouwelijke controles een kleine maar significant hogere dopamine transporterdichtheid hebben dan mannen. Een tweede bevinding was dat de dichtheid van dopamine transporters bij gezonde controles afneemt met de leeftijd, een bevinding die eerdere studies bevestigt.

In een tweede studie naar het effect van geslacht op dopamine transporterdichtheid gebruikten we een ander SPECT radioligand, [^{123}I]-β-CIT, om zowel de dopamine als de serotonine transporter af te beelden bij gezonde controles. We vonden een significant hogere dichtheid van zowel dopamine als serotonine transporters bij vrouwen dan bij mannen en een relatie tussen het dopamine en serotonine systeem bij gezonde controles (Hoofdstuk 8). Deze gevonden geslachtsverschillen kunnen implicaties

hebben voor dopamine imaging studies in aandoeningen waarbij geslachtsverschillen een rol spelen, zoals schizofrenie, maar ook bij dopamine-gerelateerde stoornissen, zoals verslaving. Aangezien een verstoorde balans tussen het dopamine en serotonine systeem een rol kan spelen bij de pathofysiologie van schizofrenie, zou het interessant zijn om deze relatie verder te onderzoeken (Kapur and Remington, 1996).

Dopamine transporters en tardieve dyskinesie

Tardieve dyskinesie is een ernstige extrapiramidale bijwerking van antipsychotische medicatie. Een van de hypothesen over de pathofysiologie van tardieve dyskinesie is de vrije-radicalen hypothese. (Lohr et al., 1988). Volgens deze hypothese is tardieve dyskinesie een neurodegeneratief proces met neuronale schade in de basale ganglia. Langdurige behandeling met antipsychotica verhoogt het dopamine metabolisme en de dopamine afbraak. Bij dit proces komen vrije radicalen vrij. Deze overdadige productie van vrije radicalen kan leiden tot neuronale membraan instabiliteit en dopaminerge celdood. Om de vrije-radicalen hypothese bij tardieve dyskinesie in vivo te testen, onderzochten we de dichtheid van dopaminerge terminals bij patiënten met tardieve dyskinesie met behulp van [^{123}I]-FP-CIT SPECT (Hoofdstuk 9). Er werd geen verschil gezien in [^{123}I]-FP-CIT binding in het striatum tussen patiënten met tardieve dyskinesie en controles. Onze bevinding past bij de uitkomst van een groot gerandomiseerd klinisch onderzoek naar de effectiviteit van vitamine E, een behandeling gebaseerd op de vrije-radicalen hypothese. Dat onderzoek gaf geen aanwijzingen voor effectiviteit van vitamine E in de behandeling van tardieve dyskinesie (Adler et al., 1999). Een recentelijk meer dominerende theorie over de pathofysiologie van tardieve dyskinesie is de dopaminerge hyper-sensitiviteitstheorie, deze verklaart tardieve dyskinesie door een upregulatie van dopamine receptor als gevolg van de langdurige blokkade door antipsychotica. Ondersteuning van deze theorie werd gegeven door een zeer recente studie die upregulatie van striatale D$_2$ receptoren

aantoonde bij patiënten met antipsychotica, waarbij de hoogste upregulatie gezien werd bij patiënten met tardieve dyskinesie (Silvestri et al., 2000).

Algemene overwegingen over dopamine transporter imaging

Dopamine transporter imaging is recent klinisch beschikbaar gekomen door de registratie van een nieuw radioligand, DaTSCAN, voorheen [^{123}I]-FP-CIT. Dit radioligand is ontwikkeld voor klinische studies bij patiënten met dopaminerge degeneratie, zoals patiënten met M. Parkinson. Er is echter een beperking die relevant kan zijn bij fundamenteel onderzoek. [^{123}I]-FP-CIT is niet volledig selectief voor de dopamine transporter, het bindt ook aan de serotonine transporter. Aangezien de serotonine transporterdichtheid in het striatum slechts een fractie van de dopamine transporterdichtheid bedraagt, zal dit geen grote klinische invloed hebben. Bij fundamentele studies kan een klein serotonerg effect echter niet compleet uitgesloten worden bij het onderzoeken van de striatale [^{123}I]-FP-CIT binding.

Deel 3

Muscarine receptorbezetting

Naast het dopamine systeem bestudeerden we bijwerkingen aan de hand van de bezetting van muscarine receptoren door de antipsychotica olanzapine en risperidone (Hoofdstuk 10). In vitro studies lieten zien dat olanzapine een hoge bezetting van muscarine receptoren veroorzaakt, in tegenstelling tot risperidone, dat nauwelijks affiniteit voor deze receptoren bezit. In deze studie werd [^{123}I]-IDEX gebruikt, een radioligand afgeleid van dexetimide, een anticholinergicum dat onder andere wordt gebruikt bij M. Parkinson. SPECT imaging 8 uur na toediening resulteerde in een duidelijk bindingspatroon met hoge stapeling van activiteit in het striatum

en in de cortex van gezonde controles. Bij schizofreniepatiënten die behandeld werden met olanzapine was de [^{123}I]-IDEX binding in alle regio's sterk verlaagd, vergeleken met controles. Dit wordt verklaard door een substantiële bezetting van muscarine receptoren door olanzapine, en sluit aan bij in vitro gegevens.

Onverwacht werd bij patiënten met risperidone een significant lagere binding van [^{123}I]-IDEX in het striatum gezien dan bij controlepersonen. In vitro studies toonden echter dat risperidone een zeer lage affiniteit voor de muscarine receptor in het striatum heeft (Schotte et al., 1996). Daarom is de lage [^{123}I]-IDEX binding bij patiënten met schizofrenie mogelijk geen effect van risperidone. Een andere verklaring voor de lagere [^{123}I]-IDEX binding is dat de dichtheid van muscarine receptoren bij patiënten met schizofrenie lager is dan bij controles. Dit werd ook gesuggereerd in een andere recente SPECT studie (Raedler et al., 2000), en was een bevinding bij recente post-mortem studies (Crook et al., 1999, 2000). Mogelijk is schizofrenie zelf een verklaring voor de lagere striatale [^{123}I]-IDEX binding bij patiënten met risperidone. Deze interessante bevinding verdient verder onderzoek, met name bij patiënten met schizofrenie die nooit eerder met antipsychotica behandeld zijn, om mogelijke medicatie effecten uit te sluiten.

Algemene Conclusie

Verschillende studies werden uitgevoerd binnen dit project "SPECT imaging bij jonge patiënten met schizofrenie", waarvan de meeste het dopamine systeem betroffen. SPECT bleek een betrouwbaar, niet-invasief, instrument om het centrale dopamine systeem te onderzoeken bij jonge patiënten met schizofrenie. Presynaptische en postsynaptische delen van dit systeem kunnen adequaat onderzocht worden met verschillende radioliganden.

De dopamine hypothese is, sinds de ontwikkeling ervan in het midden van de 20ste eeuw, verbazingwekkend interessant gebleven (Davis et al., 1991). Dat is opvallend, gezien de vele pogingen om deze hypothese te falsificeren. Een overweldigend aantal studies die de interactie tussen neurotransmitters beklemtonen (bijvoorbeeld het serotonine systeem (Lieberman et al., 1998), en het cholinerge systeem (Tandon, 1999)) hebben de hypothese onder vuur gelegd, maar deze niet gefalsificeerd. Het klinische effect van antipsychotica, die allemaal dopamine receptor antagonisten zijn, is een belangrijke basis voor de dopamine hypothese. Verder is het psychotogene effect van dopamine agonisten een relevant argument voor de dopamine hypothese. De dopamine hypothese blijft daarom de belangrijkste hypothese voor schizofrenie.

In dit project hebben we ons vooral gericht op bijwerkingen van antipsychotica en op het presynaptische systeem. Onze presynaptische studie bij patiënten met schizofrenie toonde echter geen veranderingen aan in de presynaptische dopamine transporter, en lijkt de dopamine hypothese daarom niet te ondersteunen. Echter, wanneer we onze bevinding plaatsen tussen die van andere recente studies naar het presynaptische dopamine systeem, past deze in de laatste theorie van een hypersentitief dopamine systeem, dat een toegenomen dopamine vrijkomen ondersteunt, ondanks een normaal aantal dopaminerge zenuw-terminals (Duncan et al., 1999).

Een fundamenteel aspect van schizofrenie is de variatie in symptomen en beloop van de ziekte. Daarom zou een specificatie van symptomen, of ernst van de ziekte van patiënten in de studie een verhelderende bijdrage kunnen geven aan imaging studies. Een groter aantal patiënten in een studie zou het mogelijk maken om analyses van sub-groepen te maken, bijvoorbeeld bij patiënten met voornamelijk negatieve symptomen.

Een ander karakteristiek aspect van schizofrenie is de aanvangsleeftijd, deze is bij de meeste patiënten in de adolescentie.

De meerderheid van SPECT en PET studies bij schizofrenie is ook uitgevoerd bij jonge patiënten. Deze groep patiënten wordt echter onderzocht met de nadruk op een korte periode van behandeling met antipsychotica, of de antipsychotica-naïeve status van de patiënt, maar niet op specifieke eerste episode schizofrenie aspecten. Net als in deze andere imaging studies, en aangezien zowel dopamine receptoren als transporters afnemen met de leeftijd, besloten wij alleen jonge patiënten te includeren met een kleine leeftijdsspreiding. Daarbij includeerden we ook een subgroep met antipsychotica-naïeve patiënten. Het onderzoeken van typische klinische aspecten van deze jongre patiëntengroep was niet het doel van dit project. Echter in het debat over de vraag of schizofrenie alleen ontwikkelingsgebonden pathologie betreft of dat er ook een progressieve neurodegeneratieve component meespeelt , zou de vergelijking van aspecten van het dopamine systeem in eerste episode patiënten en in chronische patiënten met schizofrenie erg interessant zijn.

Een algemene opmerking over alle schizofrenie imaging studies is dat zelfs studies die significante verschillen aantonen tussen patiënten met schizofrenie en gezonde controles, altijd een grote overlap bestaat tussen de groepen, zodat geen diagnose gesteld kan worden door een individuele patiënt te scannen (Figuur 1).

Controls   Schizophrenics
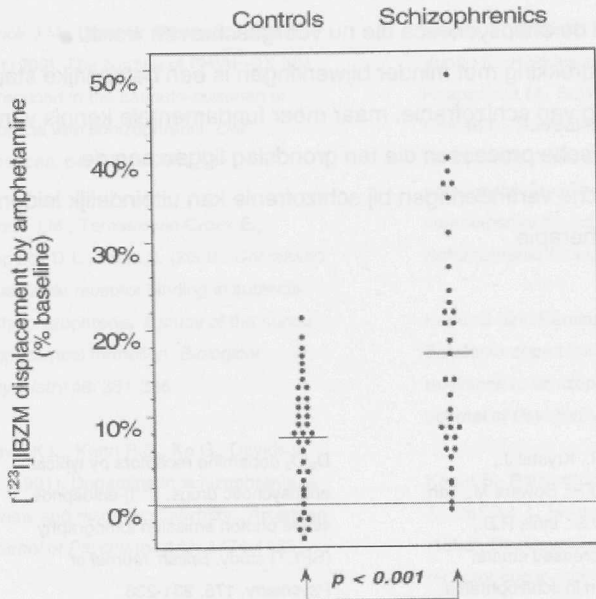
Figuur 1. Het effect van amfetamine op de binding van $[^{123}I]$-IBZM bij gezonde controles en schizofreniepatiënten. De Y-as toont het percentage verlaging van de $[^{123}I]$-IBZM bindingspotentiaal door amfetamine, hetgeen een maat is voor de toegenomen bezetting van D_2 receptoren door dopamine na amfetamine toediening. (M. Laruelle et al. Biological Psychiatry 1999; 46:56-72, overgenomen met toestemming).

De combinatie van dopamine SPECT met andere imaging modaliteiten, zoals Magnetische Resonantie Spectroscopie (MRS) zal waarschijnlijk meer succes hebben in het verschaffen van aanvullende inzichten in de pathofysiologie van deze invaliderende aandoening. Bijvoorbeeld, de bevinding van een correlatie tussen de D_2 receptordichtheid in het striatum en prefrontale neuronale pathologie, een recente gecombineerde $[^{123}I]$-IBZM SPECT en MRS studie (Bertolino et al., 1999), is mogelijk een trendsetter voor toekomstige imaging strategieën.

Tenslotte, geen totaalbeeld van schizofrenie zal mogelijk zijn door enkel neuro-imaging technieken. Alleen in samenwerking met andere onderzoeksvelden als neuropsychologie, neurochemie, en farmacologie zal meer inzicht bereikt worden, resulterend in een meer fundamentele

behandeling dan de antipsychotica die nu voorgeschreven wordt.
Symptoomonderdrukking met minder bijwerkingen is een belangrijke stap
in de behandeling van schizofrenie, maar meer fundamentele kennis van
de pathofysiologische processen die ten grondslag liggen aan de
neuropsychiatrsiche veranderingen bij schizofrenie kan uiteindelijk leiden
tot een causale therapie.

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Curriculum Vitae

Jules Lavalaye werd geboren op 21 mei 1970 te Sint Michielsgestel. In 1988 behaalde hij het atheneum diploma aan het Monseigneur Frencken College te Oosterhout. Hij volgde zijn studie geneeskunde aan de Universiteit van Amsterdam (artsexamen 1997), met diverse niet-medische bijvakken. Aansluitend werkte hij als aio en later als arts-onderzoeker bij de Adolescentenkliniek van de afdeling Psychiatrie van het Academisch Medisch Centrum, in samenwerking met de afdeling nucleaire geneeskunde. In april 2000 werd gestart met de opleiding Nucleaire Geneeskunde in het Academisch Medisch Centrum (opleider dr. B.L.F. van Eck-Smit).

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Chapter 6 Figure 1. [123I]FP-CIT SPECT images of an antipsychotic-naive patient with schizophrenia. Transverse slices of the striatum with overlaid Volumes of Interest for striatum and occipital cortex. The level of radioactivity is color encoded from low (black) to high (white).