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

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

Key words: [123I]FP-CIT – Dopamine transporter imaging – Animal studies – Dopaminergic medication – Parkinson's disease – Single-photon emission tomography

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Introduction

The development of positron emission tomography (PET) and single-photon emission tomography (SPET) radiotracers for quantification of the dopaminergic nigrostriatal pathway has been successful. Especially quantification of dopamine (DA) transporters, situated in the membrane of presynaptic neurons, is now possible. One of the tracers for SPET imaging of this transporter is [123I]FP-CIT (*N*-ω-fluoropropyl-2β-carbomethoxy-3β-[4-iodophenyl]nortropane). DA transporter imaging has been mainly focussed on studying parkinsonian syndromes. These studies showed loss of striatal DA transporters in Parkinson's disease, an observation in line with post-mortem findings [1]. Imaging of DA transporters has also been applied to investigate other neuropsychiatric diseases such as schizophrenia.

Due to the practical applicability of [123I]FP-CIT SPET, 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 SPET 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 [123I]FP-CIT to striatal DA 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 DA 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 classic neuroleptic (haloperidol, obtained from Janssen Pharm.). The selective serotonin reuptake inhibitor fluvoxamine was a generous gift from Solvay Pharmaceuticals. The potent selective DA 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.

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Table 1. Medication administered to rats for 2 weeks (sub-chronic administration) or on the day of imaging (acute administration)

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

[^{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.

In vivo distribution in rats

Drug administration. Male Wistar rats (Broekman Institute B.V., Someren, The Netherlands; body weight 250–350 g) were used. Sub-chronic administration was performed in groups of four or five rats per compound, for 2 weeks with 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 for 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 of the procedure as described previously [2].

The striatum was chosen as an area of binding to DA transporters due to its high concentration of DA reuptake sites. The hypothalamus was chosen as an area representative of binding to serotonin transporters because it contains many serotonin, but few DA transporters [3]. 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 the 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 the striatum-to-cerebellum binding ratio.

After acute administration of all drugs under study, the absolute striatal binding of [^{123}I]FP-CIT was not significantly different from that in controls (Table 3). The striatum-to-cerebellum binding ratio was 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 [^{123}I]FP-CIT binding in this region.

Hypothalamus binding

Sub-chronic administration of all compounds showed no statistical significant effect on absolute [^{123}I]FP-CIT binding in the hypothalamus or on the hypothalamus-to-cerebellum binding ratio (Table 2).

Acute administration of fluvoxamine resulted in a significant decrease in absolute [^{123}I]FP-CIT binding in the hypothalamus, as well as in the hypothalamus-to-cerebellum binding ratio (Table 3). None of the other compounds induced a significant change in absolute [^{123}I]FP-CIT binding in the hypothalamus, or in the hypothalamus-to-cerebellum uptake ratio.

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

	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 four or five 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 into two sessions with separate control groups

Table 3. [123I]FP-CIT binding in groups of four or five rats after acute administration of pergolide, olanzapine, Sinemet, risperidone, haloperidol, fluvoxamine and selegiline[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 four or five rats. Drugs were administered orally 2 h before injection of [123I]FP-CIT and radioactivity was measured 2 h p.i.

Table 4. [123I]FP-CIT binding in groups of four rats after acute administration of GBR12,909[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 four or five rats. Drugs were administered i.v. 5 min before injection of [123I]FP-CIT and radioactivity was measured 2 h p.i.

Discussion

Sub-chronic administration

In this study, only small changes in absolute [123I]FP-CIT binding in rat striatum and hypothalamus were found after sub-chronic administration of dopaminergic medication or fluvoxamine. None of these changes were statistically significant. Ratios of striatum- and hypothalamus-to-cerebellum binding were also not significantly 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 DA transporters was found in humans [4] and animals [5]. While no effect of selegiline administration on in vivo radioligand binding to striatal DA transporters was found in humans [4], animal studies showed inconsistent results [5, 6].

We found no significant influence of pergolide on [123I]FP-CIT binding to DA transporters. In agreement with this, no effect of treatment with the DA agonist apomorphine on striatal [3H]WIN 35,428 binding was recently reported [5]. However, the finding in the latter study of decreased striatal [3H]WIN 35,428 binding after haloperidol administration [5] was not confirmed in our study. In addition, our study showed no significant influence of sub-chronic administration of olanzapine and risperidone on striatal [123I]FP-CIT binding. Nevertheless, significant influences of chronic administration of these drugs cannot be excluded conclusively by means of the employed experimental paradigm.

Acute administration

Absolute striatal [123I]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 [3H]WIN 35,428 binding has been shown to be insensitive to acute haloperidol administration [6]. Our present data also show that acute administration of the atypical antipsychotics olanzapine and risperidone had no influence on striatal [123I]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 [123I]β-CIT binding [7]. Moreover, using [99mTc]TRODAT-1, Dresel et al. [8] 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 in striatal [3H]WIN 35,428 binding in mice [6]. However, this decrease was not found when levodopa was administered more than 2.5 h before injection of [3H]WIN 35,428. In our study, 100 mg/kg levodopa was administered orally 2 h before injection of [123I]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 [123I]FP-CIT binding in the striatum and the hypothalamus, respectively [9]. These findings suggest that this animal model can be used to detect possible effects of medication on [123I]FP-CIT binding to dopamine or serotonin transporters.

The finding of a decrease in [123I]FP-CIT binding in the hypothalamus after acute administration of fluvoxamine is in line with a report that clomipramine decreases [123I]β-CIT binding in the hypothalamus [10]. By contrast, the finding in that study of enhanced striatal [123I]β-CIT binding after clomipramine administration was not matched in our study with fluvoxamine and [123I]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 was 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 doses, do not affect the results of DA transporter imaging with [123I]FP-CIT in humans. Therefore it seems unnecessary to interrupt dopaminergic therapy for [123I]FP-CIT SPET imaging.

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