Iodine-123 labelled nor-β-CIT binds to the serotonin transporter in vivo as assessed by biodistribution studies in rats

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Abstract. Iodine-123 labelled 2β-carbomethoxy-3β-(4-iodophenyl)nortropane (nor-β-CIT), a radioiodinated cocaine analogue, was evaluated as an agent for the in vivo labelling of serotonin transporters by biodistribution studies in rats. Intravenous injection of [^{123}I]nor-β-CIT resulted in high accumulation of radioactivity in brain areas with high densities of serotonin (hypothalamus) and dopamine transporters (striatum), although the binding was less pronounced in the hypothalamus. While binding of [^{123}I]nor-β-CIT in the hypothalamus was blocked significantly by fluvoxamine (a selective serotonin transporter blocker) but not by GBR12,909 (a selective dopamine transporter blocker), the opposite was observed in the striatum. The results of this study indicate that [^{123}I]nor-β-CIT, although not being a selective radioligand, binds specifically to serotonin transporters in the hypothalamus in vivo and thus suggest that [^{123}I]nor-β-CIT promises to be a suitable radioligand for single-photon emission tomography imaging of serotonin transporters in humans.

Key words: 2β-Carbomethoxy-3β-(4-iodophenyl)nortropane – Serotonin transporter – Biodistribution studies – Hypothalamus

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Introduction

Disturbances of the serotonergic neurotransmitter system have been implicated in the pathophysiology of a number of diseases of the nervous system. For instance, changes in serotonin (5-HT) levels in the central nervous system have been considered to play a role in the aetiology of depression and other neuropsychiatric disorders. Reduction in the number of central 5-HT neurons has been reported in post-mortem studies performed in patients with Alzheimer's disease, and recent studies suggested neurotoxic effects on 5-HT neurons of the widely used recreational drug ecstasy (3,4-methylenedioxymethamphetamine; MDMA) [1, 2].

Visualisation and quantification of 5-HT neurons in the living human brain by means of imaging techniques such as positron emission tomography (PET) and single-photon emission tomography (SPET) facilitate the detection of degeneration of 5-HT neurons. The 5-HT transporter is considered to be a reliable marker of 5-HT neurons. The plasma membrane 5-HT transporter is located on the presynaptic 5-HT nerve terminal, and plays a key role in the regulation of the 5-HT concentration in the synaptic cleft.

Several potential tracers have been tested for their in vitro ability to bind to the 5-HT transporter. Among these tracers, the cocaine analogue 2β-carbomethoxy-3β-(4-iodophenyl)nortropane (nor-β-CIT) showed high affinity for the 5-HT transporter (IC_{50}=0.36 n*M*) [3]. Interestingly, the results of recent studies showed that ^{123}I-labelled nor-β-CIT promises to be a novel SPET tracer for the 5-HT transporter [4, 5].

The goal of the present study was to obtain additional information on the in vivo characterisation of [^{123}I]nor-β-CIT. Selectivity of [^{123}I]nor-β-CIT as an in vivo label for the central 5-HT transporters was investigated by performing biodistribution studies in rats.

Materials and methods

Chemicals and radiolabelling of nor-β-CIT. The selective 5-HT blocker fluvoxamine [6] was a generous gift from Solvay Pharmaceuticals (Weesp, The Netherlands). The selective dopamine uptake blocker GBR12,909 [7] was obtained from Research Biochemicals International (RBI, Natick, Mass., USA). ^{123}I labelling of nor-β-CIT was performed by oxidative radioiododestannylation (Amersham Cygne, Technical University Eindhoven, The Netherlands) of its trimethylstannyl precursor. [^{123}I]nor-β-CIT had a specific activity of >200 MBq/nmol and a radiochemical purity >97%. [^{123}I]nor-β-CIT was dissolved in a sodium acetate buffer (pH 4.7) with 5% ethanol, passed through a 0.22-μm membrane filter, and subsequently diluted to the appropriate concentration for intravenous (i.v.) injections in rats.

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In vivo distribution studies. Male Wistar rats (obtained from Broekman Institute B.V., Someren., The Netherlands) received an injection of approximately 1.85 MBq [^{123}I]nor-β-CIT/0.4 ml buffer into the tail vein under ether anaesthesia.

In a first study, groups of rats (n=3; 250–350 g body weight) were sacrificed at several time points after injection of [^{123}I]nor-β-CIT to investigate the time course of binding in rat brain. The hypothalamus was chosen as an area representative of binding to the 5-HT transporter because it contains many 5-HT but few dopamine transporters [8]. The striatum was chosen as a brain area reflecting binding to dopamine transporters due to its high concentration of dopamine transporters. The cerebellum has a low density of 5-HT and dopamine transporters [9, 10] and was consequently chosen as a brain area reflecting free and non-specific [^{123}I]nor-β-CIT binding in rat brain.

In blocking experiments, rats (n=4–6; 300–400 g body weight) received 5 min prior to injection of [^{123}I]nor-β-CIT an i.v. injection with an excess of blocker (fluvoxamine or GBR12,909; 5–10 mg/kg body weight dissolved in 0.4 ml buffer), whereas a control group of rats (n=4–6) received an i.v. injection of 0.4 ml buffer. Rats were sacrificed 4 h after injection of the radiotracer, since the time course study (see Results) showed high specific binding in the striatum and hypothalamus at that particular time point.

Rats were killed by bleeding via heart puncture under ether anaesthesia. Blood, several brain regions (striatum, hypothalamus and cerebellum) and pieces of various peripheral tissues (lung, heart, liver, kidney, fat and muscle), were rapidly excised and weighed. ^{123}I radioactivity was assayed in a gamma counter. The data were corrected for radioactivity decay, and the amount of radioactivity was expressed as percentage of injected dose, multiplied by the body weight in kilograms, per gram tissue or blood [11, 12].

Statistical analysis. Differences between groups were analysed by analysis of variance (ANOVA). In the case of multiple comparisons, the Bonferroni correction method was used. In all statistical analyses, probability values <0.05 were considered significant.

Results

Injection of [^{123}I]nor-β-CIT resulted in high absolute binding in hypothalamus and striatum in comparison with cerebellum (Table 1). For measuring [^{123}I]nor-β-CIT binding to 5-HT transporters in the hypothalamus and to dopamine transporters in the striatum, the cerebellum appeared to be a representative brain area for non-specific binding, since fluvoxamine and GBR12,909 were not able to block absolute [^{123}I]nor-β-CIT binding in the cerebellum (Table 2).

Uptake of [^{123}I]nor-β-CIT in the hypothalamus was fast in time, resulting in high hypothalamus-to-cerebellum ratios of approximately 4.5 and 5.1 at 4 h and 6 h post-injection, respectively. Uptake of the radiotracer was also fast in the striatum, resulting in high striatum-to-cerebellum ratios of approximately 6.8 and 8.4 at 4 h and 6 h post-injection, respectively. The binding ratios of [^{123}I]nor-β-CIT were higher for dopaminergic than for serotonergic brain areas (Table 1).

Pre-injection of fluvoxamine, but not of GBR12,909, significantly decreased the absolute binding of [^{123}I]nor-

Table 2. Blockade of ^{123}I radioactivity uptake by fluvoxamine or GBR12,909 in hypothalamus, striatum and cerebellum after i.v. injection of [^{123}I]nor-β-CIT in mature rats[a]

	Control	Fluvoxamine	GBR12,909
Hypothalamus	0.506±0.044	0.157±0.036*	0.392±0.092
Striatum	0.827±0.146	0.644±0.096	0.451±0.098*
Cerebellum	0.115±0.013	0.094±0.014	0.094±0.012

* Statistically significant different from controls

[a] Data are given as %ID×kg/g and represent the mean±SD of four to six rats. The drugs were injected i.v. 5 min before injection of the radioligand and radioactivity was measured at 4 h p.i.

Table 1. Biodistribution of ^{123}I radioactivity and hypothalamic and striatal uptake ratios at different times after intravenous injection of [^{123}I]nor-β-CIT in mature rats[a]

	15 min	30 min	1 h	2 h	3 h	4 h	6 h	24 h
Hypothalamus	0.672±0.024	0.679±0.014	0.691±0.068	0.594±0.032	0.486±0.120	0.422±0.024	0.320±0.051	0.016±0.020
Striatum	0.820±0.040	0.869±0.012	0.940±0.079	0.908±0.041	0.785±0.146	0.639±0.029	0.528±0.070	0.043±0.038
Cerebellum	0.457±0.048	0.406±0.017	0.316±0.027	0.175±0.007	0.122±0.016	0.094±0.002	0.063±0.007	0.009±0.005
Blood	0.176±0.016	0.143±0.013	0.090±0.008	0.083±0.001	0.060±0.012	0.053±0.002	0.049±0.003	0.012±0.002
Fat	0.046±0.017	0.076±0.021	0.093±0.007	0.081±0.004	0.057±0.009	0.049±0.006	0.036±0.003	0.006±0.003
Muscle	0.069±0.015	0.061±0.009	0.051±0.005	0.039±0.002	0.032±0.003	0.029±0.001	0.025±0.002	0.005±0.001
Kidney	0.604±0.045	0.494±0.069	0.314±0.016	0.241±0.013	0.191±0.039	0.171±0.087	0.127±0.008	0.023±0.017
Liver	0.407±0.040	0.394±0.018	0.478±0.023	0.508±0.027	0.489±0.037	0.516±0.070	0.532±0.076	0.294±0.009
Lung	2.600±1.198	1.430±0.267	0.782±0.041	0.494±0.048	0.323±0.035	0.284±0.043	0.191±0.022	0.170±0.245
Heart	0.356±0.083	0.253±0.044	0.145±0.005	0.108±0.008	0.079±0.005	0.069±0.002	0.055±0.004	0.011±0.003
Hypothalamus/ cerebellum	1.488±0.198	1.766±0.154	2.200±0.281	3.400±0.310	3.944±0.582	4.500±0.339	5.122±1.154	1.355±1.200
Striatum/ cerebellum	1.811±0.253	2.200±0.112	2.977±0.074	5.199±0.392	6.400±0.631	6.811±0.201	8.422±1.397	4.199±2.045

[a] Data are given as the %ID×kg/g and represent the mean±SD of three rats

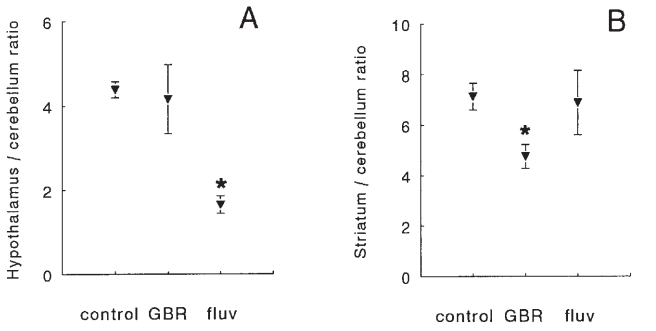

Fig. 1A, B. Blockade of [^{123}I]nor-β-CIT binding in rat hypothalamus by injection of fluvoxamine but not by that of GBR12,909, and the opposite for blockade of binding in rat striatum. Drugs were injected i.v. 5 min before injection of the radiotracer, and radioactivity was measured at 4 h p.i. Control rats received buffer injections. The data are expressed as radioactivity ratios of hypothalamus-to-cerebellum binding (**A**) and striatum-to-cerebellum binding (**B**) (mean ± SD of four to six rats). *Significantly different from controls

β-CIT in the hypothalamus (Table 2) and the hypothalamus-to-cerebellum binding ratio (Fig. 1). Intravenous injection of fluvoxamine did not significantly change the absolute binding of [^{123}I]nor-β-CIT in the striatum (Table 2) or the striatum-to-cerebellum ratio (Fig. 1). Preinjection of GBR12,909, however, significantly decreased the absolute striatal binding (Table 2) and the striatum-to-cerebellum ratio of [^{123}I]nor-β-CIT (Fig. 1).

Besides high binding in the brain, [^{123}I]nor-β-CIT also rapidly accumulated in liver, lung and kidney. For each peripheral organ, except for adipose tissue, binding of the radiotracer declined gradually after 15 min postinjection (Table 1). Injection of fluvoxamine, but not GBR12,909, significantly blocked [^{123}I]nor-β-CIT binding in lung (data not shown). However, neither GBR12,909 nor fluvoxamine blocked [^{123}I]nor-β-CIT binding in the other peripheral organs.

Discussion

In vivo binding of [^{123}I]nor-β-CIT in the hypothalamus of the rat is mediated by the 5-HT transporter since only fluvoxamine, and not GBR12,909, was able to block the binding of the radiotracer. This observation is in line with the results of recent studies. An autoradiographic study performed in human brain showed binding of [^{125}I]nor-β-CIT in the thalamus (a 5-HT-rich brain area) to be fully inhibited by the selective 5-HT reuptake blocker citalopram but not by GBR12,909 [4]. In addition, PET and SPET studies showed that the in vivo binding of radiolabelled nor-β-CIT in 5-HT-rich brain areas of monkey and human brain was significantly reduced by pre-treatment with citalopram [4, 5]. In this study, binding of [^{123}I]nor-β-CIT in the rat hypothalamus was not fully blocked by pre-treatment with a high dose of fluvoxamine. This finding is in contrast to the finding of the above-mentioned autoradiographic study [4] which showed that binding of [^{125}I]nor-β-CIT in the human thalamus was fully inhibited by 1 μ*M* citalopram. However, in in vivo experiments the percentage of blockade is dependent not only on the concentration of the blocker but also, for example, on kinetic differences between the blocker and the radiotracer.

In this study, we showed a high and long-lasting uptake of [^{123}I]nor-β-CIT in the lung of rats, which is in agreement with data obtained in humans [5]. Importantly, pre-treatment with fluvoxamine blocked the [^{123}I]nor-β-CIT binding in the lung significantly, which was expected since the lungs are 5-HT-rich regions [5].

The results of the present study show that the ratio of hypothalamus-to-cerebellum binding of [^{123}I]nor-β-CIT in rat brain is approximately 5.1 at 6 h post-injection. This ratio is much higher than that recently reported for [^{123}I]FP-CIT in rat brain (hypothalamus-to-cerebellum ratio of approximately 2.0 at 2 h post-injection [12]). Interestingly, Hiltunen and co-workers (1998) compared the in vivo binding of [^{123}I]nor-β-CIT and [^{123}I]β-CIT to central 5-HT transporters in humans. They showed that specific [^{123}I]nor-β-CIT binding at the level of the midbrain (a 5-HT transporter-rich region) was 33% higher compared with that of [^{123}I]β-CIT. So, [^{123}I]nor-β-CIT, in comparison with the cocaine analogues [^{123}I]β-CIT and [^{123}I]FP-CIT, might be the tracer with the greatest potential for visualisation of 5-HT transporters with SPET. However, it may be of interest to examine in future studies whether, apart from higher specific binding, there are additional advantages of [^{123}I]nor-β-CIT over other cocaine analogues for the visualisation of 5-HT transporters with SPET.

In addition to cocaine analogues, [^{123}I]nitroquipazine has been studied as a potentially useful SPET ligand for visualisation of 5-HT transporters. However, a relatively low target-to-non-target ratio (2.3) was observed in primates [13]. Additionally, [^{123}I]nitroquipazine was metabolised rapidly, and a high non-specific binding was observed after injection of the radiotracer [14]. Consequently, [^{123}I]nitroquipazine seems not to be a useful tracer for SPET imaging of 5-HT transporters.

Recent PET and SPET studies performed in monkeys and humans showed relatively high striatal binding of [^{11}C]nor-β-CIT and [^{123}I]nor-β-CIT, respectively [4, 5]. An autoradiographic study performed in human brain also showed high striatal binding of [^{125}I]nor-β-CIT [4], which is in line with the observation that nor-β-CIT showed not only high affinity for the 5-HT transporter (IC_{50}=0.36 n*M*) but also for the dopamine transporter (IC_{50}=0.69 n*M*) [3]. In addition, Bergström and co-workers (1997) demonstrated that striatal [^{125}I]nor-β-CIT binding is due to binding to the dopamine transporter since pre-treatment with GBR12,909 did significantly block the in vitro striatal binding. As anticipated by the assumption that the concentration of striatal dopamine transporters in rats is approximately tenfold higher than the concentration of striatal 5-HT transporters [15], the

results of the present study show that the in vivo [^{123}I]nor-β-CIT binding in rat striatum is predominantly mediated by the dopamine transporter since GBR12,909, but not fluvoxamine, significantly blocked striatal binding of the radiotracer. Moreover, this result suggests that [^{123}I]nor-β-CIT is not a selective in vivo tracer for the 5-HT transporter.

The present investigation shows that in vivo binding of [^{123}I]nor-β-CIT is higher in the striatum than in the hypothalamus, which is in line with results of other studies [4, 5]. Although [^{123}I]nor-β-CIT shows a high target-to-non-target ratio, future studies should focus on the development and evaluation of radiotracers for the 5-HT transporter that show even higher in vivo selectivity for this transporter. It is also of interest to determine whether radioactive metabolites of newly developed radiotracers for the 5-HT transporter pass the blood-brain barrier and have affinity to monoamine transporters.

In conclusion, the results of this study indicate that [^{123}I]nor-β-CIT, although not being a selective radioligand, binds in vivo specifically to 5-HT transporters in the hypothalamus (a brain area known to be rich in 5-HT transporters) and thus suggest that [^{123}I]nor-β-CIT promises to be a suitable radioligand for SPET imaging of 5-HT transporters in humans.

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