Short Communication

Comparative In Vivo Study of Iodine-123-Labeled β-CIT and nor-β-CIT Binding to Serotonin Transporters in Rat Brain

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ABSTRACT Both iodine-123-labeled β-CIT (β-carbomethoxy-3β-(4-iodophenyl)tropane) and nor-β-CIT (β-carbomethoxy-3β-(4-iodophenyl)nortropane) have shown to be suitable radioligands for imaging serotonin (5-HT) transporters. [123I]nor-β-CIT has the highest in vitro affinity for 5-HT transporters among β-CIT analogs reported so far. However, no direct comparison-studies of these two radiotracers as to their in vivo binding to 5-HT transporters have been reported so far. Therefore, it is still unclear which of the two radiotracers is more suitable for single photon emission computed tomography (SPECT) imaging of 5-HT transporters. The purpose of this study was to compare directly in a controlled design the in vivo [123I]β-CIT and [123I]nor-β-CIT binding to 5-HT transporters under the same conditions in rats with the focus on brain kinetic characteristics by means of a two-compartment analysis. We observed that [123I]β-CIT has a higher binding potential and faster kinetics for 5-HT transporters than [123I]nor-β-CIT, suggesting that [123I]β-CIT may be a more suitable radioligand than [123I]nor-β-CIT for imaging 5-HT transporters with SPECT. Synapse 34:77–80, 1999.

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 etiology of depression and other neuropsychiatric disorders. Reduction in the number of central 5-HT neurons has been reported in postmortem studies performed in patients with Alzheimer’s and Parkinson’s disease, whereas recent studies suggested neurotoxic effects on 5-HT neurons of the widely used recreational drug Ecstasy (3,4-methylenedioxymethamphetamine; MDMA), and of the anorectic drug dexfenfluramine. Visualization and quantification of 5-HT neurons in the living human brain using positron emission tomography (PET) or single photon emission computed tomography (SPECT) could potentially allow the detection of degeneration of 5-HT neurons. The 5-HT transporter is considered a reliable marker of 5-HT neurons. The plasma membrane 5-HT transporter is located on the presynaptic 5-HT terminal.

In recent years, extensive efforts have led to the development of suitable radioligands for imaging the serotonin (5-HT) transporter. One of these radioligands is iodine-2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT), which can be used simultaneously for 5-HT and dopamine (DA) transporter imaging in the living human brain with single photon emission computed...
tomography (SPECT) (Kuikka et al., 1993, 1995; Brücke et al., 1993; Bergström et al., 1994). Recent studies confirmed differential kinetics of $^{[123I]}\beta$-CIT binding to 5-HT and DA transporters. The hypothalamus contains much more 5-HT than DA transporters (Kuhr et al., 1972), whereas the striatum contains much more DA transporters than 5-HT transporters (Innis et al., 1993). Therefore, assessment of the binding to both transporters can be performed in one series of experiments (Laruelle et al., 1993; Fujita et al., 1996).

Another suitable radioligand for imaging the 5-HT transporter is nor-$\beta$-CIT (2$\beta$-carbomethoxy-3$\beta$-(4-iodophenyl)nortropane), which is a desmethyl analog of $^{[123I]}\beta$-CIT. $^{[123I]}$nor-$\beta$-CIT has the highest in vitro affinity for 5-HT transporters among the $\beta$-CIT analogs reported so far. The in vitro affinity of $^{[123I]}$nor-$\beta$-CIT is tenfold higher ($IC_{50} = 0.36$ nM) when compared with $^{[123I]}$\beta-CIT ($IC_{50} = 4.2$ nM) (Boja et al., 1994). However, no direct comparison studies of these two radiotracers as to their in vivo binding to 5-HT transporters have been reported so far. In addition, the kinetics of $^{[123I]}$nor-$\beta$-CIT has not been studied extensively. Therefore, it is still unclear which of the two radiotracers is more suitable for SPECT imaging of 5-HT transporters. The purpose of this study was to directly compare in a controlled design the in vivo $^{[123I]}$\beta-CIT and $^{[123I]}$nor-$\beta$-CIT binding to 5-HT transporters under the same conditions in rats, with the focus on brain kinetic characteristics by means of a two-compartment analysis. In addition, in vivo binding to DA transporters was studied.

Fig. 1. Time–activity curves for specific and nonspecific brain binding of $^{[123I]}\beta$-CIT and $^{[123I]}$nor-$\beta$-CIT (24 h data not shown) in hypothalamus (A), striatum (B), and cerebellum (C). Regional activities were normalized to injected dose multiplied by the body weight per gram tissue (%ID \times kg/g tissue). Binding ratios of $^{[123I]}\beta$-CIT and $^{[123I]}$nor-$\beta$-CIT (24 h data not shown) in hypothalamus (D) and striatum (E), as expressed as hypothalamus-to-cerebellum and striatum-to-cerebellum, respectively (mean ± SE of three rats).
**MATERIALS AND METHODS**

Groups of male Wistar rats (n = 3) were intravenously injected with either approximately 1.85 MBq \[^{123}\text{I}]\text{nor-}\beta\text{-CIT}\) or \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) and sacrificed at several timepoints after tracer injection (up to 24 h pi), as described previously (Booij et al., 1998). The brains were quickly removed, dissected into cerebral cortex, striatum, thalamus, hypothalamus, hippocampus, and cerebellum, and weighed. The radioactivity of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) in each region was measured and radioactivity concentrations were expressed as percent injected dose, multiplied by the body weight per gram tissue weight (%ID × kg/g tissue), as described previously (Rijks et al., 1996). For both radioligands, the cerebellum was used as a reference region for the estimation of free and nonspecifically bound radioligand. The hypothalamus was chosen as an area of binding of the 5-HT transporter, whereas the striatum was chosen as an area of binding of the DA transporter. The specific binding at each timepoint was estimated by subtraction of radioactivity in cerebellum from total radioactivity in the region of interest.

**RESULTS**

Figure 1 shows the time-course of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) and \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) specific and nonspecific binding and binding ratios. \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) showed higher specific binding in the hypothalamus than \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\). However, the kinetics of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) seem to be faster in this brain area. \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) binding reached a plateau in binding beginning at 1 h after the injection, lasting up to 4 h after injection, whereas \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) reached its peak 2 h after injection (Fig. 1A). Highest specific binding for both tracers was detected in the striatum (Fig. 1B). \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) showed higher specific binding in the striatum than \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\). Moreover, the kinetics of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) in the striatum was faster for \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\). Peak striatal specific binding of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) and \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) was reached approximately 2 h and 3 h after the injection of the radiotracer, respectively (Fig. 1B). In the cerebellum, washout of both tracers was faster than in the hypothalamus and striatum. Nonspecific binding of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) was higher than that of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) (Fig. 1C).

Kinetic differences of the two tracers under study were further clarified by a two-compartment analysis, as previously described by Fujita et al. (1996) (Fig. 2). The rate constants \(k_3\) and \(k_4\) were estimated by the least squares method (Table I). With regard to the estimated \(k_3\) values, both tracers showed the highest and lowest values in striatum and hippocampus, respectively. With regard to estimated \(k_4\) values, for both tracers the occipital cortex showed the highest values. Lowest \(k_4\) values were calculated for \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) in the hypothalamus and in the striatum for \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\). With the exception of the frontal cortex, the binding potential (BP; unitless, \(k_3/k_4\)), was overall higher for \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) than for \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\), although statistical significance was reached only in the striatum (Table I).

In this study, binding in the hypothalamus and striatum was assumed to reflect binding to 5-HT and DA transporters, respectively. This assumption is based on results of recent blocking and displacement studies performed in rats, which showed that in vivo binding of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) in the hypothalamus and striatum is mediated by 5-HT and DA transporters, respectively (Fujita et al., 1996; Laruelle et al., 1993; Booij et al., 1998).

**DISCUSSION**

In this study, we showed that the beginning of peak specific binding of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) in the hypothalamus and striatum started 1 h and 3 h after injection of the radiotracer, respectively. The differential kinetics of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) binding to 5-HT and DA transporters was clarified by the two-compartment kinetic analyses. This analysis revealed that \(k_3\) in the striatum was almost twice \(k_3\) in the hypothalamus, which is in line with the results reported by Fujita et al. (1996). They suggested that this differential kinetic reflects the differential kinetics of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) binding to 5-HT and DA transporters. In this study, peak specific binding and peak in binding ratios (Fig. 1D,E) of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) was approximately at the same timepoint in the hypothalamus as in the striatum. In line with this finding, we previously reported hypothalamus- and striatum-to-cerebellum ratios to both be highest 6 h after injection of \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) (Booij et al., 1998). However, estimated \(k_3\) values, as obtained in the present study, in the striatum were almost three times \(k_3\) values in the hypothalamus. Finding different \(k_3\) values in the striatum and hypothalamus suggests that \[^{123}\text{I}]\text{[123I]nor-}\beta\text{-CIT}\) displays different binding kinetics to 5-HT and DA transporters.
Interestingly, Hiltunen et al. (1998) recently reported different timepoints of the peak ratio in specific [123I]nor-
-β-CIT binding in the human hypothalamus and striatum (4 and 21 h p.i., respectively), which support differences in kinetics of [123I]nor-β-CIT binding to 5-HT and DA transporters. Considering that the calculation of $k_3$ values is more “reliable” than the calculation of peak specific binding, we suggest that [123I]nor-β-CIT displays different binding kinetics to 5-HT and DA transporters in rats. Therefore, it may be justified to assess the concentration of 5-HT and DA transporters at different timepoints after injection of [123I]nor-β-CIT (Fujita et al., 1996).

The BP is a frequently used outcome measure for experiments, since the BP corresponds to the product of the site density ($B_{\text{max}}$) and affinity ($1/K_D$, where $K_D$ is the equilibrium dissociation constant of the receptor-ligand complex). In this study, we report a 36% higher binding potential of [123I]β-CIT to the 5-HT transporter than [123I]nor-β-CIT in rat brain. In contrast to our findings, Hiltunen et al. (1998) reported that the ratio of in vivo specific binding of [123I]nor-β-CIT in the human midbrain was 33% higher as compared to [123I]β-CIT. A higher specific [123I]nor-β-CIT binding to 5-HT transporters was also observed in primate brain. The relatively low BP of [123I]nor-β-CIT to 5-HT transporters observed in the present study as compared to the above-mentioned studies may be due to a species difference. Another possibility is that the lower observed BP derives from the model used to estimate kinetic parameters. However, we calculated the estimated kinetics of [123I]β-CIT and [123I]nor-β-CIT with the model described by Fujita et al. (1996). By using this model, we found similar kinetics of [123I]β-CIT binding in rat brain as reported by Fujita, which give support to the observed kinetic characteristics of [123I]nor-β-CIT.

[123I]β-CIT and [123I]nor-β-CIT are both nonselective radiotracers for labeling 5-HT and DA transporters in vivo. Interestingly, they can be used in clinical studies to examine both transporters in one series of examinations, by estimating the binding in the hypothalamus (to 5-HT transporters) and in the striatum (to DA transporters), respectively. In this study, we observed that in rats [123I]β-CIT has a higher BP than [123I]nor-β-

CIT for both transporters. This finding suggests that [123I]β-CIT may be a more suitable radioligand than [123I]nor-β-CIT for imaging both monoamine transporters. Future comparative studies in humans will be needed to examine whether [123I]β-CIT is indeed a more suitable radioligand than [123I]nor-β-CIT for imaging of 5-HT transporters with SPECT.

### REFERENCES


### TABLE I. Kinetics and volumes of distribution of [123I]nor-β-CIT and [123I]β-CIT in individual brain regions

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Frontal cortex</th>
<th>Occipital cortex</th>
<th>Striatum</th>
<th>Thalamus</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_3$ (min$^{-1}$)</td>
<td>0.056 ± 0.013</td>
<td>0.038 ± 0.012</td>
<td>0.077 ± 0.005</td>
<td>0.046 ± 0.007</td>
<td>0.027 ± 0.006</td>
<td>0.016 ± 0.002</td>
</tr>
<tr>
<td>$k_4$ (min$^{-1}$)</td>
<td>0.035 ± 0.006</td>
<td>0.046 ± 0.013</td>
<td>0.036 ± 0.008</td>
<td>0.023 ± 0.002</td>
<td>0.015 ± 0.003</td>
<td>0.016 ± 0.016</td>
</tr>
<tr>
<td>BP (unitless)</td>
<td>1.56 ± 0.07</td>
<td>0.82 ± 0.06</td>
<td>2.14 ± 0.18</td>
<td>2.04 ± 0.10</td>
<td>1.78 ± 0.11</td>
<td>1.64 ± 0.05</td>
</tr>
<tr>
<td>$k_3$ (min$^{-1}$)</td>
<td>0.010 ± 0.004</td>
<td>0.011 ± 0.003</td>
<td>0.038 ± 0.011</td>
<td>0.0093 ± 0.002</td>
<td>0.022 ± 0.009</td>
<td>0.0091 ± 0.002</td>
</tr>
<tr>
<td>$k_4$ (min$^{-1}$)</td>
<td>0.008± ± 0.004</td>
<td>0.011 ± 0.001</td>
<td>0.003 ± 0.001</td>
<td>0.0043 ± 0.001</td>
<td>0.0099 ± 0.001</td>
<td>0.0062 ± 0.001</td>
</tr>
<tr>
<td>BP</td>
<td>1.19 ± 0.03</td>
<td>1.03 ± 0.10</td>
<td>12.41 ± 2.55</td>
<td>2.15 ± 0.58</td>
<td>2.42 ± 0.92</td>
<td>1.48 ± 0.24</td>
</tr>
</tbody>
</table>

1Data are expressed in mean ± SE values.
2Statistical significant difference in binding potential (BP) between [123I]nor-β-CIT and [123I]β-CIT (unpaired Student’s t-test; $P < 0.01$).