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 (2β-carbomethoxy-3β-(4-iodophenyl)tropane) and nor-β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)nortropane) have shown to be suitable radioligands for imaging serotonin (5-HT) transporters. [^{123}I]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 emisison computed tomograpy (SPECT) imaging of 5-HT transporters. The purpose of this study was to compare directly in a controlled design the in vivo [^{123}I]β-CIT and [^{123}I]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 [^{123}I]β-CIT has a higher binding potential and faster kinetics for 5-HT transporters than [^{123}I]nor-β-CIT, suggesting that [^{123}I]β-CIT may be a more suitable radioligand than [^{123}I]nor-β-CIT for imaging 5-HT transporters with SPECT. **Synapse 34:77–80, 1999.** © 1999 Wiley-Liss, Inc.

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

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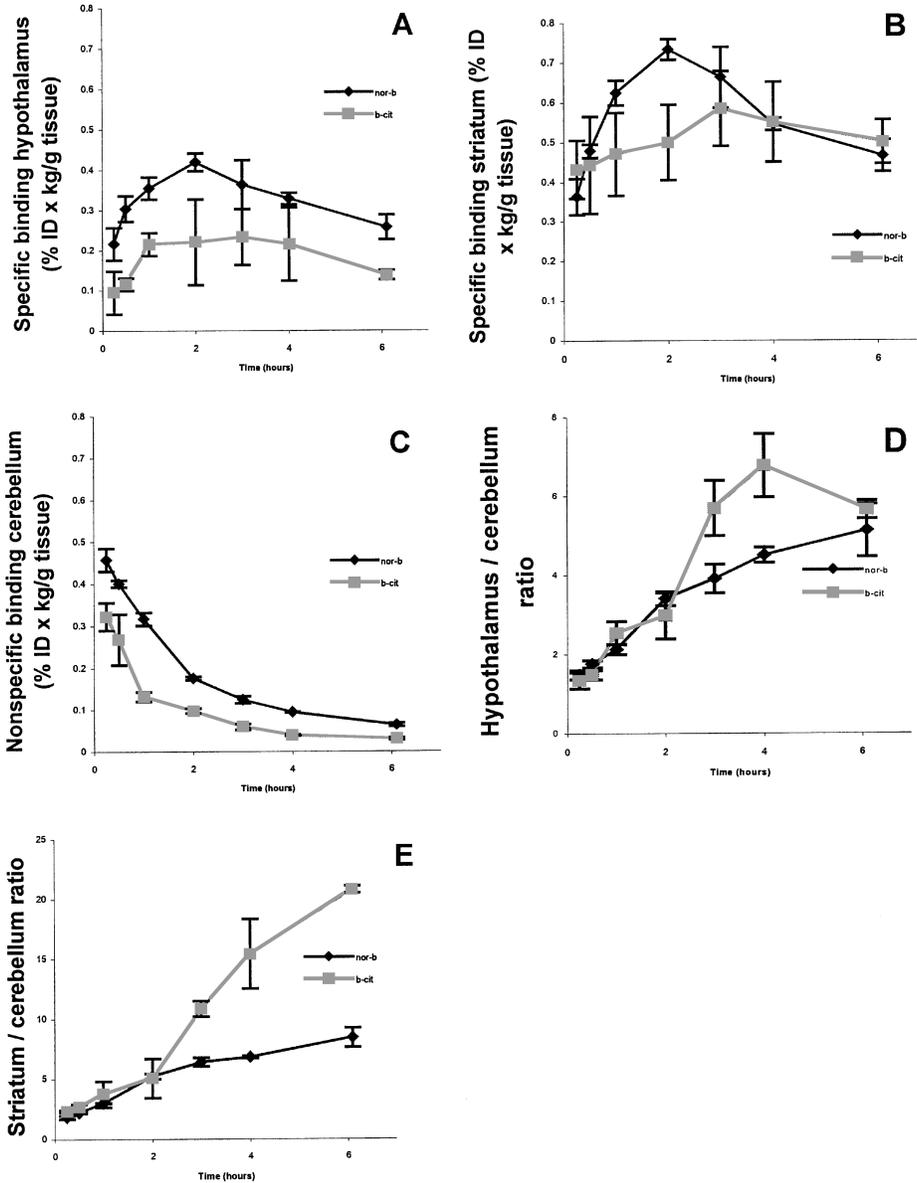
Fig. 1.   Time–activity curves for specific and nonspecific brain binding of [^{123}I]β-CIT and [^{123}I]nor-β-CIT (24 h data not shown) in hypothalamus (**A**), striatum (**B**), and cerebellum (**C**). Regional activities were normalized to injected dose multiplied by the body weight per gram tissue (%ID × kg/g tissue). Binding ratios of [^{123}I]β-CIT and [^{123}I]nor-β-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).

tomography (SPECT) (Kuikka et al., 1993, 1995; Brücke et al., 1993; Bergström et al., 1994). Recent studies confirmed differential kinetics of [^{123}I]β-CIT binding to 5-HT and DA transporters. The hypothalamus contains much more 5-HT than DA transporters (Kuhar 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-β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)nortropane), which is a desmethyl analog of [^{123}I]β-CIT. [^{123}I]nor-β-CIT has the highest in vitro affinity for 5-HT transporters among the β-CIT analogs

reported so far. The in vitro affinity of [^{123}I]nor-β-CIT is tenfold higher (IC$_{50}$ = 0.36 n*M*) when compared with [^{123}I]β-CIT (IC$_{50}$= 4.2 n*M*) (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 [^{123}I]nor-β-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 [^{123}I]β-CIT and [^{123}I]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. In addition, in vivo binding to DA transporters was studied.

MATERIALS AND METHODS

Groups of male Wistar rats (n = 3) were intravenously injected with either approximately 1.85 MBq [123I]nor-β-CIT or [123I]β-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 [123I]nor-β-CIT or [123I]β-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 [123I]nor-β-CIT and [123I]β-CIT specific and nonspecific binding and binding ratios. [123I]nor-β-CIT showed higher specific binding in the hypothalamus than [123I]β-CIT. However, the kinetics of [123I]β-CIT seem to be faster in this brain area. [123I]β-CIT binding reached a plateau in binding beginning at 1 h after the injection, lasting up to 4 h after injection, whereas [123I]nor-β-CIT reached its peak 2 h after injection (Fig. 1A). Highest specific binding for both tracers was detected in the striatum (Fig. 1B). [123I]nor-β-CIT showed higher specific binding in the striatum than [123I]β-CIT. Moreover, the kinetics of [123I]nor-β-CIT in the striatum was faster than for [123I]β-CIT. Peak striatal specific binding of [123I]nor-β-CIT and [123I]β-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 [123I]nor-β-CIT was higher than that of [123I]β-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 [123I]nor-β-CIT in the hypothalamus and in the striatum for [123I]β-CIT. With the exception of the frontal cortex, the binding
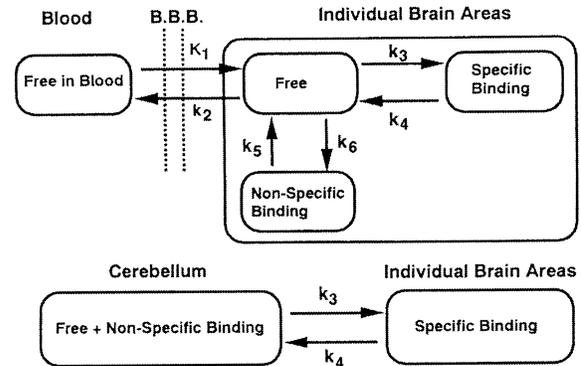

Fig. 2. Two-compartment model to determine the forward (k_3) and dissociation (k_4) rate constants of [123I]β-CIT and [123I]nor-β-CIT in individual brain regions in vivo. The cerebellum was used as a reference region for the estimation of free and nonspecifically bound radioligand. Specific binding in individual brain regions was determined by subtracting the radioactivity in the cerebellum.

potential (BP; unitless, k_3/k_4), was overall higher for [123I]β-CIT than for [123I]nor-β-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 [123I]β-CIT and [123I]nor-β-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 [123I]β-CIT in the hypothalamus and striatum started 1 h and 3 h after injection of the radiotracer, respectively. The differential kinetics of [123I]β-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 [123I]β-CIT binding to 5-HT and DA transporters. In this study, peak specific binding and peak in binding ratios (Fig. 1D,E) of [123I]nor-β-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 [123I]nor-β-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 [123I]nor-β-CIT displays different binding kinetics to 5-HT and DA transporters.

TABLE I. Kinetics and volumes of distribution of [123I]nor-β-CIT and [123I]β-CIT in individual brain regions[1]

	Brain region					
	Frontal cortex	Occipital cortex	Striatum	Thalamus	Hypothalamus	Hippocampus
[123I]Nor-β-CIT						
k_3 (min^{-1})	0.056 ± 0.013	0.038 ± 0.012	0.077 ± 0.005	0.046 ± 0.007	0.027 ± 0.006	0.016 ± 0.002
k_4 (min^{-1})	0.035 ± 0.006	0.046 ± 0.013	0.036 ± 0.008	0.023 ± 0.002	0.015 ± 0.003	0.016 ± 0.016
BP (unitless)	1.58 ± 0.07	0.82 ± 0.06	2.14 ± 0.18	2.04 ± 0.10	1.78 ± 0.11	1.04 ± 0.05
[123I]β-CIT						
k_3 (min^{-1})	0.010 ± 0.004	0.011 ± 0.003	0.038 ± 0.011	0.0093 ± 0.002	0.022 ± 0.009	0.0091 ± 0.002
k_4 (min^{-1})	0.0081 ± 0.004	0.011 ± 0.001	0.003 ± 0.001	0.0043 ± 0.001	0.0090 ± 0.001	0.0062 ± 0.001
BP	1.19 ± 0.03	1.03 ± 0.10	12.41 ± 2.55[2]	2.15 ± 0.58	2.42 ± 0.92	1.48 ± 0.24

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

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_{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.

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