Comparative evaluation of two serotonin transporter ligands in the human brain: [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine

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Abstract. Serotonin (5-HT) is considered to be an important transmitter underlying mood and behaviour. Abnormalities of the 5-HT transporter have been suggested in mood disorders, since it is one of the major binding sites of antidepressants. A number of ligands have been developed to visualise the 5-HT transporter in vivo, but only a few have successfully visualised specific binding in vivo. In this study, we comparatively evaluated two ligands for 5-HT transporter, [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine, in the human brain. Brain uptake of [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine was measured with PET in 15 healthy volunteers. Second PET scans were performed after pretreatment with the potent 5-HT reuptake inhibitor clomipramine. Data were analysed as regional brain uptake as well as whole brain uptake. In six healthy volunteers uptake of the two ligands was also measured in the lung since it is one of the high-uptake organs in the body. In the brain, high accumulation was observed in the thalamus and striatum, the regions known to contain high densities of 5-HT transporter, for both [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine. The average ratio of thalamus to cerebellum uptake at 90 min after the tracer injection was approximately 1.6 for [^{11}C](+)McN5652 and 1.7 for [^{11}C]cyanoimipramine, while the ratios obtained after pretreatment with clomipramine were approximately 1.2. However, the whole brain uptake of [^{11}C](+)McN5652 was approximately twice that of [^{11}C]cyanoimipramine, while the lung uptake of [^{11}C](+)McN5652 was approximately half that of [^{11}C]cyanoimipramine. Both [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine showed sufficient specific binding for performance of a quantitative analysis in the brain. [^{11}C](+)McN5652 could be superior because of its higher distribution to the brain.

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Introduction

The serotonin (5-HT) system plays an important role in mood and behaviour. Abnormalities of serotonergic transmission have been suggested in mood disorders and several psychiatric disorders [1]. 5-HT transporter has been reported to be one of the major binding sites for a number of antidepressants, especially selective serotonin reuptake inhibitors (SSRIs) [2, 3]. Several SSRIs, such as fluoxetine, paroxetine and citalopram, have been labelled with carbon-11 or fluorine-18 to visualise the 5-HT transporter in vivo [4, 5, 6, 7, 8]. However, most ligands were not suitable for visualisation of the 5-HT transporter owing to difficulties in evaluating their specific binding in vivo. Recently a series of *N*-methyl-2-(arylthio)benzylamine analogues were synthesised and radiolabelled with ^{11}C for 5-HT transporter ligand [9, 10]. Some of these compounds showed nanomolar affinity for 5-HT transporter and one of them, [^{11}C]DASB ([^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulphanyl)-benzonitrile), gave promising results in human studies [11]. [^{11}C]cyanoimipramine was developed in the late 1980s [12]. Although Suhara et al. have reported the lung uptake with the ligand, it has not been evaluated thoroughly as a 5-HT transporter ligand in the brain [13]. [^{11}C](+)McN5652 is a selective 5-HT reuptake inhibitor that has nanomolar potency for 5-HT transporter and is currently being used as a positron emission tomography (PET) tracer for 5-HT transporter [14, 15, 16, 17, 18, 19, 20].

The aim of this study was to compare the two ^{11}C-labelled compounds, [^{11}C](+)McN5652 and [^{11}C]cyanoim-

ipramine, as brain 5-HT transporter ligands, and to examine their characteristics.

Materials and methods

Subjects

Twenty-one healthy male volunteers (age 19–34 years; mean±SD, 22.9±4.0 years) participated in this study. Fifteen subjects were included in the brain uptake study and six in the lung uptake study. All subjects were recruited from the university campus, were well screened, and had no history of present or past psychiatric, neurological or somatic disorders. They also had no alcohol- or drug-related problems. They had not taken any kind of medication for at least the 2 weeks prior to the start of the study. The study was approved by the ethics and radiation safety committees of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from each subject.

Radioligands

[^{11}C](+)McN5652 was synthesised by S-methylation of the corresponding desmethyl precursor with [^{11}C]iodomethane by using an automated synthesis module [15]. The desmethyl precursor was prepared by demethylation of non-radioactive McN5652 and successive stabilisation by the addition of a protecting agent for the SH group, dithiothreitol (DTT), into the reaction medium immediately after the reaction. The radiochemical purity was higher than 95% and the specific radioactivity was more than 40 TBq/mmol at the time of injection in all PET scans except one lung scan, in which the specific radioactivity was 8.8 TBq/mmol at the time of injection. [^{11}C](+)McN5652 was injected intravenously at a dose of 179–759 MBq (mean±SD, 581±200 MBq).

[^{11}C]cyanoimipramine was labelled with [^{11}C]CH_3I by N-methylation reaction on *N*-desmethyl-cyanoimipramine (supplied by F Hoffman-La Roche Ltd., Basel, Switzerland) [13]. The radiochemical purity was higher than 95% and the specific radioactivity was more than 37 TBq/mmol at the time of injection. [^{11}C]cyanoimipramine was injected intravenously at a dose of 360–721 MBq (mean±SD, 592±124 MBq).

PET study

Brain uptake study

Nine subjects took part in the [^{11}C](+)McN5652 study and six subjects in the [^{11}C]cyanoimipramine study. The brain PET scans were carried out with an ECAT 47 (CTI-Siemens, Knoxville, Tenn., USA) scanner, which provides 47 planes and a field of view of 16.2 cm. A head fixation device with an individual mouthpiece was used during the brain scans (Fixster Instruments, Stockholm, Sweden). A 10-min transmission scan was performed to correct for attenuation. Dynamic PET scans with [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine were carried out for 90 min (2 min ×15, 4 min ×5, 8 min ×5) in 2D mode. Because of the limited availability of technical staff, arterial blood samples were taken only from three of the nine subjects in the [^{11}C](+)McN5652 study.

Pretreatment with the 5-HT reuptake inhibitor clomipramine without arterial blood sampling. Three of the nine subjects with [^{11}C](+)McN5652 and three of the six subjects with [^{11}C]cyanoimipramine were studied again after the administration of the potent 5-HT transporter inhibitor clomipramine. Each subject was orally administered 50 mg of clomipramine 5 h before the PET scan, as the peak plasma level has been reported to occur 2–4 h after a single oral dose [21].

Brain uptake study with arterial blood sampling. Three of the nine subjects with [^{11}C](+)McN5652 underwent serial arterial blood sampling. Arterial blood samples were taken ten times during the initial 3 min after tracer injection, then eight times during the next 17 min, and then once every 10 min until the end of the scan. Metabolite analysis was carried out at ten time points during the scans. The subjects then underwent second scans 5 h after being pretreated with 10 mg, 25 mg or 50 mg of clomipramine, again with arterial blood sampling.

Metabolite analysis. The supernatant obtained by centrifuging the plasma fraction with acetonitrile was analysed by radio-HPLC analysis (column, μBondapak C18; mobile phase, 50/50 acetonitrile/0.1 *M* ammonium formate).

Data analysis of the brain uptake study. Brain scans were reconstructed with a Ramp filter cut-off frequency of 0.5. The full-width at half-maximum (FWHM) in the transaxial direction was 6.0 mm at the centre and 6.7 mm at 10 cm offset from the centre, which was determined with a line source. With reference to a brain atlas and individual magnetic resonance imaging, circular (10 mm diameter) regions of interests (ROIs) were drawn manually on transverse slices of summed images in the following areas: cerebellum, striatum, frontal cortex and thalamus. The whole brain volume of interest was drawn manually on all 47 slices including the cerebellum and cerebrum. Regional radioactivity was calculated for each frame, corrected for decay and plotted against time. The injected radioactivity was normalised to 370 MBq. The whole brain uptake was expressed as the average radioactivity (kBq/ml) multiplied by the whole brain volume of interest. The average radioactivity of the whole brain uptake was compared between [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine using the areas under the time activity curves from 0 to 90 min.

The distribution volumes of the cerebellum, frontal cortex, thalamus and striatum were calculated using metabolite-corrected arterial plasma data [22]. Kinetic analysis was performed based on the two-compartment model. The two-compartment configuration consisted of the metabolite-corrected arterial plasma compartment (C_a) and one tissue compartment (C_t) that included the ligand free and non-specifically bound and specifically bound compartments. The distribution volume (DV) (ml of plasma/g of tissue) was defined as the ratio of the unidirectional forward rate constant K_1 (ml g^{-1} min^{-1}) and backward rate constant k_2 (min^{-1}) between C_a and C_t:

$$\mathrm{DV} = \frac{K_1}{k_2}$$

The percent changes of distribution volumes were calculated as follows:

$$\%\mathrm{change} = 100 \times (\mathrm{DV_d} - \mathrm{DV_c}) / \mathrm{DV_c}$$

where DV_d is the distribution volume with clomipramine and DV_c the distribution volume without clomipramine.

Lung uptake study

Three subjects each took part in the [^{11}C](+)McN5652 study and the [^{11}C]cyanoimipramine study. PET scans for the lung were carried out with an ECAT 47 scanner, except for one [^{11}C](+)-McN5652 lung scan done with an ECAT EXACT HR+ (CTI-Sie-

mens, Knoxville, TN, USA); the latter provides 63 planes, a field of view of 15.5 cm and an FWHM of approximately 5.2 mm. After a 10-min transmission scan, radioactivity was measured for 40 min (10 s ×18, 20 s ×9, 30 s ×10, 60 s ×23, 120 s ×3) in 2D mode without cardiac or respiratory gating. During the scans, the subjects' arms were placed outside the imaging field. Lung scans were reconstructed with a Ramp filter cut-off frequency of 0.5. Thoracic volumes of interests were delineated on the boundaries of bilateral lungs on all slices. The injected radioactivity was normalised to 370 MBq. Lung uptake was calculated as the average radioactivity (kBq/ml) of the measured volumes of interest. Lung uptake was compared by the areas under the lung uptake curves from 0 to 40 min.

Whole-body scan

Whole-body scans were performed in the six subjects after the lung scans. Five minutes after the dynamic scans of the lung, radioactivity was measured for 30 min, starting from the head segment, requiring 5 min for each 16.2-cm segment. No transmission scans were done to correct for attenuation.

In vitro binding

The characteristics of in vitro binding of (+)McN5652 and cyanoimipramine were investigated in comparison with clomipramine and (-)McN5652, the pharmacologically inactive enantiomer [14, 20].

The cerebral cortex from male Sprague-Dawley rats (250–300 g) was homogenised and centrifuged at 45,000 × *g* for 10 min at 4°C. Pellets were suspended in the same buffer and recentrifuged [23]. Aliquots of membrane suspension were incubated with [^{3}H]citalopram (NEN Life Science Products, Inc., Boston, Mass., USA) at 22°C in a final volume of 1 ml for 90 min. The homogenates were rapidly filtered through Whatman GF/B filters pretreated with 0.5% polyethyleneimine. The filters were washed with three 4-ml aliquots of ice-cold buffer. The radioactivity trapped by the filters was measured with a liquid scintillation counter (Aloka, Japan). Non-specific binding was estimated in the presence of 1 μ*M* 6-nitroquipazine. The values of the dissociation constant (K_d), and K_i (K_i=IC_{50}/(1+[L]/K_d), where [L] is the concentration of [^{3}H]citalopram used and IC_{50} the concentration that results in 50% inhibition of specific binding, were calculated using the EBDA and LIGAND programs (Biosoft, Cambridge, UK).

Chemical characteristics

pKa measurement

The pKa values of cyanoimipramine and (+)McN5652 were estimated by the gradual addition of NaOH into the ligand solutions to be neutralised [24]. This part of the study was conducted by the Toray Research Center, Inc. (Otsu, Japan).

LogP measurement

The logP values were measured using a standard shake flask method. The sample (McN5652, 3.5 mg; cyanoimipramine, 2.3 mg) was well shaken with a mixture of 1-octanol (2.5 ml) and phosphate buffer (2.5 ml, pH=7.4) for 30 min at 23°±1°C. After centrifugation (3,000 rpm × 10 min) of the mixture, the two layers were separated and the concentration of partitioned substance in each layer was quantified by HPLC (CAPCELL PAK C_{18}UG80, 4.6 mm × 250 mm, SHISEIDO; MeOH/H_2O/triethylamine= 90/10/0.05). The mean logP values were determined after three separate determinations.

Results

Brain uptake study

The uptake of both [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine was highest in the thalamus and striatum, lower

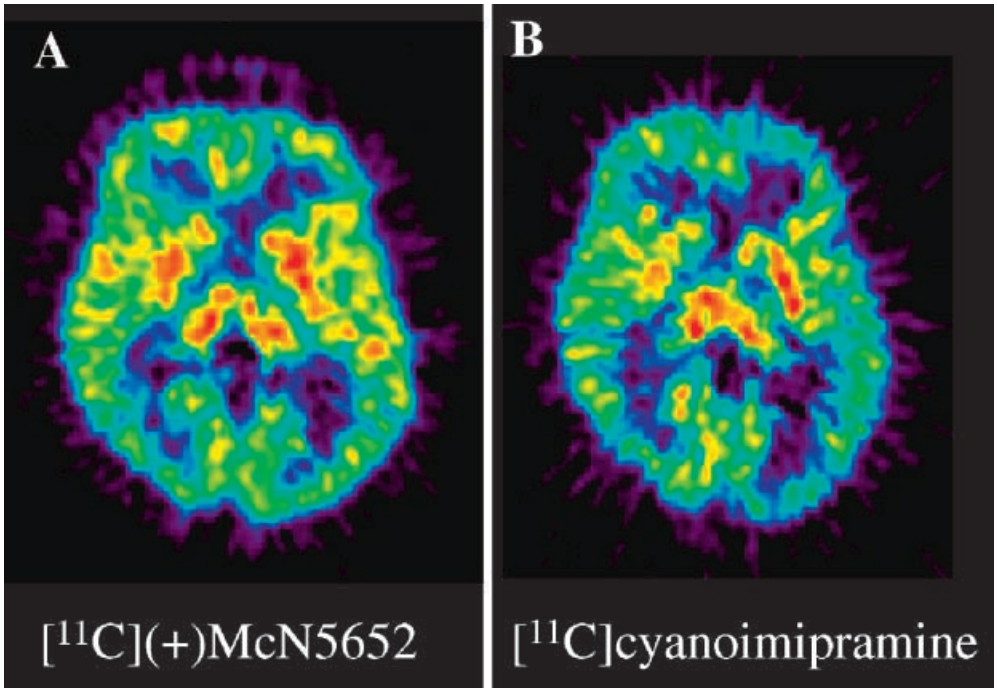

Fig. 1. The summation images of [^{11}C](+)McN5652 (**A**) and [^{11}C]cyanoimipramine (**B**) for 90 min. High uptake of both ligands was seen in the thalamus and striatum

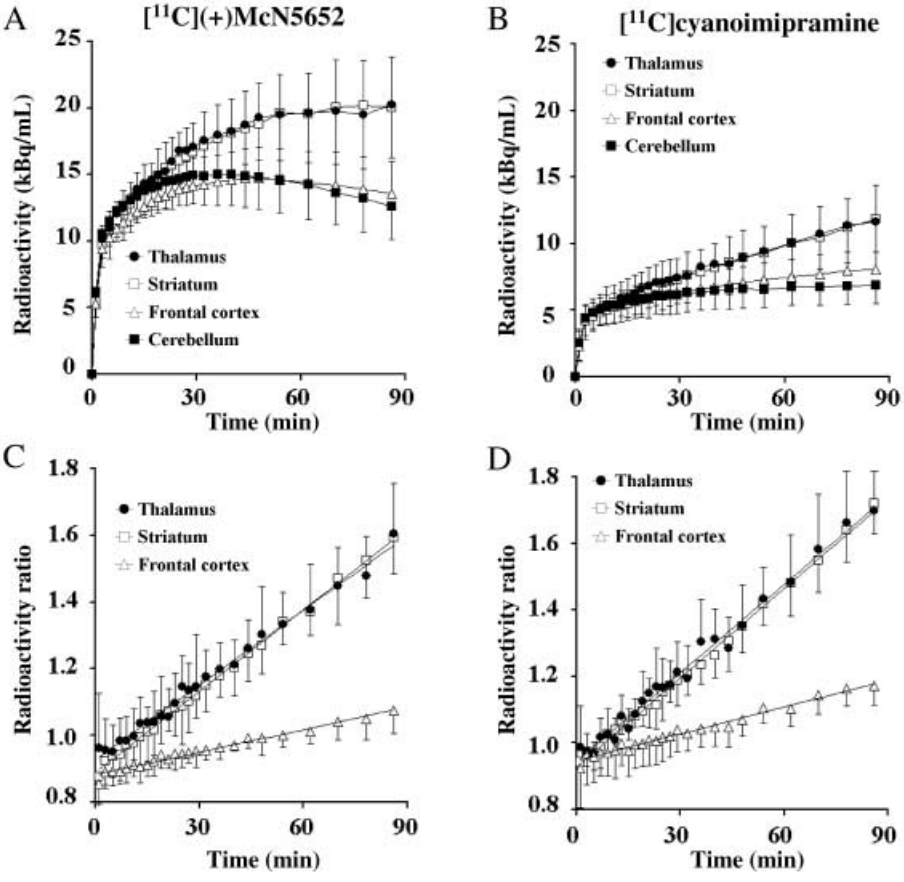

Fig. 2. A, B Average time-activity curves of [11C](+)McN5652 (**A**) and [11C]cyanoimipramine (**B**) in various brain regions. Each curve is based on the results in six subjects. Error bars indicate 1SD. The radioactivity was corrected for decay and the injected dose was adjusted to 370 MBq. **C, D** Time course of the region-to-cerebellum ratios for [11C](+)McN5652 (**C**) and [11C]cyanoimipramine (**D**). Again, each curve is based on findings in six subjects, and error bars indicate 1SD. The ratios in the three regions increased during the scan for both ligands

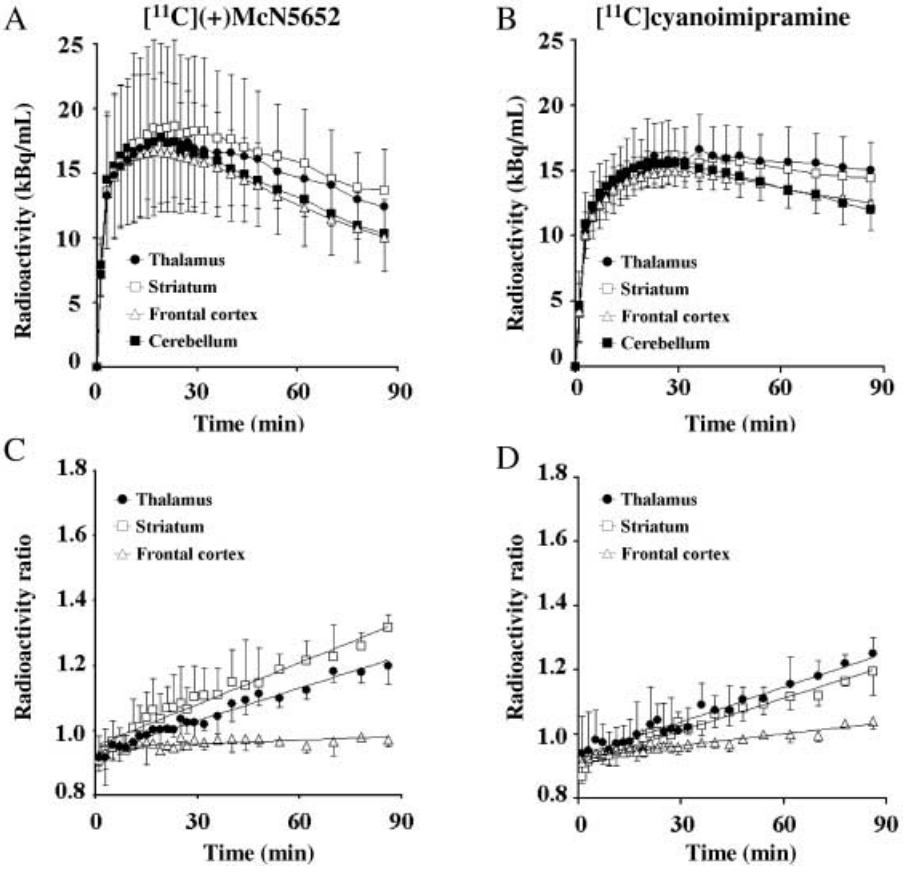

Fig. 3. A, B Average time-activity curves of [11C](+)McN5652 (**A**) and [11C]cyanoimipramine (**B**) after pretreatment with 50 mg of clomipramine. Each curve is based on the results in three subjects. Error bars indicate 1SD. The radioactivity was corrected for decay and the injected dose was adjusted to 370 MBq. **C, D** Time course of the region-to-cerebellum ratios for [11C](+)McN5652 (**C**) and [11C]cyanoimipramine (**D**) after pretreatment with 50 mg of clomipramine. Again, each curve is based on findings in three subjects, and error bars indicate 1SD

Table 1. The effect of clomipramine on the distribution volume of [11C](+)McN5652

Region	Dose (mg)	Distribution volume		% change
		Control	Pretreatment with clomipramine	
Cerebellum	10	21.8	21.8	0
	25	21.1	22.9	9
	50	20.3	22.5	11
Frontal cortex	10	22.5	21.5	–4
	25	23.1	22.7	–2
	50	20.1	21.5	7
Thalamus	10	34.5	29.5	–14
	25	37.3	27.0	–27
	50	36.0	25.8	–28
Striatum	10	36.2	30.8	–15
	25	39.0	31.1	–20
	50	36.5	28.8	–21

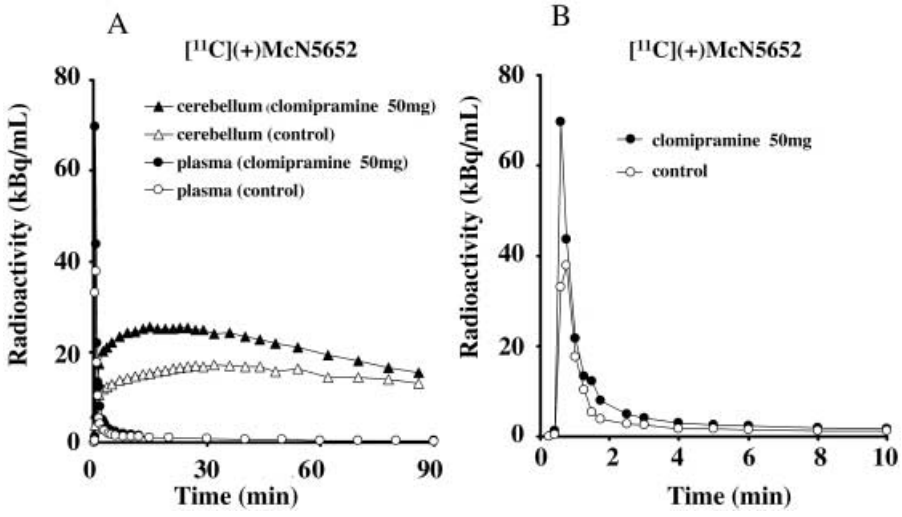

Fig. 4. A Time-activity curves of [11C](+)McN5652 in the cerebellum and metabolite-corrected plasma input with and without pretreatment with clomipramine 50 mg. B Metabolite-corrected plasma input during the initial 10 min with and without pretreatment with 50 mg of clomipramine. The radioactivity was corrected for decay and the injected dose was adjusted to 370 MBq

in the frontal cortex and lowest in the cerebellum (Figs. 1, 2). The uptake of [11C](+)McN5652 in the thalamus and striatum increased up to 60 min and then reached a plateau, while that in the cerebellum and frontal cortex rose until approximately 40 min and then began a moderate decline (Fig. 2A). The uptake of [11C]cyanoimipramine in each region continued to increase during the PET scan (Fig. 2B). The region-to-cerebellum ratios of both ligands were increased in the thalamus and striatum during the PET scan (Fig. 2C, D). The average ratios of the thalamus to cerebellum, the striatum to cerebellum and the frontal cortex to cerebellum at 90 min after the injection were 1.61±0.15, 1.59±0.10 and 1.08±0.07 with [11C](+)McN5652, while those with [11C]cyanoimipramine were 1.70±0.13, 1.72±0.18 and 1.17±0.12. After blocking the 5-HT transporter with clomipramine, the uptake of both ligands in the thalamus and striatum became closer to that in the cerebellum compared with the results in the control study. The radioactivity of both ligands declined from 30 min after the injection in

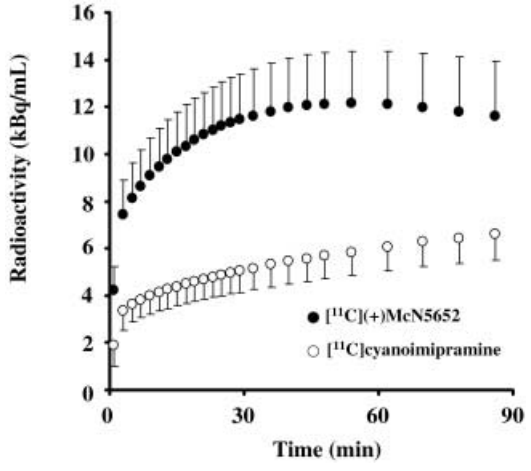

Fig. 5. Time-activity curves of the average radioactivity of the whole brain ROIs for [11C]cyanoimipramine and [11C](+)McN5652. Each curve is based on findings in six subjects, and error bars indicate 1SD. The radioactivity was corrected for decay and the injected dose was adjusted to 370 MBq

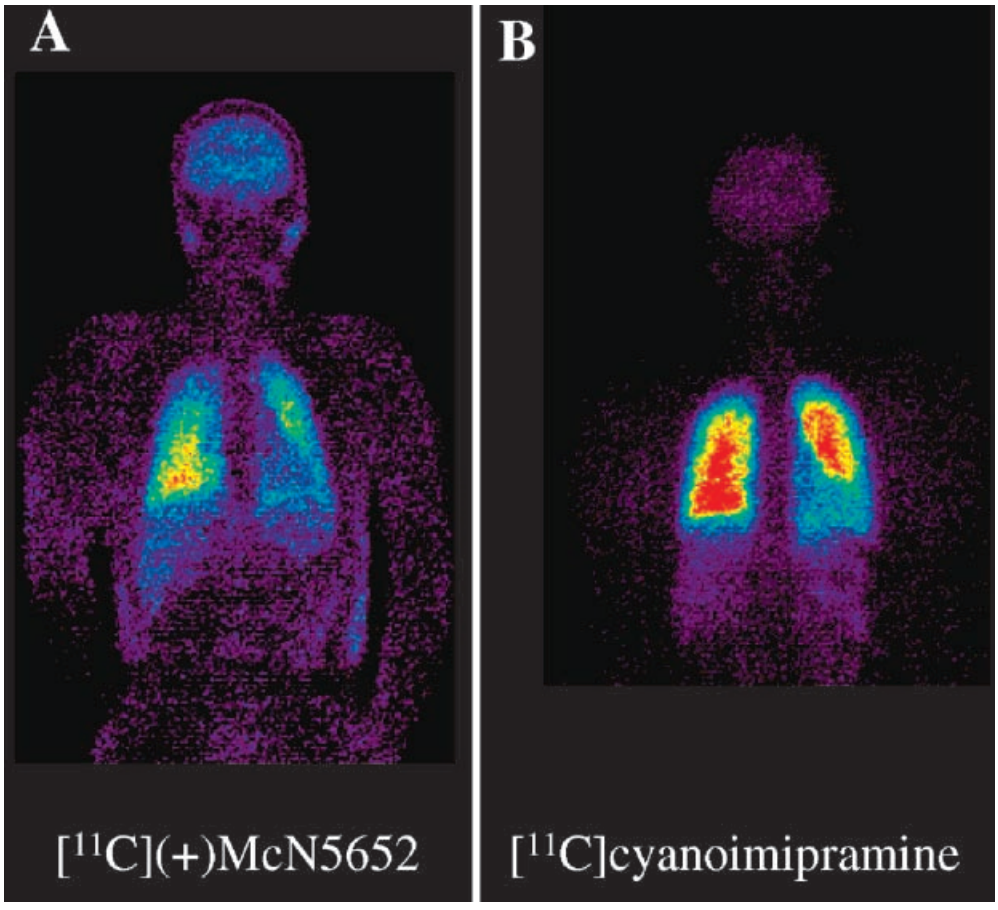

Fig. 6. Whole-body images of [11C](+)McN5652 (A) and [11C]cyanoimipramine (B). The images represent the late phase of accumulation after dynamic scan. Organs with the highest accumulation are red and those with low accumulation are purple

both the thalamus and the striatum (Fig. 3A, B). The average ratios of the thalamus to cerebellum, the striatum to cerebellum and the frontal cortex to cerebellum at 90 min after the injection were 1.20±0.06, 1.31±0.05 and 0.98±0.03 with [11C](+)McN5652, while those with [11C]cyanoimipramine were 1.25±0.05, 1.19±0.07 and 1.04±0.02 (Fig. 3C, D). The distribution volumes of [11C](±)McN5652 in the cerebellum and frontal cortex were not markedly changed after the clomipramine treatment, but those in the thalamus and striatum were clearly decreased after the treatment (Table 1).

The cerebellar uptake of [11C](+)McN5652 after pretreatment with 50 mg of clomipramine was higher than in the control study, with the magnitude being more prominent in the initial uptake phase (Fig. 4A). The metabolite-corrected arterial plasma input was higher in the clomipramine pretreatment experiment than in the control study, and it was more pronounced in the initial 10 min (Fig. 4B).

Ninety minutes after the injection, 4.8%±0.7% of the injected [11C](+)McN5652 was accumulated in the whole brain, as compared with only 2.7%±0.5% of the injected [11C]cyanoimipramine. The area under the average brain uptake curve during 90 min of [11C](+)McN5652 was approximately twice that of [11C]cyanoimipramine (Fig. 5).

Lung uptake study

Both ligands showed significant accumulation in the lung (Figs. 6, 7). The dose-corrected lung uptake of [11C](+)McN5652 was lower than that of [11C]cyanoimipramine (Figs. 6, 7). The distribution pattern in the whole-body images was similar among the subjects for each ligand. The time-activity curve of [11C](+)McN5652 showed a fast wash-out compared with the accumulation of [11C]cyanoimipramine. The area under the lung uptake curve of [11C](+)McN5652 was 53% of that of [11C]cyanoimipramine.

In vitro study

The results are summarised in Table 2. The IC_{50} and K_i values of (+)McN5652 were slightly higher than those of cyanoimipramine. The pKa value of (+)McN5652 was lower than that of cyanoimipramine. The mean values of logP were 3.8±0.2 for (+)McN5652 and 4.2±0.1 for cyanoimipramine.

Table 2. The characteristics of cyanoimipramine and (+)McN5652

Drug	[3H]citalopram binding			pKa	logP
	IC_{50} (n*M*)	K_i (n*M*)	nH		
Cyanoimipramine	1.58	0.83	1.05	9.48 (22°C)	4.2±0.1 (*n*=3)
(+)McN5652	2.02	1.25	1.08	8.52 (21°C)	3.8±0.2 (*n*=3)
Clomipramine	10.14	5.56	1.16		
(–)McN5652	1391	912	0.73		

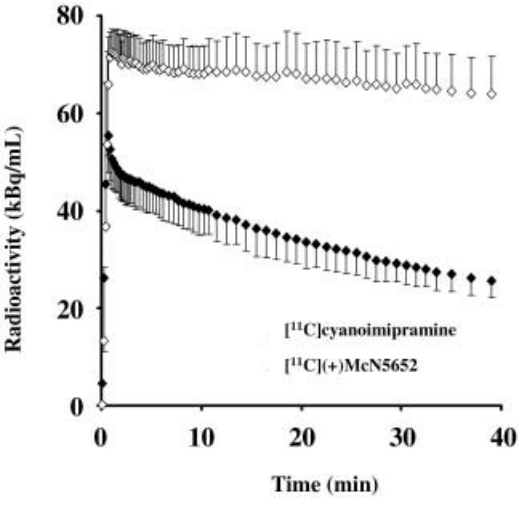

Fig. 7. Lung uptake of [11C](+)McN5652 and [11C]cyanoimipramine, each in three subjects, with error bars indicating 1SD. Radioactivity was the average of all ROIs within the field of view. The radioactivity was corrected for decay and the injected dose was adjusted to 370 MBq

Discussion

Both [11C](+)McN5652 and [11C]cyanoimipramine showed high uptake in the thalamus and striatum and, to a lesser extent, in the neocortex and cerebellum (Figs. 1, 2). The cerebellum was used as the reference region because it has been reported to have very low densities of [3H]imipramine and [3H]paroxetine binding sites in vitro [25, 26]. The region-to-cerebellum ratios of the thalamus and striatum increased to approximately 1.7 at 90 min after the tracer injection for both ligands (Fig. 2C, D). In this study, clomipramine was used to block 5-HT transporter, a tricyclic antidepressant and potent inhibitor of 5-HT reuptake (K_i=5.56 n*M*) (Table 2), and until recently the only such drug available in Japan. After pretreatment with clomipramine, the uptake of the two ligands in the thalamus and striatum became closer to that in the cerebellum, and to a similar extent (Fig. 3A, B). Therefore, the binding of both ligands in the thalamus and striatum was considered to represent the specific binding to the 5-HT transporter. After pretreatment with clomipramine, the uptake in the frontal cortex of both ligands changed slightly (Fig. 3), and the distribution volume of [11C](+)McN5652 showed almost no change (Table 1). Because the uptake curve of the frontal cortex was close to that of the cerebellum, quantifying the specific binding in the frontal cortex would seem to be rather difficult.

Although the uptake of both ligands was lowest in the cerebellum (Fig. 2A, B), the relative uptake compared with the plasma input was high (Fig. 4A). The distribution volume of [11C](+)McN5652 in the cerebellum was 21.1±0.8 (Table 1), a much higher value than those in the cerebellum for other ligands without specific binding in the cerebellum, such as 0.54±0.11 for the 5-HT_{1A} receptor ligand [11C]WAY100635 [27] and 0.42±0.06 for the dopamine D_2 receptor ligand [11C]raclopride [28]. This may suggest a possibly higher non-specific binding portion for [11C](+)McN5652 binding in vivo. The shapes of the time-activity curves of the cerebellum with and without clomipramine pretreatment differed with both ligands (Figs. 2, 3, 4). The wash-out was quicker with clomipramine pretreatment, as if there were specific binding in the cerebellum (Figs. 3, 4). However, the values of the distribution volume of [11C](+)McN5652 in the cerebellum with and without clomipramine pretreatment were not markedly different, whereas those in the thalamus and striatum were dose-dependently decreased after the pretreatment with clomipramine (Table 1). The change in the shape of the time-activity curves of the cerebellum can be attributed to the change in plasma input (Fig. 4), and similar results can be expected for [11C]cyanoimipramine [13]. The difference in the input with and without clomipramine can be explained by the specific binding in the peripheral organs [13].

In this study, we observed significant lung uptake for both [11C](+)McN5652 and [11C]cyanoimipramine (Figs. 6, 7). Several uptake mechanisms in the lung have been suggested for the basic amine compound with affinity for the 5-HT transporter. Large amounts of 5-HT transporter are expressed on pulmonary membranes for the reuptake of circulating amines in the blood [29]. One mechanism may consist in specific binding to the peripheral 5-HT transporter, and another may be a non-specific accumulation mechanism for basic amines with a high pKa value [30]. Basic amines are thought to be taken up by pulmonary alveolar macrophages and possibly by type II epithelial cells [31, 32]. Cyanoimipramine had

slightly higher affinity to 5-HT transporters than (+)McN5652, and it had slightly basic characteristics compared with (+)McN5652 (Table 2). The difference in the magnitude of lung uptake might be attributed to their different chemical characteristics.

The whole brain uptake of [^{11}C](+)McN5652 was approximately twice that of [^{11}C]cyanoimipramine (Fig. 5), and this may result in better counting statistics at the same injected dose for [^{11}C](+)McN5652. The difference in peripheral accumulation between [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine (Fig. 6) could be one of the factors responsible for the difference in brain uptake between them. The peripheral uptake also affected the kinetics of both ligands, and the release of the ligands from the lung would be responsible for the protracted duration of the peak uptake in the brain. The slow kinetics of both ligands might also be influenced by the high non-specific binding [33]. However, a recent study indicated that there was no firm relation between logP values of the compounds and the degree of non-specific binding in PET/SPET studies [34]. For the quantification of these ligands, compartment model-based methods would be required that include the effects of prolonged input and high non-specific binding [35]; the peripheral uptake of radioligands might be an important factor to be considered in the development of ligands, such as 5-HT transporter ligands [36, 37].

Conclusion

Both [^{11}C](+)McN5652 and [^{11}C]cyanoimipramine were developed as PET tracers to examine the 5-HT transporter. [^{11}C](+)McN5652 was considered a better ligand than [^{11}C]cyanoimipramine because of its higher brain distribution. However, the fact that both ligands had high peripheral binding indicates that, in the case of the development of 5-HT transporter ligands, the peripheral uptake of the radioligand needs to be taken into consideration.

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