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ARTICLE

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PET measurement of serotonin transporter occupancy: a comparison of escitalopram and citalopram

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Abstract

The selective serotonin reuptake inhibitor (SSRI) citalopram (R,S-citalopram) is a racemic compound of two enantiomers. On the basis of in-vitro studies, inhibition of the human serotonin transporter (5-HTT) is achieved by the S-enantiomer (S-citalopram or escitalopram). The aim of the present PET study was to compare 5-HTT occupancy after single equimolar doses (with respect to S-enantiomer) in humans in vivo using R,S-citalopram (20 mg) and S-citalopram (10 mg) using PET and the radioligand [^{11}C]MADAM. The design was a single-dose, double-blind, two-way crossover study in eight healthy male subjects. The 5-HTT binding potential at baseline and after single doses of study drugs was used to calculate 5-HTT occupancy in seven brain regions. Serum concentrations of the study drugs were determined in order to calculate the apparent inhibition constant ($K_{i,app}$), a secondary parameter of interest for the comparison. In all brain regions examined, occupancy was numerically higher after treatment with R,S-citalopram [66 ± 19% to 78 ± 17% (mean ± S.D.) depending on the region] than after S-citalopram (59 ± 15% to 69 ± 13%; overall comparison: $F = 14.8$, d.f. = 1, 90, $p < 0.001$). In line with this the apparent inhibition constant was significantly lower for R,S-citalopram than for S-citalopram (overall comparison: $F = 6.7$, d.f. = 1, 90, $p < 0.05$). The small but significant difference in occupancy and $K_{i,app}$ found between R,S-citalopram and S-citalopram suggests that not only S-citalopram but also R-citalopram to some degree occupies the 5-HTT in the human brain in vivo.

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Key words: Escitalopram, human, 5-HTT, [^{11}C]MADAM, PET.

Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are well established for the treatment of anxiety and mood disorders (Vaswani et al., 2003). The SSRI citalopram is a racemic 1:1 mixture of the S- and R-enantiomer (escitalopram and R-citalopram) (Hyttel et al., 1992). In-vitro studies have shown that the activity of R,S-citalopram (R,S-Cit) resides in the S-enantiomer since S-citalopram (S-Cit) has a 30-fold higher affinity for the 5-HT transporter (5-HTT) than has R-citalopram (R-Cit) (Owens et al., 2001). It is thus expected that S-Cit and not R-Cit is primarily responsible for 5-HTT occupancy in the brain.

More recently, S-Cit has become available for clinical use. Interestingly, when the amounts of S-Cit are equal the onset of effect of S-Cit alone has been shown to be faster compared to that of R,S-Cit, both in a non-clinical study using a rat behavioural model and in a clinical study on patients with major depressive disorder (Montgomery et al., 2001). This difference in time to response, and in the proportion of responders has been replicated in several clinical studies (Burke et al., 2002; Colonna et al., 2005; Lepola et al., 2003; Moore et al., 2005). Moreover, without affecting the extracellular levels of S-Cit, R-Cit has been shown to counteract the S-Cit-induced increase of extracellular 5-HT levels in the frontal cortex of freely moving rats. Furthermore, R-Cit has been shown to reduce the

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anxiolytic- and antidepressant-like effect of S-Cit in three different animal models (Mork et al., 2003; Sanchez, 2003; Sanchez et al., 2003a,b). The exact molecular mechanism for this effect of R-Cit on the pharmacodynamics of S-Cit is not fully understood. One suggested mechanism is that R-escitalopram attenuates the binding of S-Cit to the 5-HTT (Sanchez et al., 2004), possibly due to a conformation change resulting from R-Cit binding to an allosteric site (Chen et al., 2005).

Positron emission tomography (PET) has made it possible to quantify drug targets within the central nervous system in humans in vivo (Farde, 1996). [^{11}C]MADAM is a promising radioligand with nanomolar affinity for the 5-HTT, and is thus potentially suitable for the quantification of 5-HTT in humans in vivo (Lundberg et al., 2005). Quantification of the 5-HTT with PET and [^{11}C]MADAM in control subjects has been shown to have good reliability and to be sensitive to pre-treatment with reference compounds for the 5-HTT (Halldin et al., 2005; Lundberg et al., 2006).

The aim of the present PET study was to compare the 5-HTT occupancy of R,S-Cit and S-Cit. This was done in order to examine the hypothesis derived from in-vitro studies that 5-HT reuptake inhibition or 5-HTT occupancy by R,S-Cit in humans in vivo is primarily due to S-Cit. Eight healthy male subjects were examined with PET and [^{11}C]MADAM before and after treatment with a single dose of 20 mg R,S-Cit and of 10 mg S-Cit in a crossover design. The apparent inhibition constant ($K_{i,app}$) was the secondary parameter of interest for the present comparison.

Materials and methods

Subjects and design

The study was approved by the Ethics and the Radiation Safety committees of the Karolinska Hospital. Nine male subjects (age 22–33 yr, weight 68–98 kg) participated after giving verbal and written informed consent. All subjects were healthy based on medical history, psychiatric interview, physical examination, electrocardiogram, blood and urine analysis, and magnetic resonance imaging (MRI) of the brain. They were not being treated with any medication and they were all non-smokers.

All subjects were examined with PET four times on 4 d. On day 1, a baseline measurement was performed at noon. On day 2, 1–14 d later, the first measurement after administration of the first study drug was made. On day 3, 14–21 d later, the second measurement after administration of the second study drug was made. On day 4, 28–56 d later, a second baseline experiment was carried out to determine the reliability of the method and the intra-individual baseline variation. The results of this reliability examination have been recently published (Lundberg et al., 2006).

The study had a single-dose, randomized, double-blind, two-way crossover design. The blinding was kept until the analysis was finalized. All subjects were examined with PET and [^{11}C]MADAM after a single oral dose of either 10 mg S-Cit (Cipralex; H. Lundbeck A/S, Valby, Denmark) or 20 mg R,S-Cit (Cipramil; H. Lundbeck A/S) given in randomized order. Thus, the amount of S-Cit administered was identical in each treatment. Due to the unavailability of [^{11}C]MADAM, one subject was not examined with PET after treatment with S-Cit, and was therefore excluded from the analysis. Subjects were monitored regularly with regards to adverse events.

On the day of study drug treatment, the drug was given at 08:00 hours and the PET measurement started at 14:00 hours, i.e. ~6 h after dosing, when drug serum concentration was expected to be at a more constant level. Blood samples for determination of serum concentration of S-Cit and R-Cit were taken at 0, 1, 2, 3, 4, 6, 6.5, 7, 7.5, 8, 12, 24 and 48 h post-dose. The actual sampling times were recorded.

MRI and the head fixation system

The MRI system used was Signa, 1.5 T (GE Medical Systems, Milwaukee, WI, USA). T_2-weighted and T_1-weighted images (matrix $256 \times 256 \times 156$; pixel size $1.0156 \times 1.0156 \times 1.0$ mm) were obtained. To allow the same head positioning in the two imaging modalities, a head fixation system with an individual plaster helmet was used both in the PET and MRI measurements (Bergstrom et al., 1981).

Radiochemistry

[^{11}C]MADAM was obtained by methylation of the appropriate desmethyl precursor using [^{11}C]methyltriflate, as described previously (Tarkiainen et al., 2001). Between 298 and 313 MBq was injected intravenously. The specific radioactivity of the radioligand injected varied between 658 and 3359 Ci/mmol, corresponding to an injected mass between 0.7 and 3.4 μg ($n = 21$; in three of the PET measurements, analysis of specific activity failed for technical reasons).

PET experimental procedure

The PET system used was ECAT EXACT HR 47 (Siemens, Berlin and Munich, Germany), which was

run in the 3D mode (Wienhard et al., 1994). The in-plane and axial resolution is ~3.8 and 4.0 mm, respectively, full width at half maximum (FWHM). The reconstructed volume was displayed as 47 sections with a centre-to-centre distance of 3.125 mm.

In each PET measurement, the subject was placed recumbent with his head in the PET system. A sterile physiological phosphate buffer (pH 7.4) solution containing [^{11}C]MADAM was injected as a bolus during 2 s into a cannula inserted into the right antecubital vein. The cannula was then immediately flushed with 10 ml saline.

Brain radioactivity was measured in a series of consecutive time-frames for 93 min. The frame sequence consisted of three 1-min frames, four 3-min frames and thirteen 6-min frames. After correction for attenuation, random and scatter events, images were reconstructed using a Hann filter (2 mm FWHM). The reconstructed volume was displayed as 47 horizontal sections with a centre-to-centre distance of 3.125 mm and a pixel size of 2.02×2.02 mm^2.

Co-registration

The head fixation system allows for repositioning with an accuracy of <3 mm (Bergstrom et al., 1981). A co-registration procedure was applied to further align the PET and MRI datasets. For each subject, the MR image was adjusted to position the anterior–posterior (AC–PC) commissural line in the horizontal plane, and the inter-hemispheric plane orthogonal to the AC–PC plane. The MR image was resampled and cropped to generate a $256 \times 256 \times 141$ matrix with 1 mm^2 pixels before it was used for manual definition of regions of interest (ROIs). The PET images were resampled to a $2 \times 2 \times 2$ mm voxel size and co-registered to a corresponding MRI half-resolution dummy. Co-registration was done using the normalized mutual information method implemented in SPM2 (Maes et al., 1997).

Regions of interest

ROIs were defined manually by the first author (J.L.) using the Human Brain Atlas software (Roland et al., 1994) and according to anatomical boundaries for frontal cortex, temporal cortex, anterior cingulate, hippocampal complex, insula, putamen, raphe nuclei and cerebellum. All ROIs except for the raphe nuclei were delineated in 8–10 consecutive sections on the MR images and transferred to the corresponding reconstructed and co-registered PET images. The raphe nuclei cannot be differentiated from surrounding tissue on MR images. Therefore, these ROIs were delineated directly on the PET images in 3–5 sections, according to a method previously applied in PET studies of radioligand binding in the raphe nuclei (Andree et al., 2003).

Quantification of [^{11}C]MADAM binding

To obtain the average radioactivity concentration for the whole volume of interest, data for each ROI were pooled. Regional radioactivity was calculated for each frame, corrected for decay, and plotted vs. time, thus providing time–activity curves (TACs) for each region. For bilateral ROIs, right and left regions were averaged.

Regional binding potential (BP) values (Mintun et al., 1984) were calculated by means of the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996). The application of SRTM for interpretation of [^{11}C]MADAM binding using cerebellum as the reference region has been described in detail elsewhere (Lundberg et al., 2005).

5-HTT drug occupancy (%) was calculated according to equation (1):

$$\text{occupancy} = \left(1 - \frac{\text{BP}_{\text{treatment}}}{\text{BP}_{\text{baseline}}}\right) \times 100, \quad (1)$$

where $\text{BP}_{\text{baseline}}$ refers to the BP calculated at the baseline condition and $\text{BP}_{\text{treatment}}$ to the BP calculated after treatment with either R,S-Cit or S-Cit.

Determination of serum concentrations

Serum concentrations of R-Cit and S-Cit were analysed at the Department of Early Development Pharmacokinetics (H. Lundbeck A/S) by means of an enantioselective high-performance liquid chromatography method with tandem mass spectrometry (HPLC MS/MS) detection (Gutierrez et al., 2003). Alkalinized serum was extracted with 1.5% isoamyl alcohol in n-heptane, and back-extracted into dilute hydrochloric acid. Mass-spectrometric detection was performed on a Quattro LC (Micromass, Manchester, UK) using positive electrospray ionization in the multiple reaction-monitoring mode. The lower limit of quantification in serum was 3.08 nmol/l for R-Cit and S-Cit.

Calculation of $K_{\text{i,app}}$

The relationship between occupancy and drug serum concentration can be described by a curvilinear function given by the following equation as derived from the law of mass action:

$$B = \frac{B_{\max} \times F}{K_{\text{i, app}} + F}, \quad (2)$$

where B is the concentration of ligand bound to receptor, B_{max} the number of available receptors, F the concentration of unbound ligand and $K_{i,app}$, the apparent inhibition constant. The affinity is called apparent when serum concentration is used as an estimate for free fraction in blood (f_1), and when the concentration of endogenous ligand (5-HT) and non-specific binding in brain (f_2) is not corrected for.

In drug occupancy studies, equation (2) may be rewritten as follows:

$$\text{occupancy} = \frac{\text{occ}_{max} \times C_s}{K_{i,app} + C_s}, \quad (3)$$

where occ_{max} is the maximal occupancy induced by the drug and C_s is the serum concentration of the drug. This is done under the assumption that there is a linear relationship between drug concentration in the brain and serum so that F may be substituted with C_s. In the analysis, occ_{max} was fixed at 100%. The calculated 5-HTT occupancy was related to the mean serum concentration of S-Cit at 6–8 h post-dose, i.e. during the PET measurement, and $K_{i,app}$ was determined by means of a least-squares minimization procedure. In this way, $K_{i,app}$ for each brain region examined was determined after treatment with R,S-Cit ($K_{i,app,RS}$) and S-Cit ($K_{i,app,S}$) for each of the eight subjects.

Statistics

Both occupancy and $K_{i,app}$ values for each study drug were compared using a general linear mixed model containing fixed terms for region, sequence, period, and treatment, and a random term for subject nested within sequence. Based on this model, tests for treatment differences were made. Initially, a model including an interaction term between region and treatment was used to establish that treatment differences did not differ significantly between regions.

Results

Both study drugs were well tolerated. The serum concentrations of S-Cit reached a peak (t_{max}) at 3.4 and 2.8 h after administration of R,S-Cit and S-Cit, respectively (mean values). This difference in t_{max} was not statistically significant. t_{max} for R-Cit was reached 3.9 h after administration of R,S-Cit (Table 1). The PET measurement started after t_{max}, i.e. 6 h after dosing and the serum concentrations were thus in slow decline.

At the first PET measurement, which was made at baseline conditions (i.e. without treatment with the study drug), there was a rapid and high uptake of radioactivity in all brain regions examined, apart from the cerebellum where the radioactivity was low (Figure 1). After treatment with R,S-Cit or S-Cit, the uptake of radioactivity was more rapid than at baseline conditions. The uptake in the target regions was decreased vs. cerebellum, which was slightly higher after treatment with the study drugs when compared with the baseline condition (Figures 1 and 2). The mean occupancy values were numerically higher in all examined regions after treatment with R,S-Cit [66 ± 19% to 78 ± 17% (mean ± S.D.) depending on the region] than after S-Cit (59 ± 15% to 69 ± 13%). The difference was most evident for insula and the hippocampal complex (Table 2). There was a statistically significant overall treatment difference ($F = 14.8$, d.f. = 1, 90, $p < 0.001$). With the exclusion of insula and the hippocampal complex, which showed the largest inter-subject variability, the difference was smaller but remained statistically significant ($F = 6.1$, d.f. = 1, 62, $p < 0.05$). The overall test for treatment x region interaction was non-significant ($F = 0.7$, d.f. = 6, 90, $p = 0.638$).

The apparent inhibition constant for S-Cit ($K_{i,app,S}$), was numerically higher than for R,S-Cit ($K_{i,app,RS}$) (Table 3). This difference was most apparent for insula and the hippocampal complex. The hippocampal complex also showed the largest inter-individual differences. Statistically, there was a significant overall treatment difference ($F = 6.7$, d.f. = 1, 90, $p < 0.05$). With the exclusion of insula and the hippocampal complex the difference was smaller but remained statistically

Table 1. Serum drug concentrations after single oral dose of 20 mg R,S-Cit or 10 mg S-Cit (mean ± S.D., nmol/l)

Nominal time (h)	R,S-Cit *n*	R,S-Cit S-Cit	R,S-Cit R-Cit	S-Cit *n*	S-Cit S-Cit	S-Cit R-Cit
0	8	0	0	8	0	n.a.
1	8	9.5 ± 13.4	10.4 ± 13.7	8	14.1 ± 10.0	n.a.
2	8	22.1 ± 11.5	23.5 ± 11.0	8	23.9 ± 5.4	n.a.
3	8	26.7 ± 6.4	28.5 ± 6.2	7	25.0 ± 6.4	n.a.
4	8	25.8 ± 6.8	28.2 ± 6.3	8	25.0 ± 4.5	n.a.
6	8	23.3 ± 4.1	26.3 ± 3.9	8	21.6 ± 3.6	n.a.
6.5	8	22.3 ± 5.3	25.4 ± 4.5	8	20.4 ± 4.5	n.a.
7	8	22.3 ± 4.3	25.0 ± 3.7	7	20.3 ± 5.0	n.a.
7.5	8	21.6 ± 4.3	24.2 ± 3.6	8	20.1 ± 4.1	n.a.
8	8	22.4 ± 4.4	26.2 ± 4.3	7	20.2 ± 4.5	n.a.
12	8	20.6 ± 4.8	24.7 ± 4.7	8	18.6 ± 3.9	n.a.
24	8	14.8 ± 4.9	20.2 ± 3.1	8	13.4 ± 4.2	n.a.
48	7	8.5 ± 2.9	13.2 ± 2.5	7	8.1 ± 3.8	n.a.

n.a., Not applicable.

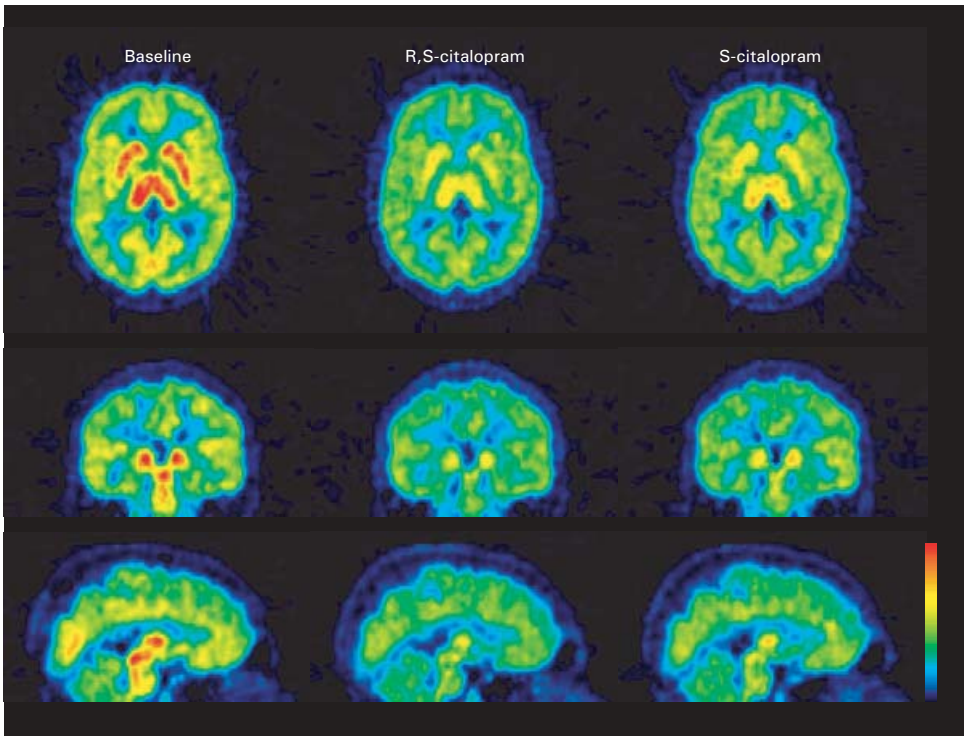

Figure 1. Summation images based on data from frames 6–20 showing regional radioactivity after intravenous injection with [^{11}C]MADAM at baseline conditions (left), after treatment with 20 mg R,S-Cit (middle) and after treatment with 10 mg S-Cit (right). The projections are transaxial (top), coronal (middle) and sagittal (bottom).

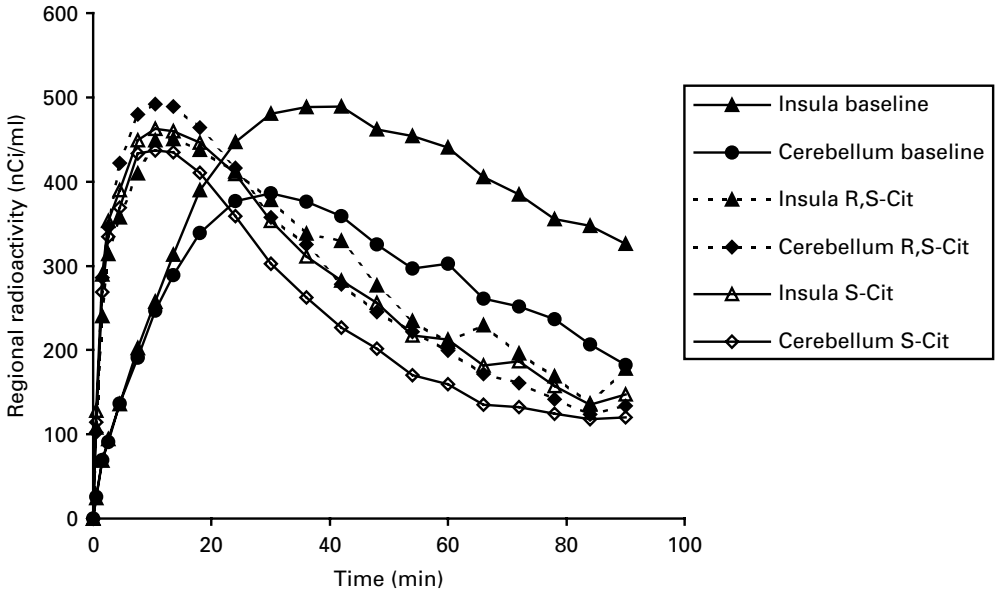

Figure 2. Example of time–activity curves for two of the regions examined at baseline and the two study drug treatment conditions.

Table 2. 5-HTT occupancy (%) after a single oral dose of 20 mg R,S-Cit or 10 mg S-Cit (nmol/l) (%)

	A cingulate		Frontal cortex		Temporal cortex		Insula		Hippocampus		Putamen		Raphe nuclei	
Subject	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit
1	46	52	63	56	40	42	78	45	68	14	61	62	70	49
2	97	74	66	64	66	78	72	71	57	57	68	55	81	79
3	85	61	91	83	85	95	81	80	73	43	73	65	87	82
4	63	81	53	87	44	51	63	53	63	66	73	72	62	58
5	67	71	80	62	59	57	59	55	94	64	67	67	66	60
6	90	47	76	52	94	92	80	38	100	60	62	55	88	88
7	76	70	88	65	80	35	81	73	100	77	76	78	76	67
8	78	66	86	66	63	52	67	56	70	89	66	55	75	70
Mean	75	65	75	67	66	63	73	59	78	59	68	64	76	69
S.D.	16	11	14	12	19	23	9	15	17	23	5	9	9	13

Table 3. 5-HTT $K_{i,app}$ after a single oral dose of 20 mg R,S-Cit or 10 mg S-Cit (nmol/l)

	A cingulate		Frontal cortex		Temporal cortex		Insula		Hippocampus		Putamen		Raphe nuclei	
Subject	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit	R,S-cit	S-cit
1	22.5	18.8	11.2	16.1	28.8	28.4	5.2	24.9	8.9	126.8	12.1	12.1	8.1	20.0
2	0.7	7.1	14.1	11.6	14.3	5.8	10.7	8.6	20.9	15.5	13.1	17.0	6.5	5.6
3	4.8	18.7	2.7	5.9	5.0	1.4	6.4	7.1	10.3	38.1	10.0	15.7	4.1	6.5
4	13.0	4.9	2.7	3.1	29.0	19.7	13.4	18.4	13.3	10.7	8.3	8.1	13.5	15.0
5	8.7	6.2	5.5	9.5	12.1	11.7	11.9	12.4	1.2	8.6	8.5	7.5	9.0	10.1
6	2.4	21.7	5.5	17.9	1.5	1.6	5.6	31.3	0.0	12.7	13.6	16.1	3.1	2.7
7	8.1	9.6	3.1	12.0	6.6	42.0	5.9	8.5	0.0	6.6	8.2	6.2	8.2	11.1
8	4.9	9.0	4.3	8.9	10.1	15.9	8.4	13.6	7.3	2.2	8.9	14.1	5.7	7.3
Mean	8.1	12.0	6.1	10.6	13.4	15.8	8.4	15.6	7.7	27.6	10.3	12.1	7.3	9.8
S.D.	7.0	6.7	4.2	4.9	10.4	14.0	3.2	8.7	7.3	41.5	2.2	4.3	3.3	5.6

significant ($F=4.3$, d.f.$=1$, 62, $p<0.05$). The overall test for treatment x region interaction was non-significant ($F=1.1$, d.f.$=6$, 90, $p=0.378$).

Discussion

Regional [^{11}C]MADAM binding to 5-HTT was measured by PET in eight healthy male subjects at four times: first under baseline conditions and then, in randomized order, in a double-blind design after single dose treatment with a 20 mg R,S-Cit or 10 mg S-Cit. These doses are the recommended therapeutic doses for treatment of major depressive disorder when given as multiple doses. The fourth PET experiment was performed as part of a reliability examination of the method, the results of which have been recently published (Lundberg et al., 2006).

The regional occupancy values were between 66% and 78% for R,S-Cit (mean values) and between 59% and 69% for S-Cit. The results are consistent with those from in-vitro and animal experiments that show that S-Cit has a higher affinity for 5-HTT and is the active enantiomer of R,S-Cit in humans in vivo (Hyttel et al., 1992; Sanchez et al., 2004).

The occupancy values were, however, numerically higher (mean values) for R,S-Cit in all regions examined (Figure 2 and Table 2), and although this was not true for all subjects in all regions, the overall difference was statistically significant ($F=14.8$, d.f.$=1$, 90, $p<0.001$). The apparent inhibition constant of S-Cit ($K_{i,app,S}$), was accordingly higher than for R,S-Cit ($K_{i,app,RS}$), and this difference was also statistically significant ($F=6.7$, d.f.$=1$, 90, $p<0.05$; Table 3). One explanation for the higher occupancy and lower

$K_{i,app}$ of R,S-Cit may be a small but detectable occupancy of R-Cit, although R-Cit has low affinity to the 5-HTT in vitro and in animal models (Sanchez et al., 2004). Interestingly, an observation in the same direction was recently made in a study using single photon emission computer tomography (SPECT) and [^{123}I]ADAM to compare 5-HTT occupancy after single oral doses of R,S-Cit and S-Cit. The reported midbrain occupancy 6 h after administration was ~8% lower than in the present study (64% after 10 mg S-Cit and 70% after 20 mg R,S-Cit), a result that may be attributed to the generic limitation for quantitative estimations of the SPECT methodology as well as the lower spatial resolution compared to PET. The authors reported no statistically significant difference in occupancy after 10 mg of each study drug, as well as after 20 mg of each study drug, indicating R-Cit to give a measurable effect on the occupancy of [^{123}I]ADAM (Klein et al., 2006).

In the present study, all subjects were given the same amount of S-Cit, resulting in small inter-individual variations in drug serum concentration and occupancy. In addition, to allow for a statistical comparison between the treatment conditions, $K_{i,app}$ was calculated based on a single occupancy value. This approach gave a marked variance in $K_{i,app}$ values between subjects within each brain region and treatment condition (Table 3). It should be noted that the more robust approach using all eight occupancy values in the determination of $K_{i,app}$ for each region gave similar results. A better estimate of $K_{i,app}$ would require additional occupancy data at lower S-Cit concentrations.

Assuming that S-Cit binds only to the 5-HTT, BP values for [^{11}C]MADAM at baseline conditions and after treatment with the study drugs were used as input values in the estimation of $K_{i,app}$ for S-Cit. However, if R-Cit does interfere with [^{11}C]MADAM binding in vivo in man, the $K_{i,app}$ values for S-Cit calculated after dosing of R,S-Cit, i.e. $K_{i,app,RS}$, may be underestimated.

It has recently been suggested that the differences between R,S-Cit and S-Cit described in vitro in animal models and in clinical trials might be explained by an effect of R-Cit on the 5-HTT binding of S-Cit (Sanchez et al., 2004). However, in human subjects the plasma concentration of R-Cit at steady state has been reported to be ~1.5–2 times higher than that of S-Cit with daily dosing of R,S-Cit (Rochat et al., 1995; Sidhu et al., 1997). In our sample, the R-Cit:S-Cit ratio was around 1:1 (Table 1). It is thus possible that 5-HTT occupancy could be different with a higher R-Cit:S-Cit ratio than was the case in our study. This hypothesis could be examined with a study design with multiple dosing of R,S-Cit and S-Cit.

The question whether the binding of R-Cit take place at an allosteric or primary binding site as for S-Cit could not be addressed in this study as the effect of R-Cit on [^{11}C]MADAM binding in man in vivo is unknown. A recent in-vitro study suggests that R-Cit has a stabilizing effect on the [^{11}C]MADAM/5-HTT complex (Chen et al., 2005). The effect of R-Cit on S-Cit binding may thus be explained by mechanisms other than direct competition for the same binding site.

The detected degree of occupancy after a single oral therapeutic dose in the present study was somewhat lower than the ~80% that has been reported for R,S-Cit and other SSRIs after continuous dosing (Meyer et al., 2004). S-Cit serum concentrations were also lower than the ~100 nmol/l (mean value) found in patients on continuous dosing of R,S-Cit (20–80 mg/d) for treatment of major depression (Rochat et al., 1995). However, the pharmacokinetics of both R,S-Cit and S-Cit is linear (Burke, 2002). This allows for a prediction of 5-HTT occupancy at clinically relevant S-Cit serum concentrations (27–326 nmol/l, mean 100 nmol/l; Rochat et al., 1995) by entering these values together with the calculated $K_{i,app}$ from the present study in to equation (3) and assuming that the change in ratio between R-Cit and S-Cit from 1:2 to 2:1 does not affect the binding. The calculated occupancy is between 75% and 97% (mean 92%) for R,S-Cit and between 65% and 95% (mean 87%) for S-Cit, i.e. in good agreement with the 5-HTT occupancy detected with PET at steady-state conditions of clinical doses of SSRIs (Meyer et al., 2004). A generalization of the results to the clinical setting should, however, still be done with caution as the study is based on a limited sample of young healthy male subjects. It should also be noted that repeated SSRI dosing in rodents has been shown to result in decreased 5-HTT concentration (Benmansour et al., 1999). It is not known if this phenomenon equally affects 5-HTT occupancy in human subjects after repeated dosing of S-Cit and R,S-Cit, respectively.

In both study-drug treatment conditions there was a more rapid uptake of [^{11}C]MADAM in the brain compared with baseline. The difference was statistically significant (Table 4). A previously described effect in both rodents and humans on cerebral blood flow and metabolism, which is induced by changes in 5-HT concentration, may provide an explanation for this observation (Cohen et al., 1996).

The concentration of radioactivity in the cerebellum was statistically significantly higher after treatment with the study drug compared to the baseline

Table 4. Time to peak radioactivity (t_{max}) and the peak radioactivity concentration (C_{max}) in cerebellum

Condition	t_{max} (min)	C_{max} (nCi/ml)
Baseline	19±7	355±61
R,S-Cit	8±3***	461±58***
S-Cit	8±3***	446±43**

*** $p<0.001$, ** $p<0.01$ (paired t test, two-tailed, comparison between baseline and treatment conditions. No significant difference between treatment conditions.)

condition (Table 4). This phenomenon has also been observed in experiments in a monkey model. It was noted that the ratio of radioactivity in cerebellum to blood did not change and that the increased radioactivity in the cerebellum represented non-displaceable [^{11}C]MADAM (Halldin et al., 2005). The increased fraction of non-displaceable [^{11}C]MADAM detected after treatment with a compound with affinity for 5-HTT is thus most probably due to occupation of 5-HTT in extracerebral compartments, such as binding to platelets. Another compartment of quantitative significance is lung tissue, as has previously been reported in studies on human subjects (Suhara et al., 1998). As there was no statistical difference in the time to peak uptake of radioactivity or in the concentration of radioactivity in the cerebellum between PET examinations with the two study drugs, this phenomenon should not affect the result of the study (Table 4). Moreover, there was no statistical difference between the area under the curve of the TACs for cerebellum after treatment with S-Cit (17.0±2.6 mCi . min/ml, mean±S.D.) or R,S-Cit (17.3±2.9 mCi . min/ml, $p=$ 0.54, paired t test, two tailed).

In conclusion, S-Cit was found to have higher $K_{i,app}$ than R,S-Cit. The difference is most probably due to a small 5-HTT occupancy of R-Cit, although the results of the present study warrants experimental studies on the effect of R-Cit on [^{11}C]MADAM binding. This observation may be related to the more rapid onset of effect noted for S-Cit compared to R,S-Cit in several clinical studies on treatment of major depression.

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Statement of Interest

Three of the authors (J.S.C., K.B.P., H.L.) are employed by Lundbeck A/S, Denmark.

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