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Evaluation of *S*-[^{11}C]Citalopram as a Radioligand for *In Vivo* Labelling of 5-Hydroxytryptamine Uptake Sites

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The biologically active *S*-enantiomer of [*N*-methyl-^{11}C]citalopram was evaluated as a radioligand for *in vivo* labelling of the 5-hydroxytryptamine uptake site in brain, using *ex vivo* tissue counting in rats and positron emission tomography in man. In rats, the maximal signal for total versus non-specific binding was approx. 2 at 60–120 min after radioligand injection. Subsequent studies in man failed to identify a specific signal over a 90 min scanning period, due to prolonged retention of non-specific label.

Introduction

Positron emission tomography (PET), in providing quantitative regional measurements of biochemical parameters in living man, has considerable potential for studying the chemical bases of neurologic and psychiatric disorders. However, its use for investigating the serotonergic system, for example in the pathogenesis of depression, dementia, schizophrenia and other disorders of higher function, is limited by the paucity of positron-emitting radioligands which have an *in vivo* selectivity for 5-hydroxytryptamine (5-HT) receptors or uptake sites. The present study was part of an ongoing programme to develop a positron-emitting ligand as a marker for the latter.

Many 5-HT uptake inhibitors with antidepressant activity bind to the neuronal 5-HT transporter, at the "high-affinity imipramine binding site" (Langer, 1987). Although [*N*-methyl-^{11}C]imipramine itself has been prepared and its biodistribution in mice described (Nakamura *et al.*, 1989), the heterogeneity of its binding limits its potential as an *in vivo* marker. In contrast, citalopram is recognized as a highly selective inhibitor for 5-HT uptake (Hyttel, 1982; d'Amato *et al.*, 1987; Plenge *et al.*, 1990). In a previous paper, racemic [*N*-methyl-^{3}H]citalopram was shown in rats to fulfil many crucial criteria for an *in vivo* ligand, including selective biodistribution and saturability, together with loss of specific binding after chemical lesioning of the serotonergic projections (Hume *et al.*, 1991).

Recently, the racemate of citalopram has been labelled with carbon-11 ($t_{1/2}$ = 20.2 min) (Dannals *et al.*, 1990; Ram, 1990; Hume *et al.*, 1991) and its *in vivo* uptake measured in rats (Hume *et al.*, 1991). Although the ratios of counts in selected brain tissues relative to cerebellum, the latter representing a region devoid of specific binding (d'Amato *et al.*, 1987), were only of the order of 1.4, preliminary data suggested that this ratio could be increased by radiolabelling the *S*-enantiomer, since the *S*-enantiomer only is a potent 5-HT re-uptake inhibitor (Boegesoe, 1989). The present paper describes the evaluation of *S*- and *R*-[*N*-methyl-^{11}C]citalopram in rats and their *in vivo* biodistribution in man, measured using PET.

Materials and Methods

Radiochemistry

No-carrier-added [^{11}C]iodomethane was reacted with either *S*- or *R*-*N*-desmethyl-citalopram oxalate in ethanol containing 2,2,6,6-tetramethylpiperidine, for 5 min at 95°C, to give *S*- or *R*-[*N*-methyl-^{11}C]citalopram, respectively, in up to 60% radiochemical yield, decay-corrected (Hume *et al.*, 1991). The radioactive product was formulated for intravenous (i.v.) injection by dissolution in normal saline and sterile millipore filtration.

Ex vivo biodistribution

Male Sprague–Dawley rats (body weight 260–280 g) were each given 10 MBq of either *S*- or *R*-[*N*-methyl-^{11}C]citalopram i.v. in ~300 μL saline (sp. act. range = 4–12 GBq μmol^{-1}, at time of injection). Details of the method are reported in Hume *et al.* (1991). Decay-corrected radioactivity in the brain tissues listed in Table 1 was measured post-mortem, at times ranging between 5 and 120 min

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Table 1. Kinetic parameters for S-[N-methyl-^{11}C]citalopram binding in rat brain

Tissue	R_1 (K_1/K_1')	BP (k_3/k_4)
Olfactory bulbs	0.91 ± 0.08	0.18 ± 0.06
Frontal cortex	1.14 ± 0.09	0.51 ± 0.06
Striatum	0.91 ± 0.08	0.47 ± 0.07
Thalamus	0.96 ± 0.07	0.77 ± 0.06
Hippocampus	0.78 ± 0.05	0.43 ± 0.05
Medulla	1.03 ± 0.09	0.42 ± 0.05

Parameters (±SE) fitted using a cerebellum input function in the reference tissue model schematically illustrated in Fig. 1.

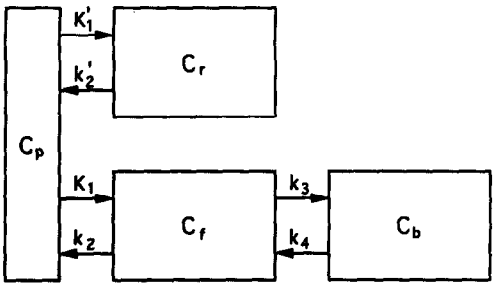

Fig. 1. Schematic diagram of the reference tissue compartment model used for analysis of rat data. C_p, C_r, C_f and C_b represent concentrations (Bq g^{-1}) of radioactivity in plasma, reference tissue and the "not-specifically bound" and "specifically bound" compartments within the tissue of interest. K_1 and K_1' represent the clearance (mL g^{-1} min^{-1}) from the plasma to the free compartments in tissue and reference tissue, respectively. k_2, k_2', k_3 and k_4 are the rate constants (min^{-1}) for transfer between the compartments shown.

after injection of the radioligand. To account for variations in injectate and body weight, the radioactive content was expressed in units of "uptake", defined as: (Bq per g tissue)/(injected Bq per g body weight), as described by Cremer *et al.* (1992). The animal studies were carried out by licensed investigators in accordance with the *British Council's Guidelines on the Use of Living Animals in Scientific Research*. Animal numbers were kept to a minimum by using only one rat per time point, but with a sufficient number of times to allow satisfactory kinetic analysis of the data. Previous studies using [N-methyl-^{3}H]citalopram showed a standard deviation of approx. 5% using 3–8 animals (Hume *et al.*, 1991).

PET studies

PET scans were carried out using an ECAT 931-08/12 camera (CTI, Knoxville, Tenn., U.S.A.) (Spinks *et al.*, 1988), on 2 normal subjects who gave informed consent. The first subject (male, 35 years) was given a bolus i.v. injection of S-[N-methyl-^{11}C]citalopram either alone (scan 1A) or following treatment with Cipramil (citalopram) (scan 1B). Cipramil (Lundbeck A/S), was given orally: 3 doses of 40 mg over 36 h, the last given 2 h prior to the scan. The second subject (male, 28 years) was scanned with each enantiomer (scans 2A and 2B for the S- and R-isomers, respectively).

The mean injected dose (±SD) for the S-isomer studies was 352 ± 89 MBq (range 255–430 MBq), with a specific radioactivity ranging from 6 to 10 GBq μmol^{-1}, at time of injection. For the R-isomer scan, the injected dose was 136 MBq (sp. act. 2 GBq μmol^{-1}). Sequential scans were collected over a period of 90 min, with continuous arterial sampling. The fractions of labelled plasma metabolites were measured at 4 times during each study, using a modification of the method described by Frost *et al.* (1989). The regions of interest (ROI), listed in Table 2, were defined (12.3 mm dia) with reference both to brain structures thought to be involved in the pathogenesis of depressive illness and also to allow comparison with the tissue dissected in the rat studies. Scan 1A was immediately preceded by a cerebral blood flow (CBF) study, using inhalation of [^{15}O]carbon dioxide, as described elsewhere (Lammertsma *et al.*, 1990).

Data analysis

The *ex vivo* counting data from the rat studies were fitted to a compartmental model, using cerebellum as a reference tissue. The model, schematically illustrated in Fig. 1, provided best estimates for $R_1 (= K_1/K_1')$, k_2, k_3 and binding potential,

Table 2. Regional kinetic analysis of PET studies using a single tissue compartment model with metabolite-corrected plasma input

	Patient and scan*			
	1A	1B	2A	2B
(a) K_1 (±SE)†				
Fronto-temporal cortex‡	0.23 ± 0.01	0.20 ± 0.01	0.27 ± 0.01	0.24 ± 0.01
Striatum	0.23 ± 0.01	0.19 ± 0.01	0.27 ± 0.01	0.22 ± 0.01
Thalamus	0.29 ± 0.01	0.22 ± 0.01	0.29 ± 0.01	0.25 ± 0.01
Brain stem	0.23 ± 0.01	0.18 ± 0.01	0.23 ± 0.01	0.18 ± 0.01
Cerebellum	0.27 ± 0.01	0.23 ± 0.01	0.32 ± 0.01	0.25 ± 0.01
(b) V_d (±SE)†				
Fronto-temporal cortex‡	25 ± 3	27 ± 4	21 ± 2	43 ± 11
Striatum	34 ± 4	41 ± 8	26 ± 2	53 ± 17
Thalamus	35 ± 4	31 ± 6	27 ± 2	34 ± 7
Brain stem	26 ± 3	21 ± 3	21 ± 2	34 ± 11
Cerebellum	24 ± 1	24 ± 1	22 ± 1	29 ± 2

*Scan details are given in Methods.
†SE = standard error of the fitted estimate.
‡The cortex ROI is the average of 2 frontal and 2 temporal ROIs.

BP ($=k_3/k_4$), assuming that the K_1/k_2 ratios are equal between tissues (Cunningham *et al.*, 1991). Errors due to inter-rat variation were reduced since each "specific" datum point had a corresponding (i.e. from the same animal) cerebellar "input".

For the PET studies, time–radioactivity curves were fitted to a single tissue compartment model using a metabolite-corrected plasma input function, with the arterial whole blood curve as a second input function for the estimation of cerebral blood volume within an ROI, essentially as described in Lammertsma *et al.* (1991). The model gave best estimates for K_1 and apparent volume of distribution of the label, $V_d(=K_1/k_2)$.

Results

Rat studies

With the exception of cerebellum, where radioactive content decreased following the initial extraction, all the brain tissues dissected showed some retention or further accumulation of radioactivity with time after injection of *S*-[*N*-methyl-^{11}C]citalopram. Examples of time–radioactivity curves are given in Fig. 2(a), for thalamus and cerebellum. In order to account for regional variations in blood flow, a kinetic modelling approach was used to quantify the binding characteristics, as described in Methods. Tissue values for $R_1(K_1/K_{1\,\text{cerebellum}})$ and BP(k_3/k_4) are listed, rostrocaudally, in Table 1. At 60–120 min after injection of *S*-[*N*-methyl-^{11}C]citalopram, the ratios of counts in dissected tissue relative to cerebellum were constant and mean values over this period showed a highly significant correlation with BP ($P < 0.001$).

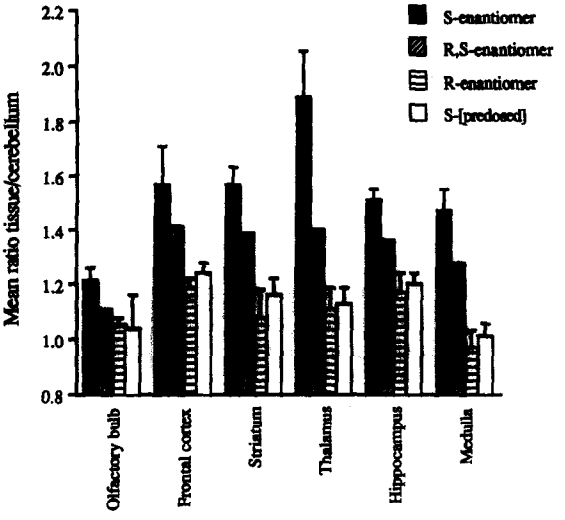

Fig. 3. Mean ratios (with SD) of counts in rat brain tissue relative to cerebellum over the period 60–120 min after injection of either *S*-[^{11}C]citalopram ($n = 4$) or *R*-[^{11}C]citalopram ($n = 4$), or after predosing with citalopram hydrobromide (2 mg kg^{-1} i.v., 10 min prior to injection of radioligand) ($n = 3$). The ratios obtained using *R,S*-[^{11}C]citalopram (60 min only, $n = 2$ with both values shown) were taken from Hume *et al.* (1991).

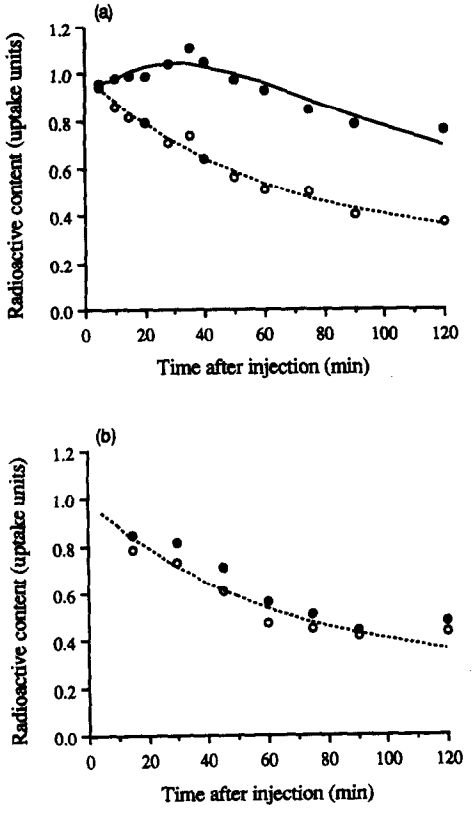

Fig. 2. Radioactive content of thalamus (●) and cerebellum (○) following a tracer i.v. injection of either (a) *S*-[*N*-methyl-^{11}C]citalopram or (b) *R*-[*N*-methyl-^{11}C]citalopram. Each time point represents one rat. In (a), the solid line represents the best fit to the data using the model illustrated in Fig. 1. The dashed line, a multiexponential fit to the cerebellum data, is also shown in (b) for direct comparison.

In contrast to the biodistribution of the *S*-enantiomer, radioactivity was not differentially retained following injection of *R*-[*N*-methyl-^{11}C]-citalopram, as illustrated in Fig. 2(b) for thalamus and cerebellum. The regional variations in the ratios of tissue counts relative to cerebellum at equilibrium (60–120 min) are summarized in Fig. 3, for each enantiomer. Also shown, for comparison, are ratios obtained using racemic [*N*-methyl-^{11}C]citalopram, taken from Hume *et al.* (1991). All regions showed a significant increase in signal (total compared with non-specific binding) for *S*- versus *R*-enantiomer (Student's *t*-test, $P < 0.001$–0.002) and all showed an increase for *S*-enantiomer versus racemate. Predosing the rats with non-radioactive citalopram (2 mg kg^{-1}, i.v. 10 min prior to *S*-[*N*-methyl-^{11}C]citalopram) decreased the ratio to the order of that obtained with the *R*-isomer, in all regions dissected.

PET studies

In contrast to the rat data, the PET scans showed similarly shaped time–radioactivity curves for all selected ROIs. The kinetic parameters, estimated using a single tissue compartment model with

metabolite-corrected plasma input, are presented in Table 2, for each of the scans. The values for the rate constant K_1 correlated closely with CBF ($r^2 = 0.900$), indicating a similar extraction of the radioligand (of approx. 40%) in all of the selected ROIs. The cerebellar V_d was high and was not significantly different from that in other ROIs. The regional distribution of radioactivity was little changed by using the inactive *R*-isomer instead of the *S*-isomer (scan 2B compared with scan 2A) and, under the conditions used, predosing with citalopram (scan 1B compared with scan 1A) did not significantly affect the calculated values of either V_d or K_1 in any region.

Discussion

In the search for a PET radioligand for the 5-HT uptake site in man, several tricyclic and non-tricyclic antidepressants, with various degrees of selectivity, have been evaluated using a combination of *in vitro* and *ex vivo* autoradiography together with tissue counting. Included are radiolabelled cyanoimipramine (Wolfe *et al.*, 1987; Hashimoto *et al.*, 1987), sertraline (Hume *et al.*, 1989), paroxetine (Scheffel and Hartig, 1989; Hume *et al.*, 1989; Hashimoto and Goromaru, 1990a), nitroquipazine (Hashimoto and Goromaru, 1990b) and, more recently, the racemate of citalopram (Hume *et al.*, 1991). However, although *in vitro* autoradiography may identify suitable candidates which are subsequently used as potent and selective ligands to study pathology (e.g. Lawrence *et al.*, 1990; Plenge *et al.*, 1990), a major problem in achieving an adequate specific signal *in vivo* is the retention of a high degree of non-specific label over the relatively short times useful for PET scanning.

Although the present studies and those reported previously (Hume *et al.*, 1991) demonstrated a degree of non-specific radioactivity in rat brain following injection of [^{11}C]citalopram, there was sufficient temporal separation between specific and non-specific binding to give a quantifiable signal at 60 min after radioligand injection (Table 1). It should therefore be possible to further use radiolabelled citalopram as an *in vivo* probe to investigate serotonergic function in animal models. However, even using the biologically active *S*-[*N*-methyl-^{11}C]citalopram, the PET studies showed no specific signal in human brain. There was no marked difference in the shape of time–radioactivity curves for any of the defined ROIs and there was no statistical difference between regional distributions of the *S*- and *R*-isomers of [*N*-methyl-^{11}C]citalopram, supporting the view that the majority of the tissue radioactivity measured in the PET studies was non-specific.

The reasons for the species difference in retention of the non-specific label are not clear but recent studies of racemic [^{3}H]citalopram binding to human compared with rat brain membranes demonstrated a greater degree of non-specific binding relative to total binding in the human membranes, even under *in vitro* conditions (Plenge and Mellerup, 1991). The marked species differences seen *in vivo* may also be related to the slower rate of metabolism of citalopram in man compared with rat (Fredricson-Øverø, 1978).

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