

Radioiodinated SB 207710 as a radioligand in vivo: imaging of brain 5-HT₄ receptors with SPET

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Abstract. Single-photon emission tomography (SPET) and positron emission tomography (PET), when coupled to suitable radioligands, are uniquely powerful for investigating the status of neurotransmitter receptors in vivo. The serotonin subtype-4 (5-HT₄) receptor has discrete and very similar distributions in rodent and primate brain. This receptor population may play a role in normal cognition and memory and is perhaps perturbed in some neuropsychiatric disorders. SB 207710 [(1-butyl-4-piperidinyl-methyl)-8-amino-7-iodo-1,4-benzodioxan-5-carboxylate] is a selective high-affinity antagonist at 5-HT₄ receptors. We explored radioiodinated SB 207710 as a possible radioligand for imaging 5-HT₄ receptors in vivo. Rats were injected intravenously with iodine-125 labelled SB 207710, euthanised at known times and dissected to establish radioactivity content in brain tissues. Radioactivity entered brain but cleared rapidly and to a high extent from blood and plasma. Between 45 and 75 min after injection, the ratios of radioactivity concentration in each of 12 selected brain tissues to that in receptor-poor cerebellum correlated with previous measures of 5-HT₄ receptor den-

sity distribution in vitro. The highest ratio was about 3.4 in striatum. SB 207710 was labelled with iodine-123 by an iododestannylation procedure. A cynomolgus monkey was injected intravenously with [¹²³I]SB 207710 and examined by SPET. Maximal whole brain uptake of radioactivity was 2.3% of the injected dose at 18 min after radioligand injection. Brain images acquired between 9 and 90 min showed high radioactivity uptake in 5-HT₄ receptor-rich regions, such as striatum, and low uptake in receptor-poor cerebellum. At 169 min the ratio of radioactivity concentration in striatum to that in cerebellum was 4.0. In a second SPET experiment, the cynomolgus monkey was pretreated with a selective 5-HT₄ receptor antagonist, SB 204070, at 20 min before [¹²³I]SB 207710 injection. Radioactivity in all brain regions was reduced almost to the level in cerebellum by 176 min after radioligand injection. These findings show that [¹²³I]SB 207710 is an effective radioligand for imaging brain 5-HT₄ receptors in vivo.

Keywords: [¹²³I]SB 207710 – 5-HT₄ receptors – Brain – Imaging – Single-photon emission tomography – Radioligand

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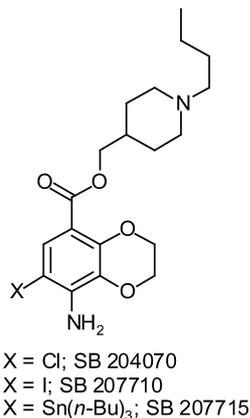
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Introduction

Molecular imaging techniques, such as single-photon emission tomography (SPET) and positron emission to-

Fig. 1. Structures of SB 204070, SB 207710 and SB 207715



mography (PET), when coupled to suitably effective radioligands, can be used to measure regional neurotransmitter receptor concentrations in living subjects and to monitor the receptor binding of unlabelled ligands, whether endogenous (neurotransmitter) or exogenous (e.g. therapeutic or drug of abuse) [1, 2, 3, 4, 5]. This methodology is of general importance for elucidating how various neuropsychiatric disorders unfold in human subjects and for establishing the mechanism and efficacy of drug treatment strategies. Investigation of the serotonergic system, which is strongly implicated in several neuropsychiatric conditions (e.g. depression, anxiety, schizophrenia and Alzheimer's disease) and is a target for several effective drugs (e.g. antidepressants), has until recently been hampered by a lack of effective radioligands for the several serotonin binding sites and receptors [6, 7].

The serotonin subtype-4 (5-HT₄) receptor is well characterised, both structurally and pharmacologically, as a G-protein coupled receptor [8, 9, 10, 11, 12]. It is found abundantly in several organs and tissues, including brain. The distribution of the receptor in rodent [13, 14, 15, 16, 17, 18, 19, 20, 21], monkey [18] and human brain [19, 20, 21, 22, 23, 24, 25, 26] is discrete, being mainly localised to the limbic and striatonigral regions, and is very similar between species. This receptor population is implicated in dopamine, serotonin and acetylcholine release [8, 9, 10, 11, 12, 27, 28] and possibly plays a role in normal cognition and memory [8, 9, 10, 11, 12]. Rather limited investigations of post-mortem human brains have indicated that this receptor population is perturbed in neuropsychiatric disorders, such as Alzheimer's disease [29] and Huntingdon's disease [29], but not in Parkinson's disease [29] or schizophrenia [30]. The validity and significance of these sparse findings might be explored extensively by molecular imaging techniques if an effective radioligand were available for use *in vivo*. SB 207710 [(1-butyl-4-piperidinylmethyl)-8-amino-7-iodo-1,4-benzodioxan-5-carboxylate] (Fig. 1) is a selective antagonist with sub-nM affinity for 5-HT₄ receptors [31, 32]. Iodine-125 labelled SB 207710 has also previously been shown to be an effective radioligand *in vitro*

[26, 33]. In view of these properties of SB 207710, we set out to assess the ability of iodine-123 labelled SB 207710 to act as a radioligand for the SPET imaging of 5-HT₄ receptors *in vivo*.

Materials and methods

Materials. (1-Butyl-4-piperidinylmethyl)-8-amino-7-*tri-n*-butylstannyl-1,4-benzodioxan-5-carboxylate (SB 207715; Fig. 1) and (1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate (SB 204070; Fig. 1) were gifts from SmithKline Beecham Pharmaceuticals (Harlow, UK). A solution of [¹²⁵I]SB 207710 ([¹²⁵I](1-butyl-4-piperidinylmethyl)-8-amino-7-iodo-1,4-benzodioxan-5-carboxylate; specific radioactivity 74 TBq/mmol; 3.7 MBq/ml) in methanol-aqueous buffer (95:5 v/v) was supplied by SmithKline Beecham Pharmaceuticals (prepared by Amersham International plc) and stored at -4°C. [¹²³I]Sodium iodide was obtained from MAP Technologies. Columns for reverse phase high-performance liquid chromatography (HPLC) were purchased from Waters Associates Inc. (μ-Bondapak C18 and Novapak C18) or Phenomenex Ltd (Ultrapak ODS). Acetonitrile and methanol (each HPLC grade) were purchased from Fisons Ltd. All chemicals were from commercial sources and of analytical grade.

Synthesis of SB 207710. A mixture of SB 207715 (100 mg; 0.29 mmol), acetic acid (0.30 ml) and *N*-iodosuccinimide (65 mg; 0.29 mmol) was stirred and kept between 0°C and room temperature (RT) for 3.5 h. Water and ethyl acetate were added and the product extracted into the ethyl acetate layer, dried over anhydrous magnesium sulfate, filtered and then concentrated to give a dark brown oil (20 mg). HPLC purification of this oil on a μ-Bondapak C18 column (300×7.8 mm i.d.) eluted with acetonitrile-water-acetic acid (70:30:1 by vol.) at 3 ml/min gave SB 207710 (10 mg, 14%; retention time, 9.33 min). ¹H-NMR (400 MHz, CD₃CN): δ 7.29 (1H, s), 4.09 (2H, d, *J*=7.1 Hz), 3.40 (4H, d, *J*=6.1 Hz), 2.98 (4H, dd, *J*=10.9, 5.6 Hz), 2.64 (1H, m), 1.95 (2H, t, *J*=6.9 Hz), 1.68 (4H, m), 1.54 (2H, tt, *J*=6.97, 7.3 Hz), 1.32 (2H, tq overlapping, *J*=7.3, 7.4 Hz), 0.91 (3H, *J*=7.4 Hz). MS (CI +ve, NH₃): 475 [M+H]⁺. HRMS: Found 475.107265. [C₁₉H₂₈N₂O₄I]⁺ requires 475.109385.

Radiosynthesis of [¹²³I]SB 207710. SB 207715 (0.1 mg) in methanol (20 μl) was incubated with [¹²³I]sodium iodide solution (10 μl), 0.2 M hydrochloric acid (20 μl) and chloramine-T (1 mg/ml; 20 μl) for 5 min at RT. The reaction was then quenched with acetonitrile-0.01 M phosphoric acid (3:7 v/v; 0.3 ml). This mixture was injected onto a μ-Bondapak C18 column (300×7.8 mm i.d.; 10 μm particle size) eluted at 6 ml/min with acetonitrile-0.01 M phosphoric acid (3:7 v/v) with eluate monitored for absorbance at 254 nm and radioactivity. [¹²³I]SB 207710 (retention time 9.0 min) was formulated for intravenous injection by rotary evaporation to dryness, dissolution in sterile physiological saline and finally sterile filtration (0.22 μm pore size; Millipore Inc.).

Analysis of [¹²³I]SB 207710. The radiochemical purity, chemical purity and specific radioactivity of each batch of [¹²³I]SB 207710 were determined by HPLC on a μ-Bondapak C18 column (300×7.8 mm i.d.; 10 μm particle size) eluted at 6 ml/min with either acetonitrile-0.01 M phosphoric acid (3:7 v/v) (retention time, 9.0 min) or a gradient of 10–70% acetonitrile-0.01 M phosphoric

acid over 6.5 min and then isocratic elution to 9 min (retention time, 7.0 min), with eluate monitored for absorbance at 254 nm and radioactivity.

Animals. Procedures were in strict accordance with recommendations from the EEC (86/909/CEE) for the care and use of laboratory animals. Experiments in rats (adult male Sprague-Dawley; mean±SD of body weight=297±17 g; Harlan Olac Ltd, Bicester, UK) were carried out by licensed investigators in accordance with the UK Home Office's "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986" (HMSO, Feb. 1990). The SPET experiments in cynomolgus monkeys were approved by the Animal Ethics Committee (Northern Stockholm) and performed in Sweden.

Determination of [¹²⁵I]SB 207710 distribution in rats. A known procedure [34] was used to determine the biodistribution of radioactivity in rats following an intravenous injection of radioligand. Briefly, a tail artery and vein were catheterised with the rat under isoflurane anaesthesia. Rats were kept under light restraint after recovery. At about 2 h after catheterisation, each rat was heparinised and 15 min later injected in the tail vein with [¹²⁵I]SB 207710 (~222 kBq; 37 GBq/μmol) in physiological saline (0.25 ml). At graded times after radioligand injection, samples of blood were taken via the tail artery (six per rat). At designated times between 1 and 75 min after radioligand injection, rats were given an intravenous injection of Euthatal and the brains rapidly removed post mortem. Sixteen tissues were dissected, namely olfactory bulb plus tubercles, frontal cortex, anterior cingulate cortex, striatum, septum, parietal cortex, thalamus, hypothalamus, hippocampus, amygdala plus piriform cortex, temporal cortex, superior colliculi, inferior colliculi, entorhinal cortex, medulla with pons and cerebellum. The tissue dissection was based on a published procedure [35]. The amount of radioactivity per gram of brain, blood, or plasma was measured using a gamma counter (LKB Wallac) with automatic correction for physical decay and expressed as a percentage of the total radioactivity injected per rat, i.e., [(cpm per gram tissue/cpm injected) × 100].

Measurement of radioactivity in whole monkey blood and plasma. During a SPET experiment of a cynomolgus monkey injected intravenously with [¹²³I]SB 207710 (see below), arterial/venous blood samples were taken at known times after radioligand injection. The radioactivity concentration in kBq/ml or % injected dose (ID)/g in whole blood and plasma was measured and corrected for background radioactivity and physical decay.

SPET experiments with [¹²³I]SB 207710 in cynomolgus monkeys. A triple-headed gamma camera having a low-energy collimator (Trionix Research Laboratory Inc.) was used. The spatial resolution was about 11 mm full-width at half-maximum at the centre of the field of view. The energy window (20%) was centered on the photo-peak of ¹²³I. During a 360° rotation, 90 views were collected. Images were reconstructed with a slice thickness of 2.2 mm (voxel size: 2.2×2.2×2.2 mm³). Attenuation correction was made numerically by assuming the object shape to be an ellipse and the attenuation coefficient to be uniform (11 mm). Correction for scattered photons was not performed.

A male cynomolgus monkey (8 kg) was anaesthetised with repeat intramuscular injection of a mixture of ketamine (Ketalar, 2.5–5 mg/kg) and xylazine (Rompun, 1–2 mg/kg per hour). The monkey was positioned supine with the head fixed in the scanner to orient the imaging plane parallel to that defined by the meatus

acusticus externus and the lateral angle of the orbita. NCA [¹²³I]SB 207710 (86 MBq) was injected intravenously as a bolus through a cannula into the sural vein. The cannula was flushed with physiological saline (10 ml) after the radioligand injection. Thirteen sequential scans (in the order 3×2.5 min, 3×5 min, 2×7.5 min and 5×22.5 min) were obtained starting immediately after the injection of the radioligand over a period of ~3 h (i.e. with some discontinuities between scan frames). Brain and regional cerebral uptake of radioactivity uptake were corrected for physical decay, taking the midpoints of scan frames to determine elapsed time. The experiment was repeated in the same monkey given the selective and potent 5-HT₄ receptor antagonist, SB 204070 (0.5 mg/kg) [36, 37, 38, 39], intravenously at 20 min before the administration of [¹²³I]SB 207710 (64 MBq). Fourteen sequential scans (in the order 3×2.5 min, 3×5 min, 3×7.5 min and 5×22.5 min) were obtained starting immediately after the injection of the radioligand over a period of ~3 h.

The percentage of injected radioactivity in whole brain at various times, represented by the mid-points of scan frames, was calculated from the radioactivity concentration (C_R) in Bq/ml, the brain volume (V , ~65 ml) and injected radioactivity (R) in Bq, using the relationship:

$$\text{Brain radioactivity uptake (\%)} = 100[(C_R \times V)/R]$$

Regions of interest were drawn manually on the SPET images for the whole brain, the neocortex (frontal, temporal and occipital), the striatum and the cerebellum, according to an atlas of cryosectioned cynomolgus monkey head [40].

Regional radioactivity was calculated for each scan, corrected for physical decay, normalised to an injected dose of 80 MBq and plotted versus time after radioligand injection, represented by the mid-points of scan frames.

Results

Radiosynthesis and analysis of [¹²³I]SB 207710. NCA [¹²³I]SB 207710 was synthesised in useful radioactivities (~100 MBq). Radiochemical yields were 60–90% decay corrected. The radiochemical purity of [¹²³I]SB 207710 on average exceeded 99%. Preparations were free of precursor (SB 207715) and otherwise chemically pure. Formulated [¹²³I]SB 207710 was radiochemically stable over the period of use.

[¹²⁵I]SB 207710 distribution in rats. Blood and plasma showed a rapid loss of radioactivity, to a level of ~0.3% per gram, within 1 min after radioligand injection (Fig. 2). At the earliest time of measurement (~10 s), the radioactivity content in the blood cell fraction (assuming a haematocrit of 40%) was about twice that of plasma. This fell to ~0.1 of plasma counts over the first 5 min after radioligand injection. From 5 to 75 min after radioligand injection, the ratio of radioactivity concentration in whole blood to that in plasma was almost constant, at 0.65±0.02 (Fig. 2).

Only a small proportion of the injected radioactivity was present in brain at 1 min after radioligand injection (~0.3% per gram). There was a positive correlation of the initial regional uptake of [¹²⁵I]SB 207710 with published

[41] blood volume values but not blood flow (data not shown). Following the initial uptake of radioactivity, there was no further accumulation in any sampled brain region, although the rate of radioactivity loss varied regionally and was slower than from the blood cell fraction. Tissue radioactivity concentration as a function of time after radioligand injection is shown in Fig. 3A for four of the sampled tissues, namely receptor-rich striatum, hypothalamus and hippocampus and receptor-poor cerebellum.

The initial ratios of radioactivity concentration in each chosen tissue relative to that in cerebellum depended on regional differences in radioligand extraction, but a “pseudo-equilibrium” was achieved by about 45 min after radioligand injection and was maintained over the following 30 min of the experiment (Fig. 3B). This may be termed the “specific signal”. Mean values for the specific signal in all sampled brain regions are presented in Fig. 4. The highest signal was found in striatum (~3.4).

The correlation between the signal obtained from [125 I]SB 207710 in vivo and measures of 5-HT₄ receptor

density in rat brain obtained with [3 H]GR 113808 in vitro [15] is shown in Fig. 5.

Clearance of radioactivity from monkey blood and plasma. After the intravenous injection of [123 I]SB 207710 into monkey, radioactivity cleared from whole blood and plasma at a similarly slow rate (Fig. 6). The ratio of radioactivity concentration in whole blood to that in plasma was between 0.63 and 0.68 from 11 min after radioligand injection.

SPET brain imaging of [123 I]SB 207710. SPET images of horizontal brain slices, obtained in cynomolgus monkey by summation of the acquired attenuation-corrected data between 9 and 90 min after intravenous injection of [123 I]SB 207710 (86 MBq), revealed high radioactivity uptake in striatum and cortical regions (Fig. 7A) and low radioactivity uptake in cerebellum (Fig. 7B).

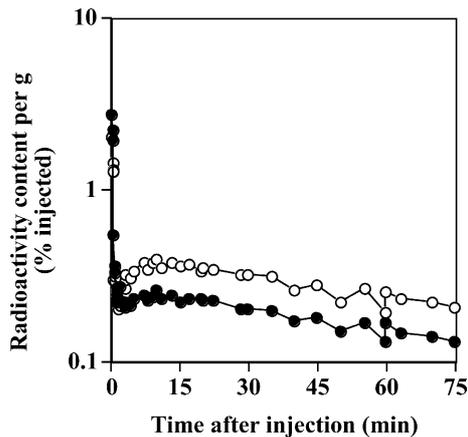


Fig. 2. Radioactivity content (% injected per gram) of blood (●) and plasma (○) as a function of time after intravenous injection of [125 I]SB 207710 into rat. The curve is a composite of data from seven of the ten rats used in the experiment, with six data points from each rat

Fig. 3. A Tissue radioactivity concentration as a function of time after [125 I]SB 207710 injection into rats for brain tissue samples, namely receptor-rich striatum (○), hippocampus (□) and hypothalamus (▲), and receptor-poor cerebellum (●). **B** Tissue to cerebellum radioactivity concentration ratio as a function of time after radioligand injection in rats for striatum (○), hippocampus (□) and hypothalamus (▲)

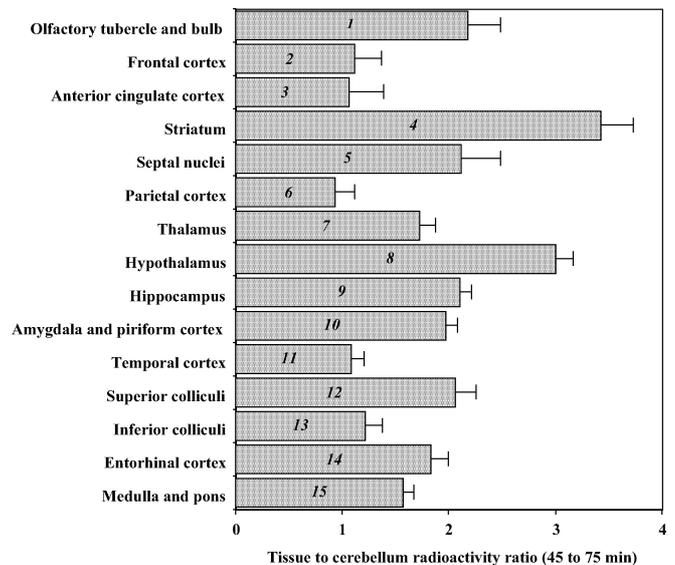
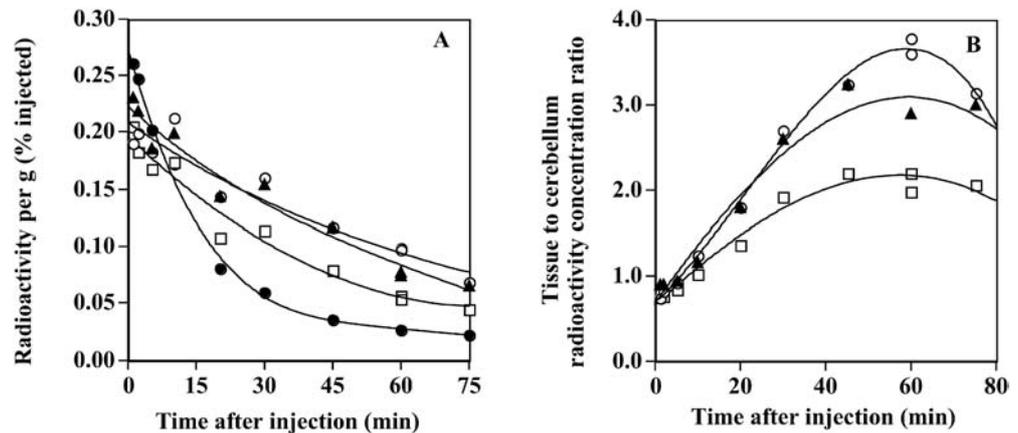


Fig. 4. “Specific signals” (mean tissue to cerebellum radioactivity ratios) for rats killed either 45, 60 (two rats) or 75 min after [125 I]SB 207710 injection. Error bars are \pm SD

$$y = 0.65039 + 1.52136 \cdot 2x \quad R^2 = 0.704$$

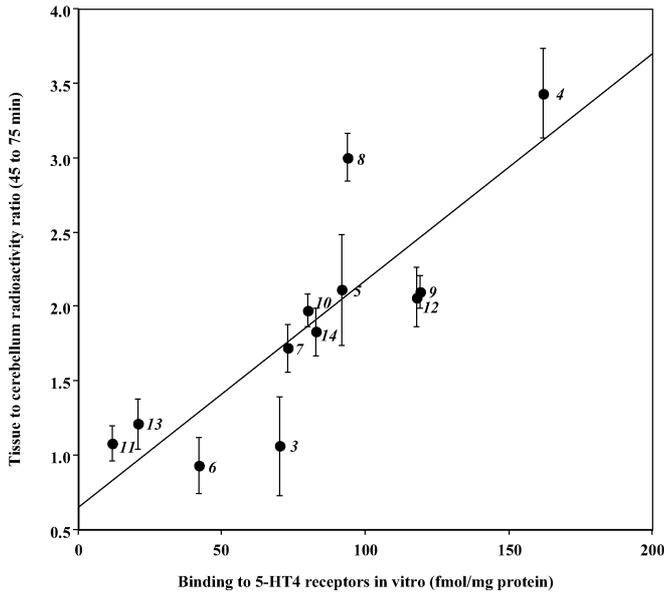


Fig. 5. Correlation between “specific signal” in rat brain in vivo following intravenous injection of [125 I]SB 207710 and the binding measured with autoradiography in rat brain in vitro using [3 H]GR 113808 [15]. Key: 3, anterior cingulate cortex; 4, striatum; 5, septal nuclei; 6, parietal cortex; 7, thalamus; 8, hypothalamus; 9, hippocampus; 10, amygdala and piriform cortex; 11, temporal cortex; 12, superior colliculi; 13, inferior colliculi; 14, entorhinal cortex. (Equation of line: $y=0.65+0.0152x$; $r=0.84$)

In all regions, radioactivity reached a maximum at between 8 and 20 min after radioligand injection and then cleared slowly at a similar rate (Fig. 8A). At 34 min, the ratios of radioactivity concentration in striatum, frontal cortex and temporal cortex to that in receptor-poor cerebellum were 2.75, 2.4 and 2.3, and at 169 min the ratios became 4.0, 3.1 and 2.5, respectively. The whole brain radioactivity uptake maximally reached an average of 0.031 counts/pixel per second at 18.25 min after radioligand injection, representing 2.28% of the administered dose (Fig. 8A).

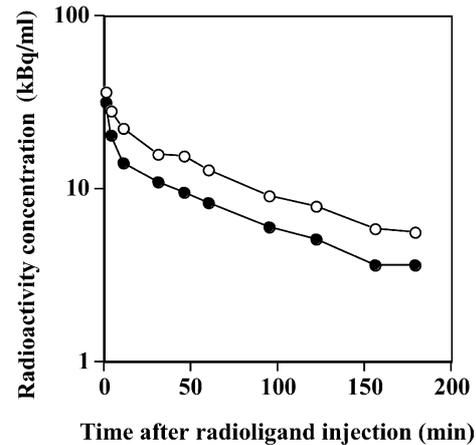


Fig. 6. Clearance of radioactivity (kBq/ml) from cynomolgus monkey whole blood (●) and plasma (○) after intravenous injection of [123 I]SB 207710

Fig. 7A–D. Horizontal SPET images of cynomolgus monkey brain at the levels of the striatum and cerebellum acquired between 9 and 90 min after intravenous injection of [123 I]SB 207710 alone (at level of striatum, A; at level of cerebellum, B) and after pretreatment with SB 204070 (0.5 mg/kg) (at level of striatum, C; at level of cerebellum, D). The front of the brain is uppermost in each image

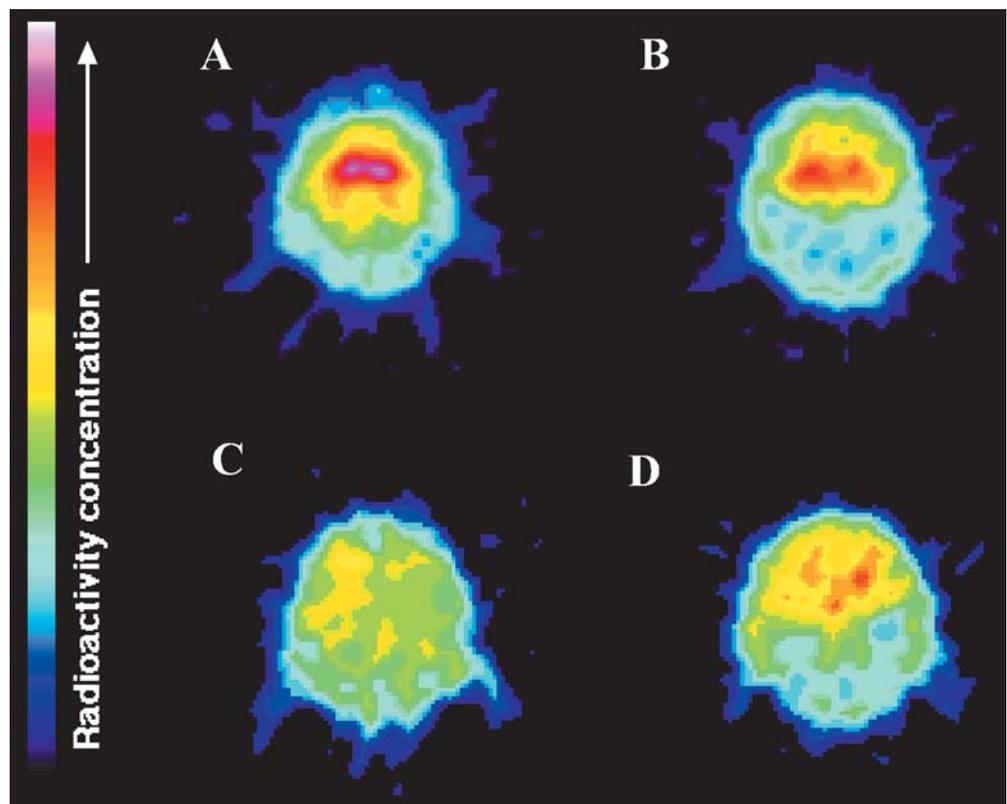
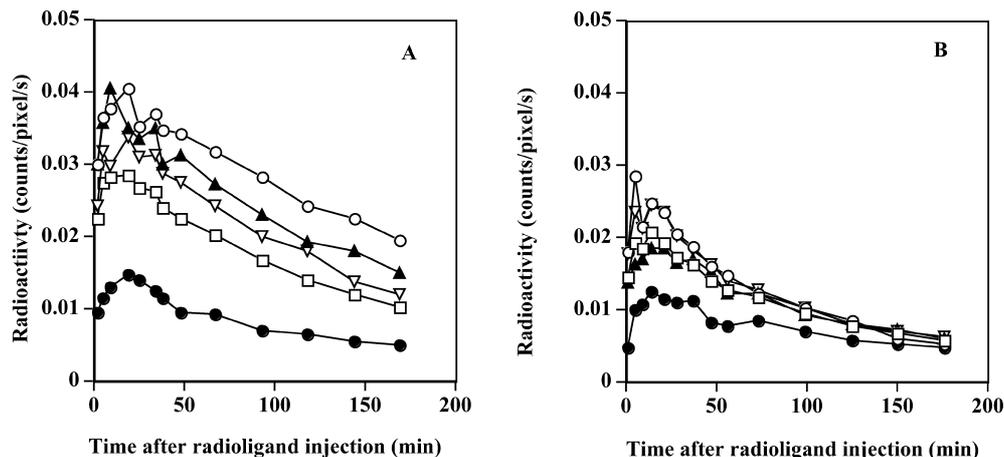


Fig. 8A, B. Time-course of radioactivity in whole brain (□) and in brain regions [striatum (○), frontal cortex (▲), temporal cortex (△) and cerebellum (●)] of a cynomolgus monkey given an intravenous injection of [¹²³I]SB 207710, without pretreatment (A) and beginning at 20 min after an intravenous injection of SB 204070 (0.5 mg/kg) (B). Data are normalised to an injected dose of 80 MBq



In a corresponding experiment, in which the 5-HT₄ receptor antagonist, SB 204070, was administered 20 min before [¹²³I]SB 207710 (64 MBq), horizontal SPET images at striatal and cerebellar levels showed a uniformly low level of radioactivity at 90 min after radioligand injection (Fig. 7C, D). Radioactivity concentrations in all measured brain regions peaked at between 5 and 15 min after radioligand injection and then gradually reduced to a level only marginally above that in cerebellum at the end of the experiment (Fig. 8B). Thus, at 176 min the ratios of radioactivity concentration in striatum, frontal and temporal cortex to that in cerebellum were 1.1, 1.4 and 1.3, respectively. The whole brain radioactivity uptake maximally reached an average of 0.0165 counts/pixel per second at 14.5 min after radioligand injection, representing 1.7% of the administered dose (Fig. 8B).

Discussion

Experiments were initially performed in rats to test the behaviour of the available [¹²⁵I]SB 207710 as a radioligand in vivo. After intravenous injection of [¹²⁵I]SB 207710 into rats, there was a rapid loss of radioactivity from blood within 1 min (Fig. 2). Over the first 5 min there appeared to be a rapid loss of radioactivity from the cell fraction and a gradual recuperation of radioactivity in plasma. Thereafter the ratio of radioactivity concentration in whole blood to plasma was constant at 0.65±0.02 (Fig. 2), indicating insignificant binding of radioactivity to blood cells. The radioactivity concentration in plasma was retained at a relatively high and constant level at ~0.3% of that injected per gram from 10 min after radioligand injection, possibly because of binding of the radioligand to blood proteins. Calculations with the program Pallas 3.0 predict that unionised SB 207710 has a high lipophilicity (logP=4.12), which might lead to high protein binding. The logD (logP at pH 7.4) is, however, predicted to be moderate at 1.77. Only a low percentage of the injected radioactivity was found in rat brain at 1 min after radioligand injection

(Fig. 3). Possible binding of the radioligand to blood proteins might account for this low brain extraction. The early regional distribution of radioactivity in brain correlated strongly with regional blood volume but not with published [41] values for blood flow. In all the sampled brain regions there was no later radioactivity uptake, although the rate of loss of radioactivity was regionally dependent, being greatest from the cerebellum (Fig. 3A).

While there are some 5-HT₄ receptors in the granular layer of rat cerebellum, this tissue is otherwise devoid of 5-HT₄ receptors [15, 16]. The gross samples of cerebellum taken in these experiments are therefore assumed to represent a “reference region” with negligible concentrations of 5-HT₄ receptors [13, 21]. The radioactivity in cerebellum is expected to represent the total non-specific binding of radioligand plus any radioactive metabolites. Relative to cerebellum, rat striatum and hypothalamus are highly abundant in 5-HT₄ receptors [15, 16]. The clearance of radioactivity from these regions was significantly slower than from cerebellum (Fig. 3A) and resulted in appreciable ratios of radioactivity concentration in these regions relative to that in cerebellum (Fig. 3B). The remainder of tissues sampled showed graded specific binding (Fig. 4) with the magnitude of these ratios (averaged between 45 and 75 min) correlating strongly with the reported [15] levels of 5-HT₄ receptors, as determined in vitro with the 5-HT₄ receptor-selective ligand, [³H]GR 113808 (Fig. 5). The high signals observed in striatum, hypothalamus and hippocampus and the low signals in frontal and temporal cortex are generally consistent with in vitro findings on the regional distribution of 5-HT₄ receptors in rat brain [13, 14, 18]. These results show that [¹²⁵I]SB 207710 acts as an effective radioligand for 5-HT₄ receptors in rat brain in vivo and suggested to us the need to prepare [¹²³I]SB 207710 for evaluation as a SPET radioligand in a primate.

Chloramine-T-mediated radioiodostannylation of SB 205177 gave [¹²³I]SB 207710 as a single major product, which was easily separated by HPLC. Its identity was verified by analytical HPLC, calibrated with authentic

SB 207710. Radiochemical yields of 60–90% were observed for the single-pot process.

After intravenous injection of NCA [^{123}I]SB 207710 into a cynomolgus monkey, radioactivity cleared sharply from whole blood and plasma in the first 10 min and thereafter more gradually to a relatively low level at 180 min (Fig. 6). The ratio of whole blood radioactivity to plasma radioactivity was between 0.63 and 0.68 from 11 min after injection, indicating no significant binding to blood cells (Fig. 6). Although the appearance of radioactive metabolites in plasma has not yet been studied thoroughly, in a preliminary experiment we were able to detect the rapid appearance of polar radioactive metabolites by radio-HPLC. Such polar compounds would not be expected to enter brain to any great extent.

Horizontal SPET images at descending levels through the brain, constructed from the sum data acquired between 9 and 90 min after radioligand injection, showed relatively high uptake in striatum and in frontal and temporal cortex and low uptake in occipital cortex and cerebellum (Fig. 7). These images are consistent with determinations of the distribution of 5-HT₄ receptors in other primates in vitro, such as the pig-tail macaque monkey [17] and human [19, 20, 21, 22, 23, 24, 25, 26]. High concentrations of 5-HT₄ receptors have been found in primate striatum and almost none in cerebellum. The human brain, unlike the rat brain, contains significant concentrations of 5-HT₄ receptors in frontal cortex [24, 26]. In vitro autoradiography of whole human brain hemispheres with two selective radioligands has also revealed a distinct laminar distribution of 5-HT₄ receptors throughout the entire cortex [24, 26].

In this SPET experiment, radioactivity entered the whole brain to a small extent (maximally 2.3% of the injected dose at 18 min) (Fig. 8A). The regional kinetic data showed that maximal radioactivity uptake in all examined regions occurred between 8 and 20 min. The maximal concentration of radioactivity in cerebellum was less than the maximal concentration in striatum or cortical regions and fell to a low level by the end of the experiment. Substantially higher radioactivity concentrations were retained in putatively 5-HT₄ receptor-rich striatum, frontal cortex and temporal cortex than in cerebellum. The ratio of radioactivity concentration in striatum to that in cerebellum was about 3 soon after radioligand injection and slowly reached 4 at 169 min after injection. This pattern was also apparent for other brain regions, such as frontal and temporal cortex, though ratios at 169 min were somewhat lower (Fig. 8A). The higher ratio in striatum relative to that in cortical areas is consistent with the expected higher concentration of 5-HT₄ receptors in this tissue. The high ratios soon after injection may indicate different levels of non-specific binding between cerebellum and other regions and may invalidate the use of cerebellum as a reference region for brain non-specific binding.

In a second SPET experiment, in which the same cynomolgus monkey was pre-dosed with the highly selec-

tive 5-HT₄ receptor antagonist, SB 204070 (0.5 mg/kg), at 20 min before intravenous injection of [^{123}I]SB 207710, scans acquired between 9 and 90 min after injection showed lower radioactivity content with a less discrete distribution (Fig. 7C, D). Radioactivity concentrations in all measured brain regions, including striatum, frontal cortex and temporal cortex, were reduced almost to the level in cerebellum by 176 min from radioligand injection (Fig. 8B). The pretreatment had little effect on the kinetics of radioactivity in cerebellum. These data show that SB 204070 blocks the retention of radioactivity in putatively 5-HT₄ receptor-rich regions of cynomolgus monkey brain. They confirm the 5-HT₄ receptor selectivity of [^{123}I]SB 207710 binding to striatum, frontal cortex and temporal cortex of monkey brain in vivo. The maximal uptake of radioactivity in whole brain (1.7% of ID at 14.5 min) was appreciably lower in this experiment than in the radioligand alone experiment and is consistent with a lower level of receptor-specific binding at this time.

In summary, radioiodinated SB 207710 behaves as an effective radioligand for 5-HT₄ receptors in rats and monkey in vivo, giving a sizeable signal in 5-HT₄ receptor-rich regions. In cynomolgus monkey, the potent and selective antagonist SB 204070 blocks the signal. Thus, [^{123}I]SB 207710 warrants further evaluation as a radioligand for SPET studies of 5-HT₄ receptors in human brain. Analogues of SB 207710 may also serve for the development of candidate radioligands for PET imaging of brain 5-HT₄ receptors, as recently demonstrated [42]. They show the feasibility of imaging 5-HT₄ receptors in vivo. Ultimately, radioligands with better characteristics for SPET and PET imaging (e.g. radioligands with higher brain penetration and higher signal) may be required.

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