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Autoradiographic Localization of 5-HT_{2A} Receptors in the Human Brain Using [^{3}H]M100907 and [^{11}C]M100907

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KEY WORDS 5-HT_{2A} receptors; [^{3}H]M100907; [^{11}C]M100907; human brain; whole hemisphere autoradiography

ABSTRACT M100907 (MDL 100907, R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol) is a new selective antagonist of 5-HT_{2A} receptors. The compound has been labeled with ^{11}C and proved useful for in vivo studies of 5-HT_{2A} receptors using positron emission tomography (PET). In the present study the distribution of 5-HT_{2A} receptors was examined in the postmortem human brain using whole hemisphere autoradiography and [^{3}H]M100907 and [^{11}C]M100907. The autoradiograms showed very dense binding to all neocortical regions, whereas the hippocampus was only weakly labeled with [^{3}H]M100907. Other central brain regions, such as the basal ganglia and thalamus, showed low [^{3}H]M100907 binding, reflecting low densities of 5-HT_{2A} receptors. The cerebellum or structures of the brain stem were virtually devoid of 5-HT_{2A} receptors. [^{11}C]M100907 gave images qualitatively similar to those of [^{3}H]M100907, although with lower spatial resolution. The labeling of human 5-HT_{2A} receptors with [^{3}H]M100907 was inhibited by the addition of the 5-HT_{2A} receptor blockers ketanserin or SCH 23390 (10 μM), leaving a very low background of nonspecific binding. The 5-HT_{1A} receptor antagonist WAY-100635 and the D_2-dopamine receptor antagonist raclopride had no effect on the binding of [^{3}H]M100907. The selective labeling of 5-HT_{2A} receptors with [^{3}H]M100907 clearly shows that this compound is suitable for further studies of the human 5-HT_{2A} receptor subtype in vitro. The in vitro autoradiography of the distribution of 5-HT_{2A} receptors obtained with radiolabeled M100907 provides detailed qualitative and quantitative information on the distribution of 5-HT_{2A}-receptors in the human brain as well as reference information for the interpretation of previous initial results at much lower resolution in humans in vivo with PET and [^{11}C]M100907. **Synapse 38:421–431, 2000.**

INTRODUCTION

In addition to the well-established pharmacological connection between dopamine and schizophrenia, the involvement of serotonin in schizophrenia has received considerable attention during the last decade (Ohuoha et al., 1993; Breier, 1995). This is mainly due to the unique pharmacological profile of the atypical antipsychotic clozapine, which has a higher affinity for 5-HT_2 receptors than for D_2-dopamine receptors (Meltzer et al., 1989; Nordström et al., 1993; Meltzer, 1994) which are normally considered the site of action for antipsychotic drugs. The high affinity of clozapine for 5-HT_{2A} receptors has been suggested to contribute to its reduced side effect liability and its efficacy in therapy-resistant schizophrenia (Meltzer et al., 1989; Schmidt et al., 1995). This view is supported by recent in vivo studies using positron emission tomography (PET), which have revealed that antipsychotics like clozapine and risperidone block a significant proportion of 5-HT_2 receptor binding (Leysen et al., 1978; Nordström et al., 1993, 1995; Nyberg et al., 1993; Farde et al., 1995). The involvement of serotonin in the pathophysiology of schizophrenia has been corroborated by findings in

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TABLE I. Binding properties of M100907

Ki for 5-HT_{2A} receptor (nM)	0.85 nM[1]	(Kehne et al., 1996)
Ki for 5-HT_{2A} receptor (nM)	1.00 nM;[2] 0.70 nM[3]	(Johnson et al., 1996)
Kd for 5-HT_2 receptor (nM)	0.56 ± 0.05 nM	(Johnson et al., 1996)
Ki for 5-HT_{2C} receptor (nM)	88 nM	(Kehne et al., 1996)
Ki for other receptors (nM)	Sigma: 87 nM, α_1: 128 nM, dopamine > 500 nM	(Kehne et al., 1996)

[1]Mean of Ki determinations using [^{3}H]ketanserin (various tissues) and [^{125}I]d-LSD.
[2]Vs. [^{3}H]M100907.
[3]Vs. [^{3}H]ketanserin.

postmortem schizophrenic brains, where changes in 5-HT receptor densities have been shown in specific brain regions. Thus, 5-HT_{1A} receptor binding has been found to be increased in the prefrontal cortex in deceased schizophrenic patients (Hashimoto et al., 1993; Sumiyoshi et al., 1996) as well as in posterior cingulate, motor cortex, and hippocampus (Joyce et al., 1993). However, with regard to the 5-HT_2 receptors, the findings are so far inconsistent, as both increases (Joyce et al., 1993) and decreases (Dean and Hayes, 1996) in the 5-HT_2 receptor density have been reported in neocortices from schizophrenic patients.

M100907 (R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (previously referred to as MDL-100907) is a newly developed, highly potent, and selective 5-HT_{2A} receptor antagonist (Sorensen et al., 1993; Kehne et al., 1996). The affinity of M100907 for the 5-HT_{2A} receptor is subnanomolar (Sorensen et al., 1993; Kehne et al., 1996) and has, in contrast to previous 5-HT_2 receptor ligands, at least 300-fold lower affinity for other receptor types, including the 5-HT_{2C}, α_1-adrenergic and D_2-dopamine receptors (Sorensen et al., 1992) (Table I). Based on a number of animal studies (Sorensen et al., 1993; Carlsson, 1995; Maurel-Remy et al., 1995; Schmidt and Fadayel, 1995; Schmidt et al., 1995; Kehne et al., 1996; Moser et al., 1996; Padich et al., 1996), M100907 was suggested to have antipsychotic properties and was therefore tested in initial clinical trials (Brunner et al., 1998). However, so far no conclusive antipsychotic effects have been published. This compound has also been labeled with tritium ([^{3}H]M100907) to a high specific radioactivity (Johnson et al., 1996). Characterization of [^{3}H]M100907 binding on rat frontal cortex homogenates confirms the high affinity (K_d = 0.56 nM) and selectivity shown with the unlabeled compound (Johnson et al., 1996). [^{3}H]M100907 has also been shown to be suitable for autoradiographic studies of 5-HT_{2A} receptor distribution in the rat (Lopez-Gimenez et al., 1997) and human brain (Lopez-Gimenez et al., 1998). [^{11}C]M100907 has also been used for studies of 5-HT_{2A} receptors in vivo with PET, both in the monkey (Lundkvist et al., 1996), as well as in the human brain (Ito et al., 1998; Talvik-Lotfi et al., 2000).

Autoradiography using postmortem human brain whole hemisphere cryosections provides a high image resolution (about 50 μm) and is therefore very suitable for the detailed microanatomical description of neuroreceptor distributions (Sedvall, 1990; Persson et al., 1991; Farde and Hall, 1992; Hall et al., 1998). In the present study, we present the detailed distribution of 5-HT_{2A} receptor in postmortem human brain using whole hemisphere autoradiography with the new radioligand [^{3}H]M100907. The pharmacological characteristics of [^{3}H]M100907 binding on whole hemisphere autoradiography was also examined by competition studies. For direct comparison with PET (Farde and Hall, 1992; Sedvall et al., 1995), M100907 was also labeled with the short-lived isotope ^{11}C and used in whole hemisphere autoradiography.

Abbreviations:

5-HT	5-hydroxytryptamine
DOI	(2,5-dimethoxy-4-iodophenyl)2-aminopropane
M100907	R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidinemethanol
PET	positron emission tomography
SCH 23390	R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
WAY-100635	*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride.

MATERIALS AND METHODS

Human brain tissue

Human brains were obtained from clinical autopsy at the National Institute of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden. The study was approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. Whole hemispheres, removed at clinical autopsy (see Table II for details) were sectioned using a heavy-duty cryomicrotome (LKB 2250, LKB, Stockholm, Sweden) into 100 μm horizontal (canto-meatal) or coronal tissue sections which were transferred to gelatinized or poly-L-lysine-treated glass plates (10 × 22 cm). The sections were allowed to dry before they were stored at −25°C with dehydrating agents until use.

Autoradiography

The autoradiographic procedure was essentially as described previously (Persson et al., 1991; Hall et al., 1998). The incubation solution containing [^{3}H]M100907 (approximately 2 nM, range 1.84–2.24 nM) was applied to the sections (total volume about 14 ml, in Tris-HCl buffer pH 7.7, 0.05 M, containing 0.1% (weight/vol) ascorbic

TABLE II. Human subjects

No.	Age (years)	Hemisphere	Sex	Postmortem time (hours)	Cause of death
41.	54	Right	Female	24	Pulmonary embolism
52.	62	Right	Male	21.5	Heart infarction
60.	46	Right	Male	43	Accidentally shot during hunting
62.	53	Right	Male	15	Heart infarction
81.	40	Right	Female	?	Heart infarction

None of the brains exhibited damages, abnormalities, or neurological features.

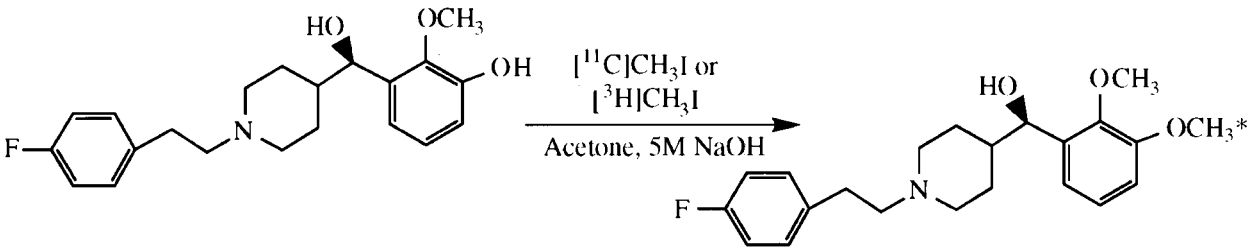

Fig. 1. Schemes for the syntheses of [^{3}H]- and [^{11}C]M100907. The asterisk denotes the position of radiolabeling.

acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$). The incubations lasted 60 min at room temperature. Washing was performed with cold buffer for 2 × 10 min, followed by a brief cold wash by dipping the sections into distilled water. The sections were dried on a warm plate and were then exposed to tritium-sensitive film (Amersham, ^{3}Hyperfilm) for 4 weeks before development (developer: Kodak D19, fixation: Kodak Fixer 3000).

For autoradiography using [^{11}C]M100907, six cryosections were mounted separately into a specially designed incubation chamber, which was kept behind lead bricks to minimize radiation exposure to personnel (Persson et al., 1991). The cryosections were incubated with 200 ml radioligand per section in Tris-HCl buffer pH 7.7, 0.05 M, containing 0.1% (weight/vol) ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ for 20 min at room temperature with different radioligand concentrations (3.7–7.5 nM, 3–6 μCi/ml at the beginning of the incubation). Although a longer incubation time is required to reach steady-state, a short incubation time is necessary for short-lived tracers like [^{11}C]M100907 (T½ of ^{11}C = 20 min), so that a higher level of radioactivity is present at the time of exposure and, thus, a better image can be achieved. After the incubation, the radioactivity was pumped out of the incubation chambers and the sections were rinsed twice for 2 min in cold buffer. The sections were then rapidly rinsed in distilled water and then placed on a warm plate in a warm, dry airflow to facilitate rapid drying. The sections were exposed to β-sensitive film (Hyperfilm-β-max, Amersham) overnight before development (developer: Kodak D19, fixation: Kodak Fixer 3000).

In competition experiments using either [^{3}H]M100907 or [^{11}C]M100907, the competing substance was added to the incubation fluid. The autoradiograms were digitized using Microtek E6 high resolution scanner connected to a Power Macintosh G3. Softwares: Photoshop 4.0 (Adobe, USA), Image 1.61 (NIH, USA) and Microsoft Power Point 4.0.

Quantitative determinations of the density of [^{3}H]M100907 binding was performed in several human brain regions. The obtained pixel values were transformed into radioactivity values and to binding density (fmol/mg original wet weight) using ^{3}H-calibrating scales (Microscales, Amersham, UK, and American Radiolabeled Chemicals Inc., St. Louis, MO, USA) without any correction for thickness, as tissue thicker than 5 μm is infinitely thick with regard to tritium β-radiation (Geary and Wooten, 1983). The calculations of approximate IC_{50}-values were performed using a rectangular hyperbolic function with KaleidaGraph™ (Synergy). Parallel, adjacent sections were stained with cresyl violet to serve as anatomical correlates for the autoradiograms.

Compounds

Synthesis of [^{3}H]M100907 and [^{11}C]M100907

[^{3}H]M100907. [^{3}H]Methyl iodide was used for labeling of the 3-methoxy position of [^{3}H]M100907 (Fig. 1). [^{3}H]Methyl iodide (≈1 mCi, 100 μl in toluene) was added to a reaction vial (1.0 ml mini-vial, Alltech) containing the 3-hydroxy precursor (MDL 105725, 1.0 mg), acetone (400 μl), and sodium hydroxide (5 M, 2 μl). The vial was sealed and heated at 80°C for about 15 min. Mobile phase (600 μl) was added before injection into the semipreparative normal-phased HPLC column. The column was eluted with dichloromethane/methanol/triethylamine (96/4/0.04 by vol.) at a flow rate of 2 ml/min. [^{3}H]M100907 eluted after 10–12 min with a retention time identical to a reference sample. The

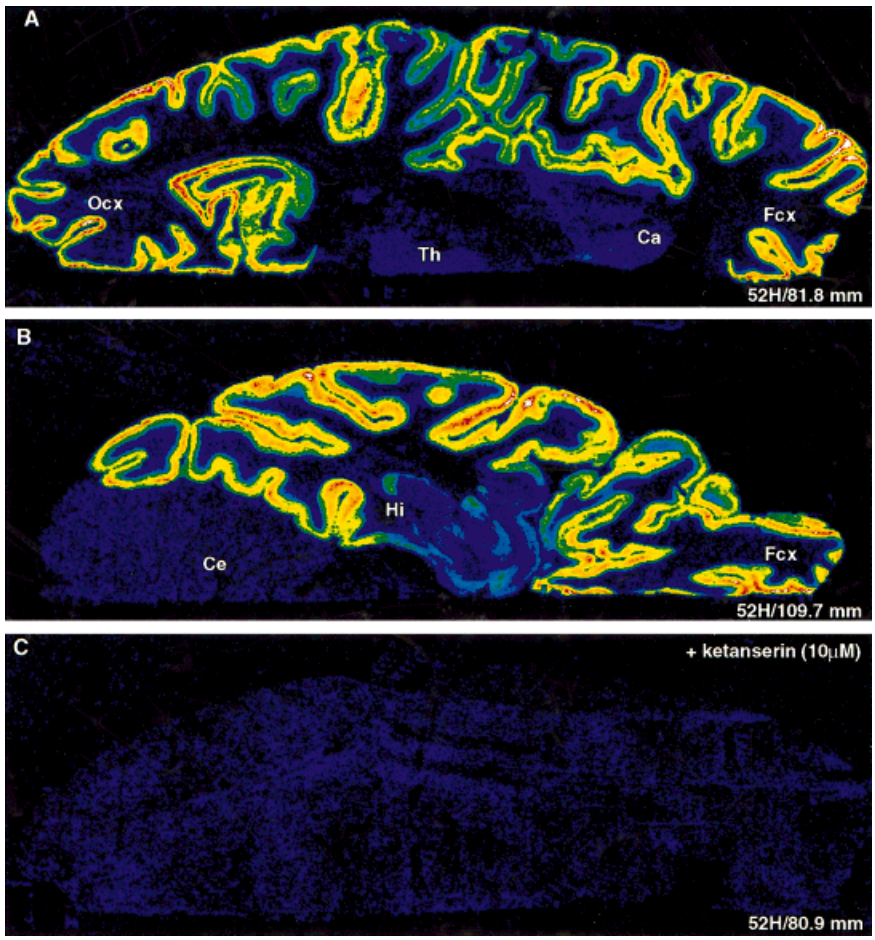

Fig. 2. Color-coded whole hemisphere autoradiograms showing binding of [^{3}H]M100907 to 5-HT$_{2A}$ receptors in two horizontal levels of the human brain. **2C** shows nonspecific binding in the presence of 10 μM ketanserin. General legend to the autoradiograms. In the horizontal autoradiograms, the frontal lobe is facing right. Number in lower right corner is internal brain number and distance from vertex in mm. Figure coding, referring to all figures: Ac, accumbens; Am, amygdala; Ca, caudate nucleus; Ce, Cerebellum; Cix, cortex cingulum, posterior; Cl, claustrum; Dg, hippocampus, dentate gyrus; Ecx, cortex entorhinalis; Fcx, cortex frontalis; Hi, hippocampus; Icx, cortex insularis; Ocx, cortex occipitalis; Pcx, cortex parietalis; Phi, parahippocampus; Po, pons; Pu, putamen; Sp, septum; Tcx, cortex temporalis; Th, thalamus.

collected fraction was evaporated and dissolved in 70% ethanol and stored at −4°C. The radiochemical yield of [^{3}H]M100907 was 40% with a radiochemical purity >98%. The specific radioactivity was 70 Ci/mmol (2.6 GBq/μmol).

[^{11}C]M100907 was labeled according to Lundkvist et al. (1996) in its 3-methoxy position using [^{11}C]methyl iodide as the labeling agent (Fig. 1). [^{11}C]Methyl iodide was trapped at room temperature in a reaction vial (1.0 ml mini-vial, Alltech), containing the 3-hydroxy precursor (MDL 105725, 0.5 mg), acetone (400 μl), and sodium hydroxide (5 M, 2 μl). The vial was sealed and heated at 80°C for 3 min. The crude product was purified with preparative normal-phased HPLC as described above. After evaporation of the mobile phase, the residue was formulated in 8 ml sterile phosphate buffered saline (pH = 7.4) and subsequently used for the autoradiographic studies. The incorporation of [^{11}C]methyl iodide to [^{11}C]M100907 was 60–80%. The total radiochemical yield of [^{11}C]M100907, calculated from end of bombardment and decay-corrected, was 40–50% with a total synthesis time of 30 min. Purification was performed by semipreparative normal-phase HPLC yielding [^{11}C]M100907 with a radiochemical purity better than 99%. The specific radioactivity of [^{11}C]M100907 obtained at time of administration was 500–1,000 Ci/mmol (18–37 GBq/μmol).

Other compounds

Ketanserin (a predominantly 5-HT$_{2A}$ receptor antagonist) was obtained from Janssen Pharmaceutica (Beerse, Belgium), M100907 and the precursor (MDL 105725) from Marion Merrell Dow, Kansas City, MO, USA (Dr. A.A. Carr), raclopride (a D$_2$/D$_3$-dopamine receptor antagonist) from Astra Arcus AB, Södertälje, Sweden, SCH 23390 (a D$_1$-dopamine and 5-HT$_2$ receptor antagonist) from Schering Plough, USA, and WAY-100635 (a selective 5-HT$_{1A}$ receptor antagonist) from Dr. I. Cliffe, Cerebrus Ltd., UK. Other chemicals were obtained from commercial sources and were of analytical grade whenever possible.

RESULTS

Distribution of [^{3}H]M100907 binding

General

[^{3}H]M100907 binding had a widespread distribution in the human brain, with highest binding densities in the neocortex (Fig. 2). The density of [^{3}H]M100907 binding was slightly higher in the frontal parts, as

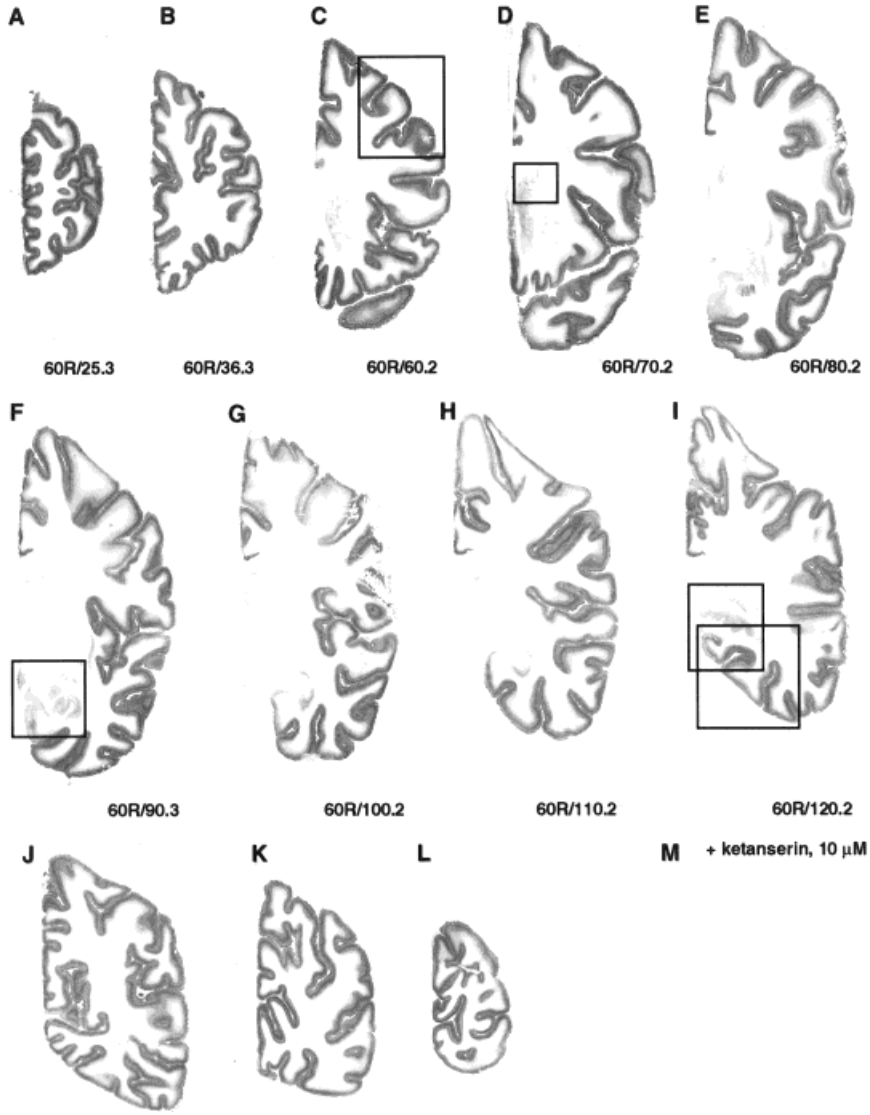

Fig. 3. Whole hemisphere autoradiograms showing binding of [^{3}H]M100907 in coronal sections of the human brain. The inset squares indicate the position of details shown in Figures 4–6.

visualized in the series of coronal sections (Fig. 3). Much lower densities of binding were seen in the central parts of brain. The binding of [^{3}H]M100907 in all brain regions was totally abolished by the excess (10 μM) addition of the 5-HT$_2$ receptor antagonist ketanserin (Figs. 2C, 3M, 6B).

Neocortex

[^{3}H]M100907 labeled the 5-HT$_{2A}$ receptors in all neocortical regions with no marked differences in binding between the regions. [^{3}H]M100907 binding in all cortical regions was seen mainly in three layers (Figs. 3, 4), where the highest binding was in medial layers (probably corresponding to layer III) and in a thin superficial layer (layer I). Weaker density of [^{3}H]M100907 binding was also seen in layer V. The density was slightly higher in the occipital cortex than in the adjacent posterior cingulate cortex (Fig. 4B). The claustrum was relatively intensely labeled with [^{3}H]M100907 (Fig. 4C).

Archicortex

The hippocampal formation was relatively weakly labeled with [^{3}H]M100907 (Fig. 5). The CA-regions of hippocampus had the highest density of binding, whereas the dentate gyrus was almost devoid of labeling. The parahippocampus and the entorhinal cortex were less densely labeled than the adjacent neocortical regions. The amygdala was heterogeneously labeled with [^{3}H]M100907, with a markedly higher density in the basal and in the lateral nuclei than in the medial and ventral nuclei (Fig. 5A–C).

Central nuclei

[^{3}H]M100907 binding was very low in basal ganglia, thalamus, and related regions. However, despite this low density patches of weak [^{3}H]M100907 binding could be seen in the caudate nucleus (Fig. 6). This patchy distribution was more distinct in the ventral

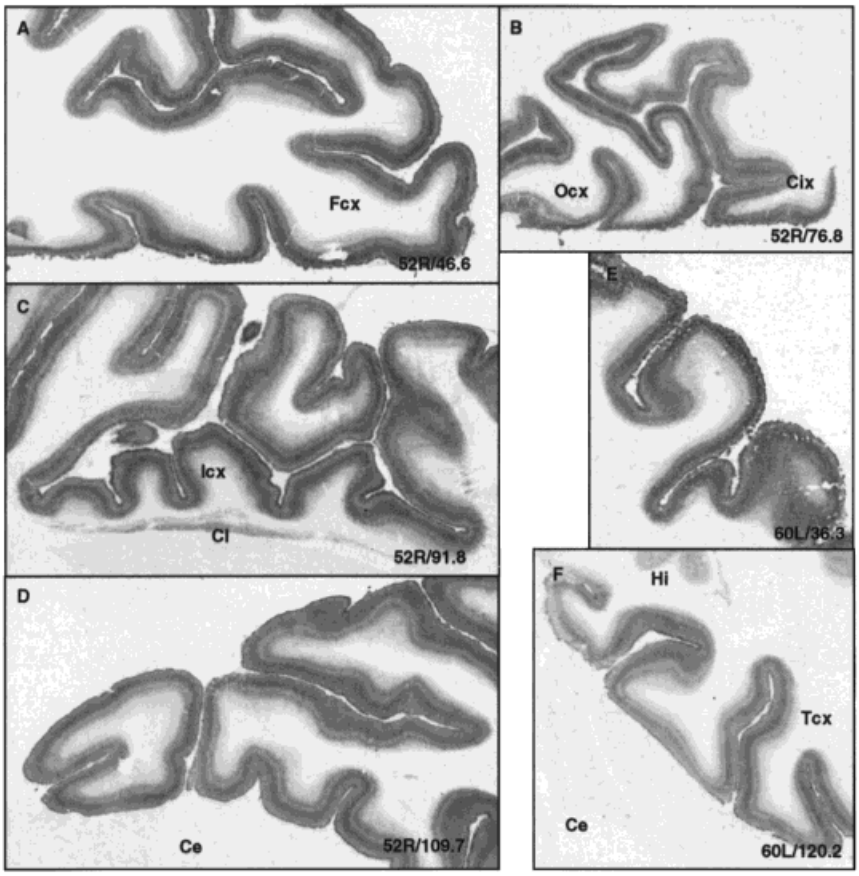

Fig. 4. Details of whole hemisphere autoradiograms showing binding of [^{3}H]M100907 in various neocortical regions. **A:** frontal cortex and anterior cingulate cortex. **B:** Occipital cortex and posterior cingulate cortex. **C:** Insular cortex. **D:** Occipital cortex. **E:** Parietal cortex. **F:** Temporal cortex. The position of **E** and **F** is shown in Figures 3E and 3I, respectively.

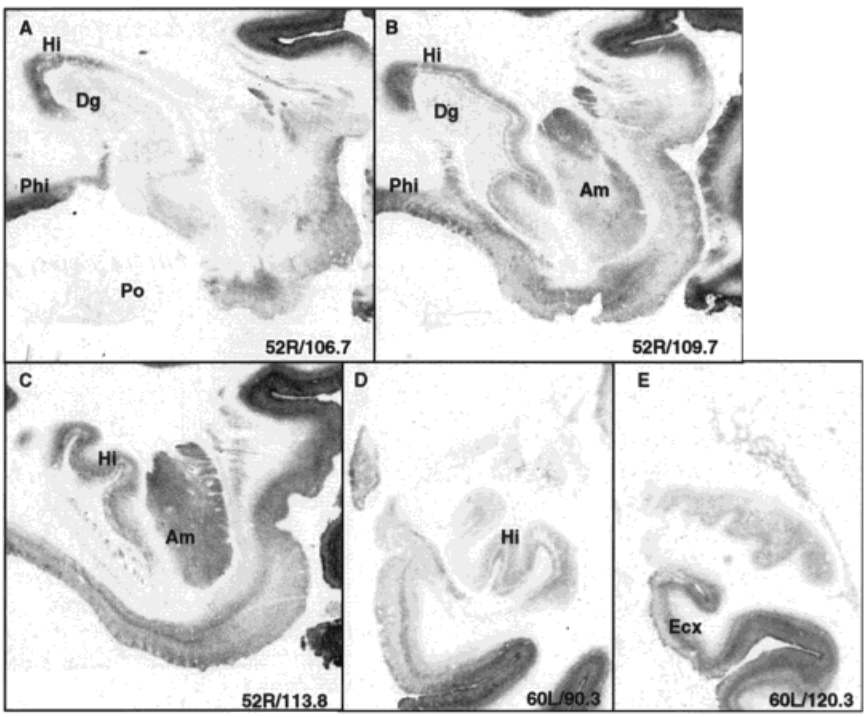

Fig. 5. Details of whole hemisphere autoradiograms showing binding of [^{3}H]M100907 to central brain regions including hippocampus, amygdala. The position of **D** and **E** is shown in Figures 3F and 3I, respectively.

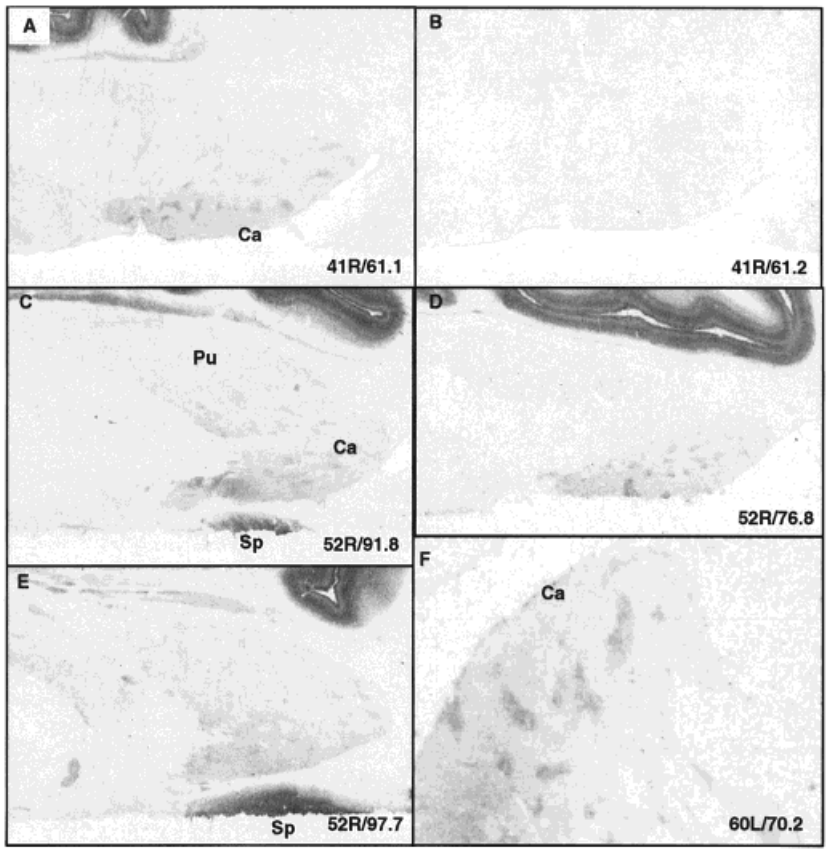

Fig. 6. Details of whole hemisphere autoradiograms showing binding of [^{3}H]M100907 in basal ganglia. The position of **F** is shown in Figure 3D.

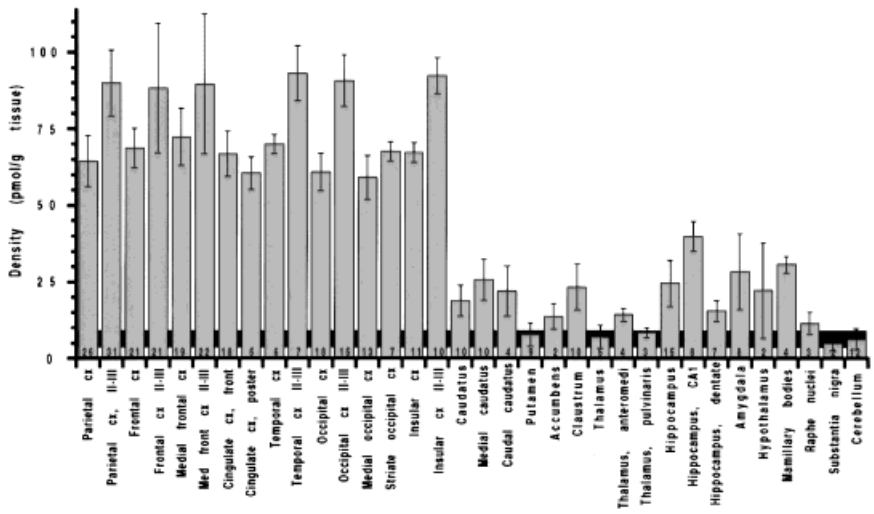

Fig. 7. Quantification of [^{3}H]M100907 binding to various brain regions as determined from whole hemisphere autoradiography showing binding density, expressed in fmol/mg original wet weight, at approximately 2 nM [^{3}H]M100907. Mean (± SEM) density of [^{3}H]M100907 binding to various brain regions as determined from whole hemisphere autoradiography. The quantifications were performed on 23 images from two brains. The mean (± SEM) nonspecific binding (two determinations on 13 regions) is shown as a horizontal line (± SEM is shown as a shaded area).

parts of caudate and in the nucleus accumbens, but were only barely visible in the ventral putamen. Ketanserin (10 μM) totally blocked the binding in these patches.

Other regions

In the brain stem, no specific [^{3}H]M100907 binding was seen in the raphe nuclei. The cerebellum was virtually devoid of 5-HT$_{2A}$ receptors in all levels (Figs. 3H–K, 4D,F). The cerebellar nuclei also failed to demonstrate any specific binding of [^{3}H]M100907.

Quantification of [^{3}H]M100907 binding

Density of [^{3}H]M100907 binding

The binding of [^{3}H]M100907 was quantified in a number of different brain regions of the different brains by transformation of the film blackness into radioactivity values and to binding density, expressed

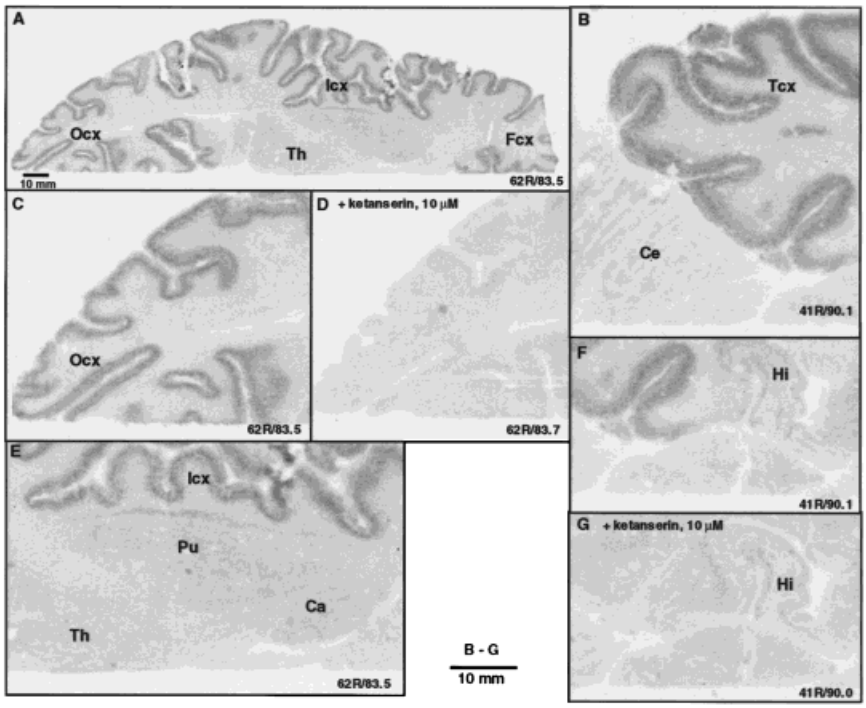

Fig. 8. Whole hemisphere autoradiograms showing binding of [^{11}C]M100907 in horizontal sections of the human brain. **A:** Whole hemisphere in the level of caudate and putamen. **D** and **G** show nonspecific binding in the presence of 10 μM ketanserin.

in fmol/mg original wet weight, at approximately 2 nM [^{3}H]M100907 using ^{3}H-calibrating microscales (Fig. 7). The nonspecific binding, as determined in the presence of ketanserin, was below 10 fmol/mg tissue in all brain regions. When determining the total density of [^{3}H]M100907 binding throughout the layers of the neocortex the obtained value was approximately 60–70 fmol/mg tissue wet weight. The total density of [^{3}H]M100907 binding in layer III of the cerebral cortex in all regions was approximately 90 fmol/mg. The caudate displayed densities slightly higher than the nonspecific binding (19–26 fmol/mg), whereas the density in the putamen was approximately 8 fmol/mg, which is in the range of the nonspecific binding obtained. The overall density in the hippocampal formation was approximately 25 fmol/mg, of which the main binding was to the CA-1 region (40 fmol/mg), being considerably lower in the dentate gyrus (15 fmol/mg). The amygdala (as a whole), hypothalamus, and the mamillary bodies all had intermediate densities (20–30 fmol/mg) of [^{3}H]M100907 binding. The cerebellum did not show any specific binding, as the total [^{3}H]M100907 binding was similar to the nonspecific binding.

Pharmacology of [^{3}H]M100907 binding

The specific [^{3}H]M100907 binding to all regions could be blocked by the excess (10 μM) addition of the 5-HT$_2$ receptor antagonist ketanserin, leaving a low proportion of nonspecific binding (approximately 7 fmol/mg, see above). Lower concentrations of ketanserin dose-dependently inhibited the binding. The IC$_{50}$ of ketanserin in a neocortical region was approximately 15 ± 1 nM. As seen with [^{11}C]M100907 binding (see below), the nonselective D$_1$-dopamine receptor and 5-HT$_2$ receptor antagonist SCH 23390 similarly blocked the binding of [^{3}H]M100907 with a relatively high affinity, with an approximate IC$_{50}$ of 63 ± 8 nM. High concentrations (1 μM) of the selective 5-HT$_{1A}$ receptor and D$_2$-dopamine receptor antagonists WAY-100635 and raclopride, respectively, only weakly (approximately 10%) antagonize the binding of [^{3}H]M100907.

Autoradiography using [^{11}C]M100907

The specific binding of [^{11}C]M100907 was relatively evenly distributed in the different neocortical regions of the human brain, with much lower densities in the central regions (Fig. 8). The binding could be totally abolished by the addition of the nonselective 5-HT$_{2A}$ receptor antagonist ketanserin (10 μM). The binding was almost uniformly distributed in the different neocortical layers, although there was an indication for higher density in the middle neocortical layers (Fig. 8). Similar to the results obtained using [^{3}H]M100907, much weaker, specific [^{11}C]M100907 binding was seen in the various parts of the hippocampus (Fig. 8F,G). The density of [^{11}C]M100907 binding sites was very low in the basal ganglia, and in contrast to the images obtained with [^{3}H]M100907, no patches could be seen using [^{11}C]M100907. Other central regions like the amygdala and thalamus were virtually devoid of [^{11}C]M100907 binding. The slight labeling in the cerebellum (Fig. 8) was not affected by the addition of ketanserin, indicating that this binding is primarily due to a nonspecific accumulation of radioligand.

Addition of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (10 μM) or the D$_2$-dopamine receptor antagonist raclopride (10 μM) did not affect the binding of [^{11}C]M100907 in any region. However, as with [^{3}H]M100907, SCH 23390 (10 μM) blocked most [^{11}C]M100907 binding.

DISCUSSION

The high affinity and selectivity of [^{3}H]M100907 together with other in vitro binding characteristics such as low nonspecific binding and the possibility of labeling this ligand to a high specific radioactivity makes this radioligand an optimal compound for use in autoradiographical studies in postmortem human brains, as has previously been shown in the rat (Lopez-Gimenez et al., 1997) and human brain (Lopez-Gimenez et al., 1998). Similarly, due to this high affinity and selectivity, [^{11}C]M100907 has been shown to be one of the best ^{11}C-labeled compounds used in whole hemisphere autoradiography for the study of the 5-HT$_{2A}$ receptor. It has also been shown in the initial PET studies performed (Lundkvist et al., 1996; Ito et al., 1998) that [^{11}C]M100907 is the ligand of choice for the study of the 5-HT$_{2A}$ receptor in the living human brain.

In the present study, we examined the detailed anatomical distribution of [^{11}C]M100907 and [^{3}H]M100907 binding in the postmortem human brain using whole hemisphere autoradiography. As previous studies with less selective radioligands have shown, the 5-HT$_{2A}$ receptor is mainly found in the neocortex, but also in lower densities in central brain structures such as hippocampus and in the patches of nucleus caudatus. The distribution in the different layers of the neocortex is different from the 5-HT$_{1A}$ receptor, which, using an identical methodology, has been shown mainly in the superficial layers (Hall et al., 1997), whereas the 5-HT$_{2A}$ receptor is found in layers II–III as well as at a lower density in layer V–VI. This distinct anatomical distribution is consistent with the different behavioral and clinical pharmacology of these two serotonin receptor subtypes. One other major difference between these receptor subtypes is their distribution in the archicortex, where there are very high densities of the 5-HT$_{1A}$ receptor in the CA-regions of the hippocampus, with much lower densities of the 5-HT$_{2A}$ receptor.

The distribution of the 5-HT$_2$ receptor in the animal and human brain has previously been studied using various less selective radioligands, such as [^{3}H]ketanserin (Leysen et al., 1982; Joyce et al., 1993; Marazziti et al., 1997), [^{3}H]N-methyl-spiperone (Nyberg et al., 1993; Hall et al., 1995), and [^{125}I]DOI (Waeber and Palacios, 1994). The use of [^{3}H]M100907 as a radioligand has allowed the selective characterization of the 5-HT$_{2A}$ receptor without binding to the other 5-HT$_2$ receptor subtypes 5-HT$_{2B}$ and 5-HT$_{2C}$. These have all shown that the 5-HT$_2$ receptor has a high density in the neocortex, with much lower densities in the central brain regions. Similarly, in situ hybridization studies have shown a similar distribution of the 5-HT$_{2A}$ receptor mRNA in the animal (Wright et al., 1995; Ward and Dorsa, 1996) and human (Burnet et al., 1995) central nervous system.

Since the early discovery that administration of D-LSD induces hallucinations, serotonin and its receptors have been suggested to play a role in schizophrenia. However, most of these studies have suggested that blockade of serotonin receptors, and especially of 5-HT$_2$ receptors, might be beneficial in the treatment by reducing the side effects (Meltzer et al., 1989). A number of combined D$_2$-dopamine/5-HT$_2$ receptor antagonists like clozapine and risperidone have been shown to have beneficial effects in their therapeutic action. Some more recent studies have also indicated that blockade of 5-HT$_2$ receptors might be of therapeutic value, especially in patients with negative symptoms (Carpenter, 1995). The 5-HT$_2$ receptor antagonists might also be useful in treating impaired cognitive function (Borison, 1996). So far, no pure 5-HT$_2$ receptor antagonist has been proven to be effective as an antipsychotic drug, although a number of animal studies have indicated that M100907 should have antipsychotic properties (Sorensen et al., 1993; Maurel-Remy et al., 1995; Schmidt et al., 1995; Kehne et al., 1996; Moser et al., 1996), in spite of the lack of D$_2$-dopamine receptor antagonism. Initial clinical trials have also been performed aiming at the development of this compound as an antipsychotic drug (Brunner et al., 1998).

The effects of the 5-HT$_2$ receptor antagonists in schizophrenia might be direct via modulation of pathways of importance in the pathophysiology of schizophrenia. The effects might also, especially with regard to the attenuation of EPS induced by the classical neuroleptics, be the result of a direct or indirect interaction between the serotonergic and the dopaminergic systems. The D$_2$-dopamine receptors are present in a low density in the neocortex, although recent findings indicate a substantially higher density in the temporal cortex than in other neocortical regions (Farde et al., 1994; Hall et al., 1996). The interaction between the 5-HT$_2$ receptors and the D$_2$-dopamine receptors in the neocortex can thus be elicited directly on cell-bodies or dendrites of neocortical neurons.

The patchy appearance of the binding of [^{3}H]M100907 in the caudate nucleus and ventral putamen, recently also reported by Lopez-Gimenez et al. (1999), suggests that the 5-HT$_{2A}$ receptors are enriched in these patches. We have not yet been able to show that these patches are identical with the patches or striosomes having higher densities of D$_1$-dopamine receptors (Brené et al., 1995). In a previous study using the 5-HT$_{2A/C}$ receptor agonist radioligands [^{3}H]LSD and [^{125}I]DOI, Waeber et al. (1994) found localization to the striosomes, as visualized by acetylcholinesterase histo-

chemistry. However, using in situ hybridization studies, 5-HT_{2C} receptor mRNA, but not 5-HT_{2A} receptor mRNA, was found to be enriched in the dynorphin-rich patches (Ward and Dorsa, 1996), which is thus in contrast to the present autoradiographic findings of the 5-HT_{2A} receptor binding sites in the striosomes.

CONCLUSIONS

The highly selective labeling with [^{3}H]M100907 of 5-HT_{2A} receptors demonstrates clearly that this radioligand is useful for further studies of the 5-HT_{2A} receptor subtype in vitro with homogenate receptor assays or autoradiography. The high affinity and low nonspecific binding of [^{11}C]M100907 is promising for the future use of this radioligand in studies of distribution, pharmacology, and pathophysiology of the 5-HT_{2A} receptors in the human brain in vivo with PET. Moreover, the demonstration of no specific [^{3}H]M100907 binding in the cerebellum allows the use of this region as a reference region in PET. [^{3}H]M100907 has been shown in the initial PET studies performed in the monkey (Lundkvist et al., 1996) as well as in the human brain (Ito et al., 1998) to be a useful ligand for the visualization of the 5-HT_{2A} receptors in vivo. The in vitro autoradiograms and data presented here are valuable for anatomical comparison and interpretation of PET data obtained at lower resolution in vivo.

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