Fluorine-18-Altanserin: A Radioligand for the Study of Serotonin Receptors with PET: Radiolabeling and In Vivo Biologic Behavior in Rats

Christian Lemaire, Robert Cantineau, Marcel Guillaume, Alain Plenevaux, and Leon Christiaens

Cyclotron Research Center, Liège University, Liège, Belgium

No-carrier-added [^{18}F]altanserin was synthesized by nucleophilic substitution of the corresponding nitro compound with [^{18}F]fluoride in the presence of kryptofix 222 and K_2CO_3. After purification by preparative HPLC, [^{18}F]altanserin was produced in less than 2 hr with a radiochemical yield of 10% (EOS) and a specific activity of 0.8–1.3 Ci/μmol. In rats, the tracer localized rapidly in the whole brain (0.5% ID/g organ) with a high binding to the frontal cortex. The frontal cortex/cerebellum ratio increased with time and reached a plateau of 11 at 2 hr postinjection. This uptake in S_2 receptor regions was saturable and could be blocked by pretreatment with various S_2 antagonists. This radiopharmaceutical appears to be more selective for S_2 receptor sites than other ligands available today and allows the study of S_2 receptors under in vivo conditions.

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In vitro studies on human brain autopsy material have shown that the serotonin receptor system is implicated in several conditions, including sleep (*1*), aging (*2*), Alzheimer's disease (*3*), affective and personality disorders (*4–6*), pain (*7*), and also extrapyramidal syndromes (*8*). In vivo study of S_2 receptors would represent a significant advance in the understanding of the various conditions where these sites are thought to play a role. Positron emission tomography (PET) allows the direct, noninvasive and repetitive measurement of neuroreceptors in regions of the brain provided that the appropriate radioligand is available at high specific activity (*9*).

A number of ^{11}C- or ^{18}F-labeled radioligands have already been synthesized as radiopharmaceuticals for mapping S_2 serotoninergic receptor sites with PET. To date, in vivo studies have been performed with several serotoninergic antagonists labeled with positron emitters such as [^{11}C]ketanserin (*10,11*), [^{18}F]spiperone (*12*) and [^{11}C]methylspiperone (*13,14*), [^{18}F]setoperone (*15,16,17*), [^{11}C]methylbromo-LSD (*18,19*), and [^{18}F]ritanserin (*20,21*).

Altanserin, like setoperone, is a fluorobenzoyl derivative structurally related to ketanserin (*22*). The in vitro binding affinity constants (Ki: n*M*) of altanserin for S_2, D_2, and α_1 receptor sites, reported by J. E. Leysen (*23*) are respectively 0.13, 62 and 4.55 (Table 1). Based on these findings, altanserin was considered as potentially interesting radiotracer for in vivo serotonin receptor binding.

In this paper, we report the radiosynthesis of [^{18}F]altanserin by nucleophilic fluorination of the corresponding nitro derivative as precursor (Fig. 1). Rat experiments also are described, including general and regional biodistributions as well as carrier and blocking effects.

MATERIALS AND METHODS

Starting Materials

Most of the organic substrates and solvents were of analytical grade from Aldrich. Methyl 2-isothiocyanatobenzoate, hydrobromic acid, hydriodic acid (57 % wt), and the gold label reagents dimethylsulfoxide and acetonitrile were purchased from Janssen Chimica and used without further purification. The aminopolyether kryptofix 222 (*4,7,13,16,21,24*) hexaoxa-1,10 diazabicyclo(8.8.8)hexacosan, potassium carbonate, and ethanol were obtained from Merck. Altanserin, bromoaltanserin, and nitrobenzoylpiperidine were a gift from Janssen Pharmaceutica. Oxygen-18-enriched water (98.5%) was obtained from Campro Benelux. All other reagents including β-chloroethyl-ethylcarbamate (*24*) were prepared according to literature methods.

HPLC System

High-performance liquid chromatography (HPLC) was conducted using a Waters system consisting of M-6000 A pump, a U6K injector and a Lambda max 481 LC U.V. spectrophotometer set up at 254 nm with a 10-mm analytical cell. A NaI (Tl) scintillation detector was used for radioactivity measurements. Lichrosorb RP Select-B columns were from Merck. Conditions (column, eluent, flow) for separations are given in the following sections.

Radiochemistry

The synthesis of [^{18}F]altanserin was performed as described in the Appendix by nucleophilic substitution of the nitro group of

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For reprints contact: Christian Lemaire, Cyclotron Research Center, Liège University, 4000 Liège, Belgium.

TABLE 1
Binding Affinity Values (Ki, nM) of Various Serotoninergic Antagonists for Different Neurotransmitter Receptor Sites (23)

	S_2 [^{3}H]Ketanserin	D_2 [^{3}H]Haloperidol	α_1 [^{3}H]WB-4101
Altanserin	0.13	62	4.55
Ketanserin	0.63	240	11
Setoperone	0.37	25	13
Ritanserin	0.28	22	35

nitroaltanserin, previously synthesized from p-nitrobenzoyl-4-piperidine.

Biodistribution Study

The in vivo biodistribution was measured in female Wistar rats (180–250 g) intravenously injected (femoral vein) under light ether anesthesia with 80–150 μCi of [^{18}F]altanserin (specific activity: 0.6–1.2 Ci/μmol). Rats were killed at various times (5 min, 1 hr, and 4 hr after injection) by cardiac excision under ether anesthesia. The organs were removed and counted with a GeLi detector connected with a multi channel analyzer. The percentages of injected dose per gram of organ were calculated by comparison with a reference solution consisting of diluted samples of the injected compound.

Brain Biodistribution Study

The rat brain biodistribution was determined by dissecting, weighing, and counting samples (automatic gamma sample changer, Berthold BF 5300) from different brain regions (frontal cortex, striatum, thalamus, cerebellum) after femoral injection of 50–100 μCi of [^{18}F]altanserin (specific activity: 0.6–1.2 Ci/μmol). Female Wistar rats (180–250 g) were injected under light ether anesthesia and killed by decapitation. The results are expressed as percentage of injected dose per gram (%ID/g) of tissue. From these data, regions-to-cerebellum ratios were calculated and compared.

Blocking Experiments

The selectivity of [^{18}F]altanserin binding to serotonin S_2 receptor sites was examined as follows. Rats were intravenously injected (femoral vein) under light ether anesthesia with S_2 antagonists such as ketanserin (2.5 mg/kg), ritanserin (2.5 mg/kg), pipamperone (10 mg/kg), and methysergide (2.5 mg/kg) or D_2 antagonists such as sulpride (40 mg/kg), halopemide (20 mg/kg), and bromolisuride (0.4 mg/kg). Spiperone, a D_2, S_2 antagonist, was also used at 2 mg/kg. The drugs were dissolved in saline containing 5% alcohol and one equivalent of tartaric acid to increase drug solubility. The animals were pretreated 1 hr before injection of tracer and killed by decapitation 2 hr post-tracer injection.

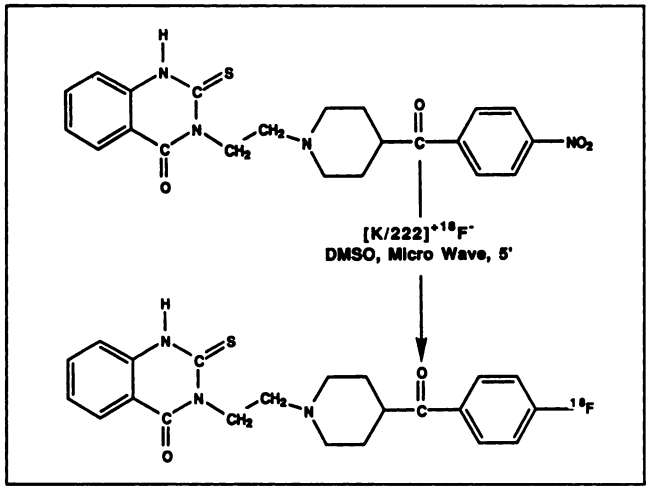

FIGURE 1. Radiolabeling of [^{18}F]altanserin starting from its nitroprecursor.

Studies on the Effect of Carrier

To check the saturability of altanserin uptake in the frontal cortex, a region rich in S_2 receptors sites, the frontal cortex-to-cerebellum ratios were determined under light anesthesia in rats after intravenous injection (femoral vein) of 100 μCi [^{18}F]altanserin at different specific activities (2 to 1000 mCi/μmol). The rats (2 for each specific activity) were killed by decapitation 2 hr after injection of the radiotracer.

Chemical Form of ^{18}F in Rat Plasma

Four rats (200 g) were intravenously injected (femoral vein) with 300 μCi of [^{18}F]altanserin under light ether anesthesia (specific activity: 0.8 Ci/μmol). The blood (±5 ml) was collected in heparinated tubes from the heart 4 hr after radiopharmaceutical injection and centrifuged for 5 min at 4000 rpm. The plasma was diluted 12-fold with water at pH 4 (0.05 *M* HOAc) and slowly passed through a C-18 Sep Pak cartridge. After washing the support with 0.1% triethylamine in water (10 ml), the radioactivity was eluted with a tetrahydrofuran and methanol mixture (25/75, 1.5 ml). The radioactive solution was then analyzed by HPLC using a Lichrosorb RP Select-B column (250 × 0.4 cm) eluted with [CH_3OH/THF/H_2O (pH 4): 13/32.5/54.5] at a flow rate of 0.7 ml/min. The extraction efficiency of the radioactivity was 95%–97%.

Chemical Form of ^{18}F in Rat Brain

Rats (200 g, n=4) were intravenously injected (femoral vein) with 300 μCi of [^{18}F]altanserin under light ether anesthesia. The animals were killed by decapitation 4 hr after injection of the radiotracer. Each brain was removed and homogenized using a Polytron tissue disrupter with 4 ml of cold methanol (*25*). The homogenate was centrifuged for 4 min and the supernatant decanted. The pellet was again homogenized with 4 ml of cold methanol and centrifuged. A small amount of unlabeled altanserin was added to the combined methalonic extracts (extraction efficiency > 90%) and an aliquot of this solution analyzed in HPLC as described for plasma analysis.

RESULTS

Radiosynthesis

The radiolabeling of [^{18}F]altanserin was carried out by nucleophilic substitution of [^{18}F]fluoride on 3-(2-(4-(4-nitrobenzoyl)-1-piperidinyl)-propyl)-1,2-dihydro-2-thioxo-4-([^{3}H])quinazolinone (nitroaltanserin (F), Fig. 1). This precursor was synthesized from p-nitrobenzoyl-4-piperidine (A) according to the route shown in Figure 2. The preparation of this substrate (A) involved a multi step synthesis starting with isonipecotic acid as previously described (*16*). This cold substrate (A) was coupled with β-chloroethylethylcarbamate followed by hydrolysis and cyclization of

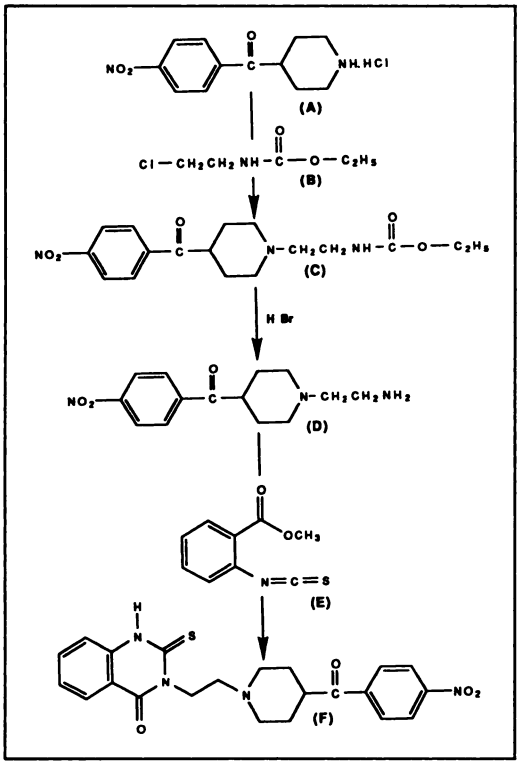

FIGURE 2. Chemical steps for the synthesis of nitroaltanserin starting from p-nitrobenzoyl-4-piperidine.

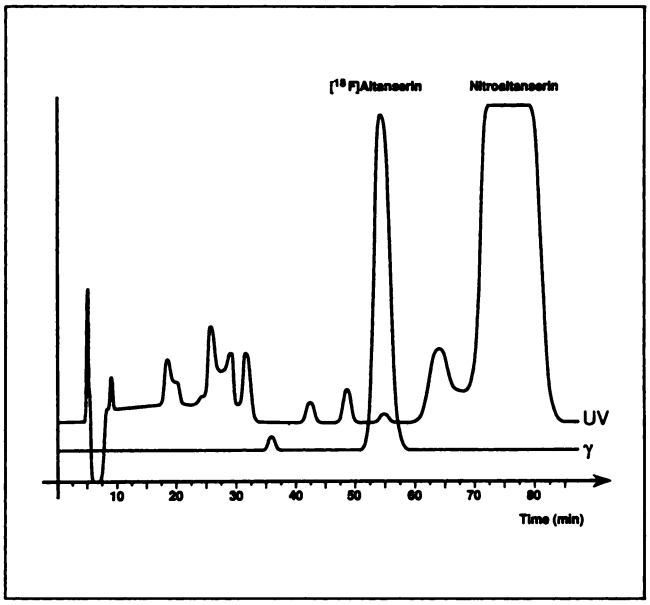

FIGURE 3. HPLC purification profile of [^{18}F]altanserin. Conditions: Select-B (7μ) column (25 × 250 mm) (Merck, Darmstadt, Germany). Eluent: [$CH_3OH/THF/H_2O$ pH 5] (12.6/32.4/55). Flow rate: 16 ml/min.

the primary amine obtained with methyl 2-isothiocyanatobenzoate leading finally to nitroaltanserin.

Fluorine-18 was produced by the conventional (p,n) reaction on ^{18}O-enriched water (30%) with a batch production yield averaging 200 mCi.

The nitro precursor allowed a one-pot fluorination step in the presence of K_2CO_3 and DMSO using the amino polyether K222 complex method. The use of microwave heating conditions as suggested by Hwang et al. (*26*) gave a radiochemical yield of 40% starting with 9 mg of nitroaltanserin in a total reaction time (purification not included) of about 5 min (Table 2).

The HPLC purification was carried out as described in the experimental section. The two chromatograms (Fig. 3) (radioactivity and UV at 254 nm) showed a difference in the two retention times of about 20 min between the radioactive compound eluting first at 55 min and the nitroaltanserin. Pure [^{18}F]altanserin was finally obtained with an overall radiochemical yield of 10% E.O.S. and a specific activity of 0.8–1.3 Ci/μmol after a total preparation time of about 110 min.

TABLE 2
Radiofluorination Yields of [^{18}F]Altanserin Obtained in Various Experimental Conditions

Substrate		Radiochemical Yield (%EOB)	
		Normal (135°C, 30 min)	Microwave (5 min)
Br-Altanserin	9 mg	1	—
NO_2-Altanserin	1 mg	<1	2
	3 mg	<2	20
	5 mg	<5	25
	9 mg	5–10	40
	15 mg	20	50

Animal Experiments

Table 3 shows the biodistribution of radioactivity in various rat tissues at 5, 60, and 240 min after injection of ^{18}F-labeled altanserin. The bulk of radioactivity concentrated at early times in the lungs, liver, and kidneys. Half a

TABLE 3
Biodistribution of [^{18}F]Altanserin in Rats

Organs	5 min	1 hr	4 hr
Blood	1.31 ± 0.04	0.46 ± 0.09	0.22 ± 0.03
Heart	0.75 ± 0.06	0.30 ± 0.05	0.15 ± 0.02
Lungs	2.04 ± 0.20	0.98 ± 0.15	0.40 ± 0.08
Liver	1.91 ± 0.08	1.36 ± 0.16	0.57 ± 0.06
Spleen	0.87 ± 0.05	0.57 ± 0.09	0.21 ± 0.04
Adrenals	2.90 ± 0.30	1.53 ± 0.20	0.62 ± 0.12
Kidneys	1.09 ± 0.12	0.87 ± 0.05	0.42 ± 0.08
Small intestine	0.77 ± 0.06	0.71 ± 0.08	0.45 ± 0.06
Ovaries	1.32 ± 0.20	0.94 ± 0.06	0.28 ± 0.04
Stomach	1.06 ± 0.20	0.47 ± 0.07	0.15 ± 0.02
Muscles	0.31 ± 0.05	0.14 ± 0.02	0.11 ± 0.06
Skin	0.42 ± 0.07	0.65 ± 0.15	0.27 ± 0.09
Thyroid	1.17 ± 0.12	0.57 ± 0.08	0.55 ± 0.15
Bone	0.18 ± 0.03	0.22 ± 0.05	0.31 ± 0.05
Brain	0.42 ± 0.04	0.54 ± 0.09	0.21 ± 0.10

%ID/g organ, n = 4.

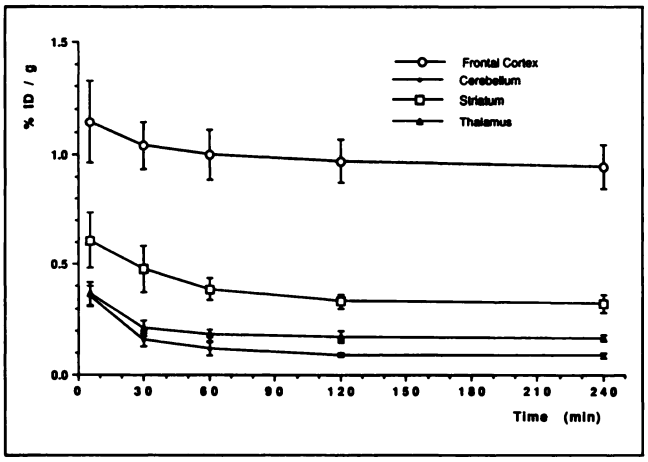

FIGURE 4. Time course of ^{18}F radioactivity in rat frontal cortex, striatum, thalamus and cerebellum after intravenous injection of [^{18}F]altanserin. Data are mean %dose/g ± s.d. (n=6).

percent of the administrated dose localized rapidly into the brain and remained constant for more than 1 hr indicating a significant retention by brain tissue. Clearance of radioactivity took place at 4 hr after injection for all tissues. Five minutes after injection, the %ID/g of bone was 0.18%. This value increased slowly with time to reach 0.31% at 4 hr postinjection. Platelets which are known to contain S_2 receptors (*27*) were isolated from 1 ml of plasma as described previously by Boyum (*28*). Values of 1.3% and 2% of the percentage of the ID/g of blood were found in platelets at 5 min and 4 hr after injection respectively.

The time course of ^{18}F radioactivity in cerebellum (CB), frontal cortex (FC), striatum (ST), and thalamus (TL) was determined in rats (n=6). As shown in Figure 4, the radioactivity in the FC decreased slowly with time from 1.14% ± 0.18% ID/g of tissue at 5 min to a value of 1.00% ± 0.11% at 60 min and remained relatively constant thereafter. The striatum and thalamus displayed lower uptakes (0.39 ± 0.05 and 0.18 ± 0.03, respectively, at 1 hr after injection). The activity in the cerebellum was low and also relatively stable from 1 to 4 hr after injection, 0.12% ± 0.03% and 0.09% ± 0.01% ID/g of tissue, respectively.

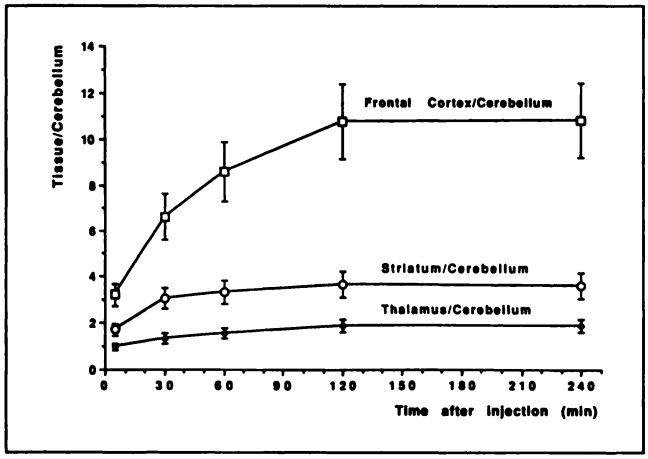

FIGURE 5. Time course of tissue-to-cerebellum ratios of ^{18}F radioactivity after intravenous injection of [^{18}F]altanserin. Data are mean ratios ± s.d. (n=6).

The time course of tissue-to-cerebellum ratios is shown in Figure 5. The ratios of striatum and thalamus to cerebellum were low (3.6 ± 0.4 and 1.8 ± 0.2, 2 hr after injection, respectively). By contrast, the frontal cortex-to-cerebellum ratio increased strongly with time and reached a plateau of 10.8 ± 1.3 at 2 hr postinjection.

The specificity of the in vivo [^{18}F]altanserin binding was evaluated by blocking experiments using S_2 and D_2 antagonists. The results are summarized in Table 4. Drugs with high affinity for serotonin S_2 receptor sites (ketanserin, pipamperone, ritanserin) (*23,29*) and spiperone, which has a mixed D_2–S_2 affinity (*23*), strongly blocked the [^{18}F] altanserin accumulation in the frontal cortex and striatum. Methysergide, which is also very specific for S_2 sites (*23*), partially prevented [^{18}F]altanserin uptake in these two brain regions. Drugs showing higher affinity for D_2 than for S_2 receptor sites such as halopemide (*30,31*) and bromolisuride (*32*) weakly decreased frontal cortex and striatum-to-cerebellum ratios. Sulpiride, a specific D_2 antagonist (*31*), did not modify significantly the FC/CB and the ST/CB ratios.

The frontal cortex-to-cerebellum ratio was drastically influenced by the specific activity of the radiolabeled compound and decreased with the addition of carrier. Ratios of 12.2 and 3.1 were found for [^{18}F]altanserin injection with specific activities of 1 Ci/μmol and 2 mCi/μmol respectively.

The in vivo stability of [^{18}F]altanserin was determined by studying the percentage of unchanged radiopharmaceutical in rat brain and blood 4 hr after radiotracer injection. Under the conditions described in the Materials and Methods section, the HPLC analysis showed in both cases a main radioactive peak corresponding to [^{18}F]altanserin. This peak contained more than 85% and 96% of the extracted radioactivity from blood and brain samples, respectively.

DISCUSSION

Synthesis

As shown in Table 2, the radiofluorination reaction with bromoaltanserin as precursor gave a very poor radiochemical yield of [^{18}F]altanserin, predicting nevertheless the possibility of a higher fluorination yield by direct nucleophilic ^{18}F-NO_2 displacement (*33*). The critical prerequisite of this work was therefore the preparation of the nitroaltanserin precursor (F), which has not been previously reported.

The radiochemical yields obtained with the nitro derivative were compared using conventional and microwave heating conditions. Although the automation of microwave heating is technically complicated, this technique led to a reduced reaction time and higher reproducible radiochemical yields. Furthermore, the use of lower starting amounts of precursor led to easier purification steps.

Optimization of the HPLC conditions was achieved on a Lichrosorb RP Select-B Merck column using the method

TABLE 4
Effect of Different Competing S_2 and D_2 Receptor Ligands on the Regional Distribution of [^{18}F]Altanserin

Cold ligand	K_i (n*M*) S_2	K_i (n*M*) D_2	Injected dose (mg/kg)	Frontal cortex/ cerebellum	Striatum/ Cerebellum	References
No competing ligand n.c.a. [^{18}F]altanserin			100 μCi (0.9 Ci/μmol)	10.3 ± 0.6	3.4 ± 0.2	
Ketanserin	0.63	240	2.5	1.6 ± 0.2	1.5 ± 0.2	23, 29
Ritanserin	0.28	22	2.5	1.5 ± 0.2	1.4 ± 0.2	23
Pipamperone	0.94	96	10	1.4 ± 0.2	1.5 ± 0.2	23, 29
Methysergide	1.30	140	2.5	3.2 ± 0.5	2.0 ± 0.3	23, 29
Spiperone	0.64	0.26	2	1.5 ± 0.2	1.3 ± 0.2	23, 29
Sulpiride	>1000	31	40	9.8 ± 0.7	3.3 ± 0.3	31
Halopemide	220	3.1	20	7.1 ± 0.7	2.9 ± 0.2	30, 31
Bromolisuride	—	0.3*	0.4	6.4 ± 0.4	2.8 ± 0.2	32

* K_d.
The rats were pretreated 1 hr before injection of tracer and killed 2 hr post [^{18}F]altanserin injection (n = 4).

previously reported (*34,35*). The pH was of critical influence on the retention time of [^{18}F]altanserin and its nitroprecursor. On the analytical scale, a good separation between the two derivatives was obtained in less than 15 min using a solvent mixture $CH_3OH/THF/H_2O$ at pH 4 (12.6/32.4/55). Unfortunately on the preparative scale, due to the appearance of side products in the labeling step, a satisfactory purification of [^{18}F]altanserin was only achieved at pH 5, which increased the retention times of the labeled and unlabeled compounds (Fig. 3).

Animal Experiments

In order to validate the use of [^{18}F]altanserin as a serotoninergic radioligand for in vivo binding assays, animal experiments were carried out.

Significant uptake of [^{18}F]altanserin was observed in the frontal cortex, a brain region known to contain high S_2 (*36*) and low α_1 receptor densities. A slight but significant accumulation of [^{18}F]altanserin was found in the striatum, which contains many more D_2 than S_2 sites. The [^{18}F]altanserin uptake in the thalamus displaying α_1 receptor density, was very low.

The critical point of this study was to differentiate the binding of [^{18}F]altanserin to S_2 from α_1 sites in the frontal cortex and the binding to S_2 from D_2 sites in the striatum. Regarding the possible binding of [^{18}F]altanserin to α_1 receptors, the only known pure α_1 antagonist prazosin, widely used in vitro, has a poor penetration into the brain (Leysen JE, *personal communication*). This compound was not used in our experiments. However, as shown in Table 1, altanserin, like ketanserin, has a much more lower binding affinity for α_1 than for S_2 receptor sites.

Regarding the differentiation between [^{18}F]altanserin binding on S_2 and D_2 sites, several serotonin S_2 and dopamine D_2 receptor blockers were chosen with respect to their in vitro biochemical properties (K_i, K_d) shown in Table 4. The doses of pretreatment were selected according to published data [Table 4, (*29,32*)]. When this information was not available, the injected doses were sufficient to induce catalepsy in rats (halopemide).

Theoretically, [^{18}F]altanserin binding on S_2 receptors sites in the frontal cortex and in the striatum should be completely blocked by pretreatment with specific S_2 antagonists. Our data clearly demonstrated that all the S_2 antagonists and particularly ketanserin (K_i: n*M* for S_2 and D_2 receptor sites: 0.63–240) strongly prevented [^{18}F]altanserin accumulation both in the frontal cortex and in the striatum, suggesting an [^{18}F]altanserin binding to S_2 receptor sites in those brain regions. Furthermore, [^{18}F]altanserin uptake in the striatum seems to occur on S_2 receptor sites since ketanserin, as demonstrated by Suehiro et al. (*37*) and Maziere et al. (*32*), did not modify in this region of the brain selective binding of specific D_2 ligands to dopamine D_2 receptor sites (N-[^{11}C]methyl-benperidol and [^{76}Br] bromolisuride). Spiperone, which has a mixed D_2–S_2 affinity, showed the same behavior. The incomplete blockage measured after pretreatment with methysergide could be explained by the lack of saturation of the S_2 sites, even with an injection of the ligand at a dose of 10 mg/kg.

In principle, the [^{18}F]altanserin binding on S_2 receptor sites in the frontal cortex and striatum should not be influenced by pretreatment with specific D_2 antagonists. The biochemical pattern of halopemide and bromolisuride (*30,31,32*) shows that these drugs exhibit more affinity for dopamine D_2 than serotonin S_2 receptors. However, a partial occupancy of S_2 sites may be expected when these ligands are used at doses required to reach saturation of D_2 receptor sites (i.e., 30 mg/kg and 0.4 mg/kg, respectively). Our results displayed this feature, but pretreatment with halopemide and bromolisuride did not influence more significantly the ST/CB than the FC/CB ratio suggesting also a selective [^{18}F]altanserin binding in the striatum on S_2 sites.

Sulpiride is highly specific but has a rather low dopamine D_2 receptor binding affinity (*31*). Its penetration into the brain is very poor (*36*) and this drug caused the same problems in our pretreatment investigations as those men-

tioned with prazosin and methysergide. In any case, very large doses of sulpiride (40 mg/kg) did not significantly modify the striatum and the frontal cortex to cerebellum ratios. According to the data of our blocking experiments in rats, [^{18}F]altanserin appeared more selective for S_2 receptors than other serotonin antagonists available for PET investigations today (Table 1) and in particular more selective than setoperone (*38*).

The frontal cortex-to-striatum ratio which appears to be a good index of the ligand specificity (S_2/D_2) is much higher for [^{18}F]altanserin and reached approximately 2.56 at 1 hr postinjection. This ratio remained relatively constant thereafter. The values calculated on the basis of the data reported by Mazière and coworkers for [^{18}F]setoperone in similar rat experiments were lower and did not exceed 1.18 (*38*). For [^{11}C]methylbromo-LSD (*19*), the greatest specificity was observed 30 min postinjection with a frontal cortex-to-striatum ratio of 1.8 in a mouse brain regional biodistribution study. Other positron-labeled S_2 ligands, such as spiperone derivatives, are less specific.

The influence of the specific activity of the radiopharmaceutical on the FC/CB ratio demonstrated the saturability of the [^{18}F]altanserin binding in the frontal cortex.

Studies of the chemical form of the ^{18}F found in blood and brain 4 hr after injection of the radiopharmaceutical demonstrated the good in vivo stability of this radiopharmaceutical in rats as also described for ketanserin by Meuldermans et al. (*39*).

CONCLUSION

In conclusion, these investigations demonstrated that [^{18}F]altanserin exhibits high specificity and selectivity for serotonin S_2 receptors, with high specific-to-nonspecific binding ratios in rats. Accordingly, [^{18}F]altanserin can be regarded as a highly promising radioligand for PET studies of serotonin S_2 receptors in the living brain.

APPENDIX: SYNTHESIS OF NITROALTANSERIN AND RADIOSYNTHESIS OF [^{18}F]ALTANSERIN

Synthesis of Nitroaltanserin

Four grams of nitrobenzoylpiperidine.HCl (14.8 mmol, A) (Fig. 2) were dissolved in 100 ml of water, neutralized with NH_4OH and extracted twice with CH_2Cl_2. The organic phase was dried over potassium carbonate and evaporated to dryness under vacuum. The resulting oil was dissolved in 100 ml of 4-methyl-2-pentanone with 3 g (19.9 mmol) of β-chloroethyl-ethylcarbamate (B), 4.7 g of K_2CO_3 and 50 mg of NaI. The mixture was heated under reflux overnight. After reaction, the hot solution was rapidly filtered and the solvent removed under vacuum.

The residue was dissolved in 5 ml of chloroform, applied to a silica gel column (50 cm × 4 cm) and eluted with chloroform. The fraction eluting between 150–175 ml was collected. This purification procedure was applied twice to ensure high purity of N-1-[2-ethoxycarbonylaminoethyl]-4-[4-(nitrobenzoyl)]piperidine (C).

The oil obtained after rotary evaporation of the chloroform was treated with 48% HBr (50 ml) under reflux for 4 hr. The solution was concentrated under vacuum, diluted with 100 ml of water, neutralized with NH_4OH and extracted twice with dichloromethane. The successive organic phases were mixed, washed with water and dried over potassium carbonate.

After removal of the solvent, the residual oil N-1-(2-aminoethyl)-[4-(nitrobenzoyl)]piperidine (D) was dissolved immediately in 10 ml of dry THF and treated with 3.2 g (16.6 mmol) of methyl 2-isothiocyanatobenzoate (E). An exothermic reaction took place and nitroaltanserin (F) crystallized immediately. After 30 min, this nitro derivative was filtered and washed with 10 ml of cold THF. The yield, starting from nitrobenzoylpiperidine was 62% (mp: 242°C). The product was identified as nitroaltanserin by ^{1}H NMR and mass spectroscopy.

Analytical HPLC using a Lichrosorb RP Select-B column (25 × 0.4 cm) under the following conditions: flow 0.8 ml/min, $CH_3OH/THF/H_2O$ (pH 4) (12.6/32.4/55), showed a purity higher than 99% (retention time ≃ 14 min).

^{1}H NMR (400 MHz, (methyl sulfoxide)-d6): δ 8.33 (d, 2H, J=8.4 Hz); 8.19 (d, 2H, J=8.4 Hz); 7.95 (d, 1H, J=8 Hz); 7.74 (t, 1H, J=7.6 Hz); 7.38 (d, 1H, J=8 Hz); 7.34 (t, 1H, J=7.6 Hz); 4.55 (t, 2H, J=7.2 Hz); 3.44 (m, 1H); 3.31 (s, 1H, NH); 3.0 (m, 2H); 2.65 (m, 2H); 2.24 (m, 2H); 1.78 (m, 2H); 1.54 (m, 2H).

MS, m/e (relative intensity): 438 (M^+, 4), 260 (22), 247 (44), 235 (41), 234 (21), 206 (15), 205 (97), 204 (100), 203 (27), 162 (26), 150 (16), 144 (24), 104 (14), 76 (17).

Fluorine-18 Production

The no-carrier-added aqueous [^{18}F]fluoride solution was produced by the ^{18}O(p,n)^{18}F reaction in a nickel target equipped with front and rear 100 μ Ti foils (*40*). A typical production required a 10 μA bombardment for 1 hr on a target containing 1.8 ml of 30% enriched [^{18}O]water. The irradiated target contents (180–200 mCi of [^{18}F]fluoride) were delivered to the laboratory through a 25-m long teflon tube (i.d.: 0.8 mm).

Radiolabeling of [^{18}F]Altanserin

To a residue of no-carrier-added $(K/222)^+{}^{18}F^-$ prepared as previously described (*41*) were added either nitroaltanserin (9 mg) or bromoaltanserin (9 mg) in 1 ml of DMSO. The vial equipped with a screw cap and silicon septum was tightly closed and either heated in a aluminium block at 135°C for 30 min or in a microwave oven (Baucknecht 150 W) for 5 min. The DMSO mixture was then diluted with 15 ml of water and the whole solution passed through a C-18 Sep Pak cartridge, which was first activated with ethanol (5ml) and then with water (5 ml). After washing the cartridge successively with 5 ml of 0.1 N HCl, 10 ml H_2O and 50 ml of a mixture methanol/water (20/80), [^{18}F]altanserin was eluted with 2 × 1 ml ethanol.

Preparative HPLC purification was carried out using a Lichrosorb RP Select-B column (250 × 25.7 mm) eluted with [CH_3OH/THF/water pH 5 (5×10^{-2} *M* HOAc)] (12.6/32.4/55) at a constant flow rate of 16 ml/min. The retention time for [^{18}F]altanserin and nitoaltanserin were 55 min and 75 min, respectively.

With the exception of the labeling step and formulation, the entire synthesis was performed with a remote control feel system. Robotic synthesis is under study for further investigations.

Formulation

The HPLC solvent containing the radioactive peak was diluted ten fold with water and passed through a C-18 Sep Pak cartridge. After washing the cartridge with water (25 ml), [^{18}F]altanserin was eluted with 1 ml ethanol. Nine milliters of NaCl (0.9%) were then added and the resulting solution was filtered on Millex-GV (0.22 μm, Millipore) to ensure sterility.

Specific Activity

The specific activity of [^{18}F]altanserin was determined on an analytical reverse-phase HPLC column (Lichrosorb RP Select-B column (250 × 4.6 mm). The mobile phase was CH_3OH/THF/H_2O pH 4 (12.6/32.4/55) with a flow rate of 0.8 ml/min. Under these experimental conditions, the retention time of [^{18}F]altanserin was 10 min. The UV detector with a 10-mm analytical cell was set at 254 nm and the area of the UV absorbance peak of [^{19}F] altanserin was determined by an automated integrator (Shimadzu C-R5A Chromatopac). A calibration curve was determined with authentic reference samples.

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