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^{123}I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB_1 receptors

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Abstract

We have investigated the binding of ^{123}I-labeled *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251), an analog of the cannabinoid receptor antagonist SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] in the mouse brain. Following intravenous injection, the peak whole-brain uptake of about 1% of the administered activity occurred at about 2 h. By 8 h radioactivity in brain had declined to about half its peak value. High-performance liquid chromatographic analysis showed that > 70% of radioactivity extracted from brain at 2 h was still present as [^{123}I]AM251. Co-injection of SR141716A inhibited the in vivo brain binding of [^{123}I]AM251 dose dependently. At 2 mg/kg, the highest dose that could be tested, inhibition was 50% at 2 h post-administration. The ED_{50} value calculated assuming that 2 mg/kg gave near-maximal inhibition was about 0.1 mg/kg. In contrast to the brain, radioactivity in other major organs (blood, liver, kidney, heart and lung) was little affected by SR141716A. The regional binding of [^{123}I]AM251 in the brain was consistent with the published distribution of cannabinoid receptors in rat brain, in that the order was hippocampus, striatum > cerebellum > brain stem. Δ^9-Tetrahydrocannabinol co-administered intravenously at 10 mg/kg, a dose which induced catalepsy and decreased locomotor activity, decreased the 2 h brain uptake of [^{123}I]AM251 by 10%, but this was not significant ($P = 0.08$). In in vitro binding assays with mouse hippocampal membranes, tetrahydrocannabinol inhibited binding of [^{123}I]AM251 with an IC_{50} value of about 700 nM, compared with about 0.2 nM for SR141716A.

Keywords: Cannabinoid receptor antagonist; SR141617A; Δ^9-Tetrahydrocannabinol

1. Introduction

Marijuana is the most commonly used illicit drug (Johnston et al., 1995). Its pleasurable subjective effects which presumably contribute to its abuse include euphoria, feelings of tranquility and altered perceptions. However, marijuana smoking is also associated with adverse effects, including impairments in memory and motor performance (Chait and Pierri, 1992). The major active ingredient of marijuana, Δ^9-tetrahydrocannabinol, exerts many of its effects via its agonist properties at the brain cannabinoid CB_1 receptor, a G-protein-coupled receptor whose gene has recently been cloned (Devane et al., 1988). A second cannabinoid receptor, termed CB_2, which is widespread in peripheral tissues, has also been described (Munro et al., 1993). The normal functions of the cannabinoid receptors are presently unclear, but an endogenous compound, anandamide, which binds to cannabinoid receptor with moderate affinity has been isolated from pig brain (Devane et al., 1992). Studies of cannabinoid receptors have been aided by the development over the last few years of high-affinity agonists, including CP 55,940 and WIN 55,212-2 (Compton et al., 1992; Devane et al., 1988). Autoradiographic studies with tritiated CP 55,940 have allowed detailed distribution studies of cannabinoid receptors in slide-mounted sections of animal (Herkenham et al., 1990) and human (Biegon and Kerman, 1995) brains. Very recently, an antagonist of the cannabinboid CB_1 receptor, SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], has been reported (Rinaldi-Carmona et al., 1994).

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This antagonist is subtype selective since, unlike tetrahydrocannabinol and the high-affinity cannabinoid receptor agonists, it is not a good ligand of the cannabinoid CB_2 receptor (Rinaldi-Carmona et al., 1994).

A ligand labeled with a radionuclide suitable for positron emission tomographic (PET) and single-photon emission computed tomographic (SPECT) imaging could in principle be used to examine the distribution of cannabinoid receptors in the living human brain, to evaluate disease and drug induced changes in cannabinoid receptor densities, and to investigate relationships between receptor occupancy by tetrahydrocannabinol and its behavioral and toxic effects. Such studies might contribute not only to our understanding of the neural basis of marijuana abuse, but also to medication development. Tetrahydrocannabinol is a prescription drug for treatment of glaucoma, and cannabinoid receptor agonists may have other potential medicinal properties (Hollister, 1986). A previous attempt to visualize tetrahydrocannabinol binding sites in baboon brain with Δ^8- tetrahydrocannabinol labeled in the ω position of the alkyl side-chain was only partially successful, probably because of a combination of high lipophilicity and low affinity (Marciniak et al., 1991). The newly developed high-affinity agonists and antagonist may serve as lead compounds for PET and SPECT radiotracers. We report here a study of the binding of ^{123}I-labeled AM251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], an analog of SR 141716A (Fig. 1), to cannabinoid receptors in the mouse brain following intravenous (i.v.) administration. In other work, AM251 has been shown to be a cannabinoid CB_1 receptor selective antagonist (unpublished).

2. Materials and methods

2.1. Radiolabeling

Preparation of [^{123}I]AM251 was accomplished by a radioiododestannylation reaction using the corresponding tributyltin compound. Details of the synthesis of this starting material and the labeling reactions will be published elsewhere. Briefly, ^{123}I-labeled iodide was purchased from Nordion International and mixed with the tributyltin precursor together with phosphoric acid and chloramine-T. The radioactive AM251 was purified by high-performance liquid chromatography (HPLC). Radioactivity and UV absorption at 260 nm were monitored. The product peak was collected and evaporated to near-dryness. Radiochemical yields were 50–60% and radiochemical purity was > 95%. The mass of AM251 determined from the UV absorption under the radioactive peak was < 1 μg. The identity of [^{123}I]AM251 was verified by rechromatography after mixing an aliquot with 5 μg of authentic AM251 and demonstrating that radioactive and UV peaks were coincident. For biological experiments the [^{123}I]AM251 was dissolved at about 50 μCi/ml in 0.9% sodium chloride containing 1% bovine serum albumin.

2.2. Tissue distribution studies

Male mice (Swiss-Webster strain, 25–35 g) were purchased from Taconic Farms (NY). They were injected with 0.2 ml of [^{123}I]AM251 solution via a tail vein. When [^{123}I]AM251 was to be co-injected with SR141716A, the non-radioactive drug was dissolved in 100% ethanol and rapidly mixed with the radiotracer solution. The final concentration of ethanol did not exceed 3.5%. Tetrahydrocannabinol was either co-administered similarly to SR141716A, or was injected i.v. as a solution in 0.1 ml of 40% cyclodextrin, 2 min prior to the radiotracer injection. Animals were killed at 1–8 h by cervical dislocation followed by decapitation. The brain was removed and in some experiments the cerebellum, brain stem, hippocampus, and a dorsal sample of striatum were removed using dissecting forceps and weighed. The lungs, liver, heart and kidneys, as well as samples of blood and urine, were also collected in some experiments. Tissue samples and aliquots of the injected [^{123}I]AM251 solution were assayed for ^{123}I using an auto gamma counter. Results were expressed as percent injected activity per organ (% IA) and percent injected activity per gram of tissue (% IA/g). Differences between groups of animals were assessed using two-tailed *t*-tests.

2.3. Analysis of radioactivity in brain and blood

Following i.v. administration of 10 μCi of [^{123}I]AM251 as described above, whole brains and samples of blood (0.1–0.2 ml) were homogenized in 3 ml of acetonitrile/

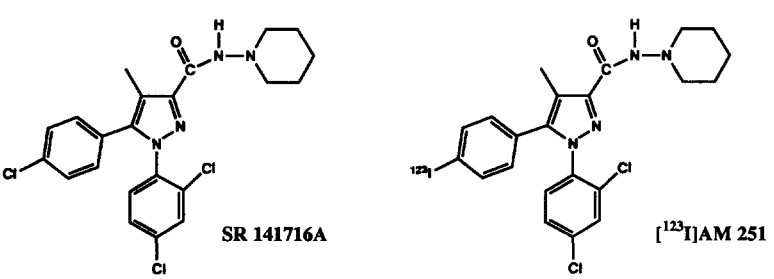

Fig. 1. Structures of SR141716A and [^{123}]AM251.

methanol (2 : 1, v/v) using a Polytron. The homogenates were centrifuged at 16000 × *g* for 2 min. Aliquots of the supernatant fractions (0.3 ml) were shaken for 1 min with a mixture of 6 ml ether, 3 ml ethanol and 3 ml water. The phases were separated and separately assayed for ^{123}I.

For HPLC analysis, 150 μl samples of the supernatants were mixed with 10 μl of a 1 mg/ml solution of AM251, and 350 μl of the HPLC mobile phase, which consisted of acetonitrile/water (2 : 1, v/v) containing 25 mM ammonium acetate. The mixture was centrifuged at 16000 × *g* for 2 min to remove particulate material, and injected onto an Alltech Econosil cyanopropyl column (250 × 4.6 mm) eluted with the above solvent at 2 ml/min. The AM251 peak in each sample was detected by its UV absorption (at about 25 min) and collected into a counting vial.

2.4. Determination of plasma-free fractions

Blood from three mice was collected in heparinized Eppendorf tubes. Red cells were sedimented at 3000 × *g* for 10 min. Aliquots (0.2 ml) of the plasma were mixed with 5 μl of [^{123}I]AM251 solution and incubated at room temperature for 15 min. The plasma samples were pipetted into Centrifree filters (Amicon) which were then centrifuged according to the manufacturer's instructions. Samples (20 μl) of the filtrate and of the plasma before and after centrifugation were counted.

2.5. Preparation of mouse hippocampal membranes

The hippocampus was removed from a single mouse brain immediately after being killed and homogenized (Polytron) in 5 ml of ice-cold buffer containing 50 mM Tris-HCl, pH 7.6, 2 mM EDTA and 5 mM $MgCl_2$, and 1% bovine serum albumin. The homogenate was centrifuged at 16000 × *g* for 8 min at 0–4°. The pellet was resuspended in 5 ml of buffer and incubated at 30° for 10 min before recentrifugation at 16000 × *g* for 8 min at room temperature. The pellet from the second centrifugation was resuspended in 20 ml buffer and stored on ice until used.

2.6. In vitro binding experiments

Incubations were initiated by addition of 0.1 ml of membrane suspension to 13 × 100 mm glass test-tubes containing about 10 nCi of [^{123}I]AM251 (specific activity > 100 Ci/mmol) and various concentrations of test drugs. The tubes were incubated for 1 h at 30° in a total volume of 1 ml. Experiments were terminated by addition of 5 ml of 50 mM Tris-HCl, pH 7.6, containing 0.25% albumin to each incubation tube. After 1 min, membranes were collected on Whatman B filter paper using a Brandel cell harvester. The membranes were then washed with 4 × 5 ml of buffer. Filters were soaked for 3 h before use in 0.5% polyethyleneimine.

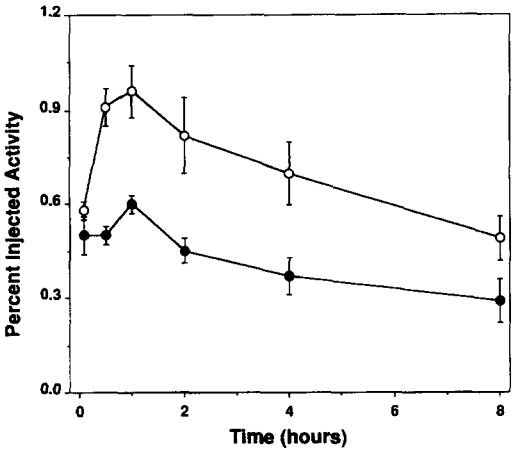

Fig. 2. Brain uptake of [^{123}I]AM251 in SR141716A-treated mice and controls. Mice (male Swiss-Webster weighing 25–35 g) were injected i.v. with [^{123}I]AM251 in vehicle alone (open circles) or together with SR141716A (1 mg/kg; closed circles), and sacrificed at the indicated times. The ^{123}I content of the whole brain was expressed as a fraction of the injected radioactivity.

2.7. Locomotor activity measurements

Groups of 5 mice were placed in activity boxes (San Diego Instruments), and monitored for 2 h following i.v. injection of THC (3 or 10 mg/kg, dissolved in 0.1 ml 40% hydroxypropyl cyclodextrin) or vehicle.

3. Results

3.1. Uptake of [^{123}I]AM251 in brain

Following i.v. injection of [^{123}I]AM251, radioactivity in the brain reached a maximum value close to 1% IA at about 1 h and declined to about one half of this value by 8 h (Fig. 2). When the radiotracer was co-injected with 1 mg/kg SR141716A, brain uptake of ^{123}I was reduced by nearly 50%. This reduction was dose dependent (Fig. 3), with an ED_{50} value of about 100 μg/kg, assuming that the effect at 2 mg/kg SR141716A was maximal.

Brain uptake was regionally specific, with greater binding in the receptor-rich areas of the cerebellum, striatum and hippocampus than in the brain stem (Table 1). The thalamic area and the rest of the brain showed intermediate levels of binding. Radioactivity in striatum and hippocampus did not decline significantly between 1 and 8 h, and radioactivity in cerebellum declined less than in the rest of the brain. The decline in cerebellum is consistent with the existence of receptor-rich and receptor-poor areas in this tissue (Herkenham et al., 1991). After co-injection with SR141716A, the ^{123}I concentration in the hippocampus, striatum and cerebellum was generally reduced to a greater extent than in the brain stem. At the 4 and 8 h time points SR141716A had relatively little effect on the binding of

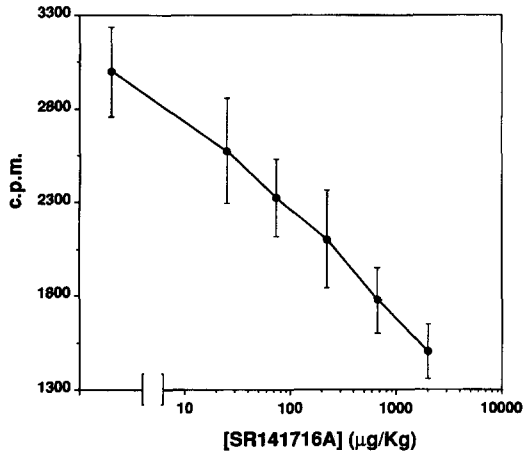

Fig. 3. Concentration dependence of inhibitory effect of SR141716A. Mice were injected i.v. with [^{123}I]AM251 in vehicle alone or together with indicated doses of SR141716A. They were sacrificed at 2 h and the ^{123}I content of the whole brain was expressed as a fraction of the injected radioactivity.

[^{123}I]AM251 in the brain stem, whereas decreases for the other regions were highly significant ($P < 0.005$).

Examination of the effect of tetrahydrocannabinol on the whole brain indicated that, in contrast to SR141716A, this compound did not significantly decrease uptake of [^{123}I]AM251 at 2 h post-injection (Table 2). The doses of 3 mg/kg and 10 mg/kg used in Expt. 2 of Table 2 were behaviorally active; in parallel experiments using activity cages (5 mice per cage), the mean number (±S.D.) of beam crossings per 10 min over the 2 h period was 3003 ± 1573, 1571 ± 874, and 1484 ± 966 after injection of vehicle, 3 mg/kg tetrahydrocannabinol, and 10 mg/kg tetrahydrocannabinol, respectively.

3.2. Uptake of [^{123}I]AM251 in other organs

Tissue concentrations in the other organs examined decreased in the order: liver, kidneys, heart, lungs, blood at all times between 1 and 8 h (Table 3). Liver contained about 10% IA at 1 h and and about 3% IA at 8 h. Unlike the brain, uptake of [^{123}I]AM251 by the other tissues was not markedly affected by co-injection with SR141716A. Maximum decreases of about 20% were seen in kidneys and liver at 4 h, but differences were no longer statistically significant at 8 h. The concentration of ^{123}I in urine was very variable for individual animals, and no systematic dependence on time or SR141716A administration could be discerned. Mean urinary concentrations were 0.5–1.8% IA/g for the eight groups of animals (data not shown).

3.3. Radioactivity in brain and blood

70–80% of ^{123}I was extracted from brain or blood samples after homogenization in acetonitrile/methanol. Nearly all (94 ± 2%, $n = 4$) of the ^{123}I extracted from brain at 2 h was extracted into ether from an aqueous phase, and HPLC analysis showed that 70 ± 5% ($n = 3$) of the initial brain extract remained in the chemical form of [^{123}I]AM251. A representative chromatogram is shown in Fig. 4. In contrast, while 64 ± 13% ($n = 4$) of activity in blood was extracted into ether, only 5–20% remained in the chemical form of [^{123}I]AM251 (not shown).

The plasma-free fraction of [^{123}I]AM251 as measured using centrifugal filters was 0.05 ± 0.04%. Recovery of

Table 1
Brain regional concentrations of ^{123}I

Time (h)		Brain region (% injected radioactivity per g)				
		CB	BS	ST	HP	RB
1	Control	2.61 ± 0.11	1.57 ± 0.56	1.98 ± 0.45	2.11 ± 0.75	2.25 ± 0.27
	SR141716A	1.71 ± 0.13	1.32 ± 0.30	1.19 ± 0.18	1.63 ± 0.34	1.36 ± 0.11
	% change	−34 [a]	−15 [b]	−40 [b]	−23 [b]	−40 [a]
2	Control	2.37 ± 0.35	1.62 ± 0.23	1.91 ± 0.33	1.98 ± 0.39	1.89 ± 0.34
	SR141716A	1.39 ± 0.09	0.92 ± 0.17	0.98 ± 0.13	1.35 ± 0.20	0.97 ± 0.14
	% change	−41 [a]	−43 [a]	−49 [b]	−32 [b]	−49 [a]
4	Control	2.13 ± 0.28	0.98 ± 0.29	1.74 ± 0.71	2.15 ± 0.33	1.61 ± 0.19
	SR141716A	1.16 ± 0.11	0.87 ± 0.14	0.71 ± 0.18	1.06 ± 0.11	0.79 ± 0.21
	% change	−46 [a]	−11 [b]	−59 [a]	−51 [a]	−51 [a]
8	Control	1.61 ± 0.18	0.94 ± 0.06	2.00 ± 0.18	1.88 ± 0.33	1.12 ± 0.20
	SR141716A	0.82 ± 0.33	0.83 ± 0.19	1.42 ± 0.14	1.12 ± 0.09	0.63 ± 0.07
	% change	−49 [a]	−12 [b]	−29 [a]	−40 [a]	−43 [a]

Values are the mean ± S.D. for 6 animals. Animals were injected i.v. with 0.2 ml saline containing 5 μCi [^{123}IAM251 alone (control group) or plus 1 mg/kg SR141716A where indicated. [a] Significant difference ($P < 0.05$). [b] Not significant ($P > 0.05$). Brain regions: CB, cerebellum; BS, brain stem; ST, striatium; HP, hippocampus; RB, rest of brain.

Table 2
Effects of Δ^9-tetrahydrocannabinol (THC) on brain ^{123}I

Expt.	Condition	Brain ^{123}I (% IA at 2 h)
1	Control	0.96 ± 0.08
	SR141716A (1 mg/kg)	0.57 ± 0.06 [a]
	THC (10 mg/kg)	0.85 ± 0.08
2	Control	0.82 ± 0.07
	SR141716A (1 mg/kg)	0.50 ± 0.03 [a]
	THC (3 mg/kg)	0.95 ± 0.12
	THC (10 mg/kg)	0.88 ± 0.08

Values (% IA) are percentage of injected radioactivity (mean ± S.D. for 5 animals) in whole brain at 2 h. In Expt. 1, drugs were co-injected with radiotracer. In Expt. 2, drugs were injected i.v. as a cyclodextrin solution 2 min before radiotracer. [a] $P < 0.001$.

radioactivity in the non-filtered fraction was 102 ± 2%, and 0.92 ± 0.09% remained on filters after six washes with water. After incubation with [^{123}I]AM251, whole blood contained approximately the same concentration of ^{123}I as plasma. After three washes, the erythrocyte fraction retained about 10% of the applied radioactivity.

3.4. In vitro binding studies

In preliminary studies with mouse hippocampal membranes, the binding of [^{123}I]AM251 was inhibited by SR141716A and tetrahydrocannabinol with IC_{50} values of about 0.2 and 700 nM, respectively (Fig. 5). The same ratio of non-specific binding to total binding (20–25% in three separate experiments) was seen at saturating concentrations of SR141716A, tetrahydrocannabinol and WIN 55,212-2 (not shown).

4. Discussion

4.1. Behavior of [^{123}I]AM251 in vivo

Two observations support the notion that [^{123}I]AM251 binds to cannabinoid receptors in the brain after i.v. administration. First, the whole-brain uptake of ^{123}I was significantly reduced by co-injection with the CB_1 receptor antagonist SR141716A at all times except the earliest time point of 5 min (Fig. 2). Although SR141716A slightly reduced the uptake of ^{123}I in other tissues (Table 3), this reduction only reached significance at 4 h for the kidneys, and at 2 and 4 h for the liver. The distribution of ^{123}I between the tissues examined, and the kinetics of clearance from each tissue were very similar in control and SR141716A-treated animals. Thus, the decrease in brain uptake does not appear to be associated with any pharmacodynamic effect of SR141716A which altered distribution to the other tissues which were examined. The failure to find a significant difference in brain uptake at 5 min further argues that SR141716A does not affect delivery of AM251 to the brain, by altering cerebral blood flow. This conclusion is supported by the reported lack of behavioral effects of SR141716A in non-cannabinoid-dependent animals (Lichtmann and Martin, 1995). The second indication for binding to cannabinoid receptors is the regional distribution of ^{123}I. The cerebellum, hippocampus and striatum contained more ^{123}I than the brain stem or the rest of the brain. This pattern is consistent with the distribution of [^{3}H]CP55,940 binding sites in autoradiographic studies of rat brain (Herkenham et al., 1991). In addition, the ^{123}I content of the brain stem, which showed the least accumulation of [^{123}I]AM251, was also the least affected by

Table 3
Tissue concentrations of ^{123}I

Tissue	Group	Tissue radioactivity concentration (% IA/g)			
		1 h	2 h	4 h	8 h
Liver	Control	14.5 ± 1.1	13.4 ± 1.4	10.3 ± 0.90	6.2 ±0.68
	SR141716A	15.4 ± 0.88	11.1 ± 0.50	8.3 ± 0.57 [a]	5.8 ±0.68
Kidneys	Control	7.3 ± 1.4	6.1 ± 0.83	5.6 ± 0.51	4.4 ±0.98
	SR141716A	6.1 ± 0.39	5.2 ± 0.26 [a]	4.3 ± 0.26 [a]	3.3 ±0.61
Heart	Control	3.7 ± 0.28	2.5 ± 0.43	2.2 ± 0.28	1.4 ±0.31
	SR141716A	3.7 ± 0.31	2.7 ± 0.20	1.9 ± 0.20	1.3 ±0.17
Lungs	Control	2.8 ± 0.22	2.0 ± 0.18	1.5 ± 0.12	1.1 ±0.24
	SR141716A	2.5 ± 0.22	2.0 ± 0.36	1.6 ± 0.20	0.88 ± 0.24
Blood	Control	2.1 ± 0.44	1.7 ± 0.54	1.0 ± 0.08	0.64 ± 0.52
	SR141716A	1.0 ± 0.42	1.3 ± 0.20	1.0 ± 0.03	0.50 ± 0.22

Values (% IA) are percentage of injected radioactivity per g of tissue (mean ± S.D. for 6 mice). Animals were injected i.v. with 0.2 ml saline containing 5 μCi [^{123}I]AM251 alone (control group) or plus 1 mg/kg SR141716A where indicated. [a] Significant difference between control and SR141716A groups ($P < 0.05$).

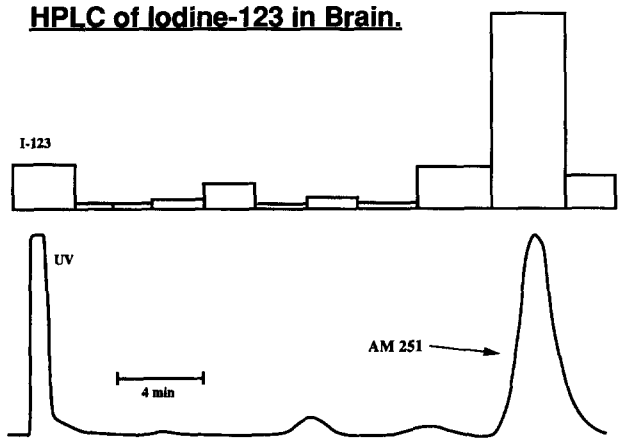

Fig. 4. HPLC analysis of radioactivity in mouse brain after administration of [^{123}I]AM251. A mouse was injected i.v. with [^{123}I]AM251 and sacrificed at 2 h. Radioactivity was extracted from the brain by homogenization in methanol/acetonitrile (1:2, v/v) containing 10 μg non-radioactive AM251, and chromatographed using a cyanopropyl column eluted with acetonitrile/water (1:2, v/v) containing 25 mM ammonium acetate at 2 ml/min.

SR141716A, as expected if the binding in the brain stem is mainly 'non-specific' in character. These experiments are consistent with the following picture. Initial uptake of [^{123}I]AM251 is non-specific in character and largely reflects solution in brain lipids. Furthermore, all brain regions may contain enough cannabinoid CB_1 receptors to bind appreciable amounts of [^{123}I]AM251 at short times after administration of the radioligand. At longer times non-specifically bound tracer leaves the brain or binds to cannabinoid CB_1 receptors, and in addition clearance of specifically bound [^{123}I]AM251 from various regions is inversely related to the cannabinoid CB_1 receptor density. Thus, ^{123}I in the striatum and hippocampus, two brain regions which contain high concentrations of cannabinoid CB_1 receptors in the rat brain, did not significantly change between 1 and 8 h. These ideas can be examined in more detail in rodent models when results of in vivo autoradiographic studies with [^{123}I]AM251 or [^{125}I]AM251 are available.

We observed dose-dependent inhibition of brain uptake of AM251 by co-injection with 25 μg/kg to 2 mg/kg SR141716A (Fig. 3). The inhibiton at 2 mg/kg may not be maximal, since the poor aqueous solubility of SR141716A hindered the administration of higher doses by the i.v. route. The estimated ED_{50} value of about 100 μg/kg should correspond to a brain concentration of about 50 nM, since about 0.5% of the injected activity is localized in the brain. This is 2 orders of magnitude higher than the IC_{50} of SR141716A observed in in vitro binding experiments with brain membranes (Fig. 5). One explanation for this discrepancy is that partioning of the lipophilic drug into brain lipids reduces the concentration of free drug able to compete with AM251 for binding to the cannabinoid CB_1 receptor.

4.2. Potential for use in SPECT experiments

^{123}I has extremely good imaging characteristics for SPECT, providing brain images with a spatial resolution of about 7 mm in the latest clinical instruments (Laruelle et al., 1995). The maximum brain uptake of [^{123}I]AM251 in mice of about 1% IA was comparable to that seen with some commonly used PET and SPECT radiotracers in this species, and, additionally, receptor-rich brain areas contained more radioactivity than receptor-poor areas. Furthermore, the mouse brain kinetics of [^{123}I]AM251, peaking at

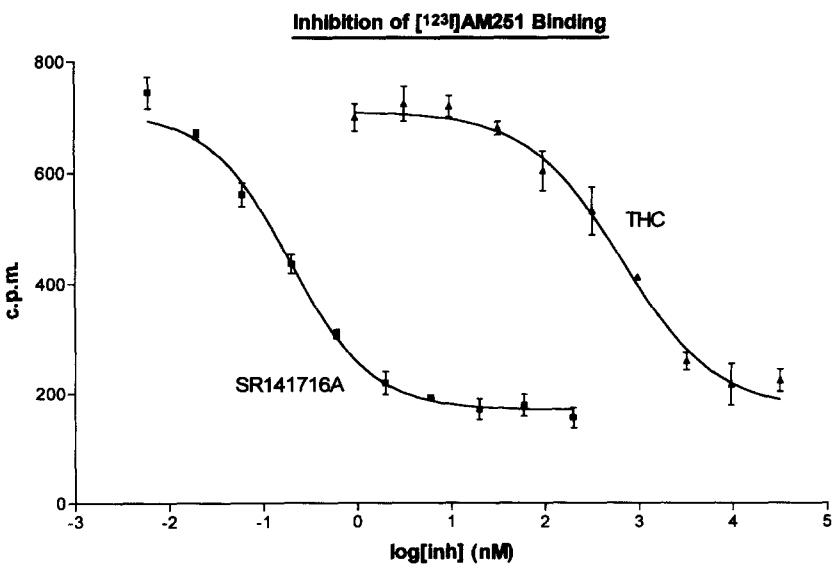

Fig. 5. Inhibition by SR141716A and Δ^9-tetrahydrocannabinol on in vitro binding of [^{123}I]AM251. Membranes were prepared from mouse hippocampus and incubated for 1 h at 30°C with [^{123}I]AM251 (< 0.1 nM) and indicated concentrations of SR141716A or Δ^9-tetrahydrocannabinol.

about 2 h and showing a half-clearance time of about 8 h, are rather similar to those of the dopamine transporter radioligand [^{123}I]RTI-55 in rodent models. In humans, [^{123}I]RTI-55 achieves an approximately equilibrium state of binding about 24 h after administration, and provides high quality images of its brain binding sites (Laruelle et al., 1994). Our data are thus encouraging for the eventual use of [^{123}I]AM251 in SPECT studies of cannabinoid CB_1 receptor densities and occupancies in the human brain.

The demonstration that most of the radioactivity in brain at 2 h still represented unmetabolized [^{123}I]AM251 (Fig. 4) is also encouraging, since the absence of recirculation of labeled metabolites to the brain greatly simplifies tracer kinetic modeling of SPECT data. SPECT studies in monkeys will ascertain whether binding kinetics are similar in rodents and primates, permit evaluation of various tracer kinetic modeling strategies for quantification of binding site concentrations, and also allow estimates of organ radiation doses which are necessary before human studies can be conducted.

4.3. Cannabinoid receptor occupancy required for behavioral effects

Tetrahydrocannabinol at a dose of 10 mg/kg did not significantly alter the brain concentration of [^{123}I]AM251 (Table 2) although it was behaviorally active as shown by a decrease in motor activity. A possible explanation for this observation is that the behavioral and subjective effects of cannabinoid receptor agonists require occupancy of only a small fraction of the available receptors. Alternatively, the failure of tetrahydrocannabinol, unlike SR 141716A, to significantly inhibit in vivo binding of [^{123}I]AM251 may be related to the surprizingly high concentration necessary for tetrahydrocannabinol to displace [^{123}I]AM251 binding in the in vitro assays when compared to published data for displacement of [^{3}H]CP 55,940 by tetrahydrocannabinol. Thus, Rinaldi-Carmona et al. (1994) reported an approximately 10-fold lower potency of tetrahydrocannabinol than of SR141716A using [^{3}H]CP 55,940, compared with a 1000-fold in the present study using [^{123}I]AM251. It could be speculated therefore that tracer doses of [^{123}I]AM251 bind predominantly to a novel, previously undocumented, binding site with a relatively low affinity for tetrahydrocannabinol compared to the agonist binding site of the CB_1 receptor. However, the possibility cannot yet be ruled out that AM251 and tetrahydrocannabinol do bind to the same site, but that even when present at tracer doses [^{123}I]AM251 can 'compete' with tetrahydrocannabinol for binding to CB_1 receptors. Other characteristics of AM251 and tetrahydrocannabinol besides in vitro affinities need to be examined in relation to the interpretation of in vivo binding experiments. These include relative systemic pharmacokinetics, kinetics of brain entry, and partitioning into brain lipids.

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