In Vivo Binding in Rat Brain and Radiopharmaceutical Preparation of Radioiodinated HEAT, an Alpha-1 Adrenocepter Ligand

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In vivo binding of [^{125}I]-2-[β-(3-iodo-4-hydroxyphenyl)ethylaminomethyl tetralone) ([^{125}I]HEAT) to alpha-1 adrenoceptors in the rat brain was determined over 4 hr. Uptake in the thalamus and frontal cortex was ~0.1% injected dose per gram tissue. Thalamus/cerebellum ratios of 10:1 and frontal cortex/cerebellum ratios of 5:1 were found at 4 hr. Pretreatment with prazosin, an alpha-1 antagonist, completely inhibited the accumulation of [^{125}I]HEAT in thalamus and frontal cortex; yet uptake of radioactivity was not significantly affected by antagonists and agonists for other receptors classes (propranolol, β-1; apomorphine, D-1; spiperone, D-2). Binding of [^{125}I]HEAT is saturable. At 4 hr, [^{125}I]HEAT or [^{123}I]HEAT was shown to be the only radioactive material in rat thalamus and frontal cortex. Iodine-123 HEAT and [^{125}I]HEAT were synthesized as radiopharmaceuticals within 3 hr in 99% radiochemical purity.

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HEAT, (2-[β-(4-hydroxyphenyl)ethylaminomethyl]tetralone) is an antihypertensive drug (*1–3*) which acts by means of peripheral and central alpha-adrenoceptor antagonism (*4*). In vitro, HEAT is 140 times more selective for alpha-1 than for alpha-2 adrenoceptors (*5, 6*), has no beta-adrenoceptor activity and negligible serotonin activity (*3*). Although iodination generally results in a drug with reduced affinity, it was found that both iodo-HEAT and [^{125}I]HEAT ([^{125}I]-2-[β-(3-iodo-4-hydroxyphenyl)ethylaminomethyl]tetralone) have 6–15-fold higher in vitro affinities for the alpha-1 adrenoceptor than does the parent compound HEAT (*7, 8*). [^{125}I]HEAT (Fig. 1) has subsequently proved to be a superior ligand for in vitro binding studies and for the mapping of alpha-1 adrenoceptors by in vitro autoradiography(*9–11*). High concentrations of alpha-1 adrenoceptors in thalamus and frontal cortex have been demonstrated by these techniques.

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In this paper we have shown by means of in vivo brain distribution, saturability, and competition studies that commercial [^{125}I]HEAT binds specifically to alpha-1 adrenoceptors in rat brain. In vivo brain distribution and stability studies with [^{123}I]HEAT, prepared in our laboratories, gave similar results. Tissue distribution studies and dosimetry estimates reported recently show that the radiation absorbed dose to man from 2 to 3 mCi [^{123}I]HEAT will be within acceptable limits (*12*). Consequently, we have developed a technique for the rapid synthesis of [^{123}I]HEAT for use as a radiopharmaceutical which should be suitable for the imaging of alpha-1 adrenoceptors in man by single photon emission computed tomography (SPECT).

MATERIALS AND METHODS

Sodium [^{125}I] in 0.1 N NaOH and [^{125}I]HEAT (2,200 Ci/mmol; 168 μCi/ml) in ethanol:0.001*M* KH_2PO_4 (1:1) were obtained commercially (Dupont Company, No. Billerica, MA). Sodium [^{123}I] in 0.1 N NaOH was supplied by Atomic Energy of Canada. Radiochemical purity of [^{125}I]HEAT, checked by thin layer chromatography (TLC) using TLC System III at weekly intervals, was >99%.

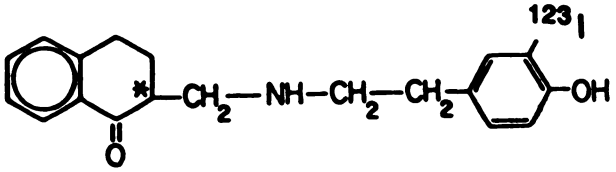

FIGURE 1
Structure of radioiodinated HEAT. * Denotes an asymmetric carbon.

Thin Layer Chromatography

The following systems were employed to determine purity of products:

System I: ammonium formate 0.05M, pH 8.4, cellulose (Eastman Kodak chromogram sheets: nonradioactive spots visualized with ninhydrin. Radioactive TLC were scanned using a Packard model 7220 Radiochromatogram Scanner, (Packard Instrument Company, Downers Grove, IL), R_f HEAT 0.51; iodo-HEAT 0.025; iodotyramine 0.61. (Literature value (*8*) R_f HEAT 0.51; iodo-HEAT 0.037; iodo-tyramine, 0.59.)

System II: ethyl acetate:methanol (50:50 v/v), silica gel (Eastman Kodak chromogram sheets); R_f HEAT 0.62; iodo-HEAT 0.53, iodo-tyramine 0.04. (Literature value—personal communication—Dupont—[^{125}I]HEAT 0.50.)

System III: chloroform:methanol (50:30 v/v), silica gel; R_f HEAT 0.62; iodo-HEAT 0.72; iodo-tyramine 0.05. (Literature value (*8*) R_f HEAT 0.75; iodo-HEAT 0.85; iodo-tyramine 0.12.)

Synthesis of Iodo-HEAT

To a solution of HEAT (0.5 ml, 2 μg/ul in 13.5 mM HCl) was added potassium phosphate buffer (2 ml, 1*M*, pH 7.6) and sodium iodide (1 ml, 57 mg/10 ml in 0.1N NaOH). The reaction was initiated by the addition of chloramine-T (0.5 ml, 0.34 mg/ml) and the solution stirred vigorously and allowed to stand at room temperature for 7 min.

The reaction was terminated by the addition of sodium thiosulfate (30 mg, 47 mg $Na_2S_2O_3 \cdot 5H_2O$), made basic with NaOH (1 ml, 0.1 N), and shaken with ethyl acetate (3 × 1 ml) containing phenol (0.01%). This volume of ethyl acetate was reduced by evaporation under a stream of dry nitrogen gas to ~30 μl. Distilled water (30 μl) was added and the residual ethyl acetate removed with nitrogen. Methanol (70 μl) containing 50 m*M* Tris-HCl (1% v/v, pH 6.5) was then added to the aqueous residue giving a methanol-water solution (70:30) suitable for high pressure liquid chromatography (HPLC).

Purification of iodo-HEAT was carried out by HPLC (Waters pump (Model 6000A), Rheodyne Injector (760), C-18 Biosphere ODS column (5 μ*M* Spherical particles, 250 × 4.6 mm), LDC Spectromonitor II uv detector) as shown in Figure 2; methanol: water:diethylamine (70:30:0.04), pH 6.4 (*13*). At a flow rate of 0.8 ml/min, HEAT and iodo-HEAT were completely resolved with retention times of 7 and 10 min, respectively. TLC (Systems II and III) was employed to confirm the purity of iodo-HEAT.

Preparation of [^{125}I]HEAT. HEAT (10 μl, 6.03 m*M* in 13.5 mM HCl), potassium phosphate buffer (200 μl, 1.0*M*, pH 7.6), carrier free sodium [^{125}I] (2 mCi, 20 μl, 40 μ*M*), and aqueous

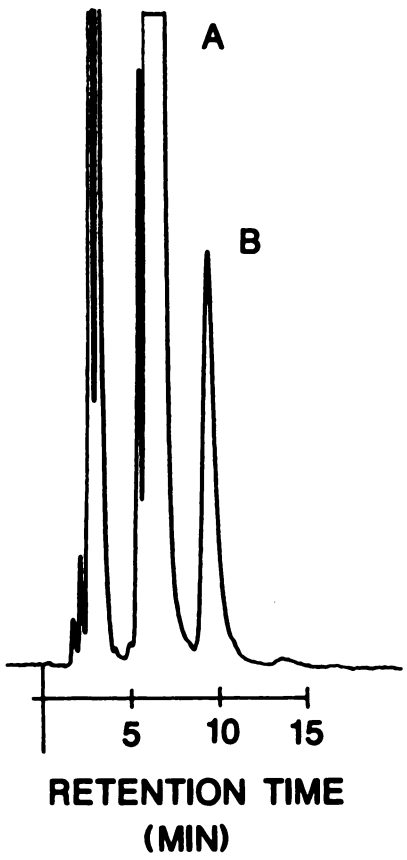

FIGURE 2
HPLC separation of HEAT (A) and iodinated HEAT (B). Free iodide is eluted with the solvent front. Column, Biophase ODS, 5 μm, C-18, 250 × 4.6 mm. Mobile phase, methanol:water:diethylamine (70:30:0.04) pH 6.5. Flow rate, 0.8 ml/min; uv detector, 254 n*M*.

chloramine-T (20 μl, 0.34 mg/ml) were reacted in a polypropylene tube. After 7 min, the reaction was stopped with an excess of aqueous sodium thiosulfate (30 μl, 0.1*M*). The [^{125}I]HEAT was extracted immediately with ethyl acetate (4 × 400 μl). These extracts were combined and the volume reduced to 30 μl under a stream of nitrogen gas. The residue was diluted as described previously and the mixture injected onto the HPLC (flow rate 0.5 ml/min to ensure complete separation of product). HPLC eluant was collected over 1-min intervals from the HPLC system and each aliquot counted to determine the location of the radioiodinated HEAT (usually contained in 2–3 ml). These aliquots were pooled and reduced in vacuo to low bulk and the residue reconstituted in normal saline (4 ml, 0.9%). Yield 50%; specific activity 2,200 Ci/mmol. TLC (Systems II and III) confirmed the product was [^{125}I]HEAT in greater than 99% radiochemical purity.

Radiopharmaceutical Preparation of [^{123}I]HEAT. HEAT (10 μl, 6.03 m*M* in 13.5*M* HCl), potassium phosphate buffer (200 μl, 1.0*M*, pH 7.6), carrier-free sodium [^{123}I] in 0.1 N NaOH (5 mCi, ~35 μl, 0.02 n*M*), sodium iodide (1.0 ng in 10 μl water, 1 pmol) and aqueous chloramine-T (20 μl, 0.34 mg/ml) were reacted for 7 min. The workup of [^{123}I]HEAT was the same as described above for [^{125}I]HEAT. Radioactive yield of desired product after the HPLC step was 75%. TLC analysis

(Systems II and III) showed this product to have a single radioactive spot at the R_f of iodo-HEAT.

This preparation was sterilized by passing through a sterile 0.22-μm nylon filter (Cameo II S, Micron Separation, Inc., Honeoye Falls, NY) into a sterile, pyrogen-free evacuated vial ready for injection. The overall yield was 55% of the original activity 5 mCi; specific activity (~2.4 × 10^4 Ci/mmol). An aliquot of this injection, used for the TLC characterization of the radiopharmaceutical containing [^{123}I]HEAT, gave R_f values identical, within experimental error, to those of iodo-HEAT. This radiopharmaceutical, when stored at 4° C for 24 hr, was found to be stable when characterized by TLC. However, decomposition began within 4 hr when stored at room temperature.

Sterility of this radiopharmaceutical was confirmed by incubating aliquots in tryptic soy broth medium (Difco Laboratories, Detroit, MI) (for fastidious and nonfastidious microorganisms) and in fluid thioglycollate medium (Difco Laboratories, Detroit, MI)(for aerobic and nonaerobic microorganisms). The Limulus Amebocyte Lysate (Pyrogent) test (Whittaker M. A. Bioproducts, Walkersville, MD) was employed to show that all preparations were pyrogen-free.

In Vivo Studies

Male Sprague-Dawley rats (150–250 g), fed on a normal diet, were used in all studies. The rats were decapitated following a lethal dose (1 grain) of sodium pentobarbital and tissues removed. The brain was dissected on a cold glass plate (*14*) and all brain and other tissue sections were placed in plastic vials, weighed and counted in a gamma counter (Beckman Model DP 5500 Counter, Beckman Instruments, Fullerton, CA).

Brain distribution studies. [^{125}I]HEAT (2–3 μCi in 0.4 saline) was administered by tail vein injection, groups of five rats being killed at 0.5, 1, 2, 3, and 4 hr after injection. The distribution of activity in the thalamus, frontal cortex, other cortex, hypothalamus, medulla, midbrain, striatum, hippocampus and cerebellum was evaluated in terms of percent injected dose per organ (Table 1) and percent injected dose per gram tissue (Table 2).

Saturation studies. Aqueous prazosin (0.01, 0.05, 0.1, 0.25, 1 and 5 mg/kg body weight) was administered intraperitoneally to each group of rats (n = 5) 30 min prior to i.v. administration of [^{125}I]HEAT and the rats were killed 4 hr later. The ratios of radioactivity in thalamus and frontal cortex (in % dose per gram) to that in the cerebellum (TH/CB and FC/CB, respectively) were determined for each concentration.

Competition studies. Spiperone, propranolol, HEAT·HCl, or apomorphine·HCl was injected intravenously into rats (n = 4) simultaneously with [^{125}I]HEAT (2–3 μCi). Rats were killed at 4 hr and the TH/CB ratio determined for each drug.

Stability of [^{123}I]HEAT in rat brain. Five rats were injected with [^{123}I]HEAT (~300 μCi) and killed at 4 hr. The thalamus and frontal cortex of each rat were excised, homogenized in methanol and centrifuged. The volume of supernatant was reduced under a stream of nitrogen gas, applied to a silica gel TLC plate and developed using Systems II and III. In all five cases, greater than 99% of the radioactivity was detected at the R_f of iodo-HEAT.

Distribution of [^{123}I]HEAT in eyes and thyroid. Eyes were removed from rats 4 hr after administration of HEAT and the % injected dose per organ and per gram were determined. Five rats were given water containing Lugol's solution 24 hr prior to being killed. Their thyroids were removed 4 hr after administration of [^{123}I]HEAT and analyzed.

RESULTS

Radiopharmaceutical Preparation

HEAT is readily radioiodinated by reaction with chloramine-T (*6, 7*) however, the yield of product is affected by a number of parameters.

In order to optimize the yield of radioactive HEAT several variables were examined. The relationship between the yield of product and the reaction time are summarized in Table 3. The optimum reaction time of 7 min for mono-iodination of HEAT gives about 70% [^{125}I]HEAT after HPLC separation (*13*). Since carrier free sodium [^{125}I] was used in this reaction, [^{125}I]HEAT isolated by HPLC is assumed to have the theoretical specific activity (2200 Ci/mmol).

In the preparation of [^{123}I]HEAT, the optimum reaction time of 7 min was used. However, the yield of this isotope containing product was substantially lower (60% maximum) than that of the [^{125}I] (82%). In order to maximize the initial incorporation of [^{123}I], exoge-

TABLE 1
Distribution of [^{125}I]HEAT in Rat Brain–Percent Dose per Organ (Mean ± s.e.m.)

Time (hr)	0.5	1	2	3	4
N	4	5	5	5	5
Thalamus	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.002	0.02 ± 0.00	0.013 ± 0.001
Frontal cortex	0.12 ± 0.03	0.07 ± 0.01	0.04 ± 0.003	0.02 ± 0.00	0.014 ± 0.001
Other cortex	0.17 ± 0.03	0.10 ± 0.01	0.06 ± 0.004	0.03 ± 0.003	0.015 ± 0.002
Hypothalamus	0.03 ± 0.01	0.01 ± 0.001	0.01 ± 0.0004	0.002 ± 0.001	0.001 ± 0.0002
Medulla	0.08 ± 0.03	0.03 ± 0.003	0.02 ± 0.001	0.01 ± 0.001	0.004 ± 0.001
Midbrain	0.05 ± 0.02	0.02 ± 0.004	0.01 ± 0.001	0.004 ± 0.001	0.003 ± 0.001
Striatum	0.04 ± 0.01	0.02 ± 0.002	0.01 ± 0.001	0.003 ± 0.000	0.002 ± 0.0004
Hippocampus	0.03 ± 0.004	0.02 ± 0.002	0.01 ± 0.001	0.002 ± 0.000	0.002 ± 0.0004
Cerebellum	0.08 ± 0.02	0.03 ± 0.003	0.01 ± 0.002	0.004 ± 0.000	0.003 ± 0.0004
Whole brain	0.62 ± 0.13	0.33 ± 0.04	0.18 ± 0.01	0.09 ± 0.01	0.056 ± 0.007

TABLE 2
Distribution of [^{125}I]HEAT in Rat Brain–Percent Dose per Gram (Mean ± s.e.m.)

Time (hr)	0.5	1	2	3	4
N	4	5	5	5	5
Thalamus	0.40 ± 0.07	0.25 ± 0.00	0.18 ± 0.01	0.10 ± 0.01	0.10 ± 0.02
Frontal cortex	0.42 ± 0.08	0.24 ± 0.03	0.16 ± 0.01	0.08 ± 0.01	0.05 ± 0.01
Other cortex	0.40 ± 0.09	0.21 ± 0.02	0.12 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Hypothalamus	0.32 ± 0.06	0.18 ± 0.02	0.09 ± 0.01	0.03 ± 0.002	0.02 ± 0.004
Medulla	0.34 ± 0.09	0.16 ± 0.02	0.09 ± 0.01	0.03 ± 0.002	0.02 ± 0.004
Midbrain	0.32 ± 0.08	0.16 ± 0.02	0.08 ± 0.01	0.03 ± 0.002	0.02 ± 0.01
Striatum	0.34 ± 0.09	0.14 ± 0.02	0.07 ± 0.01	0.03 ± 0.002	0.02 ± 0.004
Hippocampus	0.30 ± 0.07	0.14 ± 0.02	0.07 ± 0.01	0.02 ± 0.002	0.01 ± 0.002
Cerebellum	0.32 ± 0.10	0.11 ± 0.01	0.04 ± 0.01	0.01 ± 0.002	0.01 ± 0.002

nous NaI was added to the reaction. The carrier added preparation gave higher chemical yields (82%) and an overall radiopharmaceutical yield of 50–55%. Since this ^{123}I preparation is "carrier added", the specific activity is proportionally reduced.

A variety of filters were tested in order to find one suitable for sterilization purposes. Most of the filters trapped 80–90% of the radioiodinated HEAT. The most satisfactory were the 0.22 μm Cameo II S nylon filter (Micron Separation, Inc., Honeoye Falls, NY) and Millipore Swinnex-13 filter (Millipore Corp., Bedford, MA) through which were obtained pharmaceutically acceptable preparations with an overall radiochemical yield of 50%. Thus, typical radiopharmaceutical preparations of [^{123}I]-and [^{125}I]HEAT give ~50% and 55% overall yields, respectively, in 3 hr.

The results of the distribution studies in Tables 1, 2, 3, and 4 were obtained from [^{125}I]HEAT purchased from Dupont. After the optimization of experimental conditions and yields, freshly prepared batches of [^{125}I]- and [^{123}I]HEAT were assayed for regional brain accumulations. The thalamus/cerebellum and frontal cortex/cerebellum ratios obtained 4 hr after injection with these radiopharmaceuticals were the same as those obtained from the commercial material.

TABLE 3
Percent Yield of [^{125}I]HEAT from Reaction Mixture and HPLC

Time (min)	Yield of organic iodine from reaction mixture	Total yield of [^{125}I] HEAT after HPLC separation*
5	70–75%	58%–60%
7	80–82%	66%–70%
10	49–50%	40%†
15	54–55%	42%†

* The column separated [^{125}I]HEAT from other material, usually at a 80–85% yield of the total injected radioactivity, the remaining 15–20% was [^{125}I]tyramine (retention time 7 min) and iodide (at solvent front).

† At longer reaction times, the oxidative environment in the reaction tube decomposed [^{125}I]HEAT to give [^{125}I]tyramine which coelutes with unreacted HEAT.

Distribution of [^{125}I]HEAT in Rat Brain

The highest concentration of alpha-1 adrenoceptors in the brain are found in the thalamus and frontal cortex, the lowest in the cerebellum (*9*). Levels in the other regional structures fall between these extremes (*10, 11*). To determine whether [^{125}I]HEAT is bound specifically to the alpha-1 adrenoceptors in the brain, we assayed the radioactivity in nine different regions of rat brain at 0.5, 1, 2, 3, and 4 hr after injection of 2 to 3 μCi of [^{125}I]HEAT (Tables 1 and 2). At 30 min after injection, activity per gram tissue was about the same in all regions of the brain. However, at later times, the thalamus and, to a lesser extent, the frontal cortex contained significantly higher activity per gram than did the other regions. Since the cerebellar radioactivity primarily reflects the nonspecific accumulation of drug, the ratio of radioactivity in thalamus to that in cerebellum (TH/CB) is a convenient measure of the ratio of specific to nonspecific binding. This ratio increased linearly from 1.5:1 at 30 min to 10.5:1 at 4 hr (Table 4; Fig. 3) even though the concentration of [^{125}I]HEAT present in thalamus was less at the later time (Table 2). A similar increase was noted in the frontal cortex/cerebellum ratio (FC/CB).

TABLE 4
Thalamus/Cerebellum and Frontal Cortex/Cerebellum Ratios in Rat Brain After Administration of [^{125}I]HEAT (Ratio ± s.e.m.)

Time (hr)	n	TH/CB (p*)	FC/CB (p*)
0.5	4	1.5 ± 0.2	1.6 ± 0.3
1	5	2.2 ± 0.2 (<0.04)	2.0 ± 0.1 (<0.04)
2	5	4.3 ± 0.2 (<0.05)	4.0 ± 0.3 (<0.01)
3	5	7.6 ± 1.2 (<0.01)	5.5 ± 0.6 (<0.01)
4	5	10.5 ± 0.9 (<0.01)	6.3 ± 0.6 (<0.01)

* The confidence level as determined by Student's t-test at various time intervals compared to the value at 0.5 hr.

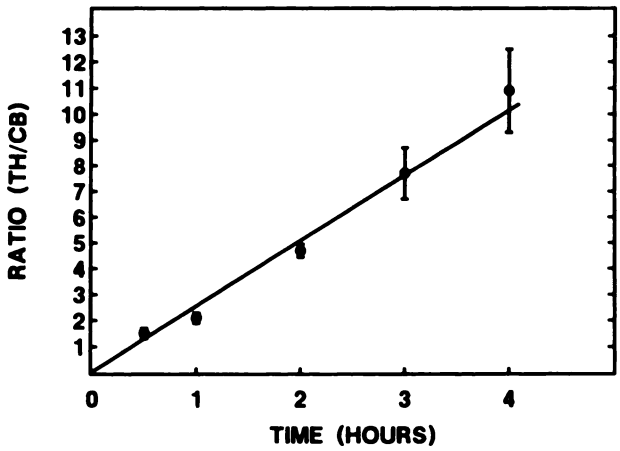

FIGURE 3
Ratio of concentration of [^{125}I]HEAT in thalamus and cerebellum at various times after intravenous injection. Data are mean (± s.e.m.) with n = 5 except for 0.5 hr where n = 4. The ratios at 3 and 4 hr are significantly different from that at 0.5 hr.

The ratios of the uptake of radioactivity per gram in various regions of the brain to that in the cerebellum were determined after 4 hr. The rank order of these ratios from our in vivo study is comparable with that obtained from the in vitro binding measurements of [^{125}I]HEAT by Jones et al. (*9*) (Table 5). This similarity in regional localization is indicative of the high degree of selectivity that [^{125}I]HEAT has for alpha-1 adrenoceptors. The TH/CB and FC/CB ratios resulting from the administration of [^{123}I]HEAT were found to be identical, within experimental error, to those obtained from [^{125}I]HEAT.

Further, it was shown that [^{123}I]HEAT does not accumulate appreciably in the eyes of the rat, the percent injected dose per gram and per organ being 0.10 ± 0.06 and 0.05 ± 0.03, respectively (n = 10). It was also determined that uptake of activity by the thyroid is greatly reduced by prior administration of KI (Lugol's solution) the % injected dose per gram and per organ being 0.24 ± 0.11 (n = 6) and 0.004 ± 0.002 (n = 6), respectively, as compared to 82% ± 10% (n = 4) and 1.3 ± 0.23 (n = 4) for the unblocked thyroid (12).

TABLE 5
Regional Accumulation of [^{125}I]HEAT at 4 hr

Region	In Vivo Ratio	In Vitro Ratio*
Thalamus	10.5	3.6
Frontal cortex	5.0	2.6
Other cortex	3.1	1.8
Hypothalamus	3.1	1.2
Medulla	2.0	1.5
Midbrain	2.2	—
Striatum	2.2	1.1
Hippocampus	1.7	1.7
Cerebellum	1.0	1.0

* Taken from Reference 9.

Saturability of the Accumulation of [^{125}I]HEAT

To show that [^{125}I]HEAT binds specifically to alpha-1 receptors, animals were injected with increasing amounts of prazosin, a highly selective alpha-1 adrenoceptor antagonist, 30 min before intravenous injection with 2–3 μCi of [^{125}I]HEAT. They were killed 4 hr later and the radioactivity measured in the various regions of the brain. Uptake of radioactivity by the thalamus and frontal cortex was significantly decreased by 0.1 mg/kg prazosin and further decreased by higher doses (Fig. 4). Concentration of activity in cerebellum was independent of the administered dose of prazosin. At the highest doses of prazosin the % injected dose per gram in all nine regions of the brain were the same within experimental error. This indicates that [^{125}I]HEAT is taken up by a finite number of specific sites.

Effect of the Administration of Various Drugs on the Accumulation of [^{125}I]HEAT in Rat Brain

If the accumulation of radioactivity in the thalamus reflects specific alpha-1 adrenoceptor binding of [^{125}I]HEAT, then drugs active at the alpha-1 adrenoceptor should prevent its binding while non-alpha-adrenergic drugs should have no effect on the accumulation of radioactivity. Several adrenergic receptor binding drugs were injected intravenously simultaneously with [^{125}I]HEAT (Table 6). The accumulation of radioactivity in the thalamus was totally blocked by the two highly specific alpha-1 antagonists prazosin and nonradioactive HEAT and was unaffected by the dopamine-1 agonist, apomorphine, and the beta-1 adrenergic antagonist propranolol. The reduced accumulation of [^{125}I]

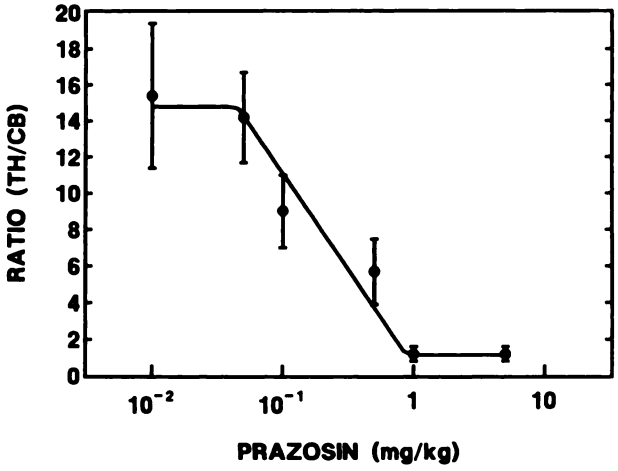

FIGURE 4
Ratio of concentration of [^{125}I]HEAT in thalamus and cerebellum at 4 hr after injection of varying amounts of prazosin. Data are mean (± s.e.m.) with n = 5. TH/CB ratios resulting from 5, 1 and 0.3 mg/kg prazosin are significantly different ($p < 0.01$) from the ratio obtained when no prazosin is administered.

TABLE 6
Effect of Drugs on Thalamus/Cerebellum Ratios of [^{125}I] HEAT Binding (n = 4)

Drug	Ratio
None (Control)	10.5
Prazosin (1 mg/kg, i.p.)	1.2
HEAT (1 mg/kg, i.v.)	1.1
Propranolol (1 mg/kg, i.v.)	7.8
Apomorphine (10 mg/kg, i.v.)	9.2
Spiperone (1 mg/kg, i.v.)	4.9

HEAT by the thalamus in the presence of dopamine-2 antagonist spiperone is due to its inherent affinity for the alpha-1 site, k_D = 18 nM (*15*). Thus it is evident that [^{125}I]HEAT binds specifically to alpha-1 sites in the brain but not to dopamine or beta sites.

Stability of [^{123}I]HEAT and [^{125}I]HEAT in Rat Brain

It is possible that radioiodinated HEAT is rapidly metabolized by the body and that the images result from some metabolic product of the original radiopharmaceutical. To determine whether this occurred, rat thalamus and frontal cortex were extracted 4 hr after administration of radioiodinated HEAT. TLC analysis of the extract (n = 7) showed a single radioactive spot at the R_f of authentic iodinated HEAT (Systems II and III). Thus there did not appear to be any significant breakdown of the [^{123}I]- or [^{125}I]HEAT bound to alpha-1 receptors in thalamus and frontal cortex.

DISCUSSION

The results presented here indicate that [^{125}I]HEAT accumulates in various regions of the rat brain by a saturable process; it distributes in these regions in the same proportions as alpha-1 adrenoceptors as found by in vitro assay. Iodine-125 HEAT is displaced by alpha-1 antagonists but not by other receptor antagonists and it is stable in vivo at 4 hr after injection. These results are in good agreement with the distribution and prazosin studies reported by O'Tuama et al. (*16*) for mice. The present study in rat found that TH/CB and FC/CB ratios 4 hr after injection of [^{123}I]HEAT were the same, within experimental, to those after administration of [^{125}I]HEAT.

Dosimetry estimates indicate that a dose of 3 mCi [^{125}I]HEAT to humans is within acceptable limits (*12*). Further, we have shown that prior administration of KI prevents appreciable uptake of radioactivity by the thyroid. The major problem with this agent for the imaging of alpha-1 adrenoceptors in the human is the low uptake, which may prevent visualization of the thalamus by planar or SPECT imaging with singlehead cameras in periods of time which are practical for patient head immobilization. If this proves to be the case, the newly developed SPECT instrument with three heads (*16*) which allows full 2π utilization of emitted photons and results in a greater than fourfold increase in sensitivity, may overcome this difficulty.

Our work complements and expands the study of [^{125}I]HEAT, an alpha-1 receptor agent, by O'Tuama et al. (*16*) and confirms the hypothesis that [^{123}I]HEAT should be an excellent ligand for imaging alpha-1 adrenoceptor in man by single photon emission computerized tomography.

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