

N-Isopropyl- ^{123}I *p*-Iodoamphetamine: Single-Pass Brain Uptake and Washout; Binding to Brain Synaptosomes; and Localization in Dog and Monkey Brain

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The kinetics of N-isopropyl- p - ^{123}I iodoamphetamine in rat brains were determined by serial measurements of brain uptake index (BUI) after intracarotid injection; also studied were its effects on amine uptake and release in rat's brain cortical synaptosomes; and its in vivo distribution in the dog and monkey. Serial BUI correspond to a first-pass extraction efficiency of 100% and a washout half-time of ~318 sec. The S-(+)- isomer inhibited norepinephrine uptake in synaptosomes as strongly as D-amphetamine, but was more potent in inhibiting synaptosomal uptake of serotonin. Both isomers were comparable to D-amphetamine in causing release of serotonin from synaptosomes, but the S-(+)- isomer promoted release of dopamine, whereas D-amphetamine did not. No specific localization in brain nuclei of the dog was seen, but there was progressive accumulation in the eyes. Rapid initial brain uptake in the ketamine-sedated monkey was noted, and further slow brain uptake occurred during the next 20 min but without retinal localization. High levels of brain activity were maintained for several hours. The quantitative initial single-pass clearance of the agent in the brain suggests its use in evaluation of regional brain perfusion. Its interaction with brain amine-binding sites suggests its possible application in studies of cerebral amine metabolism.

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In a previous study we presented structure-distribution relationships in rats for I-123-labeled iodophenylalkyl amines, which led us to select N-isopropyl- p - ^{123}I iodoamphetamine for further evaluation as a diagnostic radiopharmaceutical for brain studies (1). In support of the proposed use of the agent in evaluating regional brain perfusion, the present work provides data on single-pass clearance of the agent from arterial blood by rat brain and its initial washout. Additionally, in support of its proposed use of this agent for in vivo study

of CNS amine-binding sites, data are presented concerning the ability of this agent to enter into neurohumoral amine uptake and release from cortical synaptosomes. Finally, in vivo distribution data from dogs and a monkey are presented supporting its potential applications in nonrodent species.

MATERIALS AND METHODS

Using methods described elsewhere, the nonradioactive iodinated form of each compound studied was synthesized, purified, characterized, and exchange-labeled with I-123 (RM Baldwin and TH Lin, unpublished data).

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The extraction of the compound by the brain from arterial blood following intracarotid injection was studied by the method of Oldendorf (2). In this method, the brain uptake index (BUI) is calculated as the ratio of labeled agent to tritiated water in brain at a given time, divided by the corresponding ratio in the aliquot injected into the carotid artery. The percent extraction per pass can be calculated by multiplying the BUI for the agent at 15 sec by the brain extraction of tritiated water at 15 sec. The clearance of the agent from the brain can be calculated from serial BUI measurements corrected for the washout of tritiated water from the brain. All such measurements and calculations were performed as described in Ref. 3.

Amine uptake by synaptosomes from brain cortex was investigated as described by Coyle and Snyder (4), except that pargyline ($1.25 \times 10^{-4} M$) was used in place of nialamide. L-[3H] norepinephrine and [3H]serotonin were included in the incubation mixture at a concentration of $0.2 \mu M$; D-amphetamine, *p*-chloroamphetamine, and S-(+)-N-isopropyl-*p*-iodoamphetamine were tested at concentrations of 1, 10, and $100 \mu M$.

Amine release from synaptosomes in rat brain cortex was studied by preparing synaptosomes prelabeled with [3H]norepinephrine, [3H]dopamine, or [3H]serotonin and superfused as previously described (5).

Two unanesthetized, female mongrel dogs weighing 32 and 33 pounds were injected intravenously with 2 ml of I-123-labeled S-(+)-N-isopropyl-*p*-iodoamphetamine (2.5 mCi/mg) at a concentration of 1.97 mCi/ml saline. One dog was killed at 6 min and the other at 200 min after injection by rapid i.v. administration of 12 ml of saturated KCl solution. Selected tissues were removed at necropsy, weighed wet, and radioassayed. The brain, including brain stem, was sliced into ~5 mm thick coronal sections and the sections were imaged with a scintillation camera fitted with a 250-keV collimator or a pinhole.

A 6-pound male Capuchin monkey under ketamine hydrochloride sedation was injected with 1.3 mCi of I-123-labeled RS-N-isopropyl-*p*-iodoamphetamine (2.34 mCi/mg) in 0.5 ml of saline. Images of the brain and whole body were obtained with a mobile camera and either a pinhole collimator or a combination of a high-sensitivity, medium-resolution collimator placed on top of a 250-keV collimator. All data were collected and stored digitally, then computer-analyzed for activity and activity change in regions of interest, using routine procedures.

RESULTS

At both 5 sec and 2 min, the BUI values for R(-)-N-isopropyl-*p*-iodoamphetamine were found to be similar to those for the RS-isomer. Consequently, they were combined to yield a BUI 124 ± 13 ($n = 5$) at 5 sec

TABLE 1. CONCENTRATIONS (μM OF D-AMPHETAMINE, *p*-CHLOROAMPHETAMINE, AND S-(+)-N-ISOPROPYL-*p*-IODOAMPHETAMINE REQUIRED FOR 50% INHIBITION OF SEROTONIN AND NOREPINEPHRINE UPTAKE IN SYNAPTOSOMES

Drug	50%-inhibiting concentration (μM)	
	[3H] serotonin	[3H] norepinephrine
D-Amphetamine	37.0	5.0
<i>p</i> -Chloroamphetamine	1.5	0.94
S-(+)-N-isopropyl- <i>p</i> -iodoamphetamine	1.9	5.8

and of 257 ± 27 ($n = 6$) at 2 min. For RS-isomers the BUI values were 182 ($n = 3$) at 1 min, 423 ($n = 3$) at 3 min, and 580 ($n = 3$) at 4 min.

If we assume a brain extraction efficiency of 84% per pass for tritiated water (relative to 100% per pass for C-14 butanol), the foregoing BUI values will correspond to essentially 100% per pass for the brain extraction efficiency for N-isopropyl-*p*-iodoamphetamine.

Correcting for brain washout of tritiated water (for method see Ref. 3), the initial washout half-time for N-isopropyl-*p*-iodoamphetamine was calculated to be 318 sec (5.3 min).

Table 1 presents the concentrations (μM) of (a) D-amphetamine, (b) *p*-chloroamphetamine, and (c) S-(+)-N-isopropyl-*p*-iodoamphetamine, required to cause 50% inhibition of synaptosomal uptake of serotonin and norepinephrine. S-(+)-N-isopropyl-*p*-iodoamphetamine inhibits serotonin and norepinephrine uptake in synaptosomes. Both S-(+)-N-isopropyl-*p*-iodoamphetamine and *p*-chloroamphetamine are substantially more effective than D-amphetamine in inhibiting serotonin uptake by synaptosomes, since only about 1/20 of the molar concentration of these agents is required to produce inhibition comparable to that obtained with D-amphetamine. The ability of S-(+)-N-isopropyl-*p*-iodoamphetamine to inhibit synaptosomal uptake of norepinephrine is roughly comparable to that of amphetamine, but both are only about one fifth as potent as *p*-chloroamphetamine.

Table 2 presents the effect of $10^{-4} M$ D-amphetamine, *p*-chloroamphetamine, S-(+)- and R(-)- isomers of N-isopropyl-*p*-iodoamphetamine on the spontaneous release of norepinephrine, serotonin, and dopamine from synaptosomes. It can be seen that both isomers of N-isopropyl-*p*-iodoamphetamine are effective in causing release of serotonin. The S-(+)-isomer is also effective in causing release of dopamine. D-amphetamine and *p*-chloroamphetamine produce comparable release of serotonin from synaptosomes.

TABLE 2. EFFECT OF D-AMPHETAMINE, p-CHLOROAMPHETAMINE AND S-(+)- AND R(-)- ISOMERS OF N-ISOPROPYL-p- IODOAMPHETAMINE ON RELEASE OF NOREPINEPHRINE, SEROTONIN, AND DOPAMINE FROM SYNAPTOSOMES

Drug	% of total [³ H]amine released		
	[³ H]norepinephrine	[³ H]serotonin*	[³ H]dopamine
Control	68.1 ± 1.2	40.2 ± 1.3	63.6 ± 1.6
D-Amphetamine	64.3 ± 1.6	60.3 [†] ± 2.0	63.8 ± 1.7
p-Chloroamphetamine	68.5 ± 1.9	62.1 [†] ± 1.6	64.2 ± 1.4
S-(+)-N-isopropyl-p-iodoamphetamine	67.6 ± 1.7	61.1 [†] ± 1.7	69.9 [‡] ± 1.6
R(-)-N-isopropyl-p-iodoamphetamine	65.7 ± 1.6	62.2 [†] ± 1.7	66.7 ± 1.5

* Values represent % of total radioactivity released during initial 3 min of superfusion, and are means ± s.e.m. of six determinations. All drugs were included in superfusate at a concentration of 10⁻⁴M.

[†] p < 0.001.

[‡] p < 0.05.

TABLE 3. RELATIVE TISSUE ACTIVITY IN DOG GIVEN I-123 N-ISOPROPYL-p- IODOAMPHETAMINE

Ratio	6 min	200 min*
Gray matter/white matter	1.32	0.93
Skeletal muscle/brain	—	0.24
Heart/brain	—	0.57
Liver/brain	—	1.04
Kidney/brain	—	1.03
Adrenals (whole)/brain	2.68	1.07
Eyes (2)/brain [†]	2.57	7.92

* Whole brain contains 1.22% of administered dose at 200 min.

[†] Ratio of two whole eyes to 1 g of brain.

Table 3 shows the relative tissue activity in a dog killed at 6 min, and in one killed at 200 min, after i.v. administration of the S-(+)- isomer of N-isopropyl-p-iodoamphetamine (I-123).

Figure 1 shows scintiphotos of the head and whole body of a monkey at 90–165 min after i.v. administration

of RS-isomers of N-isopropyl-p-iodoamphetamine (I-123).

Figure 2 plots activity against time in areas of interest in brain, lung, and liver of the monkey shown in Fig. 1.

Table 3 shows that the ratio of activity in cerebral gray matter to that in white matter (both cpm/g) decreases from 1.32 at 6 min to 0.93 at 200 min; similarly, the ratio of activity in adrenals to that in whole brain decreases from 2.68 to 1.07. Conversely, the ratio of activity in two whole eyes to 1 g of brain increases from 2.57 at 6 min to 7.92 at 200 min. At 200 min, the activity/gram of skeletal muscle is one fourth of that in brain, whereas cpm/g in heart muscle is about half of that in brain. At 200 min, activity per gram in liver, kidneys, or adrenals

Distribution of I-123 N-Isopropyl-p-iodoamphetamine in a Monkey

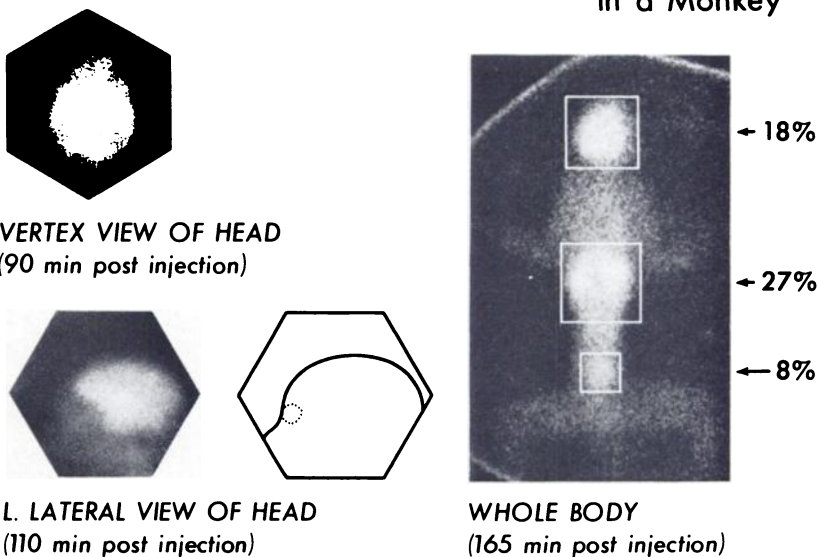


FIG. 1. Scintiphotos of head and whole body of monkey at 90 to 165 min following i.v. administration of RS- isomers of N-isopropyl-p-iodoamphetamine (I-123).

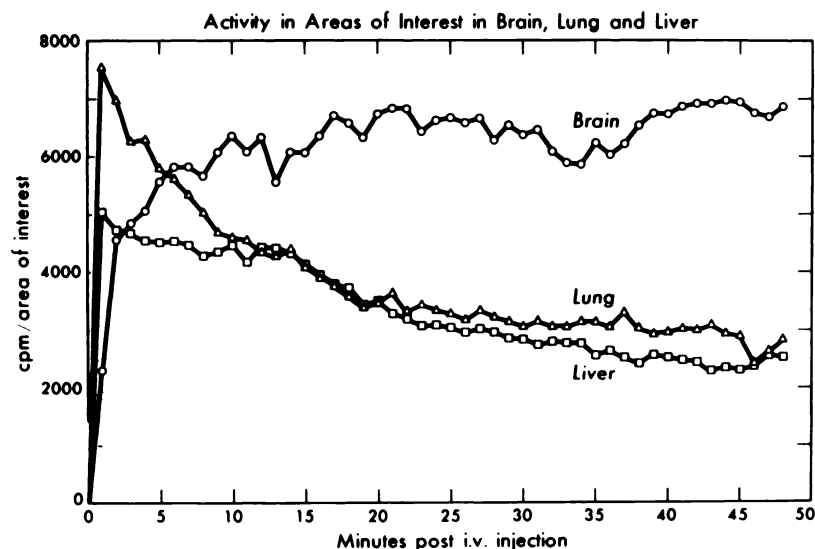


FIG. 2. Activity as function of time in areas over brain, lung, and liver of monkey shown in Fig. 1.

is about equivalent to that in brain. Scintiphotos of coronal slices through the brain and brain stem of the animal killed at 6 min confirmed the distinctly higher activity in cerebral gray matter than in white matter. Scintiphotos of the eyes also confirmed greater activity there at 200 min than at 6 min. Other than noted above, no distinct localization in specific regions of the brain was observed at 6 min or 200 min. Activity in the brain at 200 min was about 1.22% of administered dose.

From Fig. 1, it can be determined that activity in a monkey's brain can be imaged for at least several hours after administration of the agent. Activity in the brain is clearly greater than that in surrounding tissues. No concentration of activity was detected in the eyes of this monkey. Serial images of the head did not show any obvious change in pattern of distribution of brain activity during the first few hours after administration of the agent. Serial scintiphotos did show initial retention of activity in the lungs, followed by rapid clearance. The image of the whole body at 165 min after dose showed concentration of activity in the head, upper abdomen containing stomach, liver, and kidneys, and to a lesser extent in the bladder region of the pelvis. Activity in the regions of interest shown in the squares outlined in Fig. 1 (corrected for background) indicate that of the entire activity emerging from the body surface in the monkey, roughly 18% arose from within the head, 27% from within the upper abdomen, and 8% from within the pelvis. Images of the head showed that the bulk of activity in the head was in the brain. No uptake was noted in the region of the thyroid gland. A repeat whole-body scintiphoto, taken ~31 hr after dose, was qualitatively similar to that shown at 165 min.

Figure 3 shows that activity accumulates rapidly in the brain and then continues to increase slowly for the next 20–25 min, remaining at this high level for at least the first 50 min after injection. The initially high lung

activity (seen in 1-min serial scintiphotos) decreases with time, with about half of the initial activity seen at 1 min clearing with a half-time of 5–6 min and the remainder with an initial half-time of ~2.5 hr. Liver activity clears with an initial half-time of about 1 hr.

DISCUSSION

The brain's extraction efficiency of ~100% per pass, calculated from BUI data, supports the suggestion that the initial distribution of N-isopropyl-*p*-iodoamphetamine in the brain is a measure of regional brain perfusion. Similar 15-sec BUI values for S-(+)- and RS-isomers suggests that either may be used for this purpose. The brain washout $t_{1/2}$ of ~318 sec for this agent is longer than the washout $t_{1/2}$ of 171 sec for nicotine or the 93-sec washout $t_{1/2}$ for antipyrine (3). Consequently, this agent should be superior to nicotine in assessing regional brain perfusion. This is noteworthy since nicotine had previously been proposed as the best known agent for such a purpose (6).

Assuming that efflux of this agent from the brain is monoexponential, the ratio of its efflux to influx in the brain can be estimated. Efflux per gram of brain can be estimated by the product of the washout rate constant and the concentration of agent per gram of brain. Influx per gram of brain can be estimated as the product of the blood concentration of agent and the blood flow per gram of brain (assuming 100% extraction per pass). The brain-to-blood activity ratios in the rat at 5 min and 60 min have both been shown to be >10 (1). Brain perfusion in the anesthetized rat is approximately 0.68 ml/min/g of brain (4). Consequently, the ratio of efflux to influx in the brain is estimated as

$$\frac{0.693/5.3 \text{ min}^{-1}}{0.68/>10 \text{ min}^{-1}} = >2.$$

If efflux from the brain were indeed over twice the influx at 5 and 60 min, then the net activity in the brain should decrease substantially over this time period. Such is not the case. In fact, the net activity in the brain clearly increases between 5 and 60 min (1). We believe that this discrepancy occurs because the washout during the first 4 min after intra-arterial injection reflects the loss of unbound or poorly bound agent from the brain (e.g., the agent retained in the brain as a consequence of intravascular against extravascular intracerebral pH gradients, or a favorable brain lipid/blood partition coefficient (1)). The fact that net brain activity increases between 5 and 60 min in the rat suggests that some component of brain retention of activity has a much longer half-time than 5.3 min. (Unfortunately, primarily as a result of recirculation, BUI measurements beyond 4 min do not reflect brain washout alone, so the postulated prolonged brain retention of the agent cannot be simply verified from serial BUI measurements.) We believe that the postulated long retention component is related to retention of the agent in high-capacity, relatively non-specific amine-binding sites (1). One such grouping of sites appears to be the brain synaptosomes, but probably there are many others.

It is clear that this agent interacts with synaptosomal mechanisms for uptake and release of neurohumoral transmitters. The pattern and potency of such interaction differ somewhat from those of D-amphetamine and *p*-chloroamphetamine. Since the S-(+)- isomer influences synaptosomal release of dopamine at concentrations at which the R-(−)- isomer does not, the specificity and potency of the two isomers for a variety of amine-binding sites may well differ.

The specificity and affinity of the agent under study for nonsynaptosomal amine-binding sites in the brain will likely differ from those in synaptosomes. The present work, however, employing synaptosomes as a preliminary model, provides an approach for evaluating the high-capacity, relatively nonspecific, widely distributed CNS amine-binding sites postulated to interact with this agent.

The rapid (within 2 min) high-level accumulation of activity in the monkey brain is consistent with quantitative single-pass brain clearance of this agent measured in the rat and described above. Consequently, it appears likely that the initial distribution of this agent in the primate brain is a measure of relative regional brain perfusion. We have suggested that the delayed distribution is related largely to its binding to high-capacity, relatively nonspecific, amine-binding sites, in addition to influences exerted by pH gradients across the blood-brain barrier and favorable partition coefficients for the agent between blood and brain lipids (1). If such binding is the major factor in determining distribution of the agent in the brain after the first hour, then the binding sites must be widely distributed throughout the

brain. If anatomical concentration of binding sites occurs, this is insufficient for its detection by planar scintigraphic imaging or scintigraphy of brain cross-sections *in vitro*.

If, as suggested, delayed images of the brain reflect activity of widely and relatively homogeneously distributed amine-binding sites in the brain, then it may be possible to study the binding affinity and nature of these sites by using appropriate displacing agents. It is known that fluoxetine (7) and chlorimipramine (8) displace *p*-chloroamphetamine from certain brain transport and binding sites, and it is possible that they and similar agents may be used to displace *p*-iodoamphetamine derivatives, such as the agent under study, from their binding sites in a manner that can be detected and quantitated scintigraphically.

The prolonged retention of activity in the lung of the pentobarbital-anesthetized rat (1) was not seen in the present monkey study, nor was the high eye uptake of the agent in the unanesthetized dog's eye reflected in uptake of activity in the ketamine-sedated monkey's eye in the present study. Remarkable eye uptake of 2,5-dimethoxy-*p*-iodoamphetamine was noted by Sargent and Shulgin in pentobarbital-anesthetized monkeys (9). Although the differences in accumulation of activity in the eye between the ketamine-anesthetized monkey and the unanesthetized dog may be species-related, experiments are being performed to determine whether ketamine competes with our agent for one or more amine-binding sites in the lung and/or eye.

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