Effect of Antidepressant and Narcoleptic Drugs on N-Isopropyl p-Iodoamphetamine Biodistribution in Animals

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N-isopropyl p-iodoamphetamine (IMP) demonstrates a high affinity for lung and brain during the first pass following intravenous injection. Its high brain affinity has been used to advantage for cerebral perfusion imaging, but the effects of drugs on IMP distribution could affect its utility. In this study, we determined the effects of the tricyclic antidepressant imipramine and the MAO inhibitors deprenyl and phenelzine on the biodistribution of IMP. We first determined the effect of loading dose and anesthesia on the biodistribution of IMP. In rats, biodistribution was not dependent on loading dose between 0.1 and 1.1 mg/kg. Anesthesia with thiopental and chloral hydrate depressed lung and brain IMP uptake. In rats, preloading doses of imipramine depressed lung uptake but did not result in increased brain IMP uptake; postloading doses of imipramine did not release IMP from the lung. In rabbits, simultaneous or postloading doses of imipramine resulted in release of IMP from the lung with an increase in brain activity. Both mixed A and B MAO inhibitors (phenelzine) and B selective MAO inhibitors (deprenyl) did not affect IMP distribution in rats. Based on the action of imipramine on IMP uptake and clearance in the lung, we postulate that IMP uptake and metabolism within the lung is related to the mixed function oxidase (MFO) system. As the lung is rich in the MFO system in humans, we would also predict from this study that IMP distribution in patients under antidepressant therapy would not be affected by either tricyclic or MAO inhibitor agents apart from the effect of these drugs on cerebral perfusion.

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N-isopropyl p-iodoamphetamine (IMP) was first proposed for brain imaging because of its high lipophilicity and correspondingly high brain uptake (*1*) and because its initial distribution in the brain is proportional to blood flow (*2–5*). The initial lung extraction of IMP is high and its clearance is slow ($T_{1/2}$ = 60 min) (6,7). The material that is washed out of the lung goes primarily to the liver. Since the brain uptake curve does not plateau until 20 min after injection, we can assume that a small part of the activity cleared from the lung goes to the brain.

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One possible mechanism for increasing IMP brain activity would be to pharmacologically reduce lung activity or to increase blood-brain barrier penetration. Lung uptake of IMP is by way of a saturable binding system (*8*). A number of pharmaceuticals, such as the antidepressant imipramine, displace amphetamine analogs from the lung and interfere with its metabolism (*9*). Therefore, it would be interesting to see whether pharmacologic intervention with these tricyclics and narcoleptic drugs might increase IMP brain uptake. Furthermore, many depressed patients will be treated with these drugs and it is important to determine whether they interfere with IMP distribution.

We investigated the effect of the tricyclic antidepressant imipramine and the MAO inhibitors phenelzine and L deprenyl on brain, lung, and liver distribution of

IMP in rats and rabbits. To further elucidate the site of IMP binding in the lung, we studied IMP interactions with specific lung binding sites (muscarinic, beta adrenergic, and peripheral benzodiazepinic receptors).

MATERIALS AND METHODS

Stereoisomeric d-1 N-isopropyl p-iodamphetamine (IMP), labeled with ^{123}I,* was obtained with radiochemical purity > 98%. Hydrogen-3 quinuclidinyl benzilate (33 μCi/mmol), ^{3}H RO 54864 (76.6 μCi/mmol), and ^{3}H dihydroalprenolol (30 μCi/mmol) were also used.†

Biodistribution Studies in Rats

To determine the effect of anesthetic agents on IMP distribution, parenteral anesthetics were administered intraperitoneally to adult male rats weighing 250–300 g in a dose of 150 mg/kg for ketamine, 50 mg/kg for pentobarbital sodium, 100 mg/kg for thiopental, 360 mg/kg for chloral hydrate, and 2.5 mg/kg for flunitrazepam. Diethyl ether was given through a nose cone.

Biodistribution of radioactive chemicals was determined in adult male rats weighing 150–300 g. Drugs and radioactive chemicals were injected directly into a saphenous vein in a volume of 0.1–0.2 cc. Rats were killed by cardiac excision or by overdose of ether. The organs of interest (liver, lungs, heart, brain, kidneys, bladder, digestive tract, blood, muscles, and skin) were removed and weighed; aliquots were counted in a gamma well counter. Total activities of blood and muscle were calculated assuming that they were 7% and 40% of total body weight respectively.

Biodistribution Studies in Rabbits

Biodistribution of radiolabeled compounds was studied by external imaging of albino New Zealand rabbits (2.5–3 kg). Pentobarbital (50 mg/kg) was administered through an ear vein. In the rabbit studies, the experimental and control animals (no imipramine) were placed side by side under a large field-of-view Anger camera linked to a digital computer. Imipramine (2.5 mg/kg) was injected intravenously simultaneously (n = 2), 5 min (n = 2), and 15 min (n = 1) after IMP in the experimental rabbits. Regions of interest over the brain, lung, heart, and liver were delineated manually to obtain time–activity curves for these organs.

Binding Studies in Rats

For the binding studies, 3-wk-old male rats were decapitated; their lungs were removed and homogenized for 30 sec at 4°C in Tris–HCl 50 m*M*, pH 7.5 buffer, with a polytron homogenizer. Each suspension was centrifuged at 2,000 *g* for 2 min and the suspendant centrifuged again in a chilled tube for 15 min at 36,000 *g* and 4°C. Pellets were washed twice in Tris buffer and resuspended in the same buffer to give a final concentration of about 1–2 mg protein/cc. The resuspended pellets were then used in the binding assay. Receptor binding assays for muscarinic (*10*), beta adrenergic (*11*), and peripheral benzodiazepinic (*12*) receptors were carried out by incubation of labeled ligand with 200 ml of particulate suspension in the presence or absence of IMP in concentrations of 10^{-7}–10^{-4} *M*.

RESULTS

Biodistribution Studies in Rats

Figure 1 compares the organ uptake for different anesthetics 20 min after the i.v. injection of [^{123}I]IMP in Wistar male rats. Thiopental markedly depressed brain and lung uptake for IMP and chloral hydrate depressed lung uptake. These drugs therefore were not used further as anesthetic agents. Subsequent studies were performed under light ether anesthesia.

When cold carrier IMP was added to the labeled tracer, competition was demonstrated in the lung of the rats at high doses of carrier (2.7 mg/kg) (Table 1). The brain was not saturable at these high concentrations.

The MAO inhibitors, phenelzine and L deprenyl, did not inhibit or release [^{123}I]IMP from the brain, lungs, or liver at a dose of 5 mg/kg in rats (Table 2). At very high dose levels (15 mg/kg) of phenelzine in rats, lung uptake of IMP was reduced, but there was no difference in brain or liver uptake compared to low dose phenelzine administration (Table 2).

When 1 mg/kg of imipramine was injected intravenously into adult male rats 30 min before [^{123}I]IMP and 50 min before killing, lung activity was depressed significantly (Fig. 2). More marked inhibition of lung uptake was seen with 4 mg/kg doses. There was decreased brain uptake of IMP only when 4 mg/kg of imipramine was used. When imipramine was injected into rats one and 15 min after [^{123}I]IMP, there was no significant difference in lung or brain uptake compared to controls (Table 3).

There was considerable variance in the concentration

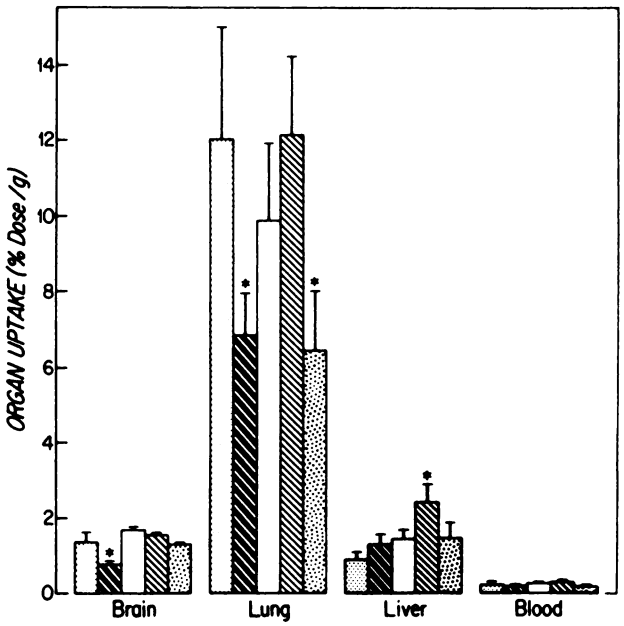

FIGURE 1
The effect of anesthetics on IMP biodistribution in the rat. (□) Nembutal; ( ) Thiopental; (□) Ether; (▨) Flunitrazepam; (□) Chloral Hydrate; *$p < 0.01$.

TABLE 1
Organ Uptake of ^{123}I IMP with Various Loading Doses of IMP in Rats

	Number of rats			
	n = 3	n = 3	n = 3	n = 3
Dose (mg/kg) of IMP	0.1 cold 0.09 hot	0.6 cold 0.09 hot	1.1 cold 0.09 hot	2.7 cold 0.09 hot
Brain	2.68 ± 0.30	2.09 ± 0.36	2.11 ± 0.05	2.48 ± 0.42
Lung	14.00 ± 4.70	11.32 ± 0.34	10.00 ± 0.39	6.65 ± 0.59*
Liver	1.16 ± 0.07	0.98 ± 0.02	1.48 ± 0.20	0.88 ± 0.17
Blood	0.22 ± 0.05	0.18 ± 0.01	0.16 ± 0.006	0.13 ± 0.01

*$p < 0.05$. Results are expressed as mean percentage of injected dose per g ± s. d. Loading (cold) doses were given intravenously and simultaneously with the radioactive (hot) doses. Rats (200–250 g) were killed 2 min after injection by cardiac excision.

of IMP in the lungs and brains of the control rats among the several reported experiments due to the differences in the times between injection and killing and differences in the weights of the rats from one experiment to another. To avoid the effects of biologic variables other than those being tested, we performed the control and experimental studies for each experiment on the same day. Thus all the rats in each experiment were from the same batch and were studied under the same laboratory conditions.

Biodistribution Studies in Rabbits

In New Zealand rabbits imipramine altered lung and brain uptake significantly not only during simultaneous injection, but also when imipramine was injected as late as 15 min after [^{123}I]IMP (Fig. 3). When the data from all five studies were summed, brain activity was significantly higher with imipramine (2,930 ± 594 cpm) than without imipramine (1,894 ± 374 cpm) ($p < 0.02$). Lung activity was significantly lower with imipramine (3,297 ± 668 cpm with imipramine; 6,664 ± 1,711 cpm with control rabbits; $p < 0.005$). Liver activity was not significantly different in the two groups (2,133 ± 352 cpm with imipramine; 2,040 ± 341 cpm for control rabbits).

Binding Studies in Rats

Binding studies on membrane preparation from rat lung demonstrated that [^{123}I]IMP was unable to release [^{3}H]quinuclidinyl benzilate (QNB) from muscarinic receptors, [^{3}H]dihydroalprenolol from beta adrenergic receptors, and [^{3}H]RO5-4864 from peripheral benzodiazepine receptors.

DISCUSSION

Basic amines ($pK_a > 8.5$) that have a high brain uptake, such as N-isopropyl p-iodoamphetamine, demonstrate a marked affinity for the lung (*13*). The physiologic significance of the pulmonary concentration of these lipophilic compounds with lung-to-plasma ratios between 20 and 200 has not yet been fully assessed (*9*).

Two major systems for amine metabolism are located in the lung. One is the monoamine oxidase (MAO) system, which is concentrated in the mitochondria of endothelial cells and plays an important role in noradrenaline and 5-hydroxytryptophane metabolism. The other, the major route of amine metabolism, is the mixed function oxidase (MFO) system. The first part of the MFO enzymatic chain produces dealkylation and

TABLE 2
Effect of Deprenyl and Phenelzine on the Biodistribution of IMP in Rats

	Deprenyl				Phenelzine				
	Controls for simultaneous injection	L deprenyl (5 mg/kg)	Controls 15 minute injection	L deprenyl (5 mg/kg)	Controls	Phenelzine (5 mg/kg)	Phenelzine (5 mg/kg)	Phenelzine (2 mg/kg)	Phenelzine (15 mg/kg)
Number of rats	n = 3	n = 3	n = 3	n = 3	n = 10	n = 7	n = 6	n = 3	n = 3
Weight of rats (g)	200–250	200–250	200–250	200–250	150–200	150–200	150–200	300–350	300–350
Time interval between drug and ^{123}I IMP injection	—	Simultaneous	—	15 min after [^{123}I]IMP	—	Simultaneous	15 min after [^{123}I]IMP	10 min before [^{123}I]IMP	10 min before [^{123}I]IMP
Dose of IMP (μg/kg)	50	50	75	75	90	90	90	60	60
Time after IMP injection to killing (min)	20	20	20	20	20	20	20	30	30
Brain	1.68 ± 0.11	1.66 ± 0.22	2.03 ± 0.17	1.99 ± 0.30	2.53 ± 0.39	2.44 ± 0.16	2.43 ± 0.20	1.1 ± 0.1	0.8 ± 0.2
Lung	3.80 ± 0.60	3.21 ± 0.40	4.47 ± 0.77	4.67 ± 1.15	7.00 ± 0.00	6.89 ± 1.05	5.78 ± 0.79	6.5 ± 1.3	3.4 ± 0.5
Liver	2.00 ± 0.40	1.51 ± 0.41	1.93 ± 0.23	1.81 ± 0.32	2.79 ± 0.56	2.71 ± 0.50	2.72 ± 0.23	1.1 ± 0.2	0.9 ± 0.1
Blood	0.16 ± 0.03	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.03	0.18 ± 0.02	0.17 ± 0.01	0.17 ± 0.03	0.14 ± 0.01	0.19 ± 0.04

*$p < 0.05$. Results expressed as mean percentage of injected dose per g ± s. d. of dose per g.

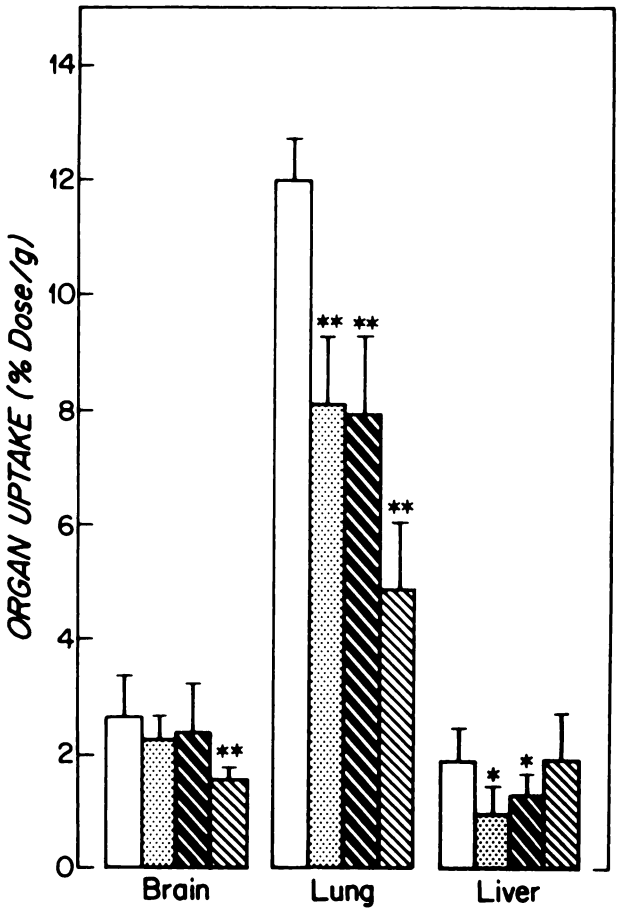

FIGURE 2
The effect of imipramine (injected 30 min before [^{123}I]IMP and 50 min before killing) on IMP biodistribution in the rat. Imipramine: (□)–, (□) 1 mg/kg; (▨) 3 mg/kg; (▨) 4 mg/kg; $^{*}p < 0.05$; $^{**}p < 0.01$.

ring hydroxylation; the second part of the chain results in N-oxidation and deamination. Mixed function oxidase systems are dependent on species, strain, organ, sex, substrate, and age (*9*). Mixed function oxidase activity is located in the microsomal fraction of pulmonary cells, including the alveolar macrophage (*14*). The mixed function oxidase enzymes are flavoprotein or NADPH; the most important of the oxidase systems requires NADPH and oxygen as cofactors and use cytochrome P450 as a component of an electron transfer chain. Amphetamine derivatives complex with cytochrome P470 (*14–17*). After dealkylation, intermediates of iodoamphetamine metabolism probably undergo C-hydroxylation to 1(4 iodophenyl), 2, aminopropane and/or N-hydroxylation to form iodo-N-hydroxylamphetamine and iodophenylacetoneoxime produce iodophenylketone and alcohol (*18*). Philpot (*9*) has demonstrated that the cytochrome P450 lung concentration is 25% of that in the liver in rabbits. Rats have a much more concentrated MFO system in the lungs.

TABLE 3
Effect of Imipramine (5 mg/kg) on the Biodistribution of IMP in Rats

	Number of Rats		
	n = 3	n = 3	n = 3
Time of imipramine injection	Control	1 min after i.v. [^{123}I]IMP	15 min after i.v. [^{123}I]IMP
Dose of IMP (μg/kg)	60	60	60
Brain	1.7 ± 0.1	1.7 ± 0.2	1.9 ± 0.1
Lung	4.2 ± 0.8	3.4 ± 0.4	4.1 ± 0.4
Liver	1.8 ± 0.1	2.1 ± 0.2	2.6 ± 0.2*

*$p < 0.05$. Results are expressed as mean percentage of injected dose per g ± s. d. Rats (230–285 g) were killed 30 min after [^{123}I]IMP injection.

A number of inhibitors or inducers affect the rate at which amines are metabolized in the intact cell. In addition, tissue binding of the substrate, its penetration to the active site of the enzyme, availability of cofactors, and the presence of competing drugs can affect the reaction rate within the cell (*16,19*).

We have found a significant alteration in lung and brain [^{123}I]IMP uptake with some anesthetics (thiopental and chloral hydrate), but not others (ether or flunitrazepam). Although depressed brain uptake may be related to decreased blood flow (*20*), decreased lung uptake with thiopental and chloral hydrate anesthesia is not. Like the thiocarbonyl compounds (disulfiram) and thiophosphonyl compounds (parathion), thiopental may be converted into its oxygen analog by an MFO system, transferring its sulfur onto cytochrome P450 and impeding ligand complex formation of amines with cytochrome (*18*). Chloral hydrate may also accumulate in the MFO lung system (*21*).

Imipramine, a tricyclic agent, potentiates the action of amphetamine in the liver by inhibiting parahydroxylation of the phenyl ring (*23*). Imipramine accumulates avidly in the lung, is poorly demethylated, and is cleared rapidly by the liver. We found that brain activity was not increased in the rat when imipramine was injected after [^{123}I]IMP; however, liver activity was increased. Our results suggest that the MFO system acts rapidly in the rat lungs, since so little of the tracer is released when imipramine is injected after IMP.

In rabbits, imipramine releases a large fraction of lung activity followed by a significant increase in brain activity. The different effects of imipramine in the rabbit and rat may be due to species differences in the MFO activity in the lungs. Since the lung MFO system in man is densely populated as it is in the rat and in most species, brain [^{123}I]IMP activity in the human will be similar to the pattern we observed in the rat and will not be enhanced by imipramine.

In the brain, paraiodoamphetamine inhibits the up-

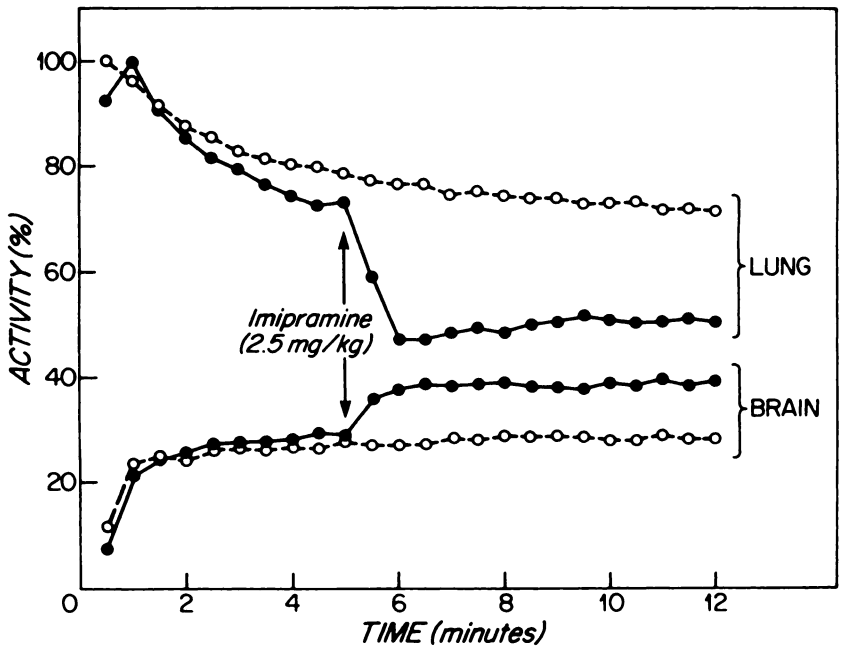

FIGURE 3
The effect of imipramine on IMP brain and lung activity in the New Zealand rabbit. The imipramine was injected 5 min after [^{123}I]IMP. (O–O) Control; (●–●) Imipramine.

take and turnover of serotonin in the mitochrondrial monoamine oxidase (MAO) system (*24*) and facilitates blood brain barrier transport of aromatic amines (*25*). We did not find, however, a significant change in [^{123}I]IMP brain uptake with MAO B or mixed A and B inhibition in rats. Assuming that our data can be extrapolated to humans, patients on MAO inhibitor therapy can be studied without fear that the therapy will affect either the uptake or cerebral distribution of the tracer independent of changes in cerebral blood flow.

NOTES

*Medi-Physics, Inc., Emeryville, CA.
†Du Pont Company, No. Billerica, MA.

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