Biological Disposition of Rigid Analogs of Amphetamine

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Abstract □ Tissue levels of amphetamine and of amphetamine analogs with a rigid conformation (2-aminoindan, 2-aminotetralin, and 2-aminobenzocycloheptene) were measured in rats by a spectrofluorometric method involving the reaction of the primary amine group with fluorescamine. All drugs were concentrated in tissues, the order of distribution being lungs > kidneys > liver = spleen = brain > muscle > fat = heart > blood. In brain, amphetamine and 2-aminoindan were present mostly in the supernatant fraction after high speed centrifugation of brain homogenates; the two higher molecular weight drugs were present at slightly greater concentrations in the particulate fraction. All four drugs disappeared from rat brain with half-lives of 1–2 hr. Iprindole pretreatment increased drug levels in brain and prolonged the half-lives by two- to threefold. The data suggest that the biological disposition of the rigid analogs resembles generally that of amphetamine and that all of the drugs are probably metabolized by ring hydroxylation in the rat.

Keyphrases □ Amphetamine and analogs—with rigid conformation, biological disposition, spectrofluorometric analysis in rat tissues, effect of pretreatment with iprindole □ Distribution, biological—amphetamine and analogs with rigid conformation, spectrofluorometric analysis in rat tissues, effect of pretreatment with iprindole □ Spectrofluorometry—analysis, amphetamine and analogs with rigid conformation, rat tissues □ Iprindole—pretreatment, effect on biological disposition of amphetamine and analogs with rigid conformations, rats □ Stimulants, central—amphetamine and analogs with rigid conformation, biological disposition, rats

Among drugs acting on the central nervous system, amphetamine has been one of the most widely investigated. Numerous structural derivatives of amphetamine have been synthesized and studied, including amphetamine analogs having a rigid conformation due to a bridge between the α-carbon and the ring. For instance, hydroxylated and methoxylated 2-aminotetralins have been of interest because of their relationship to psychotomimetic drugs such as mescaline (1, 2). 2-Aminoindans and 2-aminotetralins have been evaluated as potential antiparkinsonism drugs based on their structural similarity to dopamine (3, 4). Effects of these rigid analogs on monoamine metabolism *in vivo* and on monoamine oxidase *in vitro* have been reported (5, 6).

This paper deals with three rigid analogs of amphetamine (I), in which the α-carbon is connected to the *ortho*-position of the ring directly (2-aminoindan, II) or through one (2-aminotetralin, III) or two (2-aminobenzocycloheptene, IV) methylene units. Much is known about the metabolism and biological disposition of amphetamine in various animal species. This study compares some fundamental pharmacokinetic parameters of these rigid analogs of amphetamine in rats, including their levels in tissues and rates of disappearance from brain.

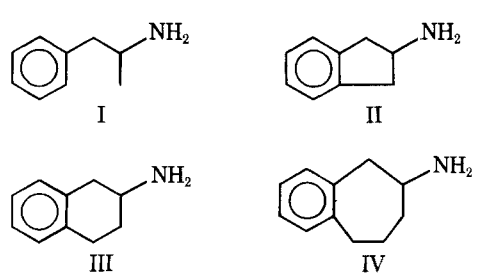

Table I—Subcellular Distribution of Amphetamine and Rigid Analogs in Rat Brain[a]

Drug	Drug Level in Brain, nmoles/g: Particulate (P)	Supernate (S)	Ratio, P/S
I	44 ± 7	75 ± 7	0.6
II	51 ± 1	90 ± 3	0.6
III	67 ± 1	62 ± 5	1.1
IV	61 ± 7	49 ± 2	1.2

[a] Drugs were injected at 0.1 mmole/kg ip 1 hr before the rats were killed. Brain homogenates (in 2.5 m*M* sucrose) were centrifuged for 30 min at 100,000×*g*. Drug levels were measured both in particulate and supernatant fractions.

EXPERIMENTAL

dl-Amphetamine hemisulfate was used as purchased[1]. The derivatives of amphetamine were synthesized[2] as hydrochloride or hemisulfate salts. All were racemic mixtures. The drugs were injected intraperitoneally in aqueous solution into male Wistar rats[3] weighing about 150 g. The rats were maintained in a room with controlled lighting and temperature and with food and water available *ad libitum*. After they were treated with drugs, the rats were killed by decapitation. Tissues were quickly removed and frozen on dry ice and then stored frozen prior to analysis.

Drug levels were measured spectrofluorometrically[4] by a previously described method (7) after extraction and reaction with fluorescamine. All data are expressed as mean values with standard errors for five rats per group. Comparisons between groups were made by the Student *t* test.

RESULTS AND DISCUSSION

The three rigid analogs of amphetamine reacted with fluorescamine to form fluorophores with characteristics similar to those of the fluorophore produced by amphetamine, *i.e.*, with maximum excitation at 390 nm and maximum emission at 490 nm. Standard curves over a 30–90-nmoles/ml concentration range established fluorescent intensity to be linearly proportional to concentration. The standard curves were similar for all four amines, so reaction with fluorescamine provided a sensitive means for assaying tissue levels of these drugs.

Figure 1 shows the distribution of the drugs in various tissues 1 hr after injection of equimolar doses. All compounds were highly concentrated in tissues, with blood levels of the drugs (not shown) being very low (about half of the levels shown for heart). The general pattern of tissue distribution was similar for the four drugs. For instance, drug levels in lung were higher in all cases than in any other tissue. Drug levels in skeletal muscle were low, and drug levels in epididymal fat and heart were even lower for all four drugs.

Some differences in relative drug levels were noticed in the remaining tissues. Compound II was present in most tissues at higher levels than amphetamine or the other derivatives with a rigid conformation. Levels of all rigid analogs tended to be higher in brain than those of amphetamine. Levels of III and IV were at least as high in brain as in liver or spleen. The ratio of drug levels in brain–skeletal muscle progressively increased with size of the drug molecule (1.3 for I, 2.0 for II, 3.2 for III, and 3.9 for IV). These differences suggest that the larger bicyclic compounds have a greater relative affinity for brain than does amphetamine.

The subcellular distribution of the drugs in brain homogenates subjected to centrifugation at 100,000×*g* is shown in Table I. As reported

[1] Chemicals Procurement Laboratories, College Park, N.Y.
[2] In the Lilly Research Laboratories.
[3] Harlan Industries, Cumberland, Ind.
[4] Aminco-Bowman spectrophotofluorometer.

Table II—Drug Levels and Half-Lives of Rigid Analogs of Amphetamine in Brains of Control Rats and Rats Treated with Iprindole to Inhibit Aromatic Hydroxylation

Drug	Iprindole	Drug Level in Brain, nmoles/g				Half-Life, hr
		1 hr	2 hr	3 hr	4 hr	
I	–	84 ± 6	49 ± 10	39 ± 4	18 ± 3	1.4
	+	109 ± 9[a]	103 ± 9[a]	83 ± 8[a]	65 ± 5[a]	3.9[a]
II	–	148 ± 6	110 ± 7	78 ± 6	44 ± 6	1.7
	+	136 ± 4	111 ± 3	91 ± 6	73 ± 4[a]	3.3[a]
III	–	120 ± 7	69 ± 8	52 ± 8	25 ± 4	1.3
	+	133 ± 9	103 ± 7[a]	87 ± 5[a]	71 ± 3[a]	3.6[a]
IV	–	100 ± 7	55 ± 4	26 ± 4	16 ± 5	1.0
	+	120 ± 7	89 ± 2[a]	78 ± 3[a]	81 ± 3[a]	3.3[a]

[a] Significant difference from corresponding group without iprindole, $p < 0.05$. Drugs were injected at zero time at a dose of 0.1 mmole/kg ip; some rats had been pretreated 1 hr earlier with iprindole (10 mg/kg ip).

previously (8), about two-thirds of the amphetamine (I) in brain was recovered in the supernatant fraction. The same distribution was found for II, but III and IV were present in the particulate fraction at slightly higher concentrations than in the supernatant fraction. This distribution may reflect, at least in part, the lipophilic character of the drug molecule, since halogenated derivatives of amphetamine have high particulate–supernatant ratios (8).

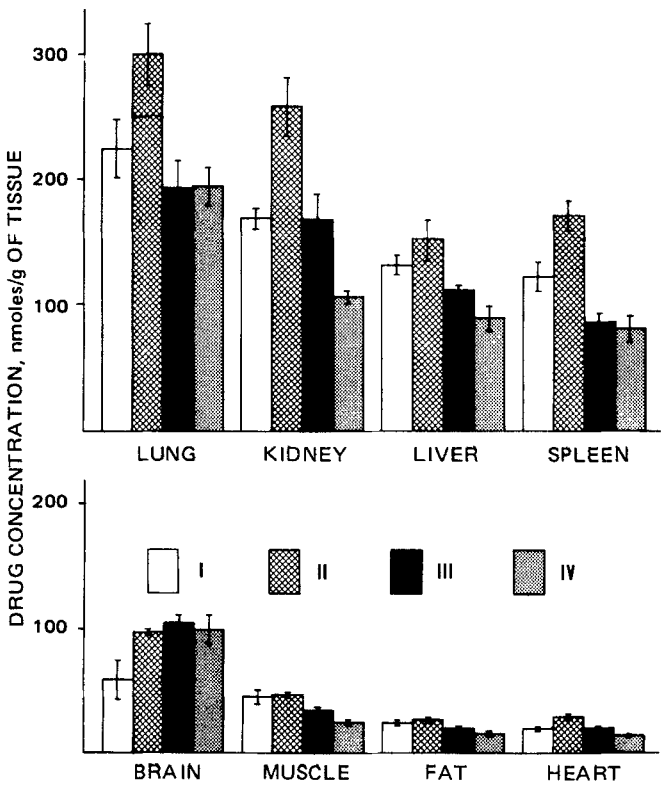

Figure 1—*Tissue distribution of amphetamine and rigid analogs in various organs of the rat. Drugs were injected at 0.1 mmole/kg ip 1 hr before the rats were killed.*

Drug levels in brain at four different times are shown in Table II. From semilogarithmic plots of drug levels *versus* time, half-life values of all four drugs were calculated to be between 1 and 2 hr. In rats pretreated with iprindole, an inhibitor of the aromatic hydroxylation of amphetamine (9), half-lives were increased two- to threefold. This finding constitutes indirect evidence that all of these drugs are metabolized by aromatic hydroxylation in the rat.

REFERENCES

(1) J. P. Green, K. P. Dressler, and N. Khazan, *Life Sci.*, **12**, 475 (1973).

(2) C. F. Barfknecht, D. E. Nichols, D. B. Rusterholz, J. P. Long, J. A. Engelbrecht, J. M. Beaton, R. J. Bradley, and D. C. Dyer, *J. Med. Chem.*, **16**, 804 (1973).

(3) Y. C. Martin, C. H. Jarboe, R. A. Krause, K. R. Lynn, D. Dunnigan, and J. B. Holland, *ibid.*, **16**, 147 (1973).

(4) J. D. McDermed, G. M. McKenzie, and A. P. Phillips, *ibid.*, **18**, 362 (1975).

(5) P. A. Crooks, H. J. Little, and J. M. H. Rees, *J. Pharm. Pharmacol.*, **25**, P124 (1973).

(6) Y. C. Martin, J. B. Holland, C. H. Jarboe, and N. Plotnikoff, *J. Med. Chem.*, **17**, 409 (1974).

(7) R. W. Fuller, K. W. Perry, J. C. Baker, C. J. Parli, N. Lee, W. A. Day, and B. B. Molloy, *Biochem. Pharmacol.*, **23**, 3267 (1974).

(8) R. W. Fuller and C. W. Hines, *J. Pharm. Sci.*, **56**, 302 (1967).

(9) J. J. Freeman and F. Sulser, *J. Pharmacol. Exp. Ther.*, **183**, 307 (1972).

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