Tissue Levels of Chloroamphetamines in Rats and Mice

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During the first 4 hr. after i.p. injection of equimolar amounts of the two drugs, more 4-chloroamphetamine than amphetamine was present in the brain and other tissues of rats and mice. The higher levels resulted from a slower disappearance of 4-chloroamphetamine. Other amphetamines substituted with chlorine in the *para* position disappeared more slowly from rat brain than did amphetamine. This may be due to blocking of a major metabolic pathway for amphetamine—*para* hydroxylation—by the *para* substitution, since amphetamines with chlorine substituted in other positions disappeared from tissues at rates comparable to that for amphetamine.

PLETSCHER *et al.* (1) first reported that the levels of 4-chloro-*N*-methylamphetamine were higher than those of amphetamine in the brains of rats given equimolar doses of the two compounds. This has led to the study of the effect of different chloro substitutions on the tissue distribution and disappearance rate of amphetamine. The results of this study, which have been presented in a preliminary form,[1] are reported in detail in this paper.

EXPERIMENTAL

The animals used were male albino rats from the Harlan strain, body weight approximately 150 Gm., or Cox standard albino mice, body weight approximately 16 Gm. Dextroamphetamine sulfate[2] was obtained from Smith Kline & French Laboratories. *l*-Amphetamine sulfate was from the Ott Chemical Co. The chloroamphetamines were synthesized as the hydrochlorides in the Lilly Research Laboratories. In all experiments, the compounds were injected intraperitoneally in aqueous solution at a dose of 0.1 mmole/Kg. (18–24 mg./Kg.). Four rats or six mice per group were used except as otherwise noted. Animals were decapitated at the time intervals indicated. The organs were quickly removed, frozen on dry ice, and stored at −15° prior to analysis. Drug levels were determined by the methyl orange assay as used by Dubnick *et al.* (2). Hydroxylated amphetamines that might be formed metabolically are not measured by this assay. An internal standard curve for each drug and each tissue was used to calculate drug levels. This standard curve was determined from control animal tissues to which drug had been added *in vitro*. Tissue blanks were low. Typical blank values were, in mμmoles/Gm.: heart and spleen, 0; blood, 1; brain, 2; liver, 6; kidney, 18.

RESULTS AND DISCUSSION

The levels of dextroamphetamine and of *dl*-4-chloroamphetamine in the brain of rats and mice after i.p. injection are shown in Fig. 1. In both species, the 4-chloro compound disappeared much more slowly than the unsubstituted amphetamine. 4-Chloroamphetamine and 4-chloro-*N*-methylamphetamine lower serotonin in the brain of rats but not in mice (1, 3). This species difference is not due to differences in concentrations of the compounds in the brain, since the levels and disappearance rates of 4-chloroamphetamine were almost identical in rats and mice. The species difference thus rests on some basis other than tissue levels of the drugs. On the other hand, drug levels may explain why 4-chloroamphetamines lower serotonin in rat brain while amphetamine does not. The former compounds persist in the brain for longer times than does amphetamine.

Disappearance rates (from rat brain) for other substituted amphetamines were calculated from plots of the type shown in Fig. 1. The results are shown in Table I. All of the compounds with a chloro substituent in the 4 position disappeared much more slowly (9–11%/hr.) from brain than did those with no chloro substitution in this position (49–73%/hr.). There seemed to be no steric effect in either group of compounds. Since amphetamine is *para* hydroxylated in rats (4–6) and mice,[3] the slower disappearance rate of the 4-substituted compounds may be closely related to the fact that this metabolic pathway has been blocked.

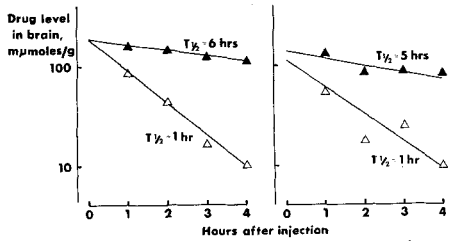

Fig. 1.—Brain levels of dextroamphetamine and *dl*-4-chloroamphetamine in rats (left) and mice (right). Key: ▲, *dl*-4-chloroamphetamine; Δ, dextroamphetamine.

To determine if binding of the drugs in the brain played a role in their rate of disappearance, the drug concentrations were measured in the supernatant and precipitated fractions after centrifugation of brain homogenates from treated rats. The centrifugation was carried out in a Spinco model L ultracentrifuge at a force of 105,000 × g. Drug levels were then determined in both supernatant and precipitated fractions. Results are shown in Fig. 2. The supernatant fraction was taken to represent "free" drug and the precipitated fraction "bound" drug. Unsubstituted amphetamine was found primarily in the soluble fraction, whereas all chlorinated amphetamines were bound to a greater degree. The association of chloroamphetamines with the

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[1] Federation of American Societies for Experimental Biology, Atlantic City, N. J., meeting, April 1966.

[2] Marketed as Dexedrine sulfate by Smith Kline & French Laboratories, Philadelphia, Pa.

[3] After injection of ^{3}H-amphetamine into mice, the authors have isolated from the urine of the mice an ether-soluble substance that was identified by paper chromatography as *p*-hydroxyamphetamine. It was not established whether this represented a major or minor metabolite.

TABLE I.—RATE OF DISAPPEARANCE OF SUBSTITUTED AMPHETAMINES FROM RAT BRAIN

Substitution	Isomer	Disappearance Rate, %/hr.
None	*d*	53
	l	49
2-Chloro-*N*-methyl	*dl*	73
2,6-Dichloro	*dl*	58
3-Chloro	*dl*	53
4-Chloro	*dl*	9
4-Chloro-*N*-methyl	*dl*	11
3,4-Dichloro	*d*	11
3,4-Dichloro	*l*	11

particulate fraction may reflect their greater lipid solubility. The degree of binding may influence the length of time that the drugs remain in the brain. Kuntzman and Tsai (7) found that the chloro derivative of norcyclizine had a longer *in vivo* half-life than did cyclizine, perhaps because of more extensive binding to plasma proteins. However, Fig. 1 and Fig. 2 show that 2,6-dichloroamphetamine had a high bound/free ratio and still disappeared rapidly. This would suggest that drug binding is not the major factor that determines disappearance rate in this case, consistent with the idea that blockade of metabolism accounts for the slower disappearance of chloroamphetamines.

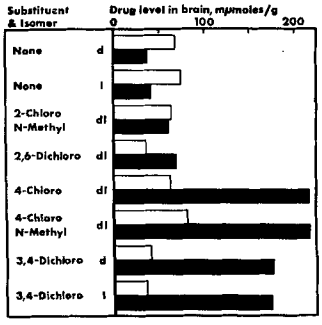

Fig. 2.—Occurrence of substituted amphetamines in free and bound forms in rat brain. Brains were analyzed 1 hr. after administration of the drugs. Key: open bar, free; closed bar, bound.

The levels of amphetamine and of 4-chloroamphetamine in other tissues of the rat, measured at 1 and 4 hr. after injection, are shown in Fig. 3. The relative distribution between the various tissues was similar for the two compounds. Large amounts of the compounds were present in the lung and kidney. (Drug levels in the lung and epididymal fat pads were not determined at 1 hr.) An important difference between the drugs existed in the blood levels. In

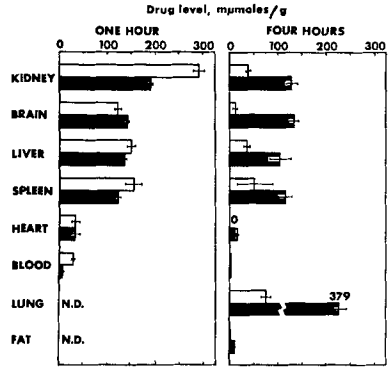

Fig. 3.— Tissue levels of dextroamphetamine and *dl*-4-chloroamphetamine in the rat. Amphetamine levels are represented by open bars, 4-chloroamphetamine by closed bars. Standard errors of the means of determinations in 5 rats per group are shown.

addition to the 60-min. time shown in Fig. 3, blood levels were determined at 15, 30, and 45 min. Levels of amphetamine in blood at these times ranged from 4 to 10 times as high as the levels of 4-chloroamphetamine. Tissue/plasma ratios were therefore much higher for 4-chloroamphetamine than for amphetamine, suggesting that the over-all tissue uptake and binding of the chloroamphetamine was greater. This is also revealed by the fact that at 4 hr., when blood levels of both drugs had dropped to the limits of detection, tissue concentrations of 4-chloroamphetamine were still amost as high as at 1 hr. In all of the tissues, the levels of 4-chloroamphetamine at 4 hr. were substantially higher than those of amphetamine, indicating that a slower disappearance rate of the chloroamphetamine occurs in other tissues as well as in the brain. Tissue distribution of the drugs in mice was very similar to that shown in Fig. 3 for the rat.

The data presented show that substitution of chlorine in the *para* position of amphetamine markedly slows the disappearance rate of the drug from tissues. It is suggested that an important reason may be the blocking of one of the metabolic pathways—namely, *para* hydroxylation—by which amphetamine is normally transformed prior to excretion from the body.

REFERENCES

(1) Pletscher, A., Bartholini, G., Bruderer, H., Burkard, W. P., and Gey, K. F., *J. Pharmacol. Exptl. Therap.*, **145**, 344 (1964).
(2) Dubnick, B., Leeson, G. A., Leverett, R., Morgan, D. F., and Phillips, G. E., *ibid.*, **140**, 85(1963).
(3) Fuller, R. W., Hines, C. W., and Mills, J., *Biochem. Pharmacol.*, **14**, 483(1965).
(4) Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **110**, 315 (1954).
(5) Dring, L. G., Smith, R. L., and Williams, R. T., *J. Pharm. Pharmacol.*, **18**, 404(1966).
(6) Ellison, T., Gutzait, L., and Van Loon, E. J., *J. Pharmacol. Exptl. Therap.*, **152**, 383(1966).
(7) Kuntzman, R., and Tsai, I., *Federation Proc.*, **25**, 418 (1966).