Biochemical Pharmacology, 1967, Vol. 16, pp. 11–16. Pergamon Press Ltd., Printed in Great Britain.

d-AMPHETAMINE LEVELS IN BRAIN AND OTHER TISSUES OF ISOLATED AND AGGREGATED MICE

RAY W. FULLER and C. W. HINES

The Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Ind., U.S.A.

(*Received* 20 *July* 1966; *accepted* 7 *September* 1966)

Abstract—The relative tissue distribution of *d*-amphetamine in the mouse after i.p. administration was: kidney > lung > liver, spleen, brain > heart > blood. Aggregation of mice, which enhances amphetamine toxicity, increased amphetamine levels in blood, heart, brain, and liver, but not in other tissues. This effect occurred at a dose of amphetamine near the LD_{50} dose in the aggregated mice. Aggregation also increased brain levels and toxicity of *dl*-4-chloroamphetamine. Chlorpromazine pretreatment prevented aggregation-induced increases in amphetamine levels in heart and brain. The possible role of increased tissue levels in the altered pharmacological effects of amphetamine produced by aggregation is discussed.

THE STIMULATION of the central nervous system and the lethality of *d*-amphetamine in the mouse are affected by environmental factors such as temperature, noise, stress, and aggregation of animals.[1–6] In addition, aggregation influences the effect of *d*-amphetamine on body temperature,[1] blood glucose and tissue glycogen levels,[7] and brain and heart catecholamine levels[8] of mice. Consolo *et al.*[9] have recently shown that amphetamine levels are higher in the brain of aggregated mice than in isolated mice. This suggested the possibility that the distribution of amphetamine within the animal or the rate of disposition of the drug might be altered by aggregation. Previous studies related to tissue distribution of amphetamine have dealt with species other than the mouse.[10–13]

The present study reports the tissue distribution and disappearance rates of amphetamine in mice and the effect of dose and of aggregation on tissue and blood levels of amphetamine.

EXPERIMENTAL

Male albino Cox/Standard mice weighing approximately 16 g were used in all the studies. There were six mice per group in each experiment except as otherwise noted. The animals were kept singly (isolated) or in groups of six (aggregated) in wooden cages 7 cm square, with wire mesh top and bottom, 10 cm in height.

d-Amphetamine-^{3}H (generally labeled) sulfate, obtained from the New England Nuclear Corp., was diluted with unlabeled *d*-amphetamine sulfate (Dexedrine) obtained from Smith, Kline and French Laboratories. The amphetamine was injected i.p. at a volume of 10 ml/kg, dissolved in distilled water. *dl*-4-Chloroamphetamine hydrochloride (Lilly) was injected in the same way as amphetamine. All doses are expressed as amount of free base for both drugs. Immediately after injection, the mice were placed in cages as described above. The mice were decapitated 1 hr later, and

tissues were removed, blotted, frozen immediately on dry ice, and stored at −15° prior to analysis. Blood was collected in heparinized tubes, and analyses were performed immediately.

Tissue and blood levels of amphetamine were determined by liquid scintillation counting in the following way. Tissues were homogenized in four volumes of 0·1 N HCl. Thirty per cent $HClO_4$ was added to tissue homogenates or to whole blood to give an end concentration of 6 per cent, and the tubes were centrifuged after thorough mixing. One ml of supernatant fraction was shaken for 10 min with 0·2 ml of 10 N NaOH and 3 ml washed benzene. After centrifugation, 2 ml of the benzene layer was added to 10 ml of scintillator solution* for counting. This extraction procedure is the same as that used in the spectrophotometric determination of amphetamine; recovery of added amphetamine was complete (>95 per cent). Hydroxylated and acidic metabolites are not extracted. The radioactivity in the benzene extract was identified as amphetamine by paper chromatography. The solvent system for descending chromatography was *n*-butanol/glacial acetic acid/water, 4/1/1.

Levels of 4-chloroamphetamine were determined by the method of Axelrod[10] as modified by Dubnick *et al.*[14]

LD_{50} values and their standard deviations were calculated by the method of Miller and Tainter.[15]

RESULTS AND DISCUSSION

The tissue distribution of amphetamine in mice as related to dose was determined prior to investigating the effects of aggregation on amphetamine levels (Table 1).

TABLE 1. EFFECT OF DOSE ON TISSUE LEVELS OF *d*-AMPHETAMINE IN MICE

Tissue	Dose of *d*-amphetamine injected		
	1·8 mg/kg	9·2 mg/kg	18·4 mg/kg
	Tissue amphetamine levels (mμmoles/g)		
Kidney	37·2 ± 2·3	136·0 ± 5·1	314·6 ± 12·2
Lung	34·8 ± 1·0	124·0 ± 10·0	323·1 ± 21·0
Spleen	15·6 ± 1·8	82·6 ± 7·4	278·8 ± 27·8
Liver	15·9 ± 0·6	82·2 ± 6·5	181·7 ± 8·8
Brain	10·8 ± 0·6	64·2 ± 1·1	188·6 ± 4·1
Heart	8·5 ± 0·7	38·2 ± 1·3	107·2 ± 11·3
Blood	1·5 ± 0·1	8·0 ± 0·7	24·3 ± 1·7

Standard errors and the means of four determinations are shown.

The relative concentration of amphetamine was: kidney > lung > spleen, liver, brain > heart > blood. This distribution resembles that reported by Axelrod in the dog.[10] Increasing the dose of amphetamine from 9·2 to 18·4 mg/kg more than doubled blood levels of amphetamine. All the tissues studied contained a higher concentration of amphetamine than did blood, indicating an ability of the organs to concentrate and/or bind amphetamine. The tissue/blood ratio in the kidney, liver, and heart

* The scintillator solution contained 15·2 g PPO (2,5-diphenyloxazole) and 0·38 g POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene] dissolved in 3·8 l. toluene.

diminished with increased doses of amphetamine. Approximately 17 to 20 per cent of the injected amphetamine was present in the tissues listed in Table 1 at 1 hr. The remainder had presumably been metabolized or excreted, or was present in other parts of the body.

In a separate experiment, amphetamine levels in tissues, after a dose of 1·8 mg/kg, were measured at 60, 75, 90, 105, and 120 min after injection and found to decrease logarithmically during this period. The disappearance rates, in per cent per hour, were: kidney, 50; lung, 47; liver, 51; brain and spleen, 52; and heart and blood, 46. The disappearance rates were therefore quite similar for all the tissues studied and for blood.

Consolo *et al.*[9] recently reported that aggregation of mice, long known to increase amphetamine toxicity, resulted in higher levels of amphetamine in the brain. Table 2

TABLE 2. *d*-AMPHETAMINE LEVELS IN THE BRAIN OF ISOLATED AND AGGREGATED MICE

Dose (mg/kg)	Amphetamine level (mμmoles/g brain)		Significance of difference
	Isolated	Aggregated	
1·8	8·0 ± 0·3	7·0 ± 0·6	n.s.
3·7	15·9 ± 1·0	19·9 ± 1·5	n.s.
5·5	26·7 ± 1·3	28·2 ± 1·7	n.s.
7·4	34·1 ± 1·5	42·9 ± 1·8	$P < 0{\cdot}005$

Standard errors and the means of six determinations are shown, n.s. = not significant.

shows the effect of dose on this response to aggregation. At the dose of 7·4 mg/kg, the data agree with the results of Consolo *et al.* At the lower doses, brain amphetamine levels were not increased by aggregation. The calculated LD_{50} values of *d*-amphetamine in our experimental conditions were 7 ± 1 and 93 ± 10 mg/kg, respectively, in aggregated and isolated mice. Thus, aggregation increased brain amphetamine levels only at a dose (7·4 mg/kg) very near the LD_{50} dose. Approximately 50 per cent of these animals, which were sacrificed at 1 hr, would have died within 4 hr.

The effect of aggregation on amphetamine levels in other tissues was determined (Fig. 1) at a dose of 7·4 mg/kg. The results show that increased levels of amphetamine in aggregated mice occurred not only in brain but also in liver, heart, and blood. The ratio of levels in aggregated mice to levels in isolated mice were: heart, 2·4; blood, 2·0; brain, 1·7; and liver 1·4. On the other hand, amphetamine levels in kidney, lung, and spleen were not affected by aggregation; the ratios of tissue/blood amphetamine levels for these three organs were thus decreased by aggregation, while those for brain, heart, and liver were not markedly changed.

Other studies in our laboratory have dealt with 4-chloroamphetamine, a compound that lowers brain serotonin in rats but not in mice.[16, 17] It was found that 4-chloroamphetamine differs from amphetamine in two respects: its disappearance rate from brain is much slower (10 per cent per hr), and it exists predominantly in a particulate-bound form in brain.[18] It was thus of interest to determine if aggregation of mice would influence brain levels of 4-chloroamphetamine. Table 3 shows that aggregation

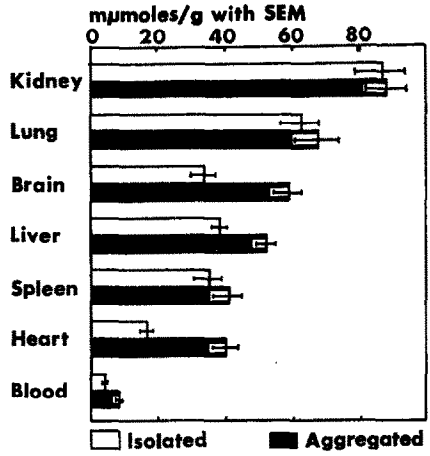

FIG. 1. *d*-Amphetamine levels in tissues of isolated and aggregated mice. *d*-Amphetamine was given at a dose of 7·4 mg/kg 1 hr prior to sacrifice. Means of six determinations are shown with their standard errors.

of mice profoundly affected the toxicity of 4-chloroamphetamine and that brain 4-chloroamphetamine levels were increased by aggregation in mice given a dose near the LD_{50} dose in aggregated mice. 4-Chloroamphetamine therefore resembles amphetamine in these experiments.

TABLE 3. LD_{50} VALUES AND BRAIN LEVELS OF 4-CHLOROAMPHETAMINE IN ISOLATED AND AGGREGATED MICE

Grouping of mice	LD_{50} (mg/kg)	Brain level (mμmoles/g)*
Isolated	84 ± 10	47·8 ± 3·6
Aggregated	8 ± 1	62·0 ± 3·8 ($P < 0·025$)

* Brain levels were determined 1 hr after a dose of 8 mg *dl*-4-chloroamphetamine/kg. Standard errors and the means of six deminations are shown.

Figure 2 shows the effect of chlorpromazine pretreatment on brain and heart amphetamine levels in isolated and aggregated mice. In this experiment, aggregation significantly increased amphetamine levels in tissues of saline-pretreated mice ($P < 0·05$), but had no significant effect on amphetamine levels in tissues of chlorpromazine-pretreated mice. Chlorpromazine caused slightly, but not significantly, increased levels of amphetamine in brain and heart of isolated mice. Moore has shown that the increased norepinephrine depletion in brain and heart produced by amphetamine in aggregated compared to isolated mice was antagonized by chlorpromazine under conditions similar to those used by us in the experiment shown in Fig. 2.[19] It seems likely that the lack of effect of aggregation in chlorpromazine-pretreated mice on amphetamine levels in brain and heart may explain the prevention of increased norepinephrine depletion.

The present study does not reveal the mechanism for the increased amphetamine levels in aggregated mice, which are obviously stressed to a greater degree than isolated mice. Their hyperactivity is so marked that they enter a stage of exhaustion which is followed by death at the higher dose levels. The extremely stressed condition of the aggregated mice may compromise their ability to excrete and/or metabolize amphetamine, by an effect on the blood flow to the kidney or liver or on the organs themselves.

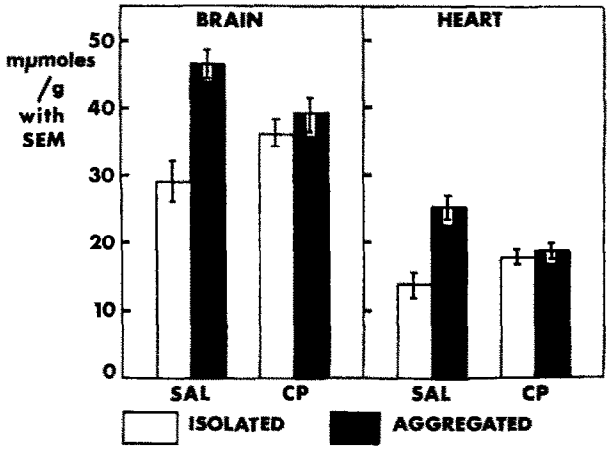

FIG. 2. Effect of chlorpromazine on *d*-amphetamine levels in the brain and heart of isolated and aggregated mice. *d*-Amphetamine was given at a dose of 7·4 mg/kg 1 hr prior to sacrifice. Chlorpromazine (2 mg/kg) or saline was injected i.p. 30 min prior to amphetamine injection. Means of six determinations are shown with their standard errors.

Amphetamine in aggregated as compared to isolated mice is more toxic, depletes brain and heart norepinephrine to a greater extent, has a greater hyperthermic effect, and lowers blood glucose and tissue glycogen levels. The relationship among these responses to aggregation is not clear, although a possible connection between the enhanced toxicity and the other effects has been considered.[7, 8, 20] It may be that the increased amphetamine levels in brain, heart, blood, and liver in aggregated animals play a role in the enhanced toxicity and in the other effects. It is clear that the increased tissue levels do not solely account for the enhanced toxicity, since higher amphetamine levels can be produced without lethal effects by administration of larger amphetamine doses to isolated animals. The increased amphetamine levels may be causally related to some of the other effects, besides enhanced toxicity, produced by aggregation. There may be a similar type of positive feedback mechanism whereby aggregation enhances the pharmacological effects of amphetamine which in turn decrease the rate of amphetamine removal and lead to higher tissue levels of amphetamine, contributing to further and prolonged pharmacological effects.

REFERENCES

1. B. M. ASKEW, *J. Pharm. Pharmac.* **13**, 701 (1961).
2. M. R. A. CHANCE, *J. Pharmac. exp. Ther.* **87**, 214 (1946).
3. M. R. A. CHANCE, *J. Pharmac. exp. Ther.* **89**, 289 (1947).
4. J. A. GUNN and M. R. GURD, *J. Physiol., Lond.* **97**, 453 (1940).

5. R. HOHN and L. LASAGNA, *Psychopharmacologia* **1**, 210 (1960).
6. B. WEISS, V. G. LATIES and F. L. BLANTON, *J. Pharmac. exp. Ther.* **132**, 366 (1961).
7. K. E. MOORE, L. C. SAWDY and S. R. SHAUL, *Biochem. Pharmac.* **14**, 197 (1965).
8. K. E. MOORE, *J. Pharmac. exp. Ther.* **142**, 6 (1963).
9. S. CONSOLO, S. GARATTINI, R. GHIELMETTI and L. VALZELLI, *J. Pharm. Pharmac.* **17**, 666 (1965).
10. J. AXELROD, *J. Pharmac. exp. Ther.* **110**, 315 (1954).
11. R. L. YOUNG and M. W. GORDON, *J. Neurochem.* **9**, 161 (1962).
12. J. J. ALLEVA, *J. med. Chem.* **6**, 621 (1963).
13. R. P. MAICKEL, R. H. COX, D. S. SEGAL and F. P. MILLER, *Fedn Proc.* **25**, 385 (1966).
14. B. DUBNICK, G. A. LEESON, R. LEVERETT, D. F. MORGAN and G. E. PHILLIPS, *J. Pharmac. exp. Ther.* **140**, 85 (1963).
15. L. C. MILLER and M. L. TAINTER, *Proc. Soc. exp. Biol. Med.* **57**, 261 (1944).
16. R. W. FULLER, C. W. HINES and J. MILLS, *Biochem. Pharmac.* **14**, 483 (1965).
17. A. PLETSCHER, G. BARTHOLINI, H. BRUDERER, W. P. BURKARD and K. F. GEY, *J. Pharmac. exp. Ther.* **145**, 344 (1964).
18. R. W. FULLER and C. W. HINES, *Fedn Proc.* **25**, 658 (1966).
19. K. E. MOORE, *J. Pharmac. exp. Ther.* **144**, 45 (1964).
20. H. H. WOLF and D. J. GEORGE, *J. pharm. Sci.* **53**, 748 (1964).