chemical Pharmacology, 1963, Vol. 12, pp. 1283–1283. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF D-AMPHETAMINE AND RESTRAINT ON THE CONTENT OF NOREPINEPHRINE AND DOPAMINE IN RAT BRAIN*

K. E. MOORE and E. W. LARIVIERE†

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, N.H., U.S.A.

(*Received* 19 *April* 1963; *accepted* 25 *June* 1963)

Abstract—Injection of D-amphetamine into rats caused a rapid reduction of norepinephrine content in brain; repletion occurred within 72 hr. Dopamine content of the brain was not affected. Restraint did not enhance the D-amphetamine-induced release of norepinephrine.

NOREPINEPHRINE and dopamine have been implicated as neurohumoral agents serving specialized functions within the central nervous system; changes in the brain concentration of these amines have been correlated with the central actions of certain drugs.[1, 2] Dopamine is located almost exclusively in the corpus striatum and is thought to be involved in the control of motor functions.[3] It is believed that norepinephrine plays a role in the central sympathetic nervous system; release of this amine is accompanied by signs of arousal or excitement.[4]

Amphetamine exerts its peripheral vasopressor effect through the release of endogenous stores of norepinephrine.[5] The mechanism(s) of its excitatory actions in the central nervous system is unknown, although studies indicate that its effects resemble those of stimulating central adrenergic neurons.[6]

The excitatory and lethal effects of amphetamine are markedly enhanced when it is administered to mice and rats that are exposed to electric shock or crowding (aggregation); pretreatment with adrenergic blocking agents prevents this facilitation of the effects of amphetamine.[7] In a preliminary communication, Moore[8] reported that D-amphetamine releases endogenous stores of norepinephrine in the brain and heart of mice and that aggregation enhances this release. It was proposed that the influence of aggregation in potentiating the ability of amphetamine to release norepinephrine is an important factor in causing the increased excitement and toxicity of this drug.

Since the responses to amphetamine are influenced by the environment in which animals are tested[9, 10] we are examining the effects that "stressful" situations, in addition to aggregation, play in the actions of D-amphetamine. The present communication reports the effects of D-amphetamine on the dopamine and norepinephrine content of rat brain and the lack of influence that physical restraint has on these effects.

* Supported by Grant A-6275 from the National Institute of Arthritis and Metabolic Diseases.

† Summer research fellow supported in part by a grant from the New Hampshire Cancer Society. Present address: University of Rochester Medical School, Rochester, N.Y.

METHOD

Female Sprague–Dawley rats weighing 180 to 220 g were used throughout the study. Drugs were injected intraperitoneally in a constant volume of 5 ml/kg, after which the animals were placed in individual cages until they were sacrificed. Animals that were restrained were placed in adjustable wire-mesh cages that prevented locomotion and changes in posture. At appropriate times animals were sacrificed by decapitation; the brains were quickly removed, weighed, and homogenized in 6 ml cold 0·4 N $HClO_4$. The homogenate was allowed to stand for 30 min in ice and then centrifuged at 14,000 × *g* for 10 min at 4°. The pellet was resuspended in 6 ml of 0·4 N $HClO_4$ and centrifuged as before. The two supernatants were combined and stored at −20° until analysed.

The tissue extracts and standards were purified on alumina-containing chromatographic columns as described previously.[11] Each day 7 to 9 columns were used (1 for norepinephrine standard, 2 for dopamine standard, and 4 to 6 for brain extracts). The amines were eluted from the alumina with 8 ml 0·2 N acetic acid. The elute from each column was neutralized to pH 6·0 to 6·5 and divided into four equal parts. Two aliquots (sample and blanks) were used for the norepinephrine assay and two aliquots (sample and blank) were used for the dopamine assay.

Norepinephrine was determined by a modification of the method described by Bertler *et al.*[12] To one 2-ml sample of eluate were added 0·3 ml phosphate buffer (0·1 M, pH 6·5), 0·05 ml 0·25% $ZnSO_4$, and 0·05 ml 0·25% potassium ferricyanide. Two minutes after adding the ferricyanide, 0·25 ml of freshly prepared alkaline ascorbate was added (1 ml 2% ascorbic acid + 9 ml 5 N NaOH). Fluorescence was measured 10 min later in an Aminco–Bowman spectrophotofluorometer at activation–emission wavelengths of 400:520 mμ (uncorrected). Blanks were included with each analysis and treated exactly as described for the standards except that 0·2 ml 5 N NaOH was added 2 min after the ferricyanide, and 0·05 ml of 1% ascorbic acid was added 5 min later. These "faded" blanks were read 10 to 15 min later. Norepinephrine standards were prepared by adding 0·5 μg norepinephrine to 6 ml 0·2 N sodium acetate, pH 8·4, and passing the mixture through the column as was done for the tissue extracts. The recovery of the norepinephrine standards was 77·7 ± 2·1 per cent (mean and standard error), and the norepinephrine content of brain extracts was calculated from these standards.

Dopamine was determined by a modification of the method of Carlsson and Waldeck.[13] To 2 ml of the neutralized eluate (pH 6·0 to 6·5) were added 0·5 ml 0·1 M phosphate buffer (pH 6·5) and 0·06 ml iodine solution (0·254 g iodine and 5·0 g KI in 100 ml water). After 5 min 0·5 ml alkaline sulfite (5·04 g $Na_2SO_3 \cdot 7H_2O$ in 100 ml 5 N NaOH) was added. After another 5 min 0·6 ml 5 N acetic acid was added. The samples were placed in a water bath at 50° for 30 min. The samples were then centrifuged, and the supernatant was added to quartz cuvet and irradiated for 20 min with an ultraviolet lamp (Mineralight model SL 2537). The fluorescence was read in an Aminco–Bowman spectrophotofluorometer at activation–emission wavelengths of 330:400 mμ (uncorrected) with a Wratten filter 80B placed in the fluorescent pathway. Blanks were prepared with each analysis by using another 2 ml of eluate and proceeding in the same manner as described for the standards except that water instead of iodine was added to the sample. It was found that if NaOH instead of alkaline sulfite was added to the blank, there resulted an erroneously low blank. The dopamine standard was

prepared by dividing one brain extract in half, adding water to one aliquot and 1·0 μg dopamine to the other; the two were passed through separate columns and then oxidized as described above. The difference in the fluorescence of these two extracts was taken as the dopamine standard used for the calculations. By this procedure the recovery of dopamine was 92·7 ± 3·9 per cent as compared with 45·6 ± 3·8 per cent when the dopamine was passed through the column as was done for the norepinephrine standard. Why the addition of dopamine to the tissue extract produced better recoveries of the standard is not known, but a similar finding was reported by Gey and Pletscher.[14]

When brain catecholamines are determined as just described, norepinephrine does not interfere with the dopamine assay nor does dopamine interfere with the norepinephrine assay. Only when one amine is present in a much greater concentration than the other should a correction for fluorescent interference be calculated (see Drujan *et al.*[15]).

With this method both norepinephrine and dopamine could be measured in a single rat brain after a simple extraction and purification process. The catecholamine content of brains from control rats and from rats treated in various ways is shown in Table 1.

TABLE 1. THE NOREPINEPHRINE AND DOPAMINE CONTENTS OF RAT BRAIN AFTER PRETREATMENT WITH VARIOUS DRUGS

Drug	Dose, mg/kg	No. of animals	Norepinephrine	Dopamine
None		6	0·42 ± 0·01	0·54 ± 0·04
Reserpine	1	4	0·04 ± 0·02	0·22 ± 0·05
DL-α-Methyl-*m*-tyrosine	400	4	0·15 ± 0·02	0·41 ± 0·03
Phenylisopropylhydrazine (JB516)	10	9	0·61 ± 0·04	0·60 ± 0·05

Animals were sacrificed 24 hr after intraperitoneal injection of drug. Catecholamine concentrations are expressed in micrograms per gram wet weight of fresh whole brain ± standard error of the mean.

The reduction of the norepinephrine and dopamine content by reserpine, the reduction of norepinephrine by α-methyl-*m*-tyrosine, and the increased contents of both amines after pretreatment with a monoamine oxidase inhibitor (JB516) are similar to the results of other workers.

The drug salts employed throughout this study were D-amphetamine sulfate and chlorpromazine hydrochloride, and doses are expressed accordingly.

RESULTS

The effect of D-amphetamine on the brain contents of dopamine and norepinephrine is shown in Fig. 1. The reduction of brain norepinephrine (4 hr after injection of D-amphetamine) was dose dependent; after 10 or 30 mg D-amphetamine/kg, the norepinephrine content was significantly different from that of saline controls ($P > 0·01$). The amount of dopamine in the brains of amphetamine-treated animals was not significantly different ($P > 0·05$) from that of control brains. The norepinephrine and dopamine concentrations in brains of restrained animals were not significantly different from those of unrestrained rats.

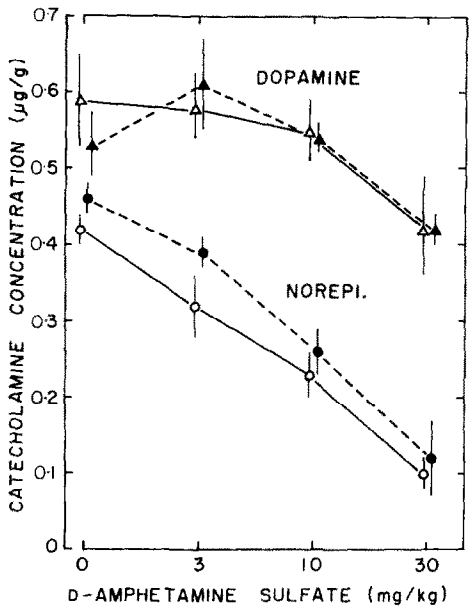

FIG. 1. Effect of D-amphetamine and physical restraint on the catecholamine content of brain. Control animals were sacrificed 4 hr after the injection of saline or various doses of D-amphetamine. Restrained animals were so maintained for 4 hr after similar injections. Each point represents the mean of 6 rats, and the vertical line through the points represents the standard error of the mean; △, control dopamine; ▲, restraint dopamine; ○, control norepinephrine; ●, restraint norepinephrine.

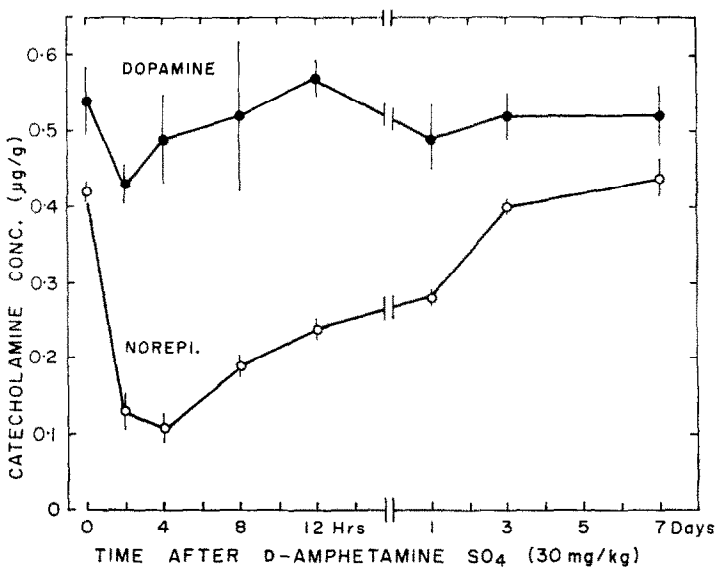

FIG. 2. Time course of the effect of D-amphetamine (30 mg/kg) on brain catecholamines. Each point represents the mean of 6 rats, and the vertical line through the points represents the standard error of the mean; ○, norepinephrine; ●, dopamine.

The time course of the effect of D-amphetamine on the brain catecholamine content is depicted in Fig. 2. Norepinephrine was reduced to its lowest level in 2 to 4 hr and returned to control values by 72 hr. The dopamine content was only slightly reduced for the first few hours but within 4 hr had returned to control levels.

DISCUSSION

Injection of D-amphetamine into rats caused a rapid but relatively short-lasting reduction in the brain content of norepinephrine; it had little effect on the brain content of dopamine. Repeated injections of D-amphetamine have been reported to reduce brain norepinephrine in rats,[16] and D-amphetamine and methamphetamine reduce norepinephrine stores in rabbit brain.[17, 18] It is not known what role the release of norepinephrine plays in the central actions of amphetamine, but many of the peripheral actions of this drug are the result of its ability to release endogenous stores of norepinephrine.[5] Amphetamine has a direct action in the brain since it causes stimulation at a time when the norepinephrine brain stores are very low—i.e. in reserpine-treated animals.[19] However, the norepinephrine that is released in the brain probably adds to the direct stimulant action of D-amphetamine. For example, the release of endogenous catecholamines by amphetamine appears to be involved in the enhanced excitement that is seen when this drug is injected into aggregated mice.[8]

It is not known how amphetamine causes the reduction of brain norepinephrine. There is some evidence that amphetamine interferes with the binding of catecholamines.[20] Sanan and Vogt[17] proposed that amphetamine might cause depletion of norepinephrine by occupying storage sites that are normally occupied by norepinephrine. The short time course of the D-amphetamine-induced norepinephrine depletion (as compared with the prolonged depletion caused by such drugs as reserpine) is consistent with this view. The release of norepinephrine could result from the stimulation of central adrenergic centers in the brain stem such that norepinephrine is released more rapidly than it can be resynthesized and stored. This mechanism might also explain why norepinephrine, which is located primarily in those areas that represent the central adrenergic centers (hypothalamus and brain stem), is depleted while dopamine, which is located almost exclusively in the basal ganglia, is not affected.

In contrast to the results of the aggregation studies in mice,[8] restraint did not enhance the lethality or norepinephrine-depleting action of D-amphetamine in rats. The short period of restraint did not appear to be a sufficiently severe type of stress and probably was a poor method to employ in this study since Höhn and Lasagna[21] found that confinement of individual mice did not enhance the toxicity of amphetamine. Furthermore, in studies with mice, aggregation was accompanied by exaggerated motor activity while the present technique served to curtail motor activity. Different types of stress have been reported to reduce the norepinephrine content in brain of rats.[22, 23] These methods of imposing stress are currently being studied to test their influences on the central stimulating, lethal, and catecholamine-depleting actions of D-amphetamine.

Acknowledgement—The authors are grateful for the technical assistance of Mrs. Louise Sawdy. The D-amphetamine sulfate and chloropromazine hydrochloride were generously supplied by Mr. C. W. French, Smith Kline and French Laboratories, Philadelphia, Pa.

REFERENCES

1. A. CARLSSON, *Pharmacol. Rev.* **11**, 490 (1959).
2. M. VOGT, *Brit. med. Bull.* **13**, 166 (1957).
3. A. BERTLER and E. ROSENGREN, *Experientia (Basel)* **15**, 10 (1959).
4. B. B. BRODIE, S. SPECTOR and P. A. SHORE, *Pharmacol. Rev.* **11**, 548 (1959).
5. J. H. BURN and M. J. RAND, *Lancet* **1**, 673 (1958).
6. C. MUNOZ and L. GOLDSTEIN, *J. Pharmacol. exp. Ther.* **132**, 354 (1961).
7. B. WEISS, V. G. LATIES and F. L. BLANTON, *J. Pharmacol. exp. Ther.* **132** 336 (1961).
8. K. E. MOORE, *Fed. Proc.* **22**, 310 (1963).
9. M. R. A. CHANCE, *J. Pharmacol. exp. Ther.* **87**, 214 (1946).
10. M. R. A. CHANCE, *J. Pharmacol. exp. Ther.* **89**, 289 (1947).
11. K. E. MOORE and T. M. BRODY, *J. Pharmacol. exp. Ther.* **132**, 6 (1961).
12. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* **44**, 273 (1958).
13. A. CARLSSON and B. WALDECK, *Acta physiol. scand.* **44**, 293 (1958).
14. K. F. GEY and A. PLETSCHER, *J. Pharmacol. exp. Ther.* **133**, 18 (1961).
15. B. D. DRUJAN, T. S. SOURKES, D. S. LAYNE and G. F. MURPHY, *Canad. J. Biochem.* **37**, 1153 (1959).
16. J. R. MCLEAN and M. MCCARTNEY, *Proc. Soc. exp. Biol. (N. Y.)* **107**, 77 (1961).
17. S. SANAN and M. VOGT, *Brit. J. Pharmacol.* **18**, 109 (1962).
18. T. HIGUCHI, T. MATSUO and K. SHIMAMOTO, *Jap. J. Pharmacol.* **12**, 48 (1962).
19. M. G. GELDER and J. R. VANE, *Psychopharmacologia (Berl.)* **3**, 231 (1962).
20. J. AXELROD and R. TOMCHICK, *J. Pharmacol. exp. Ther.* **130**, 367 (1960).
21. R. HÖHN and L. LASAGNA, *Psychopharmacologia (Berl.)* **1**, 210 (1960).
22. R. LEVI and E. W. MAYNERT, *Fed. Proc.* **21**, 336, (1962).
23. D. X. FREEDMAN, J. D. BARCHAS and R. L. SCHOENBRUN, *Fed. Proc.* **21**, 337 (1962).