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THE EFFECTS OF COCAINE, AMPHETAMINE AND SOME AMPHETAMINE-LIKE COMPOUNDS ON THE *IN VIVO* LEVELS OF NORADRENALINE AND DOPAMINE IN THE RAT BRAIN

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Abstract—The effects of D- and L-amphetamine, D-methylamphetamine, L-ephedrine, DL-phenmetrazine, D- and L-phendimetrazine and cocaine on the levels of noradrenaline and dopamine in the rat brain frozen *in situ* have been investigated. No significant effects on dopamine levels were noted. D-amphetamine, L-ephedrine and D-methylamphetamine significantly reduced and DL-phenmetrazine and L-phendimetrazine significantly increased the level of noradrenaline. The noradrenaline to dopamine ratio was significantly reduced by D-amphetamine and D-methylamphetamine but significantly increased by L-phendimetrazine. No relationship exists between the effects of these drugs on behaviour and on the brain levels of noradrenaline and dopamine. The relationship between brain levels of catecholamines and adenosine triphosphate and possible mechanisms of action of amphetamine and cocaine are discussed.

SINCE the detection of noradrenaline in brain,[1] attempts have been made to explain the mode of action of tranquillizers and anti-depressives in terms of their effects on brain levels of this amine.[2–4] D-Amphetamine,[5–7] methylamphetamine[8] and cocaine[8] reduce brain noradrenaline levels in rats and rabbits, while ephedrine[9] has no effect on the noradrenaline level in cat hypothalamus. Burn and Rand[10] suggest that the peripheral effects of amphetamine are due to noradrenaline release; this effect may also be responsible for its central actions. However, Van Rossum *et al.*[11] propose that amphetamine has a direct noradrenaline-like action on specific receptors in the brain, while cocaine has an indirect action caused by noradrenaline release.

Dopamine is present in brain[12, 13] but is localised mainly in the corpus striatum.[14] This compound, rather than noradrenaline, may be concerned with the function of the corpus striatum and thus with the control of motor activity.[14, 15] Van Rossum[15] suggests that amphetamine increases locomotor activity by reacting with dopamine receptors in the brain. Observations that amphetamine[7, 16] and cocaine[17] have no effect on brain dopamine levels in rats may be consistent with this view.

An attempt has been made to correlate the effects of amphetamine and amphetamine-like drugs on brain levels of adenine nucleotides,[18] and on those of noradrenaline and dopamine. The possibility that their effects on brain levels of noradrenaline and dopamine correlate with their stimulant potency has also been examined.

METHODS

Male albino rats (75 to 95 *g*), were placed in groups of 2 or 3 of equal body weight. One rat in each group served as control and the order of treatment was randomised using a 3 × 3 latin square design (series 2,3 and 15) or a table of random numbers (all other series). The data obtained were evaluated by the appropriate analysis of variance.[19]

Drugs used were D-amphetamine sulphate, L-amphetamine sulphate, D-methylamphetamine hydrochloride, L-ephedrine hydrochloride, DL-phenmetrazine hydrochloride [DL-3-methyl-2-phenylmorpholine hydrochloride], D-phendimetrazine bitartrate [D-3,4-dimethyl-2-phenylmorpholine bitartrate], L-phendimetrazine bitartrate and cocaine hydrochloride. All doses reported refer to these salts. The drugs were dissolved in 0·9% (w/v) sodium chloride solution, sterilised and injected intraperitoneally (0·2 ml per 100 g body weight). The same volume of 0·9% (w/v) sodium chloride solution was given to the control animals.

Immediately after injection, each animal was placed in a cylindrical cage (9·5 cm long × 6·5 cm diameter) until the end of the experimental period (1 hr in series 1, and 3 hr in all other series) when it was killed by plunging the cage into liquid nitrogen. The whole brain was dissected out in a modified glove box (−5°C), weighed and stored in liquid nitrogen for up to 48 hr, before extraction.

The frozen brains were pulverised and extracted first with 15 ml, and then with 5 ml, of ice-cold 0·4 M perchloric acid.[18] The supernatant, after each centrifugation, was filtered (4°C) into a chilled 25 ml measuring cylinder and the extract volume adjusted to 25 ml with ice-cold de-ionized water.

In series 1, one rat in each group was killed and the brain extracted as described above. The other rat in the group was decapitated, the brain quickly removed, weighed and placed in a 100 ml vortex beaker, embedded in crushed ice and containing 10 ml ice-cold 0·4 M perchloric acid. After homogenization (2 min at 14,000 rev./min., 'M.S.E. microhomogeniser'), the suspension was transferred to a centrifuge tube. The vortex beaker was washed with 5 ml ice-cold 0·4 M perchloric acid, which was also added to the centrifuge tube. The suspension was centrifuged (5 min, 0°C, 2,200 *g*) and the supernatant filtered as above. 5 ml ice-cold 0·4 M perchloric acid were added to the precipitate, stirred for 2 min, centrifuged (5 min, 0°C, 2,200 *g*), the supernatant filtered as before and the extract volume adjusted to 25 ml with ice-cold deionized water.

The catecholamines were adsorbed and eluted, as described by Crout *et al.*,[20] except that the acid-washed alumina was prepared by the method of Weil-Malherbe and Bone[21] and 5N potassium carbonate was used for the titration. Two portions of 5 ml ice-cold 0·2N acetic acid were used for the elution, the total eluate added to a chilled 20 ml flask containing 0·1 ml ice-cold 0·2 M disodium ethylenediamine-tetraacetic acid, adjusted to volume with de-ionized water, centrifuged (5 min, room temperature, 2,600 *g*) and stored (4°C) until assayed (usually 2 hr).

Noradrenaline was assayed by the method of Bertler *et al.*,[22] omitting the zinc sulphate and using only a reagent blank. 5 ml of each of the eluates (from drug- and control-treated animals) were titrated and made up to 10 ml with de-ionized water. The eluates were assayed up to 10 ml with de-ionized water. The eluates were assayed together and two samples (2 ml) were taken from each titrated solution. After oxidation and rearrangement, which were carried out at a constant temperature (25°C), the

solutions were centrifuged (10 min, room temperature, 2,600 *g*) and read in an Aminco–Bowman spectrophotofluorometer—activating wavelengths 390 mμ and 440 mμ and fluorescent wavelength 510 mμ (all uncorrected values). No correction was made for the recovery of 82 per cent for noradrenaline.

Dopamine was estimated by the method of Carlsson and Waldeck.[23] 5 ml of each eluate were titrated, and duplicate samples (2 ml) taken for assay. 1·0 μg dopamine was used as the standard and added to the portion of each eluate before titration. After oxidation (25°C) and irradiation, the solutions were centrifuged (10 min, room temperature, 2,600 *g*) and read at an activating wavelength of 315 mμ and a fluorescent wavelength of 380 mμ (both uncorrected values). No correction was made for the recovery of 83 per cent for dopamine.

In series 20 and 21, designed to examine if DL-phenmetrazine and L-phendimetrazine interferred in these assays, a quantity of drug the same as the dose given to the animals was added to 20 ml ice-cold 0·4 M perchloric acid containing 0·5 μg noradrenaline, 0·02 μg adrenaline and 1·0 μg dopamine. The mixture was adjusted to 25 ml with ice-cold de-ionized water and the amines adsorbed, eluted and assayed as above. 0·2 ml of 0·9% (w/v) sodium chloride solution was added to a similar mixture of perchloric acid and amines to serve as the control.

RESULTS

The effects of freezing the untreated animal in liquid nitrogen, D- and L-amphetamine, cocaine, D-methylamphetamine, L-ephedrine, DL-phenmetrazine and D- and L- phendimetrazine on the levels of noradrenaline and dopamine and on the ratio of noradrenaline to dopamine in the rat brain are shown in Tables 1 and 2.

None of these drugs significantly altered the dopamine level and only freezing in liquid nitrogen (Table 1, series 1), D-amphetamine 5 mg/kg (Table 1, series 3 and 4) and 10 mg/kg (Table 1, series 5), D-methylamphetamine 10 mg/kg (Table 2, series 14), each of which caused a fall, and L-phendimetrazine 40 mg/kg (Table 2, series 19), which caused a rise, significantly affected the noradrenaline to dopamine ratio. L-amphetamine 5 mg/kg used in the 3 × 3 latin square design (Table 1, series 3) also lowered the noradrenaline to dopamine ratio significantly, but this effect disappeared when the same dose level was used in pairs of animals (Table 1, series 6).

Freezing in liquid nitrogen (Table 1, series 1), D-amphetamine 5 mg/kg (Table 1, series 4) and 10 mg/kg (Table 1, series 5), L-ephedrine 20 mg/kg (Table 2, series 11) and 40 mg/kg (Table 2, series 12) and D-methylamphetamine 10 mg/kg (Table 2, series 14) significantly reduced the brain noradrenaline level, while DL-phenmetrazine 40 mg/kg (Table 2, series 17) and L-phendimetrazine 40 mg/kg (Table 2, series 19) significantly increased it. L-amphetamine, at doses up to 40 mg/kg (Table 1, series 6–9), D-phendimetrazine 40 mg/kg (Table 2, series 18) and cocaine 40 mg/kg (Table 1, series 10) were ineffective. D-amphetamine 5 mg/kg had no significant effect on the noradrenaline level when used in the 3 × 3 latin square design (Table 1, series 3), but produced a significant fall when used in groups of 2 animals (Table 1, series 4).

The significant increases in noradrenaline produced by DL-phenmetrazine and L-phendimetrazine were not due to interference with the assay procedure (Table 3, series 20 and 21). In addition, they did not interfere with the assay procedure for dopamine (Table 3, series 20 and 21).

DISCUSSION

The freezing technique was used to make the results more easily comparable with those of Lewis and Van Petten.[18] A comparison has also been made between this method and one involving decapitation and homogenization. The lower levels of noradrenaline in the brains of the frozen rats may be due to the absence of handling immediately before killing but differences in the technique may be partly responsible.

TABLE 1. EFFECTS OF FREEZING, D-AMPHETAMINE, L-AMPHETAMINE AND COCAINE ON THE LEVELS OF NORADRENALINE AND DOPAMINE IN THE RAT BRAIN

Series	Treatment	Dose (mg/kg)	No. of Animals	Concentration μg/g frozen tissue NA	DOP	Ratio NA/DOP
1	Decapitation	—	10	0·56 ± 0·06	1·80 ± 0·09	0·31 ± 0·03
	Freezing	—	10	0·43 ± 0·06‡	1·72 ± 0·11	0·24 ± 0·03*
2	Control	0	9	0·52 ± 0·04	1·46 ± 0·16	0·49 ± 0·18
	L-Amphetamine	2·5	9	0·47 ± 0·04	1·52 ± 0·16	0·32 ± 0·03
	D-Amphetamine	2·5	9	0·46 ± 0·03	1·50 ± 0·13	0·33 ± 0·05
3	Control	0	9	0·48 ± 0·03	1·36 ± 0·14	0·40 ± 0·07
	L-Amphetamine	5	9	0·43 ± 0·04	1·56 ± 0·14	0·29 ± 0·04*
	D-Amphetamine	5	9	0·40 ± 0·02	1·48 ± 0·09	0·29 ± 0·04*
4	Control	0	10	0·71 ± 0·04	1·41 ± 0·08	0·52 ± 0·04
	D-Amphetamine	5	10	0·62 ± 0·04*	1·45 ± 0·08	0·43 ± 0·03*
5	Control	0	10	0·85 ± 0·04	1·57 ± 0·10	0·57 ± 0·06
	D-Amphetamine	10	10	0·63 ± 0·05‡	1·71 ± 0·16	0·39 ± 0·05‡
6	Control	0	10	0·62 ± 0·08	1·41 ± 0·09	0·47 ± 0·07
	L-Amphetamine	5	10	0·61 ± 0·08	1·34 ± 0·08	0·47 ± 0·06
7	Control	0	10	0·64 ± 0·07	1·47 ± 0·04	0·44 ± 0·05
	L-Amphetamine	10	10	0·56 ± 0·04	1·57 ± 0·07	0·37 ± 0·05
8	Control	0	10	0·86 ± 0·09	1·43 ± 0·09	0·66 ± 0·10
	L-Amphetamine	20	10	0·77 ± 0·07	1·61 ± 0·09	0·50 ± 0·06
9	Control	0	10	0·43 ± 0·05	1·48 ± 0·11	0·29 ± 0·04
	L-Amphetamine	40	10	0·36 ± 0·03	1·37 ± 0·13	0·29 ± 0·04
10	Control	0	10	0·68 ± 0·06	0·75 ± 0·06	0·94 ± 0·09
	Cocaine	40	10	0·64 ± 0·08	0·83 ± 0·05	0·78 ± 0·10

All values are the means (± S.E.M.) of nine or ten experiments. The animals were killed 3 hr after injection of the drug or control solution except in series 1, where no treatment was given, and one rat was killed by freezing in liquid nitrogen after 1 hour in the cage, while the other rat in the group was killed by decapitation. Significance of differences from control (in series 1 the values from the decapitated animals were taken as the control): *0·05 > P > 0.025; †0·025 > P > 0·01; ‡0·01 > P > 0·001. In this and subsequent tables NA = noradrenaline; DOP = dopamine.

Care must be taken in choosing the statistical design. Using the 3 × 3 latin square design the effects of two drugs can be investigated together as compared with those of only one if the other design is employed. Thus more information can be obtained in approximately the same period of time. It was, however, unsatisfactory as D-amphetamine 5 mg/kg produced no effect on the noradrenaline level in groups of 3 rats, but significantly reduced it in groups of 2. L-amphetamine also produced a different effect

TABLE 2. EFFECTS OF L-EPHEDRINE, D-METHYLAMPHETAMINE, DL-PHENMETRAZINE, D-PHENDIMETRAZINE AND L-PHENDIMETRAZINE ON THE LEVELS OF NORADRENALINE AND DOPAMINE IN THE RAT BRAIN

Series	Treatment	Dose (mg/kg)	No. of Animals	Concentration μg/g frozen tissue NA	DOP	Ratio NA/DOP
11	Control	0	10	0·52 ± 0·04	2·89 ± 0·23	0·20 ± 0·03
	L-Ephedrine	20	10	0·42 ± 0·03*	3·04 ± 0·24	0·15 ± 0·02
12	Control	0	10	0·57 ± 0·07	1·47 ± 0·13	0·42 ± 0·06
	L-Ephedrine	40	10	0·36 ± 0·06‡	1·27 ± 0·13	0·31 ± 0·05
13	Control	0	10	0·62 ± 0·04	1·24 ± 0·08	0·52 ± 0·04
	D-Methylamphetamine	5	10	0·56 ± 0·05	1·32 ± 0·09	0·44 ± 0·05
14	Control	0	10	0·67 ± 0·06	1·24 ± 0·08	0·55 ± 0·05
	D-Methylamphetamine	10	10	0·50 ± 0·05†	1·26 ± 0·06	0·39 ± 0·03†
15	Control	0	9	0·56 ± 0·05	1·67 ± 0·10	0·34 ± 0·03
	D-Methylamphetamine	0·9	9	0·49 ± 0·02	1·72 ± 0·06	0·29 ± 0·01
	DL-Phenmetrazine	1·8	9	0·48 ± 0·04	1·52 ± 0·10	0·33 ± 0·03
16	Control	0	10	0·68 ± 0·05	1·39 ± 0·08	0·50 ± 0·04
	DL-Phenmetrazine	20	10	0·71 ± 0·07	1·36 ± 0·06	0·52 ± 0·05
17	Control	0	10	0·76 ± 0·08	1·28 ± 0·10	0·63 ± 0·07
	DL-Phenmetrazine	40	10	0·98 ± 0·08‡	1·41 ± 0·08	0·70 ± 0·05
18	Control	0	10	0·70 ± 0·08	1·26 ± 0·07	0·56 ± 0·06
	D-Phendimetrazine	40	10	0·72 ± 0·07	1·32 ± 0·06	0·56 ± 0·06
19	Control	0	10	0·74 ± 0·07	1·05 ± 0·10	0·75 ± 0·09
	L-Phendimetrazine	40	10	0·96 ± 0·06‡	1·09 ± 0·08	0·93 ± 0·10‡

All values are the means (± S.E.M.) of nine or ten experiments. The animals were killed 3 hours after the injection of the drug or control solution. Significance of differences from control: * $0{\cdot}05 > P > 0{\cdot}025$; † $0{\cdot}025 > P > 0{\cdot}01$; ‡ $0{\cdot}01 > P > 0{\cdot}001$.

TABLE 3. EFFECTS OF DL-PHENMETRAZINE AND L-PHENDIMETRAZINE ON THE ASSAY OF NORADRENALINE AND DOPAMINE

Series	Treatment	No. of experiments	Concentration μg NA	DOP
20	Control	10	0·45 ± 0·06	0·93 ± 0·08
	DL-Phenmetrazine	10	0·46 ± 0·05	0·86 ± 0·09
21	Control	10	0·37 ± 0·03	0·74 ± 0·09
	L-Phendimetrazine	10	0·36 ± 0·03	0·74 ± 0·07

All values are the means (± S.E.M.) of ten experiments. No animals were involved. These experiments were designed to investigate if DL-phenmetrazine and L-phendimetrazine interfered with the assays for noradrenaline and dopamine. The doses of the drugs which had been given to the animals in series 17 and 19 (0·2 ml of a 20 mg/ml solution) were added to perchloric acid containing 0·5 μg noradrenaline, 0·02 μg adrenaline and 1·0 μg dopamine. The amines were adsorbed, eluted and assayed in the same way as brain extracts. 0·2 ml of 0·9% (w/v) sodium chloride solution was added to a similar mixture of perchloric acid and amines to serve as the control.

in the 3 × 3 latin square design than in groups of 2 animals. It was, therefore, concluded that the latter design was better, as each drug was compared directly with the control with no possibility of the effects of a second drug interfering. The 3 × 3 latin square design is, however, suitable for investigating the effects of drugs on the levels of other substances in brain, for example, adenine nucleotides[18, 24] where there is a smaller error inherent in the technique.

From studies on locomotor activity the descending order of potency is D-methylamphetamine > D-amphetamine > DL-phenmetrazine > D-phendimetrazine > L-ephedrine > L-amphetamine.[25–27] If the reduction in the noradrenaline level is the criterion of potency, D-amphetamine is more potent than D-methylamphetamine which is more potent than L-ephedrine. L-amphetamine and D-phendimetrazine have no effect on the noradrenaline level, and DL-phenmetrazine and L-phendimetrazine increase it. Thus, there is no correlation between behavioural stimulant potency and effects on the noradrenaline levels in the rat brain. Similarly, since all the drugs used, except L-phendimetrazine, which has no significant effect, increase brain levels of adenosine triphosphate (ATP),[18] there is no correlation between their effects on the adenine nucleotide and noradrenaline levels in rat brain. The close relationship between ATP and catecholamines in the adrenal medulla[28–30] and in splenic nerves[31] is not therefore found in brain.

As dopamine is the precursor of noradrenaline,[32] the effects on the noradrenaline to dopamine ratio were studied as a fall in this might indicate decreased noradrenaline synthesis. Goldstein and Contrera[33] have shown that amphetamine inhibits dopamine β-oxidase, which converts dopamine to noradrenaline. This should decrease the noradrenaline level and increase that of dopamine, causing the ratio of noradrenaline to dopamine to fall. Of the drugs investigated, D-amphetamine and D-methylamphetamine reduced the ratio while L-phendimetrazine caused it to rise.

Amphetamine may increase locomotor activity by reacting with brain dopamine receptors.[15] If this is so, then the dopamine levels should be unchanged or slightly raised. None of the drugs used altered the dopamine levels in the rat brain significantly, hence this possibility cannot be excluded.

Amphetamine may also act on the noradrenaline receptors in the brain.[11] If this were so, no effect on the noradrenaline level would be expected. However, only L-amphetamine and D-phendimetrazine failed to alter the rat brain noradrenaline levels. Those drugs which lower noradrenaline may produce their central actions in the manner suggested by Burn and Rand[10] for their peripheral actions, but since this effect is not consistent throughout the series and some drugs increase the brain noradrenaline level, it seems unlikely. The fall in noradrenaline may be due to the stimulation instead of *vice versa*, but the rise in noradrenaline caused by some compounds is not consistent with this view. If the catecholamines act by catalysing the cyclization of ATP to adenosine-3′,5′-phosphate,[34] a fall in the noradrenaline level might be expected since the drug could release noradrenaline which then acts on the receptor site, producing stimulation. It is doubtful if the drugs investigated produce their effects in this way, since DL-phenmetrazine and L-phendimetrazine increase the brain noradrenaline levels. Also, the formation of adenosine-3′,5′-phosphate should reduce the amount of ATP present while in practice these drugs increase it.[18]

The mechanism by which DL-phenmetrazine and L-phendimetrazine increase brain levels of noradrenaline is not clear. DL-phenmetrazine is a weak inhibitor of mono-

amine oxidase,[35] but it is unlikely that this effect is responsible for changes of the magnitude observed. Inhibition of monoamine oxidase cannot explain the action of amphetamine since this would be expected to raise brain noradrenaline while it actually produces a fall. The latter is not due to an effect on catechol-0-methyl transferase since amphetamine does not enhance the activity of this enzyme.[36]

Cocaine may produce its central stimulant effects by releasing brain noradrenaline[11] but as it does not lower rat brain noradrenaline levels, it is unlikely that this can explain its actions.

The very large doses of the drugs used required to alter the noradrenaline level in the rat brain, compared with those to produce maximum increase in locomotor activity[25, 37] and to increase the adenine nucleotide levels,[18] and the absence of any consistent effect on the noradrenaline levels makes it unlikely that their action is mediated in this manner. Furthermore, in locomotor experiments[26] and in increasing ATP levels[18] D-phendimetrazine is more potent than L-phendimetrazine, but in our experiments L-phendimetrazine has the greater effect on the brain noradrenaline levels. That DL-phenmetrazine and L-phendimetrazine produce the opposite effect to D-amphetamine, although they have similar pharmacological actions, is difficult to explain.

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