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Blockade of biogenic amine synthesis: its effect on the responses to leptazol and dexamphetamine in rats

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1. The convulsive effects of leptazol in the rat are potentiated by prior treatment with dexamphetamine.

2. An intact dopamine synthesis is necessary for the potentiation of the convulsive action of leptazol.

3. An intact noradrenaline synthesis is not necessary for this action of amphetamine, as long as the dopamine synthesis is intact.

4. An intact 5-hydroxytryptamine synthesis is not necessary for the potentiation to be shown.

5. Blockade of either noradrenaline, dopamine or 5-hydroxytryptamine synthesis has no direct effect on leptazol convulsions.

6. It is possible that it is an intact 3-4 dihydroxyphenylalanine (dopa) synthesis rather than an intact dopamine synthesis that is involved.

Results from previous studies of the effects of dexamphetamine on leptazol-induced convulsions are conflicting. It has been reported to have no effect (Wolf & Stock, 1966) or to be proconvulsant (Friebel & Klatt, 1959). We have found dexamphetamine to be proconvulsant with leptazol in both rats and mice (Turner & Spencer, 1968).

Dexamphetamine-induced hyperactivity has recently been the subject of a number of investigations. α-Methyl-*p*-tyrosine (α-MPT), which is reported to block noradrenaline synthesis by inhibition of the enzyme tyrosine hydroxylase (Spector, Sjoerdsma & Udenfriend, 1965), has been used in several investigations, and as a result there is now overwhelming evidence that dexamphetamine exerts its central stimulant effects via endogenous catecholamines. More specifically, an intact catecholamine synthesis appears to be necessary for these effects, rather than high catecholamine concentrations (Weissman, Koe & Tenen, 1966 ; Dingell, Owens, Norvich & Sulser, 1967 ; Weissman & Koe, 1967 ; Frey & Magnussen, 1968 ; Sulser, Owens, Norvich & Dingell, 1968). It has also been proposed that only small concentrations of catecholamines are necessary for the central action of amphetamine and that this action might include release of catecholamines from nerve terminals in the brain (Hanson, 1967). Other investigations have attempted to differentiate

further between stereotypy and hyperactivity after amphetamine. It has been demonstrated that dopamine might be responsible for stereotypy and noradrenaline for motor excitation (Scheel-Kruger & Randrup, 1967 ; Randrup & Munkvad, 1967), although this hypothesis conflicts with the earlier evidence of Van Rossum (1963).

The *p*-chloro analogues of amphetamine which selectively deplete central 5-hydroxytryptamine (5-HT) (Pletscher, Burkard, Bruderer & Gey, 1963 ; Pletscher, Burkard & Gey, 1964 ; Fuller, Hines & Mills, 1965 ; Lippman & Wishnick, 1965) have also been used in these investigations. Thus, 5-HT has been shown to be necessary for the central stimulant effects of *p*-chloroamphetamine by Frey & Magnussen, (1968) who postulated that noradrenaline liberated by 5-HT was the final mediator of this central stimulant action.

We have investigated which of these three central amines—noradrenaline, dopamine or 5-HT—is necessary for the amphetamine-induced potentiation of leptazol, and also if any of these amines promotes the convulsions due to leptazol alone. Three selective synthesis blocking agents have been used: α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor (Spector *et al.*, 1965) ; diethyldithiocarbamate (DDC) a dopamine-β-oxidase inhibitor (Hashimoto, Ohi & Imaizmi, 1965 ; Goldstein, 1966 ; Carlsson, Lindqvist, Fuxe & Hökfelt, 1966 ; Carlsson, Fuxe & Hökfelt, 1967) ; and *p*-chlorophenylalanine (p-Cl Ph A), a tryptophan hydroxylase inhibitor which selectively depletes 5-hydroxytryptamine (Koe & Weissman, 1966).

Methods

Animals

Adult male Wistar albino rats weighing 200–250 g were used. They were maintained on a 41B cube diet and water until 4 hr before the leptazol challenge. Twenty-four hours before the experiment, the animals were transferred to a temperature controlled room maintained at 20° ±0·5° C where the experiments were made.

Leptazol convulsions

Convulsions were produced in groups of five rats by subcutaneous injection of leptazol 65 mg/kg in 0·9% w/v sodium chloride solution (0·5 ml./100 g) and the number of clonic phases counted during the next 30 min. Observation of rats treated with high doses of leptazol showed the following seizure pattern: (i) myoclonic jerks ; (ii) facial clonus followed by clonus of the whole body (first clonic phase) ; (iii) phase of depression ; (iv) clonus of the whole body (second clonic phase) ; (v) forelimb flexor tonus.

In all cases the seizure pattern was similar in that there were always two clonic phases in each rat if the convulsion went to completion. Hence, this was chosen as the parameter by which the severity of the seizure was measured.

Thus the maximum number of possible clonic phases in each group of five rats was ten (two phases per rat). The numbers of clonic phases in the test groups were expressed as a percentage of the maximum (percentage of maximum clonic convulsions). Dexamphetamine sulphate and *p*-chlorophenylalanine methyl ester hydrochloride were dissolved in 0·9% w/v sodium chloride solution ; α-methyl-*p*-tyrosine was suspended in 0·9% w/v sodium chloride solution and the suspension

was stirred before withdrawal of each dose. These drugs were administered intraperitoneally in a dose volume of 0·5 ml./100 g. All doses are in terms of free base or amino-acid.

Spectrophotofluorometric determinations of biogenic amines

Animals were killed by cervical dislocation, the brains were dissected out, weighed, and homogenized in 4 ml. of 0·4 N perchloric acid at 0° C. The homogenate was centrifuged at 15,000 *g* for 8 min at 0° C and the supernatant stored at 0° C. A second homogenization using a further 2 ml. 0·4 N perchloric acid was performed on the original sample and recentrifuged as before, the second supernatant being bulked with the initial supernatant. The total clear supernatants from two brains were combined, shaken and divided into two equal portions, one for dopamine and noradrenaline determination and the other for 5-hydroxytryptamine determination. Known amounts of the three amines were added to some extracts as a check on the recovery of these amines.

Noradrenaline and dopamine estimation

The aliquot of clear supernatant was titrated to pH 6·5 using 5 N potassium carbonate at a pH meter. The precipitate of potassium perchlorate thus produced was removed by centrifugation at 15,000 *g* for 6 min at 0° C and the clear supernatant passed onto a Dowex 50 W.X.8 resin (100 mg dry weight) column which had been washed previously with: (1) 8 ml. 2 N hydrochloric acid ; (2) 10 ml. distilled water ; (3) 5 ml. 0·5 M phosphate buffer pH 6·5 ; (4) 10 ml. distilled water ; (5) two further 10 ml. volumes of water. The dimensions of the washed resin column were 4 mm diameter and 12–15 mm in length.

The supernatant was passed through the resin at a flow rate not exceeding 1 ml. in 2 min. After adsorption of the amines, the columns were washed with 10 ml. distilled water. Then, after passing 0·5 ml. 0·4 N hydrochloric acid on to the column to displace the water, the noradrenaline was eluted with 8 ml. 0·4 N hydrochloric acid at a flow rate not exceeding 1 ml. every 2 min. The dopamine was then eluted with 8 ml. 2 N hydrochloric acid at the same flow rate (having first displaced any 0·4 N hydrochloric acid with 0·5 ml. 2 N hydrochloric acid). This procedure was a modification of that used by Bertler, Carlsson & Rosengren, (1958).

The noradrenaline was assayed by a trihydroxyindole method evolved from those of Euler & Floding (1955) and Bertler *et al.* (1958). Phosphate buffer was used instead of acetate buffer, and zinc sulphate was omitted from the method. In the alkaline ascorbate, sodium borohydride was found to stabilize fluorescence (Gerst, Odd, Steinsland & Walcott, 1966), although it was necessary to use a concentration of sodium borohydride ten times higher than that suggested by these workers. This stabilized the fluorescence of noradrenaline for at least 60 min. The fluorescence of noradrenaline was read at the activation and emission wavelengths 395/500 mμ respectively in an Aminco Bowman spectrophotofluorimeter.

Dopamine was assayed by the method of Carlsson & Waldeck (1958), with the modification of Carlsson & Lindqvist (1962). However, only 0·05 ml. iodine solution was used instead of 0·1 ml. in the oxidation and maximum fluorescence developed without the use of ultraviolet irradiation. The fluorescent principle produced by this procedure was unstable in that it faded rapidly when subjected to the

activation light in the fluorimeter, but if the tubes were immersed in a boiling water bath for 5 min immediately after the oxidation and then allowed to cool to room temperature the dopamine fluorescence was stabilized at its maximum for at least 60 min. The fluorescence was then read at the activation and emission wavelengths 325/378 mμ respectively.

5-Hydroxytryptamine estimation

The 5-HT aliquot was neutralized with 5 N potassium carbonate and centrifuged as for the catecholamine determination and the clear supernatant passed on to a column of Dowex 50 W.X.8 resin (100 mg dry weight) previously prepared in the sodium form with (1) 8 ml. 1 N sodium hydroxide; (2) 15 ml. distilled water; (3) 15 ml. 0·1 N sodium hydroxide containing 0·2% w/v EDTA; (4) 10 ml. distilled water; (5) Two further 10 ml. volumes of distilled water. The clear supernatant was passed through this column at a flow rate not exceeding 1 ml. every 2 min. The 5-HT was then eluted from the column with 15 ml. 0·1 N sodium hydroxide (containing 0·2% w/v EDTA) into 1·5 ml. sodium acetate buffer, pH 4·6, and read directly in the spectrophotofluorimeter at the activation and emission wavelengths 295/345 mμ respectively. This method is similar to that used by Cox & Potkonjak (1967), but these authors eluted the catecholamines from this column with 1 M potassium chloride before elution of 5-HT. In our hands the presence of potassium chloride gave rise to higher blanks and significantly reduced the sensitivity of the 5-HT assay.

The recoveries of the three amines by the methods outlined above were:

Noradrenaline	91·5% ± 7·6%
Dopamine	79·3% ± 14·4%
5-HT	78·4% ± 13·6%

All values given are uncorrected for recovery.

Results

*Effects of α-methyl-*p*-tyrosine*

Whole brain biogenic amine concentrations were determined in rats after 4 hr pretreatment with α-methyl-*p*-tyrosine, 100 mg/kg intraperitoneally. The results are shown in Table 1.

This dose of α-MPT inhibited tyrosine hydroxylase activity since it depleted noradrenaline concentrations to 50% of controls and dopamine concentrations to 30% of controls. There was no significant effect on 5-HT concentrations.

TABLE 1. *Effect of 4 hr pretreatment with α-methyl-*p*-tyrosine (100 mg/kg) on whole brain biogenic amine concentrations in rats*

Pretreatment	Noradrenaline (ng/g)	Dopamine (ng/g)	5-HT (ng/g)
Intraperitoneal saline (controls) (4 hr)	197±37 (9)	526±121 (4)	382±38 (5)
Intraperitoneal α-methyl-*p*-tyrosine 100 mg/kg (4 hr)	99+14 (9)	158±31 (5)	387±44 (5)
	$P<0·05$	$P<0·025$	

The figures in parenthesis indicate the number of determinations made; data are expressed as mean±standard deviation.

This same dose of α-MPT was used to investigate the effects of leptazol after pretreatment with α-MPT and dexamphetamine. Rats received α-MPT 3·5 hr before dexamphetamine, 5 mg/kg intraperitoneally, which was followed 30 min later by the leptazol challenge. Controls were set up by substituting saline for α-MPT and/or dexamphetamine. In this way each drug was tested alone and in combination with the other drugs. The percentage of clonic convulsions after leptazol are summarized in Fig. 1.

The leptazol controls showed only 43% of maximum clonic phases, whereas dexamphetamine pretreatment raised this to 89%. However, when α-MPT was given before the dexamphetamine, this potentiation was blocked. When α-MPT was given alone it had no effect on the subsequent leptazol convulsions.

Diethyldithiocarbamate

Whole brain biogenic amine concentrations were determined in rats after 4 hr pretreatment with diethyldithiocarbamate (DDC) 400 mg/kg intraperitoneally. The

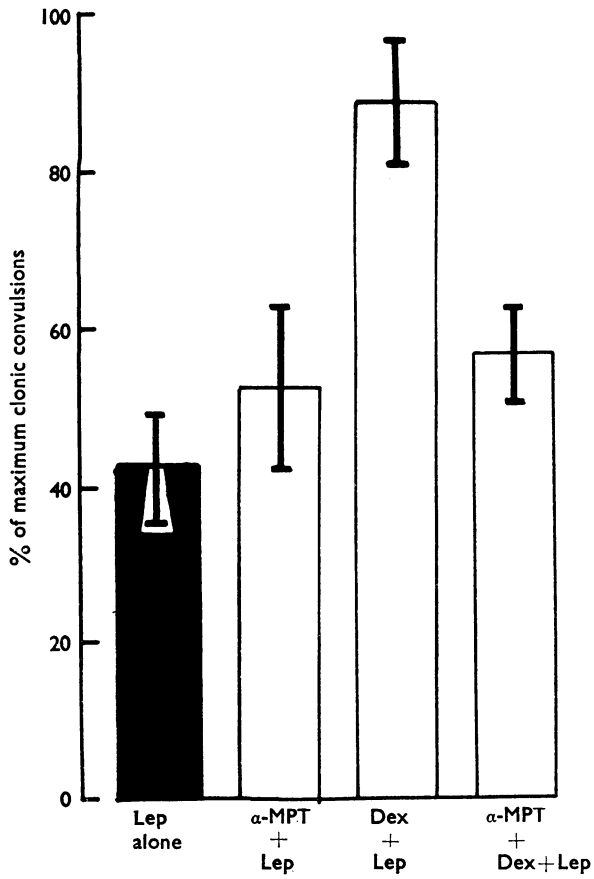

FIG. 1. Effect of pretreatment with α-methyl-*p*-tyrosine on convulsions produced in rats by leptazol alone or leptazol plus dexamphetamine. Lep, leptazol 65 mg/kg subcutaneously; α-MPT, α-methyl-*p*-tyrosine 100 mg/kg intraperitoneally, 4 hr before the leptazol challenge; Dex, dexamphetamine 5 mg/kg intraperitoneally, 30 min before the leptazol challenge. Vertical bars indicate standard deviations.

results are given in Table 2. DDC produced a depletion of noradrenaline to 43% of control concentrations and an elevation of dopamine to 124% of control concentrations. 5-HT concentrations showed no significant change after DDC pretreatment.

TABLE 2. *Effect of 4 hr pretreatment with diethyldithiocarbamate (400 mg/kg) on whole brain biogenic amine concentrations in rats*

Pretreatment	Noradrenaline (ng/g)	Dopamine (ng/g)	5-HT (ng/g)
Intraperitoneal saline (controls) (4 hr)	217±32 (13)	590±15 (5)	416±48 (20)
Intraperitoneal diethyldithiocarbamate 400 mg/kg (4 hr)	92±30 (17)	730±86 (5)	419±42 (30)
	$P<0.01$		

The figures in parenthesis indicate the number of determinations made; data are expressed as mean±standard deviation.

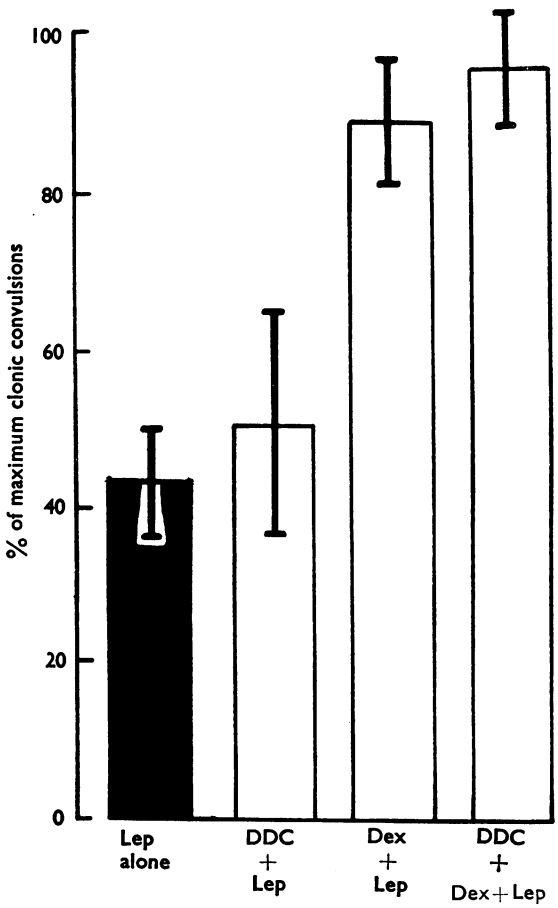

FIG. 2. Effect of pretreatment with diethyldithiocarbamate on convulsions produced in rats by leptazol alone or leptazol plus dexamphetamine. Lep, Leptazol 65 mg/kg subcutaneously; DDC, diethyldithiocarbamate 400 mg/kg intraperitoneally, 4 hr before the leptazol challenge; Dex, dexamphetamine 5 mg/kg intraperitoneally, 30 min before the leptazol challenge. Vertical bars indicate standard deviations.

This treatment was repeated to examine its effects on the dexamphetamine potentiation of leptazol, and also on the response to leptazol alone. The experiment was performed in the same way as described for α-MPT incorporating similar controls.

The percentage of maximum clonic convulsions are plotted as before in Fig. 2. DDC did not alter significantly the percentage of maximum clonic convulsions due

TABLE 3. *Effect of* p-*chlorophenylalanine* (320 *mg/kg*) (3 *days' pretreatment*) *on whole brain biogenic amine concentrations in rats*

Pretreatment	Noradrenaline (ng/g)	Dopamine (ng/g)	5-HT (ng/g)
Intraperitoneal saline (controls) (3 days)	268±67 (5)	593±32 (5)	440±48 (5)
Intraperitoneal *p*-chlorophenylalanine 320 mg/kg (3 days)	311±40 (5)	500±51 (5)	147±37 (5)
			$P<0{\cdot}005$

The figures in parenthesis indicate the number of determinations made; data are expressed as mean±standard deviation.

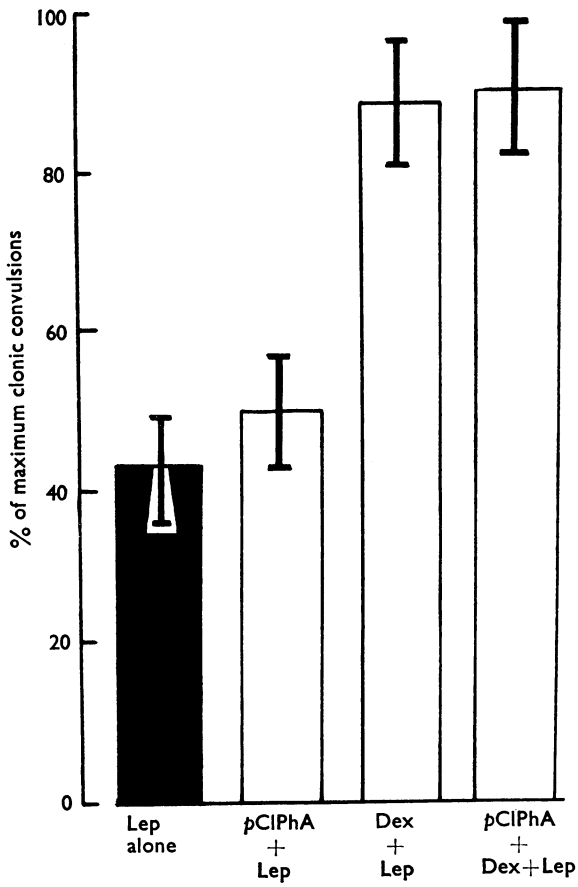

FIG. 3. Effect of pretreatment with *p*-chlorophenylalanine on convulsions produced in rats by leptazol alone or leptazol plus dexamphetamine. Lep, Leptazol 65 mg/kg subcutaneously; *p*ClPhA, *p*-chlorophenylalanine 320 mg/kg intraperitoneally, 3 days before the leptazol challenge; Dex, dexamphetamine 5 mg/kg intraperitoneally, 30 min before the leptazol challenge. Vertical bars indicate standard deviations.

to leptazol and, in contrast to α-MPT, neither did DDC pretreatment block dexamphetamine potentiation of leptazol.

p-*Chlorophenylalanine*

The effect of *p*-chlorophenylalanine, 320 mg/kg 3 days after injection (Koe & Weissman, 1966), was examined on whole brain biogenic amine concentrations. The results are as shown in Table 3. *p*-Chlorophenylalanine reduced 5-HT concentrations to 30% of control levels with no significant effect on either noradrenaline or dopamine concentrations.

This treatment was repeated using dexamphetamine and leptazol and the appropriate controls in the same way as described for α-MPT and DDC, and the percentage of maximum clonic convulsions plotted as before (see Fig. 3). *p*-Chlorophenylalanine had no effect on leptazol convulsions and neither did it have any effect on dexamphetamine induced potentiation of leptazol.

Discussion

Interruption of noradrenaline synthesis with α-MPT caused a noradrenaline depletion to 50%, and for dopamine a depletion to 30%, of control concentrations. At the same time the potentiation of leptazol by dexamphetamine was blocked. This supports the hypothesis that an intact noradrenaline synthesis is necessary for dexamphetamine to exert its effect (Weissman *et al.*, 1966 ; Dingell *et al.*, 1967 ; Weissman & Koe, 1967 ; Frey & Magnussen, 1968 ; Sulser *et al.*, 1968). However, since dopamine was also significantly depleted, it may be that only an intact dopamine synthesis is necessary for amphetamine to exert its effect. Inhibition of dopamine β-oxidase by DDC decreased noradrenaline to 43% of the control concentrations and produced a slight but statistically not significant rise in dopamine concentrations. In these conditions dexamphetamine was still able to potentiate leptazol. This indicates that it was a depletion of dopamine which was associated with the block of the potentiation by dexamphetamine. This result is in agreement with the work of Van Rossum (1963) but contradicts previous work in which it is suggested that noradrenaline is responsible for hyperactivity and dopamine is responsible for stereotypy after administration of dexamphetamine (Scheel-Kruger & Randrup, 1967 ; Randrup & Munkvad, 1967). We consider the potentiation of leptazol is unlikely to be stereotypy but more likely to be associated with hyperactivity. The block of the dexamphetamine-induced potentiation of leptazol could have been due to either low dopamine levels or to inhibition of dopamine synthesis. The latter is more likely, since other workers have shown that a similar dopamine depletion produced by α-methyl-*meta*-tyrosine or reserpine without affecting synthesis, had no effect on the actions of dexamphetamine (Van Rossum, Van Der Schoot & Hurkmans, 1962 ; Weissman *et al.*, 1966). Alternatively, it is possible that only an intact 3,4-dihydroxyphenylalanine (dopa) synthesis is necessary to allow dexamphetamine to potentiate leptazol. To confirm this it would be necessary to inhibit the enzyme dopa decarboxylase specifically. The only drug at our disposal would have been α-methyl dopa, but this compound is not a specific dopa decarboxylase inhibitor (Hess, Connamacher, Ozaki & Udenfriend, 1961). It replaces noradrenaline with a less active "false transmitter" which mimics the actions of noradrenaline and masks any effects due to depletion (Carlsson & Lindqvist, 1962 ; Day & Rand, 1963, 1964, 1967). Consequently, we were unable to

determine the effect of dopa decarboxylase inhibition. Blockade of 5-HT synthesis with *p*-chlorophenylalanine produced a depletion of 5-HT to 33% of control concentrations with no effect on the potentiation of leptazol by dexamphetamine. Hence, neither normal tissue concentrations nor an intact synthesis of 5-HT appeared to be necessary for amphetamine to produce this potentiation of leptazol.

The apparent dependence of dexamphetamine upon intact dopamine stores or dopa synthesis, for potentiation of the effects of leptazol, suggests that the convulsive actions of leptazol alone might be mediated in some way by changes in endogenous biogenic amines. However, the data reported in this study shows that changes in biogenic amine levels and synthesis do not modify the effects of leptazol per se. Thus the actions of dexamphetamine appear to be mediated at some site away from the precise locus of action of leptazol, the hyperactivity generated by dexamphetamine simply rendering the animal more sensitive to leptazol.

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