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Desipramine and amphetamine metabolism

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Desipramine (2·5–5 mg/kg) increases the urinary excretion of amphetamine given intraperitoneally at doses of 7·5–15 mg/kg and it decreases the excretion of *p*-hydroxyamphetamine. The rate of removal of brain amphetamine is decreased by desipramine (5 mg/kg, i.p.) when amphetamine is injected intraperitoneally but not when it is injected intracerebrally. It is suggested that desipramine impairs the hydroxylation of amphetamine in the liver thereby increasing the level of circulating amphetamine and eventually of brain amphetamine.

IMIPRAMINE and other tricyclic antidepressant agents are known to increase and prolong the pharmacological effects of (+)-amphetamine (Carlton, 1961; Lapin & Shchelkunov, 1963; Stein, 1964, 1966; Morpurgo & Theobald, 1965). Recently Valzelli, Consolo & Morpurgo (1966) showed that imipramine and desipramine prolong the hyperthermia induced by amphetamine in rats and that they also increase the amphetamine levels in brain and liver.

These findings prompted an investigation to establish whether desipramine was able to affect the formation of *p*-hydroxyamphetamine, the major metabolite of amphetamine in rats (Axelrod, 1954a, b; Alleva, 1963; Dring, Smith & Williams, 1966).

Materials and methods

Male Sprague-Dawley rats, 165 ± 5 g, were kept in Makrolon cages at constant room temperature (22°) and relative humidity (60%). (+)-Amphetamine sulphate and desipramine were injected intraperitoneally and the urines were collected 24 hr after dosing.

Amphetamine and *p*-hydroxyamphetamine were determined in urine and amphetamine in brain by the method of Axelrod (1954 a, b). Free *p*-hydroxyamphetamine was determined in urines before acid hydrolysis. Suitable controls indicated that desipramine did not interfere with the determination of urinary amphetamine or *p*-hydroxyamphetamine. The brains of animals pretreated with desipramine (5 mg/kg i.p.) did not affect the recovery of (+)-amphetamine added *in vitro*.

In some experiments (+)-amphetamine was introduced into the brain by the method of Valzelli (1964).

Results

Table 1 gives the results obtained on measuring amphetamine and *p*-hydroxyamphetamine in urines of rats treated with different doses of amphetamine alone or with desipramine. It is evident that desipramine treatment changed the pattern of amphetamine metabolism by increasing the urinary excretion of amphetamine and decreasing that of *p*-hydroxyamphetamine.

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TABLE 1. AMPHETAMINE AND *p*-HYDROXYAMPHETAMINE CONTENT IN RAT URINE 24 HR AFTER DIFFERENT DOSES OF (+)-AMPHETAMINE ALONE OR WITH DESIPRAMINE. Desipramine was always given 1 hr before (+)-amphetamine. Each figure is the average of at least 8 determinations.

Treatment	mg/kg i.p.	ml of urine/ rat in 24 hr	μg/ml of urine (± s.e.)	
			Amphetamine	Total *p*-hydroxyamphetamine
Amphetamine	7·5	4	72·5 ± 1·7	67·6 ± 13·6
Desipramine .. .. Amphetamine	2·5 + 7·5	4	178·5 ± 1·4	3·2 ± 1·1
Amphetamine	10·0	4	80·0 ± 0·9	80·8 ± 13·9
Desipramine .. .. Amphetamine	2·5 + 10·0	4	158 · 5 ± 2·0	n.m.*
Amphetamine	15	3	126·8 ± 3·7	135·2 ± 8·6
Desipramine .. .. Amphetamine	5 + 15	3	200·0 ± 0·5	n.m.*

* n.m. = not measurable

The pH of urines was in all instances about 6·5. This measurement was made since according to Asatoor, Galman, Johnson & Milne (1965) and Beckett, Rowland & Turner (1965) the amount of amphetamine excreted unchanged by the rats increases with acidity of the urine.

TABLE 2. TOTAL EXCRETION IN URINE AND RECOVERY OF AMPHETAMINE AND *p*-HYDROXYAMPHETAMINE AFTER ADMINISTRATION OF AMPHETAMINE ALONE OR IN COMBINATION WITH DESIPRAMINE TO RATS

Desipramine μg/rat i.p.	Amphetamine base μg/rat i.p.	Amphetamine μg/24 hr urine	Free *p*-OH-amphetamine μg/24 hr urine	Total *p*-OH-amphetamine μg/24 hr urine	Amphetamine + *p*-OH-amphetamine μg/24 hr urine	% hydroxylation relative to excretion	Recovery % amphetamine + *p*-OH-amphetamine
—	730	290	42	271	561	48	76
416	730	714	16	16	730	2	100
—	960	320	52	324	644	50	67
416	960	632	15	15	647	2	67
—	1460	380	79	405	785	52	53
832	1460	600	15	15	615	2	42

The total excretion of amphetamine, and both free and total *p*-hydroxyamphetamine, is showed in Table 2 which also includes the percentage hydroxylation relative to excretion and the percentage recovery of drug and metabolite. From these data it is even more evident that desipramine almost completely blocks the hydroxylation of amphetamine. The percentage of the dose of amphetamine excreted decreases with increase in dose while the percentage hydroxylation remains constant. Both the percentage recovery and hydroxylation are in good agreement with data recently published using [^{14}C]amphetamine (Dring & others, 1966).

To establish whether desipramine was able to modify the level of brain amphetamine because of its inhibition of the hydroxylation process or by a direct effect on the disposition of brain amphetamine, the rate of decrease of brain amphetamine was determined. This was done both in the presence and absence of desipramine, when (+)-amphetamine was

injected intraperitoneally or intracerebrally and desipramine intraperitoneally 1 hr previously. Fig. 1 summarizes the results. The rate of removal of brain amphetamine was decreased by desipramine when amphetamine was injected intraperitoneally but not intracerebrally.

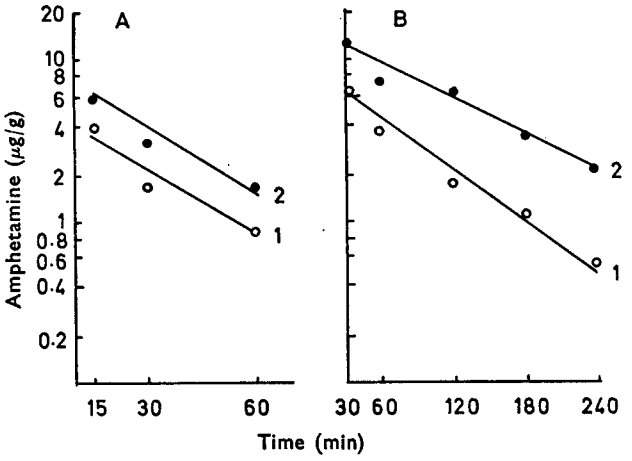

FIG. 1. A. Level of brain amphetamine after intracerebral injection of 50 μg of (+)-amphetamine (0·1 ml) alone (1) or following a pretreatment with desipramine (5 mg/kg i.p. 1 hr before) (2). B. Level of brain amphetamine after i.p. injection of 7·5 mg/kg of (+)-amphetamine alone (1) or following a pretreatment with desipramine alone (1) or following a pretreatment with desipramine (5 mg/kg i.p. 1 hr before) (2). The percentages removed (K) were:

$$K_{1A} = 3{\cdot}2;\ K_{2A} = 2{\cdot}7;\ K_{1B} = 1{\cdot}2;\ K_{2B} = 0{\cdot}76$$

The variance analysis shows that the lines 1A and 2A are not significantly different from parallelism. The lines 1B and 2B are significantly different from parallelism ($P < 0{\cdot}01$).

Discussion

The increase and prolongation of the pharmacological effects of amphetamine in the presence of tricyclic antidepressant agents, may be explained, at least in rats, by a difference in the disposition of amphetamine. Previous work established that the levels of amphetamine in brain and liver were increased by imipramine and similar agents (Valzelli & others, 1966). This suggested that impairment of the hepatic metabolism of amphetamine would increase circulating amphetamine and therefore give rise to higher levels of brain amphetamine. Since it is well known that the major metabolite of amphetamine in rats is *p*-hydroxyamphetamine, it should be at the hydroxylation stage that the tricyclic antidepressant agents exert their inhibiting effect. Our findings corroborate this by showing that the urinary excretion of *p*-hydroxyamphetamine is markedly decreased by desipramine. Indeed amphetamine derivatives substituted in the *para* position by chlorine or an hydroxyl group do not have their hyperthermic activity potentiated by tricyclic antidepressant agents (Valzelli & others, 1966).

The block of this hydroxylation probably occurs at the level of the hepatic microsomes. Desipramine does not show any major effect on the rate of removal of brain amphetamine when this amine is injected directly into the brain. It remains to be established whether desipramine inhibits the microsomal enzymes or prevents the uptake of amphetamine by preventing a contact between the enzyme and the substrate.

Imipramine and desipramine are hydroxylated in the 2 and 10 positions (Herrmann & Pulver, 1960; Bickel, 1966; Crammer & Scott, 1966). Since desipramine is rapidly formed from imipramine but is slowly metabolized in rats (Dingell, Sulser & Gillette, 1964) it might be expected that a competitive inhibition with the hydroxylation of amphetamine would be effective *in vivo* for several hours. Other investigations have recently shown that tricyclic antidepressants inhibit the metabolism of tremorine, presumably by a similar mechanism (Sjoqvist & Hammer, 1966). The hydroxylation of tyramine to dopamine is also inhibited by desipramine in rabbits (Lemberger, Kuntzman, Conney & Burns, 1965). A possible inhibition of the uptake of amphetamine at the microsomal level is supported by the finding that tricyclic antidepressant agents prevent the uptake of several drugs (Costa, Boullin, Hammer & others, 1966).

References

Alleva, J. J. (1963). *J. mednl Chem.*, **6**, 621–624.

Asatoor, A. M., Galman, B. R., Johnson, J. R. & Milne, M. D. (1965). *Br. J. Pharmac. Chemother.*, **24**, 293–300.

Axelrod, J. (1954a). *J. Pharmac. exp. Ther.*, **110**, 21.

Axelrod, J. (1954b). *Ibid.*, **110**, 315–326.

Beckett, A. H., Rowland, M. & Turner, P. (1965). *Lancet*, **1**, 303.

Bickel, M. H. (1966). Symposium on Antidepressant Drugs. Amsterdam: Excerpta Medica Foundation (in the press).

Carlton, P. L. (1961). *Psychopharmacologia*, **2**, 364–376.

Costa, E., Boullin, D. J., Hammer, W., Vogel, W. & Brodie, B. B. (1966). *Pharmac. Rev.*, **18**, 577–597.

Crammer, J. L. & Scott, B. (1966). *Psychopharmacologia* (Berl.), **8**, 461–468.

Dingell, J. V., Sulser, F. & Gillette, J. R. (1964). *J. Pharmac. exp. Ther.*, **143**, 14–22.

Dring, L. G., Smith, R. L. & Williams, R. T. (1966). *J. Pharm. Pharmac.*, **18**, 402–405.

Herrmann, B. & Pulver, R. (1960). *Chimica*, **14**, 30.

Lapin, I. P. & Shchelkunov, E. L. (1963). Second Int. Pharmacological Meeting, **1**, 205. Oxford: Pergamon.

Lemberger, L., Kuntzman, R., Conney, A. H. & Burns, J. J. (1965). *J. Pharmac. exp. Ther.*, **150**, 292–297.

Morpurgo, C. & Theobald, W. (1965). *Medna. Pharmac. exp.*, **12**, 226–232.

Sjoqvist, F. & Hammer, W. (1966). Symposium on Antidepressant Drugs. Amsterdam: Excerpta Medica Foundation (in the press).

Stein, L. (1964). *Fedn Proc. Fedn Am. Socs exp. Med.*, **23**, 836–850.

Stein, L. (1966). Symposium on Antidepressant Drugs. Amsterdam: Excerpta Medica Foundation (in the press).

Valzelli, L. (1964). *Medna exp.*, **11**, 23–26.

Valzelli, L., Consolo, S. & Morpurgo, C. (1966). Symposium on Antidepressant Drugs. Amsterdam: Excerpta Medica Foundation (in the press).