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THE EFFECTS OF SOME HALLUCINOGENIC DRUGS UPON THE METABOLISM OF NORADRENALINE

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In a previous publication (1), the effects of the hallucinogenic drugs lysergic acid diethylamide (LSD-25), mescaline, phencyclidine and Ditran upon the metabolism of 5-hydroxytryptamine (5-HT) in rat brain were reported and discussed. The distribution of 5-HT in the brain suggests that the amine may be important in those emotional and behavioural responses which are manifestly disturbed during hallucinogenesis. The distribution of the catecholamines, noradrenaline (NA) and dopamine (DA), in the brain is similar to that of 5-HT. Several investigators have reported changes in brain noradrenaline levels following the administration of hallucinogenic drugs (2,3,4,5), and it has been suggested that LSD-25 may produce its effects by actions on both 5-HT and catecholamine metabolism (6). It was therefore of interest to examine the effects on catecholamines of four drugs which have shown to affect 5-HT metabolism (1).

Methods

Wistar rats (90-110g) of either sex were used and all drugs were administered by intraperitoneal injection. The animals were killed by decapitation at intervals after injection; brains and hearts were rapidly removed, freed from superficial blood clots and homogenised in 10-12ml of ice-cold 0.4N perchloric acid. Estimations of NA and DA were carried out

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on the clear supernatant, obtained following centrifugation, by a combination of the methods of Anton and Sayre 1962 (7) and Anton and Sayre 1964 (8).

Normetanephrine (NM) was estimated by the method of Anton and Sayre (9) with the following modifications:

Whole rat brain was homogenised in 6ml NHCl. The homogenate was centrifuged at 30,000g for 30 mins and the clear supernatant transferred to a 10 ml beaker containing 2 ml of M Tris buffer (pH 7-9). The pH was adjusted to 6.8-7.2 and 2 ml of borate buffer (pH 10) was added. 14g of K_2HPO_4 powder was added with thorough mixing. Since n-butanol is a better solvent for NM than ether (except in the presence of interfering substances which do not occur in significant amounts in the brain (9), and is more easily handled), this solvent was substituted at this stage. 8 ml of n-butanol was added and the mixture was shaken for 10 mins, centrifuged and the organic layer removed. The aqueous phase was shaken again with 4 ml n-butanol and, after centrifugation, the butanol extracts were pooled and shaken with 4 ml of 0.1 CHl and 18 ml n-heptane. The butanol-heptane phase was discarded and the acid phase was mixed with 9 g K_2HPO_4 and 1 ml of borate buffer. The resultant saturated solution was shaken with 15 ml of ether. The ether layer was removed and shaken with 1.5 ml of 0.01 ml N HCl. Aliquots of the acid phase were then used for fluorophor formation as described by Anton and Sayre (9), except that 0.4 ml aliquots were used and volumes of other reagents were adjusted accordingly. The use of n-butanol in place of ether gave better recoveries in our hands, principally because of smaller losses incurred in handling this solvent.

As endogenous levels of NM in the brain are low and variable (9), twice the dose of each hallucinogen known to affect NA levels was injected and estimations were carried out at a time when effects would be expected to be maximal (30 mins after injection).

2 mg/kg reserpine was injected by the intraperitoneal route into 4 groups of rats. Animals in two of the groups also received injections of 20 mg/kg phencyclidine simultaneously with the reserpine. The dose of phencyclidine was chosen in order to prolong the action of the drug sufficiently to allow significant depletion of NA and DA by reserpine.

Five groups of rats received injections of 300 mg/kg alpha-methyl-p-tyrosine (MPT) intraperitoneally. Animals in four of the groups also received injections of phencyclidine (10 mg/kg), or Ditran (10 mg/kg), or LSD-25 (200 µg/kg) or mescaline (20 mg/kg). All animals were killed 3 hrs after the injection of MPT or MPT + hallucinogen.

Eight groups of rats were injected with alpha-methyl-m-tyrosine (MMT) 300 mg/kg by the intraperitoneal route. Animals in two groups received simultaneous injections of phencyclidine (10 mg/kg). Animals in two other groups received Ditran (10 mg/kg). Animals in two of the remaining groups received either LSD-25 (200 µg/kg) or mescaline (10 mg/kg). Animals were sacrificed either two or three hours after the injection of MMT.

The statistical significance of the results was calculated using Student's t test.

Results

1) Effects of hallucinogens upon the levels of noradrenaline and dopamine in rat brain.

Figures (1) and (2) illustrate the effects of the four hallucinogens upon the brain levels of NA and DA. No significant changes in the levels of NA and DA in the hearts of these animals could be detected. (Heart levels of DA are very low and it is doubtful whether estimations in this range are of value. However, estimations were carried out for the sake of completeness). The heart levels were: NA : 1.94 ± 0.14 mµ moles/g and DA : 0.019 ± 0.008 mµ moles/g.

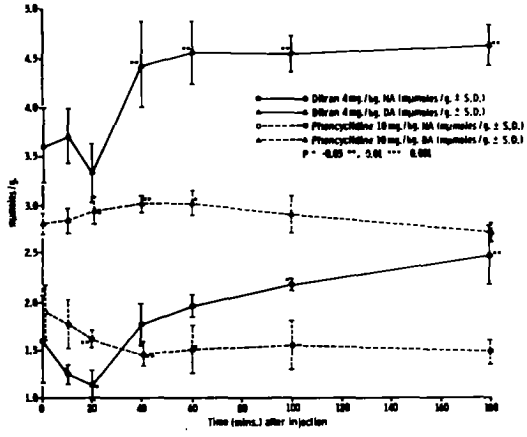

FIG. 1

Effect of ditran (upper graphs) and phencyclidine (lower graphs) on brain dopamine (Δ) and noradrenaline (.) Each point represents the mean ± standard deviation: 5 rats per group. The significance of the difference between the control and experimental group is given by: *P <0.05: ** P <0.01: *** P <0.001.

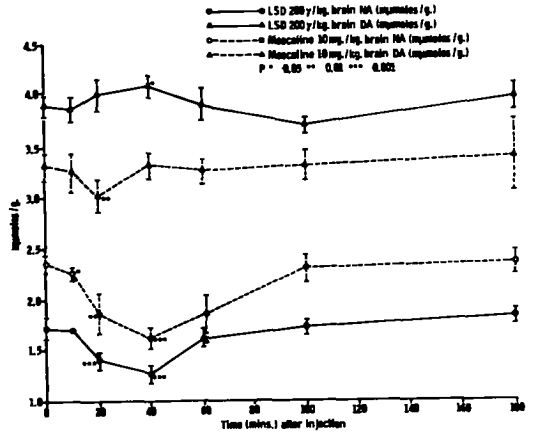

FIG. 2

Effect of mescaline (upper graphs) and LSD (lower graphs) on brain dopamine (Λ) and noradrenaline (.). All other details as in Fig. 1.

2) Effect of phencyclidine upon depletion of NA by reserpine.

The results of this experiment are summarised in Table 1

TABLE 1

Treatment	Interval between injection and sacrifice	NA m μmoles/g ± S.D.	Significance of difference (p)
Reserpine 2 mg/kg	120 mins	1.42 ± 0.30 (5)	
Reserpine 2 mg/kg + Phencyclidine 20 mg/kg	120 mins	0.71 ± 0.15 (5)	<0.0025
Reserpine 2 mg/kg	240 mins	0.58 ± 0.09 (5)	
Reserpine 2 mg/kg + Phencyclidine 20 mg/kg	240 mins	0.39 ± 0.06 (5)	<0.025

Level of NA in brains of animals injected with saline: 1.92 ± 0.28 m μmoles/g (5). Figures in parentheses indicate number of animals in group.

These results show that phencyclidine accelerates the depletion of NA caused by reserpine.

Further experiments were carried out using MPT, which causes depletion of NA by inhibiting the rate-limiting enzyme tyrosine hydroxylase (10), or MMT, the metabolite of which causes selective depletion of NA by a replacement of the amine (11).

3) Effects of hallucinogens upon the depletion of NA and DA of the brain by MPT.

It was apparent from the results of these experiments that none of the hallucinogenic drugs influenced the depletion of either NA or DA produced by alpha-methyl-p-tyrosine. NA levels were reduced by 40% and DA levels by

64% 2 hrs. after an injection of 300 mg/kg MPT. Saline-injected animals NA: 2.56 ± 0.10 mμmoles/g and DA 4.17 ± 0.27 m μmoles/g.

4) Effects of hallucinogenic drugs upon the depletion of brain NA and DA by MMT.

The results are summarised in Table 2.

TABLE 2

Treatment	Interval between injection and sacrifice	Catecholamines (m μmoles/g + S.D.)	Significance of difference (P)
MMT 300 mg/kg	120 mins	NA: 1.62 ± 0.08 DA: 1.28 ± 0.09 (5)	
MMT 300 mg/kg + phencyclidine 10 mg/kg	120 mins	NA: 1.36 ± 0.19 DA: 1.11 ± 0.06 (5)	0.025 n.s.
MMT 300 mg/kg + Ditran 10 mg/kg	120 mins	NA: 1.40 ± 0.23 DA: 1.11 ± 0.06 (5)	0.05 n.s.
MMT 300 mg/kg + LSD-25 200 μg/kg	120 mins	NA: 1.325 ± 0.14 DA: 1.01 ± 0.12 (5)	0.0025 0.005
MMT 300 mg/kg + mescaline 20 mg/kg	120 mins	NA: 1.35 ± 0.13 DA: 1.07 ± 0.12 (5)	0.0025 0.025
MMT 300 mg/kg	180 mins	NA: 0.864 ± 0.14 DA: 1.2 ± 0.07 (5)	
MMT 300 mg/kg + phencyclidine	180 mins	NA: 0.53 ± 0.27 DA: 0.72 ± 0.03 (5)	0.0025 0.0005
MMT 300 mg/kg + Ditran 10 mg/kg	180 mins	NA: 0.41 ± 0.14 DA: 0.72 ± 0.03 (5)	0.0025 0.0005

Saline-injected animals : NA : 2.72 ± 0.29 m μmoles/g and DA : 2.55 ± 0.14 m μmoles/g (5).

All four hallucinogenic drugs increased the depletion of brain catecholamines produced by alph-methyl-m-tyrosine.

5) Effects of hallucinogenic drugs upon normetanephrine levels in rat brain

The results of this experiment are summarised in Table 3.

TABLE 3

Treatment	NM (m μmoles/g + S.D.)	Significance of difference from saline-injected animals (P)
Saline 0.4 ml.	0.25 ± 0.08 (5)	
Phencyclidine 20 mg/kg	0.242 ± 0.038 (5)	n.s.
Ditran 8 mg/kg	0.182 ± 0.013 (5)	0.05
LSD-25 400 μg/kg	0.213 ± 0.018 (5)	n.s.
Mescaline 20 mg/kg	0.173 ± 0.044 (5)	0.05

From these results it is apparent that none of the hallucinogenic drugs increases the level of NM in rat brain.

DISCUSSION

Both 5-HT and NA have been implicated in the process of hallucinogenesis (12). The suggestive evidence for this theory has been referred to in the introduction to this paper and in earlier work (1). In the series of experiments described here, an attempt has been made to establish whether hallucinogens from different chemical classes all affect the catecholamines of brain in the same way. If a pattern is demonstrable, it is reasonable

to suggest that actions on catecholamines may be relevant to hallucinogenesis.

Each of the four drugs used in this study produced a decrease in the level of NA in rat brain. Changes in brain DA levels were less consistent, but a slight rise usually accompanied the fall in NA. A fall in brain levels of NA could result from a direct stimulation of monoamine oxidase (MAO) activity. Such an action is unlikely as 5-HT levels in the brain are elevated simultaneously with the fall in NA and no stimulation of MAO activity could be detected in homogenates of the brains from animals treated with the same four hallucinogens (1). Inhibition of aromatic amino acid decarboxylase (AAAD) activity might account for the decreased NA levels, but such an action is also unlikely (a) because this is not the rate-limiting step in the synthesis of NA (13)

(b) because DA levels are either unaffected or slightly elevated during the period of drug action;

(c) no stimulation of AAAD activity could be demonstrated in earlier experiments (1).

It has been suggested that the turnover rate of NA is accelerated by LSD-25 (6), and the experiments summarised in sections 1-4 were designed to determine how such an acceleration might occur.

All four hallucinogens increased the depletion of NA and DA produced by MMT. Surprisingly, no significant effect could be detected upon the degree of depletion of the amines following the administration of MPT. This does not agree with the findings of Anden et al.(6). No reason for this discrepancy can be offered at present.

MPT causes depletion of brain NA and DA by blocking their synthesis at the rate-limiting step of tyrosine hydroxylation (11). It would therefore be expected that a drug which increased the depletion of the catecholamines

would act by causing the release of the NA and DA already formed.

MMT produces depletion by the replacement of NA and DA in the neuronal storage granules with metaraminol (14), the metabolite of MMT. An augmentation of this effect could be produced by

(a) synthesis blockade;

(b) a selective block in the re-uptake process for NA, thus reducing the competition between NA and metaraminol for the storage sites;

(c) an increased catabolism of NA either by MAO or catechol-o-methyl transferase (COMT), since metaraminol is not metabolised by these enzymes and (as in (b)) competition for the binding sites would be reduced.

No action of hallucinogenic drugs upon any of the enzymes involved in the synthesis of NA has been demonstrated. There may be some inhibition at the dopamine-beta-oxidase step, reflected by the slightly elevated DA levels, but this could not account for the relatively large depletion of NA.

A selective block in the uptake of NA into either the neurone or the storage granule is unlikely since both NA and metaraminol are taken up by the same transport mechanisms (14).

No increase in the levels of NM could be demonstrated following the administration of hallucinogenic drugs; indeed, slight but significant decreases were observed with Ditran and mescaline. These results suggest that the depletion of NA is not a consequence of an increased release of the amine from the neurone, since an increased release would elevate NM levels, as would a decreased re-uptake into the neurone (15).

The remaining possibility is that the depletion observed results from an increased catabolism of NA within the neurone by MAO. This need not necessarily reflect a direct stimulation of MAO activity, but may indicate an increased release of NA from the bound form without a corresponding increase in the release of the amine from the neurone.

If this is so, it may be suggested that a part of the mechanism of action of hallucinogenic drugs is to increase the release of NA from the bound to the free form, whilst at the same time inhibiting its release from the neurone. The net result of this action and of the action on 5-HT previously reported (1) would be that normal stimulation of both adrenergic and tryptaminergic receptors in the brain would be disturbed by hallucinogenic drugs.

SUMMARY

The actions of phencyclidine, Ditran, LSD-25 and mescaline upon the catecholamines of rat brain have been investigated. Decreased levels of NA, accompanied by slightly elevated DA levels, were observed with all four drugs. Phencyclidine increased the degree of depletion of NA produced by reserpine. All four drugs increase the depletion of NA and DA by MMT, but had no effect upon the depletion produced by MPT. NM levels either were unaffected (phencyclidine and LSD-25) or were slightly decreased (Ditran and mescaline), indicating that the fall in NA is not a consequence of an increased release from neurones. It is suggested that part of the mechanism of action of hallucinogenic drugs may be to inhibit the release of NA from the neurone, hence allowing its catabolism by MAO.

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