Life Sci. 8, 805-814 (1969). LSD 1960

Life Sciences Vol. 8, Part 1, pp. 805-814, 1969. Pergamon Press
Printed in Great Britain

THE EFFECTS OF SOME HALLUCINOGENIC DRUGS UPON THE METABOLISM OF 5-HYDROXY-TRYPTAMINE IN THE BRAIN

Sally R. Tonge and B. E. Leonard*

Department of Pharmacy, University of Nottingham,

University Park, Nottingham.

(Received 14 April 1969; in final form 5 May 1969)

The discovery that lysergic acid diethylamide (LSD-25) is a potent inhibitor of the actions of 5-hydroxytryptamine (5-HT) at peripheral sites suggested the possibility that the drug may owe its hallucinogenic activity to an interaction with this amine in the central nervous system (1,2,3). A rise in the level of 5-HT in rat brain following the administration of LSD-25 has been reported (4). More recently, it has been found that both the metabolism (5) and the release (6) of 5-HT are affected by LSD-25.

The purpose of the experiments described in this paper was to establish whether other hallucinogenic compounds have similar effects upon 5-HT metabolism in brain and to what extent such effects are related to the pharmacological actions of these compounds. The mechanisms by which these actions might occur are also discussed.

The drugs chosen for this investigation are representatives of four structurally dissimilar chemical groups, related only by the ability to induce hallucinations: LSD-25, Mescaline, Phencyclidine ("Sernyl", Parke Davies and Co.) and Ditran (Lakeside Laboratories). The latter two are of particular interest; they have previously been studied largely with reference to their effects upon acetylcholine metabolism as they are

*Present address: I.C.I. Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

structurally related to the atropine-like compounds.

Methods

All drugs were administered to rats of the Wistar strain (inbred over several generations) by the intraperitoneal route.

In the absence of a specific test for hallucinogenic activity in rats, gross behaviour pattern was used as an approximate guide to the course of action of the drugs. Animals were killed by decapitation at intervals after the administration of each drug to establish whether any relationship between neurochemical changes and the behaviour could be observed.

5-HT was estimated in whole brain, blood, lung and gut of each animal either by the method of Snyder et al (7), or by the method of Bogdanski et al (8) when estimations were carried out in the presence of p-chlorophenylalanine.

5-hydroxyindolyl-acetic acid (5-HIAA) was estimated by the method of Giacolone and Valzelli (9) with the following modifications.
Whole rat brain was homogenised in N HCl containing 2% ascorbic acid. The homogenate was centrifuged at 3,000g until clear and the supernatant filtered through glass wool when necessary. The supernatant was saturated with NaCl and shaken with three 2.5ml volumes of n-butyl acetate. The pooled n-butyl acetate was shaken with 2ml of 0.1N HCl containing 2% ascorbic acid. After thorough centrifugation, the aqueous layer was removed as completely as possible and the organic phase was shaken with 1.2ml of phosphate buffer, pH 7.0, for 10 mins. The organic layer was then removed and a 1ml aliquot of the buffer was used for the determination of 5-HIAA. 0.5ml 9N HCl was added to the 1ml aliquot and the fluorescence at 540-550 mμ resulting from activation at 295 mμ (uncorrected wavelengths) was read. The method as published by Giacolone and Valzelli (9) gave poor recoveries of 5-HIAA in our hands, apparently due to a failure to extract the acid from the

homogenate. Where estimations of both 5-HT and 5-HIAA were carried out on the same homogenate, it was necessary to pool the brains from two animals.

Monoamine oxidase and aromatic amino acid decarboxylase activities were measured by the method of Bennett and Giarman (10), except that tranylcypromine (0.2ml of a 250 microgram/ml solution in a final volume of 3ml) was used as a monoamine oxidase inhibitor in place of the choline p-tolyl ether recommended.

A dose of each drug which consistently produced pronounced behavioural effects in the animals was administered (phencyclidine 10mg/kg, Ditran 4mg/kg, mescaline 10mg/kg and LSD-25 200µg/kg), and animals were killed both during the period of maximal drug effect and during apparent recovery.

Reserpine (2mg/Kg, intraperitoneal injection) was administered to four groups of rats. Animals in two of the groups received phencyclidine (20mg/Kg, i.p.) simultaneously with the reserpine. The dose of phencyclidine injected was chosen to ensure that its effects would persist for a time sufficient to allow a significant depletion of 5-HT by the reserpine. The animals were killed by decapitation at the times shown in Table 1.

Five groups of rats were injected intraperitoneally with 400mg/Kg of p-chlorophenylalanine, a specific depletor of 5-HT (11). Animals in four of the groups also received injections of hallucinogenic drugs. Since no significant depletion of 5-HT occurs until several hours after an injection of p-chlorophenylalanine, it was necessary to give more than one injection of each hallucinogen in order to extend the duration of action. All animals were killed nine hours after the injection of p-chlorophenylalanine. The hallucinogens were administered at 0, 3 and 6 hours after p-chlorophenylalanine.

Statistical significance was calculated using the Student's *t* Test.

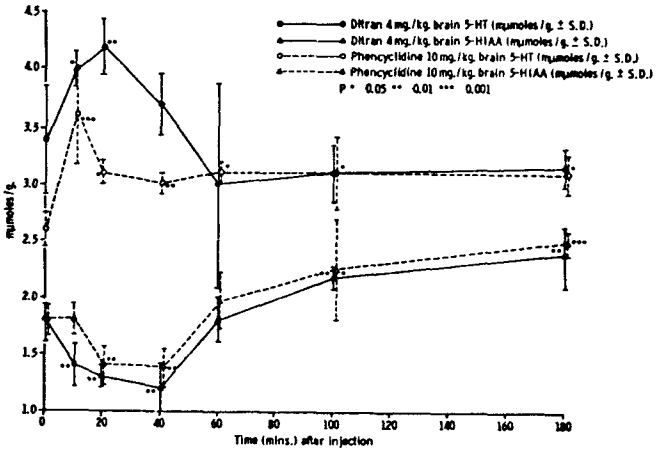

FIG. 1

Effect of ditran (upper graphs) and phencyclidine (lower graphs) on brain 5-HT (■) and 5-HIAA (▲). Each point represents the mean ± deviation: 5 rats per group. The significance of the difference between the control and experimental groups is given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

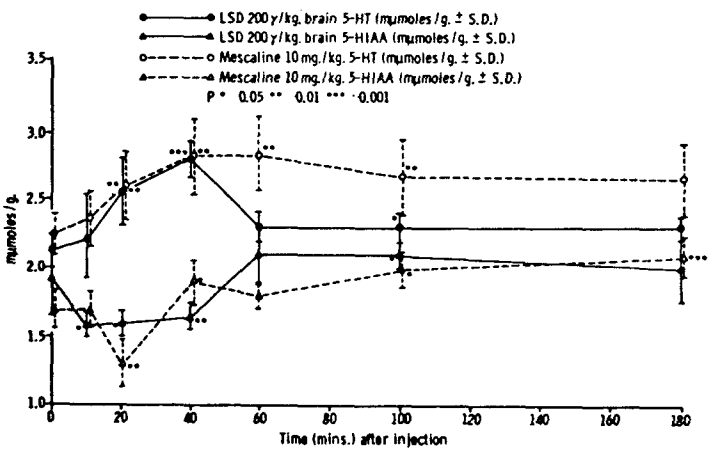

FIG. 2

The effect of Mescaline (upper graph) and LSD-25 (lower graph) on brain 5-HT (●) and 5-HIAA (▲). Details as given for Fig. 1.

Linear regression lines were drawn relating changes in 5-HT to changes in 5-HIAA both during the phase of drug action in which 5-HT rises and 5-HIAA falls and during later phases where the substances rise almost in parallel. Correlation coefficients were calculated for both phases.

Results

1) Effects of hallucinogens upon brain levels of 5-HT and 5-HIAA.

Figures 1 and 2 illustrate the effects of the four hallucinogenic drugs upon brain levels of 5-HT and 5-HIAA. No significant changes in the levels of 5-HT could be detected in other tissues from the same animals (blood: 0.98 ± 0.22 mμ moles/g, lung: 4.58 ± 0.26 mμ moles/g, gut: 8.94 ± 0.37 mμ moles/g).

In all cases, the maximal rise in 5-HT corresponded well to the peak behavioural effects. Excitement, with hyperactivity and stereotyped behaviour was observed in varying degrees with all four drugs. The animals showed marked fear reactions, except in the case of phencyclidine where backward circling movements and ataxia were the predominant symptoms.

2) Effects of hallucinogens upon monoamine oxidase and aromatic amino acid decarboxylase activity.

An increase in brain levels of 5-HT could be caused either by stimulation of aromatic amino acid decarboxylase activity or by inhibition of monoamine oxidase activity. Inhibition of monoamine oxidase might also explain the decreased levels of 5-HIAA found in animals treated with hallucinogens. However, neither phencyclidine nor Ditran produced any alterations in the activities of these two enzymes in rats killed at intervals after the administration of the drugs. (Aromatic amino acid oxidase activity expressed as μmoles 5-HT formed/g. brain/hr. $\pm$ S.D. = 3.3 ± 0.67; monoamine oxidase activity expressed as μmoles 5-HT destroyed/g. brain/hr. = 5.6 ± 0.52). Since neither enzyme was affected, it seemed likely that the elevation of 5-HT

resulted from an alteration in the release of the amine. The effect of phencyclidine upon the depletion of 5-HT by reserpine was therefore investigated.

3) Effect of phencyclidine upon depletion of 5-HT by reserpine.

The results of the experiment are summarised in Table 1.

Table 1.

Treatment	Interval between injection and sacrifice	5-HT (mμ moles/g brain)	Significance of difference (P)
Reserpine 2mg/Kg	120 mins.	1.54 ± 0.31 (5)	
Reserpine 2mg/Kg + Phencyclidine 20mg/Kg	120 mins.	1.91 ± 0.27 (5)	< 0.05
Reserpine 2mg/Kg	240 mins.	0.56 ± 0.09 (5)	
Reserpine 2mg/Kg + Phencyclidine 20mg/Kg	240 mins.	0.93 ± 0.09 (5)	< 0.05

The level of 5-HT in the brain of rats injected with saline only was 2.57 ± 0.17 mμ moles/g. (5) (figures in parentheses indicate number of animals in the group).

Since mechanisms by which reserpine causes depletion of 5-HT is the subject of some controversy, further experiments on 5-HT depletion were carried out

using p-chlorophenylalanine. This substance specifically depletes 5-HT by inhibiting the enzyme tryptophan hydroxylase (11).

4) Effects of hallucinogens upon the depletion of 5-HT by p-chlorophenylalanine (PCPA).

The results of this experiment are summarised in Table 2.

Table 2.

Treatment	5-HT (mμ moles/g brain ± S.D.)	Significance of the difference (P)
PCPA 400mg/Kg	0.94 ± 0.19 (5)	
PCPA 400mg/Kg + Phencyclidine 10mg/Kg	1.47 ± 0.2 (5)	<0.0005
PCPA 400mg/Kg + Ditran 4mg/Kg	1.22 ± 0.22 (5)	<0.005
PCPA 400mg/Kg + LSD 200μg/Kg	1.27 ± 0.08 (5)	<0.0005
PCPA 400mg/Kg + Mescaline 20mg/Kg	1.2 ± 0.07 (5)	<0.0025

The levels of 5-HT in the brains of saline-injected animals was 1.634 ± 0.17 mμ moles/g. brain.

Discussion

The experiments described above suggest that hallucinogenic drugs may owe their central effects, at least in part, to an ability to modify the normal functions of 5-HT in the brain. It is generally believed that 5-HT has a role in chemical transmission in the central nervous system, and the

occurrence of the amine in relatively large amounts in those parts of the brain known to be concerned with emotional and behavioural response provides suggestive evidence of a link between the perceptual confusion produced by hallucinogenic drugs and an altered 5-HT metabolism.

5-HT is believed to exist in at least two pools in the brain, one "bound" and the other "free"(12). The decrease in 5-HIAA levels accompanying the increase in 5-HT levels in the brains of rats treated with hallucinogenic drugs suggests that a failure to release 5-HT from the "bound" form, thus preventing its metabolism by monoamine oxidase, might account for the observed elevation. However, a linear regression line plotted for levels of 5-HT and 5-HIAA for all four hallucinogens during the early phases of drug action shows that only about 4% of the increase in 5-HT can be accounted for by a decreased oxidative metabolism of the amine ($R = -0.177$, $P = 0.05$). During later phases of drug action, i.e. when changes in 5-HT and 5-HIAA levels were almost parallel, the correlation coefficient relating the two substances is significantly higher ($R = 0.5$, $P = 0.001$). This suggests that the normal relationship between 5-HT and 5-HIAA is disturbed during the early phase of drug action and begins to return to normal as recovery from the effects of the drug proceeds.

All four drugs very significantly decreased the rate of depletion of 5-HT following the administration of p-chlorophenylalanine. Rats treated with hallucinogens showed only 10% (phencyclidine), 25% (Ditran), 23% (LSD-25) and 26% (mescaline) depletion as compared with a 43% depletion when p-chlorophenylalanine alone was given. Preliminary experiments suggest that both phencyclidine and Ditran increase the particulate : supernatant 5-HT ratio in a manner comparable to that observed with LSD-25 (4, 5). These findings support the theory that the turnover rate of 5-HT is decreased during hallucinogenic drug action and may account for the observed rise in 5-HT. It has been suggested that LSD-25 may act directly upon 5-HT receptors

in the central nervous system, decreasing the turnover rate by a feed-back mechanism (6). The theory of a direct action upon 5-HT receptors is supported by the observation that phencyclidine, Ditran, LSD-25 and mescaline all produce the full range of behavioural effects in rats even after depletion of 5-HT with p-chlorophenylalanine.

Studies in which more than one hallucinogenic drug is used have the advantage of distinguishing between those effects which are common to all, and may therefore have some bearing upon the mode of action, and those which may be side effects associated with a particular chemical structure. The four drugs chosen for this study have widely different structures and may well act in different ways to produce the same net neurochemical changes. All apparently influence the release of 5-HT, and investigations are now in progress to determine whether this action in itself may account for hallucinogenesis or is a reflection of a more basic effect.

Summary

The effects of the four hallucinogenic drugs (phencyclidine, Ditran, LSD-25 and mescaline) upon the metabolism of 5-HT in the brain were investigated. All four drugs caused increases in the levels of 5-HT, accompanied in the early stages by decreases in the levels of 5-HIAA, suggesting that the relationship between the two substances which exists in untreated animals had been altered. No effect upon either monoamine oxidase or aromatic amino acid decarboxylase activity could be detected. The depletion of 5-HT which follows the administration of either reserpine or p-chlorophenylalanine was significantly reduced by all four drugs. These results support the theory that LSD may owe at least part of its hallucinogenic activity to an ability to alter the capacity of the tissues to bind 5-HT. Further, the demonstration that four hallucinogens of widely different structure all affect the binding of 5-HT strongly supports the implication of this action in hallucinogenesis.

Acknowledgement

The authors wish to thank Dr. J. Crossland for his interest in this project. Lakeside Laboratories and Parke Davis & Co. Ltd. are gratefully acknowledged for the gifts of Ditran and phencyclidine respectively.

References

1. Gaddum, J.H. J. Physiol., 121, 15P (1953).
2. Amin, A.H., Crawford, T.B.B. and Gaddum, J.H. J. Physiol., 126, 596-618 (1954).
3. Woolley, D.W. and Shaw, E. Proc. Nat. Acad. Sci., 40, 228-231 (1954).
4. Freedman, D.X. and Giarman, N.J. Ann. N.Y. Acad. Sci., 96, 98-106 (1962).
5. Rosecrans, J.A., Lovell, R.A. and Freedman, D.X. Biochem. Pharmacol. 16, 2011-2021 (1967).
6. Andén, N.-E., Corrodi, H., Fuxe, K. and Hökfelt, T. Brit. J. Pharmacol. 34, 1-7 (1968).
7. Snyder, S.H., Axelrod, J. and Zweig, M. Biochem. Pharmacol. 14, 831-835 (1965).
8. Bogdanski, D.F., Pletscher, A., Brodie, B.B. and Udenfriend, S. J. Pharmacol. Exp. Ther., 117, 82-88 (1956).
9. Giacolone, E. and Valzelli, L. J.Neurochem., 13, 1265-1266 (1966).
10. Bennett, D.S. and Giarman, N.J. J. Neurochem. 12, 911-918 (1965).
11. Koe, B.K. and Weissman, A. J. Pharmacol. Exp. Ther., 154, 499-516 (1966).
12. Bloom, F.E. and Giarman, N.J. Ann. Rev. Pharmacol. 229-258 (1968).