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EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON THE METABOLISM OF BRAIN 5-HYDROXYTRYPTAMINE*

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Abstract—The effects of *d*-lysergic acid diethylamide (LSD), in doses ranging from 200 to 1300 $\mu\text{g/kg}$, on the time course of the changes in brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) have been studied in male Sprague-Dawley rats. The results show that LSD influences 5-HT metabolism in brain in a complex way. After i.v. administration of 200 $\mu\text{g/kg}$ LSD, brain 5-HT increases to a maximum of 15–20 per cent at 25 min, while 5-HIAA decreases 15–20 per cent at 20 min. After i.p. administration, which prolongs the total period during which LSD is found in the brain, the time course is spread out and is triphasic: (1) in the first 30 min after 520 or 1300 $\mu\text{g/kg}$, brain 5-HT increases 15–20 per cent and brain 5-HIAA decreases 15–20 per cent; (2) from 30 to 60 min, 5-HT still increases, while the 5-HIAA level is returning to normal; (3) after 60 min, both 5-HT and 5-HIAA levels decrease at a parallel rate.

Both differential and density gradient centrifugation show that 5-HIAA is not present in any particulate fraction, but 5-HT is localized in both the Whittaker B (nerve-ending particle) and the microsomal fractions. Thirty min after 520 $\mu\text{g/kg}$ LSD i.p., the 5-HT content in both fractions is increased 25–30 per cent.

These results are consistent with increased binding of 5-HT or an effect of LSD on control mechanisms that regulate 5-HT turnover in the rat brain, or both.

FREEDMAN¹ initially observed that i.p. doses above 130 $\mu\text{g/kg}$ of lysergic acid diethylamide (LSD) induced a small but consistent and significant increase in brain 5-HT levels of male Sprague-Dawley rats, an increase which was clearly evident after reserpine pretreatment. It was further demonstrated by Freedman and Giarman² and by Schanberg and Giarman³ that this increase in 5-HT occurred mainly in the particulate fraction of homogenates subjected to ultracentrifugation. Siva Sankar *et al.* found that, after pretreatment of rabbits with ¹⁴C-5-hydroxytryptophan, LSD caused an increase of labeled 5-HT in brain (in all areas except the cerebrum),⁴ and that LSD decreased the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) in the albino rat.⁵ Such findings seemed to indicate an effect of LSD on 5-HT metabolism. However, with the techniques then available, no significant effect *in vitro* of LSD on the synthesis, binding or metabolism of 5-HT was evident.¹ It was tentatively concluded that LSD induced the increase in brain 5-HT by increasing the capacity of

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tissues *in vivo* to bind 5-HT. Subsequent work has shown that LSD at 5 min after administration to rats is concentrated in the particulate fraction and that after the drug leaves this fraction (the half-life is calculated at 20 min), 5-HT levels begin to rise; that is, at the time of maximal increase in 5-HT, the LSD concentration in the brain is at or approaching its lowest level.^{6, 7} Such findings demonstrate at the least that there is some interaction of LSD and brain 5-HT, although the nature or mechanism remains obscure. In order to further clarify this interaction, the time course of the changes in brain 5-HT and 5-HIAA was studied.

METHODS

Design of experiments. Male Sprague-Dawley rats (150–200 g) from Charles River Breeding Farms were used. Experimental rats received LSD (LSD-25, Sandoz) either i.p. or i.v. and were sacrificed by decapitation at various time intervals after administration. After decapitation, brains were immediately removed and homogenized in either 4 vol. of 0.1 N HCl (5-HT and 5-HIAA experiments) or 9 vol. of 0.3 M sucrose (centrifugation studies). Control animals received saline. All data were compared with control information obtained during the same experiment; each individual experiment was designed so that a minimum of 25 per cent of the animals were controls. No more than 16 animals were studied in any single experiment. When LSD was given i.p., results were more consistent if the animals were fasted for 16 hr prior to drug administration. Student's *t*-test was used for determining statistical significance.

5-HT and 5-HIAA assays. Both 5-HT and 5-HIAA were estimated in the same brain samples after protein precipitation. This procedure involved a combination of the methods for 5-HT⁸ and for 5-HIAA.⁹ Brain homogenates (4 vol. of 0.1 N HCl) were deproteinized in one of two ways. In experiments involving whole brain assays, zinc sulfate (1 ml of 20% solution/900 mg brain) and sodium hydroxide (0.5 ml of 1 N NaOH/900 mg brain) were added separately, followed by 10 sec of vigorous shaking by a Vortex mixer. This procedure resulted in a total brain weight of 150 mg/ml of supernatant fraction, after centrifugation at 15,000 g for 10 min at 0–4°. In experiments involving differential centrifugation and density gradients, proteins were precipitated with concentrated perchloric acid (0.6 ml/10 ml), after an initial addition of HCl to a concentration of 0.1 N.

Deproteinized brain samples were then adjusted to pH 1.0–1.5 with either 5 N NaOH or 6 N HCl, and 5-HIAA was determined fluorometrically by the method of Udenfriend *et al.*⁹ in the Aminco-Bowman spectrophotofluorometer. After the initial extraction of 5-HIAA with diethyl ether, 5-HT was determined by the method of Bogdanski *et al.*⁸

In this procedure, which combined both methods for 5-HT and for 5-HIAA, no loss of 5-HT was experienced and both 5-HIAA and 5-HT were selectively extracted; this was true regardless of the sequence of the ether and the butanol extractions. Both compounds were identified by their individual fluorescent spectral properties. Ascorbic acid (100–200 µg) had to be added to all aqueous phases throughout the procedure to insure maximal 5-HT or 5-HIAA recovery. The per cent recovery of 100–200 µg added to brain tissue was 85.2 ± 8.6 and 74.1 ± 10.8 ($\pm$ S.D., $N = 8$) for 5-HT and 5-HIAA respectively. The sensitivity of this assay was 25–50 µg for 5-HIAA and 10–25 µg for 5-HT when the proper standards were used; 4–6 standards, ranging

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between 50 and 600 μg , were passed through the procedures to give standard curves for each assay.

Protein methods. The method routinely used was a modified biuret procedure. An aliquot of brain homogenate or suspension of brain particulate (1–4 ml, depending upon the amount of protein present) was mixed with 3 vol. of 1 N NaOH and 6 vol. of diethyl ether were added. This mixture was shaken 30 min to extract the lipid material. After separation of the ether layer, 2 ml of the aqueous layer was thoroughly mixed with 3 ml of biuret reagent, and the usual biuret procedure was then followed; bovine serum albumin was used to prepare the standard curve. Standards were analyzed each time the assay was performed. When the protein content of subcellular fractions was low, the method of Lowry *et al.*¹⁰ was used.

Particulate-supernatant distribution. Separation of particulate and supernatant fractions was achieved by the method of Gillis *et al.*,¹¹ except that the centrifugation was carried out in the International B-60 centrifuge (A-321 rotor, 130,000 g). Concentrated perchloric acid was added to the resulting supernatant fraction, to a final concentration of 0.4 N. The precipitated protein was centrifuged at 30,000 g, and the resulting supernatant fraction was reserved for 5-HT and 5-HIAA assay. The particulate protein was precipitated and centrifuged as described above, and the resulting supernatant was reserved for 5-HT and 5-HIAA assay.

Subcellular distribution of 5-HT and 5-HIAA. Rats were decapitated and the brain immediately removed, weighed, and homogenized according to Gillis *et al.*¹¹ All the following operations were carried out at 0–4°. An aliquot of the homogenate was removed for determination of the total brain 5-HT and 5-HIAA. The remainder of the homogenate was centrifuged at 1000 g for 10 min. The residue (nuclear fraction) was washed twice and supernatant and washings were combined and centrifuged at 12,000 g for 20 min. The resulting crude mitochondrial fraction was resuspended in 10 ml of the original sucrose medium and either reserved for density gradient subfractionation or processed as such for protein or 5-HT and 5-HIAA assay. The supernatant from the crude mitochondrial fraction was centrifuged at 100,000 g for 60 min to give the so-called "microsomal" fraction and the true supernatant. The volume of the latter was measured and suitable aliquots were taken for the various assays. The microsomal particulate was resuspended and processed for assay.

For subfractionation of the crude mitochondrial fraction, various procedures with sucrose¹² and Ficoll¹³ gradients were tried in preliminary experiments. Eventually two discontinuous sucrose gradient techniques were used: the method of Glowinski *et al.*¹⁴ and that of Gray and Whittaker.¹⁵ The latter method was used in the bulk of the experiments reported here; this method results in separation into the myelin layer (A), the nerve-ending particle layer (B), and the true mitochondria (C).

LSD assay. The LSD concentration in the brain was measured spectrophotofluorometrically by the method of Axelrod *et al.*¹⁶

Electron microscopy. Samples of subcellular fractions to be subjected to electron microscopy were first centrifuged at 100,000 g to form a firm pellet. The pellet was fixed for 2 hr in phosphate-buffered 5% glutaraldehyde (pH 7.4) and allowed to stand for 1 hr with 1% osmium tetroxide. The pellet was then dehydrated in graded ethanols and embedded in Maraglas-DER 732. Sections were then cut in a MT-2 Servall microtome, double-stained with uranyl acetate and lead acetate, and examined in the RCA 3-F electron microscope.

RESULTS

Electron microscopic analysis

The electron micrographs of the various subcellular fractions demonstrated the following:*

Nuclear fraction. This fraction contained a wide variety of nervous elements including unbroken cells; perhaps it should be more realistically termed a "low-speed sediment fraction".

Microsomal fraction. This fraction contained many small vesicles, small nerve endings, small axons and dendritic processes, and various structures of the endoplasmic reticulum. This fraction basically represents small fragmental contaminants from other fractions, in addition to the true microsomes.

Sucrose density gradients. The separations here were good and were in complete accord with the results of Whittaker: Subfraction A. This contained only myelin fragments. Subfraction B. This contained about 80 per cent nerve-ending particles, with a few scattered mitochondria. The nerve-endings were in good condition, with minimal disruption. Subfraction C. Almost 100 per cent mitochondria, plus a small amount of contaminants from the B layer, were obtained.

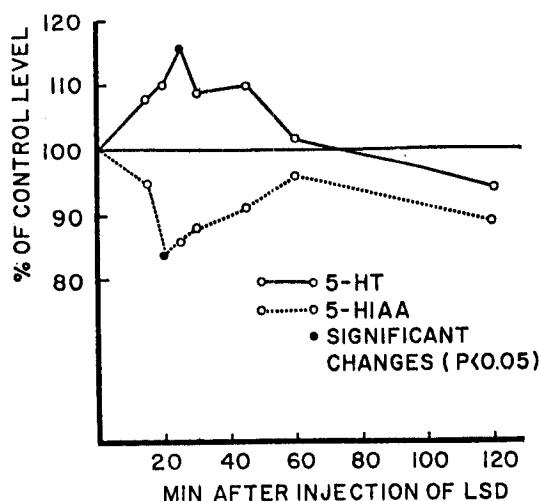


FIG. 1. Time course of the effects of an i.v. dose (200 µg/kg) of LSD on the metabolism of 5-HT in the brain. The changes in 5-HT and 5-HIAA levels are plotted as per cent of the control levels at each time. Control values are as follows: 5-HT, 537 ± 15 ; 5-HIAA, 454 ± 18 (mµg/g brain $\pm$ S.E., $N = 16$). Each point represents the mean of at least 6 animals.

Effects of intravenous LSD on brain 5-HT and 5-HIAA levels

These experiments confirmed that LSD induces a significant increase in brain 5-HT levels within 30 min after i.v. administration; in the same period, there is a decrease in brain 5-HIAA levels. These effects did not occur simultaneously, as Fig. 1 shows; the acid metabolite effect occurs just prior to the 5-HT effect.

* The electron micrographs were prepared and interpreted by Dr. F. E. Bloom and Dr. G. K. Aghajanian, Department of Anatomy, Yale University School of Medicine.

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Effects of route of administration and dose of LSD on brain 5-HT and 5-HIAA levels

Various differences between the i.v. and the i.p. routes of administration of LSD were noted: (1) a slightly different half-life of the drug in the brain; (2) a difference in the duration of the chemical effects; and (3) differences in the effects on behavior.

A comparison of the half-life of LSD in the brain after the two routes of administration is revealing. Freedman^{6, 7} has demonstrated previously that, after an i.v. dose (200 $\mu\text{g/kg}$) of LSD, maximal concentration of the drug in the brain is reached within 1–2 min, and that more than 50 per cent of the maximal concentration leaves the brain within 10–15 min. In the present experiments, after an i.p. dose of 520 $\mu\text{g/kg}$, maximal concentrations of LSD in the brain are not reached until 15 min (Fig. 2);

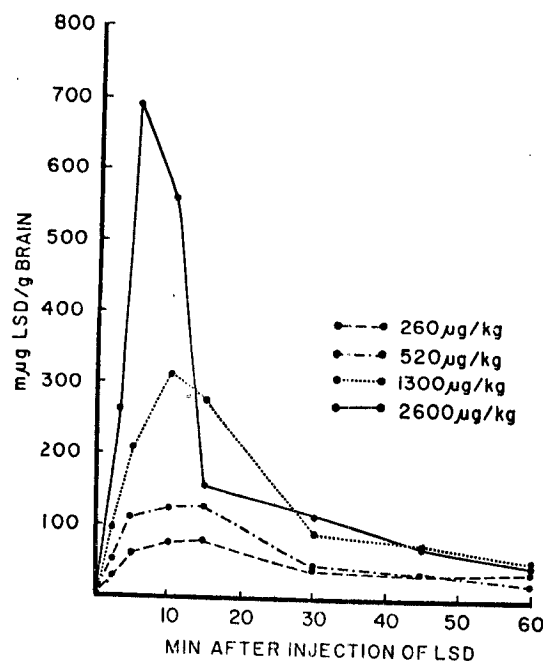


FIG. 2. Time course of the clearance of LSD from the brain after various i.p. doses. The LSD concentrations in the brain are expressed as $\mu\text{g/g}$ brain.

by 30 min, the drug is essentially cleared from the brain. Even after a dose as large as 2600 $\mu\text{g/kg}$ i.p., LSD is removed from the brain within 30 min.

The i.p. route, when compared with the i.v. route, appeared to spread out the changes in the 5-HT and 5-HIAA levels over a greater period of time. The duration of the biochemical events also appeared to correlate with the gross behavioral manifestations of the drug; as the behavioral effects are terminating, the biochemical changes tend to be maximal. Thus the onset of drug effects is indicated first by nose twitches with distended and trembling whiskers and generalized piloerection, especially around the head and neck; tachypnea quickly supervenes and splayed hind-quarters and periods of hypersensitivity to noise are then evident throughout the period of acute drug effect. With respect to these signs, 200 $\mu\text{g/kg}$ LSD administered i.v. was roughly equivalent to a dose of 520 $\mu\text{g/kg}$ given i.p. The onset of these effects

after i.v. LSD occurred sooner than after LSD administered i.p., but the duration of behavioral effects was shorter than after the i.p. dose. Without more controlled observations,¹⁷ it is obviously not reliable to attempt to quantify the behavioral effects that follow the two modes of administration, but differences in the onset of and recovery from behavioral effects are quite easily distinguished.

The i.p. route was chosen for this study of the changes in the brain after LSD. There is a definite dose-response relationship with respect to brain levels of 5-HT and 5-HIAA (Fig. 3). A dose of 260 $\mu\text{g}/\text{kg}$ i.p. appeared to be the threshold dose,

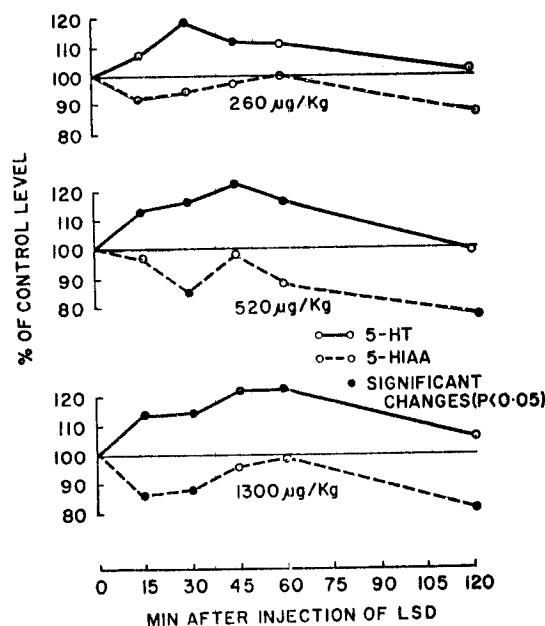


FIG. 3. Time course of the effect of various i.p. doses of LSD on the metabolism of brain 5-HT. The changes in 5-HT and 5-HIAA levels are plotted as per cent of the control levels at each time. The same number of animals was used in the control group as in the experimental group for each dose: 15 min, 6; 30 min, 12; 45 min, 6; 60 min, 4; and 120 min, 4.

which produced significant effects on 5-HT while no concurrent significant decrease in 5-HIAA was evident. A dose of 520 $\mu\text{g}/\text{kg}$, however, induced significant changes in both 5-HT and 5-HIAA, and these significant effects were observed concurrently. The highest dose of LSD studied was 1300 $\mu\text{g}/\text{kg}$. The effects of this dose were somewhat different and of a greater magnitude than those of the lower doses. A more sustained effect on 5-HIAA was observed, and the maximal increase in 5-HT developed over a longer period of time.

As is evident in Fig. 3, there are three phases in the time course of the changes following the higher i.p. doses of LSD. (1) There is an initial phase of 30 min in which the brain 5-HT level increases, with a concurrent quantitative decrease in the acid metabolite; this is especially evident after 1300 $\mu\text{g}/\text{kg}$, but is apparently nonexistent (or not detectable) with the lowest dose (260 $\mu\text{g}/\text{kg}$). (2) In the second phase, brain 5-HT continues to increase, while 5-HIAA is returning to its normal level; both amine and

metabolite in the initial phase of both amine and metabolite.

The effects

The purity and specificity of the results demonstrated that the sucrose homogenate was detectable (synaptosomes) and 5-HIAA is relatively washed out of synaptosomes by centrifugation. This amine was not there, even after centrifugation.¹⁸

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metabolite are changing at a parallel rate, in contrast to the divergent effects observed in the initial phase. (3) The last phase represents a return of 5-HT level to normal; both amine and metabolite levels are decreasing at a parallel rate.

The effects of LSD on the subcellular localization of 5-HT and 5-HIAA

The purpose of this series of experiments was to determine the subcellular location and specificity of the changes after LSD with respect to both 5-HT and 5-HIAA. The results demonstrated that 5-HIAA is present in the supernatant fraction of a brain sucrose homogenate but not in any particulate subcellular fraction. 5-HT, however, was detected in various subcellular particulates, especially in the nerve-ending particle (synaptosome) fraction of Whittaker. It was found, in separate experiments, that any 5-HIAA in a particulate fraction of brain homogenate in sucrose could be quantitatively washed out merely by resuspending the particulate several times in fresh portions of sucrose. Thus, neither in differential centrifugation nor in density gradient centrifugation was 5-HIAA found in any fraction except the supernatant fraction, provided the particulate fractions were washed. With 5-HT, however, 75 per cent of this amine initially present in particulate matter from a brain homogenate remained there, even after 5 washings, in agreement with the results of Giarman and Schanberg.¹⁸

The significant decrease in 5-HIAA 30 min after 520 µg/kg LSD was observed both in the supernatant fraction from a crude particulate-supernatant separation and in the supernatant fraction after differential centrifugation (Table 1). For 5-HT,

TABLE 1. EFFECTS OF LSD ON THE SUBCELLULAR LOCALIZATION OF BRAIN 5-HIAA*

Tissue preparation	5-HIAA levels†		Per cent of control
	Control animals	LSD-treated animals	
Whole brain homogenate	303 ± 40 (18)	258 ± 30 (12)‡	86
Supernatant§	231 ± 27 (4)	199 ± 10 (4)‡	86
Particulate§	78 ± 10 (4)	60 ± 8 (4)	79
Supernatant after differential centrifugation	532 ± 61 (8)	412 ± 70 (8)‡	79

* The animals were decapitated, and the brains removed and homogenized 30 min after 520 µg/kg LSD i.p.

† Each value represents the mean 5-HIAA level for the number of animals in parentheses ± S.E.

‡ P < 0.05.

§ The supernatant and particulate were prepared by centrifuging 1 g whole brain (homogenate) at 130,000 g for 30 min.

|| This supernatant was prepared by first removing the nuclear and mitochondrial fractions from the homogenate and then by centrifuging at 100,000 g for 60 min; the units here are mµg 5-HIAA/brain.

increases were observed in the crude particulate and in the particulate fractions after differential centrifugation (Table 2). Several points should be noted about the data in Table 2: (1) a 20-30 per cent increase in 5-HT was observed in the microsomal particulate and in the Whittaker B fraction (nerve-ending particle fraction); (2) there

is an increase in the ratio of bound 5-HT/free 5-HT in the subcellular particulates after LSD; (3) no specific particulate fraction was observed to be entirely responsible for these effects. With respect to statistical significance, the total increase in the whole brain after a dose of 520 $\mu\text{g/kg}$ LSD i.p. is never large (about 85 m μg), and given the

TABLE 2. EFFECTS OF LSD ON THE SUBCELLULAR LOCALIZATION OF BRAIN 5-HT*

Tissue preparation	Control animals		LSD-treated animals		Per cent of control
	(m μg 5-HT†/g brain or mg protein)	Bound 5-HT‡/free 5-HT	(m μg 5-HT†/g brain or mg protein)	Bound 5-HT‡/free 5-HT	
Whole brain	525 $\pm$ 46 (17)		610 $\pm$ 67 (12)§		116
Particulate (prepared as in Table 1)	312 $\pm$ 28 (6)	2.50	375 $\pm$ 34§ (4)	3.00	120
Supernatant	125 $\pm$ 12		125 $\pm$ 38		100
Microsomal fractions: Particulate	7.7 $\pm$ 2.9 (8)	1.84	9.9 $\pm$ 5.5 (8)	2.75	128
Supernatant	4.2 $\pm$ 1.7		3.6 $\pm$ 1.2		86
Mitochondrial fractions:	(5)		(7)		
A	2.5 $\pm$ 1.2	0.60	2.9 $\pm$ 1.7	0.81	110
B	6.0 $\pm$ 1.8	1.42	7.8 $\pm$ 1.5	2.50	130
C	1.3 $\pm$ 0.5	0.31	2.6 $\pm$ 1.8	0.73	200

* The animals were decapitated, and their brains removed for homogenization and assay 30 min after 520 $\mu\text{g/kg}$ LSD i.p.

† Each value represents the mean 5-HT level for the number of animals in parenthesis $\pm$ S.E.

‡ The bound/free ratio was determined by analysis of particulate and supernatant obtained in each procedure. In the case of the mitochondria fractions, the microsomal supernatant was used as an indication of the free portion of 5-HT.

§ $P < 0.05$.

|| $P < 0.2$ (see text).

normal variations, statistically significant differences in the 5-HT content in subcellular fractions would be difficult to demonstrate.

DISCUSSION

With evidence suggesting an effect of LSD on 5-HT metabolism *in vivo*, the question arises whether there is any real connection between the effects on binding and this effect on metabolism. The initial effect of LSD after it enters the brain is to alter somehow the dynamic relationship between 5-HT and its metabolite, an effect which results during the first 30 min in an increase in 5-HT level and a concurrent, almost quantitative, decrease in 5-HIAA; these changes reach a peak as certain excitatory behavioral effects are terminating.¹⁹ Over a period of 2 hr, subsequent shifts in amine and metabolite levels occur and, with the spread of effects over time observed with i.p. dosage, a triphasic effect of LSD is clearly apparent. These effects are of greater magnitude as the dose is increased from 260 to 1300 $\mu\text{g/kg}$.

Whatever the influence of route of administration and the rate of clearance of drug from brain, the initial disturbance of normal 5-HT metabolism might be explained by: (1) interference of LSD with the storage or release mechanisms, or both (assuming a dynamic equilibrium between stored or bound 5-HT and released or free 5-HT);

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(2) specific enhancement or facilitation of binding induced by LSD; (3) some direct or indirect mode of inhibition of monoamine oxidase (MAO) activity. Each could explain an increase in 5-HT accompanied by a decrease in 5-HIAA. The suggested effect of LSD on binding, however this is induced,²⁰ could involve an initial and reversible interference with the normal accessibility of enzyme (MAO) and substrate; temporary interference with MAO is a major factor to be tested in subsequent experiments. Of course, LSD produces a central excitation and release of norepinephrine²¹ and there are factors which could complicate this analysis of drug action.

After 30 min, 5-HT continues to increase, but the 5-HIAA level now appears to follow the 5-HT change. This second phase, which also lasts about 30 min, conceivably could result from a stimulation of the rate of 5-HT synthesis, with the newly synthesized 5-HT now being accessible to degradation by MAO.

Finally, in the third phase, the 5-HT level is returning to normal and both 5-HT and 5-HIAA are decreasing at a parallel rate. This latter fact suggests the possibility of some kind of mechanism to cut back the rate of 5-HT synthesis. This seems unlikely when one considers that much higher levels of 5-HT can be achieved with MAO inhibitors. Yet the situation is somewhat different from that with classic MAO inhibition, since MAO is apparently functionally operative, at least after the first 30 min. It has been suggested that intraneuronal MAO is at least indirectly involved in the control of catecholamine synthesis; elevated catecholamine levels after MAO inhibition are accompanied by a substantial reduction in amine synthesis, possibly by feedback inhibition of tyrosine hydroxylase.²²

Recent studies of 5-HT metabolism in the developing chick brain²³ and in rats after tryptophan loading²⁴⁻²⁶ have provided evidence to suggest that a feedback mechanism affecting the biosynthesis of 5-HT is operative at the tryptophan hydroxylase step in the 5-HT synthetic pathway. That the hydroxylation of tryptophan has a role in 5-HT metabolism in the brain has been well documented,²⁷⁻²⁹ and studies suggest that tryptophan 5-hydroxylase is primarily a mitochondrial enzyme located 'within' the nerve-ending particle,³⁰ a site which can thus contain mechanisms for the total synthesis and storage of 5-HT. Although a feedback mechanism involving tryptophan hydroxylase and operating at the level of subcellular storage sites could be one factor in the control of the relative amounts of free and bound 5-HT, it remains entirely speculative whether inhibition of MAO or direct stimulation of tryptophan hydroxylase by LSD are factors involved in the observed shifts of 5-HT metabolism.

With respect to subcellular localization of these effects, the data support the whole brain data in suggesting a facilitation of binding. The level of 5-HT does not increase in the supernatant fraction, whereas the 5-HIAA level decreases significantly. Thus, as 5-HT increases, a portion is bound in some subcellular compartment which may protect it from MAO. These experiments did not specifically localize the site of this binding, but most of the changes did occur in both the nerve-ending particle and microsomal fractions, which fractions contain most of the vesicles and neuronal membrane fragments from the whole brain. Recent studies³¹ support the conclusion that the presence of 5-HT in nerve-ending preparations, frequently described,³²⁻³⁶ and in microsomal fractions^{32, 36-39} is not an artifact. It was found that ³H-labeled 5-HT, injected into the lateral ventricle of the rat, is selectively distributed within the neuropil; intense peridendritic autoradiographic activity strongly suggested localization at nerve terminals.³¹ Whether the 5-HT is present in the microsomal fraction

because of contamination from smaller or fragmented nerve-ending particles^{38, 39} is still an open question. The electron micrographs of our microsomal fractions also show some such contamination, which may explain the 5-HT in this fraction. Localization and interactions of LSD with enzyme and substrate in these fractions might aid in elucidating the specific sequence of mechanisms underlying the observed changes in 5-HT metabolism.

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