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EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON THE UPTAKE AND RETENTION OF BRAIN 5-HYDROXYTRYPTAMINE *IN VIVO* AND *IN VITRO**

MICHAEL G. ZIEGLER, RICHARD A. LOVELL and DANIEL X. FREEDMAN

Department of Psychiatry, The University of Chicago, 950 East 59th Street, Chicago, Ill. 60637, U.S.A.

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Abstract—The effects of D-lysergic acid diethylamide (LSD) and other drugs on brain 5-hydroxytryptamine (5-HT) *in vivo* and *in vitro* and on ^{3}H-5-HT administered intraventricularly have been studied in male Sprague–Dawley rats. The results *in vivo* show that the retention of ^{3}H-5-HT in the brain is increased by treatment (i.p.) with LSD and *p*-chlorophenylalanine (PCPA) and decreased by reserpine. This LSD effect *in vivo* can be reversed by intraventricular ouabain (given together with ^{3}H-5-HT) in amounts which cause brief convulsions. *In vitro*, LSD added to the medium initially (0–10 min) decreased uptake of ^{3}H-5-HT in brain slices but enhanced its retention at 60 min. These effects of LSD were not observed either after pargyline treatment (i.p.) or in the presence of pargyline *in vitro*. These results are consistent with the idea that LSD decreases 5-HT turnover through an inhibition of neuronal firing, which can be overcome by neuronal depolarization sufficient to cause convulsions. It is suggested that the decreased 5-HT turnover seen after LSD is somehow mediated by a mechanism which protects 5-HT from deamination by monoamine oxidase, and that an effect on 5-HT uptake may be a mechanism involved in the interaction of LSD with brain 5-HT.

IN A CONTINUING investigation of the effects of psychotomimetic or hallucinogenic drugs on brain amine metabolism, we have described characteristic dose- and time-dependent effects of D-lysergic acid diethylamide (LSD) on brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA): an increase in 5-HT (largely confined to particulate fractions) and a concomitant decrease in 5-HIAA. With respect to brain 5-HT, these changes have been interpreted as resulting from an enhanced binding.[1,2] Other laboratories have provided evidence in support of the idea that acute doses of LSD caused a decreased 5-HT turnover.[3–6]

Studies of brain 5-HT metabolism *in vivo* in our own and in other laboratories have utilized the technique of intraventricular or intracisternal administration of labeled 5-HT. Light microscopic autoradiography of intraventricularly injected ^{3}H-5-HT suggested an accumulation of the amine in nerve terminals,[7,8] as did fluorescence histochemistry.[9] In support of these results, it was shown that ^{14}C-5-HT is rapidly taken up from the ventricles *in vivo*, probably by an active transport mechanism;[10] the ^{14}C-5-HT declined in two phases with half-lives of 45 min and 3 hr.[11] Intracisternally injected 5-HT is quickly taken up *in vivo*[12] and is pharmacologically manipulable, i.e. its metabolism is altered by psychoactive drugs such as pargyline, reserpine,

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imipramine, chlorpromazine,[5] and diazepam.[13] Corroborative studies of 5-HT uptake or transport mechanisms in brain slices have demonstrated the accumulation of ^{3}H-5-HT,[14] its inhibition by LSD,[14] and the facilitated uptake of ^{14}C-5-HT with the characteristics of active transport.[15] Brain slices also release 5-HT both spontaneously[16] and upon electrical-field stimulation.[17,18] LSD,[17–19] as well as ouabain,[18–20] was found to inhibit this electrically induced release.

LSD is known also to inhibit the firing of 5-HT-containing raphe neurons *in vivo*,[21,22] and to antagonize the excitatory but not the inhibitory effects of 5-HT on a sample of single neurons in the brain stem of the decerebrate cat.[23] To investigate further the mechanism of action of LSD on brain 5-HT and to begin to correlate the electrophysiological and the neurochemical effects of LSD, we undertook further studies of the uptake and metabolism of 5-HT *in vivo* and *in vitro*.

METHODS

Male Sprague–Dawley rats (200–250 g, Madison, Wis.) were given systemic drugs by intraperitoneal (i.p.) injection in a volume of 2·5 ml/kg, except for *para*-chlorophenylalanine (PCPA), which was given orally (p.o.). Control animals for each experiment with an i.p. drug were given equivalent amounts of 0·9% NaCl. PCPA controls received the gruel used as a vehicle for PCPA but without the drug. In the initial experiments, animals received intraventricular injections of 0·1 μg ^{3}H-5-HT in 5 μl of 0·9% NaCl under light chloral hydrate anaesthesia. The skull was exposed, a hole drilled in the skull 2·5 mm lateral to and 1 mm posterior to the bregma, and the injection was made 6 mm below the surface of the skull. The data from these initial

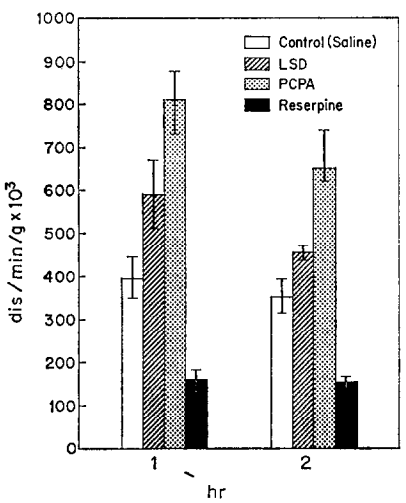

FIG. 1. Effects of LSD, PCPA and reserpine on the amount of ^{3}H-5-HT in brain 1 and 2 hr after intraventricular ^{3}H-5-HT injection of animals under chloral hydrate anaesthesia. Radioactivity (^{3}H-5-HT) is represented as dis./min/g of brain. LSD was given in a single i.p. dose of 520 μg/kg simultaneously with the intraventricular injection of ^{3}H-5-HT. PCPA was given in doses of 100 mg/kg p.o. on days 1, 2 and 3; animals received ^{3}H-5-HT and were sacrificed on day 5. Reserpine was given in two doses of 2·5 mg/kg i.p. at 24 and 12 hr before the ^{3}H-5-HT. Means ± S.E. are shown for the following numbers of animals at 1 and 2 hr respectively: controls, 14 and 7; LSD, 14 and 4; PCPA, 6 and 5; reserpine, 13 and 7. All drug effects shown are significantly different from controls ($P < 0·05$), as determined by Student's *t*-test.

experiments are shown in Fig. 1. All other intraventricular injection experiments (see Figs. 2–4) were performed on animals with a permanent cannula implanted into the lateral ventricle according to the method of Hayden *et al.*[24] The rats were allowed to recover from cannula implantation for a minimum of 3 days and 0·1 μg ^{3}H-5-HT alone or together with 2·5 μg ouabain in 5 μl of 0·9 % NaCl was injected atraumatically without anaesthesia. Intraperitoneal injections of LSD or saline were given simultaneously with the intraventricular injection of ^{3}H-5-HT or ^{3}H-5-HT and ouabain. The dose of LSD was always 520 μg/kg.

5-HT and 5-HIAA assays. 5-HT was estimated by the-method of Bogdanski *et al.*[25] When 5-HIAA was estimated simultaneously in the same brain sample as 5-HT, a modification[1] of the methods for 5-HT[25] and 5-HIAA[26] was used. An aliquot of the final acid extract was taken for determination of ^{3}H-5-HT and ^{3}H-5-HIAA radioactivity by liquid scintillation counting with Bray's phosphor solution[27] in a Packard

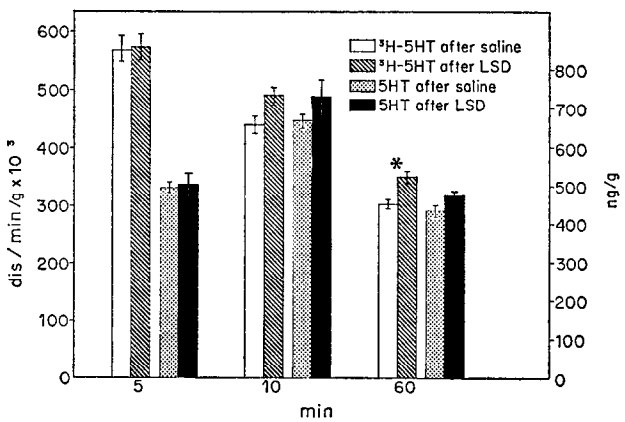

FIG. 2. Brain ^{3}H-5-HT levels and total 5-HT levels in animals which received i.p. LSD, 520 μg/kg, or saline simultaneously with ^{3}H-5-HT given intraventricularly. All animals had permanently implanted cannulae and were conscious when the ^{3}H-5-HT and saline or LSD were administered. Each bar represents the mean for five to six animals ± S.E. The asterisk above a pair of bars indicates a significant difference ($P < 0·05$) between that pair of values.

3375 spectrometer. For each sample, counting efficiency was determined with an automatic external standard system, and counts were converted to disintegrations by means of a quenching curve experimentally constructed using tritiated water standards.

Methods in vitro. Slices, 0·5 mm thick and weighing approx. 30 mg, were cut from cerebral cortex and midbrain with a Stadie–Riggs slicer and incubated in air in a Dubnoff shaker at 37° in 5 ml of Krebs–Ringer bicarbonate containing 0·2 % glucose.[28] The concentration of 5-HT in the medium was 5 ng/ml. After incubation, slices were removed from the solution and rinsed twice in fresh, cold saline. In 5-HT uptake and retention experiments, the tissue was dissolved in 1 ml Soluene (Packard) and 10 ml Bray's phosphor solution was then added for measurement of radioactivity. Determination of 5-HT production from tryptophan was estimated by counting ^{3}H-5-HT extracted from 300 mg of slices incubated in a medium containing ^{3}H-tryptophan. The total 5-HT content of the slices was determined by a modification[29] of the method

of Bogdanski *et al.*[25] An aliquot of the final acid extract was counted in Bray's phosphor solution and the remainder was used for the measurement of fluorescence. Total 5-HT and ^{3}H-5-HT in the incubation medium were measured by the same methods.

Materials. Reserpine was obtained from CIBA-GEIGY Pharmaceutical Company (Serpasil). *p*-Chlorophenylalanine (PCPA), ouabain and 5-hydroxytryptamine creatine sulfate were obtained from Schwarz–Mann. Pargyline hydrochloride was the gift of Abbott Laboratories, North Chicago, Ill. D-Lysergic acid diethylamide (LSD) tartrate was supplied by the Center for Studies of Narcotic and Drug Abuse, N.I.M.H. 5-Hydroxytryptamine-^{3}H (G) creatinine sulfate, specific gravity 8·5 Ci/m-mole, was purchased from Amersham/Searle Corp., Arlington Heights, Ill. DL-Tryptophan-2,3-^{3}H (N), specific activity 179 mCi/m-mole, was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Retention of ^{3}H-5-HT in the brain and drug effects in vivo. After intraventricular injection of ^{3}H-5-HT, brain levels of labeled amine decrease steadily from 1 to 8 hr, and then more slowly thereafter. We were primarily interested in the events within the first 2 hr after the LSD was administered, since it is within this period that the changes in endogenous 5-HT and 5-HIAA occur[1,2] and since the drug is essentially cleared from the brain within 2 hr after i.p. injection.[1]

In the initial experiments, carried out with rats under chloral hydrate anaesthesia, there was an increase in the amount of ^{3}H-5-HT retained in the brain 1 and 2 hr after the simultaneous administration of LSD (i.p.) and ^{3}H-5-HT (intraventricular), as shown in Fig. 1. Pretreatment with PCPA markedly increased the ^{3}H-5-HT present, and pretreatment with reserpine significantly decreased the labeled amine in the brain at 1 and 2 hr (Fig. 1). These results indicate that the ^{3}H-5-HT in the brain was pharmacologically manipulable and hence support the idea that the labeled 5-HT could in fact be mixed with the endogenous pool(s) of 5-HT.

Both the animals which received ^{3}H-5-HT intraventricularly under anaesthesia and the animals which received ^{3}H-5-HT through cannulae chronically implanted in the lateral ventricle showed a similar elevation of ^{3}H-5-HT at 60 min after the LSD injection (Figs. 1 and 2). Neither endogenous 5-HT nor exogenous ^{3}H-5-HT was affected by LSD at 5 min; both showed some evidence of an elevation at 10 and 60 min, with a significant rise in ^{3}H-5-HT at 60 min (Fig. 2). Our earlier work had shown that endogenous 5-HT is also significantly increased at 30–45 min, but not at 10 or at 60 min after this dose.[1]

Ouabain in a dose of 2·5 μg given intraventricularly along with ^{3}H-5-HT or ^{3}H-5-HT and LSD resulted in convulsive behavior in 2–3 min, which ranged from wild running to tonic-clonic jerking. Respiration continued in all animals, none appeared cyanotic, and the convulsive behavior lasted approximately 1 min. Ouabain decreased the total ^{3}H-5-HT in brain at 5 and 60 min, and caused a decrease in the label at 5 min and a reversal of the LSD-induced elevation of ^{3}H-5-HT at 60 min. In fact, the ^{3}H-5-HT in the brains of the ouabain–LSD-treated animals was actually less than with ouabain alone (Fig. 3). Endogenous levels of 5-HT were also lower in animals treated with ouabain and LSD than in those given ouabain alone; thus LSD did not significantly alter ^{3}H-5-HT specific activity (Fig. 4).

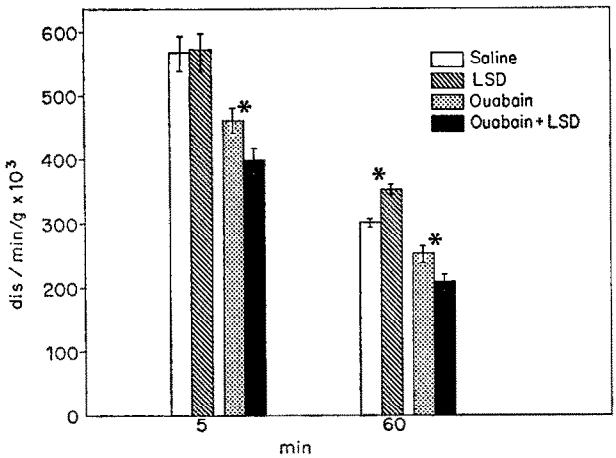

FIG. 3. Brain ^{3}H-5-HT levels in animals which received LSD, 520 μg/kg, or saline i.p. simultaneously with ^{3}H-5-HT or ^{3}H-5-HT and ouabain intraventricularly. Each bar represents the mean for five to six rats $\pm$ S.E. Asterisks over a pair of bars represent significant differences between the two values in that pair ($P < 0{\cdot}05$).

Effects of LSD and ouabain on the uptake and retention of ^{3}H-5-HT by brain slices in vitro. Blackburn *et al.*[15] showed that, with 5 mμg/ml of 5-HT in Krebs–Ringer bicarbonate medium containing 0·2% glucose, 5-HT uptake by brain cortex slices is linear for the first 5 min. We confirmed this and found that LSD (10^{-6} M) inhibited

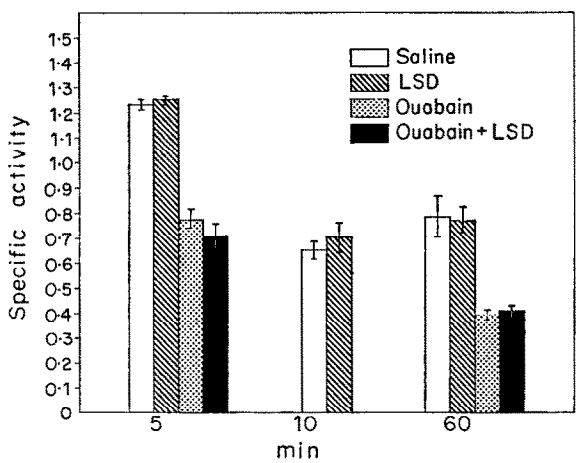

FIG. 4. Specific activity of brain ^{3}H-5-HT expressed as dis/min of ^{3}H-5-HT $\times$ 10^3/mμg 5-HT/g of brain. Data are from experimental and control animals used for Figs. 2 and 3.

this initial uptake about 9 per cent at 5 min, but caused a significant enhancement of retention of radioactivity at 1 hr (Fig. 5). In the presence of ouabain (10^{-4} M), ^{3}H-5-HT uptake was substantially reduced at 5 min, and the retention of ^{3}H-5-HT at 60 min was markedly diminished. In addition, ouabain *in vitro* abolished the LSD-induced increased retention of 5-HT (Fig. 5).

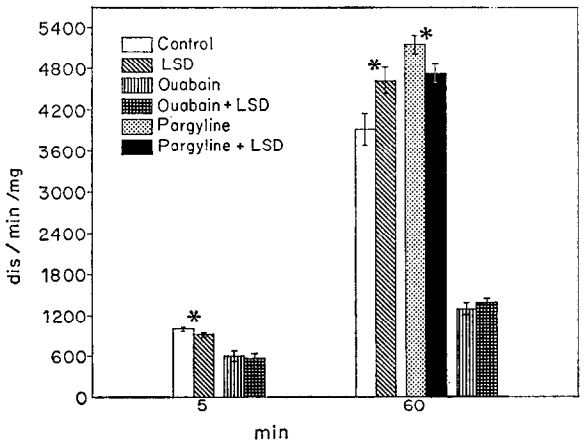

FIG. 5. Radioactivity (^{3}H-5-HT) expressed as dis/min/mg in cerebral cortex slices incubated for the times indicated in Krebs–Ringer bicarbonate medium containing glucose and ^{3}H-5-HT (5 ng/ml). LSD (10^{-6} M) or ouabain (10^{-4}) was added at time 0. Pargyline (10^{-4}) was added for a 10-min preincubation before addition of ^{3}H-5-HT alone or with LSD at time 0. Each bar represents the mean of five animals ± S.E., except in the pargyline experiment, in which each bar represents ten rats. An asterisk over a pair of bars indicates a significant difference ($P < 0.05$) between the two values in the pair.

Goldstein *et al.*[30] have shown a marked increase in catecholamine biosynthesis in brain slices incubated with a concentration of ouabain which inhibited membrane ATPase and allowed catecholamines to leak out of the cells. This effectively diminished the intracellular concentration and thus stimulated synthesis. There has been interest in the possibility of a similar feedback control of 5-HT biosynthesis and 5-HT levels. Some evidence for such a feedback control at high brain 5-HT levels produced by monoamine oxidase (MAO) inhibition has been advanced,[31] but conflicting reports have also appeared[32,33] and the matter remains controversial. A relationship between the steady-state concentration of 5-HT and 5-HT biosynthesis is implied if such feedback control exists. To see if the presence of ouabain was increasing biosynthesis of 5-HT, midbrain and cortex slices were incubated in the presence of ^{3}H-tryptophan, and ^{3}H-5-HT production was measured with and without ouabain (10^{-4} M) in the medium. 5-HT production was not stimulated. Ouabain tended to deplete the midbrain slices of 5-HT and raise the 5-HT content of the medium, but these effects were not statistically significant ($P < 0.05$; Table 1). ^{3}H-5-HIAA was not measured in this experiment, so the possibility that both 5-HT synthesis and degradation were accelerated by ouabain cannot be ruled out.

Effects of LSD and pargyline in vitro *and* in vivo. The presence of an MAO inhibitor in the medium (pargyline, 10^{-4} M) results in the greatest ^{3}H-5-HT retention at 60 min by brain cortex slices (Fig. 5). With pargyline and LSD together in the medium, ^{3}H-5-HT retention at 60 min was not increased over that observed with pargyline alone, but was in fact decreased; no more ^{3}H-5-HT was retained by the slices than with LSD alone (Fig. 5).

These findings *in vitro* prompted the following experiments *in vivo*. Rats were divided into three groups: those which received saline (controls), pargyline only (50 mg/kg i.p.), and pargyline together with LSD (520 μg/kg i.p.). These drugs were

TABLE 1. SYNTHESIS OF ^{3}H-5-HT FROM ^{3}H-TRYPTOPHAN IN RAT BRAIN SLICES*

	^{3}H-5-HT (dis/min/mg or ml)		5-HT (ng/mg or ml)	
	Slice	Medium	Slice	Medium
Midbrain				
Control	373 ± 33	292 ± 3	1·36 ± 0·01	0·96 ± 0·19
Ouabain (10^{-4} M)	348 ± 9	300 ± 10	1·01 ± 0·31	1·26 ± 0·27
Cortex				
Control	394 ± 12	308 ± 16		
Ouabain (10^{-4} M)	359 ± 31	306 ± 14		

* The ^{3}H-5-HT and total 5-HT content of midbrain slices and their medium and the ^{3}H-5-HT content of cerebral cortex slices and their medium were measured after a 15-min incubation with ^{3}H-tryptophan in Krebs–Ringer bicarbonate–glucose medium. Ouabain was added to a concentration of 10^{-4} M at zero time. Data are shown with standard errors of the mean. Each value represents the mean for three experiments. Animals used for midbrain slices received 60 mg/kg of pargyline i.p. 2 hr before sacrifice. Animals used for cortex slices received 20 mg/kg of pargyline i.p. 1 hr before sacrifice.

administered i.p. at zero time and brain 5-HT and 5-HIAA subsequently determined in rats sacrificed at 10-min intervals from 0 to 60 min (Fig. 6). In relation to the rats which received pargyline only, LSD did not further increase 5-HT nor further decrease 5-HIAA when given together with pargyline. The distinctive effects of LSD may be

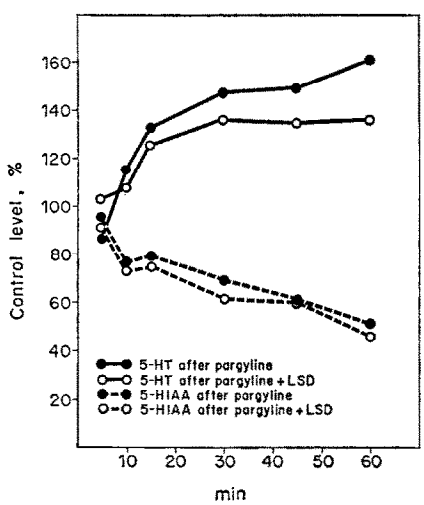

FIG. 6. Brain 5-HT and 5-HIAA shown as per cent of control animals which received no drugs. Pargyline (50 mg/kg i.p.) was given to all experimental animals at time 0. LSD (520 μg/kg i.p.) was given to half the animals at time 0 along with the pargyline. Each point represents at least seven rats in both experimental and control groups. None of the points represents a significant difference between the pargyline and pargyline + LSD groups.

masked by the action of the MAO inhibitor. In a further experiment *in vivo*, animals were pretreated with 50 mg/kg of pargyline 16 hr before receiving 520 μg/kg of LSD or saline (Fig. 7). LSD again resulted in no significant alteration in brain levels of 5-HT and 5-HIAA from 0 to 120 min, in relation to the rats which received the pargyline pretreatment only (Fig. 7).

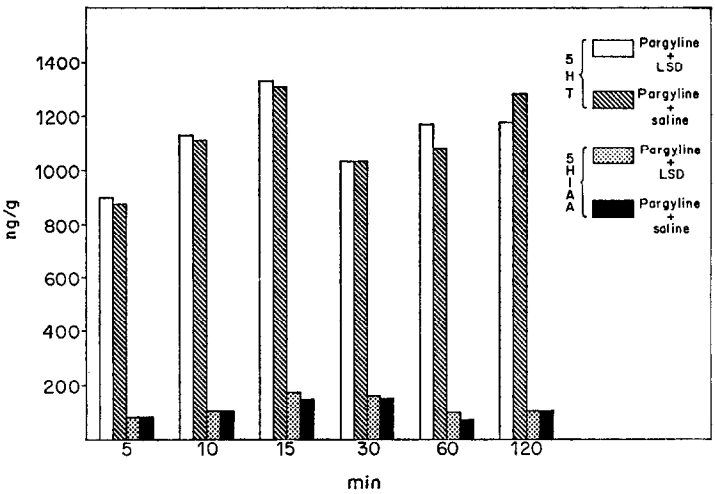

FIG. 7. Brain 5-HT and 5-HIAA shown in mμg/g of brain. Pargyline (50 mg/kg i.p.) was given 16 hr before time 0. LSD (520 μg/kg i.p.) was given to the experimental group at time 0.

DISCUSSION

Uptake of 5-HT in amounts 2000 times greater than those used in these experiments from the rat ventricular system was found to each maximal levels in 5–10 min,[12] but it is likely that, at such high concentrations, 5-HT uptake by brain tissue occurs almost entirely by diffusion.[15,34] A rapid, facilitated transport seen at lower concentrations of 5-HT has been demonstrated.[14,15,35] Autoradiographic localization of intraventricularly injected ^{3}H-5-HT showed penetration of radioactivity in the neuropile surrounding the ventricles, and particularly in nerve endings and unmyelinated axons.[7,8] In addition to this biochemical and morphological evidence that intraventricular or intracisternal 5-HT is taken up by brain neurons, the susceptibility of the ^{3}H-5-HT in the brain to the action of reserpine and PCPA in these experiments supports our assumption that the labeled amine can mix with endogenous, functional 5-HT, and therefore is a useful index of changes in 5-HT disposition and/or metabolism resulting from interaction of LSD with 5-HT in brain. Pretreatment of the animals with reserpine, which impairs amine storage[36] but not neuronal uptake,[14,15,35] decreased brain ^{3}H-5-HT in these experiments by 60 per cent 1 hour after intraventricular injection. Endogenous 5-HT is lowered more than 60 per cent by reserpinization;[37] thus, some of the intraventricularly administered ^{3}H-5-HT was apparently reserpine-resistant. Pretreatment with PCPA, which lowers 5-HT by synthesis inhibition,[38] increased retention of ^{3}H-5-HT, presumably because the label can penetrate the depleted storage sites. These storage sites for 5-HT apparently protect the amine from MAO and are normally occupied by endogenous 5-HT to a significant degree.

Is the LSD-induced elevation of ^{3}H-5-HT observed at 1 hr in both anaesthetized and conscious animals a secondary result of some primary effect of the drug which occurs earlier such as, for example, inhibition of serotonergic neuronal firing?[21,22] The results of our experiments *in vitro* might suggest that this is the case, since initially LSD acts to inhibit 5-HT uptake and only later (at 1 hr) is an enhanced retention of 5-HT seen. There is a good deal of evidence *in vivo* that 5-HT turnover is slowed at

certain time intervals after LSD;[3,4,6] the enhanced retention of ^{3}H-5-HT may be a reflection of this slowed turnover. This enhanced retention of labeled 5-HT may also be seen as a reflection of what we have previously called "enhanced binding" (increased 5-HT in particulate fractions after LSD).[39] Whether this effect (enhanced binding) is casual or secondary to mechanisms leading to decreased turnover is as yet unclear. It may be that turnover slows because the 5-HT is somehow prevented by the action of LSD from being deaminated by MAO. This too could account for the enhanced retention of labeled ^{3}H-5-HT. Thus, how LSD prevents access of 5-HT to MAO is interesting because the drug cannot be shown to inhibit MAO either *in vivo* or *in vitro*.[2,40]

The results of the ouabain experiments reported here can be considered from two points of view: from the point of view of ouabain's well known inhibitory effects on membrane uptake mechanisms, and from the point of view of ouabain considered as a potent neuronal depolarizing agent. Ouabain inhibits membrane ATPase,[41,42] and inhibits 5-HT uptake by brain slices [14,15,43] and by synaptosomes;[44,45] its inhibition of synaptosomal 5-HT uptake was shown to be secondary to inhibition of Na^+, K^+-ATPase.[44] Because of these known inhibitory effects on 5-HT uptake, we tested ouabain *in vivo* to find out what effect it would have on the enhanced retention of ^{3}H-5-HT at 60 min caused by LSD. The amount of intraventricular ouabain used (2·5 μg) caused brief convulsions and reversed the enhanced retention of ^{3}H-5-HT 1 hr after LSD. This reversal may be related to the neuronal depolarization ouabain caused, which was sufficiently generalized to result in convulsions. The overall decrease in retained 5-HT observed in animals receiving ouabain may be due to decreased active transport, resulting from ouabain's effect on membrane ATPase. It is also possible that it may simply be a reflection of increased cerebrovascular blood flow caused by a mild hypercapnia incident to the animal's seizures. At the time our experiments were being completed, we were unaware of the work of Doggett and Spencer,[46] which showed that amounts of intraventricular ouabain less than 0·4 μg have depressant effects, whereas amounts greater than 0·4 μg have convulsant effects. In these experiments, we observed the ouabain reversal of the enhanced retention of ^{3}H-5-HT caused by LSD. These results suggest that one phase of the action of LSD on 5-HT may involve uptake. Recent experiments in our laboratory with chlorimipramine, an uptake inhibitor more specific for 5-HT,[47,48] support the idea that interference with 5-HT uptake may be involved as one of the mechanisms of the LSD effect on brain 5-HT.

Concomitant with its effect on Na^+, K^+-ATPase and the Na^+-pump mechanism, ouabain depolarizes neurons and leads to neuronal discharge. Thus, its effects on the LSD–5-HT interaction may also be considered in relation to neuronal firing. LSD is known to inhibit the firing of 5-HT-containing neurons,[21,22] to block the release of 5-HT from brain slices under electrical field stimulation,[19] and to antagonize excitatory but not inhibitory responses to iontophoretically administered 5-HT.[23] If we consider generalized convulsions in animals given intraventricular ouabain as indicative of generalized neuronal discharge, then ouabain may be thought of as counteracting the LSD-induced inhibition of 5-HT neurons. If, as has been suggested,[21,22,49] there is a relationship between the rate of firing of serotonergic neurons and the turnover rate of 5-HT therein, then the LSD-induced decrease in turnover may no longer be evident when these neurons are depolarized by ouabain. In other words, LSD may be

unable to inhibit or prevent firing in the presence of ouabain, and so the slowing of 5-HT turnover no longer occurs, and thus the LSD-induced increase in retention of ^{3}H-5-HT is prevented. Another way of looking at the ouabain effect is in terms of 5-HT release. If 5-HT is released from firing serotonergic neurons, then LSD, which inhibits this firing, slows 5-HT release as well as turnover. The result is an increased retention of 5-HT. Ouabain counteracts the inhibitory effect of LSD, fires the neurons, and so less ^{3}H-5-HT is retained in the brains of LSD-treated animals when given ouabain. The data reported here do not distinguish between these alternate hypotheses of ouabain action. In order to do so, further experiments are needed such as, for example, to test the effect of neuronal depolarization, induced by other depolarizers (K^+, glutamate), on the LSD–5-HT interaction.

Pargyline together with LSD was no more effective than LSD alone in maintaining radioactivity in brain slices at 1 hr. In fact, there was less retention with both pargyline and LSD than with pargyline only in the medium (Fig. 5). LSD at 10^{-6} M effectively reduced 5-HT metabolism *in vitro*, while having only a minor effect on 5-HT uptake. *In vivo*, the LSD effect of enhanced retention or binding of 5-HT is not apparent when 5-HT is markedly elevated because its deamination is blocked. Thus, MAO inhibition and LSD have qualitatively similar effects on 5-HT turnover both *in vitro* and *in vivo*, but their effects must involve different mechanisms, since LSD has no inhibitory action on MAO.[2,40]

These results suggest that there may be different mechanisms or different phases in the time course of LSD effects on brain 5-HT. We know that LSD affects raphe neurons by diminishing their firing.[21,22] The ouabain experiments support the idea that this diminished firing results in a decreased 5-HT turnover, since ouabain apparently counteracts the inhibitory effect of LSD on neuronal firing and reverses the enhanced retention or binding of 5-HT after LSD. However, the precise mode of action of ouabain in this system has not been elucidated. Since the LSD-induced increase in 5-HT occurs chiefly in brain particulate fractions,[1,39] the effect of neuronal firing may be to release bound 5-HT from sites which are included in these fractions. The experiments *in vitro* also provide further evidence for the still unexplained effect of LSD having to do with unavailability or lack of access of 5-HT to MAO. The additional presence of pargyline in the medium with LSD resulted in no more ^{3}H-5-HT being retained than with LSD alone. Furthermore, similar effects on brain 5-HT levels are observed after LSD alone or after either MAO inhibition alone or MAO inhibition combined with LSD treatment. Finally, these experiments have provided new evidence suggesting that an uptake mechanism may be involved in the action of LSD. The task ahead then is clear: to distinguish these different phases or mechanisms as they operate in the time course of LSD effects on brain 5-HT and to show how they are interrelated.

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