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Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide

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1. Lysergic acid diethylamide (LSD) and the 5-hydroxytryptamine (5-HT) precursor, 5-hydroxytryptophan produced similar functional effects in rat spinal cord and brain to the 5-hydroxytryptamine precursor 5-hydroxytryptophan, which indicates that LSD stimulates central 5-HT receptors.

2. By means of combined histochemical and biochemical techniques it was found that LSD reduced the turnover rate of brain and spinal cord 5-HT, studied after inhibition of the tryptophan hydroxylase by α-propyldopacetamide. The turnover of brain noradrenaline but not dopamine was somewhat accelerated.

3. The functional and chemical effects by LSD were related to dose and to time. They were not observed after the LSD analogues 2-bromo-LSD and methysergide.

4. The retardation of the 5-HT turnover by LSD may be due to negative feed-back mechanisms evoked by direct stimulation of the central 5-HT receptors.

The potent psychotomimetic drug (+)-lysergic acid diethylamide (LSD) has a 5-hydroxytryptamine (5-HT) blocking effect on rat uterus and it has therefore been speculated that the hallucinogenic effects observed in man were the result of a similar action in the central nervous system (Gaddum, 1957; Woolley & Shaw, 1957; Giarman & Freedman, 1965). In animal experiments, however, LSD can cause effects similar to those observed after drugs which cause increased concentrations of 5-HT in the central nervous system (see review by Mantegazzini, 1966). In view of these findings it is not unlikely that LSD interferes with the 5-HT neurotransmission in the central nervous system, especially because previous studies have also revealed an increase in the 5-HT contents of rat brain produced by LSD (Freedman, 1961). In the current investigation the effect of LSD on the activity of the central monoamine neurones has been studied, utilizing monoamine synthesis inhibitors. It is known that the amine depletion obtained with synthesis inhibitors is highly dependent on the rate of flow of nervous impulses (Andén, Corrodi, Dahlström, Fuxe & Hökfelt, 1966). The inhibitors used have been the α-methyl-tyrosine methylester (H 44/68) which inhibits tyrosine hydroxylase (Spector, Sjoerdsma & Udenfriend, 1965; Corrodi & Hanson, 1966; Andén, Corrodi *et al.*, 1966) and α-propyl-dopacetamide (H 22/54), which inhibits both tyrosine and tryptophan

hydroxylase (Carlsson, Corrodi & Waldeck, 1963). Furthermore, the effect of LSD and 5-hydroxytryptophan (5-HTP) on the hind limb reflexes of acutely spinalized rats have been studied. The effects of the closely related non-hallucinogenic lysergic acid derivatives, 2-bromo-(+)-lysergic acid diethylamide (BOL) and (−)-methyl-(+)-lysergic acid butanolamide (methysergide) have also been studied in a similar way.

Methods

Adult Sprague-Dawley rats (150–250 g) were used. The rats were killed by decapitation under light chloroform anaesthesia. In all experiments the rectal temperature was frequently monitored and was found to be between 36.5° and 38° C. The following salts were used: LSD tartrate, BOL bitartrate, methysergide bimaleate. All doses refer to the base.

Biochemical studies

For dosage and time-intervals, see tables. All drugs were given intraperitoneally. 5-HT, noradrenaline (NA) and dopamine (DA) were determined fluorimetrically after cation exchange chromatography (Bertler, 1961; Andén & Magnusson, 1967; Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962). The same number of saline, drug, H 22/54 or H 44/68-treated rats were used as controls in each experiment. The concentrations of monoamines found in rat brain and spinal cord are expressed as a percentage of normal values. These are for brain (means ± S.E.M. of ten experiments) NA, 0.45 ± 0.009 μg/g; DA, 0.72 ± 0.018 μg/g; 5-HT, 0.46 ± 0.019 μg/g; for spinal cord (means ± S.E.M. of five experiments) 5-HT, in the cranial half 0.50 ± 0.027 μg/g and in the caudal half 0.84 ± 0.072 μg/g. The statistical significance was calculated by Student's *t* test.

Histochemical studies

In the experiments with H 44/68, LSD (2 mg/kg, i.p.) was given 30 min before administration of H 44/68 (250 mg/kg, i.p. 4 hr before killing). An additional dose of LSD (2 mg/kg, i.p.) was sometimes given 2 hr before killing. In the experiments with H 22/54, LSD, BOL or methysergide was administered 3.5 and 1.5 hr before killing, the H 22/54 (500 mg/kg, i.p.) being given 3 hr before killing. At both time-intervals either 2 or 1 mg/kg was administered i.p. Some rats were injected with only one dose of LSD (1 mg/kg) 0.5 hr before H 22/54. As controls rats which had received H 22/54, H 44/68, LSD, BOL or methysergide alone in the same way as described above were used. Various parts of the brain and the spinal cord were taken for histochemical analysis of DA, NA and 5-HT (Falck *et al.*, 1962; see review by Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967).

Functional studies

About 100 rats were acutely spinalized in the midthoracic region under ether anaesthesia. The spinal hind limb reflexes were observed after nialamide (50 mg/kg, i.p.) plus DL-5-HTP (10–75 mg/kg i.v., 2 hr after nialamide), LSD (0.5–2 mg/kg i.p.), BOL (2 mg/kg i.p.) and methysergide (2 mg/kg i.p.). In some experiments phenoxybenzamine (20 mg/kg i.p.), haloperidol (10 mg/kg i.p.) or chlorpromazine (10 mg/kg i.p.) was injected one hour before LSD or 5-HTP.

Results

Chemical studies

Experiments with H 22/54 (Tables 1, 2, 3 and 4). The 5-HT depletion in the whole brain and in the intact spinal cord was markedly retarded under the influence of LSD but not after BOL or methysergide as observed both biochemically and histochemically. This effect was dose-dependent, highly significant after 2 hr (Table 4) but not significant with low doses of LSD (250 and 50 μg/kg, i.p.). Histochemically, a marked retardation of the disappearance of the yellow fluorescence was observed in the various 5-HT nerve terminal systems of the brain (Figs. 1 and 2) and the spinal cord. Thus a marked decrease in fluorescence intensity of the 5-HT nerve terminals could only be seen in the rats treated with H 22/54 alone.

TABLE 1. *5-HT levels in rat brain*

Treatment	Dose LSD (mg/kg i.p.)	Dose H 22/54 (mg/kg i.p.)	Number exp.	5-HT (%)
Untreated	—	—	5	100·0±3·9
LSD	2×2*	—	3	96·2±6·5
H 22/54	—	500	4	43·5±1·5 (1)
LSD+H 22/54	2×2*	500	4	77·5±5·3 (2)
LSD+H 22/54	1	500	4	67·5±4·7 (3)
LSD+H 22/54	0·25	500	4	49·6±2·9 (4)
LSD+H 22/54	0·05	500	4	46·6±4·3 (5)

Statistical significance between: (1) and (2), $P<0·001$; (1) and (3), $P<0·005$; (1) and (4), (5), $P>0·05$ (not significant).

* The second dose of LSD was given 2 hr after the first.

The animals received LSD in varying doses i.p. and 15 min later H 22/54 was given i.p. (500 mg/kg as a 3% solution in saline). The animals were killed 3 hr later. 5-HT given as percentage of normal values (see **Methods**). Mean±S.E.M.

TABLE 2. *5-HT levels in the cranial and caudal half of the intact rat spinal cord*

Half of the spinal cord	Treatment	Dose LSD (mg/kg i.p.)	Dose H 22/54 (mg/kg i.p.)	Number of exp.	5-HT (%)
Cranial	Untreated	—	—	5	100·0±5·4
	LSD	2×2	—	5	100·0±9·2
	H 22/54	—	500	5	38·0±4·4 (1)
	H 22/54+LSD	2×2	500	5	56·0±3·4 (2)
Caudal	Untreated	—	—	5	100·0±8·6
	LSD	2×2	—	5	104·8±9·0
	H 22/54	—	500	5	42·9±1·9 (3)
	H 22/54+LSD	2×2	500	5	54·8±3·2 (4)

Statistical significance between: (1) and (2), $P<0·025$; (3) and (4), $P<0·025$.

The animals were treated with H 22/54 (500 mg/kg, i.p. as a 3% solution in saline) and LSD (0·5 and 2·5 hr after the H 22/54 injection). The rats were killed 2 hr after the last injection of LSD. 5-HT as percentage of normal values (see **Methods**). Mean±S.E.M.

TABLE 3. *5-HT levels in rat brain*

Treatment	Number of exp.	5-HT (%)
Untreated	5	100·0±4·1
BOL	3	78·2±6·0
Methysergide	3	86·8±4·6
H 22/54	4	37·7±3·7 (1)
BOL+H 22/54	4	42·1±0·5 (2)
Methysergide+H 22/54	4	38·2±0·7 (3)

Statistical significance between: (1) and (2), $P>0·1$; (1) and (3), $P>0·1$.

2-Bromo-LSD (4 mg/kg, i.p.) and methysergide (2 mg/kg, i.p.), respectively, was given 15 min before H 22/54 (500 mg/kg, i.p.). The animals were killed 3 hr later. 5-HT as percentage of normal value. Means±S.E.M.

TABLE 4. *5-HT levels in rat brain*

Treatment	5-HT (%)
1 *hour*	
Untreated	100·0±3·9
LSD	109·0±3·6
H 22/54	75·0±5·8 (1)
LSD+H 22/54	85·0±7·8 (2)
2 *hours*	
Untreated	100·0±3·9
H 22/54	53·4±2·3 (3)
LSD+H 22/54	79·3±6·2 (4)
3 *hours*	
Untreated	100·0±3·9
H 22/54	45·5±1·8 (5)
LSD+H 22/54	69·5±4·7 (6)

Statistical significance between (1) and (2), not significant; (3) and (4), $P<0·001$; (5) and (6) $P<0·005$.

The animals received LSD (1 mg/kg i.p.) 15 min before H 22/54 (500 mg/kg i.p. as a 3% solution in saline). They were killed 1, 2 and 3 hr later. 5-HT as percentage of normal values (see **Methods**). Mean±S.E.M. of four experiments.

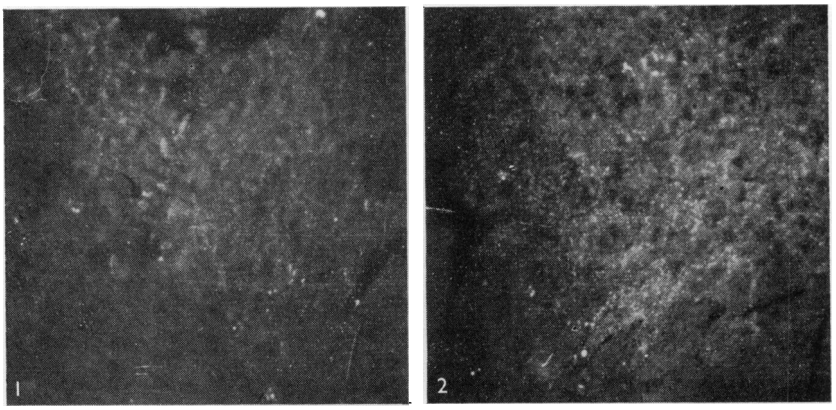

FIG. 1. Nuc. suprachiasmaticus of rat treated with H 22/54 (500 mg/kg, i.p., 3 hr before killing). Weakly fluorescent 5-HT nerve terminals are observed. (×200.)

FIG. 2. Nuc. suprachiasmaticus of rat treated with LSD (2+2 mg/kg, i.p.) 15 min before and 1.5 hr after H 22/54 (see text to Fig. 1). Densely packed, very fine 5-HT nerve terminals are observed exhibiting a moderate yellow fluorescence intensity due to a decreased rate of disappearance of neuronal 5-HT after synthesis inhibition plus LSD. (×200.)

TABLE 5. *NA and DA levels in rat brain*

Treatment	Dose LSD (mg/kg i.p.)	Dose H 44/68 (mg/kg i.p.)	Number of exp.	DA (%)	NA (%)
Untreated	—	—	5	100·0±2·5	100·0±2·0
LSD	2×2*	—	3	94·6±5·8	94·7±6·0
H 44/68	—	250	4	25·2±2·2	47·6±2·6 (1)
LSD+H 44/68	2×2*	250	4	31·1±1·9	30·4±0·9 (2)
LSD+H 44/68	1	250	4	21·6±1·4	42·6±2·6 (3)

Statistical significance between: (1) and (2), $P<0·001$; (1) and (3), $P>0·05$.

* The second dose of LSD was given 2 hr after the first.

LSD was given in two different doses 15 min before H 44/68 (250 mg/kg i.p. as a 3·5% solution in saline). The animals were killed 4 hr later. DA and NA as percentage of normal values (see **Methods**). Mean±S.E.M.

Experiments with H 44/68 (Table 5). Biochemically, an acceleration of the NA but not of the DA depletion was seen in whole brain after H 44/68, provided that high doses of LSD (2+2 mg/kg) were used.

Histochemically, a clear-cut acceleration of the amine depletion was observed in the various NA nerve terminals of the brain under the influence of LSD. This effect was only observed after two doses (2+2 mg/kg) of LSD. The depletion rate of amine from the DA nerve terminals seemed to be unchanged by LSD.

Functional studies

In the acutely spinalized rat, treatment with nialamide plus 5-HTP caused a dose-dependent appearance of athetoid movements and hyperextension in the hind legs and, as in the intact rats, a tremor in the forelimbs and movements of the head. Similar effects were seen after LSD in a dose-dependent manner. BOL and methysergide did not produce any detectable functional actions by themselves, nor did they block the effects of 5-HTP and LSD. LSD and 5-HTP produced these changes even after pretreatment with reserpine (10 mg/kg, i.p., 20 hr before) plus H 22/54 (250 mg/kg, i.p., 1 hr before). The effects of LSD or 5-HTP could not be blocked by phenoxybenzamine, haloperidol or chlorpromazine, which probably act by blockade of catecholamine but not 5-HT receptors. Neither 5-HTP nor LSD caused a clear-cut increase of the flexor reflex as was seen after administration of the catecholamine precursor dihydroxyphenylalanine.

Discussion

The present studies showed that 5-HTP and LSD produced the same actions on spinal reflexes. Also other central effects as observed on the gross behaviour of the rats were remarkably similar after administration of the two drugs. All these actions of LSD still appeared after treatment with reserpine plus H 22/54. This treatment in all probability removed all the 5-HT in the neurones, so it is very unlikely that the effects of LSD were due to a release of 5-HT from nerve terminals. It can therefore be assumed that LSD directly stimulates 5-HT receptors of the postsynaptic neurones.

In the chemical studies it was shown that LSD markedly reduced the rate of amine depletion in the various 5-HT nerve terminal systems of the brain and the spinal cord after inhibition of the 5-HT synthesis by H 22/54. This finding indicates that LSD decreases the activity of the central 5-HT neurones because the 5-HT nerve terminals caudal to a spinal transection lacking nerve impulses are not depleted of its amine content after synthesis inhibition (Andén, Fuxe & Hökfelt, 1966 ; *cf.* introduction). It is possible that such a reduced activity of the 5-HT neurones by LSD was caused by a receptor stimulation. It can be speculated that 5-HT receptor stimulation induces a negative feedback mechanism on the presynaptic 5-HT neurones via a neurone chain. Such a compensation should lead to a reduced impulse flow and a slower turnover of the transmitter in the 5-HT neurones.

Thus the present results indicate that a 5-HT receptor stimulation may be responsible for some pharmacological effects of LSD and perhaps also for the hallucino-

genic features. This view is strengthened by the fact that the non-hallucinogenic lysergic acid derivatives did not produce the chemical and functional changes observed after LSD. The results may also imply that the central 5-HT neurones have mental functions in the brain.

In contrast to Freedman (1961), we could not observe any significant increase in the concentration of 5-HT in the rat brain after treatment with LSD. The increases in Freedman's study were small, however, and they were found only shortly after the injection of LSD. Practically all our 5-HT determinations were performed more than an hour after the administration of LSD and this difference in time may be one reason for the discrepancy.

In a higher dose LSD has an effect not only on 5-HT neurones but probably also on central NA neurones, for an increased rate of depletion of intraneuronal NA was observed in all parts of the brain after tyrosine hydroxylase inhibition under the influence of LSD. This indicates an increased activity in these neurones. It may be that the changes observed in the activity of the central 5-HT and NA neurones are related. No simple explanation can be given for the increased activity in the central NA neurones observed after LSD, but this may indicate that both NA and 5-HT neurones are involved in the central actions of LSD.

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