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ACTIONS OF D-LYSERGIC ACID DIETHYLAMIDE (LSD) AND ITS DERIVATIVES ON 5-HYDROXYTRYPTAMINE RECEPTORS IN THE ISOLATED UTERINE SMOOTH MUSCLE OF THE RAT

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Several metabolites of D-lysergic acid diethylamide (LSD) such as D-lysergic acid ethyl, 2′-hydroxyethylamide (LEO), D-lysergic acid ethyl,vinylamide (LEV), D-lysergic acid ethylamide (LAE) and D-norlysergic acid diethylamide (norLSD) are formed by liver tissue and by Streptomyces. These metabolites and synthetic N^6-alkyl substituted derivatives such as N^6-ethyl-, N^6-propyl, N^6-allyl and N^6-hexyl-D-norLDS (ethyl-, propyl-, allyl- and hexyl-norLSD, respectively) were studied for their anti-5-hydroxytryptamine (anti-5-HT) and oxytoxic activities on isolated rat uteri. The LSD derivatives had less anti-5-HT activity than LSD. Except for hexyl-norLSD, N^6-alkyl substituted derivatives of LSD had higher oxytocic activities than LSD, LEO or LAE. Minimum effective concentrations as well as ED_{50} values for oxytocic activities of ethyl-norLSD, propyl-norLSD and allyl-norLSD were lower than that of LSD whereas those of the metabolites (LEO, LAE and norLSD) were higher than that of LSD. The oxytocic activity of allyl-norLSD was effectively antagonized by LSD and cyproheptadine, suggesting that this activity was due to its 5-HT-like action. These results suggest that N^6-alkyl substituted derivatives (ethyl-, propyl-, allyl-norLSD) have higher, but the metabolites of LSD have lower affinity for 5-HT receptors than LSD, and that N^6-substituted derivatives except for hexyl-norLSD have higher intrinsic activity than LSD.

Anti-5-hydroxytryptamine effect | Isolated rat uterus | LSD derivatives | LSD
5-Hydroxytryptamine receptors | LSD metabolites | Oxytocic action

1. Introduction

Axelrod et al. (1956, 1957) have found 2-hydroxy-D-lysergic acid diethylamide to be a metabolite of D-lysergic acid diethylamide (LSD). Subsequently, 12-hydroxy- and 13-hydroxy-LSD were reported to be metabolic products of LSD (Slaytor and Wright, 1962; Szara, 1963). Niwaguchi et al. (1974) have recently reported that D-lysergic acid ethylamide (LAE) and D-norlysergic acid diethylamide (norLSD) were formed by liver homogenates of experimental animals. Hayashi et al. (1974) have studied the dealkylation of LSD by several strains of Streptomyces and found new metabolites such as D-lysergic acid ethyl-, 2′-hydroxyethylamide (LEO) and D-lysergic acid ethyl,vinylamide (LEV) besides LAE and norLSD. Pharmacological activities of these metabolites have however not been studied.

The present investigation was undertaken to study the anti-5-hydroxytryptamine (anti-

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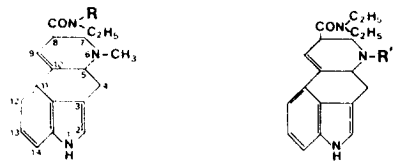
5-HT) and oxytocic activities of these metabolites on rat uteri. Since Baker et al. (1973) suggested that the N^6-methyl group of LSD played an important role in psychotomimetic actions, we also studied the pharmacological activities of synthetic N^6-alkyl substituted derivatives of LSD.

2. Materials and methods

2.1. Materials

The following reagents were used: 5-hydroxytryptamine creatinine sulfate, cyproheptadine hydrochloride and ergometrine maleate (Nakarai Chem. Ltd.); acetylcholine chloride and atropine sulfate (Merck); estradiol benzoate (Teikokuzoki Ltd.); D-lysergic acid diethylamide tartrate (Sandoz). LSD derivatives were used as the tartrate and are listed in fig. 1. LAE was synthesized from D-lysergic acid according to the procedure of Nakahara and Niwaguchi (1974). LEO and LEV were isolated from the culture broth of Streptomyces roseochromogenes (Hayashi et al., 1974). NorLSD was obtained from LSD by the procedure of Nakahara and Niwaguchi (1971). N^6-alkyl substituted derivatives of LSD were synthesized from norLSD by the procedure of Niwaguchi et al. (1976).



R =			R' =	
$-CH_2-CH_3$	LSD		$-H$	nor LSD[a]
$-CH_2-CH_2OH$	LEO[a]		$-CH_2-CH_3$	ethyl-nor LSD[b]
$-CH=CH_2$	LEV[a]		$-CH_2-CH_2-CH_3$	propyl-nor LSD[b]
$-H$	LAE[a]		$-CH_2-CH=CH_2$	allyl-nor LSD[b]
			$-C_6H_{13}$	hexyl-nor LSD[b]

Fig. 1. Chemical structures and abbreviations for derivatives of LSD. (a) Metabolites of LSD; (b) synthetic compounds.

2.2. Anti-5-HT activity and anti-acetylcholine activity

Virgin Wistar rats (180—220 g) were injected s.c. with 1 mg/kg of estradiol on the day prior to the experiment. Animals were sacrificed by a blow on the head and the uterine horns isolated. The isolated uterus was suspended in a modified Krebs—Henseleit solution in which the Ca^{2+} concentration was lowered to 0.4 mM. The Krebs—Henseleit solution was maintained at 30°C and oxygenated by bubbling a mixture of O_2—CO_2 (95 : 5). Lowering the Ca^{2+} concentration and the temperature of the bath fluid abolished the spontaneous contractions of the muscle and also reduced the contractions due to LSD derivatives. Similar conditions were used for anti-5-HT activity assay (Cerletti and Doepfner, 1958). Isotonic contractions were magnified 6-fold and recorded kymographically. The resting tension on the muscle was 0.5 g. 5-Hydroxytryptamine (5-HT) was applied at 15 min intervals and used at a concentration of 5×10^{-7} M which produced contractions about 90% of the maximum. The dose of a compound necessary to produce a 50% reduction of a 5-HT response (ID_{50}) was calculated according to the probit procedure. The inhibitory effect of LSD derivatives on the response to 5×10^{-7} M acetylcholine (Ach) was also tested at a concentration of 1×10^{-7} M under the same conditions as for the anti-5-HT activity assay.

2.3. Oxytocic activity

Prior to the measurement of the oxytocic activities of LSD and its derivatives, the response of the uterus to LSD derivatives was examined at different estrus stages. It was found that the proestrus uterus had a high sensitivity to LSD derivatives and did not show any spontaneous activity in the absence of drugs. Therefore, proestrus uteri were employed for the present experiments. The proestrus uterus of virgin Wistar rats (180—220 g) was suspended in Krebs—Henseleit solution

which contained the normal concentration of Ca^{2+} at 37°C and aerated as above. The contractions were kymographically recorded. After allowing the organ to equilibrate in the Krebs—Henseleit solution for 20 min, the contraction with 5×10^{-7} M 5-HT was recorded twice, at a 15 min interval. 20 min after washing out the 2nd 5-HT, an LSD derivative was added to the bath and the contraction recorded continuously for 10 min. The area (S) covered under the tracing of contraction, the height (H) of the contraction induced by 5-HT and the distance (L) covered in 10 min were measured on the kymogram. Oxytocic activity was calculated as follows:

$$\text{Activity} = S \times 100/(L \times H)$$

(see also fig. 3). As shown in fig. 3, the initiation of contractions by drugs which have low oxytocic activities or by low concentrations of drugs was rather slow; oxytocic activity was thus measured for 10 min rather than for a shorter period. In order to avoid auto-inhibition, one uterine horn was used for each concentration of an LSD derivative. When the effect of an antagonist on this oxytocic action was tested, two uterine horns from the same animals were used, one in the presence of and another in the absence of an antagonist. The antagonist was added 10 min before the addition of an LSD derivative.

3. Results

3.1. Anti-5-HT activity

Table 1 shows ID_{50} values for LSD and some of its derivatives. The activity of the amide-substituted derivatives was obviously lower than that of LSD and the decreasing order of activity was LSD, LEO, LEV and LAE. N^6-Alkyl substituted derivatives such as nor-LSD, N^6-ethyl-D-norLSD (ethyl-norLSD), N^6-propyl-D-norLSD (propyl-norLSD) and N^6-allyl-D-norLSD (allyl-norLSD) themselves induced contractions. ID_{50} values of these derivatives could therefore not be determined precisely. However, these compounds clearly inhibited the 5-HT response at concentrations above 4×10^{-8} M (fig. 2). The inhibitory effect of norLSD on the 5-HT response was observed at concentrations above 1×10^{-7} M. N^6-hexyl-D-norLSD (hexyl-norLSD) itself did not produce contractions and its ID_{50} value was higher than that of LSD. The amide-substituted derivatives and hexyl-norLSD did not inhibit the response to 5×10^{-7} M Ach at a concentration of 1×10^{-7} M.

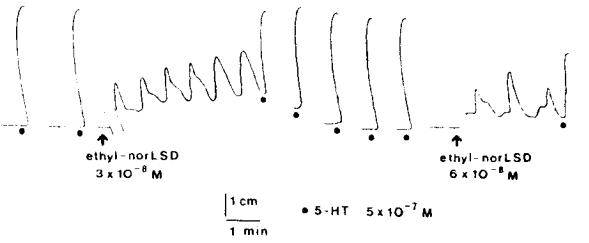


Fig. 2. Anti-5-HT action of ethyl-norLSD in the isolated rat uterus.

3.2. Oxytocic activities of LSD derivatives

Typical responses to LSD derivatives are shown in fig. 3. Propyl-norLSD (1×10^{-8} M) produced remarkable contractions. LEO (5×10^{-8} M) which did not produce contractions under the conditions used for the anti-5-HT assay experiments, also produced contractions. The response to LSD derivatives faded within 10 min as shown in fig. 3. However, the oxytocic activities calculated as mentioned above were dose-dependent. The dose—response curves of LSD derivatives are shown in fig. 4. LSD (3×10^{-9} M) showed little activity in three out of four uterine preparations and significant activity in one preparation. At 1×10^{-8} M, the oxytocic activity of LSD was observed with all the preparations tested. LSD showed its maximum response at 5×10^{-8} M, and the ED_{50} was estimated from fig. 4 to be 1.0×10^{-8} M. LAE and LEO were inactive at concentrations below 1×10^{-8} M. The ED_{50} values of LAE and LEO were 2.2

TABLE 1

Anti-5-HT activity of LSD derivatives in the rat uterus.
ID_{50}: the dose necessary to produce a 50% reduction of the 5×10^{-7} M 5-HT response.

Drugs	LSD	LEO	LEV	LAE	Hexyl-norLSD
$ID_{50} \pm$ S.E.M. ($\times 10^{-8}$ M)	1.2 ± 0.2	1.5 ± 0.1	5.4 ± 0.4	7.8 ± 1.5	6.1 ± 0.4
Number of experiments	6	7	6	6	6

TABLE 2

Effect of LSD on the responses to allyl-norLSD, ergometrine and Ba^{2+}.
N: number of experiments; N.S.: not significant.

Drugs (concentration, M)	n	Oxytocic activity (mean ± S.E.M.)	*p* value
Allyl-norLSD (1×10^{-8})	6	44.5 ± 3.3	
LSD (5×10^{-8}) + allyl-norLSD (1×10^{-8})	6	5.9 ± 3.4	<0.001
Ergometrine (3×10^{-7})	4	38.0 ± 3.3	
LSD (5×10^{-8}) + ergometrine (3×10^{-7})	4	15.0 ± 3.0	<0.001
Ba^{2+} (1×10^{-4})	5	34.2 ± 1.0	
LSD (5×10^{-8}) + Ba^{2+} (1×10^{-4})	5	41.4 ± 2.7	N.S.

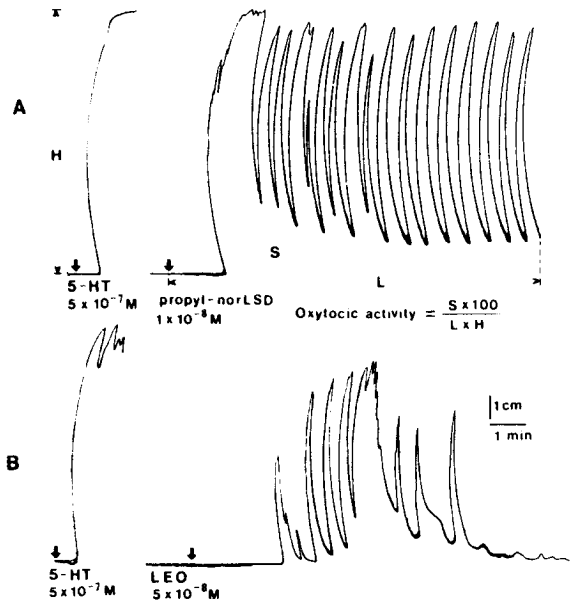


Fig. 3. Oxytocic actions of propyl-norLSD (A) and LEO (B) in the isolated rat uterus. The calculation of the oxytocic activity is also shown.

$\times 10^{-8}$ M and 1.7×10^{-8} M respectively. Nor-LSD was inactive at 1×10^{-8} M, but showed remarkable activities at higher concentrations. Ethyl-norLSD, allyl-norLSD and propyl-nor-LSD showed high activity even at a concentration of 3×10^{-9} M, and the ED_{50} values of these derivatives were smaller than that of LSD. The maximum activity of these compounds was far higher than that of LSD. Hexyl-norLSD was however inactive.

3.3. Effect of LSD on the oxytocic activity of allyl-norLSD

The oxytocic activity of allyl-norLSD and of ergometrine, one of the oxytocics used clinically, was inhibited by 5×10^{-8} M LSD (table 2, fig. 5). However, the same concentration of LSD did not significantly affect the response to Ba^{2+}.

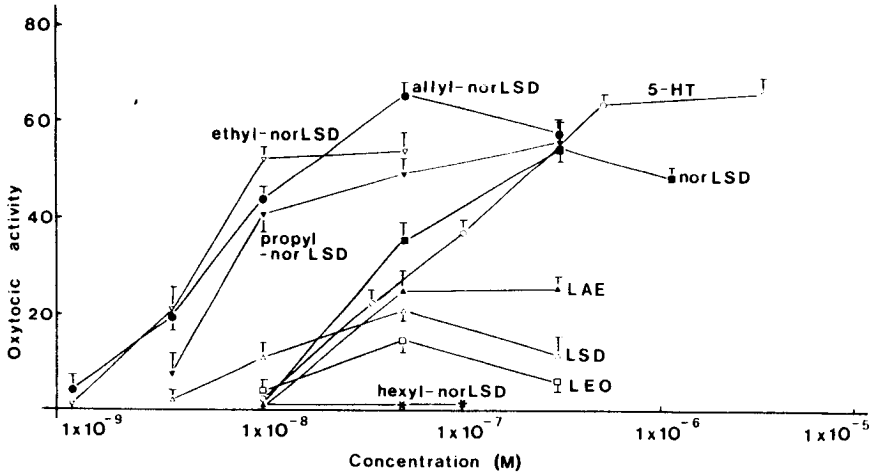


Fig. 4. Oxytocic activity of LSD derivatives in the isolated rat uterus. Each point is the mean of 4—19 experiments. The bars in the curves indicate the S.E.M.

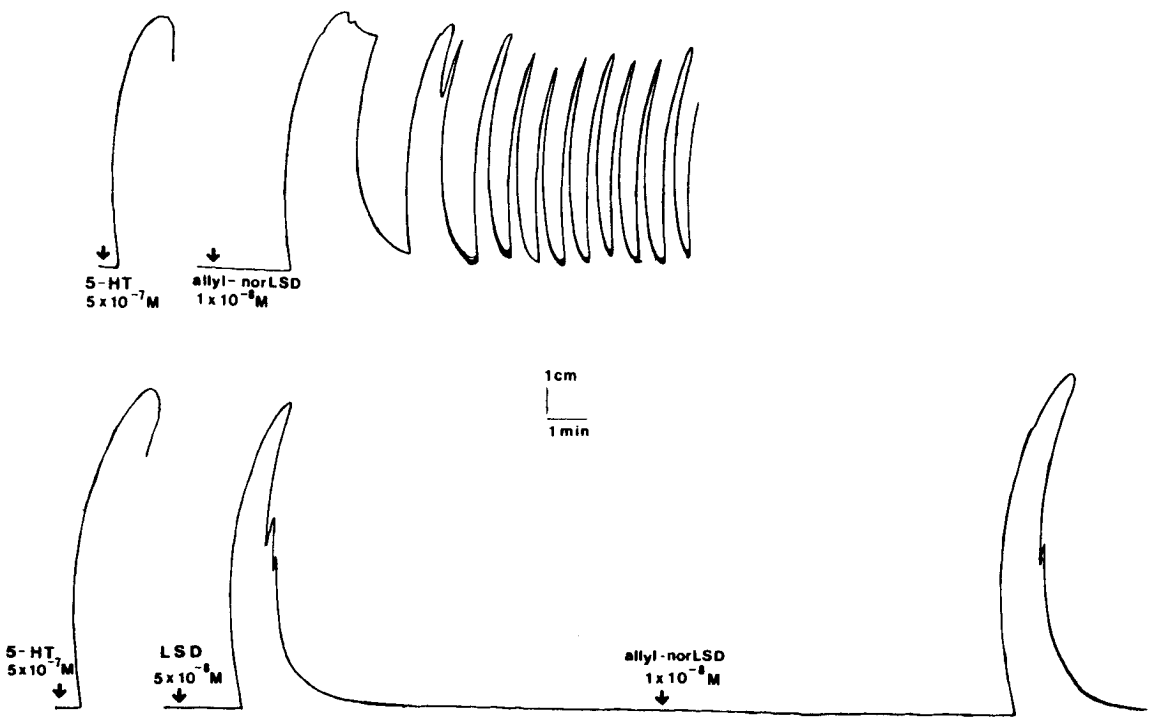


Fig. 5. Inhibitory effect of LSD on the allyl-norLSD response in the rat uterus. Top: control response; bottom: the response in the presence of LSD. LSD was added 10 min before the addition of allyl-norLSD.

3.4. Effects of cyproheptadine and atropine on the oxytocic activity of allyl-norLSD

The results are shown in table 3. A 5×10^{-8} M concentration of an anti-5-HT drug, cyproheptadine, inhibited the responses to 1×10^{-8} M allyl-norLSD and to 3×10^{-7} M ergometrine. The response to Ach was also significantly inhibited by cyproheptadine. Atropine (1×10^{-8} M) which completely inhibited the Ach response did not affect the allyl-norLSD response. The K^+ or Ba^{2+} responses were also significantly inhibited by cyproheptadine. However, the inhibition of these responses was less than that of the allyl-norLSD response.

4. Discussion

All the LSD derivatives tested had some anti-5-HT activity, but less than did LSD. Cerletti and Doepfner (1958) showed that the substitution of the amide group of LSD resulted in a low anti-5-HT potency. The present results are therefore consistent with the findings of these authors. Although N^6-alkyl substituted derivatives such as norLSD, ethyl-norLSD, propyl-norLSD and allyl-norLSD showed anti-5-HT activity, the exact values of the ID_{50} could not be determined as the derivatives themselves induced contractions, probably due to their high oxytocic activity.

All the derivatives except hexyl-norLSD showed oxytocic activity; the N^6-alkyl-substituted derivatives were moreover potent oxytocics. The oxytocic response to allyl-norLSD was strongly antagonized by LSD and cyproheptadine. Inhibition of the Ba^{2+} or K^+ response by these anti-5-HT drugs was weak, and atropine did not affect the response to allyl-norLSD. These results suggest that the oxytocic effects of allyl-norLSD and probably also of other LSD derivatives resemble the effect of 5-HT. The action of ergometrine was inhibited by both cyproheptadine and LSD, indicating that the action of allyl-norLSD was very similar to that of ergometrine.

The N^6-substituted derivatives other than hexyl-norLSD had maximum 5-HT like activities far higher than that of LSD, suggesting that the N^6-methyl group has an important

TABLE 3

Effects of cyproheptadine and atropine on the allyl-norLSD response in the rat uterus.
N: number of experiments; N.S.: not significant.

Drugs (concentration, M)	n	Oxytocic activity (mean ± S.E.M.)	*p* value
Allyl-norLSD (1×10^{-8})	6	41.5 ± 3.8	
Cyproheptadine (5×10^{-8}) + allyl-norLSD (1×10^{-8})	6	2.0 ± 2.0	<0.001
Ergometrine (3×10^{-7})	5	35.3 ± 2.5	
Cyproheptadine (5×10^{-8}) + ergometrine (3×10^{-7})	5	6.5 ± 2.8	<0.001
5-HT (5×10^{-7})	5	64.2 ± 2.0	
Cyproheptadine (5×10^{-8}) + 5-HT (5×10^{-7})	5	1.8 ± 1.3	<0.001
Acetylcholine (3×10^{-7})	5	74.0 ± 4.7	
Cyproheptadine (5×10^{-8}) + acetylcholine (3×10^{-7})	5	29.5 ± 2.8	<0.001
Allyl-norLSD (1×10^{-8})	6	42.5 ± 3.1	
Atropine (1×10^{-8}) + allyl-norLSD (1×10^{-8})	6	38.9 ± 1.0	N.S.
K^+ (22.5×10^{-3})	4	70.5 ± 5.5	
Cyproheptadine (5×10^{-8}) + K^+ (22.5×10^{-3})	4	52.4 ± 3.8	<0.05
Ba^{2+} (1×10^{-4})	5	35.4 ± 2.2	
Cyproheptadine (5×10^{-8}) + Ba^{2+} (1×10^{-4})	5	27.0 ± 2.2	<0.05

role in the action of LSD on 5-HT receptors. On the other hand, the minimum effective concentration as well as the ED_{50} of norLSD for the 5-HT like activity was higher than that of LSD or the N^6-substituted derivatives, suggesting that the N^6-alkyl group contributes at least partially to the high affinity of the latter compounds for 5-HT receptors. Hexyl-norLSD, however, showed no 5-HT like activity. The hexyl group is a long carbon chain and more lipophilic than the ethyl or the propyl group. These properties of the hexyl group may explain why hexyl-norLSD acted differently from other N^6-substituted derivatives. The amide-substituted derivatives such as LAE and LEO had a maximum 5-HT like activity not much different from that of LSD, indicating that the alkyl groups in the amide group may not largely affect the intrinsic activity of LSD. However, the ED_{50} values of LEO and LAE for the 5-HT like activity were higher than that of LSD, suggesting that the amide group of LSD contributes to the high affinity of LSD for 5-HT receptors. The fact that the ID_{50} values of amide-substituted derivatives for the anti-5-HT activity were greater than that of LSD supports this idea.

The 5-HT like activity of hallucinogenic drugs has been demonstrated using umbilical vasculature (Dyer and Grant, 1973; Dyer, 1974), dorsal metatarsal vein (Cheng et al., 1974) and in the central nervous system (Andén et al., 1968). There is some evidence to suggest a causal relationship between the 5-HT like effects of hallucinogenic drugs and their hallucinatory effects (Andén et al., 1968; Andén et al., 1971; Wallach et al., 1972; Cheng et al., 1974). As we have not tested the hallucinogenic activities of LSD metabolites (LAE, LEO, LEV and norLSD), the question of whether or not these compounds contribute to the hallucinogenic action of LSD is still unsettled. It has however been demonstrated that LAE, which has a low affinity for 5-HT receptors, showed a low psychotomimetic activity in man (Isbell et al., 1959). On the other hand, N^6-alkyl substituted derivatives such as ethyl-, propyl- and allyl-norLSD may have high pharmacological activities in the central nervous system as even in low concentrations these derivatives showed high 5-HT like activity. We have recently found that the hyperthermic effect of allyl-norLSD administered i.v. to rabbits was higher than that of LSD. The effects of these compounds on the central nervous system are now under investigation.

References

Andén, N.E., H. Corrodi, K. Fuxe and T. Hökfelt, 1968, Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide, Brit. J. Pharmacol. 34, 1.

Andén, N.E., H. Corrodi and K. Fuxe, 1971, Hallucinogenic drugs of the indolealkylamine type and central monoamine neurons, J. Pharmacol. Exptl. Therap. 179, 236.

Axelrod, J., R.O. Brady, B. Witkop and E.V. Evarts, 1956, Metabolism of lysergic acid diethylamide, Nature 178, 143.

Axelrod, J., R.O. Brady, B. Witkop and E.V. Evarts, 1957, The distribution and metabolism of lysergic acid diethylamide, Ann. N.Y. Acad. Sci. 66, 435.

Baker, R.W., C. Chothia and P. Pauling, 1973, Molecular structures of hallucinogenic substances: lysergic acid diethylamide, psilocybin and 2,4,5-trimethoxyamphetamine, Mol. Pharmacol. 9, 23.

Cerletti, A. and W. Doepfner, 1958, Comparative study on the serotonin antagonism of amide derivatives of lysergic acid and of ergot alkaloids, J. Pharmacol. Exptl. Therap. 122, 124.

Cheng, H.C., J.P. Long, D.E. Nichols and C.F. Barfknecht, 1974, Effects of psychotomimetics on vascular strips: Studies of methoxylated amphetamines and optical isomers of 2,5-dimethoxy-4-methylamphetamine and 2,5-dimethoxy-4-bromoamphetamine, J. Pharmacol. Exptl. Therap. 188, 114.

Dyer, D.C., 1974, Evidence for the action of d-lysergic acid diethylamide, mescaline and bufotenine on 5-hydroxytryptamine receptors in umbilical vasculature, J. Pharmacol. Exptl. Therap. 188, 336.

Dyer, D.C. and D.W. Grant, 1973, Vasoconstriction produced by hallucinogens on isolated human and sheep umbilical vasculature, J. Pharmacol. Exptl. Therap. 184, 366.

Hayashi, M., H. Ishii, T. Niwaguchi and Y. Nakahara, 1974, The microbial transformation of lysergic acid diethylamide (LSD) and its related compounds in streptomycetes, Proceedings of the 94th

Annual Meeting of the Pharmaceutical Society of Japan, April, Sendai, Japan, III, 114.

Isbell, H., E.J. Miner and C.R. Logan, 1959, Comparison of the reactions induced by psilocybin and LSD-25 in man, Psychopharmacologica 1, 20.

Nakahara, Y. and T. Niwaguchi, 1971, Studies of lysergic acid diethylamide and related compounds. I. Synthesis of d-N^6-demethyl lysergic acid diethylamide, Chem. Pharm. Bull. 19, 2337.

Nakahara, Y. and T. Niwaguchi, 1974, Studies on lysergic acid diethylamide and related compounds. III. Improvement of amidation of lysergic acid, Yakugaku Zasshi 94, 407.

Niwaguchi, T., T. Inoue and Y. Nakahara, 1974, Studies on enzymatic dealkylation of d-lysergic acid diethylamide (LSD), Biochem. Pharmacol. 23, 1073.

Niwaguchi, T., Y. Nakahara and H. Ishii, 1976, Studies on lysergic acid diethylamide and related compounds. IV. Synthesis of various amide derivatives of norlysergic acid and related compounds, Yakugaku Zasshi 96, 676.

Slaytor, M.B. and S.E. Wright, 1962, The metabolites of ergometrine and lysergic acid diethylamide in rat bile, J. Med. Pharm. Chem. 5, 483.

Szara, S., 1963, Enzymatic formation of a phenolic metabolite from lysergic acid diethylamide by rat liver microsomes, Life Sci. 9, 662.

Wallach, M.B., E. Freedman and S. Gershon, 1972, 2,5-dimethoxy-4-methylamphetamine (DOM), A Neuropharmacological examination, J. Pharmacol. Exptl. Therap. 182, 145.