

Antagonism of histamine-activated adenylate cyclase in brain by D-lysergic acid diethylamide

(histaminergic antagonists/adenosine 3':5'-cyclic monophosphate/H₂-receptors/ergots/D-2-bromolysergic acid diethylamide)

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ABSTRACT D-Lysergic acid diethylamide and D-2-bromolysergic acid diethylamide are competitive antagonists of the histamine activation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); E.C. 4.6.1.1] in broken cell preparations of the hippocampus and cortex of guinea pig brain. The adenylate cyclase is linked to the histamine H₂-receptor. Both D-lysergic acid diethylamide and D-2-bromolysergic acid diethylamide show topological congruency with potent H₂-antagonists. D-2-Bromolysergic acid diethylamide is 10 times more potent as an H₂-antagonist than cimetidine, which has been the most potent H₂-antagonist reported, and D-lysergic acid diethylamide is about equipotent to cimetidine. Blockade of H₂-receptors could contribute to the behavioral effects of D-2-bromolysergic acid diethylamide and D-lysergic acid diethylamide.

Evidence is growing that histamine may function as a neurotransmitter (see reviews, refs. 1-4). Histamine has a nonuniform regional distribution in brain. Most of the histamine is found in subcellular fractions containing nerve endings. Brain contains specific enzymes for histamine formation and metabolism. Histamine appears to turn over rapidly. Potassium ions release histamine from brain slices by a calcium-dependent process. Brain lesions result in a fall in the activity of histidine decarboxylase in areas distal to the lesion. Neurons respond to histamine. Histamine stimulates the activity of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); EC 4.6.1.1] and this effect is blocked by histamine H₁-antagonists (5, 6) and histamine H₂-antagonists (6, 7). We show here that D-lysergic acid diethylamide (D-LSD) and D-2-bromo-LSD (D-BrLSD) are competitive antagonists of histamine in the activation of the H₂-receptor linked to adenylate cyclase in the hippocampus and cortex of the brain.

MATERIALS AND METHODS

Preparation of Tissue. Membrane-bound adenylate cyclase was prepared from brain by homogenization in a Potter-Elvehjem glass-Teflon vessel in 0.32 M sucrose/5 mM Tris-HCl/1 mM ethylene glycol bis(β -aminoethyl ether)-N,N'-tetraacetate (EGTA)/1 mM dithiothreitol pH 7.4. The homogenate was centrifuged at 1000 \times g and the supernatant fraction was centrifuged again at 27,000 \times g. The pellet from the second centrifugation was washed twice by resuspension in the same medium and collected by centrifugation. The final pellet was suspended in the same medium at a protein concentration of 0.5-0.7 mg/ml. The membranes could be quickly frozen with dry ice/acetone and stored at -70°. Freezing and storage invariably resulted in an increase in basal activity and a decrease in maximal histamine activation, but the agonist potencies

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(ED₅₀; amount necessary to produce half-maximal response) and antagonist affinities (pA₂) were not altered.

Adenylate Cyclase Assay. The assay system has been described (8). All assays were performed in triplicate. All additions were made to the assay tubes on ice. They were then transferred to a 30° shaking incubator and preincubated for 5 min to allow the enzymatic activity to reach a steady state and to eliminate the influence of any lag periods in hormone activation. After the preincubation period, 25 μ l of [α -³²P]ATP (1-2 μ Ci) were added and in most cases the reaction was allowed to proceed for 10 min, when it was stopped by adding 100 μ l of 1% sodium dodecyl sulfate. After addition of 650 μ l of [³H]cyclic AMP ([³H]cAMP; 5000-10,000 cpm) to monitor recovery, the labeled cAMP was isolated with an alumina and Dowex column (9). The reaction was linear with protein concentration (10) in the range used and for at least 15 min after addition of the [α -³²P]ATP.

Treatment of the Data. Curve fitting techniques (11) were used to estimate the apparent ED₅₀ values, maximum stimulation by agonists, and parallelism of the dose-response curves. Antagonism was analyzed by Schild plots (12) in which antagonism is expressed by the dose-ratios (DR) of agonist needed to produce half-maximal responses in the presence and absence of different concentrations of antagonists (B). Simple competitive antagonism results in a straight line of slope 1 when log (DR - 1) is plotted against log B; and the intercept with the abscissa is -log K_B, where K_B is the apparent dissociation constant for the antagonist-receptor interaction; -log K_B is referred to as pA₂.

RESULTS

Histamine Receptor Linked to Adenylate Cyclase. Histamine stimulated adenylate cyclase activity in all areas of the guinea pig brain that were examined—cortex, hippocampus, thalamus, striatum, hypothalamus, and central grey; cortex and hippocampus were more sensitive than the other regions, as shown by others (7). Rat hippocampal adenylate cyclase was also stimulated by histamine (Table 1), but it was less sensitive to histamine than guinea pig hippocampal adenylate cyclase.

The ED₅₀ values for histamine, dimaprit, 4-methylhistamine, 2-methylhistamine, and N α ,N α -dimethylhistamine were 14 \pm 1, 6.4 \pm 0.45, 24, 120, and 21 \pm 2.3 μ M, respectively. Previous studies of histamine, 4-methylhistamine, and 2-methylhistamine on adenylate cyclase activity in broken cell preparations of the guinea pig hippocampus yielded similar ED₅₀ values (7). It is especially noteworthy that dimaprit, a compound with

Abbreviations: D-LSD, D-lysergic acid diethylamide; BrLSD, D-2-bromo-LSD; cAMP, cyclic AMP; ED₅₀, amount of agonist necessary to produce half-maximal response.

Table 1. Effect of histamine on rat hippocampal adenylate cyclase activity

Animal no.	Basal*	% increase in activity (\pm SEM) at histamine concentration		
		10^{-5} M	10^{-4} M	10^{-3} M
1	21.5 \pm 0.2	13.8 \pm 2.4 [†]	29.4 \pm 1.4 [†]	39.7 \pm 2.2 [†]
2	19.6 \pm 0.4	9.5 \pm 0.3 [†]	27.6 \pm 0.3 [†]	37.8 \pm 1.1 [†]
3	19.4 \pm 0.2	14.6 \pm 1.4 [†]	33.7 \pm 2.2 [†]	42.4 \pm 0.5 [†]

* The amount of homogenate protein was 193, 187, and 191 μ g, respectively, for the three animals. Activity is expressed as pmol of cAMP formed/min per mg of protein (\pm SEM).

[†] Significantly different ($P < 0.001$) from basal activity.

[‡] Significantly different ($P < 0.05$) from basal activity.

considerable H_2 -agonist activity but with less than 0.0001% of the activity of histamine on H_1 -receptors (13), was active in the same concentration range as was histamine.

Although measuring the relative potencies of agonists has been useful in classifying histamine receptors (14), these measurements are not dependable tools: the potencies of dimaprit and other agonists (13, 15) can vary 50-fold relative to histamine on different H_2 -receptors. More persuasive evidence for defining receptors comes from studies of antagonists (14). Fig. 1 shows typical dose-response curves for the effect of histamine on guinea pig hippocampal adenylate cyclase activity in the presence and absence of different concentrations of the H_2 -antagonist, cimetidine. Cimetidine caused a parallel shift in the dose-response curve. Also shown in Fig. 1 is the Schild plot for cimetidine with histamine or dimaprit as agonists. The fact that the dose-response curves of both histamine and the H_2 -selective dimaprit are shifted to the same degree by cimetidine clearly establishes that both agonists were reacting solely with H_2 -receptors linked to adenylate cyclase. The slope of the Schild plot (0.90 ± 0.07) did not differ significantly from the value of 1.0 which is predicted by assuming simple competitive kinetics. The pA_2 value was 6.22 ± 0.03 . The pA_2 value for cimetidine on dimaprit-stimulated adenylate cyclase activity in two preparations of membranes of the guinea pig neocortex was similar, 6.45 ± 0.19 . These are very close (Table 2) to the pA_2 values obtained (16–18) on other H_2 -receptors. Other H_2 -antagonists caused a parallel shift in the dose-response curves. The pA_2 values of these H_2 -antagonists on the histamine-activated adenylate cyclase activity in hippocampal membranes were also in agreement with the pA_2 values (16, 19, 20) on H_2 -receptors in other organs (Table 2).

At high concentration, the H_1 -antagonists (mepyramine and triptellamine) also blocked the histamine-activation of hippocampal adenylate cyclase activity. The pA_2 values of these antagonists on histamine-activated adenylate cyclase differed markedly from the pA_2 values on the H_1 -receptor (Table 2). These differences provide evidence that in blocking histamine-activation of the cyclase in the hippocampus, the H_1 -antagonists are not acting on H_1 -receptors. Importantly, the activation of the cyclase by dimaprit, a compound virtually devoid of H_1 -activity, was also blocked by H_1 -antagonists. On the hippocampal enzyme, the pA_2 value of mepyramine with histamine as agonist was 5.18 (Table 2); with dimaprit as agonist on two preparations, the pA_2 value was not significantly different, 5.04. These findings suggest that at these high concentrations the H_1 -antagonists block the H_2 -receptor.

We compared, on the hippocampus from two guinea pigs, the effects of histamine, norepinephrine, isoproterenol, and dopamine in the activation of adenylate cyclase. At a concentration of 10^{-4} M, activation averaged 107.5 ± 4.2 (SEM)% for

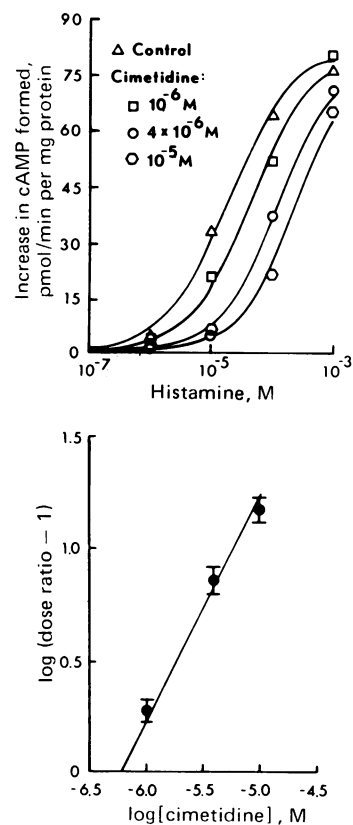


FIG. 1. (Upper) Increase in adenylate cyclase activity of the guinea pig hippocampus with varying concentrations of histamine in the absence and presence of cimetidine. Each point is the mean of triplicate determinations. Cimetidine alone did not affect basal activity, which was 79.6 ± 1.1 pmol/min per mg of protein. (Lower) Schild plot for inhibition by cimetidine of histamine-stimulated adenylate cyclase activity in the guinea pig hippocampus. Ten determinations were made on four preparations with histamine as the agonist, and two determinations on one preparation with dimaprit as agonist. The dose ratios were calculated as the ratio of the ED_{50} of the agonist in the presence of cimetidine to the ED_{50} in the absence of cimetidine. The line has unit slope and an x -intercept (pA_2 value) of 6.22.

histamine, $37.0 \pm 2.5\%$ for norepinephrine, $23.7 \pm 0.7\%$ for isoproterenol, and $24.7 \pm 2.6\%$ for dopamine. Cimetidine, 10^{-4} M, reduced histamine activation by $65.5 \pm 1.5\%$, without reducing activation by the three catecholamines.

Effects of D-LSD and Related Compounds. D-LSD blocked both dimaprit- and histamine-activated guinea pig hippocampal and neocortical adenylate cyclase without affecting basal activity (Fig. 2). The Schild plot (Fig. 2), resulting from 17 measurements on 10 hippocampal preparations, had a slope of 1.09 ± 0.11 , implying competitive inhibition. The pA_2 value for D-LSD on the hippocampal enzyme was 5.95 ± 0.03 and on the neocortical enzyme, 6.07 ± 0.12 . Thus, D-LSD has a potency on H_2 -receptors similar to that of the potent H_2 -antagonists, metiamide and cimetidine. The L-isomer of LSD at 10^{-4} M was without effect on histamine-activated adenylate cyclase. BrLSD was about 10 times more potent than D-LSD. The Schild plot for BrLSD had a slope of 1.14 ± 0.10 and the pA_2 value, derived from 17 determinations on seven preparations, was 7.16 ± 0.04 . BrLSD, unlike D-LSD, consistently depressed basal activity, up to 20% at the highest concentrations tested; this decrease was subtracted in the analysis. Neither psilocin (10^{-4} M) nor mescaline (10^{-3} M) blocked histamine-activation of hippocampal adenylate cyclase activity.

Table 2. pA₂ values of histamine antagonists on adenylate cyclase in the hippocampus compared with pA₂ values on pharmacological preparations

Antagonists	pA ₂ on adenylate cyclase activity: hippocampus, guinea pig	PA ₂ on pharmacological preparations		
		H ₂ -receptors		H ₁ -receptor:
		Atrium, guinea pig	Uterus, rat	Ileum, guinea pig
H₂-antagonists				
Imidazolylpropyl-methylthiourea	3.33 ± 0.02 (2)	3.5 (ref. 16)	—	—
N ^α -Guanylhistamine	4.14 ± 0.13 (2)	3.9 (ref. 16)	—	3.8 (ref. 16)
Imidazolylpropyl-guanidine	5.50 ± 0.02 (2)	4.65 (ref. 17)	—	—
Thiaburimamide	5.62 ± 0.07 (2)	5.49 (ref. 18)	5.49 (ref. 18)	—
Metiamide	6.06 ± 0.14 (2)*	6.04 (ref. 18)	6.12 (ref. 18)	—
Cimetidine	6.22 ± 0.03 (12)	6.10 (ref. 20)	6.09 (ref. 20)	3.4 (ref. 20)
H₁-antagonists				
Mepyramine	5.18 ± 0.04 (8)	5.3 (ref. 21)	—	9.4 (ref. 12)
Tripellennamine	5.49 ± 0.03 (3)	—	—	8.5 (ref. 22)
Cyproheptadine	7.43 ± 0.04 (5)	—	—	8.3 (ref. 23)

Values are means ± SEM. In parentheses are the number of animals studied.
* Hegstrand *et al.* (7) found 6.06.

DISCUSSION

Histamine Receptor. Histamine-sensitive adenylate cyclase was prominent in the hippocampus and cortex, which contain far less histamine than other parts of the brain, e.g., hypothalamus, thalamus, and central grey (see refs. 1 and 2) that showed relatively little histamine-stimulated adenylate cyclase activity. The receptors for histamine may be either more abundant or the adenylate cyclase may be more sensitive in the hippocampus and cortex than in other areas of the brain.

Unlike others (7), we found that adenylate cyclase in the rat hippocampal homogenates was also stimulated by histamine (Table 1). Our assay medium differed in containing dithiothreitol, cAMP, a system to generate ATP, theophylline (4 mM) instead of 3-isobutyl-1-methylxanthine (1 mM), half the concentration of EGTA, 10⁻⁵ M of GTP rather than 10⁻⁴ M, and a slightly lower pH (7.4 rather than 7.8); the basal activity in our experiments on rats was about one-third that found by others (7). Activation by histamine of adenylate cyclase in the rat hippocampus was less than that in guinea pig hippocampus, in accord with the relative insensitivity of rats to many effects of histamine (24).

The histamine-stimulated adenylate cyclase in broken cell preparations of the hippocampus of the guinea pig is linked to an H₂-receptor, as previously shown (7). The H₂-antagonist competitively blocked this effect of histamine, shifting the dose-response curve in a parallel manner, as shown for cimetidine (Fig. 1). Dimaprit, an H₂-agonist with almost no H₁-activity (13), activated the cyclase, and cimetidine blocked this effect of dimaprit. The unit slope of the Schild plot for cimetidine, with either histamine or dimaprit as agonist (Fig. 1), implies simple competition (12). The pA₂ values of a series of known H₂-antagonists in inhibiting the histamine- or dimaprit-activated cyclase are the same as the pA₂ values on known H₂-systems (Table 2)—the histamine receptor linked to atrial rate, uterine relaxation, and gastric acid secretion (16–20). All of these were classified as H₂-systems mainly because the pA₂ values of H₂-antagonists were very nearly the same on all three preparations (14).

The H₁-antagonists also blocked histamine-activated cyclase, shifting the dose-response curves in a parallel manner. However, the pA₂ values on the histamine-linked cyclase were low, i.e., the concentration of H₁-antagonist needed to block this

effect was far higher than that needed to block the H₁-receptor in the guinea pig ileum (Table 2). At these concentrations, the H₁-antagonists are therefore not acting on the H₁-receptor but rather on another one, almost certainly the H₂-receptor: only

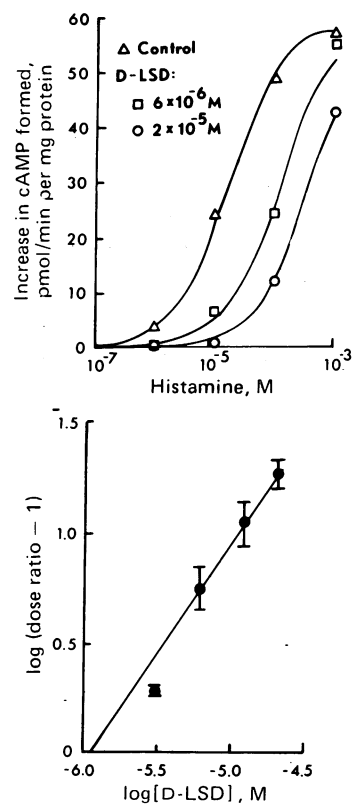


FIG. 2. (Upper) Increase in adenylate cyclase activity of the guinea pig hippocampus in response to varying concentrations of histamine in the absence and presence of D-LSD. Each point is the mean of triplicate determinations on a single enzyme preparation. D-LSD alone did not affect basal activity, which was 92.1 ± 1.7 pmol/min per mg of protein. (Lower) Schild plot for inhibition by D-LSD of histamine-stimulated adenylate cyclase activity in the guinea pig hippocampus. The dose ratios were calculated as the ratio of the ED₅₀ of histamine in the presence of D-LSD to the ED₅₀ in the absence of D-LSD. The line has unit slope and an x-intercept (pA₂ value) of 5.95.

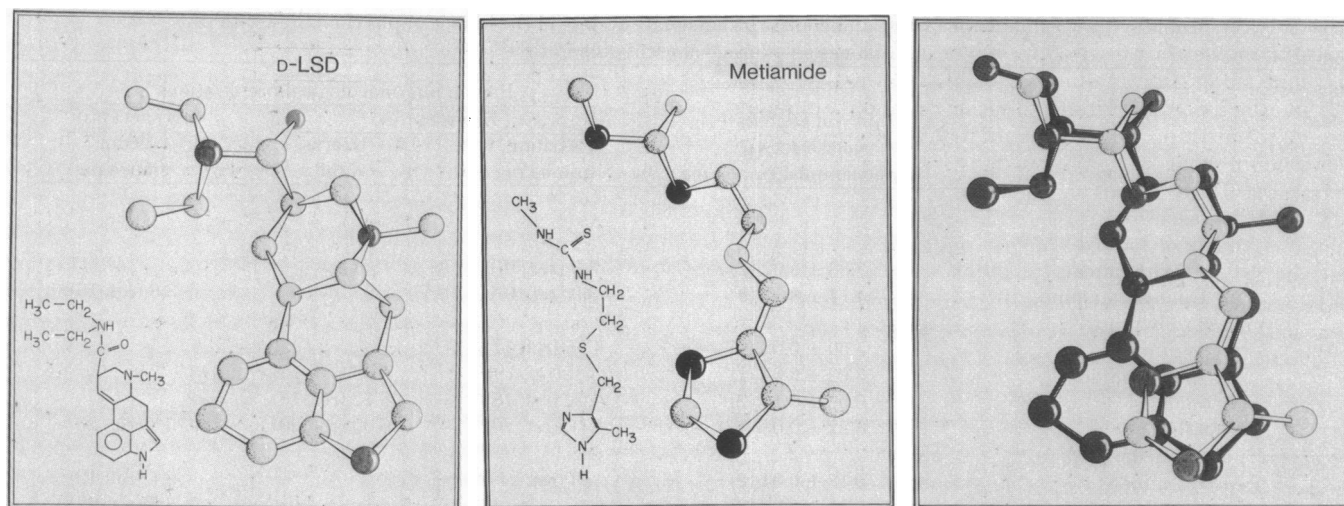


FIG. 3. Structure of (Left) D-LSD and (Center) metiamide and (Right) superimposition of the two.

an H_2 -receptor could be shown in these broken cell preparations (ref. 7; Table 2), and second, mepyramine blocked the activation by dimaprit, which is almost exclusively an H_2 -agonist.

In many previous studies of histamine activation of adenylate cyclase, notably those on brain slices, both H_1 - and H_2 -antagonists were observed to block the effect of histamine in stimulating adenylate cyclase activity. These observations prompted the conclusion that both H_1 - and H_2 -receptors mediate the stimulation. The H_1 -receptor may be active in slices and not in broken cell preparations. However, in many of the experiments on brain slices, only one concentration of antagonist was used and in none were the antagonist affinities estimated to compare with their affinities for well-defined H_1 - and H_2 -receptors.

Histamine and Pharmacology of D-LSD and BrLSD. D-LSD is a competitive antagonist of histamine at the H_2 -receptor. The pA_2 value of D-LSD was 5.95, very nearly that of cimetidine, 6.22, and of metiamide, 6.06, the two potent H_2 -antagonists. The L-isomer, which has no measurable central effects, did not antagonize the histamine-activated adenylate cyclase. BrLSD ($pA_2 = 7.16$) was about 10 times more potent than D-LSD in inhibiting histamine-stimulated adenylate cyclase activity.

BrLSD is active, though less potent than D-LSD, on many behavioral (25, 26) and electrophysiological (27, 28) systems, but it has about the same affinities as does D-LSD for the high-affinity binding sites for D-LSD (29, 30) and a greater affinity for the haloperidol binding sites (31). BrLSD was also more potent than D-LSD in increasing levels of dopa (32) and a dopamine metabolite, 3,4-dihydroxyphenylacetic acid (33), in rat striatum. Thus, agonist activity of BrLSD at dopaminergic (26) and serotonergic (27) sites is less than that of D-LSD but the antagonist activity at dopaminergic sites appears to be greater than that of D-LSD (31–33). Further evidence that D-LSD and BrLSD have affinities for the same receptors is that BrLSD blocks the effect of D-LSD on isolated tissue (34) and its behavioral effect in mice (35). Some of the receptors for which BrLSD has affinity are likely to be implicated in the hallucinations produced by D-LSD, for BrLSD blocks the hallucinogenic effect of D-LSD (36–38). BrLSD does not produce hallucinations but it causes other psychic effects qualitatively the same as LSD, including confusion, sensations of unreality, and depersonalization (38–40).

Antagonism of H_2 -receptors could contribute to the central and other pharmacological effects of D-LSD and BrLSD. The

affinities of histamine antagonists for the histamine receptor linked to adenylate cyclase are strikingly similar to their affinities for physiologically functioning receptors (Table 2). This agreement suggests that the action of other drugs on the cyclase reflects these physiological effects. Experiments in different species show that the concentration of D-LSD in brain may be sufficient to occupy some of the H_2 -receptors. In laboratory animals (25–28, 32, 33, 35), larger doses of D-LSD are required to elicit central effects than are needed in human beings (36–42). In rats (43) [as in cats (44)], after administration of 1 mg of D-LSD/kg of body weight the concentration in brain is 0.6 $\mu\text{g/g}$ or 2 $\mu\text{mol/kg}$; after 0.2 mg of D-LSD/kg of body weight, the concentration is 140 ng/g or 0.5 $\mu\text{mol/kg}$ (45, 46). Guinea pigs require a dose 5–12 times higher than do rats to exhibit a similar behavioral response (47), and the measured K_B of D-LSD for the histamine receptor is 1.1 μM . In addition, D-LSD is concentrated in the hippocampus both in monkeys (48) and rats (49). The hippocampal concentration, measured in monkeys (48) after the administration of 0.5 mg of D-LSD/kg of body weight, was 0.39 $\mu\text{g/g}$ or 1.3 $\mu\text{mol/kg}$, which is greater than the apparent K_B for occupying the H_2 -receptor linked to adenylate cyclase.

The hippocampus as a site of interaction of histamine and D-LSD may have special importance. D-LSD and lesions of the hippocampus produce similar effects. Both enhance some types of perseveration (50, 51) and both interfere with some kinds of habituation, e.g., after these treatments an animal responds to a stimulus to which it had previously been refractory (51–54). D-LSD causes discharges from the hippocampus (41, 55, 56) and enhances the response of the hippocampus to afferent stimulation (57). There is evidence that histaminergic fibers terminate in the hippocampus (58, 59) and that histamine, perhaps endogenous histamine, depresses the firing of hippocampal cells by acting on H_2 -receptors (60). D-LSD may cause discharge of the cells by blocking, among other substances, histamine; perhaps this could account for some of the more subtle effects of D-LSD, such as interference with habituation and enhancement of perseveration. It is not likely that blockade of the H_2 -receptor alone (or any other single action of these drugs) can account for all the behavioral and numerous other pharmacological effects of D-LSD or BrLSD. Affinities for different receptors were shown by biochemical studies (29–33, 61) noted above; early electrophysiological studies showed that LSD affects more than one central receptor (62).

D-LSD and H_2 -blockers have some common structural

characteristics, as shown for metiamide (Fig. 3). The residue in D-LSD that is sterically congruent with metiamide is hexanoic acid-4-aza-4-methyl-6-(3-pyrrolyl)-*N,N*-diethylamide. This and the indolyl and imidazolyl analogs and their derivatives may have H₂-antagonistic activity. Heretofore the only known compounds with H₂-blocking activity were imidazole derivatives (63, 64).

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