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Defining the Histamine H_2-Receptor in Brain: The Interaction With LSD

Jack Peter Green, Harel Weinstein, and Saul Maayani

Numerous studies have shown that LSD (D-lysergic acid diethylamide) affects serotonergic, dopaminergic, adrenergic, and tryptaminergic systems in the central nervous system (see Mandell and Geyer, 1976; Martin and Sloan 1977; Freedman and Halaris 1978). To this list is now added a histaminergic system. For LSD is a competitive antagonist of the histamine H_2-receptor in brain (Green et al. 1977).

In describing this action of LSD, we give an account of the established criteria that we followed to define the receptor with which LSD reacts. This definition fostered an analysis of the relationship between the molecular structure of known H_2-antagonists and LSD. This results in the correct prediction of a new H_2-antagonist.

The action of LSD on a histamine receptor in brain may be relevant to the neural effects of LSD. For histamine may be a transmitter in brain. Evidence for this function of histamine remains circumstantial but perhaps no more so than that offered for most other biogenic amines that are classified as putative transmitters. In fact, as recently reviewed (Green, Johnson and Weinstein 1978), studies of mammalian brain show that histamine meets most criteria for having transmitter function. It has non-uniform distribution with highest concentration in the hypothalamus. Subcellular fractions of brain containing nerve-endings are rich in histamine. Brain contains the specific enzyme that decarboxylates histidine to form histamine as well as the specific enzyme that metabolizes histamine. After destroying fibers in the median forebrain bundle or the afferent fibers to the hippocampus the activity of the specific histidine decarboxylase falls in brain regions distal to the lesion. Histamine is released from brain slices by potassium ions in a process that is dependent on ionic calcium. Histamine turns over rapidly: unlike other aromatic biogenic amines, it is not taken up by presynaptic terminals but is instead metabolized to 3-methylhistamine which is then oxidatively deaminated by monoamine oxidase B. Neurons respond to histamine, e.g., it decreases the firing rate of cerebral cortical and brainstem neurons and increases the firing rate of hypothalamic neurons. Especially provocative is the observation that electrical stimulation of

afferent fibers to the cerebral cortex or hippocampus reduces their firing rate, and part of this effect of electrical stimulation can be blocked by histamine H_2-antagonists. As described below, histamine increases adenylate cyclase activity in brain, and this effect is blocked by specific antagonists.

Evidence that LSD and D-2-bromo-LSD block the histamine H_2-receptor is presented here, and the implications are discussed.

THE CLASSIFICATION OF A RECEPTOR IN A TISSUE

Studies aimed at revealing the mechanisms of action of a drug by observing effects in intact animals are especially difficult when applied to the brain because of the awesomely complex multineuronal interactions. For example, treating rats with either LSD or mescaline decreased the firing rate of the raphe neurons but only LSD did this when applied directly to the neurons (Haigler and Aghajanian 1973), mescaline affecting the firing rate by acting indirectly (Gallagher and Aghajanian 1976). The receptors that a drug acts upon can be revealed less ambiguously by studies on isolated preparations in vitro. Towards this end, two systems have been widely used in recent years for studies of receptors in brain and other tissues. One is the study of high affinity binding of ligands to membranes. The other is the measurement of adenylate or guanylate cyclase activity which can reflect interactions of ligands with the receptor if the receptor is coupled to the enzyme. Direct actions of drugs on the cyclase can be ruled out. High affinity binding requires a ligand of high specific activity and of high affinity for the receptor. Both these criteria were met with tritiated pyrilamine, an H_1-antagonist, which was used to label the H_1-receptor in guinea pig (Hill, Young, and Marrian 1977). The low affinity of the known H_2-antagonists for the H_2-receptor requires a very highly labeled ligand to study the H_2-receptor, and this is not available. Adenylate cyclase activity was therefore used.

Whatever system is used, it is imperative that, whenever possible, the receptor regulating a physiological or biochemical function be defined, and that the kinetics of the interaction with the agonist and antagonist be described. Agonists with high selectivity for a specific receptor, e.g., isoproterenol for the β-adrenergic receptor or dimaprit for the histamine H_2-receptor, are useful but uncommon tools. The receptor may be defined by measuring the relative potencies of a series of agonists. For example, histamine stimulates rat gastric acid secretion, relaxes the rat uterus, and increases guinea pig atrial rate. The agonist activities relative to histamine of a series of agonists on these effects were indistinguishable, which suggested that the receptors were the same or at least very similar (Ash and Schild 1966; Black et al. 1972): they were classified as histamine H_2-receptors. Earlier, the relative potencies of agonists in contracting the guinea pig ileum and the rat stomach were in close agreement, implying that the receptors were very similar or the same: they were classified as histamine H_1-receptors. Such classifications demand that the slope of the dose-response curves for each agonist be the same; that all agonists

produce the same level of maximum response, differing only in affinity; and that the time of onset and offset of each agonist be similar. These criteria were met in a study on the relative potencies of a series of tryptamines in contracting the isolated stomach strip of the rat, an effect blocked by LSD and its 2-bromo analogue; the relative potencies paralleled their potencies in blocking LSD binding to brain membranes (Green et al. 1978).

While agonists have proved extremely useful in helping to classify receptors, they may yield discordant results. For example, the potency of dimaprit, an H_2-agonist, varies as much as fiftyfold relative to histamine on different H_2-receptors (Durant, Ganellin, and Parson 1975; Parsons et al. 1977). The use of competitive antagonists has provided less ambiguity in the classification of receptors. The effect of a substance directly on the tissue response, e.g., a direct action on muscle contraction is not annulled by a competitive antagonist, while the effect mediated by activation of the receptor is. Furthermore, the displacement of the dose-response curves by the antagonist is not dependent on the affinity of the agonist. Parallel displacement of the log dose-response curve by the antagonist suggests that the agonist and antagonist are acting on the same receptor. From the ED_{50} values in the presence and absence of antagonist, one can estimate the apparent dissociation constant, K_B, by estimating the concentration of antagonist required to give a dose ratio of two, i.e., the concentration of antagonist that requires doubling the concentration of agonist to obtain the same effect as obtained in the absence of antagonist. A more rigorous way to classify a receptor is to measure the displacement of the dose-response curves of an agonist by different concentrations of the antagonists. Antagonism is expressed by the dose-ratios (DR) of agonist needed to produce equal responses, e.g., the ED_{50}, in the presence and absence of different concentrations of antagonists (B). Simple competitive antagonism results in a straight line of 1 when log (DR-1) is plotted against log (B); the intercept with the abscissa is -log K_B, which is referred to as pA_2 (Schild 1947; Arunlakshana and Schild 1959). Yet more reassuring is to estimate the pA_2 value of an antagonist with different agonists. The H_1-receptor (Ash and Schild 1966) and the H_2-receptor (Black et al. 1972) were defined in this way. Agonists with very different relative potencies yielded indistinguishable pA_2 values. The use of more than one agonist - and of more than one antagonist as well - can help to avoid false inferences due to adventitious effects, e.g., effects on release, uptake, metabolizing enzymes - any of which can vitiate the estimate of pA_2.

A judicious and mindful use of these methods, this "taxonomy of receptors" (Black 1976), has led to order and understanding and fecundity. It yields new drugs such as the β-adrenergic blockers and, more recently, cimetidine, the histamine H_2-antagonist which is proving so effective in the treatment of duodenal ulcer. This and other H_2-antagonists were then used as tools to reveal the H_2-receptor and its associated functions in many tissues (Chand and Eyre 1975) and the action of known drugs on this receptor - one consequence of which was the discovery of new H_2-antagonists as described below. Knowing that a receptor for a mediator is

Table 1. Apparent dissociation constants (K_B) and pA_2 values of histamine H_2-antagonists on adenylate cyclase in the guinea pig hippocampus homogenates compared with pA_2 values on pharmacological preparations

Antagonists	Adenylate cyclase activity Hippocampus (guinea pig) K_B	pA_2	H_2-receptor in pharmacological preparations* pA_2
Imidazolylpropyl-methylthiourea	4.68×10^{-4}	3.33	3.50
N^{α}-guanylhistamine	7.94×10^{-5}	4.14	3.90
Imidazolylpropyl-guanidine	3.16×10^{-6}	5.50	4.65
Thiaburimamide	2.39×10^{-6}	5.62	5.50
Metiamide	8.71×10^{-7}	6.06	6.03
Cimetidine	6.03×10^{-7}	6.22	6.10

*Guinea pig atrium and rat uterus (Ganellin 1978)

homogeneous in different tissues provides prediction of side-effects and contraindications, e.g., that β-adrenergic blockers would be contraindicated in patients with asthma. Showing that the receptor for a mediator in different tissues is homogeneous provides convenience: the search for antagonists of histamine at the H_2-receptor was facilitated after it was established that the receptor associated with gastric secretion was indistinguishable from that associated with guinea pig atrial rate, for effects on the latter are more accurately and rapidly measured. Of more pervasive importance, the demonstration of a homogeneity of a receptor in different tissues offers the opportunity to study the receptor in a more tractable milieu. This is salient to the subject of this discussion: the histamine H_2-receptor linked to adenylate cyclase in brain is not distinguishable from that in the guinea pig heart, in the rat uterus or in the parietal cells of the stomach. The cyclase system and the peripheral systems can now be studied as a target of psychotropic drugs. And the relationship between chemical structure and biological activity becomes amenable to study on a well defined system.

Although these procedures, which rest on receptor theory, have been used for over fifty years, there appears to be a surprising innocence of them. For example, it has been repeatedly asserted that the histamine stimulated adenylate cyclase in brain slices is linked to the H_1-receptor. The evidence offered to support this is that the effect of histamine on cyclase is blocked by H_1-antagonists. In all these studies, the H_1-antagonists were used at concentrations of 10^{-6} M to 10^{-4} M. The pA_2 values for the commonly used H_1-antagonists, pyrilamine (also called mepyramine) and tripellenamine, are 9.4 and 8.5 respectively or, in K_B values, 4×10^{-10} M and 3.2×10^{-9} M. Testing an antagonist at a concentration greater than 100 times the K_B is likely to block more than the specific receptor. Non-specificity of many antagonists at high doses is well known; the H_1-antagonists in suitable concentrations are anticholinergic (see Nauta and Rekker 1978; van den Brink and Lien 1978). More to the point, at concentrations of 10^{-6} M and greater, H_1-antagonists block the H_2-receptor

*Table 2. Effect of histamine on adenylate cyclase activity in fresh membranes from different regions of the guinea pig brain**

Brain Area	Basal	Histamine Concentration, M			
		10^{-6}	10^{-5}	10^{-4}	10^{-3}
		Per cent increase in activity (±s.e.)			
Cortex	48.3±0.9	8.5±1.0†	1.1±0.4§	106.2±1.4§	117.6±2.4§
Hippocampus	73.8±0.6	5.7±0.85‡	29.1±0.76¶	86.7±1.1§	103.9±0.3§
Thalamus	18.3±0.52	7.2±1.7†	32.8±3.7¶	49.4±1.1¶	46.6±2.1¶
Striatum	89.4±1.04	3.6±1.0‡	10.5±0.8¶	20.7±5.7‖	28.0±7.4‖
Hypothalamus	42.9±0.15	3.9±0.3¶	9.6±1.4¶	15.3±2.0¶	15.0±0.5¶
Central Gray	56.8±0.7	2.2±0.7‡	4.9±1.8†	10.0±1.8‖	8.4±1.6‖

*This represents one series of experiments on membranes. Each region was studied at least twice with similar results.
†pmoles of c-AMP formed/min/mg protein (±s.e.)
‡not significantly different from basal activity
§significantly different ($p<0.001$) from basal activity
¶significantly different ($p<0.005$) from basal activity
‖significantly different ($p<0.025$) from basal activity

linked to adenylate cyclase in homogenates of both brain and heart ventricle of the guinea pig (see below).

The H_2-antagonists have lower affinity for their receptor than have the H_1-antagonists for the H_1-receptor. Cimetidine has a pA_2 value of 6.10 ($K_B = 8 \times 10^{-5}$ M). Clearly, a higher concentration of H_2-antagonist, e.g., cimetidine, is needed to block the H_2-receptor, than the concentration of H_1-antagonist needed to block the H_1-receptor. Yet in some work purporting to define a receptor, equal concentrations of each type of antagonist is used, commonly 10^{-6} M, which is near the K_B of the H_2-antagonist but a thousand times the K_B of the H_1-antagonist. Not surprisingly, at these concentrations the H_2-antagonist has no or slight effect while the H_1-antagonist blocks histamine, prompting the (false) inference that the activity is linked to the H_1-receptor. Confusion can also result from the use of only one concentration of agonist, e.g., histamine. As the antagonism is competitive and surmountable by increasing doses of agonist, one dose of agonist is unrevealing, for if the concentration is too high, it will surmount the effect of the antagonist, as shown by a glance at the dose-response curves below.

THE HISTAMINE RECEPTOR IN BRAIN

Parallel displacement of the dose-response curves to histamine by metiamide, an H_2-antagonist, indicated that histamine-stimulated adenylate cyclase in guinea pig brain homogenates is linked to an H_2-receptor. The pA_2 of metiamide on this system was the same as that on known H_2-systems. The relative potencies of two other agonists, 4-methylhistamine and 2-methylhistamine, were also consonant with stimulation of the H_2-receptor (Hegstrand, Kanof, and Greengard 1976). This conclusion was supported by observations on the same preparation with additional agonists and six H_2-antagonists. The pA_2 values of the six antagonists (Green et al. 1977) were very similar to those on the H_2-receptors in pharmacological

preparations (see Ganellin 1978) and on the H_2-receptor linked to adenylate cyclase in guinea pig cardiac ventricle (C.L. Johnson, unpublished work). Table 1 shows the results on the guinea pig hippocampus. Simple competitive antagonism was further shown by a Schild plot with cimetidine as antagonist which resulted in a straight line of slope 1. When dimaprit was used as agonist instead of histamine, the points fell on the same line (Green et al. 1977). Dimaprit is virtually an exclusive H_2-agonist, having less than 0.0001 per cent of the activity of histamine on H_1-receptors (Parsons et al. 1977). Homogenates of the cerebral cortex and hippocampus were not distinguishable in their response to agonists and antagonists. It is thus clear that histamine stimulated adenylate cyclase in homogenates of guinea pig hippocampus and cortex is linked to an H_2-receptor. (No extrapolations can be made to brain slices.)

Table 2 shows that the histamine stimulated adenylate cyclase activity is more sensitive in homogenates of the cerebral cortex and hippocampus than in other parts of the brain. Neither of these regions is especially rich in histamine. This distribution confirms previous findings (Hegstrand, Kanof, and Greengard 1976), except that we showed activity in the hypothalamus and central gray. In the cortex of guinea pigs, activity was highest in subcellular fractions containing nerve endings (Kanof, Hegstrand, and Greengard 1977). The activity was present in homogenates of the rat hippocampus (Green et al. 1977) and the rat cortex (Hegstrand, Kanof, and Greengard 1976), but the corresponding portions of guinea pig brain were more sensitive.

It may be worth noting that adenylate cyclase in the hippocampus homogenates is more sensitive to histamine than to norepinephrine and dopamine. At a concentration of 10^{-4} M, the respective percentage increases were 107.5, 37.0, and 24.7. Cimetidine, 10^{-4} M, reduced histamine activation by 65.5 per cent without reducing activation by the catecholamines (Green et al. 1977).

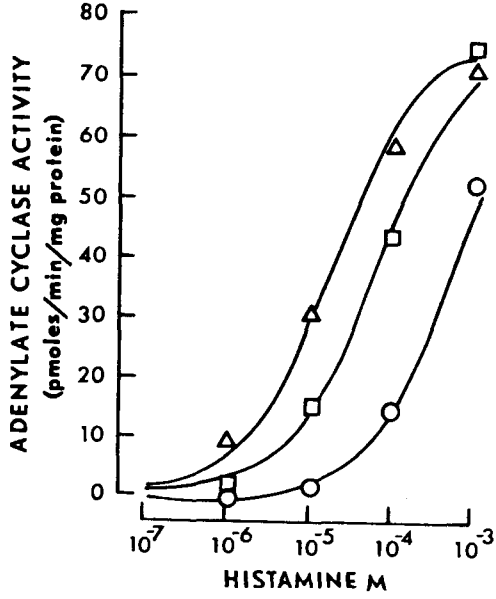
The H_1-antagonists also antagonized histamine stimulated adenylate cyclase, as shown for cyproheptadine (Fig. 1). But to do so required concentrations of pyrilamine and tripellennamine about a thousand times those required to block the H_1-receptor. For cyproheptadine, the corresponding ratio was ten times (Table 3). These differences in pA_2 values show that the H_1-antagonists were not blocking the H_1-receptor linked to cyclase. Further, the H_1-antagonists blocked stimulation of the cyclase by dimaprit which is selective for the H_2-receptor and is virtually devoid of activity on the H_1-receptor. Hence, the H_1-antagonists at high concentration block the H_2-receptor.

Cyproheptadine has greater affinity for the H_2-receptor (K_B = 3.7 x 10^{-8} M) than any substance yet described, including the H_2-antagonists that have been used clinically, metiamide and cimetidine.

CYPROHEPTADINE AS ANTAGONIST OF MANY RECEPTORS

Although often called a "serotonin antagonist", cyproheptadine has no special penchant for serotonin receptors. Table 4 shows the K_B

Fig. 1. Adenylate cyclase activity of the guinea pig hippocampus in response to varying concentrations of histamine in the absence (Δ) and presence of 10^{-7} M (□) or 10^{-6} M (o) cyproheptadine. Each point represents the increase in adenylate cyclase activity above basal level (no histamine) and is the mean of triplicate determinations on a single enzyme preparation.



(and pA_2) values of cyproheptadine on different receptors, listed in order of affinity. It has highest affinity for the H_1- and H_2-receptors and least for the serotonin receptor. The values for the serotonin and LSD receptors were estimated from published IC_{50} values as noted in the table. Cyproheptadine also antagonizes acetylcholine (Rocha e Silva and Leme 1972) and epinephrine (Stone et al. 1961).

COMPETITIVE BLOCKADE OF THE H_2-RECEPTOR BY LSD AND D-2-BROMOLYSERGIC ACID DIETHYLAMIDE (BrLSD)

Because cyproheptadine has high affinity for the H_2-receptor and it blocks 5-HT, it seemed appropriate to test LSD which also has affinity for 5-HT receptors. LSD alone did not increase adenylate cyclase activity in homogenates of the guinea pig hippocampus and cortex [as it does in rat striatum (Von Hungen, Roberts, and Hill 1975)]. But it blocked histamine or dimaprit stimulated cyclase in a competitive manner as shown by a Schild plot. The pA_2 value of LSD on hippocampal homogenates was 5.95, on cortical homogenates 6.07, not significantly different. This value is very nearly that of cimetidine, 6.22, and metiamide, 6.06, the two potent and selective H_2-antagonists. Inactive in this system were L-LSD,

Table 3. The pA_2 values of histamine H_1 antagonists on the histamine H_2 receptor linked to adenylate cyclase in guinea pig hippocampus homogenates compared with the pA_2 values on the H_1 receptor in guinea pig ileum

Antagonist	pA_2 on H_2 linked adenylate cyclase activity: hippocampus, guinea pig	pA_2 on H_1 receptor: ileum, guinea pig
Pyrilamine	5.18	9.4*
Tripellenamine	5.4	8.5†
Cyproheptadine	7.43	8.3‡

*Arunlakshana and Schild 1959
†Marshall 1955
‡Rocha e Silva and Garcia Leme 1972

psilocin and mescaline, all at 10^{-4} M. But Br-LSD was a competitive antagonist, as shown by the Schild plot. Its pA_2 value was 7.16, thus showing an affinity about tenfold that of either LSD, cimetidine, or metiamide.

PREDICTION OF A NEW H_2-ANTAGONIST

There is no ostensible similarity in chemical structure between LSD (or BrLSD) and the selective H_2-antagonists when they are drawn in the conventional way (Fig. 2). But one of the likely conformers of metiamide and cimetidine has a topological congruency with LSD, as shown for cimetidine in Fig. 3. The molecules neatly superimpose, the oxygen of the amide group of LSD being congruent with the nitrogen of the cyano group of cimetidine or the sulfur atom of the throurea group in metiamide. After making this observation, we wrote:

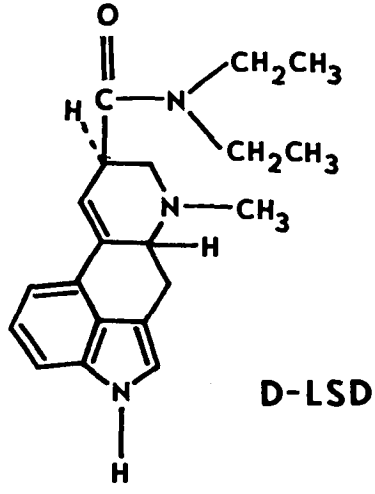
> The residue in D-LSD that is sterically congruent with metiamide is hexanoic acid-4-aza-4-methyl-6-(3-pyrrolyl)-N,N-diethylamide. This and the indolyl and imidazol analogs and their derivatives may have H_2-antagonists activity (Green et al. 1977).

We tested hexanoic acid-4-aza-4,5-dimethyl-6-(3-indolyl)-N,N-diethylamide (SK&F 10856), obtained from Dr. Carl Kaiser. The structure is shown (Fig. 4). A Schild plot proved it to be a competitive antagonist of histamine and dimaprit at the H_2-receptor linked to adenylate cyclase, with $pA_2 = 6.10$ ($K_B = 7.9 \times 10^{-7}$ M), not different from that of LSD. We plan to test the pyrrolyl and imidazoyl analogs.

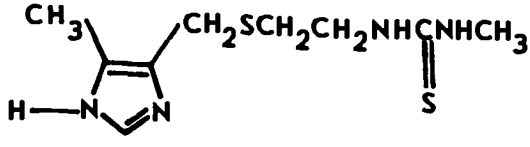
THE H_2-RECEPTOR AND THE PHARMACOLOGY OF LSD AND BrLSD

Both LSD and BrLSD are competitive antagonists of histamine at the H_2-receptor linked to adenylate cyclase in guinea pig hippocampus and cortex. The pA_2 value of D-LSD was 6.0, very nearly that of cimetidine, 6.22, and of metiamide, 6.06, the two potent H_2-antagonists. The L-isomer, which has no measureable central effects, did

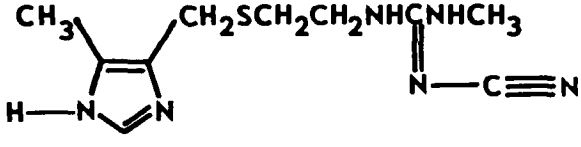
Fig. 2. The structural formulas of LSD, metiamide, and cimetidine



D-LSD



METIAMIDE



CIMETIDINE

Fig. 3. Comparison of the molecular structures of LSD and cimetidine

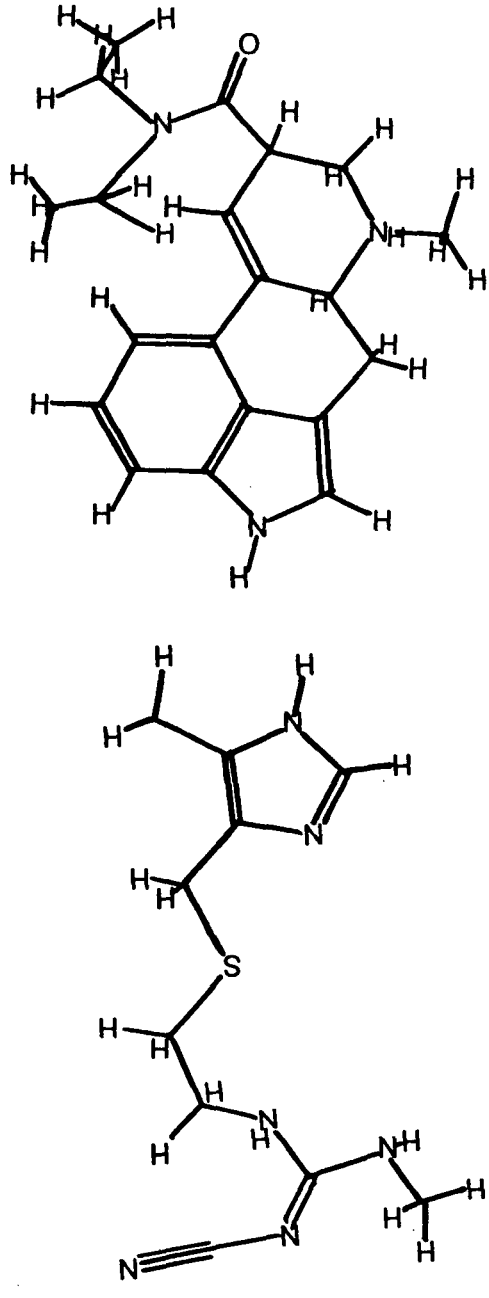


Table 4. Estimated K_B and pA_2 values of cyproheptadine for different receptors

Receptor	K_B (M)	pA_2
Histamine H_1*	5×10^{-9}	8.3
Histamine H_2†	3.7×10^{-8}	7.4
LSD‡	5.0×10^{-8}	7.3
Bradykinin*	6.3×10^{-8}	7.2
Haloperidol§	6.5×10^{-8}	7.2
Angiotensin*	2.5×10^{-7}	6.6
Dopamine§	1.0×10^{-6}	6.0
Serotonin‡	1.6×10^{-6}	5.8

*Rocha e Silva and Leme 1972
†Green et al. 1977
‡Estimated from IC50 values of high affinity binding by rat cortex, from Bennett and Snyder 1976; according to: $K_B = IC_{50}/1 + [A]/K_A$ where $[A]$ is the agonist concentration and K_A is the dissociation constant of the agonist
§Creese, Burt and Snyder 1976

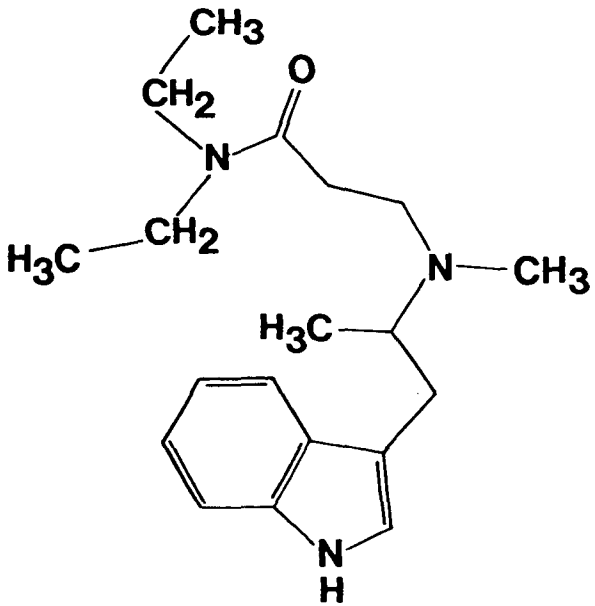
not antagonize the histamine-activated adenylate cyclase. BrLSD (pA_2=7.2) was about ten times more potent than D-LSD (or cimetidine) in inhibiting histamine-stimulated adenylate cyclase activity (Green et al. 1977).

The affinity of LSD for the H_2-receptor linked to adenylate cyclase cannot be directly compared with its affinities for other receptors linked to adenylate cyclase in brain because the pA_2 values are not available. From Table 5 it would appear that LSD and BrLSD may be more effective in blocking histamine stimulated cyclase than in blocking norepinephrine or dopamine stimulated cyclase.

However, the pA_2 values (Table 6) show that LSD has a greater affinity for the dopamine receptor than for the H_2-receptor. LSD has an even greater affinity for the serotonin receptor. BrLSD has the same pA_2 values for the serotonin, dopamine and H_2-receptors. And it has a greater affinity than LSD for both the haloperidol binding sites and for the H_2-receptor. Other work has shown that LSD and BrLSD have the same affinity for LSD binding sites in cortex (Bennett and Snyder, 1976).

Although adenylate cyclase measurements suggest that BrLSD has a greater affinity for the dopamine receptor (Table 5), high affinity binding studies of dopamine show that BrLSD has slightly less affinity than LSD for the dopamine binding sites (Creese, Burt, and Snyder, 1976; Table 6). This paradox suggests the presence of dopamine receptors that are not linked to cyclase, a receptor that has a greater affinity for LSD than for BrLSD. LSD, in contrast with BrLSD, is an agonist as well as an antagonist at striatal receptors linked to cyclase (Von Hungen, Roberts, and Hill 1975; Bockaert et al. 1976). Perhaps BrLSD has less affinity for another (presynaptic?) dopamine receptor. BrLSD has about five times more affinity for haloperidol binding sites than has LSD (Creese, Burt, and Snyder 1976). As haloperidol binds to the postsynaptic dopaminergic receptors, it was inferred that BrLSD is a better dopamine

Fig. 4. The structure of hexanoic acid-4-aza-4,5-dimethyl-6-(3-indolyl-N,N-diethylamide. It is shown in a conformation congruent with LSD and cimetidine in fig. 3.



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antagonist than is LSD. Supporting this inference are experiments in rat striatum showing that BrLSD is more potent than LSD in increasing the levels of DOPA (Persson 1977a) and of a dopamine metabolite, 3,4-dihydroxyphenylacetic acid (Persson 1977b). All these biochemical studies are consistent with observations on unit cell activity of the substantia nigra, where LSD was more effective than BrLSD in decreasing the firing rate of dopamine-containing neurons (Trulson, Stark, and Jacobs 1977; Christoph, Kuhn, and Jacobs, 1977). Perhaps correlating with this difference in affinity for postsynaptic dopamine receptors is that head twitches induced in mice by LSD (Corne and Pickering 1967) and LSD stimulated striatal cyclase (Von Hungen, Roberts, and Hill 1975) were blocked by BrLSD.

Agonist activity of LSD is also greater than that of BrLSD on the flexor reflex of the dog. LSD, like tryptamine, enhanced the flexor reflex (Martin and Sloan 1974), but a dose of BrLSD 100

Table 5. Comparative effects of LSD, L-LSD, and BrLSD in blocking the adenylate cyclase activity in broken cell preparations, stimulated by histamine, norepinephrine and dopamine, all at 10^{-4} M. Values are percentage inhibition.

Drug	Histamine*	Norepinephrine†	Dopamine‡
LSD, 10^{-4} M	-	75	81
LSD, 10^{-5} M	56	-	-
L-LSD, 10^{-4} M	0	-	0
BrLSD, 10^{-4} M	-	90	98
BrLSD, 10^{-5} M	97	-	-

*Guinea pig cortex and hippocampus (Green et al. 1977)
†Rat cortex (Von Hungen, Roberts, and Hill 1975)
‡Rat striatum (Von Hungen, Roberts, and Hill 1975)

times higher did not (W.R. Martin, personal communication).

The differences between the pA_2 values for LSD and BrLSD on the serotonin receptors could also reflect a difference between affinities for presynaptic (agonist) and postsynaptic (antagonist) serotonin sites. LSD has greater affinity for the serotonin receptor than does BrLSD. LSD, like serotonin, completely inhibits the firing of neurons in the midbrain raphe at low doses (10 µg/kg) whereas BrLSD only partially inhibits these neurons even at a dose 200 times greater (Aghajanian, Foote, and Sheard 1970). Microiontophoretically applied BrLSD also failed to totally inhibit the firing (Aghajanian 1976). Both BrLSD and LSD inhibited postsynaptic sites on the ventral lateral geniculate and amygdala (Aghajanian 1976). Some of this work recalls studies of peripheral systems. LSD and BrLSD are about equally potent antagonists at the postsynaptic serotonin receptors in the rat uterus (Cerletti and Doepfner 1958) and stomach (Vane 1959). On one peripheral tissue, the effects of LSD and BrLSD are very neatly defined. LSD is a serotonin agonist in human and sheep umbilical vasculature, and BrLSD is an antagonist of both LSD and serotonin (Dyer and Gant 1973).

It thus seems clear from studies on neural tissue using biochemical and electrophysiological techniques that LSD and BrLSD have affinities for the same receptors and that LSD has greater activity at (and perhaps a greater affinity for) functionally "presynaptic" sites, and BrLSD has greater affinity for some "postsynaptic" sites.

The sites that LSD and BrLSD share are very likely to be implicated in the hallucinogenic action of LSD. For BrLSD blocks the hallucinogenic effect of LSD in man (Ginzel and Mayer-Gross 1956; Bertino, Klee, and Weintraub 1959) at a dosage interval before cross-tolerance is seen (Isbell, Miner, and Logan 1959). The effect of a dose of LSD of 1 µg/kg was blocked by a dose of BrLSD of 32 to 64 µg/kg (Bertino, Klee, and Weintraub 1959).

Perhaps of greater interest is that BrLSD causes behavioral changes in laboratory animals and in man. Larger doses of BrLSD than LSD are needed to show changes in animal behavioral paradigms but they occur (e.g., Uyeno 1968; Uyeno and Mitoma 1969). In man there is one report of hallucinations occurring after ingestion of 0.5 mg of

Table 6. pA_2 values of LSD and BrLSD for different receptors

Receptor	LSD	BrLSD
Serotonin*	8.3	7.2
Haloperidol†	7.7	8.4
Dopamine†	7.5	7.2
Histamine-H_2‡	6.0	7.2

*Estimated from IC_{50} value of high affinity binding by rat cortex, from Bennett and Snyder 1976; according to: $K_B = IC_{50}/1 + [A]/K_A$ where [A] is the agonist concentration and K_A is the dissociation constant of the agonist
†Creese, Burt, and Snyder 1977
‡Green et al. 1977

BrLSD (Richards et al. 1958). Others have observed severe psychic effects without hallucinations. Bertino, Klee, and Weintraub (1959) wrote:

> Six of the 10 subjects receiving 128 or 256 µg./Kg. exhibited mental effects. These consisted of a "drunk feeling," "things seem bright," "devil-may-care attitude," "a feeling of tiredness, euphoria, anxiety, and impaired concentration."
>
> Psychophysiological effects of 2-brom-LSD were reported by subjects at dosages as low as 32 µg./Kg. and increased in severity with increasing dosage. The psychic effects, though milder, were qualitatively the same as those with lysergic acid diethylamide (LSD-25).

Schneckloth et al. (1957) wrote:

> Thus, when constant intravenous infusions of bromo-LSD were given to 2 normal subjects (cases 5 and 6) both experienced psychic changes, which became more severe as the infusion continued and persisted for 3 to 4 hours after the infusion was stopped. No hallucinations were noted but there were feelings initially of drowsiness, depression, anxiety and apprehension, followed by feelings of irritation, restlessness, and tenseness, and later, intensely disagreeable sensations of unreality and depersonalization, inexplicable feelings of strangeness and mild confusion.

What contribution blockade of the histamine H_2-receptor makes to the behavioral effects of BrLSD and LSD is not known. The availability of an H_2-antagonist that penetrates the brain could lead to understanding. Cimetidine, an H_2-receptor antagonist, which is now being used in humans, does not normally enter brain (Ganellin 1978). It has, however, produced rare and idiosyncratic responses that may be revealing of central function. There are reports of mental confusion after cimetidine - patients described as agitated, delirious, disoriented - all rapidly reversed when the drug was withdrawn (Delaney and Ravey 1977; Robinson and Mulligan 1977; Menzies-Gow 1977; Nelson 1977; McMillen et al. 1978; Quap 1978). Reports of

fever after cimetidine (Ramboer 1978; McLoughlin, Callender, and Love 1978) also recall that LSD raises temperature in humans (Gorodetzky and Isbell 1964; Klock, Boerner, and Becker 1975) and rabbit (Carino and Horita 1977); in some animals, H_2-receptor stimulation causes hypothermia (Cox and Lomax 1977). Further attesting to central effects is that increased plasma prolactin levels have been observed especially when the drug is given by intravenous injection (Della Fave et al. 1977; Carlson and Ippoliti 1977; Burland et al. 1978; Daubresse, Meunier and Ligny 1978); animal studies have suggested that the histamine H_2-receptor mediates events that inhibit prolactin release (Arakelian and Libertun 1977).

The H_1-antagonists at suitable concentrations interact with both H_2- (Table 3) and cholinergic receptors (e.g., Nauta and Rekker 1978; van den Brink and Lien 1978). It is therefore risky to attribute their behavioral effects to blockade of any one receptor. It may be interesting to note, however, that H_1-antagonists produce arousal in dogs (Shaw, Gershon, and Bentley 1957; Gershon and Shaw 1958). In man, high dose of the H_1-antagonists have produced disorientation, euphoria (Gott 1968), visual hallucinations (Soleymanikashi and Weiss 1970), and behavior characterized as schizophrenic-like (Nigro 1968). Diphenhydramine, an H_1-antagonist, on intravenous administration (30 or 45 mg to each male subject) produced disturbance of body image and thought. When administered at the height of the LSD reaction (25 µg of LSD by mouth), diphenhydramine intensified most of the LSD effects - especially hallucinations, depersonalization, and disturbances of body image and thinking - without prolonging the effects (Yamada, Tsunoda, and Takashina 1957).

It is becoming increasingly clear that the complex behavioral and neural effects of LSD or BrLSD cannot be explained on the basis of any single effect on any single neurophysiological system or site. The effect elicited at the lowest dose in the rat is a decreased firing of the raphe nuclei (Aghajanian 1976). This effect cannot explain the behavioral effect of LSD (Freedman and Halaris 1978; Trulson, Ross, and Jacobs 1977; Pieri et al. 1978). In humans, LSD - and, as quoted above, BrLSD - produce an array of neural effects, which range from activation of the galvanic skin response at the lowest dose (Greiner, Burch, and Edelberg, 1958), a sign of neurophysiological arousal; to marked changes in perception, affect, thought, body image; perseveration; heightened awareness; depersonalization; illusions and hallucinations (Freedman, 1968; Mandell and Geyer, 1976). One effect, hallucinations, is complex enough (Editorial, 1977). It is very unlikely that all the effects can be attributed to action at one receptor at one site. How the visual cortex processes a spot of light is still not known, but it is clear that the

> response properties of most neurones depend on subtle interactions within an intricate system of more and less specific excitatory and inhibitory influences, whose cooperative details determine most aspects of neuronal behaviour (Movshon 1978).

This complexity must apply to the action of drugs, which have access to all neurons and all receptors. Almost certainly the behavioral effects produced by any drug, e.g., LSD, BrLSD, are the final consequences of a conflation of events occurring at different neural sites and by different mechanisms. The basis for the neural and behavioral effects will be learnt after the receptor sites at which these drugs act are explicitly and rigorously delineated. Then will follow the concatenation of the neural and behavioral events.

SUMMARY

Two aspects of the complexities of the mode of action of drugs are described. One is the criteria and pitfalls of defining the interaction with specific receptors. The other is the need to consider each of the pharmacological effects of a drug as a concatenation of receptor events, because it has become clear that each drug may have substantial affinity for many specific receptors.

Illustrating these ideas is a characterization of the histamine receptor linked to adenylate cyclase in brain. The activities of a series of H_2-antagonists and H_2-agonists were shown to be the same on the histamine receptor linked to adenylate cyclase as on known H_2-receptors. The K_B values of antagonists and ED_{50} values of agonists were not distinguishable among these receptors.

Notably, at high concentrations, the H_1-antagonists are also competitive antagonists of the H_2-receptor.

Cyproheptadine has especially high affinity for the H_2-receptor. It is the most potent H_2-antagonist yet reported. Other published results are reviewed to show the variety of receptors that cyproheptadine has affinity for. Its affinity for serotonin receptors led us to examine other serotonin antagonists.

On this H_2-receptor linked to adenylate cyclase in homogenates of guinea pig hippocampus and cortex, D-LSD and D-2-bromo-LSD (BrLSD) were shown to be competitive antagonists of histamine. L-LSD, mescaline and psilocin were inactive.

Noting congruency in the molecular structure of D-LSD and known H_2-antagonists, we predicted a new H_2-antagonist. This prediction is shown to be correct: the compound has similar affinity to the H_2-receptor as has LSD.

The affinities of D-LSD and BrLSD for the H_2-receptor are compared with their affinities for other receptors. The pharmacology of D-LSD and BrLSD is reviewed. Evidence is assembled that BrLSD has considerable central effects.

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AUTHORS

Jack Peter Green, M.D., Ph.D.
Harel Weinstein, D.Sc.
Saul Maayani, Ph.D.
Department of Pharmacology
Mount Sinai School of Medicine
of the City University of New York
New York, New York 10029