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Short communication

HALLUCINOGENS ANTAGONIZE HISTAMINE H_1 RECEPTORS OF CULTURED MOUSE NEUROBLASTOMA CELLS

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Hallucinogens were competitive antagonists of histamine at the H_1-receptor of cultured mouse neuroblastoma cells. Their rank order of potency at this receptor was similar to that for their potency at eliciting subjective effects in vivo. Comparison with studies of other receptors, however, suggests that no single model of drug—receptor interaction adequately accounts for the known subjective effects of these compounds.

Histamine H_1-receptor Hallucinogen Cyclic GMP

1. Introduction

The hallucinogens (also known as psychotomimetic drugs, psychedelics, and euphorohallucinogens) are those drugs which produce anxiety, fearfulness, euphoria, a decrease in concentration and cognitive skills, marked changes in perception (most notably vivid, visual hallucinations), and autonomic effects (Wolbach et al., 1962). Lysergic acid derivatives, phenylalkylamines (mescaline and amphetamine derivatives such as 2,5-dimethoxy 4-methylamphetamine (DOM), and indolealkylamines (psilocin, psilocybin, bufotenine, DMT, and others) represent the major chemical classes of hallucinogens.

Recently, Green et al. (1977) reported the antagonism of histamine H_2-receptors by D-lysergic acid diethylamide (D-LSD) and related compounds, and concluded that such an action may contribute to their central effects. We report antagonism of histamine H_1-receptors of cultured mouse neuroblastoma cells by representatives of the major chemical classes of hallucinogens. Antagonism at the histamine H_1-receptor by these compounds appears to be more potent than that found at histamine H_2-receptors.

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2. Materials and methods

Mouse neuroblastoma clone NIE-115 cells (subculture 10-20) were grown in Dulbecco-Vogt modification of Eagle's medium (Grand Island Biological Co), supplemented with 10% (v/v) fetal calf serum (Colorado Serum Co) without antibiotics (Medium I) at 37°C in an atmosphere of 10% CO_2 in 90% humidified air. Cells were grown to confluency in flasks (75 cm^2/250 ml, Falcon Plastics, Oxnard, CA, 20 ml medium) and maintained in stationary phase for at least one week prior to use in the assay for cyclic GMP which has been described in detail elsewhere (Richelson et al., 1978). In brief, cells were washed free of medium I with phosphate buffered saline solution containing the following: 110 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 25 mM glucose, 50 mM sucrose, titrated to pH 7.3 with 0.1 N NaOH (Medium II). Next, cells were dissociated from the surface of the flask by a 15 min incubation in a modified Puck's D_1 solution and collected by low-speed centrifugation (250 × *g* for 1.5

min in a Damon/IEC CRU-5000 centrifuge), then resuspended in 2 ml of medium II at a density of approximately 5×10^6 cells/ml in a 25 ml Erlenmeyer flask to which was added [8-^{3}H]guanine (Amersham-Searly, 1 μCi/ml final concentration). The cell suspension was rotated for 45 min at 80 rpm (Gyratory Shaker, Model G2, New Brunswick), followed by dilution with medium II and distributed into the wells of a multiwell tray (Disposo trays FB16-24TC, Bellco Glass) to a final volume of 0.25 ml/well. Antagonist, 30 μl, was added to each well (except for controls which received 30 μl medium II) and incubated for 30 min in a shaker bath (model 25, GCA, Precision Scientific) at 37°C prior to stimulation with histamine (20 μl) for 30 sec. Reactions were stopped by addition of 50% (w/v) trichloroacetic acid (30 μl), and solutions were transferred to Dowex columns for isolation of cyclic GMP as previously described (Richelson et al., 1978). [8-^{14}C]-Cyclic GMP was included in each sample as internal standard. The calculated dpm of each trial was corrected for recovery of internal standard (83% average). All data represent the average of duplicate trials.

3. Results

All compounds were competitive antagonists of histamine as determined from the shape of dose-response curves (e.g., fig. 1) and, therefore, apparent equilibrium dissociation constants could be calculated by the dose-ratio method (table 1). The lysergic acid derivatives were most potent, the indolealkylamines occupied an intermediate position, and the phenylalkylamines were least potent. This rank order approximates the order of in vivo potencies (table 1), with the exception of 2-bromo-LSD. To our knowledge, there have been no in vitro studies that have adequately predicted the ratio of in vivo potencies of D-LSD to 2-bromo-LSD. Stereospecificity was amply demonstrated by the D- and L-isomers of LSD. It is interesting to note the equimolar potencies of psilocin and psilocybin as histamine H_1-antagonists. This situation holds true in vivo (note in table 1 that the ratio by dosages is approximately 1.4 : 1.0, which is the same as that of the molecular weights of the two drugs), and has not been found at other receptors in vitro (Bennett and Snyder, 1976; Burt et al., 1976). It is not known if spontaneous dephosphorylation of psilocybin to yield psilocin might have occurred, however.

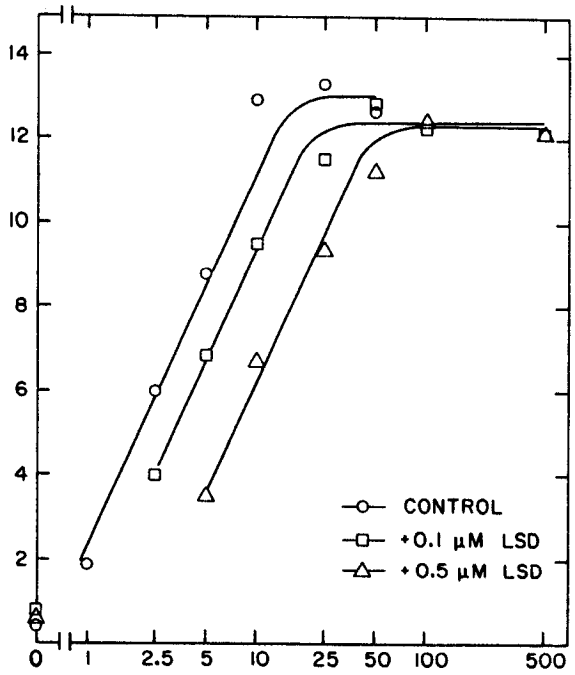

Fig. 1. Effect of D-LSD on histamine dose—response curve. Histamine-stimulated cyclic GMP formation by cultured mouse neuroblastoma cells (clone NIE-115) was assayed in the absence (control) and presence of two different concentrations of D-LSD as described in Materials and methods. Each symbol represents the mean of duplicate samples which deviated from the mean by ≤ 13%. For this experiment there were approximately 120 000 cell/well. From the dose ratio (see legend to table 1) the $K_B = 1.5 \times 10^{-7}$ M. *Ordinate:* [^{3}H]cyclic GMP ($10^{-4} \times$ DPM/10^6 cells). *Abscissa:* histamine concentration (μM).

Each drug listed in table 1 was also screened for agonist properties (30 sec incubation fol-

TABLE 1

Apparent equilibrium dissociation constants, K_B, at histamine receptors and in vivo potencies of several hallucinogens and related compounds.

	K_B, μM (±S.E.M.)		Average effective dose, mg/kg for hallucinogenic effects [3]
	H_1 [1]	H_2 [2]	
D-LSD	0.12 ± 0.02	1	0.001—0.0015
2-Bromo-LSD	0.14 ± 0.02	0.07	Inactive [4] (at 1—2)
Psilocybin	1.8 ± 0.3	—	0.075—0.15
Psilocin	1.9 ± 0.4	NE	0.05—0.10
L-LSD	25 ± 6	NE	Inactive (at 0.07)
DOM	170 ± 6	—	0.05—0.075
Mescaline	500	NE	3—5
Serotonin	500	—	Inactive

[1] $K_B = [D]/(DR - 1)$, where [D] = concentration of antagonist and DR = dose ratio of agonist. K_B values are based upon at least four independent observations.

[2] Taken from Green et al., 1977; NE = no effect; for psilocin, L-LSD at 0.1 mM; mescaline at 1 mM.

[3] Wolbach et al., 1962; Brown, 1972; Schneckloth et al., 1957.

[4] Fear, anxiety and depersonalization, but without hallucinations, have been reported.

lowed by assay of cyclic GMP). Only serotonin was found to be active. Preliminary efforts to characterize this response indicated a two-fold peak over basal level (compared with 20- to 30-fold for histamine) with a peak at 2 min (30 sec for histamine). The origin of the serotonin response, whether due to activation of possible serotonin or histamine H_1-receptors, remains unknown at this time.

In all dose—response curve experiments, basal levels of cell GMP were determined in the absence (control) and presence of the antagonist after 30 min incubation with cells (e.g., fig. 1). No changes in basal levels of cyclic GMP from control values were seen; a result which suggests that these compounds had no effect on phosphodiesterase activity.

4. Discussion

Our results show that hallucinogens competitively antagonize histamine H_1 receptors of cultured mouse neuroblastoma cells. In addition, where data are available for comparison, it is seen that these compounds are more potent as histamine H_1 antagonists than as histamine H_2 antagonists (table 1).

Utilization of mouse neuroblastoma cells as a model of the normal mammalian neuron has been well-established on the basis of morphological, biochemical, and electrophysiological studies (Richelson, 1979). These experiments were carried out on clone NlE-115 which was previously shown to possess histamine H_1 but not histamine H_2 receptors (Richelson, 1978). Some of the pharmacological properties of these receptors as determined by the affinities of several antagonists were very similar to those of the guinea pig ileum (Figge, Leonard, Richelson, unpublished data).

In recent years histamine has been identified as one of several probable neutotransmitters in mammalian brain. Both histamine H_1- and H_2-receptors have been detected in the central nervous system. Intraventricular histamine has been found to alter blood pressure, temperature, and water regulation via both histamine H_1- and H_2-receptor mechanisms (Schwartz, 1977). Effects on behavior are not as yet clear. At this time one may only conclude that mediation of certain hallucinogenic drug effects by central histamine receptors, while plausible, is certainly not supported by compelling evidence.

Hallucinogenic drugs apparently are active at other receptors as well. Studies indicating serotonin effects and antagonism are well known (see for references, Burt et al., 1976). Similar actions at the dopamine receptor have been described (Burt et al., 1976) and an effect on central norepinephrine levels has been demonstrated (Pieri et al., 1978). Yet results do not clearly favor any single receptor as the site of psychotomimetic effects, and differences in metabolism or ability to pene-

trate the blood-brain barrier do not predict potency. Such confusion reflects, in part, a lack of information on the structural units and neurotransmitter systems which mediate human behaviors. Further research regarding interactions among neurons of various types, such as already hypothesized between serotonergic and dopaminergic neurons (Maj, 1976), and which may exist between presumed histaminergic neurons and serotonergic neurons as well, may assist in the clarification of current concepts of hallucinogenic drug actions. As noted by Green et al. (1977), enthusiasm for any single receptor as the sole mode of action of these drugs does not seem warranted.

Acknowledgement

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