Infrared spectroscopy showed that fraction 1 also contained considerable amounts of 2-naphthyl sulphamate and this was confirmed by thin-layer chromatography on silica gel (Kieselgel 60: Merck, Darmstadt, F.R.G.) in the solvent system butan-1-ol: propan-1-ol: 0.1 M NH_4OH (2:1:1, v/v) [1]. The area containing the sulphamate was located by its fluorescence and eluted with 75% ethanol: the resulting solution was taken to dryness to give 4 mg of brownish powder, the infrared spectrum of which was indistinguishable from that of authentic 2-naphthyl sulphamate. Hydrolysis of 3 mg of the powder in HCl, as described above, gave 1.4 mg of 2-naphthylamine, the infrared spectrum of which was indistinguishable from that of the authentic compound.

The crude preparation of 2-naphthyl sulphamate from rat liver was extracted with 1 ml of cold ethanol to leave 7.3 mg of light brown solid which was crystallised from 0.8 ml of 95% ethanol containing 15% of potassium acetate to give 2.4 mg of glistening white platelets. The infrared spectrum of this was identical with that of authentic 2-naphthyl sulphamate.

These results confirm the existence in rat and guinea-pig livers of an enzyme or enzymes capable of converting 2-naphthylamine into 2-naphthyl sulphamate, and so confirm the existence of the activity ascribed to arylamine sulphotransferase. They do not resolve the doubts about the nature of this activity which could be due to a separate enzyme or simply a facet of the activities of other sulphotransferases, as was suggested by Banerjee and Roy [7]. This does not detract from a possible role for this activity in the metabolism of xenobiotics containing arylamine groups and more attention should therefore be given to the possible formation of sulphamates *in vivo*: it has already been shown that they can be formed from sulphonamides [11] and from metoclopramide [12].

Department of Physical Biochemistry
John Curtin School of Medical Research
P.O. Box 334
Canberra City
ACT 2601, Australia

CONNIE I. PROSSER*
ALEXANDER B. ROY

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* Present address: Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

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Direct action of D-lysergic acid diethylamide on dispersed mucosal cells from guinea pig stomach*

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Histamine receptors are classified as H_1- or H_2-receptors according to their ability to interact with specific agonists and antagonists. Those on intestinal and gastric smooth muscles are H_1-receptors, whereas the ones on gastric mucosa and atrial or uterine smooth muscles are H_2-receptors [1, 2]. In certain tissues, however (e.g. heart and brain), both H_1- and H_2-antagonists interact with histamine H_2-receptors linked to adenylate cyclase [3–5]. We reported recently that the action of histamine on guinea pig gastric mucosal cells could be blocked by H_1- and H_2-antagonists [6]. In all these preparations, the potencies of H_1-antagonists in inhibiting the histamine-induced responses were approximately 1000 times less than their potencies in inhibiting the action of histamine on functions which are mediated by H_1-receptors [1, 7]. Since D-lysergic acid diethylamide (LSD) was reported to be a competitive antagonist of histamine on H_2-receptors in the brain [3] and a partial agonist in guinea pig right atrium [8], we explored the histamine receptors in isolated gastric cells by examining the effects of LSD on [^{3}H]histamine binding, cellular cyclic AMP, and acid formation as reflected by the uptake of the weak base [^{14}C]aminopyrine.

Methods

Dispersed mucosal cells from guinea pig stomach were prepared and suspended in standard solution (Hanks' buffer, GIBCO, Grand Island, NY) as previously described [9]. Cyclic AMP was determined by radioimmunoassay [10], and uptake of [^{14}C]aminopyrine (13 mCi/mmole) and binding of [^{3}H]histamine (10 Ci/mmole) (both from the New England Nuclear Corp. Boston, MA) by centrifugation [11, 12].

Results and discussion

In dispersed mucosal cells, histamine and LSD increased cellular cyclic AMP in a dose-related fashion with half-

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Table 1. Effects of various agents on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Agent	Cellular cyclic AMP (pmoles/10^6 cells)		
	No addition	Histamine (10 μM)	Prostaglandin E_1 (10 μM)
None	0.2 ± 0.02	1.0 ± 0.07	0.5 ± 0.06
LSD (50 μM)	0.7 ± 0.10†		
Theophylline (5 mM)	1.1 ± 0.2	8.4 ± 0.6	3.1 ± 0.3
Plus LSD	3.2 ± 0.3‡	5.5 ± 0.4†	5.1 ± 0.6†
Plus LSD + cimetidine (1 μM)	1.7 ± 0.2	3.8 ± 0.5	4.0 ± 0.5
Plus LSD + promethazine (10 μM)	1.8 ± 0.3	3.6 ± 0.4	4.2 ± 0.6

* Cells were suspended in standard solution and incubated with the indicated agents for 30 min at 37°. Each value is the mean ± S.D. of three experiments. Similar results were obtained with 100 μM RO 20-1724 instead of theophylline. LSD = D-lysergic acid diethylamide.

† Significantly different ($P < 0.01$) from values without LSD by Student's *t*-test.

‡ Significantly different ($P < 0.01$) from corresponding values for LSD or theophylline alone by Student's *t*-test.

maximum effects occurring at 10 μM histamine and 15 μM LSD (Fig. 1). LSD was less efficacious than histamine and, at the maximum effective concentrations, 50 μM LSD caused approximately a 3-fold increase, whereas 200 μM histamine caused a 16-fold increase, in cyclic AMP. To explore the mechanism by which LSD increased cyclic AMP, we measured the effect of LSD in the presence of phosphodieterase inhibitors such as theophylline or RO 20-1724. These agents augmented the effect of LSD on cyclic AMP, in that the increase in cyclic AMP caused by LSD plus theophylline was greater than the sum of the increases caused by each agent alone (Table 1). Furthermore, with or without theophylline, the time-course of the increase in cyclic AMP caused by LSD was similar to that caused by histamine (results not shown). These results exclude the possibility that LSD had increased cyclic AMP by inhibiting its breakdown. Prostaglandin E_1 (PGE_1) also caused approximately a 3-fold increase in cellular cyclic AMP, and the effects of PGE_1 and LSD (with 5 mM theophylline) were additive (Table 1). In contrast, 50 μM LSD (with 5 mM theophylline) significantly inhibited the action of histamine on cellular cyclic AMP. Furthermore, the H_2-antagonists, cimetidine, as well as the H_1-antagonist, promethazine, both of which have been shown in this preparation to inhibit the action of histamine on cyclic AMP [6], also inhibited the action of LSD (with theophylline) (Table 1). These antagonists were competitive to LSD and their calculated inhibitory constants (K_i, see Ref. 6) when tested against LSD (K_i, 0.95 ± 0.2 μM for cimetidine; and K_i, 20 ± 5 μM for promethazine) were similar to those when tested against histamine [6]. Neither cimetidine nor promethazine, however, altered the response of gastric cells to PGE_1 (Table 1). Similar results were obtained with metiamide instead of cimetidine and with mepyramine or diphenhydramine instead of promethazine. These results suggest that LSD acts in parallel to PGE_1 to activate adenylate cyclase and to increase the synthesis of cyclic AMP. Whether this parallelism reflects functionally distinct catalytic moieties of adenylate cyclase in a single cell type or LSD and PGE_1, each acting on a different type of cell to activate adenylate cyclase, cannot be determined from the present studies. These observations may reflect the interaction of LSD with histamine receptors and, if so, LSD should behave as a competitive antagonist of the action of histamine on cyclic AMP. When these two agonists were tested in combination, LSD inhibited the action of histamine. In the absence of LSD, an increase in cyclic AMP was detected at 2 μM histamine and was half-maximal at 20 μM histamine (Fig. 2). In the presence of 50 μM LSD

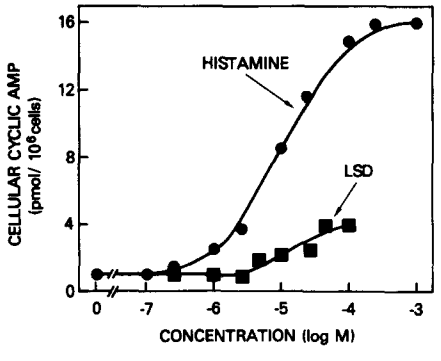

Fig. 1. Effects of histamine and D-lysergic acid diethylamide (LSD) on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach. Cells were suspended in standard solution containing 5 mM theophylline and incubated with the indicated concentrations of histamine or LSD for 30 min at 37°. This experiment is representative of three others.

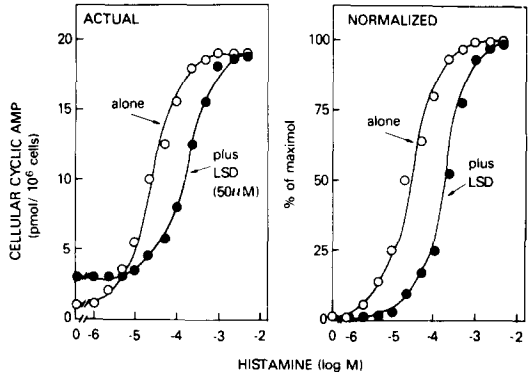

Fig. 2. Effect of D-lysergic acid diethylamide (LSD) on histamine-induced increase in cyclic AMP in dispersed gastric mucosal cells. *Left panel:* cells were suspended in standard solution containing 5 mM theophylline and incubated with or without 50 μM LSD plus the indicated concentrations of histamine. *Right panel:* cellular cyclic AMP expressed as a percentage of the maximal increase caused by histamine. This experiment is representative of three others.

Table 2. Effect of D-lysergic acid diethylamide (LSD) on [^{3}H]histamine binding to dispersed gastric mucosal cells from guinea pig*

Agent	[^{3}H]Histamine binding	
	% of total	% of saturable
Control	2.00 ± 0.10	100†
Histamine (1 mM)	0.25 ± 0.01‡	0
Histamine (10 μM)	1.15 ± 0.08‡	52
LSD (10 μM)	1.43 ± 0.12‡	67
LSD (25 μM)	1.10 ± 0.08‡	48
LSD (50 μM)	0.75 ± 0.09‡	29

* Cells were suspended in standard solution containing 20 nM [^{3}H]histamine and incubated with the indicated agents for 40 min at 37°. Results are expressed as the percentage of total radioactivity present in the incubation solution. The control value represents the total tracer binding whereas the value with 1 mM histamine represents the nonspecific binding. Histamine above 1 mM did not inhibit tracer binding beyond the inhibition caused by 1 mM histamine. Each value is the mean ± S.D. of three separate experiments.

† The value of maximum saturable binding was calculated from the total binding obtained with the tracer alone minus that with the tracer plus 1 mM histamine.

‡ Significantly different ($P < 0.01$) from control value by Student's t-test.

(which caused a 3-fold increase in cyclic AMP), 10 μM histamine was required to produce a detectable effect and 92 μM to produced a half-maximal effect (Fig. 2). The potency* of LSD calculated from results obtained with LSD alone (Fig. 1) (15 ± 3 μM, mean ± S.D. from three experiments) was not significantly different from the potency of LSD as a competitive antagonist of histamine (i.e. 11 ± 3 μM). Furthermore, cimetidine inhibited the action of LSD in a competitive fashion. The K_i for cimetidine against LSD was the same as that against histamine, suggesting that cimetidine interacts with one class of receptors (i.e. H_2-receptors). Since LSD acted as a competitive antagonist of histamine and also increased cyclic AMP, LSD can be considered as a partial agonist capable of interacting with histamine receptors.

To further support this hypothesis, we examined the effect of LSD on binding of [^{3}H]histamine to gastric cells. We previously reported that binding of [^{3}H]histamine to these cells was saturable, reversible, specific with respect to histamine agonists and antagonists, and occurred on the H_2-receptors on parietal cells [11]. Binding of [^{3}H]histamine could be inhibited by unlabeled histamine (Table 2), and there was an agreement between the action of histamine on [^{3}H]histamine binding and on the increase in cellular cyclic AMP [11]. LSD (10 μM) inhibited the saturable portion of [^{3}]histamine binding by 33% and increasing the concentration of LSD to 25 or 50 μM caused a higher inhibition of [^{3}H]-histamine binding (i.e. 52 and 71% respectively) (Table 2). These results provide evidence that LSD directly interacts with histamine binding sites on gastric cells.

We reported that, in dispersed gastric mucosal cells, the increase in cyclic AMP caused by histamine correlated with the uptake of [^{14}C]aminopyrine (AP) [12–14]. If LSD increases cyclic AMP by interacting with histamine receptors on parietal cells, LSD would be also expected to increase AP uptake. As expected, LSD caused a

* "Potency" is measured in terms of the concentration of agonist required to produce a half-maximal response. The lower this concentration the higher the potency.

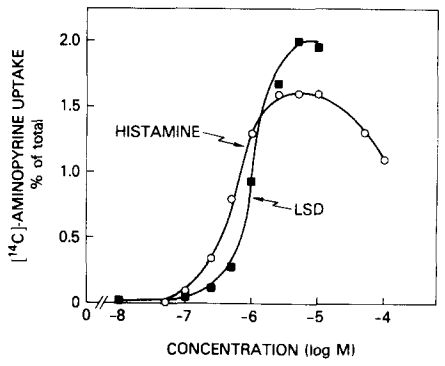

Fig. 3. Effects of histamine and D-lysergic acid diethylamide (LSD) on [^{14}C]aminopyrine uptake by dispersed mucosal cells from guinea pig stomach. Cells were suspended in standard solution containing [^{14}C]aminopyrine and 100 μM RO 20-1724 and were incubated with the indicated concentrations of histamine or LSD for 60 min at 37°. This experiment is representative of three others.

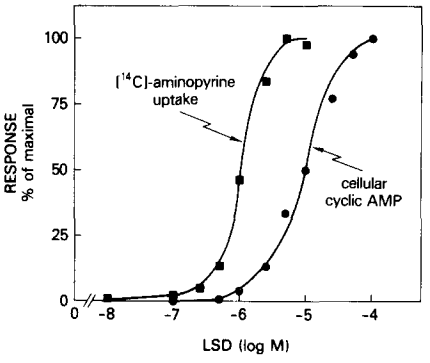

Fig. 4. Effect of D-lysergic acid diethylamide on [^{14}C]aminopyrine uptake and cellular cyclic AMP in dispersed gastric mucosal cells. Results for [^{14}C]aminopyrine uptake are expressed as the percentage of the increase obtained with 10 μM LSD (1.5 ± 0.3% of total, mean ± S.D.) and those for cyclic AMP as the percentage of the increase obtained with 100 μM LSD (3.5 ± 0.4 pmoles/10^6 cells, mean ± S.D.). The correlation coefficient between the two processes was $r = 0.89$.

concentration-dependent increase in AP uptake (Fig. 3). A significant increase in AP uptake could be detected at 0.5 μM LSD, was half-maximal at 1 μM, and maximal at 10 μM LSD (Fig. 3). Since our supply of LSD was limited, we were unable to test AP uptake at LSD concentrations above 10 μM. The potency of LSD in stimulating AP uptake was greater than its potency on cellular cyclic AMP (Fig. 4). Lower concentrations of LSD were required to cause a 50% increase in AP uptake (1 μM) than to cause a 50% increase in cellular cyclic AMP (10–15 μM). Analysis of the relationship between cyclic AMP generation and AP uptake caused by LSD revealed that there was a close correlation between these two processes ($r = 0.89$). These results on cyclic AMP and AP uptake resemble our earlier findings on the action of histamine on these two processes. We reported that histamine was approximately 10-fold more potent in stimulating AP uptake than in stimulating cyclic AMP generation [12]. Since LSD is known to be competitive antagonist of serotonin on the serotonergic receptors [1], we examined the effect of serotonin on cyclic AMP and AP uptake by gastric cells. We found that serotonin (up to 1 mM) neither increased AP uptake or cyclic AMP

production nor altered the increase in cyclic AMP caused by histamine or LSD (results not shown). These results are in agreement with the hypothesis that the increase in cyclic AMP and AP uptake caused by LSD is the consequence of LSD interacting with histamine receptors.

Since in our preparation the actions of various histamine agonists or antagonists with histamine receptors were shown to be similar to their actions on H_2-receptors in other tissues [1, 2, 6], and because of the close correlation between histamine binding and the biological response [11], we concluded that LSD interacts with histamine H_2-receptors located on the parietal cells.

Our present findings are similar to those of others [3, 4, 8], who reported that histamine receptors interact with LSD. In the brian tissue, LSD acted as a competitive antagonist of histamine [3, 4], whereas in guinea pig right atrium it acted as a partial agonist [8]. In our preparation, the affinity for LSD was reasonably close to that reported for the brian or atrium, and LSD acted as a partial agonist. Recent reports also revealed that both H_1- and H_2-antagonists inhibited competitively the histamine-activated adenylate cyclase in membrane preparations from heart [5] and brain tissues [3]. The relative potencies of histamine antagonists in those preparations were close to their relative potencies in our preparation [6], and the potencies of the H_2-antagonists were similar to their potencies on other functions which are considered to be mediated by H_2-receptors (e.g. acid secretion and contraction of uterine or atrial smooth muscle) [1, 2]. These observations suggest that the action of histamine on gastric cells as well as on heart and brain tissues is mediated by H_2-receptors. The later tissues appear to be different from other tissues (rat uterus, guinea pig atrium) in their responses to H_1-antagonists. The H_2-receptor-mediated responses of rat stomach (acid secretion) and guinea pig atrium (smooth muscle contraction) were not shown to be inhibited by H_1-antagonists [2, 7]. In contrast, in gastric cells, heart and brain tissues, H_1-antagonists are relatively high concentrations inhibited the tissue response to histamine. Whether this inhibition is of the *fully* competitive type (i.e. H_1- and H_2-antagonists interact with the histamine receptors) or of the *partially* competitive type [6] (H_1-antagonists interact with a site, distinct from the histamine receptor to reduce the affinity for histamine) remains to be determined.

In summary in dispersed mucosal cells from guinea pig stomach, D-lysergic acid diethylamide (LSD) was a partial agonist with respect to histamine. LSD, like histamine, inhibited [^{3}H]histamine binding and increased both cellular cyclic AMP and [^{14}C]aminopyrine uptake by interacting with histamine H_2-receptors on parietal cells. These processes were blocked by both histamine H_1- and H_2-antagonists and, thus, provide evidence that histamine H_2-receptors on guinea pig parietal cells resemble those in brain tissue in that they interact with LSD as well as with both classes of histamine antagonists.

Departments of Pharmacology and Surgery
Uniformed Services University of the Health Sciences, and
National Institutes of Health
Bethesda, MD 20814, U.S.A.

SHMUEL BATZRI*
JEAN DYER

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* Address all correspondence to: Shmuel Batzri, Ph.D., Uniformed Services University of the Health Sciences, Room A3014, 4301 Jones Bridge Road, Bethesda, MD 20814, U.S.A.

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Inhibitory effect of tranylcypromine on hepatic drug metabolism in the rat

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Tranylcypromine [*trans*-(±)-2-phenylcyclopropylamine], an inhibitor (MAOI) of monoamine oxidase [amine:oxygen oxidoreductase (deaminating) flavin containing; EC 1.4.3.4], is used in therapeutics as an antidepressive agent [1]. Like many other MAOI, its clinical use is impaired by its potential toxicity and its involvement in many drug interactions of which those with MAOI and hypnotics, narcotic analgesics and tricyclic antidepressants are of clinical importance [2–4]. The mechanisms of many of these drug interactions have not been elucidated, but it has been proposed that MAOI depression of the hepatic metabolizing enzyme systems responsible for the elimina-