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Comparative study of the control of basal acid output from rodent isolated stomachs

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1 Isolated, lumen-perfused, whole stomach preparations from mouse and immature rat produced a stable basal acid output which, although not blocked by histamine H_2-, acetylcholine M- or CCK_B/gastrin receptor antagonists, was almostly completely blocked by the H^+/K^+-ATPase inhibitor, omeprazole, and the metabolic inhibitor, sodium thiocyanate (NaSCN).

2 Fully-defined concentration-effect curves could be obtained on both assays with the phosphodiesterase inhibitor, isobutyl methylxanthine (IBMX) and with dibutyryl cyclic AMP.

3 On the rat stomach assay, histamine H_2-receptor blockade had no effect on the IBMX curve. In contrast, the IBMX response in the mouse was abolished by histamine H_2-receptor blockade. On both assays responses to dibutyryl cyclic AMP were resistant to H_2-receptor blockade.

4 In the absence of suprathreshold endogenous histamine, it is argued that H^+/K^+-ATPase mediated basal acid secretion from the mouse stomach assay is regulated by something other than cyclic AMP.

Keywords: Gastric acid; whole stomach preparation of mouse, rat; H^+/K^+-ATPase; phosphodiesterase inhibitors; adenylate cyclase; histamine

Introduction

The isolated, lumen-perfused stomach of the mouse is now a standard preparation for studying the pharmacology of oxyntic cell secretion (Wan, 1977; Angus & Black, 1979; Szelenyi & Vergin, 1980; Black & Shankley, 1985; Mahklouf & Schubert, 1990). Under basal conditions, this preparation lowers the pH of the unbuffered perfusate from about 6.0 to about 4.4. Treatment with the metabolic poison, sodium thiocyanate (NaSCN), abolished all but a few percent of this acidification suggesting a true basal secretion (Black & Shankley, 1985). That the oxyntic cells were the source of the secretion was implied by the abolition of this secretion by omeprazole, the H^+/K^+-ATPase inhibitor (Sachs & Wallmark, 1989).

The basal oxyntic cell secretion was not inhibited by tetrodotoxin, N-methylatropine or tiotidine thus eliminating the involvement of histamine or neurally-released acetylcholine in the activity. As gastrin (pentagastrin) stimulation is also completely suppressed by histamine H_2-receptor blockade by tiotidine, then presumably gastrin is not involved either. However, isobutyl methylxanthine (IBMX), a highly-selective inhibitor of the phosphodiesterase which hydrolyses adenosine 3':5'-cyclic monophosphate (cyclic AMP), was found to be as full an agonist as histamine in this preparation. Arguably, phosphodiesterase inhibition was potentiating the basal activity of adenylate cyclase (Black & Shankley, 1985). We concluded, therefore, that the basal oxyntic cell secretion was being driven by this basal activity of adenylate cyclase (Black & Shankley, 1985).

Earlier, Berglindh *et al.* (1980) found that IBMX was not only a full agonist in their preparation of rabbit isolated, gastric glands but also that this action could be abolished by histamine H_2-receptor antagonists. However, this suspension of gastric glands was found to have about 100 nM histamine in the supernatant (Soll & Berglindh, 1987). This pointed to the basal adenylate cyclase activity being subliminally driven by histamine in that preparation. Soll (1980) made similar observations on his suspensions of canine isolated oxyntic cells.

During the development of an isolated, lumen-perfused, stomach assay from the rat, a species which we have observed to be 3 fold less sensitive to histamine, we had occasion to re-examine and extend the analysis of the relationship between histamine, adenylate cyclase activity and basal gastric acid output.

Methods

Isolated, lumen-perfused stomach preparations

Gastric acid secretion was measured in isolated, lumen-perfused, stomach preparations essentially as described previously for the mouse (Black & Shankley, 1985). Young adult male mice (Charles River 22–26 g), fasted for 18 h prior to experimentation but with free access to water, and pre-weaned rat pups (Wistar 32–38 g) were used. Animals were killed by cervical dislocation, the abdomen opened and the oesophagus ligated close to the stomach. A polythene cannula (2 mm internal diameter) was inserted into the pylorus via the duodenal bulb, and a small incision made in the fundus through which the stomach contents were gently washed. A second cannula was tied into this incision. The stomachs were then transferred into a 40 ml organ bath containing buffered serosal solution (mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.14, $NaHPO_4$ 15.9, $CaCl_2$ 0.65, glucose 31.6) maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The preparations were continuously perfused from the fundic through the pyloric cannulae (1 ml min^{-1}) with warmed, unbuffered, mucosal solution (mM: NaCl 135, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.3, glucose 31.6) gassed with 100% O_2, and the perfusate passed over a pH-electrode system adjusted to provide 12 cmH_2O intragastric pressure.

Experimental design

Six preparations were studied simultaneously and, after a 60 min stabilisation period, any not showing stable basal responses were rejected (less than 5%). Thereafter, drugs were added to the serosal solution according to individual experimental protocols and the total vehicle volume did not exceed 1 ml.

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A randomized block design was used throughout for allocation of experimental treatments such that, as far as possible, each organ bath received each treatment within the course of an experiment.

Acid secretory responses were expressed as ΔpH, that is, the difference between basal pH, measured immediately prior to experimental intervention, and stimulated pH. Fully-defined, agonist concentration-effect curves were obtained by cumulative dosing at 0.5 $\log_{10}$ units when stable response plateaux were achieved.

Data analysis

Concentration-effect curve data from individual preparations were fitted by means of an iterative least squares minimisation programme to a general logistic function to provide estimates of the midpoint location ($\log[A_{50}]$), midpoint slope parameter (p) and upper asymptote (α) as described previously (Black & Shankley, 1985). For analysis and display purposes the individual computed parameter estimates for each treatment group were expressed as mean ± s.e.mean and single logistic curves stimulated and shown superimposed upon the experimental data.

Computed logistic curve-fitting parameters were compared by one-way ANOVA or Student's t test. Values of $P<0.05$ were considered significant. All errors quoted are s.e.mean.

Drugs

Histamine (Sigma Chemical Co. Ltd.) was dissolved in distilled water to give a 1 M stock which was back-neutralised with 25 μl 10 N NaOH ml^{-1}. Tiotidine (a gift from Imperial Chemical Industries Ltd.) was dissolved in dilute HCl to give a 0.2 mM stock solution; L365,260 (1,3-dihydro-1-methyl-3(**R**)-(3-methylphenyl)-amino-carbonylamino-6-phenyl-2H-1, 4-benzodiazepin-2-one; a gift from Dr Lotti, Merck Sharp & Dohme Research Labs.) and omeprazole (Research Biochemicals Inc.) were dissolved in 90% ethanol and SKF96356 (1-(2-methylphenyl)-4-(methylamino)-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline; a gift from SmithKline & Beecham) in 90% ethanol and 0.1 N HCl. Subsequent dilutions were made in distilled water. Isobutyl methylxanthine (IBMX) and dibutyryl cyclic AMP (db-cyclic AMP) (Sigma) and ICI 63,197 (2-amino-6-methyl-4-propyl-[1,2,4]triazolo[1,5-a]pyrimidin-5(4H) one; a gift from Imperial Chemical Industries Ltd.) were dissolved in distilled water. None of the vehicles concentrations and volumes used had an effect on basal acid output.

Results

Basal acid output

The lumen-perfusion solution for both preparations, being unbuffered, had a pH of approximately 6. Immediately after the preparations were established, the outflow pH in both preparations lay between 5 and 5.5. After 1 h of stabilization the outflow pH in the mouse was 4.64 ± 0.08 (n = 16) and in the rat was 4.86 ± 0.04 (n = 18). The outflow pH from the mouse remained stable within limits for at least 4 h although in the rat the pH tended to drift downwards after 4 h (data not shown). Blockade of histamine H_2-, acetylcholine (ACh) muscarinic (M)- and gastrin/CCK_B-receptors by tiotidine (0.1 mM), atropine (20 μM), L365,260 (1 μM), respectively, did not produce significant inhibition of the basal acid output in either preparation (Table 1). These concentrations were calculated to give dose-ratios in excess of 100 without losing receptor selectivity.

H^+/K^+-ATPase inhibition

Omeprazole (0.1 mM), an 'irreversible inhibitor of H^+/K^+-ATPase' (Fellenius *et al.*, 1981) and SKF96356 (0.1 mM), described as a 'reversible K^+-competitive inhibitor, of that enzyme (Leach *et al.*, 1992) produced significant inhibition of basal acid output in both assays (Figure 1). This inhibition attained a plateau after 60 min. We judged the concentrations of these ligands to be maximal because of the following facts. Omeprazole (10 μM) has been reported to produce greater than 90% inhibition of basal acid output in rat isolated stomachs (Coruzzi *et al.*, 1986) and we used a 10

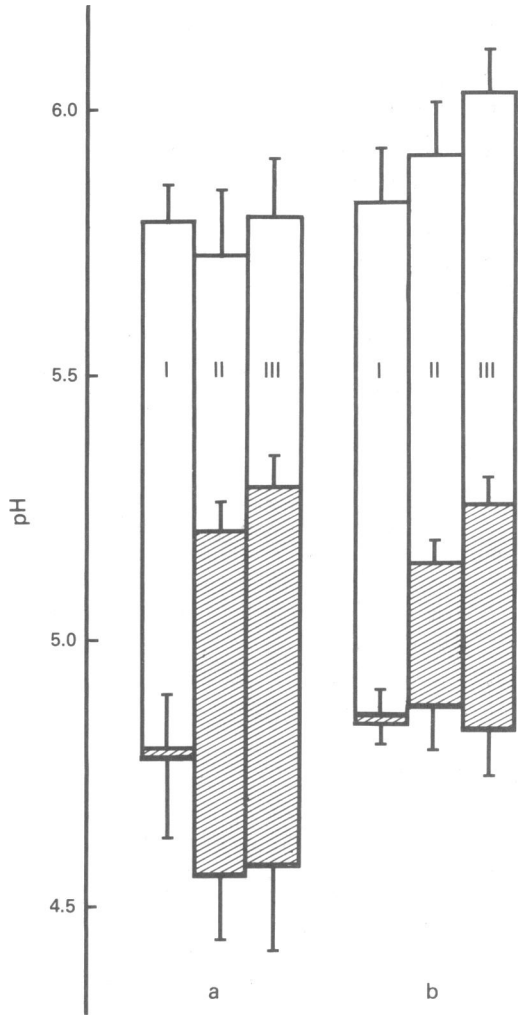

Figure 1 Inhibition of basal gastric acid secretion from isolated, lumen-perfused stomach assays of mouse (a) and immature rat (b). The histograms show the effect of (I) vehicle control followed by sodium thiocyanate (NaSCN, 30 mM), (II) 0.1 mM omeprazole followed by NaSCN (30 mM) and (III) 0.1 mM SKF96356 followed by NaSCN (30 mM). In each case the first treatment is shown as the hatched bar. All treatments were incubated for 120 min.

Table 1 The effect of tiotidine (0.1 mM), atropine (20 μM) and L365,260 (1 μM) on basal gastric acid output, expressed as ΔpH from mouse and immature rat isolated stomach assays

	Mouse	*Rat*
Control	0.04 ± 0.02	0.05 ± 0.02
Tiotidine	0.01 ± 0.03	0.02 ± 0.02
Control	0.06 ± 0.01	0.07 ± 0.02
Atropine	0.06 ± 0.01	0.05 ± 0.02
Control	0.04 ± 0.03	0.08 ± 0.02
L365,260	0.07 ± 0.04	0.08 ± 0.01

Values are given ± s.e.mean. n = 5/7.

fold higher concentration. This concentration of omeprazole abolishes the response to histamine (data not shown). A fully-defined inhibitory concentration-effect curve was obtained for SKF96356 on the rat assay which attained an asymptote at 0.1 mM (logistic curve-fitting parameters: $p[A_{50}] = 5.92 \pm 0.13$, $\alpha = 0.76 \pm 0.11$ pH, $p = 1.32 \pm 0.16$).

H^+/K^+-ATPase inhibition produced a significantly greater reduction (ΔpH) in the mouse than in the rat but Figure 1 shows that this was due to the lower starting pH in the mouse; the final equilibrium pH values of about 5.2 were not significantly different between compounds or assay species. These pH values were still significantly lower than the input pH (Figure 1). When expressed in terms of $[H^+]min^{-1}$, the acid output which was not blocked by omeprazole and SKF96356 was approximately 20% of the total basal output.

Effect of sodium thiocyanate

The residual acid output, following both omeprazole and SKF96356 treatment, was almost abolished (Figure 1) by 30 mM sodium thiocyanate (NaSCN). When NaSCN was tested on its own, two phases of inhibition were seen in most preparations. The first phase reduced the pH, after 60–90 min, to levels achieved with the maximal concentrations of the H^+/K^+-ATPase inhibitors (see Figure 2) and the second phase, beginning after about 120 min, abolished the secretion more or less completely (Figure 1).

Effects of histamine and phosphodiesterase inhibition

The above experiments established that the basal acid output was mainly due to the activity of the H^+/K^+-ATPase. This 'proton pump' can be driven by cyclic AMP leading to kinase activation (Wallmark & Sachs, 1989). Therefore, possible involvement of adenylate cyclase activity in driving basal acid output was assessed by inhibiting phosphodiesterase with isobutyl methylxanthine (IBMX) in both preparations and, additionally, by ICI63197 in the mouse preparation. Fully-defined concentration-effect curves were obtained to the compounds on both the rat and the mouse assays. These curves have been compared with histamine concentration-effect curves, which are believed (Scholes *et al.*, 1976) to be due to activation of the adenylate cyclase (Figure 3). Histamine was more potent on the mouse assay than on the rat but the midpoint slope of the concentration-effect curve in the rat was steeper (approximately double) than in the mouse. In contrast, IBMX was equipotent in both species although the midpoint slope in the rat was again approximately twice that in the mouse. Histamine and IBMX produced similar maximum responses in the mouse. However, in the rat, the maximum response to histamine was much smaller than that to IBMX. Dibutyryl cyclic AMP, the cell membrane penetrating analogue of cyclic AMP, produced a

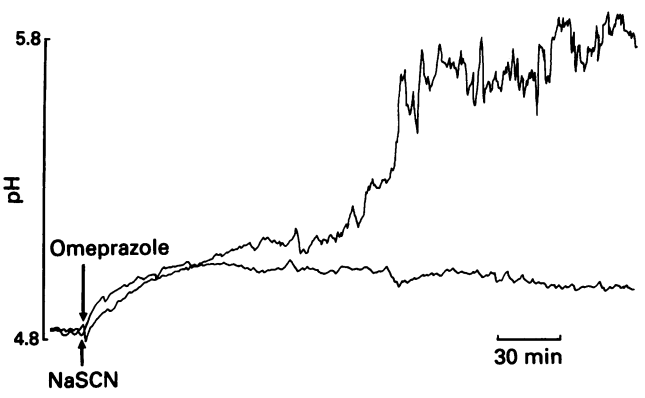

Figure 2 Examples of the time course of effect of 30 mM sodium thiocyanate (NaSCN) and 0.1 mM omeprazole on basal acid output from the lumen-perfused, immature rat stomach assay.

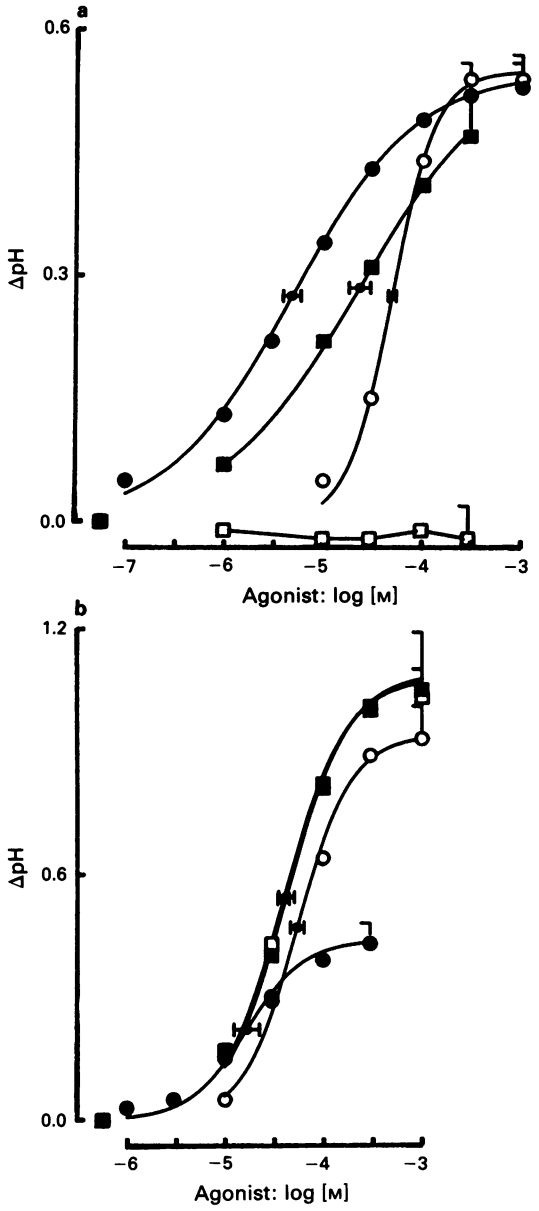

Figure 3 Concentration-effect curves to histamine (●) and dibutyryl cyclic AMP (db-cyclic AMP, ○), and to isobutyl methylxanthine (IBMX) in the absence (■) and presence (□) of 0.1 mM tiotidine obtained in the (a) mouse and (b) immature rat stomach assays. Logistic curve-fitting parameters as follows:

	n	$p[A_{50}]$	$\alpha(\Delta pH)$	*p*
Rat				
Histamine	7	4.78 ± 0.03	0.44 ± 0.05	1.48 ± 0.18
IBMX	6	4.40 ± 0.06	1.08 ± 0.14	1.38 ± 0.14
IBMX (+ tiotidine)	6	4.37 ± 0.08	1.09 ± 0.07	1.38 ± 0.19
db-cyclic AMP	6	4.26 ± 0.07	0.94 ± 0.08	1.59 ± 0.08
Mouse				
Histamine	5	5.32 ± 0.09	0.55 ± 0.04	0.70 ± 0.07
IBMX	5	4.67 ± 0.11	0.57 ± 0.09	0.63 ± 0.03
IBMX (+ tiotidine)	6	–	− 0.02 ± 0.04	–
db-cyclic AMP	6	4.37 ± 0.08	1.09 ± 0.07	1.38 ± 0.19

concentration-dependent stimulation of gastric acid output on both assays (Figure 3). The maximum response to dibutyryl cyclic AMP was not significantly different from that obtained with IBMX.

Effects of histamine H_2-receptor blockade

Tiotidine, was found to be a simple competitive antagonist of

histamine H_2-receptors in both mouse and rat preparations with pK_B values of 6.96 ± 0.11 (Black *et al.*, 1985) and 7.40 ± 0.05 (Welsh *et al.*, 1992), respectively. Tiotidine (0.1 mM) had no effect on the IBMX concentration-effect curves obtained in the rat stomach assay. However, in the mouse assay the IBMX responses were concentration-dependently inhibited by tiotidine and were abolished by 0.1 mM (Figure 3). Tiotidine (0.1 mM) had no significant effect on the dibutyryl cyclic AMP concentration-effect curves on either assay (data not shown).

Discussion

The isolated, lumen-perfused, stomach preparations from mouse and immature rat were developed for quantitative pharmacological analysis of the regulation of acid secretion (Black & Shankley, 1985). Therefore, the assay conditions were manipulated such that agonist responses could be measured from a stable basal acid secretion which was not driven by endogenous secretagogues as determined by the lack of effect on basal secretion of receptor antagonists of the natural stimulants of gastric acid secretion, histamine, gastrin and acetylcholine.

Previously, we reported that the H^+/K^+-ATPase inhibitor, omeprazole, and the metabolic inhibitor, sodium thiocyanate (NaSCN), produced similar levels of inhibition of basal acid ouput on the mouse assay (Black & Shankley, 1985). However, in this study, omeprazole produced only $58 \pm 5\%$ of the maximum pH change, equivalent to approximately 80% when the results are expressed as $[H^+]min^{-1}$, achieved by NaSCN on the mouse assay (Figure 1). The discrepancy between this result and the previous findings in the mouse preparation may be due to the biphasic timecourse of the inhibition by NaSCN. Previously the response was only studied for 60 min and the biphasicity was not identified. Consequently, the omeprazole response was compared with what we now believe to be the first plateau of the NaSCN response.

One possible explanation for the failure of omeprazole to abolish acid output would be if access to the H^+/K^+-ATPase were limited by a similar disposition mechanism to that envisaged for hormone receptor antagonists on these assays (Shankley *et al.*, 1988). On the mouse stomach assay, the underestimation of histamine H_2- and ACh M-receptor antagonist pK_B values was imagined to be due to the loss of the antagonists into the perfusate because the extent of underestimation was correlated with their log P values. Omeprazole is a relatively lipophilic compound (log $P = 2.1$ calculated by ClogP Medchem ver. 3.54, using a benzyl approximation) and therefore a candidate for such loss. However, the 'reversible' H^+/K^+-ATPase inhibitor, SKF96356, a far more lipophilic compound (log $P > 4$; C. Leach, personal communication), produced the same level of inhibition supporting the view that the inhibition of the proton pump was maximal. Moreover, in the rat assay, where we have previously shown that there is far less underestimation of antagonist affinity values (Welsh *et al.*, 1992), omeprazole and SKF96356 also produced the same submaximal inhibition of basal acid output.

Previously, we suggested that the basal acid output from the mouse stomach assay which was not blocked by omeprazole was of metabolic origin (Black & Shankley, 1985). If this assumption were correct then the biphasic nature of the NaSCN timecourse, observed in both species in this study (Figure 2), could reflect the two mechanisms of acid output inhibition by NaSCN proposed by Hersey *et al.* (1981), namely, the inhibition of oxyntic cell acid secretion which apparently occurs at lower concentrations (< 10 mM) and inhibition of mitochondrial respiration which requires higher concentrations (> 10 mM). It was as though the difference in effective concentration for the two processes were expressed in these intact assays as different rates of onset of action. However, this hypothesis may be flawed because the inhibition of mitochondrial activity in the gastric mucosa might be expected to increase rather than decrease the omeprazole-resistant basal acid output due to the release of metabolic acids. Clearly, further experimentation is required to determine the source of the NaSCN-sensitive but omeprazole-resistant basal acid output from the assays.

According to Wallmark & Sachs (1989), the oxyntic cell H^+/K^+-ATPase is driven by kinases which are activated by *inter alia*, cyclic AMP produced by adenylate cyclase. Previously, we provisionally concluded that the basal acid output from the mouse stomach preparation was related to basal activity of adenylate cyclase (Black & Shankley, 1985). The concentration-effect curves for IBMX and histamine obtained on the mouse assay had similar maxima and slopes (Figure 3). This suggested that IBMX was behaving like a post-receptor stimulant of the histamine pathway by mimicking histamine. We had assumed, in common with Soll & Berglindh (1987), that the reason Berglindh *et al.* (1980) found that the response to IBMX was abolished by histamine H_2-receptor antagonists on their assay of rabbit isolated, gastric glands was due to the presence of significant background endogenous histamine.

On the rat assay in this study, IBMX produced concentration-dependent stimulation of acid secretion which was not blocked by the histamine H_2-receptor antagonist tiotidine (Figure 3). Presumably, IBMX inhibits the phosphodiesterase-catalysed breakdown of cyclic AMP produced by the basal activity of adenylate cyclase (Figure 4). In the rat, although the curve slopes were not significantly different, the maximum response to IBMX was significantly greater than that to histamine (Figure 3). The response to IBMX was probably maximal because it was not significantly different from that obtained to dibutyryl cyclic AMP. The latter, by providing the maximum stimulus, presumably defines the maximum effect achievable via the adenylate cyclase pathway. So far as the secretory system is concerned, therefore, histamine behaves as a partial agonist in the rat with the response limitation lying between receptor occupancy and cyclic AMP production, which might be expected in view of the relatively low potency of histamine.

In this study, we have found that the response to IBMX in the mouse assay was abolished by tiotidine. That the secretagogue action of IBMX was due to phosphodiesterase inhibition was confirmed when the same result was obtained with the non-xanthine based inhibitor, ICI63197. Evidently, the IBMX stimulation of gastric acid secretion in the mouse involves the potentiation of histamine H_2-receptor-mediated acid secretion. However, if there had been any oxyntic cell adenylate cyclase activity contributing to basal acid secretion then phosphodiesterase inhibition would be expected to potentiate this and, as found in the rat assay, there would be a histamine H_2-receptor antagonist resistant component of the IBMX response. It is as though the mouse basal adenylate cyclase activity, although governed by histamine, is insufficient to produce a secretory response as judged by its being refractory to histamine H_2-receptor blockade. However, when the phosphodiesterase is inhibited, cyclic AMP can accumulate to levels which stimulate acid secretion (see Figure 4). The possibility that IBMX, by inhibiting phosphosidesterase, releases significant levels of endogenous histamine in the mouse (and rat) is excluded by the finding that db-cyclic AMP responses were refractory to histamine H_2-receptor blockade (Figure 3).

The difference in sensitivity to histamine H_2-receptor blockade of the IBMX response does not necessarily imply that the underlying biochemical regulation of basal acid secretion is fundamentally different between the species. In Figure 4, we illustrate a model which contains the minimum operators we believe are necessary to explain our results. We have shown in rectangles the three essential enzymes in the model, adenylate cyclase, phosphodiesterase and H^+/K^+-ATPase. The chemical inputs to these enzymes are shown in

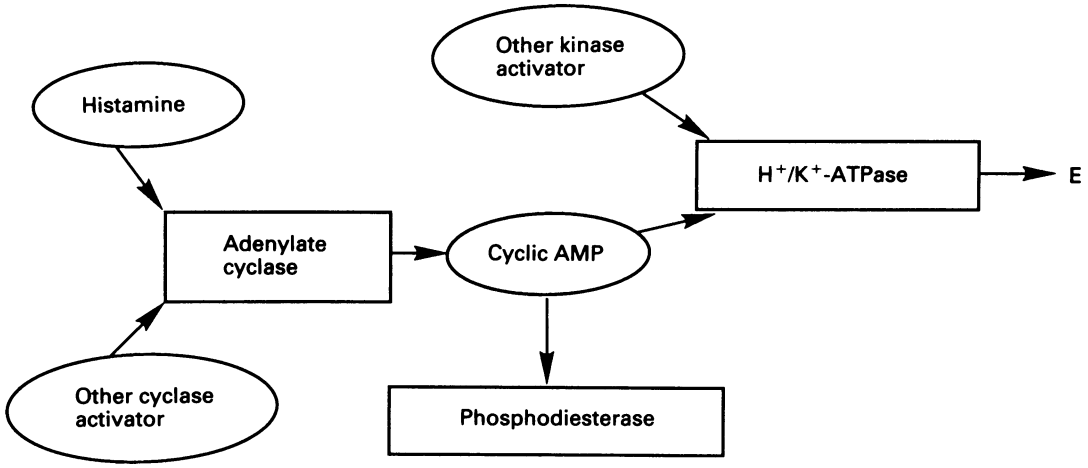

Figure 4 Schematic representation of the regulation of basal and histamine-stimulated gastric acid secretion in the mouse and immature rat stomach (see text for details).

ellipses. The H^+/K^+-ATPase is driven by both cyclic-AMP and another source of kinase activation. The adenylate cyclase can be driven by histamine and other activators not specified by our experiments. The level of cyclic AMP is determined by the ratio of the activities of adenylate cyclase and phosphodiesterase. In the rat, the inability of histamine H_2-receptor blockade to block the effects of phosphodiesterase inhibition implies that the cyclic AMP is being activated by some other factor not specified by these experiments. Similarly, the experiments do not allow us to distinguish whether cyclic AMP alone or in combination with some other source of kinase activation drives the H^+/K^+-ATPase. However, in the mouse, the fact that histamine H_2-receptor blockade abolishes the effects of phosphodiesterase inhibition but not the basal activity of the pump means that the adenylate cyclase is being driven by a level of histamine stimulation which produces a flux of cyclic AMP lower than the flux through phosphodiesterase. However, inhibition of phosphodiesterase now leads to accumulation of cyclic AMP and activation of the H^+/K^+-ATPase. The fact that histamine H_2-receptor block abolished the response to phosphodiesterase inhibition and yet had no effect on basal secretion can only happen if the adenylate cyclase is being driven solely by histamine but the pump is driven by another source of kinase activation.

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