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EFFECTS OF CALCIUM ON ACID SECRETION FROM THE RAT ISOLATED GASTRIC MUCOSA DURING STIMULATION WITH HISTAMINE, PENTAGASTRIN, METHACHOLINE AND DIBUTYRYL CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE

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1 An isolated gastric mucosal preparation from immature rats is described. The mucosal surface was superfused and acid secretion was recorded continuously by monitoring H^+-ion concentration.

2 Repeated stimulation with submaximal concentrations of histamine or dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP) produced consistent responses. Secretion induced by pentagastrin and methacholine demonstrated varying degrees of tachyphylaxis. All responses were readily reversed on washing out the secretagogue.

3 Reduction of Ca^{2+} from 3.6 mM increased responses to histamine, pentagastrin and methacholine while increase of Ca^{2+} to 7.2 mM decreased responses. Db cyclic AMP-induced secretion was not influenced by external Ca^{2+}.

4 When Mg^{2+} was raised from 1.2 mM responses to histamine and pentagastrin increased. Replacement of Ca^{2+} with Mg^{2+} produced a transient increase followed by a decrease in responses.

5 The similarity of the effects of Ca^{2+} on pentagastrin, methacholine and histamine indicates that neither pentagastrin nor methacholine act via Ca^{2+}-dependent histamine release.

Introduction

Changes in plasma Ca^{2+} concentration have been shown to affect gastric secretion in man and various other species (Barreras, 1973). Studies in rats have shown that increases in blood Ca^{2+} concentrations reduce spontaneous acid output (Kowalewski, 1968) and that calcitonin and parathyroid hormone, which regulate Ca^{2+} homeostasis, also influence secretion rates (Hotz, Goebell, & Ziegler, 1976). These effects may be caused indirectly via hormonal mechanisms, such as the release of endogenous gastrin (Becker, Konturek, Reeder, & Thompson, 1973) or histamine or by a direct action on parietal cells. Studies *in vivo* of the role of Ca^{2+} are limited by the relatively small changes in plasma concentrations that can be made, and are complicated by the presence of homeostatic mechanisms.

It has been proposed that histamine acts directly on the parietal cells and that gastrin acts indirectly by releasing histamine from local histamine-producing cells (Rosengren & Svensson, 1969). Since histamine release from rat peritoneal mast cells is critically dependent on external Ca^{2+} concentration (Foreman & Mongar, 1972) it is possible that a secretagogue which acts via the release of endogenous histamine would exhibit a greater degree of Ca^{2+}-dependence than one which acts directly. We have, therefore, investigated the effect of various external Ca^{2+} concentrations on basal and stimulated acid-secretion from the rat isolated gastric mucosa, in the absence of vascular, nervous and hormonal influences. This preparation has previously been shown to respond to agents which stimulate or inhibit gastric acid secretion *in vivo* (Hearn & Main, 1975; Main & Pearce, 1978a). Some of these results have been communicated to the British Pharmacological Society (Main & Pearce, 1977a,b).

Methods

Fed, home-bred rats (Olac strain, usually male) weighing between 80 and 120 g were anaesthetized

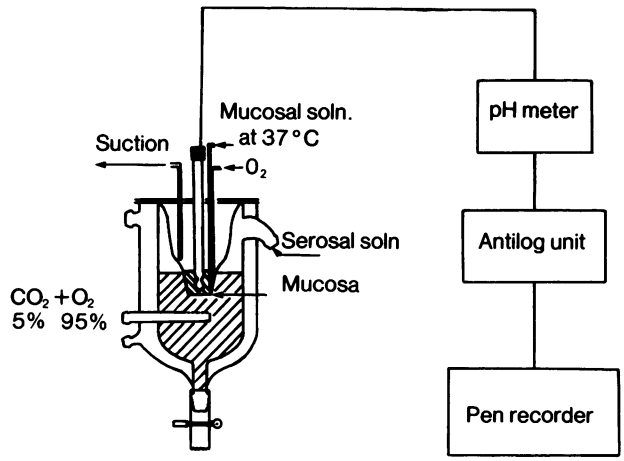

Figure 1 Experimental apparatus for studying acid secretion by the rat isolated gastric mucosa.

with pentobarbitone (60 mg/kg, s.c.). The dissection technique used, modified from that developed by Forte, Forte & Machen (1975) for the piglet, has recently been described in detail (Main & Pearce, 1978a). The non-glandular portion of the stomach was cut away and the muscle layer overlying the non-antral region separated from the mucosa by blistering. Two pieces of mucosa were obtained from one stomach and each tied, mucosal surface inwards, over a 1 cm^2 opening on a polyethylene vessel. Each tissue was placed in an organ bath at 37°C (Figure 1) containing 35 ml of a modified Krebs solution (serosal solution, SS, Sernka & Hogben, 1969) which bathed the serosal surface and was gassed vigorously with a 95% O_2 and 5% CO_2 mixture. The mucosal surface was superfused by means of a peristaltic pump (Watson-Marlow Ltd.), at the rate of 0.5 ml/min, with an unbuffered solution of similar ionic composition, gassed with 100% O_2. The volume of solution on the mucosal side was kept constant by suction via the same pump, but with larger diameter tubing, and varied between 1.6 and 2.0 ml. Secretion was recorded continuously via a dual micro-electrode in the mucosal solution (MS) connected to an antilog unit and a potentiometric pen recorder. The H^+-ion concentration was noted every 15 min and expressed as apparent secretion rate.

Solutions

For control conditions, the serosal solution (SS) contained (mM): NaCl 110.0, KCl 5.0, $CaCl_2.6H_2O$ 3.6, $MgCl_2.6H_2O$ 1.2, $NaHCO_3$ 26.0 and glucose 16.7. The mucosal solution (MS) was of similar composition with 26.0 mM $NaHCO_3$ replaced by equimolar NaCl. For test conditions, both solutions contained Ca^{2+} in concentrations ranging from 0.0 to 7.2 mM, and Mg^{2+} from 1.2 to 4.8 mM, with all other cations kept constant. All chemicals were of analytical reagent grade.

Experimental design

A series of preliminary studies with Ca^{2+} concentrations ranging from 0.0 to 14.4 mM present from the beginning of the experiment showed a wide variation in spontaneous acid output both within and between litters of rats. It was decided that an initial control period with 3.6 mM Ca^{2+} was required in each experiment in order to compare the effect of different concentrations. To reduce variation further, the mucosae from each pair were randomly allocated to different groups.

Basal acid-output was monitored for 120 min before secretory responses were investigated, although a small priming dose was usually present between 30 and 60 min. Drugs were added to the SS in volumes not exceeding 1 ml and contact times normally used were 30 min for pentagastrin and methacholine, 45 min for histamine and 60 min for dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP). A standard washout procedure was carried out after all responses. The SS was drained and replaced with warmed solution at 0, 5, 15, and 30 min after the end of the appropriate contact time and at 30 min intervals thereafter. After the control response, the tissues were washed with test solutions for 120 min before a further two responses to the same secretagogue were obtained, separated by a 45 min (histamine) or 60 min recovery period (all other drugs).

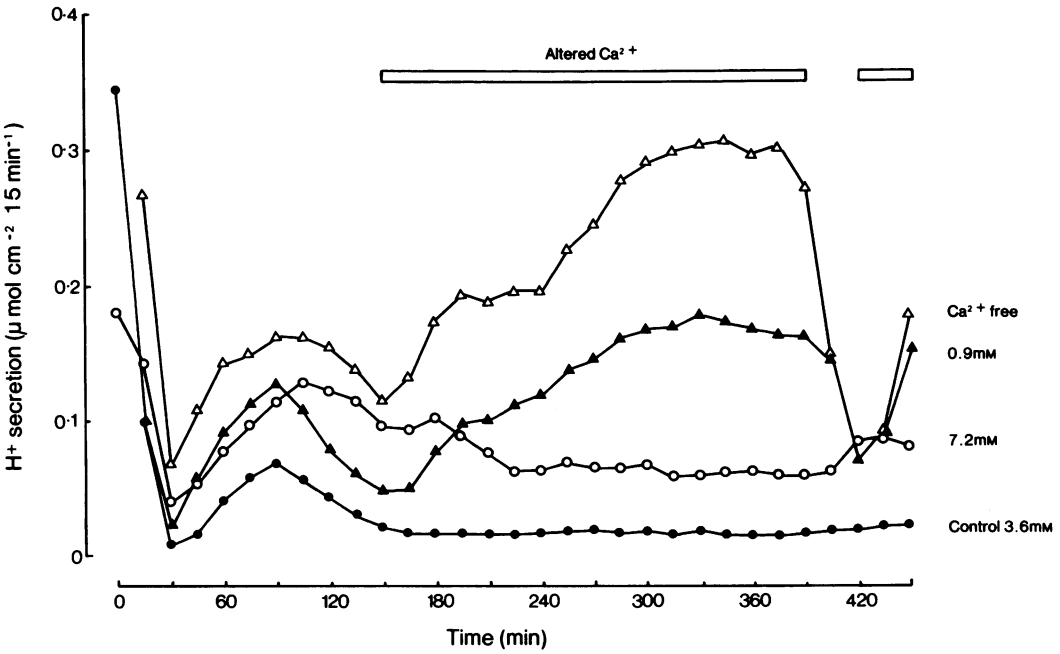

Figure 2 Effects of calcium on acid secretion from non-stimulated preparations. Solutions were changed at 150 min, and 390 and 420 min. Each point represents the mean of 6 observations. Groups are described by the following symbols: 7.2 mM (○), 3.6 mM (●), 0.9 mM (▲), Ca^{2+}-free (△).

Drug solutions

Stock solutions of drugs in 0.9% w/v NaCl solution (saline) were diluted for immediate use or stored frozen until required. The following drugs were used: pentobarbitone (Nembutal, Abbot Laboratories Ltd.), histamine acid phosphate (BDH Chemicals Ltd), pentagastrin (Peptavlon, ICI Ltd.), methacholine chloride (acetyl-β-methylcholine chloride, Sigma Chemical Co.) and dibutyryl cyclic adenosine 3',5'-monophosphate (Boehringer). Ethylene diaminetetraacetic acid, disodium salt (EDTA, BDH Chemicals Ltd.) and ethyleneglycol-bis-(β-amino-ethyl ether) *N,N'*-tetraacetic acid (EGTA, Sigma Chemical Co.) were added to Ca^{2+}-free solutions for histamine-stimulated preparations only.

Analysis of results

The secretory response is calculated as the increase in secretion rate at the peak of the response over the preceding basal rate. Results are expressed as the mean ± standard error of the mean. The value quoted is the difference in secretion rate (μmol cm^{-2} h^{-1}) between the mean of two responses under test conditions and the corresponding control value. Changes in responses were compared both within experiments (paired *t* test) and with control groups (unpaired *t* test). A *P* value of less than 0.05 was considered to be significant.

Results

Effects of altered Ca^{2+} *concentrations*

Basal acid-output In a randomized group of 24 experiments, one preparation from each pair was kept as a non-stimulated control and the other exposed to pentagastrin. In resting mucosae, acid-output decreased rapidly to a minimum within 15 to 30 min and then increased spontaneously to reach a peak at about 120 min; output then declined slightly before levelling off (Figure 2). Tissues were bathed in control solutions for the first 150 min of each experiment, and basal acid-output at 120 min was 0.40 ± 0.09 μmol cm^{-2} h^{-1} ($n = 24$). When control conditions were maintained throughout, no change in basal output was seen (−0.11 ± 0.05, $n = 6$ for each group).

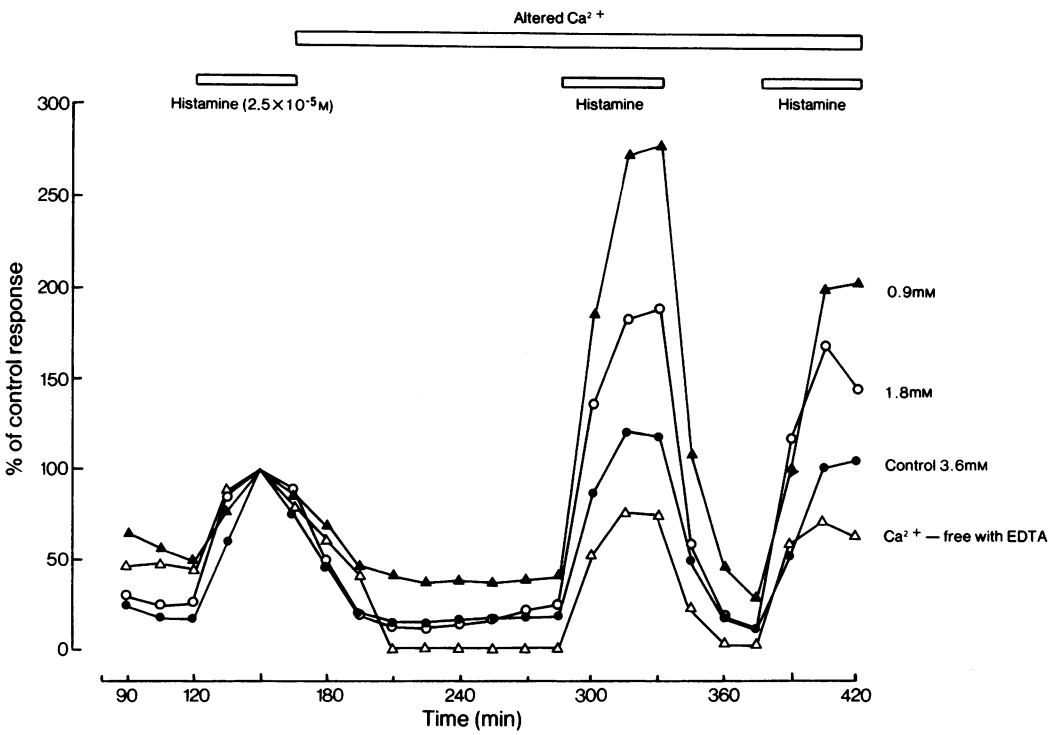

Figure 3 Effects of calcium on acid secretion from histamine-stimulated preparations. Histamine (2.5×10^{-5} M) was present at the times indicated, and solutions changed at 165 min from the start of the experiment. Each point represents the mean of 8 observations. Symbols as for Figure 2; 1.8 mM (○) and Ca^{2+}-free solutions containing 0.5 mM EDTA (SS) (△) were also used.

When Ca^{2+} was raised to 7.2 mM a significant decrease in spontaneous output occurred (-0.24 ± 0.09, $P < 0.05$). Large increases in rate were produced by reduction in concentrations to 0.9 mM or to Ca^{2+}-free ($+0.31 \pm 0.23$ and $+0.47 \pm 0.21$ respectively). Only the change in Ca^{2+}-free solutions was significantly different from that in controls ($P < 0.05$). These effects were readily reversed on washing with 3.6 mM solutions.

Although levels of basal output vary slightly between paired preparations from the same rat, the large differences in rate observed during the control period were due to variation between litters and littermates.

Stimulated acid-output

Histamine Preliminary studies with histamine demonstrated dose-dependent secretory responses (Main & Pearce, 1978a) over the range 10^{-5} to 5×10^{-5} M. The concentration used in the present experiments was 2.5×10^{-5} M, with 2.0×10^{-5} M for the priming dose.

In a randomized group of 16 experiments, both mucosae were treated with the same test solutions. Basal output was 0.97 ± 0.16 μmol cm^{-2} h^{-1} (120 min, $n = 32$) and the control response, which reached a peak after 30 min (Figure 3), was 2.01 ± 0.47. Under control conditions, responses to histamine increased slightly during the experiment ($+0.55 \pm 0.46$, $n = 8$ for each group). Reduction of Ca^{2+} to 1.8 or 0.9 mM produced significant increases in responses ($+3.68 \pm 0.41$, $P < 0.002$ and $+4.67 \pm 1.04$, $P < 0.01$ respectively). These changes were both significantly larger than seen with control solutions ($P < 0.002$, $P < 0.01$ respectively). The effect of Ca^{2+}-free solutions, with 0.5 mM EDTA in SS only, was variable. Of the 8 tissues in the group, showing a mean change of $+0.42 \pm 0.84$, 4 demonstrated an increase in responses ($+2.40 \pm 0.71$, $P < 0.05$) and 4 an almost complete cessation of secretion (-1.55 ± 0.44, $P < 0.05$). Both these changes were

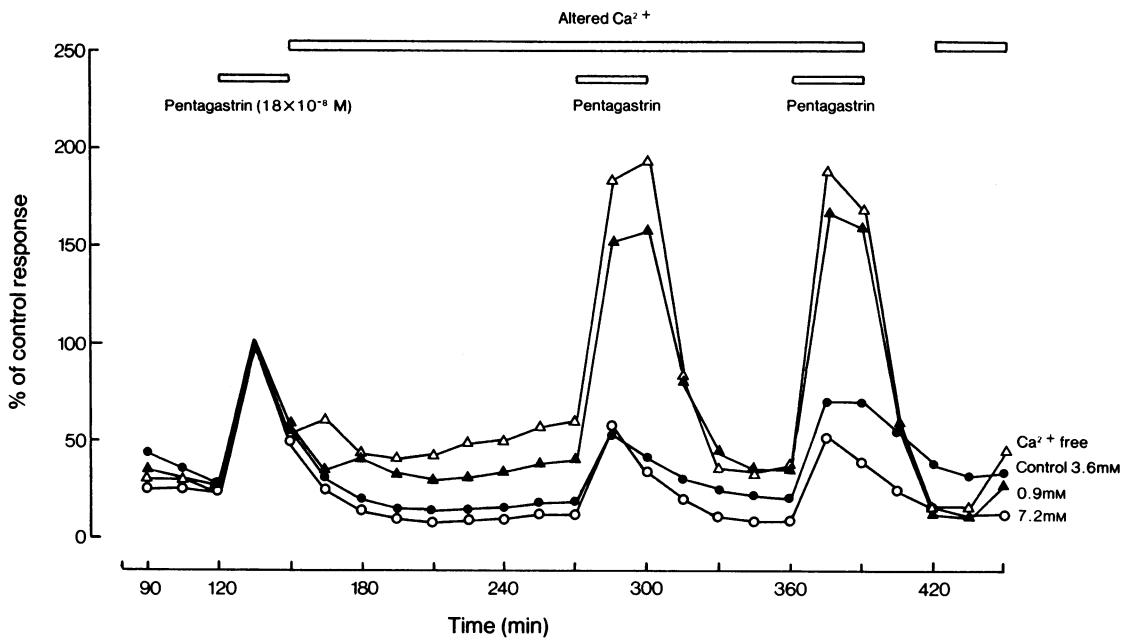

Figure 4 Effects of calcium on acid secretion from pentagastrin-stimulated preparations. Pentagastrin (1.8×10^{-8} M) was present at the times indicated and solutions changed at 150 min. Each point represents the mean of 6 observations, with 7 for Ca^{2+}-free. Symbols as for Figure 2.

significant when compared with controls ($P < 0.05$, $P < 0.02$ respectively).

Studies with EDTA or EGTA on paired mucosae from a total of 24 rats have shown that neither type of effect is dependent on the age, weight or sex of the rat used. Neither tissue from 15 rats ($n = 27$) showed more than a minimal response to histamine after the 120 min changeover period (+0.01 on 0.01). In many cases, the pH rose above that of the mucosal solution indicating increased permeability to HCO_3^- ions. Mucosae from another 9 rats ($n = 17$) still produced large secretory responses although basal output was reduced to very low levels (+2.60 on 0.02). A second response was measured for 8 of these latter preparations. The first response in Ca^{2+}-free solutions (+2.56 on 0.02) was followed 90 min later by increases of +1.86 on 0.06. Although this second response was significantly smaller ($P < 0.01$), there was no reason to suppose that a further response could not have been obtained.

Pentagastrin Preliminary dose-response studies demonstrated tachyphylaxis, the development of which was dose-related, and indicated that a relatively low concentration of pentagastrin (1.8×10^{-8} M) would be required in order to obtain reproducible responses. The experimental design shown in Figure 4 produced a satisfactory pattern of responses; the mucosae were exposed to a priming dose of pentagastrin (10^{-8} M) which appeared to reduce later variation in stimulated secretion rates.

The mucosae used for this study were the paired halves of the non-stimulated tissues used to investigate spontaneous acid-output. Basal output from the stimulated mucosae (0.36 ± 0.07 μmol cm^{-2} h^{-1} at 120 min, $n = 25$) was very similar to that from the resting preparations and was altered in the same manner by changes in external Ca^{2+}. The control response to pentagastrin which reached a peak after 15 min (Figure 4) was 1.18 ± 0.24. Under control conditions a slight decrease in responses occurred (-0.17 ± 0.08, $n = 6$ for each group except Ca^{2+}-free, $n = 7$) with a greater decrease being observed in 7.2 mM Ca^{2+} solutions (-0.61 ± 0.35). Reduction of Ca^{2+} to 0.9 mM or Ca^{2+}-free resulted in a significant increase in responses ($+0.95 \pm 0.29$, $P < 0.05$ and $+1.04 \pm 0.15$, $P < 0.002$ respectively). The changes in reduced Ca^{2+} were both significantly different from control conditions ($P < 0.01$ and $P < 0.002$, respectively).

Methacholine Dose-response studies showed that

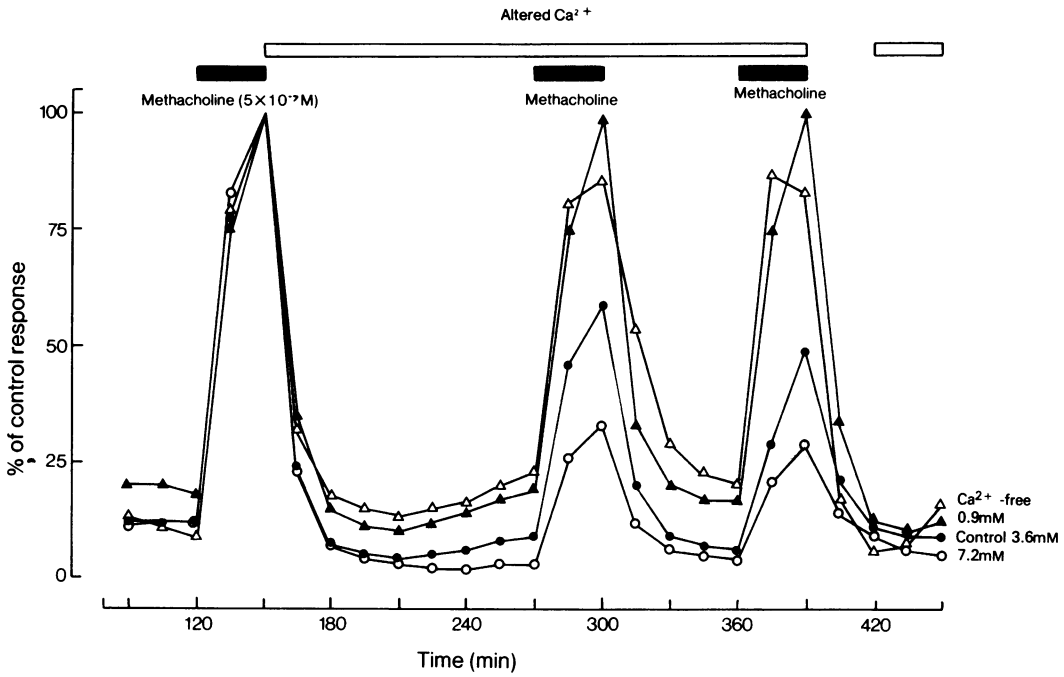

Figure 5 Effects of calcium on acid secretion from methacholine-stimulated preparations. Methacholine (5×10^{-7} M) was present at the times indicated and solutions changed at 150 min. Each point represents the mean of 6 observations with 4 for 7.2 mM Ca^{2+}. Symbols as for Figure 2.

stimulation of secretion with methacholine was accompanied by the rapid development of tachyphylaxis.

In a series of 11 experiments, the initial responses to methacholine (5×10^{-7} M) were much larger than predicted from preliminary data. This concentration did not cause maximal stimulation of acid secretion since larger responses could be produced by higher concentrations at the end of the experiment. The basal output was 1.14 ± 0.18 μmol cm^{-2} h^{-1} (120 min, $n = 22$) and the control response which reached a peak after 30 min (Figure 5) was 8.14 ± 1.26. In control solutions, secretion decreased to 55% of the initial peak (-2.93 ± 0.58, $P < 0.01$, $n = 5$) while an increase in Ca^{2+} to 7.2 mM produced larger decreases (-4.02 ± 0.73, $P < 0.02$, $n = 4$). Reduction in concentrations to 0.9 mM or Ca^{2+}-free, maintained responses near their original level (-0.11 ± 0.65 and -1.89 ± 1.37 respectively, $n = 6$ for both groups). The change in 0.9 mM solutions was significantly different from control data ($P < 0.02$).

Dibutyryl cyclic adenosine 3',5'-monophosphate Preliminary studies demonstrated that although secretory responses to db cyclicAMP were dose-related a wide variation in sensitivity existed between preparations. A concentration of 10^{-4} M was usually sufficient to produce responses of the magnitude observed with histamine and pentagastrin.

Basal output was 1.00 ± 0.10 μmol cm^{-2} h^{-1} (120 min, $n = 32$) and the initial response which reached a peak after 60 min (Figure 6) was 0.72 ± 0.09. In control solutions, responses increased during the experiment ($+0.93 \pm 0.39$, $n = 8$ for each group). Larger increases in responses were observed with 0.9 mM and Ca^{2+}-free solutions ($+1.51 \pm 0.48$, $P < 0.02$ and 1.97 ± 0.98 respectively), but these changes were not significantly greater than in the control group. In contrast to other secretagogues, responses to db cyclicAMP also increased in the presence of 7.2 mM Ca^{2+} ($+1.52 \pm 0.27$, $P < 0.002$).

Effects of raised Mg^{2+} *concentrations*

Since the previous results could have been due to changes in the relative concentrations of Ca^{2+} and Mg^{2+} ions, similar experiments were carried out with raised Mg^{2+}, keeping Ca^{2+} constant at 3.6 mM.

Histamine Basal output was 1.15 ± 0.29 μmol cm^{-2} h^{-1} (120 min, $n = 17$) and the control response was 4.68 ± 1.08 (Figure 7). When control conditions were

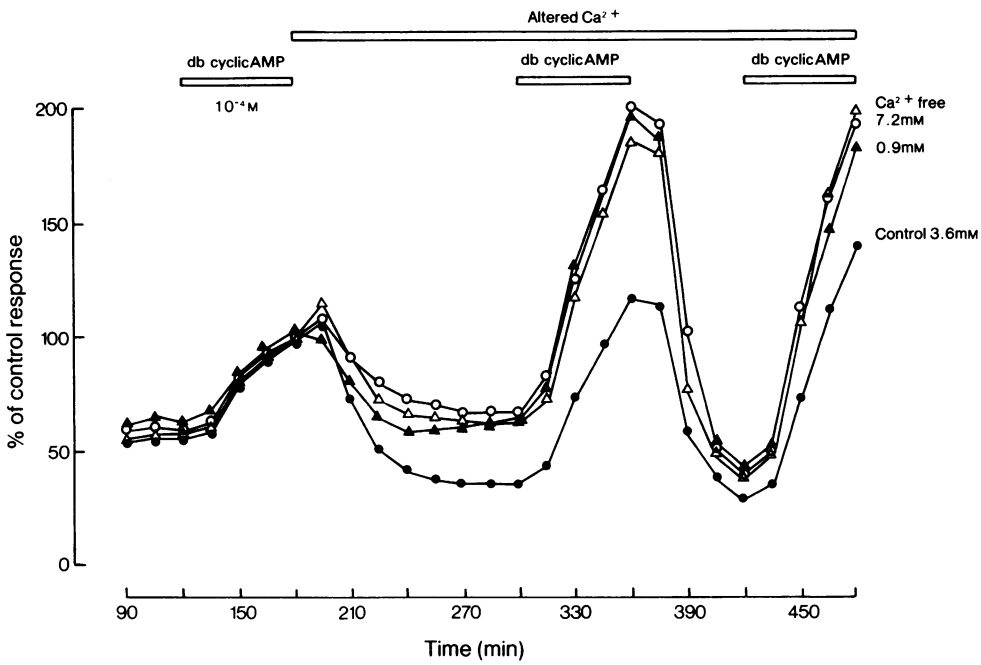

Figure 6 Effects of calcium on acid secretion from dibutyrylcyclic AMP (db cyclicAMP)-stimulated preparations. Db cyclicAMP (10^{-4} M) was present at the times indicated and solutions changed at 180 min. Each point represents the mean of 8 observations. Symbols as for Figure 2.

maintained throughout, responses increased by 1.97 ± 0.91 ($n = 6$). Larger increases were observed with 2.4 and 4.8 mM Mg^{2+} ($+4.97 \pm 1.79$, $P < 0.05$, $n = 5$ and $+2.96 \pm 1.37$, $n = 6$, respectively). These changes were not significantly different from control data.

Pentagastrin Basal output was 1.18 ± 0.21 μmol cm^{-2} h^{-1} (120 min, $n = 18$) and the control response was 3.83 ± 0.63 (Figure 8). In control solutions secretion decreased to 80% of the initial peak (-0.94 ± 0.43, $n = 6$ for each group). Although a smaller decrease was apparent with 2.4 mM solutions (-0.85 ± 0.23) this was significant within experiments ($P < 0.02$). Further increase in Mg^{2+} to 4.8 mM resulted in a larger decrease than observed with the control group (-1.22 ± 0.43, $P < 0.05$). As before, these changes were not significant when compared with control data.

Effect of raised Mg^{2+} *in the absence of* Ca^{2+}

One tissue from each of 6 pairs was exposed to histamine, the other to pentagastrin. After the control period, the mucosae were bathed with a Ca^{2+}-free solution containing 4.8 mM Mg^{2+}. The effect was biphasic and more marked on histamine-stimulated mucosae (Figure 9).

Histamine After the 120 min changeover period, spontaneous output was reduced by 0.18 ± 0.24 μmol cm^{-2} h^{-1} from the control level (1.64 ± 0.27, 120 min). The first test response, (T_1), was significantly greater ($+3.88 \pm 0.82$, $P < 0.01$) than the control response (2.52 ± 0.34). Basal output decreased rapidly during the next washout period, falling by 1.34 ± 0.20 ($P < 0.01$) to 0.11 ± 0.07. The second test response (T_2, 2.18 ± 0.99), only slightly smaller than the control, was significantly reduced from T_1 (-4.22 ± 1.48, $P < 0.05$).

Pentagastrin Basal output increased slightly from 120 min (1.67 ± 0.22) to 270 min ($+0.49 \pm 0.50$), but decreased rapidly on washing after T_1 (-1.50 ± 0.44, $P < 0.02$ to 0.66 ± 0.30). Secretory responses were less affected. The first test response was not significantly reduced from control values (-0.42 ± 0.17 from 2.16 ± 0.28) and the second was only slightly smaller (-0.11 ± 0.50 to 1.64 ± 0.65).

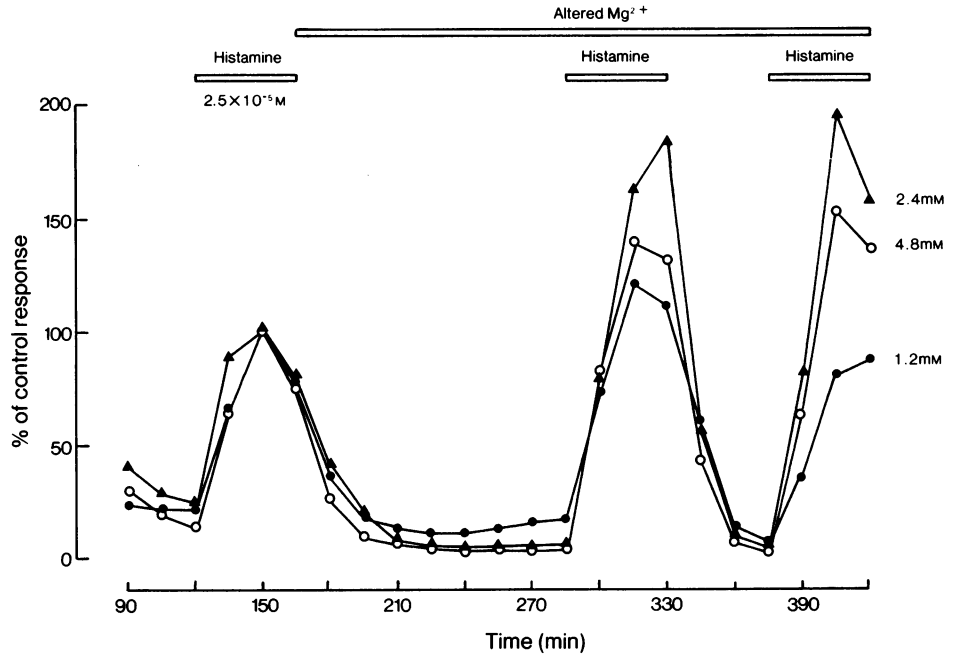

Figure 7 Effects of magnesium on acid secretion from histamine-stimulated preparations. Histamine (2.5×10^{-5} M) was present at the times indicated and solutions changed at 165 min. Each point represents the mean of 6 observations with 5 for 2.4 mM Mg^{2+}. Groups are described by the following symbols: 1.2 mM (●), 2.4 mM (▲), 4.8 mM (○).

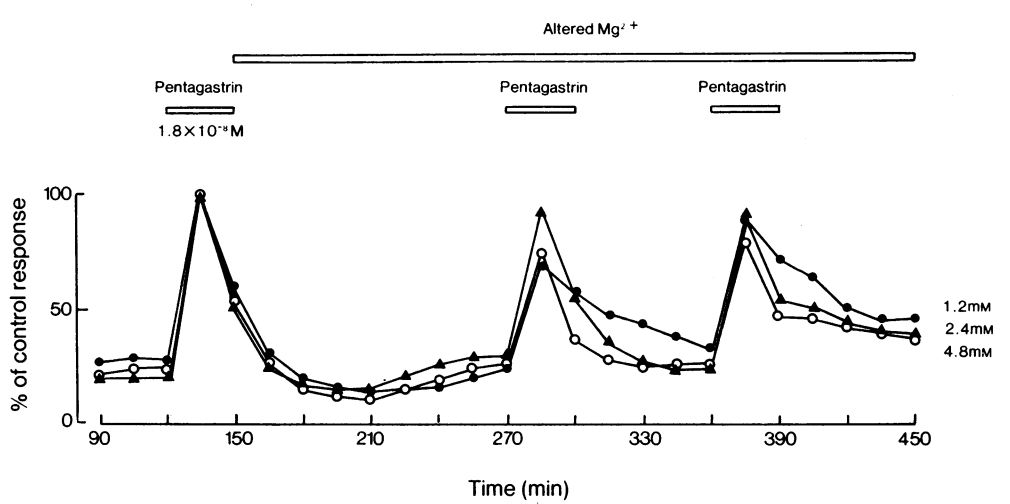

Figure 8 Effects of magnesium on acid secretion from pentagastrin-stimulated preparations. Pentagastrin (1.8×10^{-8} M) was present at the times indicated, and solutions changed at 150 min. Each point represents the mean of 6 observations. Symbols as for Figure 7.

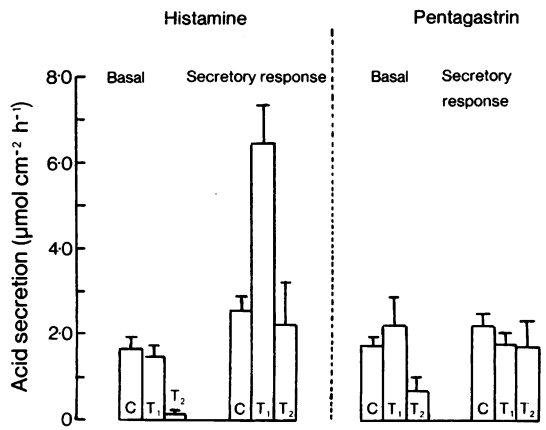

Figure 9 Effects of Ca^{2+}-free 4.8 mM Mg^{2+} solutions on basal and stimulated acid secretion from paired mucosae exposed to (a) histamine (2.5×10^{-5} M) or (b) pentagastrin (1.8×10^{-8} M). Each column represents the mean of 6 observations; vertical lines show s.e. means. The first column in each group indicates the control value (C), with T_1 and T_2 for test values.

Discussion

The present experiments confirm that the isolated gastric mucosa is a useful preparation for studying the direct effects of agents which influence acid secretion (Main & Pearce, 1978a). The preparation responds to a wide range of secretory stimulants and the use of a priming dose and an appropriate experimental design allows responses to secretagogues to be obtained for up to 10 h. The use of paired mucosae, distributed randomly between groups of experiments, helps to reduce variation and facilitates quantitative studies. Under control conditions (3.6 mM Ca^{2+}, 1.2 mM Mg^{2+}) spontaneous acid output declined slightly from an initial peak (90 to 120 min) and then remained constant. Histamine and db cyclicAMP-induced secretion increased with repeated stimulation while responses to pentagastrin or methacholine showed some tachyphylaxis.

Levels of basal acid output and secretion induced by histamine, pentagastrin or methacholine were inversely related to external Ca^{2+}-concentrations over the range 0.0 to 7.2 mM. This effect may be due to antagonism between Ca^{2+} and Mg^{2+} ions, an increase in the relative proportion of the latter increasing secretion rates. In contrast, changes in Ca^{2+} had no apparent effect on db cyclicAMP-induced secretion. The absence of an effect of Ca^{2+} may reflect an intracellular site of action for this secretagogue.

As external Mg^{2+} was raised to 2.4 mM, responses to histamine increased, with smaller changes being observed in 4.8 mM solutions. In a Ca^{2+}-free, 4.8 mM Mg^{2+} solution, responses were first increased and then greatly reduced. Similar effects were noted for pentagastrin although the magnitude of the changes was less. The potentiation observed with Ca^{2+}-free solutions and the inhibitory effect of 4.8 mM Mg^{2+} may combine to produce the pattern of responses observed with Ca^{2+}-free, 4.8 mM Mg^{2+} solutions. The observations suggest that an increased ratio of Mg^{2+} to Ca^{2+} ions causes an increase in secretion rates provided that some Ca^{2+} is present and Mg^{2+} does not exceed a critical concentration. Kowalewski (1968) has reported control levels of Ca^{2+} in rat blood of 10 mg%, corresponding to 0.5 mM Ca^{2+} (approximately 50% of which is ionised). Solutions containing 0.9 or 1.8 mM Ca^{2+} and 1.2 mM Mg^{2+} may prove optimal for future pharmacological studies on this preparation.

Exposure of bullfrog (Forte & Nauss, 1963) or frog mucosae (Jacobson, Schwartz, & Rehm, 1965) to Ca^{2+}-free solutions greatly decreased both resting electrical potential difference and basal acid output. Although the presence of EDTA or citrate usually increased the rate of development of the effect, individual mucosae varied in their sensitivity to a Ca^{2+}-free environment (Forte & Nauss, 1963). The results of more detailed studies have been described by Kasbekar (1974): during stimulation of secretion with histamine, pentagastrin, or acetylcholine, the mucosae were depleted of Ca^{2+} by repeated washing with a Ca^{2+}-free, EDTA solution until acid secretion had ceased. With continued absence of Ca^{2+}, the pH of the mucosal solution increased due to passive diffusion of HCO_3^- from the serosal solution. The replacement of 1 mM Ca^{2+} in the mucosal solution, which has been found to replenish selectively intercellular Ca^{2+} (Schwartz, Kashiwa, Jacobson, & Rehm, 1967), allowed responses to be obtained to db cyclicAMP or theophylline although the mucosae were unable to respond to the other secretagogues. Kasbekar postulated the existence of intracellular Ca^{2+}-compartments related to the process of acid secretion, in addition to those in the junctional complexes.

The similarity of the effects of Ca^{2+} on pentagastrin, methacholine, and histamine indicates that neither pentagastrin nor methacholine act via Ca^{2+}-dependent histamine release. However, our results do not exclude the possibility that the response to gastrin may be mediated by non Ca^{2+}-dependent histamine release or by increased histamine formation. An alternative hypothesis (Grossman & Konturek, 1974) that parietal cells possess separate receptors for gastrin, histamine and acetylcholine and that histamine sensitizes the cells to gastrin is supported by recent studies on O_2-uptake in canine isolated parietal cells (Soll,

1978) and by our observation that metiamide in concentrations much greater than that required to block histamine, reduced but did not abolish secretory responses to gastrin (Main & Pearce, 1978b).

In contrast to these results, Black & Welch (1977) found a differential effect of Ca^{2+} and Mg^{2+} on secretion from the mouse isolated whole stomach preparation: when compared with 0.65 mM Ca^{2+}, 60 min contact with Ca^{2+}-free solutions significantly reduced responses to pentagastrin (10^{-7} M), (101 ± 31 nmol/ml reduced to 15 ± 5, $P < 0.05$, $n = 6$ for all groups) without affecting secretion induced by histamine (10^{-5} M), (202 ± 34 control; 141 ± 32, Ca^{2+}-free; $P > 0.1$). Experiments with 5 mM Mg^{2+} also showed a selective inhibition of responses to pentagastrin (158 ± 19 reduced to 46 ± 16, $P < 0.001$; histamine, 135 ± 11 control; 114 ± 22, 5 mM Mg^{2+}; $P > 0.2$). From these data, Black & Welch postulated that pentagastrin stimulated acid secretion indirectly via histamine release, since pentagastrin was significantly more Ca^{2+}-dependent than histamine. These differences may be explained in part by experimental design and type of preparation used, but may also reflect a species-specific effect. They are unlikely to be due to inadequate equilibration between tissue and bathing solutions since in our experiments mucosae, which lack an external muscle barrier to diffusion, were incubated for 2 h before investigation of changes in responses to secretagogues. If in addition to having a direct action on parietal cells, gastrin induces a Ca^{2+}-dependent release of histamine, then differences in results may be explained by a greater interaction between histamine and gastrin in the whole stomach preparation when external muscle may prevent rapid diffusion of histamine away from the secretory cells. Studies on the output of histamine during secretion induced by different stimuli (Main & Pearce, 1977b) may help to clarify the role of histamine in acid secretion.

J.B.P. is an M.R.C. scholar.

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