Effects of non-steroidal anti-inflammatory drugs and prostaglandins on alkali secretion by rabbit gastric fundus *in vitro*

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SUMMARY The effects of non-steroidal anti-inflammatory drugs and prostaglandins E_2 and $F_{2\alpha}$ on the secretory and electrical activity of isolated rabbit fundic mucosa have been studied. Spontaneous acid secretion was inhibited by serosal side application of sodium thiocyanate ($6 \times 10^{-2} M$) and the resulting alkali secretion measured by pH stat titration. Serosal side application of indomethacin ($10^{-5} M$) or aspirin ($3 \times 10^{-3} M$) inhibited alkali secretion (0.55 ± 0.06 to $0.12 \pm 0.06 \mu mol/cm^2/h$, $n=6$, $p<0.01$ and 0.28 ± 0.06 to $0.11 \pm 0.03 \mu mol/cm^2/h$, $n=7$, $p<0.02$ respectively). Mucosal or serosal side prostaglandin E_2 (10^{-5} to $10^{-10} M$) and $F_{2\alpha}$ (10^{-4} to $10^{-10} M$) failed to alter the rate of alkalisation but secretion was significantly increased by serosal side 16,16-dimethyl-prostaglandin E_2 ($10^{-6} M$) (0.90 ± 0.20 to $1.50 \pm 0.30 \mu mol/cm^2/h$, $n=6$, $p<0.01$). Serosal side application of $10^{-6} M$ prostaglandin E_2 to fundic mucosae pretreated with either aspirin ($5 \times 10^{-3} M$) or indomethacin ($10^{-5} M$), to reduce endogenous E_2 formation, also failed to alter alkali secretion. Pretreatment of the mucosa with 16,16-dimethyl- E_2 ($10^{-6} M$) abolished the inhibitory effect of indomethacin ($10^{-5} M$) on alkali secretion ($n=6$) but did not modify the secretory response to aspirin ($3 \times 10^{-3} M$) (fall in alkali secretion with aspirin = $81 \pm 11\%$ and with aspirin plus 16,16-dimethyl- E_2 = $72 \pm 10\%$, $n=7$). In the doses used, none of the prostaglandins or non-steroidal anti-inflammatory drugs altered transmucosal potential difference or electrical resistance. These results show that the damaging agents, aspirin and indomethacin, both inhibit gastric alkali secretion but that modes of action may differ. The observation that prostaglandins, E_2 and $F_{2\alpha}$ failed to increase alkali production suggests that their protective activity against a variety of damaging agents as shown by others, may be mediated by another mechanism.

Studies in animals and man have shown that non-steroidal anti-inflammatory drugs, such as aspirin and indomethacin, cause acute gastric mucosal damage.¹⁻⁴ The precise mechanism of such damage remains unknown although early experiments by Davenport suggested that these agents may disrupt a mucosal 'barrier' to hydrogen ion diffusion.⁵⁻⁷ In recent years an alternative hypothesis has emerged which suggests that damage follows inhibition of non-parietal alkali secretion^{8,9} with subsequent failure of acid neutralisation at the

mucosal surface within the mucous gel layer.¹⁰ In support of this concept is the observation that prostaglandins, which are known to protect the stomach from damage by a variety of noxious agents,¹¹ stimulate alkali secretion and prevent the inhibition of alkalisation produced by non-steroidal anti-inflammatory drugs.¹²⁻¹⁴

Many of these observations on alkali secretion, however, have been derived from experiments on amphibian gastric mucosa.^{12,13} We have recently developed an isolated mammalian fundus preparation¹⁵ and have used this to examine the effect of the anti-inflammatory drugs, aspirin and indomethacin, and the prostaglandins, 16,16-dimethyl- E_2 , E_2 and $F_{2\alpha}$ on mammalian alkali secretion.

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Methods

EXPERIMENTAL PROCEDURE

Experiments were performed on gastric mucosa from male New Zealand White rabbits. The animals were killed by air embolus and the fundus of the stomach excised, washed with warm, unbuffered solution and the external muscle layer removed by blunt dissection in oxygenated unbuffered solution at 37°C. The mucosa was then mounted between two halves of a perspex chamber (surface area 1.8 cm²) and each surface bathed with 20 ml of solution maintained at 37°C and circulated by gas lifts (gassing with a fine bore tube). The unbuffered luminal side solution was kept at a constant pH (7.40) by infusion of either sodium hydroxide (15 mmol) or hydrochloric acid (5 mmol) using a pH stat system (ABU 80 and TTT 80, Radiometer, Copenhagen, Denmark). Secretory rate was calculated from the volume of titrants infused and expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$. Open circuit potential difference was measured using matched calomel electrodes and recorded on a high-input impedance voltmeter (Servoscribe 2s). The dc electrical resistance was determined at 5 minute intervals from the immediate fall in potential difference produced by passing a fixed external current (30 μAmps) through the tissue using silver:silver chloride electrodes.

BATHING SOLUTIONS

The serosal side solution contained Na⁺ (133.3 mmol), K⁺ (4.0 mmol), Ca²⁺ (1.8 mmol), Mg²⁺ (0.8 mmol), Cl⁻ (122.3 mmol), HCO₃⁻ (17.8 mmol), H₂PO₄⁻ (0.8 mmol), SO₄²⁻ (0.8 mmol) and glucose (20 mmol). The solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide and had a final pH of 7.20. The luminal side solution was unbuffered, the HCO₃⁻ and H₂PO₄⁻ being replaced by SO₄²⁻ (9.3 mmol), glucose omitted and mannitol added (11.3 mmol) to produce the same osmolarity as the serosal side solution. This solution was gassed with 100% oxygen, prewashed with saturated barium hydroxide to remove traces of carbon dioxide, and maintained at pH 7.40. Titrants were of similar electrolyte composition to the mucosal side solution.

DRUGS AND CHEMICALS

The following drugs and chemicals were used: sodium thiocyanate (BDH Pharmaceuticals), aspirin, indomethacin, prostaglandin E₂ and prostaglandin F_{2 α} (Sigma Chemical Co, St Louis, Mo). 16,16-dimethyl-prostaglandin E₂ was a kind gift of Dr J Pike, Upjohn, Kalamazoo, Michigan. With the exception of PGE₂ and PGF_{2 α} the compounds were

added to the serosal side solution only, the appropriate stock solution being adjusted to the correct pH immediately before use. The concentration of each stock solution was adjusted so that addition of 100 μl to the 20 ml bathing solution (in the case of aspirin 1 ml of a 60 mmol aspirin stock solution) produced the desired final concentration. In preliminary experiments, addition of up to 1 ml of 'control' solution to the nutrient side did not alter the rate of secretion by the mucosa. For mucosal side administration, stock solutions of E₂ and F_{2 α} were prepared at pH 7.4 so that 10 μl produced the desired final concentration.

STATISTICAL ANALYSIS

Secretory rate, potential difference and electrical resistance were recorded at 5 minute intervals and mean values for consecutive 15 minute periods calculated from each experiment. In the Figures, the mean and standard error of each series of experiments is presented and statistical significance calculated by comparing data obtained before and after addition of a compound using Student's paired *t* test.

Results

EFFECT OF INDOMETHACIN AND ASPIRIN ON ALKALI SECRETION

As previously described,¹⁵ serosal side application of sodium thiocyanate ($6 \times 10^{-2}\text{M}$) inhibited basal acid secretion thus allowing measurement of net alkali secretion. Secretory rates by these mucosae remained stable over a three hour period although the magnitude of alkali production showed marked variation among animals. Thus, with small numbers of animals, basal rates of alkali secretion were markedly different in some experiments and in evaluating the effect of various agents on the rate of alkalisation, each animal acted as its own control and comparisons were not made between different series of experiments.

Addition of 10^{-5}M indomethacin to the serosal side solution produced an immediate and sustained fall in the rate of alkalisation from 0.55 ± 0.06 to $0.12 \pm 0.06 \mu\text{mol}/\text{cm}^2/\text{h}$ ($n=6$, $p<0.01$; Fig. 1). 10^{-6}M indomethacin produced only a slight and insignificant fall in alkali production (results not shown). Serosal side aspirin ($3 \times 10^{-3}\text{M}$) produced a similar decrease in the rate of alkali secretion (0.28 ± 0.06 to $0.11 \pm 0.03 \mu\text{mol}/\text{cm}^2/\text{h}$, $n=7$, $p<0.02$, Fig. 2), but 10^{-4}M aspirin was without effect (results not shown). Neither drug altered transmucosal potential difference ($9 \pm 1 \text{ mV}$, $n=6$, for indomethacin and $10 \pm 1 \text{ mV}$, $n=7$, for aspirin) or electrical resistance ($44 \pm 4 \text{ Ohm}/\text{cm}^2$, $n=6$ and 38 ± 6

Fig. 1 Effect of serosal side indomethacin (10^{-5} M) on alkali secretion by rabbit fundic mucosa. Values represent means $\pm$ SE of six control (▲) and 5 16,16-dimethyl-prostaglandin E_2 pre-treatment (●) experiments. Student's paired t evaluation was carried out on pre- and post-exposure results.

Fig. 2 Effect of serosal side aspirin (3×10^{-3} M) on alkali secretion by rabbit fundic mucosa. Values represent means $\pm$ SE of seven control (▲) and 7 16,16-dimethyl-prostaglandin E_2 pre-treatment (●) experiments. Student's paired t evaluation was carried out on pre- and post-exposure values.

Ohm/cm^2 , $n=7$, respectively) of the mucosa at the doses used.

In a separate series of experiments on mucosae which had not been treated with sodium thiocyanate, 10^{-5} M indomethacin and 3×10^{-3} M aspirin did not alter basal acid secretion (control = 1.20 ± 0.20 and indomethacin = 1.30 ± 0.20 $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=6$; Control = 1.80 ± 0.60 and aspirin = 1.80 ± 0.50 $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=4$).

EFFECT OF EXOGENOUS PROSTAGLANDINS ON ALKALI SECRETION

Addition of prostaglandin E_2 to the serosal (10^{-5} to 10^{-7} M) or mucosal (10^{-7} to 10^{-10} M) side solutions did not significantly alter the rate of alkali production by fundic mucosa pretreated with sodium thiocyanate. Similar results were obtained using serosal (10^{-4} to 10^{-10} M) and mucosal (10^{-6} to 10^{-10} M) applications of prostaglandin $\text{F}_{2\alpha}$. Addition of the stable prostaglandin analogue, 16,16-dimethyl- E_2 (10^{-6} M) to the serosal side solution, however, produced a significant increase in the rate of alkalinisation from 0.90 ± 0.20 to 1.50 ± 0.30 $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=6$; $p < 0.01$ (Fig. 3). In control

Fig. 3 Effect of serosal side 16,16 dimethyl prostaglandin E_2 (10^{-6} M) on alkali secretion by rabbit fundic mucosa. Values represent means $\pm$ SE of six control (●) and six prostaglandin (▲) experiments. Student's paired t evaluation was carried out on pre- and post-exposure values.

experiments, where saline only was added to the serosal side, rates of alkali secretion remained stable (Fig. 3). None of the prostaglandins studied had any effect on transmucosal potential difference or electrical resistance of the mucosa.

EFFECT OF PROSTAGLANDIN E_2 ON ALKALI SECRETION BY FUNDIC MUCOSA PRETREATED WITH $5 \times 10^{-3} M$ ASPIRIN OR $10^{-5} M$ INDOMETHACIN

As endogenous production of prostaglandin during preparation and mounting of the mucosa may mask any stimulatory effect of exogenous prostaglandins, a series of experiments was carried out in which the mucosa was stripped in a solution containing either $5 \times 10^{-3} M$ aspirin or $10^{-5} M$ indomethacin and the mucosa subsequently exposed to serosal side $5 \times 10^{-3} M$ aspirin or $10^{-5} M$ indomethacin in the chamber for a 30 minute period. Mucosal and serosal solutions were then replaced with drug free solutions and sodium thiocyanate ($6 \times 10^{-2} M$) added to the serosal side as previously described. Basal secretory rate was recorded when alkalisation or acidification of the mucosal solution appeared stable (approximately 90 to 120 minutes after adding thiocyanate) and prostaglandin E_2 added after a 45 minute control period. Potential difference (9.2 ± 0.9 mV for aspirin and 10.1 ± 0.8 mV for indomethacin,

$n=6$) and electrical resistance (33 ± 4 and 37 ± 3 Ohm/cm² respectively, $n=6$) were not significantly different from tissues not pretreated with these agents. Some of the aspirin and indomethacin treated mucosae, however, failed to exhibit alkali secretion and secreted acid throughout the study period. Addition of prostaglandin E_2 (10^{-5} and $10^{-6} M$) to the serosal side solutions of these mucosae failed to alter the rate of alkali production ($n=6$) or acid secretion ($n=6$).

EFFECT OF 16,16-DIMETHYL-PROSTAGLANDIN E_2 ON SECRETORY RESPONSE TO INDOMETHACIN AND ASPIRIN

Pretreatment of fundic mucosa with serosal side 16,16-dimethyl- E_2 ($10^{-6} M$) for 30 minutes completely prevented the fall in alkali secretion produced by $10^{-5} M$ indomethacin (Figs. 1 and 4). The response to $3 \times 10^{-3} M$ aspirin, however, was not modified; the fall in alkali secretion immediately after aspirin exposure being $81 \pm 11\%$ in untreated mucosae and $72 \pm 10\%$ in the prostaglandin treated mucosae ($n=7$) (Figs. 2 and 4). It may be seen from Fig. 4 that the doses of aspirin and indomethacin used produced similar inhibition of basal alkali secretion.

Discussion

A number of recent observations have supported the hypothesis that gastric alkali secretion plays an important role in protecting the stomach from damage by intraluminal acid.¹⁶ Firstly, alkali secretion has been shown in a number of *in vitro* and *in vivo* gastric preparations^{8,9} and by the intact human stomach.¹⁷ Secondly, damaging agents such as non-steroidal anti-inflammatory drugs and bile salts inhibit this secretion in amphibia *in vitro* and in man *in vivo*^{13,18-20} while some 'protective' prostaglandins have been shown in amphibian species to both stimulate basal secretion¹² and prevent the inhibitory effect of non-steroidal anti-inflammatory drugs.¹³ Thirdly, studies with micro-electrodes have shown the existence of a pH gradient across the unstirred mucus gel layer covering mammalian and human gastric mucosa.^{10,21,22} The epithelial cell surface is thus bathed with fluid at a neutral pH despite the presence of intraluminal acid.

We have recently provided further support for this hypothesis by showing active bicarbonate transport in an isolated mammalian (rabbit) fundus preparation¹⁵ thus confirming the applicability of the studies in amphibia. The characteristics of alkali secretion by this mucosa are similar to those of amphibian gastric mucosa²³ and the bile salt, sodium

Fig. 4 Effect of 16,16-dimethyl-prostaglandin E_2 ($10^{-6} M$) on percent fall in alkali secretion produced by aspirin ($3 \times 10^{-3} M$) and indomethacin ($10^{-5} M$). Values represent means $\pm$ SE and were derived by comparing the rate of alkali secretion over 15 minute periods immediately before and after drug administration (n represents the number of experiments performed).

taurocholate inhibited alkali secretion in both species.^{15 19} In the present study we have shown that alkali secretion by rabbit fundic mucosa is inhibited by the non-steroidal drugs, aspirin, and indomethacin and stimulated by 16,16-dimethyl-prostaglandin E₂. In addition pretreatment with 16,16-dimethyl-PGE₂ abolished the inhibitory effect of indomethacin on alkali secretion as in amphibian experiments and all of these observations are compatible with the hypothesis that alkali secretion is a protective process controlled by endogenous prostaglandin production.

Not all of our observations, however, fit tidily with this concept. Prostaglandins E₂ and F_{2α} have been shown to protect gastric mucosa against a variety of agents but in the present study neither stimulated alkali secretion and only F_{2α} did so in amphibian fundus.¹³ It is possible that these prostaglandins were rapidly metabolised to inactive products in the rabbit mucosa leaving only the stable analogue to produce an obvious response. Even under the most favourable circumstances, however, when endogenous prostaglandin synthesis was inhibited by indomethacin or aspirin, prostaglandin E₂ in high concentration did not influence alkali secretion. The possibility thus has to be entertained that the protective properties of these prostaglandins are not mediated by stimulation of alkali secretion. Prostaglandins have been shown to increase the thickness of the mucus gel layer,^{24 25} reduce gastric mucosal cell loss²⁶ and modify mucosal blood flow²⁷ and these effects may play a more important role in their protective activity. Furthermore there is a discrepancy between the effects of 16,16-dimethyl-prostaglandin E₂ on the inhibition of alkali secretion by indomethacin and aspirin. The inhibition by indomethacin was prevented while the response to aspirin was unaffected by this analogue suggesting that in the latter case protection against mucosal damage by aspirin is not mediated by effects on alkali secretion. This observation also suggests that aspirin and indomethacin may inhibit alkali secretion by different mechanisms. Indomethacin may inhibit secretion by reducing endogenous prostaglandin production as its effect is nullified by exogenous prostaglandin analogue. Aspirin, which accumulates in epithelial cells after topical application²⁸ may, however, inhibit alkali secretion by an alternative mechanism, possibly by influencing formation or secretion of bicarbonate directly. Alternatively aspirin may inhibit production by the mucosa of other possibly more relevant, arachidonate metabolites whose action cannot be replaced by the exogenous prostaglandins used in these studies.

Thus, our study while providing further support

for the idea that alkali secretion protects the stomach from damage raises some doubt as to whether exogenous prostaglandins exert their protective effects by influencing alkali secretion.

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