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STIMULATION OF ELECTROGENIC CHLORIDE SECRETION BY PROSTAGLANDIN E_2 IN GUINEA-PIG ISOLATED GASTRIC MUCOSA

BY K. T. BUNCE AND C. F. SPRAGGS

From the Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, Hertfordshire SG12 0DJ

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SUMMARY

1. The effects of prostaglandin E_2 (PGE_2) on ion transport were investigated in guinea-pig isolated gastric mucosa.

2. Under resting conditions the mucosa produced a short-circuit current (SCC), the majority of which could be attributed to electrogenic chloride secretion. This interpretation was confirmed by the dependence of the basal SCC on extracellular chloride, and inhibition by the chloride channel blocker, diphenylamine-2-carboxylate. The mucosa also exhibited low rates of acid secretion and of sodium and rubidium absorption.

3. PGE_2 stimulated an increase in net chloride secretion which was more than sufficient to account for the concomitant increase in SCC. As with the basal SCC, the SCC response to PGE_2 was chloride dependent and inhibited by diphenylamine-2-carboxylate. PGE_2 also significantly increased acid secretion and net rubidium absorption, but these changes were not sufficient to account for SCC.

4. The H^+–K^+-ATPase inhibitor, omeprazole, inhibited basal and PGE_2-stimulated acid secretion, but did not modify the effects the PGE_2 on net chloride secretion, SCC or conductance, suggesting that these effects of PGE_2 were not related to changes in gastric acid secretion.

5. Both basal and PGE_2-stimulated SCC were dependent on extracellular sodium and inhibited by ouabain, indicating the importance of a sodium gradient and the Na^+–K^+-ATPase in maintaining the electrogenic properties of the mucosa.

6. These results are consistent with the view that PGE_2 stimulates electrogenic chloride secretion in guinea-pig gastric mucosa, and provide an ionic basis for the stimulation of secretion of sodium and chloride by prostaglandins observed in mammalian gastric mucosa *in vivo*.

INTRODUCTION

Studies *in vivo* have shown that prostaglandins stimulate non-parietal secretion from mammalian stomach (rat: Bolton, Palmer & Cohen, 1978; dog: Bolton & Cohen, 1978; cat: Gascoigne & Hirst, 1981), and that this secretion consists principally of Na^+ and Cl^- ions with smaller amounts of K^+ and HCO_3^- (dog: Miller, Henagan, Watkins & Loy, 1983; cat: Smeaton & Hirst, 1984; rat: Bunce, 1985). Studies on non-parietal secretion in mammalian isolated tissues have shown that

16,16-dimethyl-prostaglandin E_2 stimulates bicarbonate secretion in rabbit gastric mucosa (Rees, Gibbons & Turnberg, 1983), although no such secretion was observed in dog gastric mucosa (Kuo, Miller & Shanbour, 1983). In contrast, in the latter tissue, Chaudhury & Jacobson (1978) found that 16,16-dimethyl-prostaglandin E_2, rather than stimulating secretion, increased the rate of net sodium absorption across the mucosa. Thus, to date experiments on mammalian isolated gastric mucosa have not clearly established an ionic basis for the prostaglandin-induced secretion of a NaCl-rich fluid observed *in vivo*.

In the present study the effects of prostaglandin E_2 (PGE_2) on ion fluxes across guinea-pig isolated gastric mucosa have been investigated, and since changes in gastric hydrochloric acid secretion could confound interpretation of the effect of PGE_2, particularly on chloride flux, some experiments were carried out in tissues in which acid secretion was inhibited by treatment with the H^+–K^+-ATPase inhibitor, omeprazole (Larsson, Carlsson & Sundell, 1984). In addition, confirmation of our interpretation of the effect of PGE_2 has been sought using ion substitution and ion transport inhibitors. A preliminary account of this work was presented to the British Society of Gastroenterology (Spraggs & Bunce, 1985).

METHODS

Dissection and maintenance of tissue

Male guinea-pigs (250–350 g) were starved for 18–24 h with free access to water, and were anaesthetized by inhalation of a halothane–N_2O–O_2 gas mixture. The abdomen was opened, the stomach exteriorized and the muscle layers overlying the fundic region of the stomach removed by a blistering technique as described by Main & Pearce (1978). Two symmetrically paired pieces of gastric mucosa were obtained, one from the ventral and one from the dorsal side of the stomach, and these were mounted in Ussing-type flux chambers (window area 0·8 cm^2). The mucosa was bathed bilaterally with 20 ml of Krebs–Henseleit solution maintained at 37 °C and gassed with 95% O_2–5% CO_2; the composition of this solution was (in mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; $NaHCO_3$, 24·8; KH_2PO_4, 1·2; and glucose, 11·1. In experiments where acid secretion was measured the mucosal side of the tissue was bathed with an unbuffered solution. This had a composition similar to that described above but with $NaHCO_3$ and KH_2PO_4 replaced by 12·4 mM-Na_2SO_4 and 0·6 mM-K_2SO_4 respectively and tonicity maintained by addition of 13 mM-mannitol. Acid secretion was measured by pH stat titration against 0·1 M-NaOH to an end-point of pH 7·40.

Measurement of electrical parameters

Tissues were continuously voltage clamped at zero potential, with compensation for fluid resistance and differences in potential between calomel cells, using a voltage-clamp amplifier (DVC-1000, World Precision Instruments, CT, U.S.A.) and the applied short-circuit current (SCC) was continuously recorded. In some experiments transmucosal conductance was measured by clamping the mucosa transiently (1 s) at either 5 mV above or 5 mV below zero. The mean change in current from ten deflections over a 10 min period of stable SCC was used to determine conductance from Ohm's Law.

Ion flux studies

Measurement of sodium and chloride fluxes were made using ^{22}Na and ^{36}Cl. Potassium fluxes were measured using ^{86}Rb as the tracer. Pairs of gastric mucosae were bathed with normal Krebs–Henseleit solution and short-circuited. The appropriate isotope (approximately 9 kBq ml^{-1}) was added to the serosal side of one tissue for the serosal-to-mucosal flux (J_{sm}) and to the mucosal side of the other for the mucosal-to-serosal flux (J_{ms}). At least 30 min was allowed for equilibration of ^{22}Na and ^{36}Cl and at least 60 min for ^{86}Rb (Kuo & Shanbour, 1979). Unidirectional ion fluxes were determined from the appearance of isotope on the side opposite to which it was added during 30 min periods of stable SCC. Samples were also taken from the side to which the isotope was added

and were used to calculate specific activity. ^{36}Cl activity was measured by liquid scintillation β-spectrometry and ^{22}Na and ^{86}Rb by γ-spectrometry. The net flux of each ion (J_{net}) was calculated as the algebraic difference between J_{sm} and J_{ms} and compared with the corresponding charge transfer which was calculated from the area under the SCC/time traces using the Faraday relationship, where:

$$\text{charge transfer} = \frac{\text{current(A)} \times \text{time(s)}}{96500}.$$

Since net ion flux was derived from the algebraic sum of the unidirectional fluxes in paired tissues, the mean SCC from these two tissues was used for comparison with fluxes.

Ion replacement studies

The ionic dependence of SCC was determined by replacement of sodium or chloride salts in both the serosal and mucosal bathing solutions with equimolar amounts of choline or gluconate salts respectively, except $CaCl_2$ which was replaced with 2·5 mM-$CaSO_4$. In experiments on basal SCC the gastric mucosa was bathed in normal Krebs–Henseleit solution until a stable resting SCC was obtained. The Krebs–Henseleit solution was then replaced with a chloride-free or sodium-free solution and the change in SCC recorded. Reversibility of these changes was examined by returning the mucosa to normal Krebs–Henseleit solution and recording the SCC. In experiments to determine the effects of ion replacement on PGE_2-stimulated SCC, pairs of gastric mucosa were used. These were bathed in normal Krebs–Henseleit solution until stable resting SCC values were obtained. One of each mucosa pair was then bathed with a chloride-free or sodium-free solution. PGE_2 (0·1 μM) was added to both pieces of mucosa and the changes in SCC, measured after 60 min, compared in the control and test tissues.

Studies using ion transport inhibitors

Pairs of gastric mucosa were used to determine the effects of inhibitors on SCC. After establishing a stable basal SCC an inhibitor was added to the appropriate side of one of each mucosa pair. Thirty minutes later both tissues received PGE_2 (0·1 μM) and the resultant change in SCC recorded after 60 min. The effect of the inhibitor was expressed as percentage inhibition of basal or PGE_2-stimulated SCC compared with the corresponding control.

Compounds

Indomethacin (Sigma Chemical Co.) was dissolved in 1 % $NaHCO_3$ at 1 mM and was added to the serosal bathing solution at a concentration of 1 μM to inhibit endogenous prostaglandin synthesis. Prostaglandin E_2 (Prostin E, Upjohn) was diluted from a 5 mg ml^{-1} stock in ethanol with Krebs–Henseleit solution before addition to a tissue; in most experiments PGE_2 was added to both the serosal and mucosal bathing solutions. The H^+–K^+-ATPase inhibitor, omeprazole (AB Hassle), was used in some experiments to inhibit acid secretion; the compound was dissolved in polyethyleneglycol-400 and added to the serosal bathing solution. Stock solutions of ion transport inhibitors were prepared immediately prior to use. Amiloride (Merck Ltd) and ouabain (Sigma) were dissolved in distilled water at concentrations of 0·01 M. Diphenylamine-2-carboxylate (DPC) was dissolved in 0·1 M-NaOH at a concentration of 0·1 M. All compounds were added to bathing solutions in volumes of less than 0·2 ml.

Expression of results

Results are expressed as mean ± S.E. of the mean. Statistical analysis was performed using either a paired or unpaired Student's t test and P values of less than 0·05 were considered significant.

RESULTS

Effects of PGE_2 on short-circuit current and conductance

In a series of six experiments carried out under open-circuit conditions basal potential difference was 30 ± 5 mV (lumen negative), similar to values reported in this tissue by others (Klemperer, Lelchuk & Caplan, 1983). However, experiments

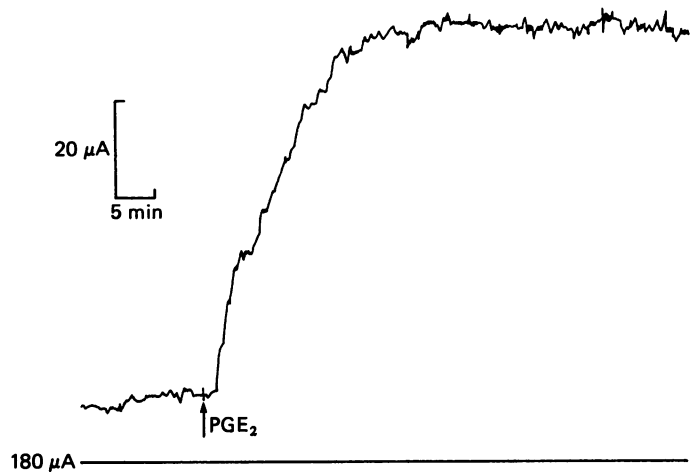

Fig. 1. Short-circuit current response to PGE_2. PGE_2 (1 μM) was added to both bathing solutions. Horizontal line indicates a SCC value of 180 μA.

were routinely carried out in short-circuited tissues, where open-circuit potential difference was not recorded. Under resting conditions guinea-pig isolated gastric mucosa reached a stable SCC of 204 ± 11 μA cm^{-2} ($n = 11$) within 60 min and this was maintained for at least 5 h. In all experiments indomethacin (1 μM) was added to the serosal bathing solution and did not affect basal SCC; in preliminary experiments gastric mucosa was bathed in fresh solutions 60 min after indomethacin treatment, and since this procedure did not modify SCC in all subsequent experiments indomethacin was added without washing. PGE_2 (1 μM) increased SCC and a typical response is shown in Fig. 1; a maximum response was obtained 40 min after drug addition and maintained for at least 2 h, therefore responses to PGE_2 were routinely measured 60 min after drug addition. In most experiments PGE_2 was added to both sides of the mucosa and a mean increase of 77 ± 6 μA cm^{-2} ($n = 6$) was obtained to 0·1 μM-PGE_2. Unilateral addition of this concentration of PGE_2 produced smaller increases in SCC: an increase in SCC of 60 ± 3 μA cm^{-2} for serosal addition and 47 ± 7 μA cm^{-2} for mucosal addition ($n = 6$ each). The gastric mucosa became desensitized to repeated additions of PGE_2 even after washing and therefore only a single response to PGE_2 was obtained in each tissue. PGE_2 (1 μM) also significantly increased transmucosal conductance by 20% from $11{\cdot}3 \pm 0{\cdot}8$ to $13{\cdot}7 \pm 0{\cdot}1$ mS cm^{-2} ($n = 5$, $P < 0{\cdot}05$) 60 min after its addition.

Effects of PGE_2 on Cl^-, Na^+ and Rb^+ fluxes

Paired tissues produced similar basal SCC values (ventral: $3{\cdot}62 \pm 0{\cdot}32$ μequiv cm^{-2} per 30 min; dorsal: $3{\cdot}72 \pm 0{\cdot}48$ μequiv cm^{-2} per 30 min, $n = 7$, $P > 0{\cdot}5$) and similar responses to PGE_2 (ventral: $4{\cdot}57 \pm 0{\cdot}32$ μequiv cm^{-2} per 30 min; dorsal: $4{\cdot}49 \pm 0{\cdot}50$ μequiv cm^{-2} per 30 min, $n = 7$, $P > 0{\cdot}5$) and therefore the use of paired tissues to determine net fluxes was considered valid. The effects of PGE_2 (1 μM) on ion fluxes are shown in Table 1. The mean SCC values from the three series of experiments were

TABLE 1. Effect of PGE_2 on ion fluxes in guinea-pig isolated gastric mucosa

	J_{sm}	J_{ms}	J_{net}	SCC	P*
^{36}Cl ($n = 7$)					
Control	9·09 ± 0·70	5·61 ± 0·55	3·45 ± 0·61	3·63 ± 0·37	n.s.
PGE_2	11·70 ± 0·57	6·34 ± 0·46	4·83 ± 0·55	4·47 ± 0·36	n.s.
Δ	2·07 ± 0·21	0·69 ± 0·22	1·38 ± 0·26	0·84 ± 0·09	n.s.
P†	< 0·005	< 0·05	< 0·005	< 0·005	—
^{22}Na ($n = 7$)					
Control	1·52 ± 0·34	2·36 ± 0·24	−0·84 ± 0·26	3·34 ± 0·23	< 0·005
PGE_2	1·55 ± 0·31	2·66 ± 0·31	−1·11 ± 0·49	4·35 ± 0·27	< 0·005
Δ	0·04 ± 0·25	0·31 ± 0·10	−0·27 ± 0·27	0·83 ± 0·09	< 0·005
P†	n.s.	< 0·05	n.s.	< 0·005	—
^{86}Rb ($n = 6$)					
Control	0·06 ± 0·01	0·28 ± 0·03	−0·22 ± 0·04	3·83 ± 0·25	< 0·005
PGE_2	0·08 ± 0·02	0·51 ± 0·03	−0·43 ± 0·02	5·77 ± 0·40	< 0·005
Δ	0·03 ± 0·02	0·23 ± 0·05	−0·21 ± 0·04	1·95 ± 0·19	< 0·005
P†	n.s.	< 0·005	< 0·005	< 0·005	—

All values are in units of μequiv cm^{-2} per 30 min. $J_{net} = J_{sm} - J_{ms}$, therefore positive values of J_{net} denote secretion (serosal to mucosal) and negative values of J_{net} denote absorption (mucosal to serosal). Δ is the difference between control and PGE_2 fluxes. P values give statistical comparisons by paired t test. P† compares control and PGE_2 fluxes and P* compares J_{net} and SCC (n.s. = not significant, $P > 0·05$).

similar and therefore a direct comparison may be made of the data from these experiments.

Under resting conditions the unidirectional fluxes of chloride were large and J_{sm} was greater than J_{ms}; this resulted in a net secretion of chloride which was not significantly different from, and able to account for, 95% of the resting SCC. Small net sodium and rubidium absorptions were also present, but were significantly different from SCC and able to account for only small proportions of resting SCC (24 and 6% respectively). PGE_2 (1 μM) stimulated SCC significantly in all three sets of experiments (Table 1). PGE_2 also significantly increased both of the unidirectional chloride fluxes, but the increase in J_{sm} was greater than the increase in J_{ms} resulting in a significant increase in net chloride secretion; net chloride secretion and SCC were not significantly different after PGE_2. PGE_2 also stimulated small increases in the net absorptions of sodium and rubidium, but again the net fluxes of these ions were not sufficient to account for SCC.

Effects of PGE_2 on gastric acid secretion

Table 2 shows the effects of PGE_2 on gastric acid secretion in short-circuited gastric mucosa. In these experiments PGE_2 was only added to the serosal side of the tissue because of concern that the presence of PGE_2 in the mucosal solution might affect the measurement of titratable acidity. PGE_2 (1 μM, serosally) produced a small but significant increase in acid secretion, which was sustained for at least 60 min. The histamine H_2 receptor antagonist, ranitidine (100 μM, serosally) was without effect on acid secretion. In contrast, the H^+–K^+-ATPase inhibitor, omeprazole (100 μM, serosally), inhibited basal acid secretion by 88 ± 4% ($n = 8$, $P < 0·005$) and

TABLE 2. Effect of omeprazole on basal and PGE_2-stimulated acid secretion in guinea-pig isolated gastric mucosa

Treatment group	Period 1	Period 2	n	P
1	$1{\cdot}97 \pm 0{\cdot}11$	$2{\cdot}00 \pm 0{\cdot}08$	6	n.s.
2	$2{\cdot}03 \pm 0{\cdot}09$	$2{\cdot}51 \pm 0{\cdot}12$	6	$< 0{\cdot}05$
3	$0{\cdot}17 \pm 0{\cdot}06$	$0{\cdot}04 \pm 0{\cdot}01$	8	n.s.

All values are in units of μequiv cm^{-2} per 30 min. Acid secretion was determined during two 30 min periods in three groups of gastric mucosa. The treatments for each group were as follows. Group 1 received no drugs, group 2 received PGE_2 (1 μM, serosally) prior to period 2, group 3 were pre-treated with omeprazole (100 μM, serosally) for 60 min prior to period 1 and received PGE_2 (1 μM, serosally) prior to period 2. P values were determined by paired t test (n.s. = not significant, $P > 0{\cdot}05$).

subsequent addition of PGE_2 (1 μM, serosally) failed to stimulate acid secretion (Table 2).

The preceding results show that as well as stimulating net chloride secretion and SCC, PGE_2 also stimulated gastric acid secretion. The possibility therefore existed that a proportion of the net chloride flux stimulated by PGE_2 was associated with hydrochloric acid secretion, particularly since the increase in net chloride secretion was greater than the increase in SCC. Further experiments were therefore carried out to investigate the effects of PGE_2 on ion fluxes in tissues where acid secretion was abolished by pre-treatment with omeprazole; the results of these experiments are described below.

Effects of PGE_2 on ion transport in omeprazole-pre-treated gastric mucosa

The effect of PGE_2 was examined following pre-treatment of the mucosae for 60 min with omeprazole.

The effect of different concentrations of PGE_2 on SCC in untreated and omeprazole-pre-treated gastric mucosa is shown in Fig. 2. In untreated mucosa, threshold effects were observed at concentrations of PGE_2 as low as 1 nM and an EC_{50} (95% confidence limits) value of 5 (2, 11) nM was calculated with a maximum response of 77 ± 6 μA cm^{-2} ($n = 6$) achieved at 100 nM. Similar results were obtained in omeprazole-pre-treated gastric mucosa (Fig. 2) with an EC_{50} value of 7 (3, 13) nM and a maximum increase in SCC of 86 ± 9 μA cm^{-2} ($n = 6$), at 100 nM. In the presence of omeprazole, PGE_2 (1 μM) also increased transmucosal conductance by 36% from $7{\cdot}0 \pm 0{\cdot}2$ to $9{\cdot}5 \pm 0{\cdot}1$ mS cm^{-2} ($n = 5$).

The effects of omeprazole pre-treatment on basal and PGE_2-stimulated ion fluxes are shown in Table 3 and are similar to the results obtained without omeprazole (Table 1). Under resting conditions net chloride secretion was able to account for 70% of SCC and these two parameters were not significantly different. PGE_2 (1 μM) stimulated significant increases both in SCC and net chloride secretion and again these two parameters were not significantly different. Also, as found in acid-secreting mucosa (Table 1), the increase in net chloride secretion stimulated by PGE_2 was more than sufficient to account for the increase in SCC. In these experiments, the increase in net chloride secretion was the result of a significant increase in J_{sm} without change in J_{ms}(Table 3).

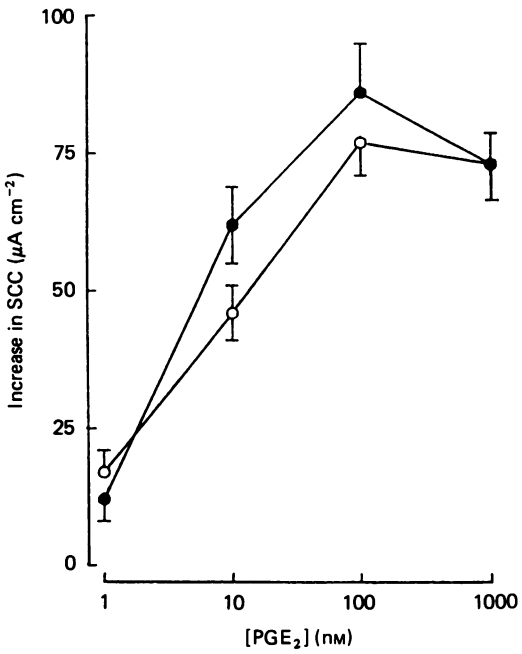

Fig. 2. Effect of omeprazole on SCC responses to PGE_2. Curves show mean ± S.E.M. of six observations each. ○: PGE_2 alone; ●: PGE_2 after treatment of gastric mucosa with omeprazole (100 μM, added to the serosal bathing solution). PGE_2 was added to both bathing solutions and the increase in SCC recorded after 60 min.

TABLE 3. Effect of PGE_2 on ion fluxes in guinea-pig isolated gastric mucosa pre-treated with omeprazole

	J_{sm}	J_{ms}	J_{net}	SCC	P*
^{36}Cl ($n = 7$)					
Control	13·92 ± 0·90	11·02 ± 0·45	2·90 ± 0·69	4·30 ± 0·39	n.s.
PGE_2	17·37 ± 1·20	11·84 ± 1·20	5·53 ± 0·63	5·36 ± 0·31	n.s.
Δ	3·45 ± 0·51	0·82 ± 1·20	2·63 ± 0·88	1·07 ± 0·11	n.s.
P†	< 0·005	n.s.	< 0·05	< 0·005	—
^{22}Na ($n = 7$)					
Control	1·04 ± 0·16	1·06 ± 0·09	−0·02 ± 0·08	3·74 ± 0·21	< 0·005
PGE_2	1·29 ± 0·19	1·56 ± 0·22	−0·27 ± 0·12	4·83 ± 0·21	< 0·005
Δ	0·25 ± 0·12	0·50 ± 0·16	−0·25 ± 0·16	1·09 ± 0·16	< 0·005
P†	n.s.	< 0·05	n.s.	< 0·005	—
^{86}Rb ($n = 6$)					
Control	0·04 ± 0·01	0·40 ± 0·06	−0·37 ± 0·06	4·64 ± 0·37	< 0·005
PGE_2	0·10 ± 0·01	0·32 ± 0·04	−0·22 ± 0·04	5·58 ± 0·34	< 0·005
Δ	0·06 ± 0·01	−0·09 ± 0·06	0·15 ± 0·06	0·94 ± 0·04	< 0·005
P†	< 0·005	n.s.	n.s.	< 0·005	—

Tissues were treated with omeprazole (100 μM, serosally) for 60 min prior to flux measurements. The layout of this Table is identical to Table 1.

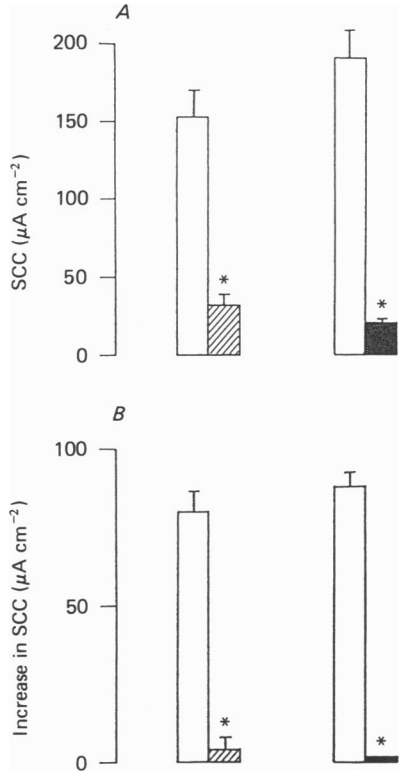

Fig. 3. Effects of sodium or chloride replacement on basal and PGE_2-stimulated SCC in guinea-pig isolated gastric mucosa. Single tissues were used for the experiments shown in *A*. Tissues were bathed in normal Krebs–Henseleit solution (open bars) on both sides until a stable basal SCC was established. A chloride-free (hatched bars) or a sodium-free solution (filled bars) was then added bilaterally and the change in SCC recorded. Paired tissues were used for the experiments shown in *B*. One tissue was bathed with Krebs–Henseleit solution (open bars) whilst its partner was bathed bilaterally in either a chloride-free (hatched bars) or a sodium-free (filled bars) solution. PGE_2 (0·1 μM, serosally) was then added to both tissues and increases in SCC recorded. Values are mean ± S.E.M.; $n = 8$ in *A* and $n = 6$ in *B*. $*P < 0.005$ by paired *t* test.

Pre-treatment with omeprazole virtually abolished net sodium absorption (Table 3), and this was achieved principally by a reduction in J_{ms} (compare control J_{ms} of Table 1, 2·36 ± 0·24 μequiv cm^{-2} per 30 min, with control J_{ms} of Table 3, 1·06 ± 0·09 μequiv cm^{-2} per 30 min). PGE_2 (1 μM) increased sodium J_{ms} significantly in omeprazole-pre-treated tissues and this resulted in an increase in net sodium absorption in five out of seven tissues, but did not achieve statistical significance. Again net sodium absorption was significantly smaller than SCC (Table 3).

Pre-treatment with omeprazole did not affect rubidium fluxes (Table 3). Under resting conditions there was a small net rubidium absorption which was not affected by PGE_2 (1 μM). Again net rubidium absorption was too small to contribute significantly to SCC.

TABLE 4. Effect of ion transport inhibitors on basal and PGE_2-stimulated SCC in guinea-pig isolated gastric mucosa

Inhibitor	Concentration (mM)	Percentage inhibition of SCC		n
		Basal	PGE_2 stimulated	
Amiloride	0·1	4±2	−4±10	4
Diphenylamine-2-carboxylate	1	75±2*	95±2*	6
Ouabain	0·1	98±2*	96±2*	6

Results are expressed as percentage inhibition of SCC compared with control values immediately prior to drug addition in single tissues (basal) or by comparison of responses in paired tissues (PGE_2 stimulated, 0·1 μM). Amiloride was added to the mucosal bathing solution whilst ouabain and diphenylamine-2-carboxylate were added serosally. *$P < 0.005$, paired t test.

Effects of ion replacement on short-circuit current

To further investigate the effects of PGE_2 on gastric ion transport sodium and chloride ions were removed from the bathing solutions and changes in SCC were measured. Figure 3 summarizes the results from these experiments. Replacement of chloride with gluconate produced a highly significant fall in basal SCC from 152±15 to 32±5 μA cm^{-2} ($n = 8$, $P < 0.005$). Re-addition of chloride ions to the bathing solutions resulted in a return of basal SCC to 185±14 μA cm^{-2} ($n = 8$). Similarly replacement of sodium with choline reduced basal SCC from 190±16 to 19±2 μA cm^{-2} ($n = 8$, $P < 0.005$) and re-addition of sodium in the bathing solutions returned basal SCC to 135±5 μA cm^{-2} ($n = 8$). Figure 3 also shows the effects of ion replacement on the response to PGE_2; the increase in SCC stimulated by PGE_2 (0·1 μM) was markedly inhibited by both chloride or sodium replacement in the bathing solutions.

Effects of ion transport inhibitors on short-circuit current

The effects of inhibitors of ion transport on basal and PGE_2-stimulated SCC are shown in Table 4. In this series of experiments, the mean basal SCC was 176±10 μA cm^{-2} ($n = 16$) and PGE_2 (0·1 μM) stimulated an increase in SCC of 70±5 μA cm^{-2} ($n = 16$). The chloride channel blocker, diphenylamine-2-carboxylate (1 mM, serosally), and the Na^+–K^+-ATPase inhibitor, ouabain (0·1 mM, serosally), produced large reductions in SCC. In contrast, mucosal addition of the sodium channel blocker, amiloride (0·1 mM), failed to modify either basal or PGE_2-stimulated SCC.

DISCUSSION

These experiments show that in the absence of electrical and chemical gradients guinea-pig gastric mucosa actively transported ions. Under resting conditions (in the absence of omeprazole) there was a large net chloride secretion which was able to account for the majority of short-circuit current (SCC). Net absorptions of sodium and rubidium and net secretion of hydrogen ions also occurred, but the net fluxes of

these ions were small and could not account for current. Additional evidence that sodium absorption did not occur through conductive channels was provided by the lack of effect of amiloride on basal SCC. Therefore, the present observations suggest that under basal conditions guinea-pig resting gastric mucosa secretes chloride ions electrogenically as described by other workers (Sernka & Hogben, 1969; Ayalon, Corcia, Klemperer & Caplan, 1980; Klemperer *et al.* 1983). Indeed, this proposition is supported by the observations that basal SCC was dependent on extracellular chloride ions and inhibited by the chloride channel blocker, diphenylamine-2-carboxylate (Distephano, Wittner, Schlatter, Lang, Englert & Greger, 1985). Electrogenic chloride secretion has also been observed in gastric mucosa from other animals such as frog (Hogben, 1955) where net chloride secretion is responsible for all of the SCC, and rat (Sernka & Hogben, 1969; Jackson & Norris, 1985), piglet (Forte & Machen, 1975) and dog (Kuo & Shanbour, 1979), where its contribution to SCC is less.

PGE_2 stimulated increases in both SCC and transmucosal conductance, and increases in these parameters are consistent with an increase in electrogenic ion flux. PGE_2 also stimulated net chloride secretion and under these conditions, whilst there was no significant difference between net chloride secretion and SCC, the increase in net chloride secretion was more than sufficient to account for the increase in SCC produced by PGE_2. This effect of PGE_2 on chloride flux was predominantly due to an increase in J_{sm}, although J_{ms} was also increased significantly. As with resting mucosa, supportive evidence that the increase in SCC stimulated by PGE_2 was due to chloride secretion was provided by the observations that the response to PGE_2 was chloride dependent and inhibited by diphenylamine-2-carboxylate. The backflux of chloride (J_{ms}) may be due to exchange diffusion since 16,16-dimethyl-PGE_2 has been shown to stimulate this process (Schiessel, Matthews, Barzilai, Merhav & Silen, 1980).

PGE_2 had lesser effects on cation fluxes, stimulating small increases in net absorption of both sodium and rubidium. However, these fluxes did not contribute significantly to SCC, and indeed, as with basal SCC, the SCC response to PGE_2 was not affected by mucosal amiloride. Despite the well-documented acid anti-secretory properties of PGE_2 (reviewed by Robert, 1981), PGE_2 increased acid secretion in the present study. This effect of PGE_2 was surprising although such a response has previously been reported for isolated gastric mucosa of the guinea-pig (Petersen, Homrighasen-Camci & Winterhager, 1985), rat (Sernka & Caplan, 1982) and frog (Takeuchi, Svanes, Critchlow, Magee & Silen, 1982). Unlike studies in the frog (Takeuchi *et al.* 1982), the stimulation of acid secretion by PGE_2 in guinea-pig gastric mucosa was not inhibited by a histamine H_2 receptor antagonist, suggesting that histamine release did not mediate this response. Both basal and PGE_2-induced hydrogen ion secretion were abolished by the H^+–K^+-ATPase inhibitor omeprazole as reported previously (Petersen *et al.* 1985).

Since hydrochloric acid is secreted by an electroneutral mechanism (Hersey, Sachs & Kasbekar, 1985), and also because the increase in net chloride secretion stimulated by PGE_2 was greater than the corresponding increase in SCC (Table 1), it was possible that part of the increase in net chloride secretion stimulated by PGE_2 was due to hydrochloric acid secretion. To facilitate interpretation of the data, the effect

of PGE_2 on ion fluxes was further examined in the presence of omeprazole. The latter compound blocked both basal acid secretion and the acid secretory response to PGE_2, but still the change in net chloride secretion produced by PGE_2 was larger although not significantly different from the corresponding change in SCC. Therefore the results in the presence of omeprazole do indicate that PGE_2 stimulated electrogenic chloride secretion by a mechanism unrelated to hydrogen ion secretion.

Comparison of the control J_{net} data in Tables 1 and 3 reveals that omeprazole had little effect on net chloride secretion even though a profound inhibition of acid secretion was achieved (Table 2) and similar results have been reported in frog gastric mucosa (Starlinger, Hollands, Rowe, Matthews & Silen, 1986; Reenstra, Bettencourt & Forte, 1987). Since omeprazole inhibits the exchange of hydrogen for potassium ions, this apparent anomaly of inhibiting H^+ secretion without affecting chloride would be resolved if the usual net secretion of hydrogen ion was replaced by potassium secretion (as measured by ^{86}Rb) in the presence of omeprazole. The data presented in Table 3 clearly show that omeprazole did not induce a net secretion of ^{86}Rb; similar results have been reported previously in guinea-pig (Bunce & Spraggs, 1986) and frog gastric mucosa (Reenstra, Bettencourt & Forte, 1986), which have led to the suggestion that the inhibition of acid secretory flow by omeprazole causes collapse of the gastric glands, preventing the efflux of ^{86}Rb into the lumen (Reenstra, Bettencourt & Forte, 1986).

In the present study algebraic summation of total net ion fluxes could not account for SCC both under control conditions and in the presence of PGE_2, and other workers have similarly failed to achieve ionic equivalence in gastric mucosa of a number of mammalian species including rat (Sernka & Hogben, 1969; Jackson & Norris, 1985), guinea-pig (Klemperer *et al.* 1983) and dog (Kuo & Shanbour, 1979). These discrepancies may arise from compounding of errors from determination of ion fluxes in different experiments. Alternatively they may be due to the electroneutral transport of a proportion of the measured ion species, or the electrogenic flux of an ion species not measured. To facilitate discussion of this problem a balance sheet of net ion fluxes and SCC has been prepared and this is shown in Table 5; only the data from omeprazole-treated tissues have been used since the low rate of hydrogen ion flux in these tissues simplifies interpretation. Under control conditions SCC was larger than the algebraic summation of ions (ΣI) which suggests that an unmeasured ion species was being secreted electrogenically; it is possible that an electrogenic secretion of bicarbonate accounts for the inequivalence in this tissue (Petersen *et al.* 1985). In contrast, in the presence of PGE_2, ΣI was greater than SCC, and comparison of difference (Δ) values highlights this effect (Table 5). In particular chloride ΔJ_{net} was larger than ΔSCC, and this may suggest that a proportion of the net chloride flux occurred by an electroneutral mechanism or alternatively that the mucosa was not being effectively voltage clamped due to its convoluted structure as suggested by Rehm (1975).

Despite the lack of ionic equivalence the present results on ion fluxes, taken together with the effects of chloride replacement and the chloride channel blocker, diphenylamine-2-carboxylate, are consistent with the view that PGE_2 stimulates electrogenic chloride secretion in guinea-pig gastric mucosa. In addition, in the present study both basal and the PGE_2-stimulated SCC were sodium dependent and

TABLE 5. Balance of net ion fluxes and SCC in omeprazole-treated guinea-pig isolated gastric mucosa

	Cl^- (s to m)	Na^+ (m to s)	Rb^+ (m to s)	H^+ (s to m)	ΣI	SCC	$\Sigma I - SCC$
Control	2·90	0·02	0·37	0·07	3·22	4·23	−1·01
PGE_2	5·53	0·27	0·22	0·04	5·98	5·26	0·72
Δ	2·63	0·25	−0·15	−0·03	2·76	1·03	1·73

Values are mean net ion fluxes in units of μequiv cm^{-2} per 30 min obtained from Tables 2 and 3. ΣI is the algebraic sum of these fluxes, which gives their contribution to charge transfer, i.e.:

$$\Sigma I = [Cl^- + Na^+ + Rb^+] - H^+.$$

The SCC values are means calculated from the three experiments in Table 3. $\Sigma I - SCC$ is the difference between the sum of fluxes and SCC; positive values show a surplus and negative values a deficit of ions relative to SCC. Δ is the difference between control and PGE_2 net fluxes.

inhibited by ouabain, and this result indicates that in gastric mucosa, as in other epithelia (Frizzell, Field & Schultz, 1979), the uptake of chloride across the basolateral membrane depends on the existence of a sodium gradient and that this is dissipated by inhibition of the Na^+–K^+-ATPase with ouabain.

These studies provide no direct information of the cell type(s) involved in the response to PGE_2. However, the observation that omeprazole did not affect SCC or net chloride secretion, taken together with the fact that inhibition of acid secretion by omeprazole causes collapse of the gastric glands (Hersey *et al.* 1985; Reenstra *et al.* 1986), makes it tempting to speculate the PGE_2 was not affecting cells within these glands, but rather stimulating cells on the surface of the gastric epithelium.

As far as the authors are aware this is the first report of such an effect of PGE_2, although electrogenic chloride secretion has been identified in resting gastric mucosa of both frog (Hogben, 1955) and mammalian species (Sernka & Hogben, 1969). Under open-circuit conditions, this stimulation of chloride secretion by PGE_2 would be accompanied by the paracellular movement of sodium down an electrical gradient and water down the osmotic gradient resulting in a secretion of a NaCl-rich fluid. The ability of prostaglandins to protect the gastric mucosa from damaging agents is well documented (Robert, Nezamis, Lancaster & Hanchar, 1976) and many mechanisms have been proposed to account for this property (reviewed by Miller, 1983). The stimulation of fluid secretion by prostaglandins may represent an important mechanism for protection from injury, serving to wash noxious agents away from the mucosal surface as suggested by Moody & Zalewsky (1981). Indeed the promotion of an unstirred water layer at the surface of an epithelium by fluid secretion results in a reduction in absorption of solutes (Thomson, 1984) and may therefore prevent the absorption of substances damaging to the gastric mucosa.

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