Prostaglandins are not mediators of the intestinal response to cholera toxin

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SUMMARY We have determined the release of prostaglandin E₂ into the lumen of closed ileal loops in the rat and have measured the capacity of the mucosa to synthesise and degrade prostaglandin E₂ both in control animals and after exposure to cholera toxin or bisacodyl. Exposure to cholera toxin caused no change in any of these measurements whereas bisacodyl caused an increase in the luminal PGE₂ content. We conclude that the secretion stimulated by cholera toxin is not mediated by locally produced prostaglandins.

The possibility that cholera toxin may stimulate intestinal secretion by activating local production of prostaglandins has been raised by the observation that non-steroidal anti-inflammatory drugs which inhibit prostaglandin production inhibit cholera toxin induced secretion.^{1,2} These anti-inflammatory drugs, however, fail to inhibit the stimulation of adenylate cyclase by cholera toxin at concentrations sufficient to inhibit prostaglandin synthesis.³ Furthermore, aspirin and indomethacin enhance absorption in non-stimulated rabbit ileal mucosa,⁴ suggesting that these drugs influence ion transport without necessarily inhibiting prostaglandin synthetic activity. Thus the role of prostaglandins in mediating secretory diarrhoea associated with cholera toxin remains uncertain. We have examined this question further by determining the synthesis and degradation of prostaglandin E₂ (PGE₂) in rat intestinal mucosal homogenates under basal conditions and after stimulation with cholera toxin. We have also determined the PGE₂ content of luminal fluid after exposure of loops of intestine to cholera toxin and to the laxative drug bisacodyl, which is believed to act by liberating local prostaglandins.

Methods

ANIMALS

Male Sprague-Dawley rats, weighing 200-250 g were anaesthetised with sodium pentobarbital (65 mg/kg subcutaneously) after a 24 hour fast. A 20 cm

segment of ileum, 5 cm proximal to the ileocaecal junction was cannulated and flushed with 20 ml of warm saline. The segment was then filled, but not distended, with approximately 2.5 ml of a solution containing NaCl 140 mM, sodium hydrogen phosphate 5 mM, pH 7.4. The volume inserted was accurately measured by filling the loops from a syringe which was weighed before and after the loops had been filled. In control loops, only the phosphate/saline solution was inserted while in other loops either cholera toxin, 5 µg/ml, or bisacodyl 100 µg/ml was included. After intestinal loops had been filled they were returned to the abdominal cavity, which was covered with a damp gauze to prevent desiccation during the subsequent incubation period. The body temperature was monitored continuously and maintained at 35°C with a heating lamp.

In the cholera toxin experiments, after a two-hour incubation period the fluid in the toxin exposed and control loops was drained off and in each instance replaced by phosphate/saline solution for measurement of absorption or secretion. An accurately measured volume of fluid was inserted and after 30 minutes the loops were removed and weighed. The fluid was then drained off and retained for PGE₂ determination. The empty segment of intestine was reweighed and the volume of fluid that had been absorbed was calculated.

In the bisacodyl experiments, incubations were continued in both control and bisacodyl-containing loops for one hour, the fluid was removed and retained for PGE₂ analysis and the extent of absorption or secretion determined as described above.

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PGE₂ DETERMINATION IN LOOP FLUIDS

A 1 ml sample of the fluid was acidified to pH 3 with 2M citric acid and 50 μ l of ³H PGE₂ (60 000 dpm) added as recovery marker. The fluid was then extracted into ether and the ether layer removed and reduced to dryness under nitrogen. The dried sample was resuspended in 500 μ l radioimmunoassay buffer and 100 μ l was taken to assess recovery of PGE₂. A further 100 μ l was used for determination of PGE₂ content by radioimmunoassay (see below).

ASSAY FOR PGE₂ SYNTHESIS AND DEGRADATION

The techniques for these determinations have been described previously,⁵ but briefly the mucosa of the loop of intestine under investigation was cut along its length and removed by scraping with a microscope slide. The mucosal scrapings (100–200 mg wet weight) were homogenised in a buffer containing potassium phosphate 100 mmol, EDTA 20 mmol, pH 7.4, at 4°C and filtered through a nylon mesh. Aliquots of homogenate were then assayed for their synthetic and degradative capacity.

SYNTHESIS

Determinations were carried out by incubating approximately 1 mg protein for 2 min at 37°C in 1 ml of phosphate/EDTA buffer containing 0.8 mM arachidonic acid, 0.06 mM noradrenaline, 2 mM glutathione, 0.04 mM hydroquinone, plus 60 μ g/ml haemoglobin and approximately 75 000 dpm [³H]PGE₂ as a recovery marker.

DEGRADATION

Determinations were carried out by incubating approximately 1 mg protein for 5 min at 37°C in 1 ml of phosphate/EDTA buffer containing 2.5 mM NAD⁺, 5 μ M PGE₂, and 0.18 μ Ci of [³H]PGE₂.

Both the synthesis and degradation assays were stopped by the addition of 100 μ l of 2M citric acid to reduce the pH to 3 followed by extraction with 3 ml diethyl ether. Blanks were prepared in which acid was added before the homogenate. For each assay 200 μ l of the diethyl ether phase was placed onto the preadsorbent area of Whatman Linear K TLC plates (LK6D), dried and the plates developed in the organic phase of ethyl acetate/iso-octane/acetic acid/water (110:50:20:100). For the degradation assay the region corresponding to the degradation product 13,14-dihydro-15-ketoPGE₂ was scraped off and counted in PCS scintillant. For the synthesis assay the region corresponding to PGE₂ was scraped off and eluted into 1 ml of radioimmunoassay buffer (100 mM KH₂PO₄, 155 mM NaCl, 15 mM NaN₃ plus 0.1% gelatin, pH 7.4) and assayed for PGE₂ using commercially available antisera (Pasteur Institute).

All biochemicals were obtained from the Sigma Chemical Co Ltd, 5,6,8,11,12,14,15(n)³H PGE₂ and PCS scintillant were obtained from the Radiochemical Centre, Amersham. PGE₂ antisera were obtained from Institut Pasteur, Paris. Bisacodyl was a gift from Boehringer-Ingelheim.

Results

Cholera toxin inhibited net absorption during the 30 minutes after exposure to the toxin: Control absorption 0.63 $\pm$ 0.133 ml/g/h (n=4); Cholera toxin 0.019 $\pm$ 0.04 ml/g/h (n=7).

Measurement of PGE₂ synthetic and degradative capacity in scrapings of intestinal mucosa taken from control and cholera toxin-exposed loops of intestine revealed that the toxin had no effect on either of these activities (Table 1). In addition, the PGE₂ content of luminal fluid was similar in control and toxin-exposed loops (Table 1).

Bisacodyl (100 μ g/ml) reduced absorption from 0.81 $\pm$ 0.14 ml/g/h (n=7) to 0.06 $\pm$ 0.075 ml/g/h (n=8), in control and bisacodyl loops respectively. In this instance, however, bisacodyl caused a significant increase in PGE₂ content of luminal fluid. PGE₂ synthesis and degradation rates were both reduced but not to a statistically significant extent (Table 2).

Discussion

Considerable evidence has accumulated to indicate that cholera toxin induces intestinal secretion by activation of epithelial adenylate cyclase,⁶ followed by an increase in intracellular cyclic AMP.⁷ In a number of isolated cell systems the toxin has been shown to bind to a specific ganglioside (GM1),⁸ followed by the penetration of the cell membrane by the A subunit of the toxin.⁹ This subunit irreversibly activates the plasma membrane adenylate cyclase by promoting the ADP ribosylation of the GTP regulatory subunit.¹⁰

Prostaglandin E₂ also stimulates intestinal secretion by activation of epithelial adenylate cyclase,⁶

Table 1 Effect of cholera toxin on mucosal PGE₂ synthesis and degradation and luminal PGE₂ content

PGE ₂ synthesis (pmol/mg protein/min)	PGE ₂ degradation (pmol/mg protein/min)	Luminal PGE ₂ content (pmol/g loop tissue)
Control 16.8 $\pm$ 3.8 (4)	462 $\pm$ 106 (4)	1.61 $\pm$ 0.34 (4)
Cholera 13.04 $\pm$ 2.8 (7)	389 $\pm$ 90 (8)	1.32 $\pm$ 0.2 (7)

Mean $\pm$ 1 SEM, numbers in parentheses = no of studies.

Table 2 Effect of bisacodyl on mucosal PGE₂ synthesis and degradation and luminal PGE₂ content

GE ₂ synthesis (pmol/mg protein/min)	PGE ₂ degradation (pmol/mg protein/min)	Luminal PGE ₂ content (pmol/g loop tissue)
Control 14.1 ± 2.8 (7)	359 ± 85 (7)	1.48 ± 0.23 (7)
Bisacodyl 8.67 ± 1.51 (7)	205 ± 45 (8)	3.14 ± 0.7 (8)*

Mean ± SEM, *p < 0.05, numbers in parentheses = no of studies.

possibly by binding to a prostaglandin receptor. In contrast to cholera toxin activation, however, prostaglandin activation of adenylate cyclase is reversible. Nevertheless similarities in action between prostaglandin and cholera toxin-induced secretion prompted the proposal that the toxin may act by releasing local prostaglandins.¹¹ The subsequent demonstration^{1,2} that prostaglandin synthesis inhibitors, such as aspirin and indomethacin, reduced the secretion caused by cholera toxin seemed to support this proposal. A more direct action on ion transport, than one involving inhibition of prostaglandin synthesis is suggested by the observation that these drugs enhance absorption in the basal state.⁴

In view of these conflicting observations we investigated further the possible involvement of prostaglandins in the action of cholera toxin in the intestine by measuring the capacity of the mucosa to synthesise and degrade PGE₂, and by measuring release of PGE₂ into the lumen after cholera toxin exposure. The results indicate that the toxin does not influence either prostaglandin synthetic or degrading enzyme activity, nor is PGE₂ released in increased amounts into luminal fluid. Similar results on the luminal levels of PGE₂ after exposure to pure cholera toxin have also been reported for rabbit ileum.¹²

As it has been suggested that bisacodyl-induced secretion is mediated by local prostaglandin release,¹³ we used this drug as a positive control. Although bisacodyl failed to influence the enzymes concerned with prostaglandin synthesis or degradation, it did significantly increase prostaglandin release into the luminal fluid as had been previously demonstrated. It is possible that increased release of PGE₂ is the result of greater availability of arachidonate, the precursor for PGE₂ synthesis. The enzyme concerned with availability of arachidonate

is phospholipase A2 and it is possible that increased activity of this enzyme is responsible for the increased PGE₂ release.

The results of these studies suggest that cholera toxin-induced secretion is not mediated by topical prostaglandin production in the mucosa.

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