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Effect of prostaglandins E_1 and E_2 on intestinal motility in the guinea-pig and rat

A. BENNETT, K. G. ELEY AND G. B. SCHOLES

Department of Surgery, King's College Hospital Medical School, London

1. Prostaglandins E_1 and E_2 affected intestinal activity both *in vitro* and *in vivo*.

2. Serosal application of prostaglandin to guinea-pig isolated ileum stimulated the longitudinal muscle but reduced peristaltic contractions of the circular muscle and the propulsion of fluid through the gut. Intraluminal application had little effect.

3. Injection of prostaglandin into the bloodstream of anaesthetized rats stimulated the longitudinal muscle of the ileum and increased the intraluminal pressure. A similar response sometimes occurred in the guinea-pig, but in general the effect was variable.

4. Release of prostaglandin in the gut wall, but probably not into the blood or into the lumen of the gut, may play a part in controlling intestinal motility.

The results of our preceding paper and the work of others show that E-type prostaglandins generally stimulate the longitudinal muscle of the isolated gut but inhibit the circular muscle (Bennett, Eley & Scholes, 1968; Bergström, Eliasson, Euler & Sjövall, 1959; Horton & Main, 1963; Bennett, Murray & Wyllie, 1968). These findings raise the question of how prostaglandins affect peristalsis, and whether they normally influence the motility of the gut. Our present experiments on the guinea-pig isolated ileum and on the anaesthetized guinea-pig and rat have been designed to study these problems.

Methods

In vitro

Peristalsis was studied by two methods. The first was similar to the Trendelenburg (1917) method except that peristalsis was recorded on a Sanborn 350 pen recorder. The effects of circular muscle contraction were registered with a Sanborn pressure transducer (No. 268B), and measurements of longitudinal muscle contraction were made with a Sanborn isotonic transducer (Type 585 DT 5).

The second (intraluminal perfusion) method was used because it allowed simultaneous measurements of intraluminal pressure, longitudinal muscle contraction,

and the propulsion of Krebs solution through the gut (see Fig. 1). A Perspex bath 18 cm long, 7 cm high and 4 cm wide was fitted with tubes in its base to remove and replace the Krebs solution. The end of the bath was provided with a gassing tube (95% oxygen, 5% carbon dioxide) and with an inlet and outlet for perfusing a glass U-tube with warm water to maintain the Krebs solution at 37° C.

The proximal end of a piece of guinea-pig ileum was tied to a fixed glass cannula immersed in the bath fluid. The other end of the cannula was connected to a Marriotte bottle through a warming coil at 37° C, and a single or a double-lumen fine polyethylene tube was passed through the cannula into the intestine. The distal end of the ileum was tied round one limb of a pivoted L-shaped cannula. Attached to the other limb was a flexible polyvinyl tube with a non-return valve made of flat Paul's tubing terminating at the same height as the inlet tube in the Marriotte bottle.

Peristalsis was induced by raising the intraluminal pressure by 1–4 cm H_2O. Longitudinal muscle contraction was measured by a Sanborn isotonic transducer attached by thread to the vertical limb of the L-shaped cannula, and pressures at the tips of the intraluminal tubes were measured with pressure transducers. The outflow from the gut was passed through a 1 ml. glass syphon with an added tube at the base connected to a pressure transducer. The rate of flow was registered as changes in the hydrostatic pressure of the fluid: the pressure rose until the syphon was full and fell abruptly when it emptied.

In vivo

Guinea-pigs (310–1,400 g) and rats (220–600 g) of both sexes were deprived of food overnight. Each animal was anaesthetized with urethane 1.5–1.75 g/kg injected either intraperitoneally or intramuscularly and the trachea was intubated. One fine polyethylene tube for the injection of drugs was tied into a jugular vein, and another was inserted into a carotid artery so that its tip lay in the aorta. A cannula containing 50 u/ml. of heparin in 0.9% w/v NaCl solution was tied into

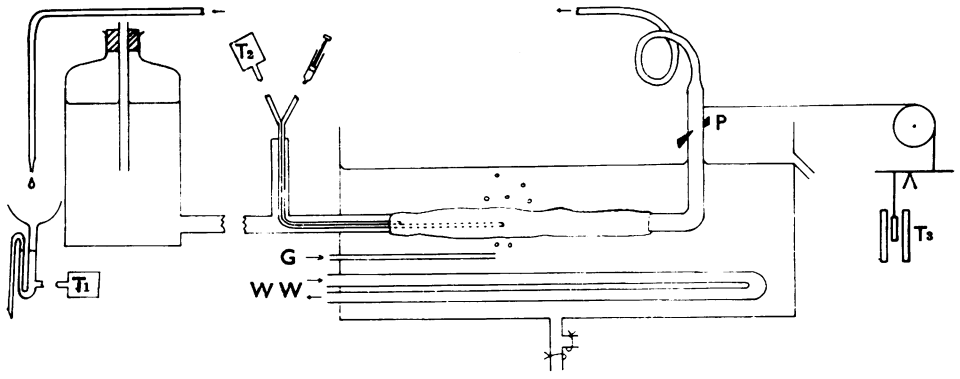

FIG. 1. Diagram of apparatus for measuring longitudinal muscle contraction, intraluminal pressure (circular muscle contraction) and propulsion of fluid during peristalsis in isolated segments of intestine. T_1 and T_2, pressure transducers; T_3, isotonic transducer; P, pivot for cannula; G, gas (O_2 95%, CO_2 5%); WW, warm water. Peristalsis is induced when the intraluminal pressure is increased by raising the Marriotte bottle. The propulsion of fluid through the gut is measured by T_1, the intraluminal pressure by T_2 and the longitudinal muscle contractions by T_3.

the other carotid artery for recording blood pressure, and the abdomen was opened by a small mid-line incision. In some experiments a saline-filled polyethylene tube was tied into the ileum, taking care to avoid ligating major blood vessels or occluding the lumen. The abdomen was then closed and the blood pressure cannula and the intraluminal tube was connected either to Bell and Howell transducers which operated a Devices pen recorder or to a Sanborn recorder.

In other experiments the contractions of the longitudinal muscle in a small loop of bowel were measured in one of two ways. In one method the loop was tied at one end to the xiphisternum without occluding the lumen, and the other end was connected to a frontal-writing isotonic lever by a thread passing under pulleys. In the other method, each end of the loop of bowel was attached to a Cushny myocardiograph (Cushny, 1910) which operated an isometric transducer (tensile sensor, Type S.T.I. Ether-Langham Thompson Ltd.) and a pen recorder.

Results

Effects of prostaglandins on peristalsis in vitro

The effects of prostaglandins E_1 and E_2 were qualitatively and quantitatively similar.

Trendelenburg method. Prostaglandin E_1 (0.1–1 μg/ml., five experiments) injected into the bath fluid surrounding the isolated intestine caused the longitudinal muscle to contract. When the intraluminal pressure was raised the phasic peristaltic contractions of the longitudinal muscle were either superimposed on the prostaglandin-induced shortening of the muscle, or the tissue first partially relaxed and then alternately contracted and relaxed. In contrast, the circular muscle contractions elicited by raising the intraluminal pressure were reduced by 10–100% (average 40%) in four experiments, but were unaffected in another in which the response of the longitudinal muscle to prostaglandin was poor.

When prostaglandin E_1 (0.5–10 μg ; four experiments) was injected into the lumen of the gut, however, there was no effect on the longitudinal muscle, and inhibition of circular muscle peristaltic contractions occurred in only one experiment in which the dose was relatively high (10 μg ; 30% reduction).

Intraluminally perfused ileum. The Trendelenburg method of studying peristalsis gives no information on the rate of propulsion of gut contents. We therefore used the intraluminally perfused ileum to determine the action of prostaglandin on the peristaltic contractions of the gut and on transport of fluid. The effects on the longitudinal and circular muscle were the same as in the Trendelenburg method. Prostaglandins E_1 (0.25 μg/ml. ; four experiments) and E_2 (0.25 μg/ml. ; two experiments) in the fluid bathing the serosal surface of the ileum caused the longitudinal muscle to contract. When the intraluminal pressure was raised, the peristaltic contractions of the longitudinal muscle were again either superimposed on the response to prostaglandin (two experiments) or the muscle first partially relaxed and then alternately contracted and relaxed (four experiments). Sometimes the peristaltic contractions of both muscle layers ceased while the pressure was raised, but the longitudinal muscle continued to shorten. Prostaglandin inhibited both the pressure increases caused by circular muscle contractions (8–90%, average 43% ; six experiments) and the propulsion of fluid along the gut (5–100%, average 45% ; Fig. 2).

Intraluminal administration of prostaglandin (E_1, 1–5 μg, three experiments; E_2, 5–20 μg, two experiments) had no effect on the longitudinal muscle, but reduced the peristaltic responses of the circular muscle in three experiments (5 μg, average 43%) and also reduced the propulsion of fluid through the gut in two of these (15 and 30%). Control injections of Krebs solution had no effect.

Effect of urethane and of temperature on the response of guinea-pig isolated ileum to prostaglandin

These investigations were made to determine a suitable anaesthetic for *in vivo* studies and to determine the effect of a fall in body temperature that can occur during anaesthesia on the response of the intestine to prostaglandin. The longitudinal muscle of guinea-pig isolated ileum was studied as described in our preceding paper (Bennett, Eley & Scholes, 1968). Urethane in a dose (2 mg/ml.; two experiments), approximately equivalent to that used *in vivo* (1.5–1.75 g/kg) reduced the responses of guinea-pig isolated ileum to acetylcholine (25 and 41%), but the response to prostaglandin was unaffected in one experiment and reduced by only 21% in the other experiment. A higher dose of urethane (6 mg/ml.), however, always reduced the contractions caused by both acetylcholine (56 and 59%) and

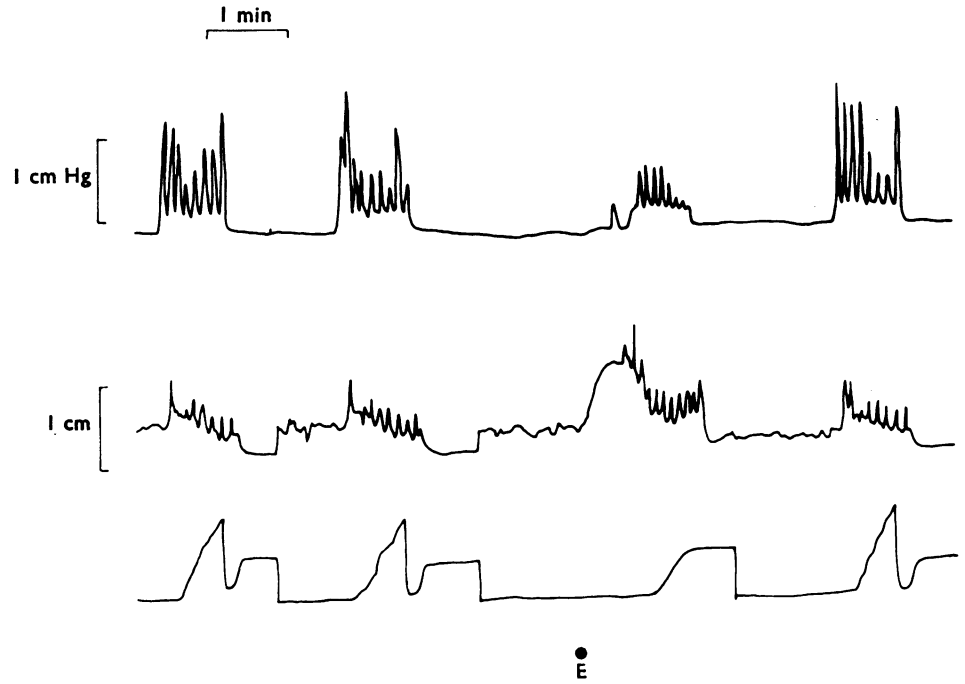

FIG. 2. Peristalsis in a segment of guinea-pig ileum perfused intraluminally. Top trace, intraluminal pressures (circular muscle contractions). Middle trace, longitudinal muscle contractions. Bottom trace, propulsion of fluid; each complete peak represents 1 ml. Peristalsis was induced by raising the intraluminal pressure from zero up to 3 mm Hg (4 cm H_2O) for 1 min every 10 min. Recorder off between responses. Prostaglandin E_2 (E, 0.25 μg/ml.) added to the bath fluid caused contraction of the longitudinal muscle, but reduced the circular muscle contractions and propulsion of fluid when the intraluminal pressure was raised. The responses returned to normal after the prostaglandin was washed out of the bath (last response).

prostaglandin E_1 (29 and 33%). Lowering of the bath temperature from 37° to 32° C had no effect on the responses to prostaglandin, although at 29° C they were reduced by 58% (two experiments).

Effect of prostaglandins E_1 and E_2 on the ileum in vivo

Guinea-pig. The basal intraluminal pressure of the small intestine varied from 0 to 4 cm H_2O with different animals. In four of eight experiments, prostaglandins E_1 and E_2 (0.05–4 μg ; four experiments each) injected into the bloodstream caused increases in the intraluminal pressure of 0.6–9 cm H_2O (0.5–7 mm Hg), depending on the animal and the dose (Fig. 3). These increases occurred 12–70 sec after the injection and remained elevated for 30–200 sec, but the duration was not related to the increase in pressure. Prostaglandin was more effective intra-arterially than intravenously, but accurate quantitative assessment was not possible because the pressure increases were not consistent. Prostaglandin had no effect on the gut in the other four guinea-pigs, although the gut responded to 5-hydroxytryptamine injected intra-arterially (2–4 μg ; two experiments). In contrast, the prostaglandins lowered the blood pressure in every experiment and the effect was 2–4 times greater with intra-arterial than with intravenous injections (Fig. 3). The increased intraluminal pressures caused by prostaglandin were apparently not caused by the fall in blood pressure or by the reflex release of hypertensive substances, because injection of histamine caused only hypotension.

In thirteen other experiments on anaesthetized guinea-pigs we measured the isotonic or isometric responses of the longitudinal muscle of the ileum to intra-arterially injected prostaglandins. The responses were not constant, even in any one experiment (Fig. 4). Sometimes the gut shortened or increased its tension, sometimes relaxation occurred, and in some instances the response was biphasic or absent (six, three, three and one experiments, respectively), although serosally applied prostaglandin (0.1–0.3 μg E_1 and E_2) always caused contraction (Fig. 4). The relaxant effect of injected prostaglandin was unaltered by blockade of α and β-adrenoceptive receptors with 0.2 ml. " Hydergine " and 0.5 mg pronethalol injected intravenously, and no contractions occurred after receptor blockade in previously unaffected loops (Fig. 4 ; five experiments).

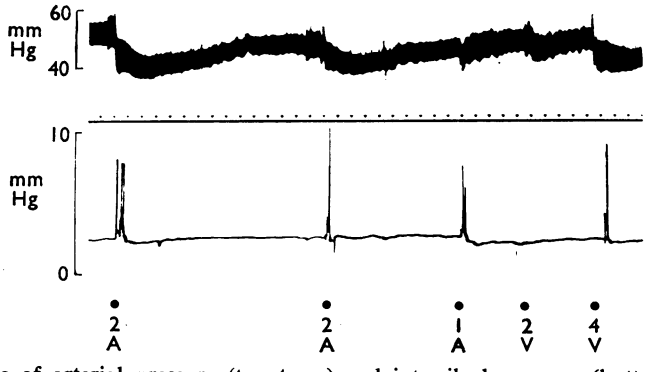

FIG. 3. Records of arterial pressure (top trace) and intra-ileal pressure (bottom trace) in an anaesthetized female guinea-pig weighing 400 g. Prostaglandin E_1 injected at each dot (numbers represent μg) caused hypotension and increased intestinal pressure. Intra-arterial (A) injection was more effective than intravenous (V) injection. Middle record, time in min.

Rat. Prostaglandins E_1 (three experiments) and E_2 (six experiments) injected intra-arterially or intravenously in doses above 0.5–2 μg (approximately 1–2 μg/kg) always increased the pressure in the lumen of the small intestine by 1–11 mm Hg (1.3–14.3 cm H_2O, Fig. 5). Doses of 0.1–4 μg also lowered the blood pressure by 5–30 mm Hg, but histamine (1–2 μg) caused hypotension without affecting the gut. The effects of prostaglandin on the bowel and on the blood pressure were 2–4 times greater with intra-arterial than with intravenous administration. Intraluminally

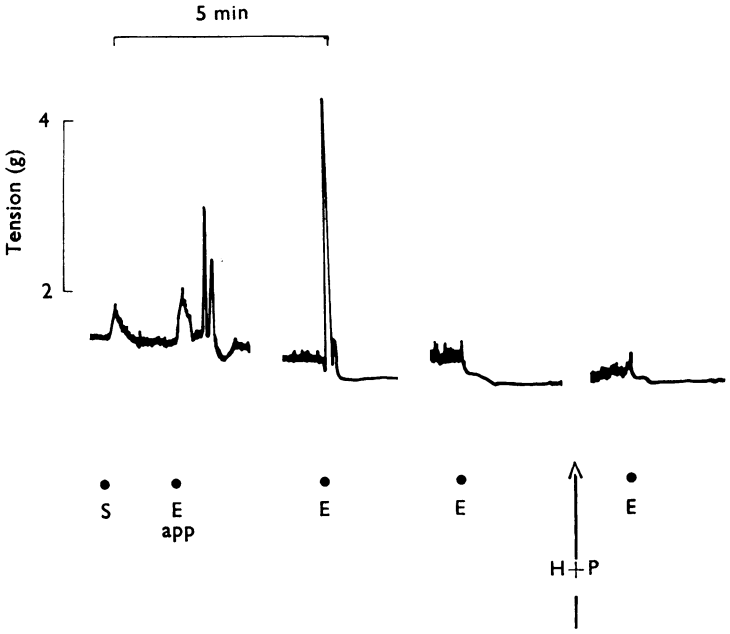

FIG. 4. Isometric responses of the longitudinal muscle of a loop of ileum in an anaesthetized female guinea-pig weighing 420 g. Prostaglandin E_1 (E app, 0.3 μg) applied to the serosa caused an increase in tension, although saline alone (S) had a small effect. The first dose of prostaglandin E_1 (E, 0.5 μg) injected intra-arterially caused a contraction followed by a long-lasting relaxation, but each subsequent dose caused only a relaxation which was unaffected by blockade of α- and β-adrenoceptive receptors with "Hydergine" (0.2 ml.) and pronethalol (0.5 mg) injected intra-arterially (H+P).

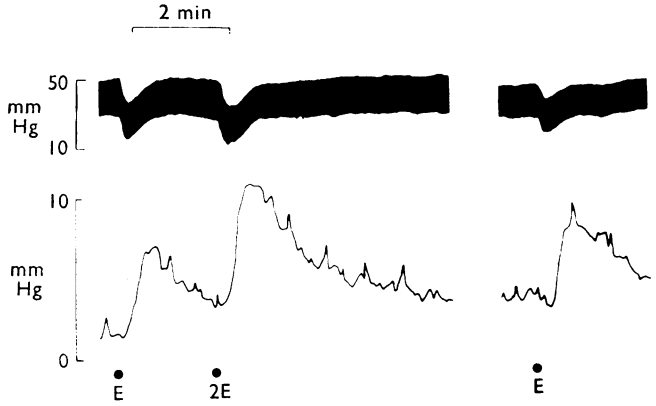

FIG. 5. Anaesthetized male rat weighing 360 g. Top trace, arterial pressure. Bottom trace, intra-ileal pressure. Prostaglandin E_2 injected intra-arterially (E, 0.5 μg) caused graded, reproducible falls in blood pressure and increases in intraluminal pressure.

applied prostaglandin (5–10 μg; two experiments) had no effect on the intestinal pressure.

As expected from the constant increases in the intraluminal pressure, blood-borne prostaglandin (0.5–2 μg injected intra-arterially) always caused contraction of the longitudinal muscle in a loop of bowel *in vivo* (seven experiments).

Discussion

The experiments with serosally applied prostaglandin on peristalsis in guinea-pig isolated ileum agree well with our previous *in vitro* results (Bennett, Eley & Scholes, 1968). In both instances prostaglandins stimulated the longitudinal muscle but inhibited the circular muscle. This effect on the circular muscle was presumably the cause of the reduced propulsion of fluid in the experiments on peristalsis. Our previous results indicate that E-type prostaglandins act directly on the circular muscle cells, so prostaglandin bathing the serosal surface of the gut must penetrate the longitudinal muscle layer before it can affect the circular layer.

We have found (unpublished observations) that a small amount of prostaglandin-like activity is released into the lumen of the guinea-pig isolated ileum during peristalsis. Diffusion of prostaglandin into the gut wall through the mucosa appears to be poor, however, for intraluminally applied material usually had no effect on peristalsis. Any E-type prostaglandin normally released into the lumen is unlikely to be reabsorbed later to alter the motility of other parts of the gut, and the same might also be true of prostaglandin released into the lumen of the rat stomach (Bennett, Friedmann, & Vane, 1967).

The experiments with the rat ileum *in vivo* gave consistent results. Prostaglandin injected into the bloodstream caused the longitudinal muscle to contract and increased the intraluminal pressure. At the same time it lowered the blood pressure, but this was not responsible for the effect on the gut because injection of histamine caused only hypotension. The incidental finding that injected prostaglandin had a greater effect intra-arterially than intravenously indicates that the rat pulmonary circulation removes or inactivates E-type prostaglandins. The same seems to be true in the guinea-pig, and Ferreira & Vane (1967) found it to be so in the dog, cat and rabbit.

In contrast to the rat, the results with guinea-pig ileum *in vivo* were variable, so that the longitudinal muscle was either contracted, unaffected or relaxed, and the intraluminal pressure was either increased or unchanged. The relaxation of the muscle was unaffected by blockade of α- and β-adrenoceptive receptors, so release of catecholamine or stimulation of adrenoceptive receptors was not involved. Perhaps the variation in responses resulted from a metabolite of prostaglandin or from changes in blood flow or oxygen supply to the gut muscle caused by the injected prostaglandin. Or perhaps prostaglandin in the bloodstream activates sites not reached through serosal application: Daniel, Sutherland & Bogoch (1959) found a similar situation with morphine which stimulated the dog ileum when injected into the vascular supply, but not when applied serosally. Both these results are consistent with the hypothesis (Bennett, 1968) that responses of intestinal muscle to a substance applied artificially to its surface, rather than responses to injection into the bloodstream, may mimic the effect that normally occurs when that substance is released physiologically in the gut wall.

Our experiments shed no light on the suggestion (Williams, Karim & Sandler, 1968) that prostaglandin might cause diarrhoea, for although the responses of human, guinea-pig and rat isolated small intestine are largely similar, the responses of the guinea-pig and rat gut to blood-borne prostaglandin *in vivo* sometimes differ. In addition, the circular muscle of human isolated ileum, unlike the guinea-pig and rat, sometimes responds to prostaglandin by a contraction which precedes the relaxation, and we have only examined the effects of prostaglandins E_1 and E_2. The effect in man therefore cannot be predicted from our results. Furthermore, changes in intraluminal pressure do not necessarily give information on changes in the propulsion of contents such as occur in diarrhoea, and we do not yet know whether the effect on the large intestine is the same as on the small intestine.

Prostaglandin in the lumen of the ileum, at least in the guinea-pig and rat, seems unlikely to be important in influencing gut motility. Blood-borne prostaglandin is also unlikely to play a physiological part in this respect, partly because it is so rapidly inactivated at other sites, and partly because the concentration necessary to affect the bowel also lowers the blood pressure. The prostaglandin in the gut wall, however, might be involved in the control of gut motility. E-type prostaglandins occur in the human and rat stomach wall and in guinea-pig ileum (Bennett, Murray & Wyllie, 1968; Coceani, Pace-Asciak, Volta & Wolfe, 1967; Bennett *et al.*, 1967; Ambache, Brummer, Rose & Whiting, 1966), and we have found prostaglandin-like activity in the human and rat ileum (unpublished observations). It is interesting to speculate that prostaglandin may also act in another way. Coceani *et al.* (1967) found that prostaglandin (together with histamine and 5-hydroxytryptamine, Paton & Vane, 1963) is released from the serosal surface of the stimulated rat stomach. Perhaps these substances are released from the serosal surface of the active human stomach and small intestine, and affect the movements of the adjacent colon. Such an action might play a part in the "gastrocolic reflex," during which increased movements of the large bowel occur after eating.

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