J. Pharm. Pharmac., 1977, **29**, 276–280 *Received July* 23, 1976

The effects of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ on guinea-pig ileal and colonic peristalsis

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Prostaglandins (PGE_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$) have been tested on the peristaltic reflex in isolated segments of guinea-pig ileum and colon using simultaneous recordings of fluid propulsion and longitudinal and circular muscle activity. Propulsion and circular muscle peristaltic activity were increased by serosally applied PGF compounds in the ileum and PGE or PGF compounds in the colon following initial contraction of the longitudinal muscle. This is consistent with a role for prostaglandins in peristalsis. Mucosally applied PGF compounds had no significant effect.

In various species PG-like material is released from the gastrointestinal tract during peristaltic activity (see reviews by Bennett & Fleshler, 1970; Bennett, 1976). Bennett, Eley & Scholes (1968b) found that PGE_1 or E_2 applied serosally to segments of guinea-pig ileum stimulated the longitudinal muscle but reduced circular muscle peristaltic contractions and propulsion. Similarly, Radmanović (1972) reported that PGE_1 generally inhibited peristalsis in this tissue but low concentrations caused transient potentiation. However, Takai, Matsuyama & Yagasaki (1974) found that PGE_1 or $F_{2\alpha}$, 10^{-7} to 2×10^{-7} g ml^{-1} ($2{\cdot}9$–$5{\cdot}8 \times 10^{-7}$ M) slightly increased peristalsis in guinea-pig ileum studied by the Trendelenberg method.

Ishizawa & Miyazaki (1973a,b) found that $PGF_{2\alpha}$ 10^{-6} M stimulated aboral propulsion in guinea-pig isolated colon, in agreement with its stimulating effect on strips of circular and longitudinal colonic muscle. PGE_1 10^{-8} M also slightly stimulated aboral propulsion, but concentrations above 10^{-7} M showed mainly initial inhibition of movement followed by one strong peak of stimulation. In agreement with data of Fleshler & Bennett (1969), PGE_1 (10^{-7} M) or PGE_2 (10^{-7}, 10^{-6} M) relaxed strips of circular colonic muscle, and contracted strips of longitudinal muscle; $PGF_{2\alpha}$ (10^{-7}, 10^{-6} M) contracted both layers. Watanabe (1972) also found that PGE_1 relaxed colonic circular muscle and $PGF_{2\alpha}$ 10^{-5} M caused contraction, but in contrast PGE_2 caused contraction and $PGF_{2\alpha}$ $<10^{-7}$ M produced relaxation. The methodology was slightly different since the circular muscle was separated from the longitudinal layer.

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To clarify the role of individual PGs in peristalsis we have investigated the effects of serosally and mucosally applied PGE_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the colon, and $F_{1\alpha}$ and $F_{2\alpha}$ in the ileum.

METHODS

Segments of mid-ileum and terminal colon (5–8 cm) were removed from freshly killed adult albino guinea-pigs of either sex, and peristalsis was studied by the method of Bennett & others (1968b) as modified by Bennett, Eley & Stockley (1976). Drugs in 0·15 M NaCl (or saline controls) were added to the Krebs solution bathing the serosal surface, or injected intraluminally over approximately 20 s in 0·5 ml. Since fresh fluid entering the gut lumen during peristalsis diluted the intraluminally injected drugs, the final concentration is not known and only the amounts injected have been stated. Muscle activity induced by raising the intraluminal pressure was designated peristaltic activity although on a few occasions there was no propulsion of intraluminal fluid. The results were analysed statistically using a two-tailed Fisher's exact probability test and differences were considered significant when $P \leqslant 0{\cdot}05$. In further experiments, PGE_1 or PGE_2 were studied on responses of spirally cut strips of colonic circular muscle to intramural nerve stimulation (electrical field stimulation, 20 s trains; 1 ms pulses; 0·5–32 Hz; 2-4 V cm^{-1} in Krebs solution) as described by Bennett, Eley & Stockley (1975).

Drugs used were acetylcholine perchlorate, potassium chloride and PGs E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ tromethamine salt. The PGs were dissolved in ethanol (0·1 ml mg^{-1}), diluted to 1 mg ml^{-1} with sodium carbonate solution (0·2 mg ml^{-1}) and the pH was adjusted to 7. The composition of the

Krebs solution was (g litre^{-1}): NaCl 7·1, $CaCl_2$, $6H_2O$ 0·55, KCl 0·35, KH_2PO_4 0·16, $MgSO_4 \cdot 7H_2O$ 0·29, $NaHCO_3$ 2·1, dextrose 1·0.

RESULTS

Details of results are given in Table 1. Saline controls never produced statistically significant effects although intraluminal injections tended to affect activity.

Effects of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ on peristalsis in guinea-pig isolated ileum. Serosally applied $PGF_{1\alpha}$ or $F_{2\alpha}$ (0·33 μg ml^{-1}) initially contracted the longitudinal muscle and slightly increased circular muscle peristaltic activity and propulsion, but there was no significant effect on longitudinal muscle peristaltic activity (Table 1, Fig. 1). Higher concentrations of intraluminally applied $PGF_{1\alpha}$ or $F_{2\alpha}$ (5 μg in 0·5 ml, Table 1) had no significant effect.

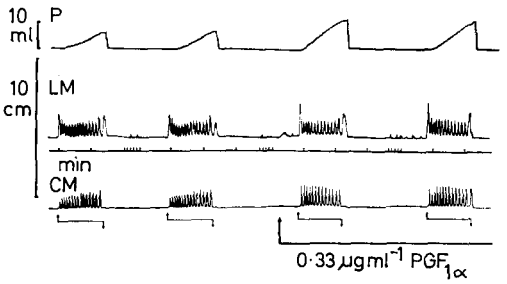

FIG. 1. Peristalsis in a segment of guinea-pig isolated ileum induced by raising the intraluminal pressure (arrows) from zero to 4 cm water pressure. The traces (from top to bottom) are propulsion (P); longitudinal muscle (LM), contraction upwards; time scale in min; circular muscle (CM). The chart speed was reduced between responses. $PGF_{1\alpha}$ 0·33 μg ml^{-1} bathing the serosal surface stimulated propulsion and circular muscle activity after a small initial contraction of the longitudinal muscle. The responses subsequently returned to normal after washing the PG from the bath.

Effects of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ on peristalsis in guinea-pig isolated colon. The peristaltic activity elicited by raising the intraluminal pressure from zero to 2–5 cm water was much slower and less regular than in the ileum (Fig. 2), and consequently more difficult to study. Serosally applied $PGF_{1\alpha}$ or, more consistently, $F_{2\alpha}$ (0·33 μg ml^{-1}) initially contracted the longitudinal muscle, but increased its peristaltic activity in only 5 and 4 experiments respectively (overall effect not statistically significant). Both PGF compounds increased circular muscle peristaltic activity and propulsion (Table 1, Fig. 2). Intraluminally applied $PGF_{1\alpha}$ or $F_{2\alpha}$ (5 μg in 0·5 ml) were ineffective.

Effects of PGE_1 and PGE_2 on peristalsis in guinea-pig isolated colon. PGE_1 or E_2 (0·33 μg ml^{-1}) bathing the serosal surface of the colon initially contracted

Table 1. *The effects of PGE_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ applied serosally (0·33 μg ml^{-1}) or mucosally (5 μg in 0·5ml) on propulsion and peristaltic activity in guinea-pig isolated intestine.* The 'number of experiments' represents the number of observations/number of tissues. The figures quoted are medians and semiquartile ranges. In the ileum and colon serosally applied PGF compounds increased circular muscle activity and propulsion (effect of $PGF_{1\alpha}$ on colonic propulsion not statistically significant). In the colon serosally applied PGE compounds stimulated circular and longitudinal muscle activity.

No. expts.	PG and route	Propulsion % change	Muscle peristaltic activity % change Circular	Longitudinal
Ileum				
14/10	$F_{1\alpha}$ Ser.	3 (0 to 18)*	13 (0 to 20)*	0 (0 to 10)
9/8	$F_{2\alpha}$ Ser.	12 (0 to 33)**	11 (6 to 15)**	0 (−30 to 10)
8/6	$F_{1\alpha}$ Muc.	0 (0 to −17)	0 (0 to −26)	0 (0 to 0)
3/2	$F_{2\alpha}$ Muc.	11 (0 to 100)	0 (0 to 0)	0 (0 to 0)
Colon				
13/13	$F_{1\alpha}$ Ser.	31 (0 to 97)	53 (10 to 309)*	0 (0 to 100)
10/8	$F_{2\alpha}$ Ser.	32 (0 to 62)*	91 (7 to 292)**	0 (−50 to 80)
11/10	$F_{1\alpha}$ Muc.	0 (0 to 6)	0 (0 to −30)	0 (0 to 0)
12/11	$F_{2\alpha}$ Muc.	5 (−4 to 25)	3 (0 to 48)	0 (0 to 0)
13/11	E_1 Ser.	3 (0 to 17)	58 (20 to 75)*	0 (0 to 50)*
18/15	E_2 Ser.	−3 (7 to 26)	29 (8 to 202)**	12 (0 to 100)**
8/8	E_1 Muc.	3 (−19 to 78)	16 (−15 to 186)	10 (10 to 100)*
11/10	E_2 Muc.	0 (−46 to 46)	25 (−25 to 62)	0 (0 to75)*

** $P < 0·01$; * $P < 0·05$ compared to saline or Krebs controls.

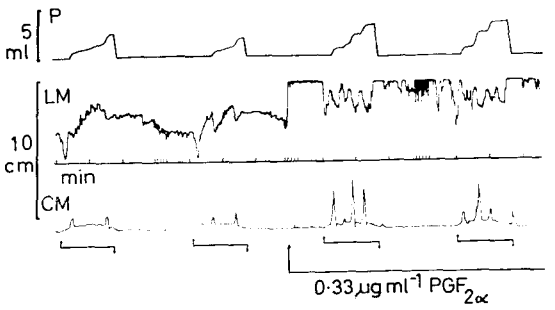

FIG. 2. Peristalsis in a segment of guinea-pig isolated colon induced by raising the intraluminal pressure (arrows) from zero to 5 cm water pressure. The traces are as in Fig. 1. $PGF_{2\alpha}$ 0·33 μg ml⁻¹ bathing the serosal surface caused a large initial contraction of the longitudinal muscle followed by big increases in all aspects of peristaltic activity.

the longitudinal muscle; peristaltic activity of the circular and longitudinal muscle increased, but the effect on propulsion was not statistically significant (Table 1, Fig. 3). On 2 occasions each, a long-lasting inhibition occurred when PGE_1 or E_2 were washed from the bath. On another occasion activity was temporarily reduced when PGE_2 was washed out.

PGE_1 or E_2 (5 μg in 0·5 ml) injected intraluminally usually contracted the longitudinal muscle, but otherwise produced variable effects with no significant change in peristaltic activity or propulsion.

Experiments to investigate the mechanism of stimulation of colonic circular muscle peristaltic activity by PGE compounds. The response of colonic circular muscle strips to electrical excitation consisted of relaxation (or inhibition of rhythmic activity in preparations lacking tone) which was followed by a rapid after-contraction immediately stimulation ceased. Following the after-contraction (which occurred regularly at 8 Hz or above, and sometimes at 2 or 4 Hz), the tissue relaxed slowly to its baseline. PGE_1 (1–2 μg ml⁻¹, 2 strips) or PGE_2 (10–800 ng ml⁻¹, 11 experiments) relaxed the strips and reduced the after-contraction. During submaximal relaxations to PGE_1 or E_2, the electrically induced maximal and submaximal relaxations were decreased, but responses were restored when the tone was raised by acetylcholine, KCl or $PGF_{2\alpha}$ (15 experiments on 10 strips).

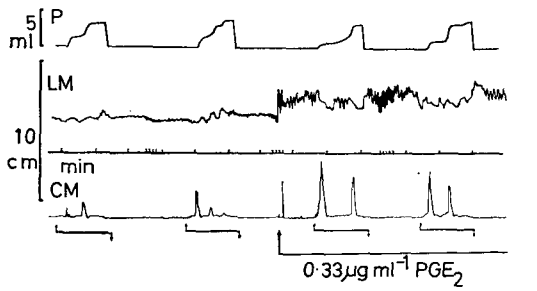

FIG. 3. Peristalsis in a segment of guinea-pig isolated colon induced by raising the intraluminal pressure (arrows) from zero to 5 cm water pressure. The traces are as in Fig. 1. PGE_2 0·33 μg ml⁻¹ bathing the serosal surface caused an initial contraction of the longitudinal muscle followed by large increases in longitudinal and circular muscle peristaltic activity but no significant change in propulsion.

DISCUSSION

PGE_1, E_2, $F_{1\alpha}$ or $F_{2\alpha}$ applied serosally affected ileal and colonic peristaltic activity, but mucosally applied PGs were virtually ineffective, possibly because of their inability to cross the mucosa. Only 0·1% of the ^{3}H-PGE_1 injected intrajejunally in rats reached the bloodstream intact (Parkinson & Schneider, 1969). The colonic longitudinal muscle contraction elicited by intraluminal PGEs might seem to argue against this, but perhaps it was due to the high concentration or to stimulation of mucosal nerves; PGE and F compounds seem to act partly on intramural nerves in contracting the longitudinal muscle of guinea-pig isolated ileum and colon (Bennett, Eley & Scholes, 1968a; Bennett & others, 1975). Nevertheless, a small effect with mucosally applied PGs would be difficult to demonstrate because control intraluminal injections tended to affect activity. The following discussion is therefore restricted to serosally administered PGs.

Ileum

The weak stimulation of ileal circular muscle activity and propulsion by $PGF_{1\alpha}$ or $F_{2\alpha}$ agrees with the findings of Takai & others (1974), and contrasts with the inhibition of peristalsis usually caused by PGE compounds in this region (Bennett & others, 1968a; Kottegoda, 1969; Radmanović, 1972). However, Takai & others (1974) obtained stimulation with 100–200 ng ml⁻¹ PGE_1 and Radmanović (1972) found that PGE_1 (10–50 ng ml⁻¹) slightly potentiated peristalsis but caused a long-lasting inhibition after washing out the PG. Perhaps this increase in circular muscle activity was due to increased intraluminal pressure following longitudinal muscle contraction; this would be more marked in a closed system (used by both groups) than in our open system. A similar mechanism might explain the slight but regular increase in circular muscle activity and propulsion elicited with

$PGF_{1\alpha}$ and $F_{2\alpha}$, whereas strips of circular ileal muscle are poorly sensitive to these PGs (Bennett & others, 1975).

The ability of aspirin or indomethacin to inhibit all aspects of peristalsis (Bennett & others, 1976) suggests that PGs are involved in peristalsis. The PGE_2 thought to be present (Ambache, Brummer & others, 1966) may contribute to the longitudinal muscle activity and/or functioning of the myenteric plexus, but substantial amounts reaching the ileal circular muscle would be expected to reduce peristalsis. An inhibitory effect of PGE_2 in the circular muscle of guinea-pig isolated ileum is indicated by the slight increase in tone and activity with indomethacin (Bennett & others, 1975). However, $PGF_{2\alpha}$ would tend to be excitatory in both muscle layers. Segments of guinea-pig ileum at rest or during electrical field stimulation appear to release only PGE_2-like material into the bathing solution (Botting & Salzmann, 1974) but Ambache & others (1966) extracted both PGE_2- and $PGF_{2\alpha}$-like material from the tissue. Perhaps only PGE_2 is generated in the longitudinal muscle (and mucosa) from which it diffuses readily into the surrounding fluid, whereas $PGF_{2\alpha}$ alone or together with PGE_2 is generated in the circular muscle and/or enteric plexuses.

Colon

Colonic peristaltic activity was substantially enhanced by PGFs. Unlike the ileum, circular muscle activity and propulsion were significantly increased possibly reflecting the sensitivity of colonic circular muscle strips to PGF compounds (Fleshler & Bennett, 1969; Bennett, 1970; Bennett & Posner, 1971; Ishizawa & Miyazaki, 1973a, b).

The stimulation of colonic activity by PGE_1 and PGE_2 was surprising since similar concentrations relaxed strips of colonic circular muscle and inhibited ileal peristalsis. However, activity was sometimes depressed after washing out the PGEs as Radmanović (1972) found in the ileum. Ishizawa & Miyazaki (1973a,b) found that $PGE_1 > 10^{-7}$ M produced mainly initial inhibition of colonic aboral propulsion followed by one strong peak of stimulation. Our experiments indicate a stimulation of non-propulsive contractions by PGE compounds rather than true peristalsis, but experiments by Ishizawa & Miyazaki (1973a,b) argue against this possibility. As suggested for the ileum, the induced circular muscle activity might be of reflex origin, initially involving longitudinal muscle or its excitatory nerves which may be cholinergic or non-cholinergic (Bennett & others, 1975). However, increased intraluminal pressure seems unlikely to be the only factor since Ishizawa & Miyazaki (1973a,b) used an open system. The effect on the longitudinal muscle could be of particular importance if PGs have difficulty in penetrating to the circular muscle to cause inhibition. PGE inhibition of circular muscle strips seems to be a direct action on the smooth muscle (Fleshler & Bennett, 1969; Watanabe, 1972; Ishizawa & Miyazaki, 1973a,b), although Radmanović (1972) considered that part of the inhibition in whole segments of ileum may be due to release of a catecholamine. Unlike strips, where PGs come directly into contact with the circular muscle at the cut edges, in segments the circular muscle is protected externally by longitudinal muscle and serosa and internally by mucosa and submucosa.

It is unlikely that PGE_1 or E_2 enhanced colonic peristaltic activity by reducing the release or actions of an inhibitory transmitter, since the reduction of electrically induced inhibition during relaxation to these compounds was reversed by restoring the tone. Another possibility is that in the colon, which unlike the ileum appears to generate circular muscle tones *in vitro*, PGEs slowly relax the circular muscle. This effect would not register on the pressure recording and greater pressure increases might occur during peristalsis because of the enlarged colonic diameter. The results of Ishizawa & Miyazaki (1973a,b) using an intraluminal ball of fixed diameter and the reduction of electrically induced after-contraction with PGE compounds, argue against this possibility. However, electrically induced responses are due mainly to postganglionic stimulation (Paton, 1955) and cannot be equated with peristaltic responses; PGEs might stimulate at an earlier stage in the reflex.

Again, it is of interest to compare the effects of adding PGs with inhibition of their synthesis. As in the ileum depression of colonic peristalsis by aspirin or indomethacin contrasted markedly with the actions of PGs, particularly of the F-series. The PGE- and PGF-like material in guinea-pig colon (Stamford, 1976) might serve different roles: perhaps the PGF generates tone in both muscle layers, whereas PGE stimulates longitudinal muscle tone and activity but inhibits the circular muscle.

Acknowledgements

We thank Mrs E. M. Charlier for technical assistance, the Wellcome Trust for financial support (EMC, HLS) and Dr J. E. Pike, Upjohn Company Ltd, U.S.A. for prostaglandins.

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