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THE CONTRIBUTION OF PROSTAGLANDINS IN THE MUSCLE OF HUMAN ISOLATED SMALL INTESTINE TO NEUROGENIC RESPONSES

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1 In strips cut parallel to the longitudinal or circular muscle, indomethacin (2–10 μg/ml) usually lowered the tone, thus probably accounting for the reduction of nerve-mediated relaxations to electrical field stimulation.

2 In longitudinal muscle strips, indomethacin enhanced contractions which occurred during electrical stimulation, probably because tone fell, but antagonized after-contractions. By contrast, in the circular muscle indomethacin reduced initial contractions but enhanced after-contractions.

3 Prostaglandin E_2 counteracted all of the effects of indomethacin in the longitudinal muscle and most of those in the circular muscle; prostaglandin $F_{2\alpha}$ restored circular muscle tone.

4 The results suggest that prostaglandins affect the muscle directly and contribute to the regulation of tone. They may also mediate non-cholinergic contraction in longitudinal muscle and suppress contractility in the circular muscle.

Introduction

Prostaglandin-like substances have been demonstrated in human stomach (Bennett, Stamford & Unger, 1973), ileum (Stamford, 1976; Bennett, Stamford & Stockley, 1977b) and colon (Bennett, del Tacca, Stamford & Zebro, 1977a; Bennett *et al.*, 1977b), and prostaglandins potently affect strips of human isolated ileal muscle (Bennett, Eley & Scholes, 1968). Studies employing inhibitors of prostaglandin action or synthesis indicate that in isolated intestine, prostaglandins contribute to the regulation of muscle tone and rhythmic activity (Bennett & Posner, 1971; Eckenfels & Vane, 1972; Ferreira, Herman & Vane, 1972; 1976; Botting & Salzmann, 1974; Willis, Davison & Ramwell, 1974; Bennett, Eley & Stockley, 1975a; Stockley & Bennett, 1976; Burleigh, 1977). *In vivo*, prostaglandin E_2 may help control human cardiac sphincter tone (Dilawari, Newman, Poleo & Misiewicz, 1975).

Prostaglandins appear to facilitate responses mediated by cholinergic nerves in the longitudinal muscle of guinea-pig isolated ileum (for discussion, see Bennett, Eley & Stockley, 1975b). We have used indomethacin to investigate the contribution of prostaglandins to neurogenic responses in the longitudinal and circular muscle of human ileum.

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Methods

Human isolated small intestine

Macroscopically normal specimens of small intestine, removed at least 6 cm from any lesion, were obtained from the operating theatre as soon as possible after surgical removal. They were placed in Krebs solution and used immediately or stored overnight at 4°C. Eleven specimens of terminal ileum and one each of jejunum and proximal ileum were studied. Tissues from all regions behaved similarly, so the results were pooled. Mesentery and mucosa were cut away, and strips 1–2 × 10–15 mm were cut through the muscle coats parallel to the longitudinal or circular fibres. The preparations were suspended under a load of 1 g between parallel 0.5 cm square silver plate electrodes 1 cm apart in Krebs solution at 37°C bubbled with 5% CO_2 in O_2. The Krebs solution had the following composition (g/l): NaCl 7.1, $CaCl_2.6H_2O$ 0.55, KH_2PO_4 0.16, KCl 0.35, $MgSO_4.7H_2O$ 0.29, $NaHCO_3$ 2.1 and dextrose 1.0.

Responses magnified five- to twenty-fold were recorded with isotonic transducers. Square-wave

pulses of alternating polarity were generated by linked stimulators (Scientific and Research Instruments Ltd. Nos. 6051 and 6053), and 20 s trains of 1 ms pulses were delivered at 4 min intervals. The voltage between the electrodes (measured in Krebs solution) was 16 volts. The stated frequency (Hz) is the total number of positive and negative pulses.

As reported previously, the responses of human ileal strips to electrical stimulation are complex (Bennett & Stockley, 1975). Contractions that occurred during stimulation have been called initial contractions to distinguish them from after-contractions (Figure 1). All contractions and relaxations were measured from the base of any spontaneous activity before stimulation, and expressed as % of controls. Results were analysed statistically with Student's *t* test for paired data.

Drugs

The following drugs were used: acetylcholine perchlorate (ACh), 5-hydroxytryptamine creatinine sulphate (5-HT), (−)-hyoscine hydrobromide, indomethacin and prostaglandins E_2 and $F_{2\alpha}$. Concentrations are expressed in terms of free base or acid.

Results

Because of the long contact periods needed with indomethacin, responses to electrical stimulation were monitored for 35–120 min in some experiments to check that they did not change spontaneously with time. Five longitudinal and 3 circular muscle strips responded consistently during these periods.

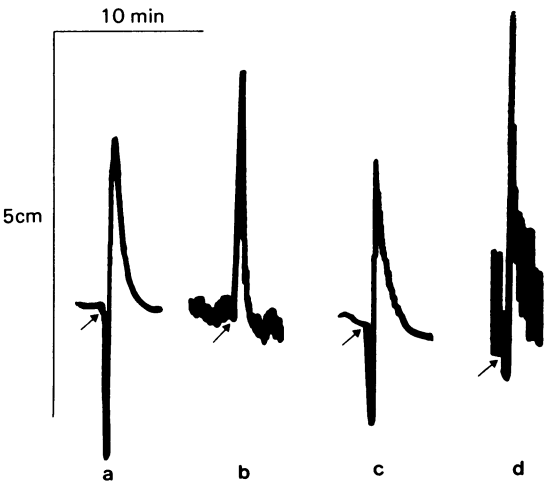

Figure 1 Responses to electrical stimulation (arrows; 20 s trains; 1 ms pulses; 16 V/cm; 4 Hz (a and b) or 8 Hz (c and d)) in human isolated ileal muscle. Strips which showed little rhythmic activity were selected to illustrate clearly the effect of indomethacin (2 μg/ml). (a) Longitudinal muscle, control responses consisting of relaxation followed by after-contraction. (b) After indomethacin, the longitudinal muscle relaxed 10.5 cm on the scale indicated and small rhythmic contractions started. The tissue contracted during stimulation (initial contraction) and rapidly relaxed the baseline after stimulation ceased. (c) Circular muscle control response consisting of relaxation and after-contraction. (d) After indomethacin, the muscle relaxed 3 cm and rhythmic contractions were initiated. Circular muscle tissue relaxation was smaller and the after-contraction bigger.

Table 1 The effect of indomethacin on the responses to electrical stimulation of 11 strips of human ileal longitudinal muscle

	Relaxation		*Initial-contraction*		*After-contraction*	
Hz	*% control*	n	*% control*	n	*% control*	n
0.5	0	2	169 (135 to 206)	6	0	1
1	0, 23	2	185 (144 to >202)	6	0	4
2	0 (0 to 0)*	5	>179 (153 to ∞)	6	0	5
4	0 to 21*	4	197 (185 to >208)	6	0	4
8	0 to 17	3	213 (197 to 280)*	5	0	4
16	0, 4	2	187 (97 to 253)	5	0, 42	2

Electrical stimulation (20 s trains; 1 ms pulses; 16 V/cm) produced various combinations of relaxations and initial- and after-contractions. *n* is the number of strips that produced a particular type of response at each frequency. Indomethacin (2 μg/ml) reduced or prevented the relaxations and almost always prevented the after-contractions. The tendency for initial contractions to increase was significant at 8 Hz. Results after indomethacin are calculated as the median % control without indomethacin in *n* strips with semiquartile ranges where $n \geqslant 5$, and with the overall range where $n < 5$. At 1, 2 and 4 Hz initial-contractions sometimes replaced relaxations so here the upper semiquartile ranges could not be calculated.
* $P < 0.05$, *t* test for paired data. Parametric statistics do not apply to the after-contraction at 0.5 to 8 Hz where the response was always prevented.

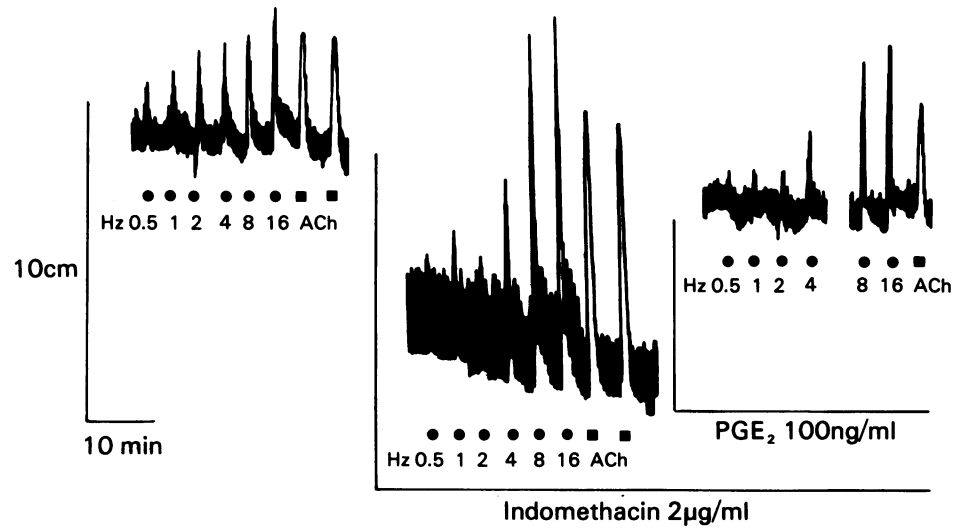

Figure 2 Human ileal longitudinal muscle contracted to acetylcholine (■, ACh, 0.25 μg/ml) and most frequencies of electrical stimulation (●, 1 ms pulses, 16 V/cm, 20 s), but responded with a small relaxation and after-contraction at 2 Hz. After incubation with indomethacin (2 μg/ml, 45 min) the tone was lowered, contractions were enhanced and relaxation was prevented. Prostaglandin E_2 (PGE_2, 100 ng/ml in the continued presence of indomethacin) partially restored the tone, reduced contractions and re-introduced a small relaxation at 2 Hz.

Longitudinal muscle

Twelve strips cut longitudinally from different specimens were used to study the effect of indomethacin (2, 5 and 10 μg/ml in 8, 3 and 5 experiments respectively). We have previously described the lowering of tone and associated enhancement of ACh-induced contractions by indomethacin in 8 of these experiments (Stockley & Bennett, 1976). Indomethacin has now been studied on responses to intramural nerve stimulation. The 3 dose levels produced similar changes but higher indomethacin concentrations usually acted more quickly (2 μg/ml 45–90 min, 5 μg/ml 30–35 min,

Table 2 The effect of indomethacin on responses to electrical stimulation of 11 circularly cut strips of human ileal muscle

	Relaxation		*Initial-contraction*		*After-contraction*	
Hz	*% control*	n	*% control*	n	*% control*	n
0.5	100 (50 to 241)	7	0	1	505 (247 to > 514)	6
1	86 (50 to 175)	9	0	2	283 (171 to ∞)*	8
2	69 (63 to 106)*	9	0	3	520 (272 to > 600)*	7
4	64 (50 to 90)*	9	0	3	353 (237 to > 400)	6
8	60 (25 to 91)*	9	0, 25	2	392 (230 to > 578)	6
16	64 (40 to 77)	7	0 to 205	3	235 (156 to 284)	5

Electrical stimulation (20 s trains; 1 ms pulses; 16 V/cm) caused mainly relaxation and after-contraction, although 3 initially contracted at some frequencies. *n* is the number of strips that produced a particular type of response at each frequency. Indomethacin (2 μg/ml) decreased the relaxations at 2, 4 and 8 Hz and tended to do so at 1 and 16 Hz. The initial contractions were decreased or prevented, but the after-contractions increased at 1 and 2 Hz and tended to do so at other frequencies. Results are presented as in Table 1. At 0.5, 1, 2, 4 and 8 Hz after-contractions were sometimes initiated in the presence of indomethacin (∞% control) so the upper semiquartile could not be calculated.

* $P < 0.05$, *t* test for paired data.

10 μg/ml 20–45 minutes). Effects of indomethacin 2 μg/ml on responses to electrical stimulation are summarized in Table 1. Indomethacin (2 or 5 μg/ml) prevented or greatly reduced the after-contractions ($P < 0.05$ at 1, 2, 4 and 8 Hz) and the relaxations ($P < 0.05$ at 2 and 4 Hz), but enhanced the initial contractions ($P < 0.05$ at 2, 4 and 8 Hz). The change in a single response from relaxation and after-contraction to initial contraction is illustrated in Figure 1 and the overall changes are shown in Figure 2. With 10 μg/ml indomethacin, after-contractions and relaxations were always prevented but whereas the initial contractions tended to be reduced at the lower frequencies, they were increased at 4, 8 and 16 Hz ($P < 0.05$). Changes in responses other than the after-contractions, might have been due to the fall in tone.

Indomethacin (5 μg/ml) enhanced submaximal contractions to 5-HT (10 or 100 ng/ml; 3 experiments). In 3 other experiments with indomethacin 5 to 10 μg/ml (in the presence of 1 μg/ml hyoscine to obtain relaxations with a wide range of frequencies), 5-HT (1, 20 or 200 ng/ml) caused a sustained submaximal contraction and increased the nerve-mediated relaxations at all frequencies ($P < 0.01$); the after-contractions remained inhibited. In contrast, prostaglandin E_2 (20–100 ng/ml) or $F_{2\alpha}$ (100 ng/ml) tended to counteract the effects of indomethacin on tone and on all three components of the electrically induced responses (Figure 2), although the effective concentration varied in different experiments.

Circular muscle

We have previously found (Stockley & Bennett, 1976) that indomethacin increases rhythmic- and ACh-induced contractions in human ileal circular muscle, and often lowers the tone. The effects of indomethacin on nerve-mediated responses in these 8 tissues and 5 further strips are now described. In circular muscle, the usual response to electrical excitation was relaxation and after-contraction. The relaxations at 2 Hz and above were reduced when the tone fell with indomethacin 2 μg/ml (7 out of 11 strips), whereas the after-contractions at all frequencies were enhanced even when tone did not change (Table 2, Figures 1 and 3). These observations were supported by 2 further experiments with 5, 10 and 20 μg/ml indomethacin studied sequentially. On the original trace the rapid after-contractions were seen to equal or exceed the amplitude of the enhanced rhythmic contractions.

In 3 experiments where 5-HT (10 ng/ml) contracted the muscle to about the pre-indomethacin level, relaxations at 0.5–16 Hz were restored and indomethacin-induced increases in rhythmic activity and after-contractions were further enhanced.

Prostaglandin E_2 (50–500 ng/ml) generally opposed all actions of indomethacin (Figure 3), although its effect on tone and relaxations were variable. The effect of prostaglandin E_2 on electrically induced relaxations varied with its effect on tone; inhibitory responses increased when prostaglandin E_2 caused contraction and vice-versa. Most contractions (ACh-induced, rhythmic, and after-contractions) were greatly reduced or prevented by

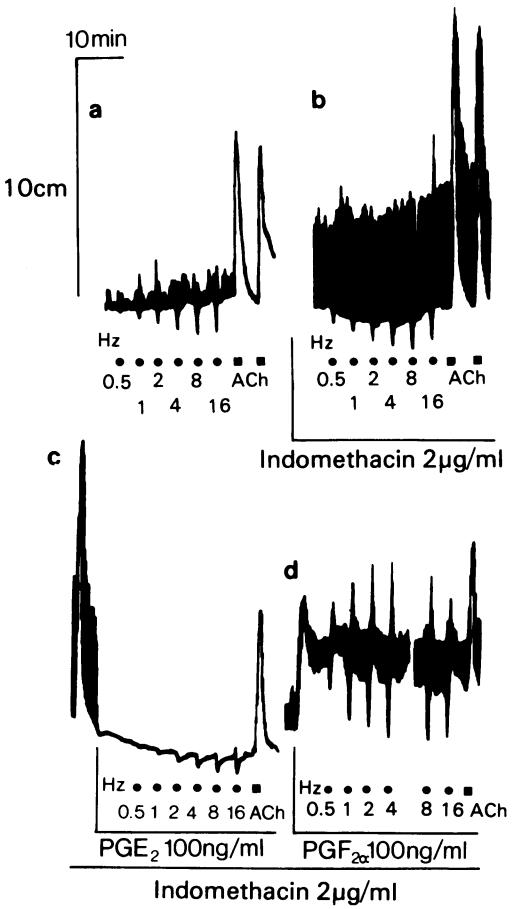

Figure 3 Responses of human ileal circular muscle to electrical stimulation (●, 1 ms pulses, 16 V/cm, 20 s) and acetylcholine (■, ACh, 0.25 μg/ml). (a) Control responses; (b) after 45 min incubation with indomethacin (2 μg/ml) rhythmic-, after- and ACh-induced contractions were enhanced, but small initial contractions were prevented. Tone and relaxations were little affected by indomethacin in this specimen. (c) Continues from (b). With indomethacin still present, prostaglandin E_2 (PGE_2, 100 ng/ml) lowered tone, prevented rhythmic- and after-contractions and reduced those to ACh. Initial contractions were re-introduced at 8 and 16 Hz and relaxations were slightly decreased. (d) Fifteen min after prostaglandin E_2 was washed out, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) raised tone, increased relaxations and reduced contraction to ACh compared to responses in the presence of indomethacin alone (trace b).

prostaglandin E_2 regardless of its effect on tone but small initial contractions were revealed at 4, 8 and 16 Hz by prostaglandin E_2 (50 or 100 ng/ml) in 2 strips in the presence of indomethacin when these relaxed to prostaglandin E_2.

In 5 experiments, prostaglandin $F_{2\alpha}$ (50 and 100 ng/ml) caused dose-related contractions, although in another strip, concentrations up to 500 ng/ml had no effect. During contractions to prostaglandin $F_{2\alpha}$ relaxations elicited at 0.5 to 16 Hz were larger than with indomethacin only (2 μg/ml, Figure 3; $n=15$, $P<0.001$). Rhythmic and after-contractions were variably affected by prostaglandin $F_{2\alpha}$ (50 or 100 ng/ml).

Discussion

The responses of intestinal muscle to prostaglandins vary with the species, the region of the gut and the muscle layer. However, in general prostaglandin E compounds contract longitudinal muscle and relax the circular muscle, whereas F compounds contract both layers (Bennett & Fleshler, 1970). The effects of prostaglandins on the human ileum in the presence of indomethacin were similar to those recorded previously in the absence of drugs (Bennett *et al.*, 1968; Bennett & Fleshler, 1970). We confirm our earlier findings (Stockley, 1974; Bennett & Stockley, 1975) that rhythmic activities and neurogenic responses in human isolated ileum are of many types, perhaps due partly to variations in quantity and type of endogenous prostaglandin-like material (Stamford, 1976; Bennett *et al.*, 1977b); the prostaglandin content of rat and guinea-pig intestine also varies between individuals (Stamford, 1976). Formation of thromboxanes and of prostaglandins other than E_2 and $F_{2\alpha}$ is inhibited by indomethacin, and these substances might also contribute to the tone and responsiveness. Since experimental damage to the tissue can increase endogenous prostaglandin release (and might even change its type), our results do not necessarily demonstrate physiological actions of prostaglandins. However, they may reflect actions of prostaglandins released in disease. The effects of indomethacin seem due substantially, or possibly entirely, to inhibition of prostaglandin synthesis, since addition of prostaglandins usually restored the responses.

Longitudinal muscle

In longitudinal muscle, indomethacin 2–10 μg/ml lowered the tone. This probably explains the reduction of nerve-mediated relaxations (both were restored by 5-HT), the enhanced contractions to ACh (Bennett & Stockley, 1975) and 5-HT, and the bigger initial contractions to stimulation. In guinea-pig ileum, indomethacin does not increase responses to electrical stimulation (Botting & Salzmann, 1974; Bennett *et al.*, 1975b) but the tissue usually has no tone and indomethacin does not cause a relaxation. Indeed indomethacin 10–40 μg/ml decreases contractions, probably non-selectively. The reduction by 10 μg/ml indomethacin of initial contractions to low-frequency stimulation in human ileum could also be non-selective.

The after-contractions appear to involve prostaglandins since they are prevented by a low concentration of indomethacin. Burnstock, Cocks, Paddle & Staszewska-Barczak (1976) obtained similar results in guinea-pig taenia caeci using high drug concentrations.

Circular muscle

The actions of indomethacin on circular muscle contrasted markedly with those on longitudinal muscle. Some types of response altered because of changes in tone, as previously found for contractions to ACh (Stockley & Bennett, 1976). Thus, when indomethacin relaxed the muscle, neurogenic relaxation decreased. However, indomethacin increased the after-contractions regardless of the change in tone, perhaps by removing an inhibitory effect of prostaglandins. The initial contractions that occurred in a few tissues were prevented by indomethacin and therefore might involve prostaglandins.

The effects of prostaglandins on human ileum would seem to depend on the prostaglandin and muscle layer concerned. Prostaglandin $F_{2\alpha}$ released within the muscle or reaching it through the bloodstream would be expected to shorten both muscle layers. However, Cummings, Newman, Milton-Thompson & Billings (1973) found that intravenous prostaglandin $F_{2\alpha}$ in man inhibited intraluminal pressure changes; perhaps it caused a maintained increase in muscle tone but increased the baseline intraluminal pressure only transiently because the intestine is an open tube. Superimposed pressure waves would therefore be smaller. Alternatively, the response might vary with the route of administration or the experimental conditions. From the data on strips of tissue, prostaglandin E_2 would be expected to shorten the longitudinal muscle and increase its responsiveness to other stimuli, but to inhibit the circular muscle and reduce its responsiveness. However, the circular and longitudinal muscle layers interact, and it is not possible to extrapolate from muscle strips to intestinal behaviour. Despite inhibition of circular muscle strips of guinea-pig colon by prostaglandin E_1 or E_2, peristaltic activity in colonic segments was increased by these compounds (Eley, Bennett & Stockley, 1977), and decreased by aspirin (Bennett, Eley & Stockley, 1976).

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