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THE EFFECTS OF PROSTAGLANDINS ON GUINEA-PIG ISOLATED INTESTINE AND THEIR POSSIBLE CONTRIBUTION TO MUSCLE ACTIVITY AND TONE

A. BENNETT, K.G. ELEY & HELEN L. STOCKLEY
Department of Surgery, King's College Hospital Medical School, London SE5 8RX

1 Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ caused contraction of the longitudinal muscle of both guinea-pig isolated ileum and colon, apparently by acting directly on the muscle and on cholinergic nerves. They had little effect on ileal circular muscle.

2 Prostaglandins E_1 and E_2 caused contraction of the longitudinal muscle of guinea-pig isolated colon, apparently by acting directly on the muscle and on excitatory nerves which are non-cholinergic. Prostaglandin E_1 seems more effective than E_2 in stimulating these nerves.

3 It seems likely that prostaglandin release *in vitro* maintains the tone of the longitudinal muscle of guinea-pig colon, whereas release of a prostaglandin E compound inhibits circular muscle tone.

Introduction

Guinea-pig ileum contains prostaglandin E_2- and $F_{2\alpha}$-like substances (Ambache, Brummer, Rose & Whiting, 1966). The tissue releases prostaglandin E_2-like activity on incubation in Krebs solution, and the quantities are increased by electrical field stimulation (Botting & Salzmann, 1974). Both prostaglandin E and F compounds contract longitudinal muscle of guinea-pig isolated ileum (Bergström, Eliasson, Euler & Sjöval, 1959; Weeks, Schultz & Brown, 1968; Bennett, Eley & Scholes, 1968) and colon (Karim, 1966; Bennett & Fleshler, 1969a; Bennett & Posner, 1971). Prostaglandin F compounds contract the circular muscle of guinea-pig colon (Fleshler & Bennett, 1969), but in contrast prostaglandin E compounds inhibit intestinal circular muscle (Bennett *et al.*, 1968; Fleshler & Bennett, 1969). On the basis of this type of evidence many have thought that prostaglandins might play a role in regulating gastrointestinal activity.

It has been suggested that prostaglandin release maintains the tone in rabbit isolated jejunum (Ferreira, Herman & Vane, 1972), and may play a similar role in the longitudinal muscle of guinea-pig isolated ileum (Davison, Ramwell & Willis, 1972; Botting & Saltzmann, 1974). Prostaglandins have also been implicated in contractions mediated by cholinergic nerves in this tissue (Ehrenpreis, Greenberg & Belman, 1973; Kadlec, Mašek & Šeferna, 1974; Bennett, Eley & Stockley, 1975). Thus, the contribution of prostaglandins to intestinal activity might depend on the types and relative amounts released, their site of release, and the intrinsic nerve activity. The aim of the present work was to determine, in those parts of the guinea-pig intestine not previously studied, the effects and mechanism of action of prostaglandins on muscle strips, and to evaluate their contribution to muscle tone by the use of drugs which inhibit prostaglandin synthesis or action.

Methods

The ileum and colon were removed from freshly killed guinea-pigs, and 1-2 cm long segments of mid-ileum, and of colon approximately 10 cm from the anus, were used to study the longitudinal muscle. Spirals were cut for studies of the circular muscle. The preparations were suspended in Krebs solution at 37°C bubbled with 5% CO_2 in O_2 under a load of 0.5-1 g, and changes in length were registered by isotonic transducers and pen recorders. Electrical field stimulation consisting of alternating square wave pulses of 1 ms duration (generated by linked stimulators 6051 and 6053, Scientific and Research Instruments Ltd.) was applied between platinum electrodes near the top and bottom of the organ bath giving a voltage drop of 3.4 V/cm measured in Krebs solution. The formula of the Krebs solution was (g/l): NaCl 7.1; $CaCl_2.6H_2O$ 0.55; KCl 0.35; KH_2PO_4 0.16; $MgSO_4.7H_2O$ 0.29; $NaHCO_3$ 2.1; dextrose 1.0.

Drugs

The following drugs were used: 1-acetyl-2-(8-chloro - 10,11 - dihydrodibenz(b,f)(1,4)oxazepine-10-carbonyl) hydrazine (SC-19220), acetylcholine perchlorate, acetylsalicylic acid, histamine acid phosphate, (−)-hyoscine hydrobromide, indomethacin, nicotine hydrogen tartrate, polyphloretin phosphate (PPP), potassium chloride, prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ tromethamine salt, and tetrodotoxin. All concentrations of salts are expressed as base or free acid. The prostaglandins were dissolved in ethanol (0.1 ml per mg) and diluted with 0.9 ml sodium carbonate solution 0.2 mg/ml. Indomethacin 1 mg or aspirin 10 mg was dissolved in 1 ml 0.9% w/v NaCl by adding solid sodium carbonate to pH7.

Results

Effects and mechanism of action of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ on the ileum

In 14 longitudinal muscle preparations from 12 animals prostaglandin $F_{1\alpha}$ (200-300 ng/ml) and $F_{2\alpha}$ (20-225 ng/ml) caused contractions. Tetrodotoxin (TTX; 0.1 or 0.2 μg/ml) prevented contractions to nicotine and reduced those to prostaglandins in both of the experiments with prostaglandin $F_{1\alpha}$ (average 16% reduction), and in 10 of 11 experiments with $F_{2\alpha}$ (overall reduction 32 ± 13% (s.e.), $P < 0.01$). Prostaglandin E_2, which has been studied previously, was used for comparison; TTX reduced its effect by 52 ± 6%, $n = 5$, $P < 0.01$. The muscle tone and contractions to acetylcholine (ACh) were not significantly altered by TTX (ACh reduced by 5 ± 10%, $n = 11$). Hyoscine (0.1 or 0.2 μg/ml), given before TTX, or after it had been washed out and contractions to nicotine had returned, prevented responses to ACh and nicotine, and consistently reduced those to prostaglandin $F_{1\alpha}$ (67 ± 12%, $n = 4$, $P < 0.05$), $F_{2\alpha}$ (60 ± 6%, $n = 9$, $P < 0.005$), and E_2 (52 ± 7, $n = 5$, $P < 0.02$) (Figure 1). The addition of TTX in the presence of hyoscine (4 experiments) and *vice-versa* (2 experiments) usually had little further effect on the responses to prostaglandin $F_{2\alpha}$.

Prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$ had little or no effect on circular muscle of the guinea-pig ileum. In one experiment prostaglandin $F_{2\alpha}$ 1.1 μg/ml caused a small contraction, but $F_{2\alpha}$ 0.3-1.1 μg/ml or $F_{1\alpha}$ 0.4-4 μg/ml had no effect in two others. In another strip prostaglandin $F_{1\alpha}$ or $F_{2\alpha}$ (both 0.5 μg/ml) caused small increases in the contraction to KCl 2 mg/ml. Neither prostaglandin $F_{2\alpha}$ (0.1-1 μg/ml, three experiments) nor $F_{1\alpha}$ (0.1-1 μg/ml, one experiment), in contact with the tissue for 5-15 min, altered submaximal contractions elicited by electrical stimulation at 1-4 Hz. Because of this lack of activity the prostaglandin F compounds were not examined further in circular ileal muscle.

Effects and mechanisms of action of prostaglandins $F_{2\alpha}$ and E_2 in colonic longitudinal muscle

In 26 experiments on tissue from 24 animals prostaglandin $F_{2\alpha}$ (75-300 ng/ml) or E_2 (20-40 ng/ml) in contact with the tissue for 2-2.5 min caused a slow contraction which was occasionally preceded by a brief relaxation. After washing prostaglandin from the bath the muscle relaxed slowly, taking 10-15 min to reach the baseline.

Hyoscine (0.1 or 0.2 μg/ml) prevented contractions to ACh and sometimes lowered the muscle tone. Nicotine-induced contractions were variably affected (prevented, reduced, no effect or increased in two, one, one and one experiments respectively, overall reduction 40 ± 26%), and nicotine-induced relaxations were enhanced in two experiments and revealed in one. Contractions to prostaglandin $F_{2\alpha}$ were mainly reduced (35 ± 16%, $n = 10$, $P < 0.05$), but responses were increased in two of these tissues whose tone was lowered by hyoscine (perhaps the increase due to lowering of tone overshadowed the effect of hyoscine). Although there was a tendency for hyoscine to reduce contractions to prostaglandin E_2, the overall effect was not statistically significant (overall reduction 19 ± 10%, $P > 0.1$). There was a reduction in 15 out of 18 experiments and an increase in three tissues; in two of these three instances the tone was reduced by hyoscine. However, if the latter two experiments are excluded because of the fall in tone, there was a significant reduction of the effect of prostaglandin E_2 by hyoscine (31 ± 7%, $n = 16$, $P < 0.01$).

TTX (0.1 to 0.4 μg/ml) sometimes reduced the tone of colonic longitudinal muscle. It always greatly reduced or prevented the response (contraction, relaxation or relaxation followed by contraction) to nicotine, but had no significant effect on contractions to ACh (reduction of 5 ± 5%, $n = 14$). It reduced contractions to prostaglandin $F_{2\alpha}$ in nine out of ten experiments (overall reduction of 39 ± 10%, $P < 0.01$) and to E_2 in 11 out of 14 experiments (overall reduction of 24 ± 9%, $P < 0.01$). In two experiments contractions to prostaglandin E_2 were unaffected, and in one experiment in which TTX greatly lowered the muscle tone contractions to both prostaglandin $F_{2\alpha}$ and E_2 were increased.

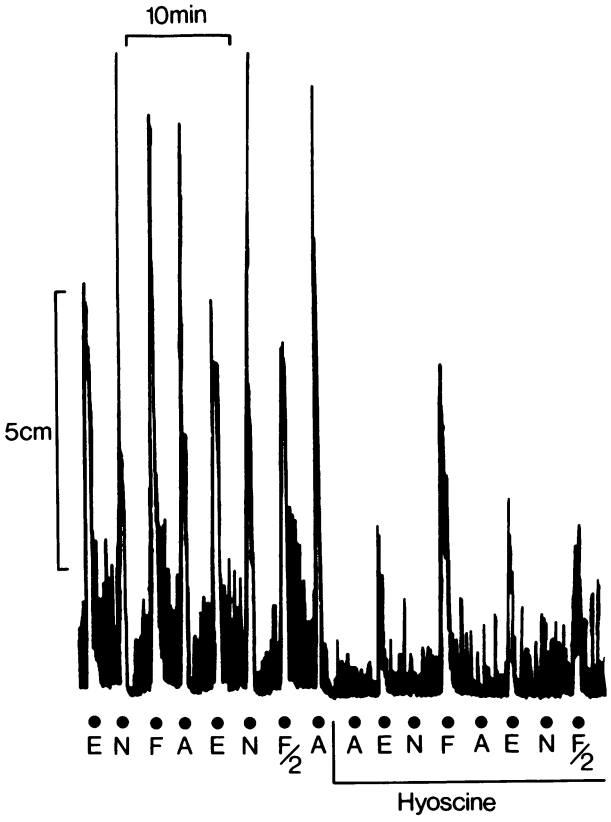

Figure 1 Hyoscine (0.1 μg/ml) prevented contractions of the longitudinal muscle of guinea-pig isolated ileum to acetylcholine (A, 20 ng/ml) and nicotine (N, 3 μg/ml), and reduced responses to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (F, 0.1 μg/ml) and E_2 (E, 10 ng/ml).

Comparison of prostaglandins E_1 and E_2 in colonic longitudinal muscle

Since the reduction with TTX of contractions to prostaglandin E_2 was less than the average of 60% reported for E_1 in this tissue (Bennett & Fleshler, 1969a), E_1 and E_2 were compared in 11 further experiments. The reduction of prostaglandin E_1 by TTX (0.1-0.4 μg/ml) alone (8 experiments) or in the presence of hyoscine (0.1-0.2 μg/ml, 3 experiments) was greater than of E_2 (54 ± 7% and 26 ± 6% respectively, $P < 0.0025$; Figure 2). There was no difference between the effect of hyoscine on prostaglandin E_1 and E_2 ($P > 0.7$) or the reduction of E_2 by TTX or hyoscine ($P > 0.5$), but the reduction of E_1 by TTX was greater than by hyoscine ($P < 0.05$).

The possible relation of prostaglandins to muscle tone

Ileal circular muscle The inhibitory effects of prostaglandins E_1 and E_2 demonstrated previously on this tissue (Bennett *et al.*, 1968) have been substantiated in further experiments which show that E_2 (0.1-1 μg/ml) inhibited electrically induced contractions of circular muscle. Aspirin (10-40 μg/ml) or indomethacin (1 μg/ml; Figure 3) (four experiments each) caused a rise (usually small) in tone, with initiation or enhancement of spontaneous contractions. Furthermore, they potentiated contractions produced by electrical field stimulation at 0.1-8 Hz. The degree of potentiation varied with the stimulating frequency, being most pronounced at low frequencies even though all responses remained submaximal. At 0.1 Hz contractions were initiated or enhanced, but this was difficult to measure because of increased spontaneous activity. There was a significant increase of 301 ± 110% at 1 or 2 Hz ($n = 14$; $P < 0.0005$), but the increase at 4 and 8 Hz (20 ± 10%) was not significant ($n = 8$; $0.05 < P < 0.1$) and in one strip the contraction at 8 Hz was reduced by 27% (possibly due to the particularly marked rise in tone caused by indo-

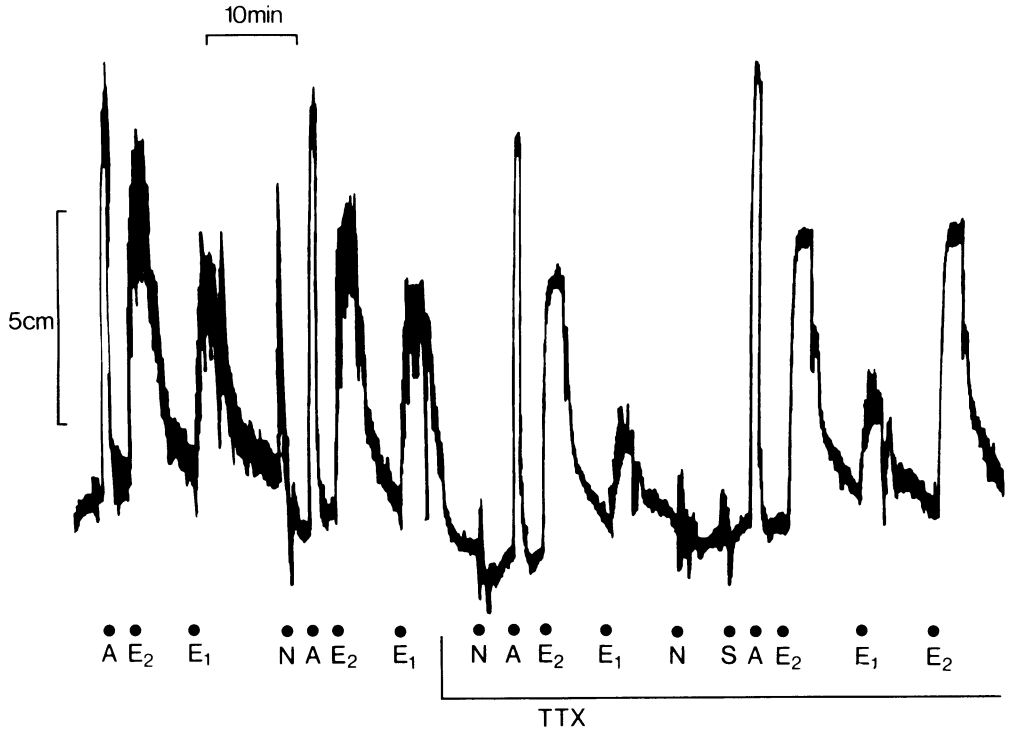

Figure 2 Longitudinal muscle of guinea-pig isolated colon. Tetrodotoxin (TTX, 0.4 μg/ml) prevented contractions to nicotine (N, 5 μg/ml), greatly reduced those to prostaglandin E_1 (E_1, 80 ng/ml), and slightly reduced those to E_2 (E_2, 10 ng/ml). Responses to acetylcholine (A, 20 ng/ml) were unaffected. S, saline 0.4 ml.

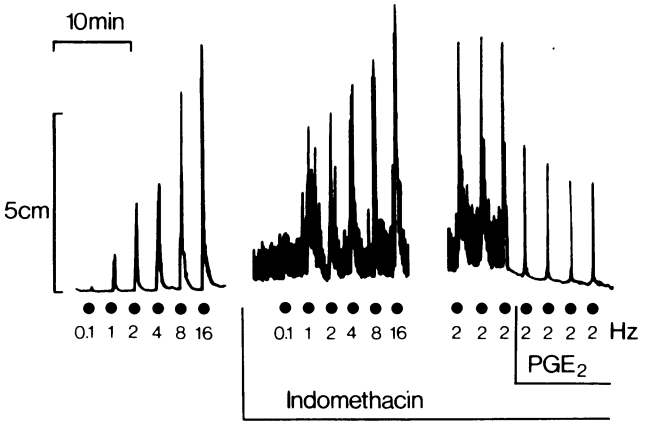

Figure 3 Guinea-pig ileal circular muscle responded to electrical excitation (1 ms pulses, 20 s trains) with contractions which were graded with the stimulating frequency. Indomethacin (1 μg/ml) initiated rhythmical activity, slightly raised the tone, and enhanced the electrically-induced contractions. Prostaglandin E_2 (PGE_2, 0.1 μg/ml) reversed all of these effects. The intervals in the trace were 60 min (incubation with indomethacin) and 75 min (before recording the effect of prostaglandin E_2).

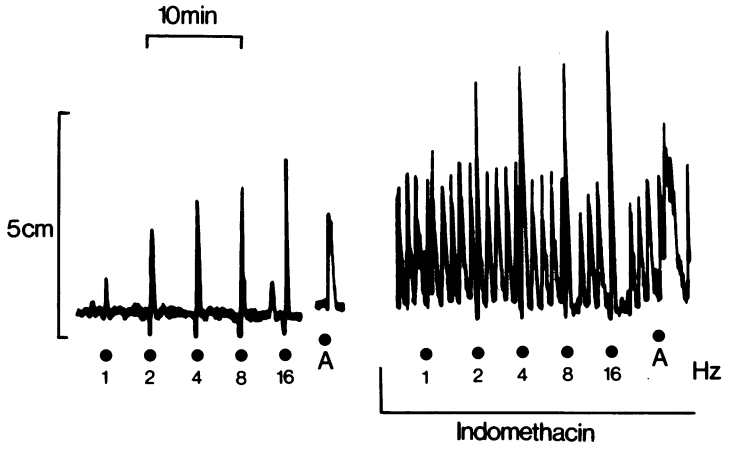

Figure 4 Electrically-induced and spontaneous contractions of the circular muscle of guinea-pig isolated colon were increased by indomethacin (1 μg/ml). The responses to acetylcholine (A, 5 μg/ml) were also increased in this and in two out of three other experiments with indomethacin, but they were unaffected by aspirin (two studies) despite larger responses to electrical stimulation.

methacin). The effects of aspirin and indomethacin were reversed by prostaglandin E_2 (0.1-1 μg/ml) as illustrated in Figure 3. Polyphloretin phosphate (PPP, 1-50 μg/ml; two experiments) or SC-19220 (10 μg/ml; two experiments) did not antagonize the inhibition of electrically stimulated contractions by prostaglandin E_2, nor did they mimic the stimulant effects of aspirin and indomethacin on muscle tone. However, these substances do not block the inhibitory effect of prostaglandin E compounds on the circular muscle (Bennett & Posner, 1971).

Colonic circular muscle Aspirin (50-100 μg/ml, five experiments) raised the tone and increased or initiated spontaneous activity in this tissue, but the effects were generally less pronounced than in the ileum. Indomethacin (1-4 μg/ml) raised the tone and increased rhythmic activity in two preparations and increased the tone in one, but failed to alter spontaneous activity in two others. Electrical stimulation at 0.5-32 Hz caused relaxation followed by an after-contraction. Both prostaglandin synthetase inhibitors regularly enhanced the after-contraction to an extent which depended on the stimulating frequency (Figure 4); responses to 1 and 16 Hz were increased respectively by 171 ± 29% and 40 ± 31% over control by aspirin, and by 136 ± 40% and 30 ± 14% by indomethacin (five experiments each). Hyoscine (5 μg/ml, six experiments) reduced the after-contractions by 25 ± 5%, and after-contractions elicited in the presence of hyoscine (5 μg/ml) were unaffected by aspirin or indomethacin (two experiments each).

Colonic longitudinal muscle Aspirin (20 or 100 μg/ml, four experiments) or indomethacin (1 or 2 μg/ml, four experiments) lowered the tone of the preparations, taking 30-90 min to reach a steady level. The gradual relaxation was accompanied by an increase in amplitude of the spontaneous activity and enhancement of submaximal contractions to ACh (10-100 ng/ml), prostaglandin E_2 (5-20 ng/ml), and contractile components of responses to electrical excitation at 0.1-8 Hz (Figure 5). The effect on contractions is expressed quantitatively in Table 1. The potentiation of contractions by indomethacin was less for ACh than for electrical stimulation ($P < 0.01$) but this effect did not reach statistical significance with aspirin ($0.05 < P < 0.1$). After the addition of prostaglandin E_2 (2 or 4 ng/ml) the tone and responses to electrical stimulation were not significantly different from the controls before addition of synthetase inhibitor. However, responses to ACh were significantly smaller (by 30 ± 9%, $P < 0.05$).

Discussion

Effects of prostaglandins on longitudinal and circular muscle preparations

The responses conform in general to the pattern of contraction of both muscle layers with prostaglandin F compounds, but longitudinal muscle contraction and circular muscle inhibition with the prostaglandin E compounds (Bennett & Fleshler, 1970). In the longitudinal muscle of guinea-pig

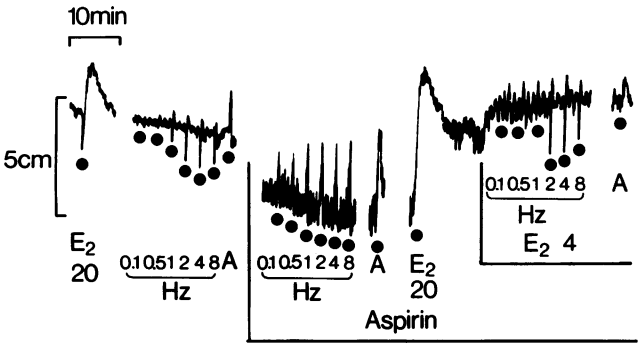

Figure 5 Guinea-pig colonic longitudinal muscle gave biphasic responses (relaxation then contraction) to prostaglandin E_2 (E_2, 20 ng/ml) and electrical stimulation (1 ms pulses; 10 s trains), and contracted to acetylcholine (A, 10 ng/ml). In the presence of aspirin (100 μg/ml for 1 h) the tone fell and spontaneous and acetylcholine-induced contractions increased. The responses to electrical stimulation and prostaglandin E_2 were converted to contractions which were bigger than the previous contractile component of the biphasic responses. After washing out prostaglandin E_2 (20 ng/ml) in the continued presence of aspirin, the tone remained elevated at almost the control level. Subsequent addition of a lower concentration of prostaglandin E_2 (E_2, 4 ng/ml) restored responses to electrical excitation and acetylcholine to approximately their control values.

ileum prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ behaved much like E_1 and E_2 in previous studies (Bennett *et al.*, 1968; Harry, 1968) in that the responses were reduced by TTX and hyoscine. It therefore seems that the prostaglandin E and F compounds have two sites of action, the cholinergic nerves and the muscle cells.

The similar pA_2 values for prostaglandins E_2 and $F_{2\alpha}$ with the antagonist SC-19220 (Bennett & Posner, 1971) are consistent with the possibility that both act at the same receptor sites. Although the reduction of contractions to prostaglandin $F_{2\alpha}$ by hyoscine seemed greater than that by TTX, the difference was not significant ($0.05 < P < 0.1$).

In the circular muscle strips of guinea-pig ileum prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ had little effect on their own or on responses to electrical stimulation, suggesting that prostaglandins of the F-series are unlikely to play a role in regulating the motility of this muscle layer.

In the longitudinal muscle of guinea-pig colon prostaglandins $F_{2\alpha}$ and E_2 caused slow contractions, occasionally preceded by a brief relaxation. With both prostaglandins a neural contribution was indicated since TTX reduced the responses. The reduction of contractions to prostaglandin $F_{2\alpha}$ by hyoscine suggests that cholinergic nerves may be involved, but the picture with prostaglandin E_2 is less clear. A significant reduction by hyoscine was seen only if two experiments were excluded from the calculations. In these two instances the responses to prostaglandin E_2 actually increased after hyoscine, but this might have been due to the lowering of muscle tone. In previous experiments (Bennett & Fleshler, 1969a) evidence was obtained that prostaglandin E_1 stimulated only non-cholinergic excitatory nerves. It seems that prostaglandin E_1 is more effective than E_2 in stimulating these nerves, since we have shown that TTX, but not hyoscine,

Table 1 Guinea-pig longitudinal distal colonic muscle. Enhancement of contractions (% increase over control ± s.e.) to electrical stimulation, acetylcholine (ACh) or prostaglandin E_2 (PGE_2) by aspirin (20 or 100 μg/ml) or indomethacin (1 or 2 μg/ml), and reversal of the effect by prostaglandin E_2 (2 or 4 ng/ml).

	Aspirin	*Indomethacin*	*PGE_2 plus aspirin or indomethacin*
1,2 and 4 Hz	194 ± 44 (12) **	120 ± 18 (12) **	17 ± 10 (18) NS
ACh 10-100 ng/ml	80 ± 33 (4) NS	46 ± 16 (4) *	−30 ± 9 (6) *
PGE_2 5-20 ng/ml	140 ± 73 (4) *	97 ± 18 (4) **	

The numbers in brackets represent the numbers of experiments. (NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$).

caused a significantly greater reduction of the effect of E_1 than of E_2. The presence of non-cholinergic excitatory nerves was confirmed by our experiments with nicotine. As reported previously with nicotine and dimethylphenylpiperazinium (Bennett & Fleshler, 1969b, 1970; Bennett, 1971), TTX virtually prevented the contractions to nicotine, whereas hyoscine had a variable effect on the responses and complete blockade of contractions occurred in only one experiment. The only difference in the present study was that responses to nicotine sometimes consisted of relaxations or biphasic changes and not just contractions. The reason for the difference is not known.

Prostaglandins and intestinal muscle tone

The results with the prostaglandin synthesis inhibitors suggest that an E-type prostaglandin might be released continuously to reduce or prevent the generation of tone and spontaneous activity in the circular muscle of the ileum and colon. Stimulation by these substances of spontaneous and electrically evoked contractions was more pronounced in the ileum than in the colon. In both regions prostaglandin E compounds are potent inhibitors of circular muscle activity, but only colonic circular muscle is sensitive to the excitatory effect of F compounds. Thus, if both types of prostaglandins are normally generated simultaneously in the colon and exert mutually antagonistic effects on the circular muscle, inhibition of their synthesis might have little overall effect. The prostaglandin antagonists PPP and SC-19220 did not mimic the response to the synthesis inhibitors in the circular muscle of the ileum, presumably because they do not antagonize the inhibitory effect of prostaglandins E_1 and E_2 on alimentary circular muscle (Bennett & Posner, 1971).

Potentiation by aspirin or indomethacin of electrically induced after-contractions in the colonic circular muscle was prevented by hyoscine, suggesting the involvement of a cholinergic mechanism. Prostaglandin F compounds do not seem to stimulate the cholinergic nerves in this tissue (Fleshler & Bennett, 1969), but removal of inhibition by an E-type prostaglandin might be responsible. Perhaps prostaglandin inhibits the release of ACh from the cholinergic nerves, as has been suggested in the rabbit heart (Wennmalm & Hedqvist, 1971). Indomethacin would then increase the activity of the cholinergic system. A similar mechanism might occur with cholinergic nerve stimulation in ileal circular muscle.

On the other hand, Botting & Salzmann (1974) concluded that in the longitudinal muscle of guinea-pig ileum prostaglandin was unlikely to inhibit ACh release, because ACh output was the same or reduced by indomethacin. The relatively high concentrations of indomethacin (10 and 20 μg/ml) used by Botting & Salzmann (1974) might have depressed ACh release non-selectively, thus masking a potentiation due to removal of prostaglandin. However, indomethacin reduced responses to electrical stimulation of this tissue in other studies, and added prostaglandin E_2 potentiated responses to cholinergic nerve stimulation (Ehrenpreis *et al.*, 1973; Kadlec *et al.*, 1974; Bennett *et al.*, 1975).

There is circumstantial evidence that prostaglandins contribute to the tone of the longitudinal muscle of guinea-pig colon. Aspirin or indomethacin lowered the tone and this was restored by low concentrations of prostaglandin E_2. A non-selective depressant action of the prostaglandin synthetase inhibitors seemed unlikely, since responses to ACh and electrical stimulation actually increased. However, the increases, which were probably due mainly to the lower tone, might have overshadowed a non-selective depression. When the tone was restored by prostaglandin E_2 in the presence of indomethacin, responses to ACh were smaller than normal. Perhaps indomethacin depresses the responses to ACh but increases the effect of electrical stimulation, so that there is no net change in the response to cholinergic nerve activation.

Taken together with the data of Davison *et al.* (1972) and Botting & Salzmann (1974), our results suggest that prostaglandins contribute to the tone of the longitudinal muscle of guinea-pig isolated ileum and colon. In contrast, prostaglandin E_2 might tend to keep intestinal circular muscle in a relaxed state. It remains to be determined whether prostaglandins exert these effects only when released in 'dying' isolated preparations, or whether they play a physiological role in controlling gut motility.

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