Br. J. Pharmac. (1974), 51, 565-570

A MODULATING ROLE OF PROSTAGLANDINS IN CONTRACTIONS OF THE GUINEA-PIG ILEUM

O. KADLEC, K. MAŠEK & I. ŠEFERNA

Institute of Pharmacology, Czechoslovak Academy of Sciences and Department of Pharmacology, Faculty of Paediatrics, Charles University, Albertov 4, 12 00, Prague 2, Czechoslovakia

1 The role of prostaglandins in contractions of the guinea-pig ileum evoked either directly by acetylcholine or indirectly by angiotensin and by coaxial stimulation has been investigated.

2 Prostaglandin E_2 in low concentration (6 nM) slightly augmented both types of contraction. Indomethacin, an inhibitor of prostaglandin synthesis, markedly reduced the indirectly evoked contractions but did not affect contractions in response to acetylcholine. The addition of prostaglandin E_2 to the preparation treated previously with indomethacin restored the effect of indirect stimulation.

3 The pretreatment of the preparation with guanethidine or α-methyl-*p*-tyrosine prevented the inhibitory effect of indomethacin on indirectly evoked contractions. Prostaglandin E_2 addition to such preparations considerably augmented both types of contraction.

4 The stimulation of non-cholinergic, non-adrenergic inhibitory nerves in the taenia coli and ileum preparations evoked hyperpolarization and relaxation of the preparations followed by action potentials and contraction. These responses were not changed by indomethacin pretreatment and prostaglandin E_2, but rebound contraction was sometimes augmented by the prostaglandin.

5 Two mechanisms for the effects of prostaglandin E_2 are suggested: a direct effect on smooth muscle, and an indirect action through the sympathetic nerves which by release of noradrenaline affect the acetylcholine release from parasympathetic nerve endings.

Introduction

Several studies have demonstrated that prostaglandins of the E series evoke contraction of the longitudinal smooth muscle of the intestine. Moreover, these compounds in small doses may potentiate the contractions elicited by other stimulants (Clegg, 1966; Bennett, Eley & Scholes, 1968; Harry, 1968; Eckenfels & Vane, 1972). A blockade of prostaglandin synthesis by indomethacin has been shown to decrease the tone and motility of the intestine (Vane, 1971; Ferreira, Herman & Vane, 1972).

The present paper was prompted by a recent communication of Chong & Downing (1973) who reported that angiotensin-induced contraction of the guinea-pig ileum was selectively inhibited by indomethacin whereas the contraction evoked by acetylcholine was not affected. Since the stimulatory action of angiotensin is at least partly mediated by cholinergic nerves, in contrast to the direct effect of acetylcholine, we decided to investigate the role which prostaglandins may play in these contractions of the guinea-pig ileum. Three types of stimulation have been used in these experiments: electrical stimulation and stimulation induced by acetylcholine or angiotensin administration. The results of our experiments seem to indicate that prostaglandin E_2 modulates the indirectly evoked contractions through its effect on the sympathetic nerves in addition to its direct potentiation of smooth muscle contractions.

Methods

Experimental procedures

Male and female guinea-pigs weighing 400-800 g were used in these experiments. The animals were killed by a blow on the head and bled from the carotid arteries. A piece of the middle part of the ileum, 4 cm long, was dissected and placed in a 20 ml bath containing Krebs solution (37° C). The ileum was stimulated electrically by means of two platinum electrodes, one placed in the lumen of the preparation and the other in the bath (coaxial stimulation; Paton, 1955). Supramaximal rectangular pulses at a frequency of 0.1 Hz were of 0.4 ms duration. Two doses of angiotensin

(1-30 nM) and of acetylcholine (0.6-60 nM), left in contact with the preparation for 60 or 30 s respectively were used to evoke submaximal contractions that were about twice and four times higher than those evoked by coaxial stimulation. To standardize the responses of the guinea-pig ileum to angiotensin, the drugs were added at 10 min intervals. Prostaglandin E_2, indomethacin, atropine and guanethidine were always added to the preparations 20 and 30 min before the actual measurement. α-Methyl-*p*-tyrosine (α-MPT) was administered intraperitoneally 24 and 16 h before the animals were killed, in a dose of 400 mg/kg each time (Robinson & Gershon, 1971). The contractions were recorded on a smoked drum by an isotonic lever. The responses of the preparation after various experimental procedures are expressed as percentages of the respective control responses obtained at the beginning of the experiment. For statistical analysis the results obtained with the two doses of each stimulant were pooled.

In some experiments a sucrose-gap method (Burnstock & Straub, 1958) was used. Taenia coli in this case was stimulated transmurally by means of two platinum electrodes that were 4 mm apart; and the nearer one, the cathode, was 6 mm from the Krebs-sucrose interface. The details of the method are described elsewhere (Kadlec & Šeferna, 1972).

Solution and drugs

The Krebs solution had the following composition (mM): NaCl, 120; KCl, 5.9; $CaCl_2$, 2.5; $NaHCO_3$, 15.4; $MgCl_2$, 1.2; and glucose, 11.5; it was bubbled with air.

The drugs used and their sources were as follows: angiotensin (Hypertensin) and guanethidine sulphate (Ismelin), CIBA; tetrodotoxin, Sankyo; α-methyl-*p*-tyrosine, Merck; acetylcholine, Germed; disodium salt of ATP, Reanal; prostaglandin E_2, Upjohn; and indomethacin, atropine sulphate, and noradrenaline hydrotartrate, Spofa.

Results

Excitatory response of the ileum

The contractions of the guinea-pig ileum induced either by coaxial stimulation or by the addition of angiotensin (1-30 nM) were completely blocked by previous administration of tetrodotoxin (0.6 μM) or atropine (0.4 μM). To evoke a contraction by direct stimulation of smooth muscle an increase in the concentration of angiotensin by one to three orders was required. A typical experiment is presented in Figure 1.

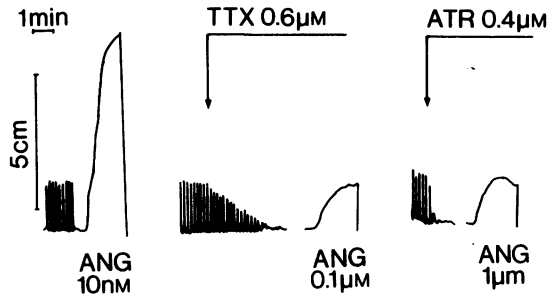

Fig. 1 Guinea-pig isolated ileum. Effect of tetrodotoxin (TTX) and atropine (ATR) on mechanical responses to electrical postganglionic nerve stimulation at a frequency of 0.1 Hz and to angiotensin (ANG). Vertical scale: 5 cm pen movement (magnification 5:1).

The addition of prostaglandin E_2 (6 nM) to the bathing fluid resulted in a slight, but significant increase in the contractions evoked by all three types of stimulation (Table 1a). A small increase in tone was observed in some experiments. Ten times higher concentrations of prostaglandin E_2 (60 nM) always increased the tone of the preparation, the contractions were decreased in amplitude and their relaxation was slowed down. Still higher concentrations of prostaglandin E_2 (0.6 μM) evoked a slowly developing contraction, the full relaxation from which took 10 min or more.

Pretreatment of the preparations with indomethacin (1 μM) did not significantly change the responses to acetylcholine, but responses to coaxial stimulation and angiotensin were significantly decreased. In some experiments the contractions evoked by drugs were not well maintained in the presence of stimulants and oscillations at the height of contraction appeared. The addition of prostaglandin E_2 (6 nM) to indomethacin-treated preparations overcame the inhibition caused by indomethacin and the responses to coaxial stimulation were even augmented (Table 1b). A typical experiment is presented in Figure 2.

Pretreatment of the ileum with guanethidine (3 μM) enhanced the contractions evoked by coaxial stimulation whereas contractions elicited by drugs were unchanged. The addition of indomethacin did not decrease the enhanced response to coaxial stimulation (Table 1c).

The addition of indomethacin also failed to decrease any contraction of preparations taken from guinea-pigs treated previously with α-MPT. The addition of prostaglandin E_2 in this case,

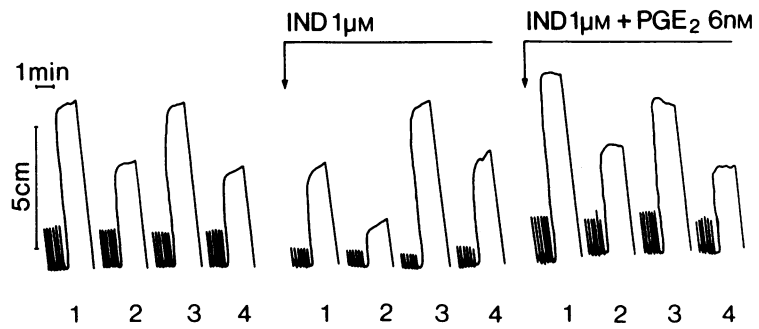

Fig. 2 Guinea-pig isolated ileum. Effect of indomethacin (IND) and prostaglandin E_2 (PGE_2) on mechanical responses to electrical postganglionic nerve stimulation to angiotensin 3 nM, (1); 1 nM, (2); and to acetylcholine, 6 nM, (3); 0.6 nM, (4). Vertical scale: 5 cm pen movement (magnification 5:1).

however, augmented all types of contractions (Table 1d). A typical experiment is shown in Figure 3.

Inhibitory response of the ileum

In seven experiments cholinergic and adrenergic blockade by atropine (0.4 μM) and guanethidine (3 μM) or by α-MPT pretreatment was carried out and responses were measured to supramaximal electrical stimulation with pulses of 1 ms duration (0.1 Hz continuously; 5 Hz for 30 s; 30 Hz for 5 s) and to addition of ATP (5 μM). The results suggest that indomethacin did not change the amplitude of relaxation during electrical stimulation or in the presence of ATP nor did it decrease the amplitude

Table 1 Relative changes in contractions of guinea-pig isolated ileum treated with prostaglandin E_2 (PGE_2), indomethacin and guanethidine in normal (a, b, c) and α-methyl-*p*-tyrosine (α-MPT) pretreated preparations (d)

Treatment	*Stimulation*	n	*Mean with s.e. mean*
(a) PGE_2 (6 nM)	Coaxial	53	126.1** ± 7.2%
	Angiotensin	27	121.9** ± 6.3%
	Acetylcholine	26	119.7** ± 6.0%
(b) Indomethacin (1 μM)	Coaxial	32	76.8** ± 6.5%
	Angiotensin	20	67.6** ± 3.5%
	Acetylcholine	12	103.7 ± 2.5%
Indomethacin (1 μM) + PGE_2 (6 nM)	Coaxial	32	114.1* ± 4.8%
	Angiotensin	20	103.4 ± 2.5%
	Acetylcholine	12	98.9 ± 4.9%
(c) Guanethidine (3 μM)	Coaxial	28	128.2** ± 4.7%
	Angiotensin	14	100.2 ± 5.8%
	Acetylcholine	14	100.9 ± 1.8%
Guanethidine (3 μM) + indomethacin (1 μM)	Coaxial	26	144.1** ± 9.4%
	Angiotensin	13	114.5* ± 6.4%
	Acetylcholine	13	106.4 ± 4.2%
(d) α-MPT pretreated Indomethacin (1 μM)	Coaxial	40	102.7 ± 2.5%
	Angiotensin	20	98.3 ± 4.8%
	Acetylcholine	20	101.4 ± 1.3%
PGE_2 (6 nM)	Coaxial	37	175.1** ± 4.9%
	Angiotensin	18	153.4** ± 13.5%
	Acetylcholine	19	158.8** ± 11.3%

The contractions were evoked by supramaximal coaxial stimulation, angiotensin (1-30 nM) or acetylcholine (0.6-60 nM) and were compared with the responses before the drug treatment (100%). Values significantly different from controls by $P < 0.05$ and $P < 0.005$ are denoted by one or two asterisks respectively; *n*, number of individual measurements in each group of at least 5 experiments.

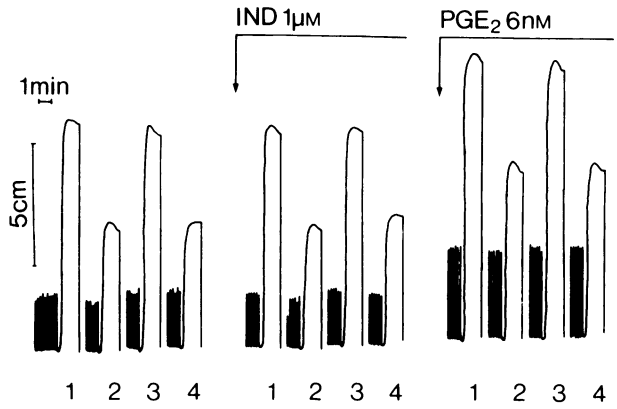

Fig. 3 Guinea-pig isolated ileum pretreated with α-methyl-*p*-tyrosine (400 mg/kg i.p, 24 and 16 h before killing). Effect of indomethacin (IND) and prostaglandin E_2 (PGE_2) on mechanical responses to electrical postganglionic nerve stimulation at a frequency of 0.1 Hz, to angiotensin, 10 nM (1); 3 nM (2); and to acetylcholine, 60 nM (3); 6 nM (4). Vertical scale: 5 cm pen movement (magnification 5:1).

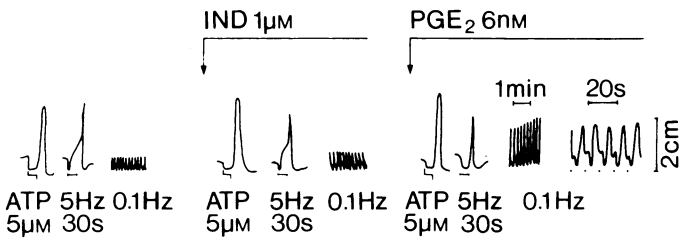

Fig. 4 Guinea-pig isolated ileum pretreated with atropine (1 μM) and guanethidine (3 μM). Effect of indomethacin (IND) and prostaglandin E_2 (PGE_2) on mechanical responses to adenosine triphosphate (ATP, 5 μM) and electrical postganglionic nerve stimulation at a frequency of 5 Hz (for 30 s) or 0.1 Hz. Relaxation was always the first component of the response to any of these types of stimulation. Time: 1 min (left section); 20 s (right section). Vertical scale: 2 cm pen movement (magnification 5:1).

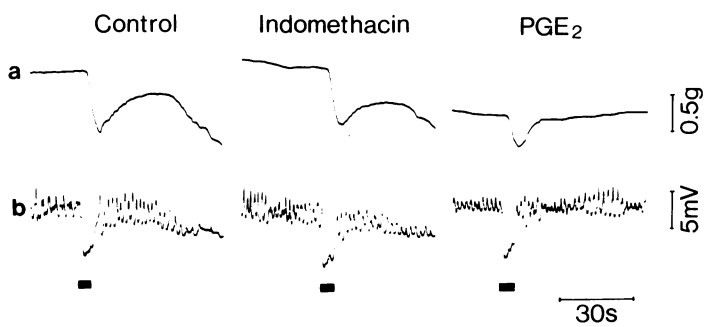

Fig. 5 Inhibitory junction potentials recorded in taenia coli of the guinea-pig in response to transmural nerve stimulation. Effect of indomethacin (1 μM) and prostaglandin E_2 (PGE_2, 60nM) on (a) mechanical response and on (b) changes in membrane potential. Electric stimulation at a frequency of 30 Hz for 5 s is indicated by a solid bar below the tracing.

of the rebound contraction. In four experiments the addition of prostaglandin E_2 (6 nM) increased the height of the rebound contraction (Figure 4).

Inhibitory response of the taenia coli

Similar experiments were also performed on the taenia coli preparation and both mechanical and electrical parameters of smooth muscle activity were recorded. The electrical stimulation of the taenia with 1 ms pulses at a frequency of 30 Hz for 5 s resulted in a hyperpolarization, cessation of spikes and a relaxation followed by rebound potentials and contraction. This type of response was not suppressed by atropine (1 μM) and guanethidine (3 μM) or α-MPT pretreatment, but it was inhibited by tetrodotoxin (1 μM). Indomethacin (1 μM) and prostaglandin E_2 (30 nM) did not change this non-cholinergic, non-adrenergic inhibitory response to electrical stimulation (Figure 5).

Discussion

The finding that contractions evoked indirectly either by coaxial stimulation or by angiotensin were completely abolished by tetrodotoxin or by atropine support the suggestion of Robertson & Rubin (1962) that the stimulant effect of angiotensin is at least partly mediated by cholinergic nerves. Therefore, we suspect that the effect of indomethacin is through the cholinergic nerve supply rather than a selective anti-angiotensin effect (Chong & Downing, 1973).

Small concentrations of prostaglandin E_2 potentiated both the contractions evoked directly by stimulation of smooth muscle and indirectly via the parasympathetic nerve supply in control as well as in α-MPT pretreated guinea-pigs. Thus, the ability of prostaglandin E_2 to potentiate all types of contractions is in good agreement with the view that the action of prostaglandin involves a direct smooth muscle component (Clegg, Hall & Pickles, 1966; Harry, 1968; Bennett *et al.*, 1968; Eckenfels & Vane, 1972; Eagling, Lovell & Pickles, 1972). In addition Bennett *et al.* (1968) described an indirect component in the potentiation of guinea-pig ileum contractions by prostaglandins E_1 and E_2. This component was ascribed to the stimulation of cholinergic nerves by prostaglandins. The pretreatment of the guinea-pig ileum with indomethacin significantly reduced only the indirectly evoked contractions and this was reversed by the addition of small doses of prostaglandin E_2. Since indomethacin is known to inhibit the synthesis of prostaglandins in tissues (Vane, 1971) the results suggest that the normal concentration of prostaglandin is important for these contractions. A direct inhibition of smooth muscle contraction by indomethacin does not seem to be probable since a small dose of indomethacin was used (Northover, 1971) and moreover the effect of actylcholine was not affected.

The possibility of the involvement of non-cholinergic, non-adrenergic inhibitory nerves of the intestine (Burnstock, 1972) in the action of indomethacin on indirectly evoked contractions was also investigated. The inhibitory responses to the stimulation of these nerves in guinea-pig ileum and taenia coli were not markedly changed either by indomethacin or prostaglandins. Only the rebound excitation (Burnstock, Satchell & Smyth, 1972) in the ileum preparation was sometimes augmented by prostaglandin E_2.

Blockade of the sympathetic nervous system by guanethidine (Burnstock *et al.*, 1972) or depletion of noradrenaline stores by administration of α-MPT (Robinson & Gershon, 1971) prevented the effect of indomethacin on indirectly evoked contractions. From the results mentioned above it might thus be inferred that the action of indomethacin (1) is mediated through the effect on prostaglandin synthesis, (2) requires the normal function of sympathetic nerves, and (3) involves the indirect contractions evoked by stimulation of parasympathetic nerves.

Wennmalm & Hedquist (1971) suggested that exogenous prostaglandin E may decrease the release of acetylcholine in parasympathetic neurotransmission to the rabbit heart by a mechanism similar to the prostaglandin-mediated inhibition of noradrenaline release from the sympathetic nerve endings in guinea-pig vas deferens (Hedquist & von Euler, 1972). Fredholm & Hedquist (1973) moreover reported that the treatment of stimulated vas deferens with indomethacin increased the release of noradrenaline.

With these theories of the mechanism of action of prostaglandin in mind our findings might be speculatively explained in the following way: prostaglandin E_2 inhibits the release of noradrenaline from sympathetic nerve endings. The blockade of prostaglandin synthesis by indomethacin prevents this inhibition of the sympathetic system with a subsequent increase of noradrenaline release. This might in turn decrease the release of acetylcholine from parasympathetic nerve terminals. Such a view is also supported by the results of Paton & Vizi (1969) who reported the inhibition of acetylcholine release from parasympathetic nerves of guinea-pig ileum by noradrenaline. This hypothesis could be tested by experiments designed to determine whether

indomethacin treatment increases noradrenaline release in guinea-pig ileum (as in the vas deferens) and whether this change leads to the decrease of acetylcholine release from parasympathetic nerves.

The authors are grateful to Dr J. Pike of the Upjohn Co., Kalamazoo Mich. for supplying the prostaglandin E_2.

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(Received December 21, 1973)