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# Regional differences in the responses to prostanoids of circular muscle from guinea-pig isolated intestine

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The effects of prostaglandins (PGs) $D_2$, $E_2$, $F_{2\alpha}$, an epoxymethano analogue of $PGH_2$ (U-46619), prostacyclin ($PGI_2$), 6-keto-$PGF_{1\alpha}$ and thromboxane (Tx) $B_2$ were tested on spirally-cut strips of guinea-pig isolated ileum or colon. In the ileum no prostanoid exerted a marked effect on the resting tissue, but $PGD_2$, $PGE_2$ or $PGI_2$ 1 $\mu$g ml$^{-1}$ inhibited submaximal contraction to KCl. U-46619 1 $\mu$g ml$^{-1}$ either inhibited or increased contractions to KCl, but $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 $\mu$g ml$^{-1}$ had no significant effect. $PGE_2$ relaxed colonic strips whereas the other prostanoids caused contraction, except for $TxB_2$ which had no effect. The PG antagonist SC-19220 blocked colonic contractions to the prostanoids, and a residual inhibitory effect of $PGD_2$, U-46619 or $PGI_2$ was demonstrated by the reduction of submaximal contractions to acetylcholine. Our results suggest that prostanoid receptors mediating inhibitory responses of circular muscle predominate in the ileum, whereas in the colon both excitatory and inhibitory prostanoid receptors occur.

Prostaglandin $E_2$ ($PGE_2$) relaxes circular muscle strips from guinea-pig ileum or colon, whereas $PGF_{2\alpha}$ causes contraction (Bennett et al 1968; Fleshler & Bennett 1969). Similar observations have been made in other species (see Bennett & Fleshler 1970). The actions of more-recently identified prostanoids have not been fully investigated, and we therefore studied some of these compounds on circular muscle in spirally cut strips of guinea-pig ileum or colon. Preliminary observations were reported to the British Pharmacological Society (Bennett & Sanger 1978).

## METHODS

Male albino guinea-pigs, about 400 g, were stunned and bled, and segments of distal ileum and distal colon were excised. Spiral strips approximately 3 mm wide and 3 cm long were cut from the ileum at least 8 cm proximal to the caecum, and from the colon at least 3 cm from the anus. Each strip was suspended in a 10 ml tissue bath under a load of 1 g in Krebs solution (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 and dextrose 1·0 g litre$^{-1}$) maintained at 37°C and bubbled with 5% $CO_2$ in $O_2$. Isotonic responses were measured using transducers and pen recorders.

Drugs used were $PGD_2$, $PGE_2$, $PGF_{2\alpha}$ tromethamine salt, (15S)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z, 13E-dienoic acid (U-46619), sodium $PGI_2$, 6-keto-$PGF_{1\alpha}$, thromboxane $B_2$, acetylcholine perchlorate, potassium chloride and 1-acetyl-2-(8-chloro-10,11-dihydrobenz(b,f)(1,4) oxazepine-10-carbonyl) hydrazine (SC-19220).

$PGI_2$ was dissolved in 1 M Tris pH10 (121 mg ml$^{-1}$), and freshly diluted with 50 mM Tris which had been adjusted to pH7·9 with HCl. U-46619 was dissolved in ethanol (10 mg ml$^{-1}$), diluted to 100 $\mu$g ml$^{-1}$ with 0·9% NaCl and further diluted with Krebs solution. All other prostanoids were dissolved in ethanol (5 mg ml$^{-1}$) and diluted with 0·9% NaCl. SC-19220 was dissolved in polyethylene glycol 400. Concentrations refer to the acids or salts listed above.

Results are expressed as means ± s.e.m. and analysed statistically using Student's *t*-test.

## RESULTS

*Ileum.* The circular muscle strips of guinea-pig ileum contracted only weakly to ACh, but responded well to KCl. No effect was seen with $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, $PGI_2$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 ng ml$^{-1}$-10 $\mu$g ml$^{-1}$, but in some experiments U-46619 0·01–1 $\mu$g ml$^{-1}$ caused a very small contraction. Since this tissue has no muscle tone, an inhibitory effect of prostanoids was looked for against consistent submaximal contractions to KCl (1-6 mg ml$^{-1}$; 30s contact time). $PGD_2$, $PGE_2$ or $PGI_2$ 1 $\mu$g ml$^{-1}$ usually reduced the contraction to KCl given 1 min later, $PGE_2$ being by far the most potent (Fig. 1). U-46619 1 $\mu$g ml$^{-1}$ either reduced or potentiated the contraction to KCl, whereas $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 $\mu$g ml$^{-1}$ had little or no effect (Fig. 1).

*Colon.* $PGE_2$ in concentrations as low as 1–100 ng ml$^{-1}$ relaxed guinea-pig colonic circular muscle

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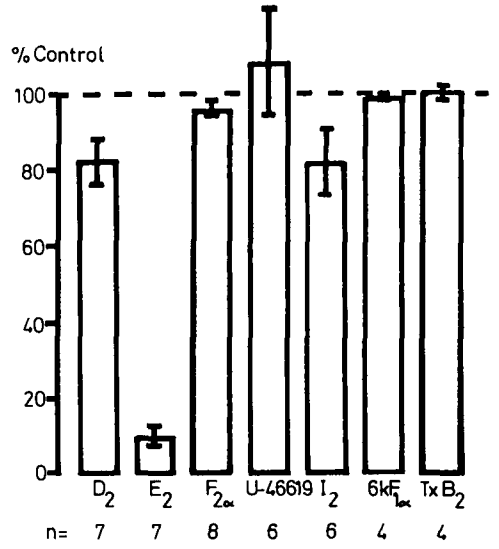


FIG. 1. $PGD_2$, $PGE_2$ or $PGI_2$ 1 $\mu$g ml$^{-1}$ reduced submaximal contractions of guinea-pig ileal circular muscle to KCl. $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 $\mu$g ml$^{-1}$ had little or no effect, whereas U-46619 1 $\mu$g ml$^{-1}$ caused either an increase or a reduction of contractions to KCl. Results are expressed as a percentage of control contractions to KCl. The columns represent the mean values, with standard errors shown by the bars; n = number of experiments.

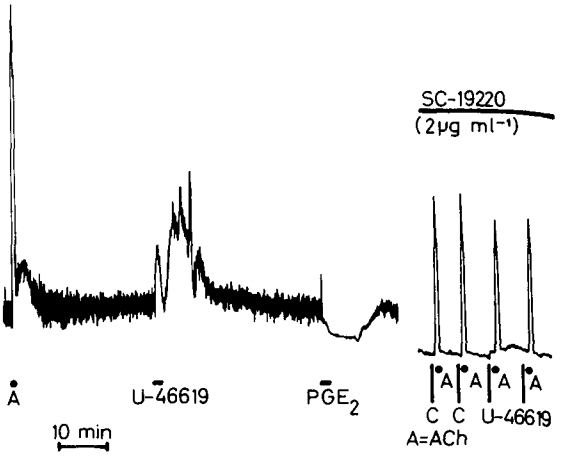


FIG. 2. Contraction of guinea-pig colonic circular muscle to U-46619 1 $\mu$g ml$^{-1}$ was long-lasting but not as strong as that to acetylcholine 1 $\mu$g ml$^{-1}$ (A). $PGE_2$ 1 $\mu$g ml$^{-1}$ caused a transient contraction, followed by a marked, sustained relaxation. SC-19220 2 $\mu$g ml$^{-1}$ reduced both the muscle tone and the contraction to U-46619 1 $\mu$g ml$^{-1}$ which then reduced contractions to A. (Contact times: A 30 s; U-46619 and $PGE_2$, 2 min. U-46619 or its vehicle control (C) were added 1 min before addition of A).

(Table 1), although these or lower concentrations sometimes caused contraction alone or preceeding the relaxation. Increasing the concentration of $PGE_2$ caused a dose-dependent increase in muscle relaxation and progressively reduced any initial contraction until only relaxation occurred. $TxB_2$ had no effect, but all the other prostanoids caused contraction. $PGF_{2\alpha}$ was the most potent excitatory prostanoid (Table 1), but compared with ACh no prostanoid was a consistently strong agonist (e.g. Fig. 2).

Table 1. Effects of prostanoids on guinea-pig colonic circular muscle, and their approximate threshold concentrations. n = number of experiments.

| | Response | Approximate threshold concentration (ng ml$^{-1}$) | n |
|---|---|---|---|
| $PGD_2$ | Contraction | 10–100 | 9 |
| $PGE_2$ | Relaxation, preceeded in 3 tissues by contraction | 1–100 | 4 |
| $PGF_{2\alpha}$ | Contraction | 1–25 | 9 |
| U-46619 | Contraction | 1–10 | 4 |
| $PGI_2$ | Contraction | 1–100 | 4 |
| 6-keto-$PGF_{1\alpha}$ | Contraction | 1–100 | 4 |
| $TxB_2$ | No effect | — | 4 |

SC-19220 (0·8–2 $\mu$g ml$^{-1}$) in the bathing fluid lowered the muscle tone and reduced or prevented contractions to the prostanoids. Inhibitory effects of some prostanoids seemed to be unmasked, as judged by the reduction of submaximal contractions to ACh (0·1–2 $\mu$g ml$^{-1}$; 30 s contact) given 1 min later, by $PGD_2$, U-46619 or $PGI_2$ 1 $\mu$g ml$^{-1}$. This reduction sometimes occurred even when SC-19220 only partially inhibited prostanoid-induced contraction (Figs 2,3). 6-Keto-$PGF_{1\alpha}$ 5 $\mu$g ml$^{-1}$ reduced contractions to ACh by 30 ± 13% (n=4), but $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 $\mu$g ml$^{-1}$ had no significant effect. With $PGF_{2\alpha}$ in concentrations greater than 1 $\mu$g ml$^{-1}$ the contractions were poorly reduced by SC-19220 (up to 10 $\mu$g ml$^{-1}$) so that an inhibitory effect of higher concentrations of $PGF_{2\alpha}$ could not be looked for.

## DISCUSSION

The longitudinal muscle of guinea-pig isolated intestine contracts to PGE and PGF compounds, $PGD_2$, 6-keto-$PGF_{1\alpha}$, $PGH_2$, epoxymethano analogues of $PGH_2$ and $TxA_2$ (Horton & Main 1963, 1965; Bennett & Posner 1971; Hamberg et al 1975; Bennett et al 1978; Engineer et al 1978; Bennett et al 1980a; Coleman et al 1980). However, the effects of prostanoids on gastrointestinal circular muscle has received little attention. $PGE_2$ usually relaxes circular muscle,

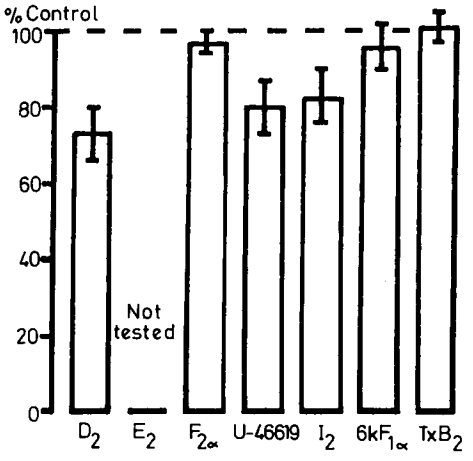


FIG. 3. $PGD_2$, U-46619 or $PGI_2$ 1 $\mu$g ml$^{-1}$ reduced submaximal contractions of guinea-pig colonic circular muscle to acetylcholine, following complete or substantial block by SC-19220 of contractions caused by 1 $\mu$g ml$^{-1}$ of these prostanoids (experimental procedure as for Fig. 2). $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$ 1 $\mu$g ml$^{-1}$ had little or no effect; $PGE_2$ was not tested. Results are expressed as a percentage of control contractions to acetylcholine. The columns represent the mean values, with standard errors shown by the bars.

whereas $PGF_{2\alpha}$ usually causes contraction (Bennett & Fleshler 1970). More recently, $PGD_2$ has been shown to contract guinea-pig colonic circular muscle (Bennett et al 1980a).

The circular muscle of guinea-pig ileum is unusual since it has no tone and responds poorly to many agonists (see Kosterlitz & Lees 1964). Of the prostanoids tested, only U-46619 was found to contract the circular muscle of guinea-pig isolated ileum, but even then the effect was very weak and not always present. Although U-46619 is an analogue of $PGH_2$, it may act on receptors for $TxA_2$ (Coleman et al 1980). We used contractions to KCl to investigate the inhibitory properties of prostanoids, and found $PGE_2$ to be 4–5 times as potent as $PGD_2$ or $PGI_2$. This contrasted with the lack of effect of $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ or $TxB_2$, and with the variable response to U-46619.

In guinea-pig colonic circular muscle low concentrations of $PGE_2$ usually caused an initial contraction and then a relaxation, whereas higher concentrations caused only relaxation. The stimulant activity of $PGE_2$ is not clearly understood. It might be due to contraction of longitudinal muscle fibres in the spirally cut strip, but in whole segments of guinea-pig isolated colon various concentrations of $PGE_2$ can stimulate circular muscle contractions during peristalsis (Eley et al 1977). The other prostanoids usually caused either a weak contraction or no effect. In general, these results are similar to those found in human isolated colonic or gastric circular muscle. An exception is $PGI_2$ which relaxes both muscle layers in these human tissues (Bennett & Sanger 1980; Bennett et al 1980b and unpublished).

Inhibitors of prostanoid synthesis usually increase or initiate spontaneous activity in strips of circular muscle from guinea-pig isolated intestine (Bennett et al 1975; Bennett et al 1980a and unpublished). Thus $PGE_2$, the most potent inhibitory prostanoid in this tissue, may exert a tonic suppression of isolated circular muscle activity. Since in other tissues inhibitory prostanoids can preferentially antagonize certain excitatory prostanoids (Bennett & Sanger 1979, 1980) the excitatory actions of prostanoids in circular muscle might be weak because they are inhibited by endogenous $PGE_2$. This could be studied using inhibitors of PG synthesis.

We confirmed that the PG antagonist SC-19220 (Sanner 1969) blocks contractions of guinea-pig colonic circular muscle to $PGF_{2\alpha}$ (Bennett & Posner 1971). In addition, SC-19220 antagonized contractions to 1 $\mu$g ml$^{-1}$ $PGD_2$, U-46619, $PGI_2$ or 6-keto-$PGF_{1\alpha}$, after which an inhibitory effect of $PGD_2$, U-46619 or $PGI_2$ 1 $\mu$g ml$^{-1}$ was demonstrated. Thus, in this tissue $PGD_2$, U-46619 or $PGI_2$ exerted a predominant excitatory activity which may have overshadowed an inhibitory response. However, inhibition by PGs of contractions to ACh could be seen even when some agonist activity remained. A similar observation was seen with $PGE_2$ in normal, but not diseased, human isolated taenia coli (Crofts et al 1979), so perhaps certain excitatory PGs inhibit contractions to ACh even in the absence of SC-19220.

Our results suggest that there are regional differences in the distribution of prostanoid receptors in guinea-pig intestinal circular muscle. In the ileum prostanoid receptors mediating inhibition predominate, and these can be activated by $PGE_2$, $PGD_2$, $PGI_2$ or sometimes by U-46619. $PGF_\alpha$ compounds or $TxB_2$ may be unable to activate receptors in this tissue, or perhaps an excitatory action is suppressed by endogenous inhibitory prostanoids. In the colon, receptors mediating both inhibition and excitation are present and can be activated by $PGD_2$, U-46619 or $PGI_2$.

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