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PROSTAGLANDINS AND THE CONTRACTILE ACTION OF BRADYKININ ON THE LONGITUDINAL MUSCLE OF RAT ISOLATED ILEUM

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1 The effect of the prostaglandin synthesis inhibitors aspirin and indomethacin (Ind) was investigated upon contractions of the perfused isolated terminal ileum of the rat to bradykinin (Bk) or potassium chloride (KCl). The release of a prostaglandin-like substance (PGLS) from the ileum was simultaneously assayed on the rat fundus strip (RFS).

2 Aspirin (610 μM) or Ind (28 μM) reduced contractions of the ileum to Bk perfused over the mucosal surface but not to Bk perfused over the serosal surface.

3 After perfusion of either ileal surface the effluent potentiated the contractions of the RFS to Bk. No potentiation was observed in the presence of either Ind or aspirin.

4 Contractions of the RFS to Bk were potentiated by prostaglandin E_2 (PGE_2) (0.7×10^{-12} M and 1.4×10^{-12} M) but not by either a higher (2.8×10^{-12} M) or a lower (0.35×10^{-12} M) concentration of PGE_2. No potentiation was observed with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at the concentrations studied.

5 Only the contractile action of Bk acting on the mucosal surface of the rat isolated perfused ileum seems to involve prostaglandin synthesis.

Introduction

There is evidence of a relationship between bradykinin (Bk) and prostaglandins in several systems including the gastrointestinal tract (Crocker & Willavoys, 1976; Crocker, Walker & Wilson, 1978; Edery & Shemesh, 1978).

Bk stimulates the release of prostaglandins from dog kidney (McGiff, Terragno, Malik & Lonigro, 1972) dog spleen *in vitro* and *in vivo* (Moncada, Ferreira & Vane, 1972; Ferreira, Moncada & Vane, 1973b) cat isolated spleen (Ferreira, Moncada & Vane, 1973a), dog knee joint (Moncada, Ferreira & Vane, 1975), guinea-pig and rat isolated lungs (Palmer, Piper & Vane, 1973; Damas & Deby, 1976), rabbit isolated heart (Needleman, Key, Denny & Marshall, 1976) and rabbit isolated perfused ear (Juan & Lembeck, 1976). In addition a spontaneous release of prostaglandin-like substance (PGLS) has been reported from several organs including the gastrointestinal tract (for references see Bennett, 1976).

In most isolated gastrointestinal preparations, Bk causes a brief relaxation followed by a contraction, although a predominant relaxation has been described in some preparations (see Walaszek, 1970). The mechanism of action of Bk on gastrointestinal muscle is poorly understood. Crocker & Willavoys (1976) have suggested that prostaglandins may participate in the contraction of the longitudinal muscle of rat isolated ileum to Bk. However, it has recently been reported that in longitudinal muscle strips of cat isolated ileum, Bk has a direct stimulant action upon specific receptors (Barabé, Drouin, Regoli & Park, 1977).

In the present study we have extended our previous investigation into the possible involvement of prostaglandins in the contractile action of Bk. The effect of the prostaglandin synthesis inhibitors aspirin and indomethacin (Ind) (Vane, 1971), was investigated on contractions of the perfused isolated terminal ileum of the rat to Bk or KCl. Released PGLS from the ileum was bioassayed simultaneously upon the rat fundus strip.

Methods

Preparation

Male Wistar rats (180 to 250 g) were killed by a blow on the head and a 4 cm section of terminal ileum, cleaned of mesentery and fat, was removed and flushed through with Krebs solution. The proximal end was fastened to polypropylene tubing (1.5 mm internal diameter) mounted horizontally in a heated water jacket (internal diameter 1 cm) at 37°C. The

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caecal end of the ileum was connected by thread over a pulley to an isometric transducer under 1 g tension. The ileum was perfused through the lumen with Krebs solution (K_1) at 37°C previously gassed with 5% CO_2 in O_2 at 3 ml/min by a Watson Marlow pump (Type MHRE 7). For serosal perfusion the ileum was everted over a glass rod before introduction into the apparatus.

Bioassay of prostaglandin-like substance

Prostaglandin-like activity was assayed on a rat fundus strip (RFS) (Vane, 1957) suspended in a perfusion jacket (Vane 1964) under 2 g tension.

The RFS was superfused with the effluent from the ileum (3 ml/min) and a Krebs solution (K_2) containing antagonists and Ind to increase the specificity and sensitivity of the assay tissue to prostaglandins, also at 3 ml/min. To obtain the recommended concentration of antagonists (Gilmore, Vane & Wyllie, 1968) and Ind (2.8 μM) superfusing the RFS, adjustments were made to the Krebs (K_2) reservoir to compensate for dilution by the effluent from the ileum.

Bk and KCl were diluted in Krebs solution immediately before use and were injected at a constant volume (0.2 ml) into the Krebs solution stream before perfusion of the ileum.

All contractions were recorded on isometric transducers (Type UFI) coupled to a Devices (Type M2) recorder.

Drugs

With the exception of Bk, all drug solutions were freshly prepared immediately before use. Bk was dissolved in bi-distilled water, sealed in sterile ampoules and stored at −16°C for up to 3 weeks. The following chemicals were used: aspirin (Sigma); bradykinin triacetate (Sigma); (−)-hyoscine hydrobromide (BDH); indomethacin (Merck, Sharpe and Dohme); methysergide hydrogenmaleinate (Sandoz); mepyramine maleate (May and Baker); phenoxybenzamine hydrochloride (Smith, Kline and French); potassium chloride (BDH, Analar); propranolol (ICI); prostaglandins E_2 and $F_{2\alpha}$ tromethamine salt (Upjohn). The Krebs solutions contained (mM); NaCl 94, KCl 4.69, $CaCl_2.2H_2O$ 1.9, KH_2PO_4 1.16, $NaHCO_3$ 24.9, $MgSO_4.7H_2O$ 4.46 and glucose 11.

Statistical analysis

Contractions to Bk and KCl bathing the mucosal or serosal surface are expressed as a percentage of their respective maximum responses. All data (except regression analysis) are presented as a mean ± s.e. mean and analysed by Student's t test. Regression lines were calculated by the method of least squares, and after showing parallelism the lines were analysed by the t test for difference between regression lines (Mood, Graybill & Boes, 1974) calculated at the concentration of Bk or KCl giving a response of 50% in their respective control experiment (EC_{50}).

Results

Bioassay on rat fundus strip of prostaglandin-like substance(s) released from the rat ileum during contractions to bradykinin or KCl

The contraction of the RFS to Bk perfused over the ileal mucosal surface was greater than control responses obtained to Bk perfused in the absence of the ileum. Figure 1a represents the Bk-induced contractions of the RFS presented as regression lines constructed from the concentrations of bradykinin within the linear part of the log dose-response relationship. Perfusion of Bk over the ileal mucosal surface caused a displacement of the regression line to the left of the control line with a significant difference at the EC_{50} of 14.0 ± 6.8% ($n = 174$, $P < 0.05$). In the presence of either Ind (28 μM) or aspirin (610 μM), contractions of the RFS to Bk were reduced towards the control values. No significant difference was observed between the regression lines at the EC_{50} in the presence of either 28 μM Ind, 10.5 ± 5.3% ($n = 96$), or 610 μM aspirin, 7.0 ± 4.1% ($n = 102$), (both $P > 0.05$) compared with control. Ind (28 μM) or aspirin (610 μM) perfused in the absence of the ileum had no effect upon the contractions of the RFS to Bk.

Similar results were obtained with Bk bathing the ileal serosal surface (Figure 1b). The regression line was displaced to the left of control with a significant difference at the EC_{50} of 10.3 ± 4.5% ($n = 200$, $P < 0.05$). In the presence of either Ind (28 μM) or aspirin (610 μM) contractions of the RFS to Bk were reduced towards the control values and there was no significant displacement calculated at the EC_{50}, 8.3 ± 5.6% ($n = 96$) and 6.5 ± 4.9% ($n = 144$) (both $P > 0.05$) respectively. Perfusion of KCl over either ileal surface caused no significant displacement of the regression lines from the control line obtained in the absence of the ileum (Figures 2a,b). Addition of Ind (28 μM) to the perfusate of either surface also had no significant effect.

During either mucosal or serosal perfusion of the ileum the resting tension of the RFS was significantly increased by 0.6 ± 0.1 g and 0.56 ± 0.13 g respectively (both $n = 35$, $P < 0.001$) and this was maintained throughout the experiment (≃3 h). This increase was abolished by addition of either Ind (28 μM) or aspirin (610 μM).

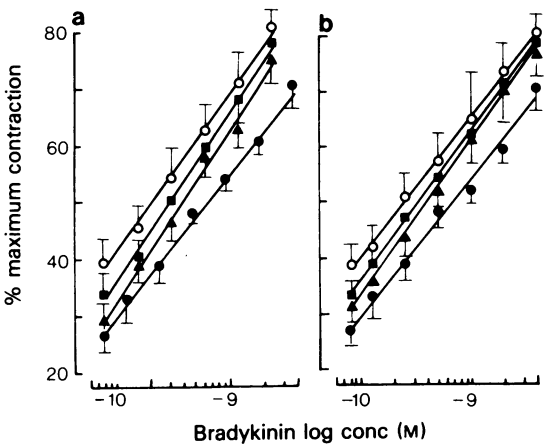

Figure 1 (a) Log dose-response regression lines for contractions of rat fundus strip (RFS) to bradykinin (Bk) following mucosal perfusion of the rat ileum. Contractions to Bk injected in the absence of the ileum (●, $n = 10$), to Bk injected into the mucosally perfused ileum (○, $n = 19$), and to Bk injected into the ileum mucosally perfused with either 28 μM indomethacin (■, $n = 6$) or 610 μM aspirin (▲, $n = 6$). (b) Log dose-response regression lines for contractions of RFS to Bk following serosal perfusion of the rat ileum. Contractions to Bk injected in the absence of the ileum (●, $n = 10$), to Bk injected into the serosally perfused ileum (○, $n = 20$), and to Bk injected into the ileum serosally perfused with either 28 μM indomethacin (■, $n = 6$) or 610 μM aspirin (▲, $n = 14$). Each point represents the mean, and the vertical line 1 s.e. mean.

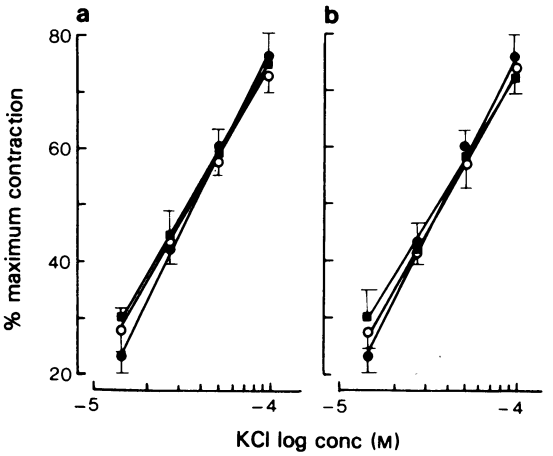

Figure 2 Log dose-response regression lines for contractions of rat fundus strip (RFS) to KCl following mucosal perfusion of the rat ileum. Contractions to KCl injected in the absence of the ileum (●, $n = 10$), to KCl injected into the mucosally perfused ileum (○, $n = 15$), and to KCl injected into the ileum mucosally persused with 28 μM indomethacin (■, $n = 10$) added to the perfusate. (b) Log dose-response regression lines for contractions of RFS to KCl following serosal perfusion of the rat ileum. Contractions to KCl injected in the absence of the ileum (●, $n = 10$), to KCl injected into the serosally perfused ileum (○, $n = 15$), and to KCl injected into the ileum serosally perfused with 28 μM indomethacin (■, $n = 10$) added to the perfusate. Each point represents the mean and the vertical line 1 s.e. mean.

Interaction on the rat fundus strip between bradykinin prostaglandin E_2 or $F_{2\alpha}$

The interaction between prostaglandins and Bk on the RFS was investigated to determine whether or not it was possible to quantify the release of PGLS.

Figure 3a demonstrates the effect of four concentrations of prostaglandin E_2 (PGE_2) (0.35, 0.7, 1.4 and 2.8×10^{-12} M) and Figure 3b the effect of four concentrations of $PGF_{2\alpha}$ (0.26, 0.53, 1.05 and 2.1×10^{-12} M) on the contractions of RFS to Bk (2.4×10^{-10} M), the concentration giving a 40% maximal response. Contractions were measured from the baseline immediately prior to the contraction, and the increases in resting tension caused by the prostaglandins are shown. Contractions to Bk in the presence of the lowest concentration of PGE_2 (0.35×10^{-12} M) were $40 \pm 6.0\%$ ($n = 10$) of maximum and were not significantly different from the control contractions of $39 \pm 3.1\%$ ($n = 14$, $P > 0.05$) obtained in the absence of prostaglandins. However, with 0.7×10^{-12} M and 1.4×10^{-12} M PGE_2, there was a significant increase in the response of the RFS with Bk to $45 \pm 2.0\%$ ($n = 10$, $P < 0.05$) and $58 \pm 3.5\%$ ($n = 14$, $P < 0.001$) respectively of maximum. There was no significant difference between contractions to Bk in the presence of PGE_2 (2.8×10^{-12} M) $40 \pm 4.1\%$ ($n = 10$, $P > 0.05$), when compared with the control responses. At all concentrations of $PGF_{2\alpha}$ studied, there was no significant increase in the response of the RFS to Bk compared to control responses in the absence of $PGF_{2\alpha}$.

PGE_2 or $PGF_{2\alpha}$ had no significant effect upon the contractions of the RFS to KCl at any of the concentrations studied (Figure 4a and 4b respectively).

Prostaglandin synthesis and the response of ileal longitudinal muscle to bradykinin or KCl

The serosal surface was more sensitive than the mucosal surface to Bk (Figure 5) the mean EC_{50} being 1.5×10^{-10} M and 8.1×10^{-10} M respectively. The maximal contraction to serosally applied Bk (5.0 ± 0.3 g, $n = 6$) was greater than after mucosal perfusion (2.4 ± 0.2 g, $n = 8$) $P < 0.01$. Responses of

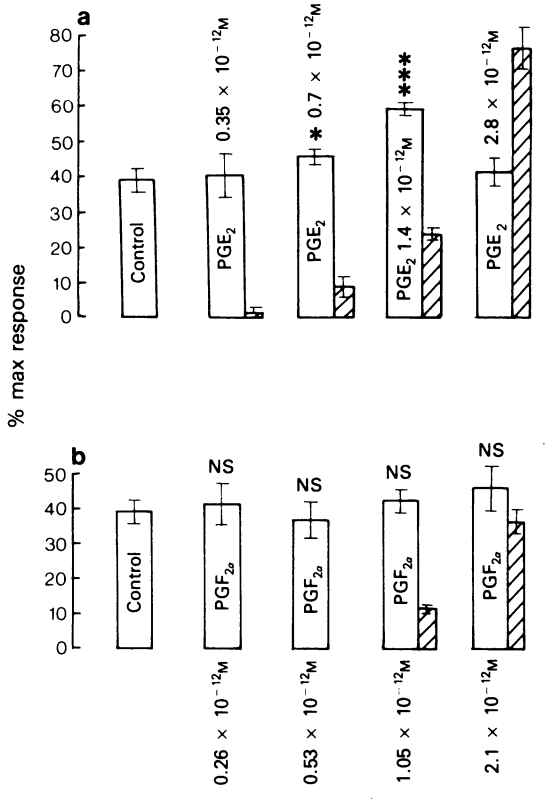

Figure 3 Response to bradykinin (Bk, 2.4×10^{-10} M) of the rat fundus strip (RFS) continuously perfused with prostaglandin E_2 (PGE_2) in (a) or $PGF_{2\alpha}$ in (b). Wide histograms represent contractions of RFS to Ek, narrow histograms (hatched) represent changes in baseline tension of the RFS during prostaglandin perfusion. All responses are expressed as a percentage of the maximal control response to Bk, where the control is the response of RFS to Bk in the absence of prostaglandins. * $P < 0.05$; *** $P < 0.001$; NS, not significant; compared with control response. Vertical lines represent 1 s.e. mean (n = 10–14).

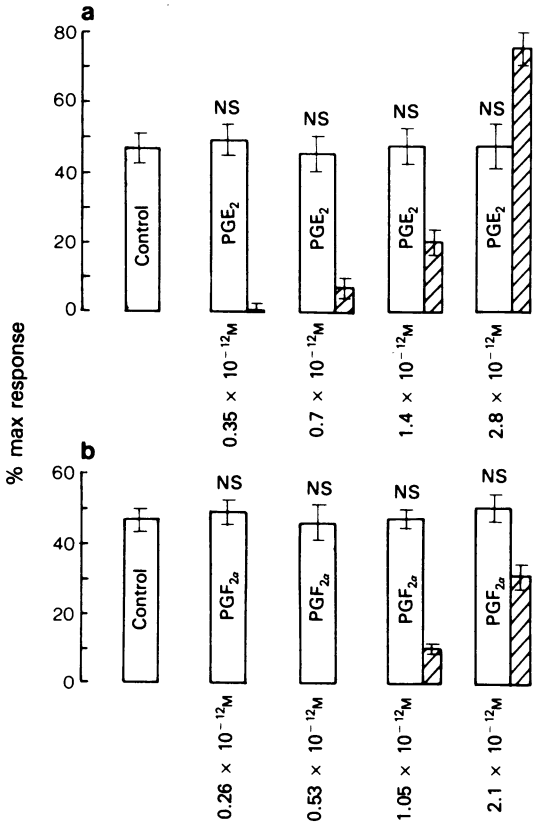

Figure 4 Response to KCl (3.2×10^{-5} M) of the rat fundus strip (RFS) continuously perfused with prostaglandin E_2 (PGE_2) in (a) or $PGF_{2\alpha}$ in (b). Wide histograms represent contractions of RFS to KCl, narrow histograms (hatched) represent changes in baseline tension of the RFS during prostaglandin perfusion. All responses are expressed as a percentage of the maximal control response to KCl, where the control is the response of RFS to KCl in the absence of prostaglandins. NS, not significant compared with control response. Vertical lines represent 1 s.e. mean (n = 10–14).

longitudinal muscle to Bk perfused over the mucosal surface were depressed by either Ind (28 μM) or aspirin (610 μM) with the maximal responses reduced by 80 ± 5% and 53 ± 6% respectively (both n = 5, $P < 0.001$). However, responses to Bk bathing the serosal surface were not significantly changed by either Ind (28 μM) or aspirin (610 μM) and the maximum responses were 100 ± 4% and 100 ± 6% (both n = 6, $P > 0.05$) respectively of control.

There was no difference between the maximal ileal contraction to mucosally applied KCl (4.7 ± 0.4 g), compared with serosally applied KCl (4.8 ± 0.3 g) both n = 15, $P > 0.05$, (Figure 6). Responses to KCl bathing either surface were not significantly changed by Ind (28 μM), the maximal responses were 100 ± 8% and 99 ± 10% (both n = 10, $P > 0.05$) on the mucosal and serosal surface respectively.

Discussion

Prostaglandins may participate in the contractile action of Bk on rat isolated terminal ileum since contractions to Bk were antagonized by Ind or aspirin

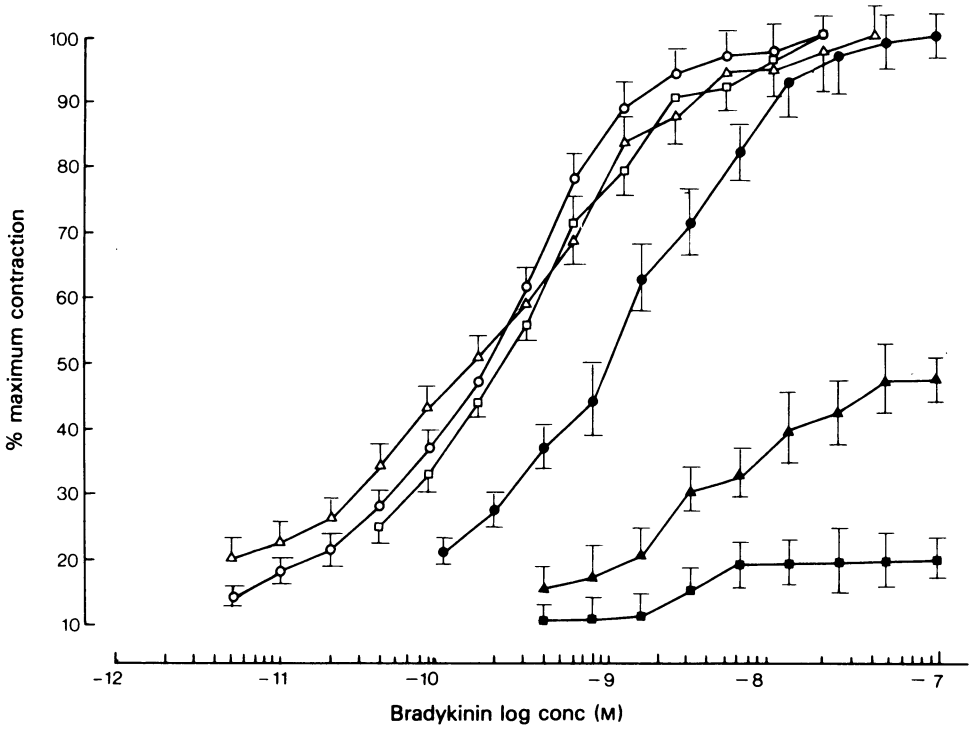

Figure 5 Log dose-response relationships of longitudinal muscle of rat ileum to bradykinin (Bk): (○) represent serosally perfused ileum with 28 μM indomethacin (□) or 610 μM aspirin (△) in the perfusate; (●) represents mucosally perfused ileum with 28 μM indomethacin (■) or 610 μM (▲) in the perfusate. Each point represents the mean, and the vertical line 1 s.e. mean (n = 5–8).

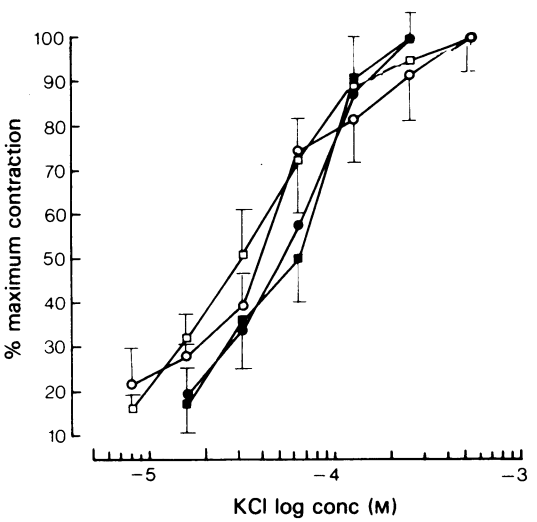

Figure 6 Log dose-response relationships of longitudinal muscle of rat ileum to KCl: (○) represent serosally perfused ileum with 28 μM indomethacin (□) in the perfusate; (●) represent mucosally perfused ileum with 28 μM indomethacin (■) in the perfusate. Each point represents the mean, and the vertical line 1 s.e. mean (n = 10–15).

(Crocker & Willavoys, 1976). Recently Edery & Shemesh (1978) reported that the release of PGLS mediates the potentiation of Bk by bradykinin potentiating factor in rat isolated ileum. Other workers have reported the release of PGLS by Bk from a variety of preparations (see Introduction). In the present study, introduction of an ileum into the Krebs stream superfusing a RFS increased the resting tension of the assay tissue regardless of the surface of the ileum perfused and this effect was abolished by Ind or aspirin. This suggested that PGLS was released from the resting ileum. Similarly, Bk but not KCl perfused over either surface of the ileum, contracted the RFS more than when the ileum was absent. The reduction by Ind or aspirin of this increased contraction to Bk is consistent with an involvement of PGLS.

The increased contraction of the RFS to Bk may have been due to either a potentiation of Bk by the basal release of PGLS, to an increased release of PGLS by Bk or to a combination of both effects. Continuous perfusion with PGE_2 (0.7×10^{-12} M and 1.4×10^{-12} M), probably the main PGLS produced by compression of rat gut (Collier 1974), increased the contraction of the RFS to Bk. However, PGE_2 (0.35×10^{-12} M and 2.8×10^{-12} M) and all concen-

trations of $PGF_{2\alpha}$ used, caused no increase in the contraction to Bk. There was no apparent relationship between this increase in the contraction of the RFS to Bk and the change in the baseline tension due to the prostaglandin. However, the marked increase in baseline tension produced by PGE_2 (2.8×10^{-12} M) might account for the failure to observe an increase in the contraction to Bk at this concentration.

Potentiation of responses to spasmogens by prostaglandins, particularly of the E series, has been described by Hall & Pickles (1963). This property of prostaglandins made it difficult to bioassay the PGLS released from the ileum during exposure to Bk. However, in the perfused rabbit ear, an organ lacking a continuous release of PGLS, the PGLS released by Bk can be quantified (Juan & Lembeck, 1976).

The potentiation of contractions of RFS by Bk was similar after either serosal or mucosal perfusion of the ileum. However, the mechanism of contraction of the longitudinal muscle of the ileum was dependent upon the surface studied. The serosal surface was more sensitive than the mucosal surface to Bk and contractions were unaffected by the prostaglandin synthesis inhibitors, Ind and aspirin. Whereas the synthesis inhibitors markedly reduced the contractions to Bk during mucosal perfusion, contractions to KCl were similar on both surfaces and were unaffected by Ind or aspirin.

These results suggest a dual action of Bk on rat isolated terminal ileum. The results following mucosal perfusion support a role for a PGLS, possibly of the E series, in the contraction to Bk and are consistent with the previous findings of Crocker & Willavoys (1976). However the failure of the prostaglandin synthesis inhibitors to reduce contractions to Bk following serosal perfusion is consistent with the findings of Barabé *et al.* (1977) who proposed that Bk had a direct action on longitudinal muscle strips from cat ileum, and segments of rat uterus. A similar dual action for Bk on the rat isolated uterus has been reported by Whalley (1978) who suggested that Bk has a direct action on the myometrium and an indirect action via release of PGLS from the endometrium.

In conclusion, the rat isolated terminal ileum releases similar amounts of PGLS after perfusion of either the mucosal or serosal surface. However, it appears that contractions of the ileum to Bk involve prostaglandin synthesis only with mucosal perfusion.

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