

# Differential peristaltic motor effects of prostanoid (DP, EP, IP, TP) and leukotriene receptor agonists in the guinea-pig isolated small intestine

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**1** Since the role of prostanoid receptors in intestinal peristalsis is largely unknown, the peristaltic motor effects of some prostaglandin (DP, EP, IP), thromboxane (TP) and leukotriene (LT) receptor agonists and antagonists were investigated.

**2** Propulsive peristalsis in fluid-perfused segments from the guinea-pig small intestine was triggered by a rise of the intraluminal pressure and recorded *via* the intraluminal pressure changes associated with the peristaltic waves. Alterations of distension sensitivity were deduced from alterations of the peristaltic pressure threshold and modifications of peristaltic performance were reflected by modifications of the amplitude, maximal acceleration and residual baseline pressure of the peristaltic waves.

**3** Four categories of peristaltic motor effects became apparent: a decrease in distension sensitivity and peristaltic performance as induced by the EP<sub>1</sub>/EP<sub>3</sub> receptor agonist sulprostone and the TP receptor agonist U-46,619 (1–1000 nM); a decrease in distension sensitivity without a major change in peristaltic performance as induced by PGD<sub>2</sub> (3–300 nM) and LTD<sub>4</sub> (10–100 nM); a decrease in peristaltic performance without a major change in distension sensitivity as induced by PGE<sub>1</sub>, PGE<sub>2</sub> (1–1000 nM) and the EP<sub>1</sub>/IP receptor agonist iloprost (1–100 nM); and a decrease in peristaltic performance associated with an increase in distension sensitivity as induced by the EP<sub>2</sub> receptor agonist butaprost (1–1000 nM). The DP receptor agonist BW-245C (1–1000 nM) was without effect.

**4** The peristaltic motor action of sulprostone remained unchanged by the EP<sub>1</sub> receptor antagonist SC-51,089 (1 μM) and the DP/EP<sub>1</sub>/EP<sub>2</sub> receptor antagonist AH-6809 (30 μM), whereas that of U-46,619 and LTD<sub>4</sub> was prevented by the TP receptor antagonist SQ-29,548 (10 μM) and the cysteinyl-leukotriene<sub>1</sub> (cysLT<sub>1</sub>) receptor antagonist tomelukast (10 μM), respectively.

**5** These observations and their pharmacological analysis indicate that activation of EP<sub>2</sub>, EP<sub>3</sub>, IP, TP and cysLT<sub>1</sub> receptors, but not DP receptors, modulate intestinal peristalsis in a receptor-selective manner, whereas activation of EP<sub>1</sub> seems to be without influence on propulsive peristalsis. In a wider perspective it appears as if the effect of prostanoid receptor agonists to induce diarrhoea is due to their prosecretory but not peristaltic motor action.

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**Keywords:** Intestinal peristalsis; prostaglandins; thromboxanes; leukotrienes; prostaglandin receptors; thromboxane receptors; leukotriene receptors; peristaltic pressure threshold; distension sensitivity; peristaltic performance

**Abbreviations:** LT, leukotriene; PG, prostaglandin; PPT, peristaltic pressure threshold

## Introduction

Prostaglandins (PGs) and thromboxanes are known to modify gastrointestinal motility, but the cell surface receptors mediating these prostanoid actions have not been fully identified. PGE receptors of the EP<sub>2</sub> type, but also of the EP<sub>1</sub>, EP<sub>3</sub> and EP<sub>4</sub> type, are expressed in the gut of several species including man (An *et al.*, 1993; Bastien *et al.*, 1994; Morimoto *et al.*, 1997; Northey *et al.*, 2000). Cellular localization studies indicate that the external muscle layers bear EP<sub>1</sub>, EP<sub>2</sub> and EP<sub>3</sub> receptors and that some EP<sub>3</sub> receptors are also associated with enteric neurons of the rat and mouse intestine (Morimoto *et al.*, 1997; Northey *et al.*, 2000), while pharmacological investigations have shown that circular

muscle cells of the guinea-pig ileum exhibit EP<sub>1</sub> and EP<sub>3</sub> receptors which on activation by agonists mediate contraction, whereas EP<sub>2</sub> receptors bring about relaxation (Botella *et al.*, 1993). From receptor knockout studies it would appear that activation of receptors for PGD (DP), PGF, PGE (EP<sub>1</sub>, EP<sub>3</sub>, EP<sub>4</sub>), PGI (IP) and thromboxane (TP) alters the motor activity of the mouse ileum, either by inducing contraction or relaxation (Okada *et al.*, 2000). In the longitudinal muscle of the guinea-pig ileum, PGE causes contraction most likely *via* activation of EP<sub>1</sub> and EP<sub>3</sub> receptors on muscle cells and EP<sub>2</sub> receptors on myenteric neurons (Lawrence *et al.*, 1992). IP receptor agonists may lead both to a nerve-mediated contraction and, through a direct action on myocytes, to a relaxation of the muscle (Lawrence *et al.*, 1992).

Consistent with these effects is the ability of PGs to influence intestinal peristalsis, the clinically most relevant

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motor pattern of the gut. Thus, PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>1 $\alpha$</sub>  and PGF<sub>2 $\alpha$</sub>  modify motility in the guinea-pig isolated ileum and colon in a manner that has been interpreted as peristaltic motor stimulation (Bennett *et al.*, 1976; Eley *et al.*, 1977; Fontaine *et al.*, 1977; Sanger & Watt, 1978). In addition, PGE<sub>1</sub>, PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  can induce peristalsis-like contractions of the circular muscle in the guinea-pig intestine (Ishizawa & Miyazaki, 1975; Grbovic & Radmanovic, 1987) and reverse the antiperistaltic action of indomethacin and acetylsalicylic acid (Bennett *et al.*, 1976), although the effect of nonsteroidal anti-inflammatory drugs to disturb peristalsis (Bennett *et al.*, 1976; Fontaine *et al.*, 1977; Bruch *et al.*, 1978) is not necessarily related to cyclo-oxygenase inhibition (Shahbazian *et al.*, 2001). However, PGE<sub>1</sub> has also been reported to inhibit peristalsis-like motility in the guinea-pig ileum *via* activation of sympathetic neurons (Radmanovic, 1972), and the PGE<sub>2</sub> analogue enprostil has been found to impair antroduodenal motility in man (Hausken *et al.*, 1991).

From these divergent reports it is difficult to deduce how activation of distinct prostanoid receptors modifies propulsive peristalsis in the gut. Since this nerve-coordinated pattern of intestinal motility involves both excitatory and inhibitory motor reflexes (Costa *et al.*, 2000), it is likewise impossible to envisage which impact the contractile and relaxant motor effects of prostanoid receptor activation have on propulsive motility. Thus, the first aim of this study was to characterize the peristaltic motor actions of PGA<sub>2</sub>, PGD<sub>2</sub>, PGE<sub>1</sub>, PGE<sub>2</sub>, the PGI<sub>2</sub> analogue iloprost, the EP<sub>1</sub>/EP<sub>3</sub> receptor agonist sulprostone, the EP<sub>2</sub> receptor agonist butaprost and the DP receptor agonist BW-245C in fluid-perfused segments from the guinea-pig small intestine. By analysis of four aspects of propulsive peristalsis and by use of some prostanoid receptor antagonists, we sought to distinguish between specific patterns of peristaltic motor changes caused by DP, EP<sub>2</sub>, EP<sub>3</sub> and IP receptor activation. The second aim was to extend this analysis to thromboxane A<sub>2</sub> and leukotriene (LT) D<sub>4</sub>, because cysteinyl-LTs (cysLTs) such as LTD<sub>4</sub> are able to contract intestinal smooth muscle (Gardiner *et al.*, 1990; Jonsson, 1998), whereas thromboxane A<sub>2</sub> is rather ineffective in altering muscle activity in the guinea-pig intestine (Sanger & Bennett, 1980; Sametz *et al.*, 2000). Therefore, we investigated whether the TP receptor agonist U-46,619 and LTD<sub>4</sub> influence intestinal peristalsis and whether the peristaltic motor responses to U-46,619 and LTD<sub>4</sub> are blocked by selective antagonists for TP and cysLT<sub>1</sub> receptors, respectively.

## Methods

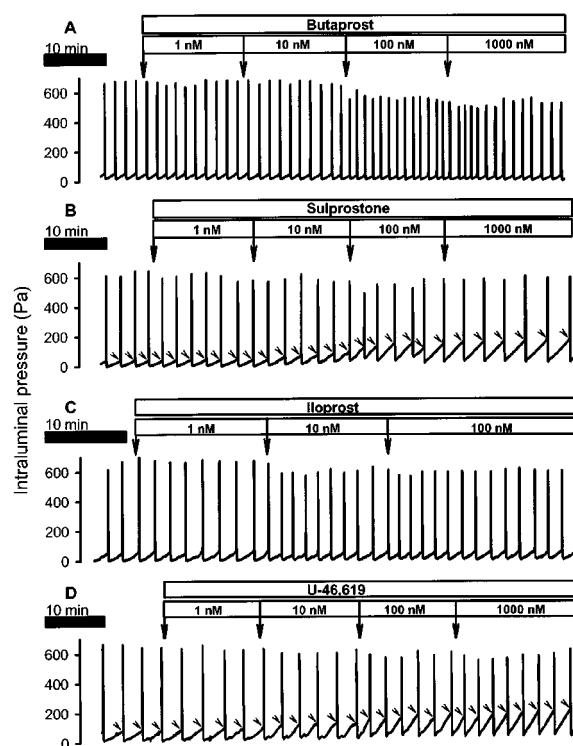
### Recording of peristalsis

The small intestine (jejunum and ileum) of adult guinea-pigs (TRIK strain, either sex, 350–450 g body weight) was isolated, flushed of luminal contents and placed, for up to 4 h, in Tyrode solution kept at room temperature and oxygenated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (Shahbazian *et al.*, 2001). The composition of the Tyrode solution was (mM): NaCl 136.9, KCl 2.7, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1.0, NaHCO<sub>3</sub> 11.9, NaH<sub>2</sub>PO<sub>4</sub> 0.4 and glucose 5.6. The small intestine (jejunum and ileum) was divided into eight segments, each being approximately 10 cm long. Since the

baseline peristaltic parameters recorded here (see below) did not significantly differ between segments taken from the proximal jejunum and distal ileum ( $n=9$ ), the segments were assigned randomly to the pharmacological treatments under study. Four intestinal segments were set up on parallel and secured horizontally in organ baths containing 30 ml of Tyrode solution at 37°C. In order to elicit propulsive peristalsis, pre-warmed Tyrode solution was continuously infused into the lumen of the segments at a rate of 0.5 ml min<sup>-1</sup> (Shahbazian *et al.*, 2001). The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, *via* an analogue/digital converter, fed into a personal computer and recorded and analysed with the software Peristal 1.0 (Heinemann *et al.*, 1999). The fluid passing through the gut lumen was directed into a vertical outlet tubing which ended 4 cm above the fluid level in the organ bath. When fluid was infused, the intraluminal pressure rose slowly until it reached a threshold at which peristalsis was triggered (Figure 1). The aborally moving wave of peristaltic contraction resulted in a spike-like increase in the intraluminal pressure (the peristaltic wave), which caused emptying of the segment if the maximal pressure of the peristaltic wave exceeded the level of 400 Pa as set by the position of the outlet tubing.

### Experimental protocols

The preparations were equilibrated in the organ bath for a period of 30 min, during which they were kept in a quiescent



**Figure 1** Recordings of the effects of butaprost (A), sulprostone (B), iloprost (C) and U-46,619 (D) on peristalsis. The drugs were administered to the organ bath at the specified concentrations. As can be seen, sulprostone and U-46,619 increased the peristaltic pressure threshold (indicated by arrow heads) whereas butaprost lowered it. Iloprost tended to lower the amplitude of the peristaltic waves.

state. Thereafter the bath fluid was renewed and peristaltic motility initiated by intraluminal perfusion of the segments. After baseline peristalsis had been recorded for a 30 min period, the drugs to be tested were added to the bath, i.e., to the serosal surface of the intestinal segments, at volumes not exceeding 1% of the bath volume. The corresponding vehicle solutions were devoid of any effect. Three sets of experiments were performed. Firstly, the motor effects of BW-245C, butaprost, PGA<sub>2</sub>, PGE<sub>1</sub>, PGE<sub>2</sub>, sulprostone (1–1000 nM), PGD<sub>2</sub> (3–300 nM) and iloprost (1–100 nM) were studied, these substances being added in a cumulative manner at 15 min intervals (Heinemann *et al.*, 1999; Shahbazian *et al.*, 2001). These intervals were long enough for agonist effects to reach a maximum, but too short for agonist effects to decline before the segments were exposed to the next agonist concentration (Figure 1). Furthermore, the test period of 15 min enabled us to observe agonist effects on at least 4 peristaltic waves, even when the frequency of peristaltic waves was as low as 0.3 Hz (Figure 1).

Secondly, the motor responses to PGD<sub>2</sub> (3–300 nM), butaprost, PGE<sub>1</sub>, sulprostone, U-46,619 (1–1000 nM) and LTD<sub>4</sub> (10–100 nM) were studied in the presence of vehicle, the EP<sub>1</sub> receptor antagonist SC-51,089 (1 μM), the DP/EP<sub>1</sub>/EP<sub>2</sub> receptor antagonist AH-6809 (30 μM), the TP receptor antagonist SQ-29,548 (10 μM) or the cysLT<sub>1</sub> receptor antagonist tomelukast (10 μM). The receptor antagonists or their vehicle were added to the organ bath 15 min before the recording of the cumulative concentration–response curves for the agonists was begun. Thirdly, the susceptibility of the peristaltic motor effects of PGE<sub>1</sub> and U-46,619 (1–1000 nM) to the α<sub>2</sub> adrenoceptor antagonist yohimbine (0.3 μM, pre-exposure time 15 min) were investigated. Each protocol was carried out with at least five segments from five different guinea-pigs.

### Evaluation of peristalsis

The recordings of peristalsis were analysed with the software Peristal 1.0 with regard to four different parameters: the peristaltic pressure threshold (PPT), the amplitude (maximal pressure) of the peristaltic waves, the maximal acceleration of the peristaltic waves, and the residual baseline pressure. PPT (Pa) is the intraluminal pressure at which a peristaltic wave is triggered. The residual baseline pressure (Pa) equals the minimal intraluminal pressure that is achieved after completion of each peristaltic wave and thus reflects the emptying capacity of the peristaltic waves (Shahbazian *et al.*, 2001). Further indices of peristaltic performance are the amplitude of the peristaltic waves (Pa) and the maximal acceleration of the peristaltic waves (Pa s<sup>-2</sup>), which is determined not only by the speed with which the muscle contracts but also by the speed with which the contraction moves aborally to empty the segments. The peristalsis parameters of three consecutive peristaltic waves that took place immediately before administration of any substance were averaged to determine the characteristics of peristalsis at baseline. An analogous procedure was applied to calculate the responses to the receptor antagonists and agonists under study. Thus, the influence of receptor antagonists on peristalsis was deduced from the mean parameters of three peristaltic waves that were recorded 15 min post-administration, immediately before the recording of the cumulative agonist concentration–response

curves was begun. The motor effects of receptor agonists were evaluated by measuring the peak changes that occurred during the 15 min observation periods and averaging the parameters of three peristaltic waves at the peak effect.

### Drugs and solutions

The sources of the drugs used here were as follows. PGD<sub>2</sub>, PGE<sub>1</sub>, PGE<sub>2</sub>, LTD<sub>4</sub>, [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptanoic acid (SQ-29,548) and yohimbine hydrochloride were obtained from Sigma (Vienna, Austria). Butaprost, (±)-3-(3-cyclohexyl-3-hydroxypropyl)-2,5-dioxo-4-imidazolidineheptanoic acid (BW-245C), 9,11-dideoxy-9α,11α-methanoepoxy-PGF<sub>2α</sub> (U-46,619), 6-isopropoxy-9-oxoxanthene-2-carboxylic acid (AH-6,809), PGA<sub>2</sub> and sulprostone were bought from Cayman (Ann Arbor, MI, U.S.A.). 8-Chlorodibenz[b,f][1,4]oxazepine-10(11H)-carboxylic acid, 2-[1-oxo-3(4-pyridinyl)propyl]hydrazide monohydrochloride (SC-51,089) and tomelukast (LY-171,883) were purchased from Biomol (Hamburg, Germany). Iloprost was a gift of Schering AG (Berlin, Germany). The drugs were dissolved with appropriate media, the concentrations given hereafter in parentheses referring to the stock solutions. BW-245C (3 mM), butaprost (1 mM), PGD<sub>2</sub> (3 mM), SQ-29,548 (10 mM), sulprostone (1 mM) and tomelukast (10 mM) were dissolved in ethanol, SC-51,089 (10 mM) in distilled water, yohimbine hydrochloride (1 mM) in Tyrode solution, AH-6,809 and U-46,619 (10 mM) in dimethyl sulphoxide, and PGE<sub>1</sub> (1 mM) and PGE<sub>2</sub> (1 mM) in methanol. PGA<sub>2</sub> (1 mM) was supplied in a methanol/ammonium acetate buffer of pH 5.6, while iloprost was provided in aqueous solution (100 μg ml<sup>-1</sup>). The stock solutions were diluted with Tyrode solution as required. Care was taken that none of the organic solvents reached concentrations higher than 0.3% in the bathing solution.

### Statistics

The data are presented as means ± s.e. mean of *n* experiments, *n* referring to the number of guinea-pigs used in the test. The results were evaluated with Student's paired *t*-test or one-way analysis of variance (ANOVA) for repeated measures followed by Dunnett's test, as appropriate. Probability values of *P* < 0.05 were regarded as significant.

## Results

### Baseline peristalsis

Quantitative estimates of the peristalsis parameters at baseline were: PPT, 71 ± 2 Pa; residual baseline pressure, 13 ± 1 Pa; maximal pressure of peristaltic waves, 622 ± 7 Pa; and maximal acceleration of peristaltic waves 317 ± 7 Pa s<sup>-2</sup> (*n* = 147).

### Peristaltic motor effects of selective PG receptor agonists

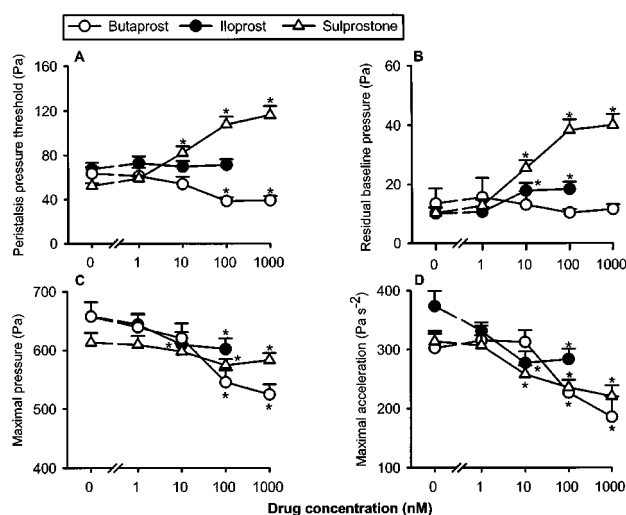
The EP<sub>1</sub>/EP<sub>3</sub> receptor agonist sulprostone, the EP<sub>2</sub> receptor agonist butaprost and the EP<sub>1</sub>/IP receptor agonist iloprost caused distinct and concentration-dependent changes in the

peristalsis parameters under study (Figures 1A,B,C and 2). Thus, PPT was decreased by butaprost (1–1000 nM), left unchanged by iloprost (1–100 nM), and increased by sulprostone (1–1000 nM). The maximal pressure (amplitude) and the maximal acceleration of the peristaltic waves was concentration-dependently attenuated by sulprostone, butaprost and iloprost (Figures 1A,B,C and 2). A further parameter of peristaltic performance, the residual baseline pressure, remained unaltered by butaprost but was enhanced by sulprostone and iloprost (Figure 2B). It should not go unnoticed that all peristaltic motor changes elicited by iloprost were quantitatively minor (Figures 1C and 2).

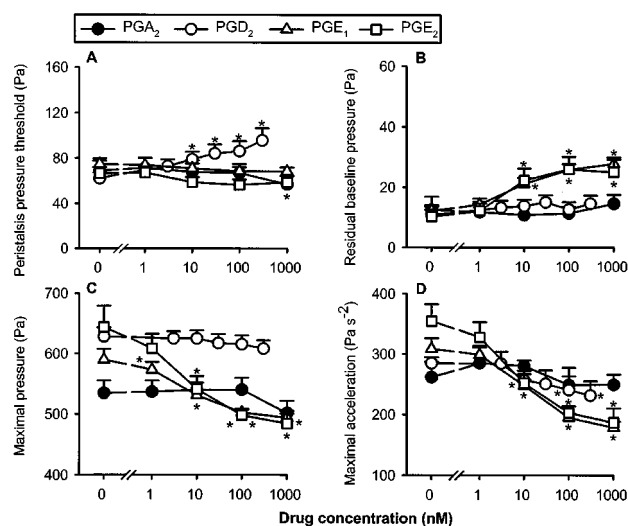
The selective DP receptor agonist BW-245C (1, 10, 100 and 1000 nM) failed to alter PPT, the residual baseline pressure and the maximal acceleration of the peristaltic waves ( $n=6$ , data not shown). The maximal pressure of the peristaltic waves was also left unchanged by BW-245C at the concentrations of 1–100 nM, while 1000 nM BW-245C enhanced this parameter to  $109 \pm 3.4\%$  of that measured under baseline conditions ( $n=6$ ,  $P < 0.05$ , ANOVA for repeated measures followed by Dunnett's test).

#### Peristaltic motor effects of natural PG receptor agonists

Exposure of the intestinal segments to  $\text{PGA}_2$ ,  $\text{PGD}_2$ ,  $\text{PGE}_1$  and  $\text{PGE}_2$  caused various patterns of peristaltic motor changes (Figure 3). PPT remained practically unchanged by  $\text{PGA}_2$ ,  $\text{PGE}_1$  and  $\text{PGE}_2$  (1–1000 nM) and was slightly, but significantly, increased by  $\text{PGD}_2$  (3–300 nM). The maximal pressure of the peristaltic waves was attenuated by  $\text{PGE}_1$  and  $\text{PGE}_2$ , but not significantly altered by  $\text{PGA}_2$  and  $\text{PGD}_2$ , whereas the maximal acceleration of the peristaltic waves was decreased by  $\text{PGE}_1$ ,  $\text{PGE}_2$  and  $\text{PGD}_2$  but not  $\text{PGA}_2$  (Figure 3).  $\text{PGE}_1$  and  $\text{PGE}_2$  also increased the residual baseline



**Figure 2** Concentration-dependent effects of butaprost, iloprost and sulprostone to alter the peristaltic pressure threshold (A), residual baseline pressure (B), maximal pressure (C) and maximal acceleration (D) of the peristaltic waves. The concentration–response curves were recorded in a cumulative manner at 15 min intervals. The values represent means  $\pm$  s.e.mean,  $n=12$  for butaprost,  $n=13$  for iloprost,  $n=16$  for sulprostone. \* $P < 0.05$  versus parameters recorded immediately before drug administration when the drug concentration was '0' (ANOVA for repeated measures followed by Dunnett's test).



**Figure 3** Concentration-dependent effects of prostaglandin  $\text{A}_2$  ( $\text{PGA}_2$ ),  $\text{PGD}_2$ ,  $\text{PGE}_1$  and  $\text{PGE}_2$  to alter the peristaltic pressure threshold (A), residual baseline pressure (B), maximal pressure (C) and maximal acceleration (D) of the peristaltic waves. The concentration–response curves were recorded in a cumulative manner at 15 min intervals. The values represent means  $\pm$  s.e.mean,  $n=5$  for  $\text{PGA}_2$ ,  $n=12$  for  $\text{PGD}_2$ ,  $n=27$  for  $\text{PGE}_1$ ,  $n=10$  for  $\text{PGE}_2$ . \* $P < 0.05$  versus parameters recorded immediately before drug administration when the drug concentration was '0' (ANOVA for repeated measures followed by Dunnett's test).

pressure, while  $\text{PGA}_2$  and  $\text{PGD}_2$  were without significant influence on this parameter (Figure 3B).

#### Peristaltic motor effects of PG receptor agonists in the presence of PG receptor antagonists

Exposure of intestinal segments to the  $\text{EP}_1$  receptor antagonist SC-51,089 (1  $\mu\text{M}$ ) failed to alter baseline peristalsis to any significant degree, as deduced from a lack of effect on PPT (Table 1) and the other parameters of peristalsis under study (not shown). In contrast, the DP/ $\text{EP}_1$ / $\text{EP}_2$  receptor antagonist AH-6809 (30  $\mu\text{M}$ ) increased PPT (Table 1) and decreased the maximal pressure and acceleration of the peristaltic waves to a significant extent ( $P < 0.01$ ,  $n=22$ , data not shown). Neither SC-51,089 nor AH-6809 was able to modify the peristaltic motor effects of sulprostone, i.e., to enhance PPT and the residual baseline pressure and to attenuate the maximal pressure and the maximal acceleration of the peristaltic waves (Figure 4). SC-51,089 likewise failed to alter the peristaltic motor changes elicited by 1–1000 nM butaprost ( $n=7$ , data not shown) and 1–1000 nM  $\text{PGE}_1$  ( $n=7$ , data not shown). The peristaltic motor responses to 3–300 nM  $\text{PGD}_2$  were not modified by AH-6809 ( $n=10$ , data not shown).

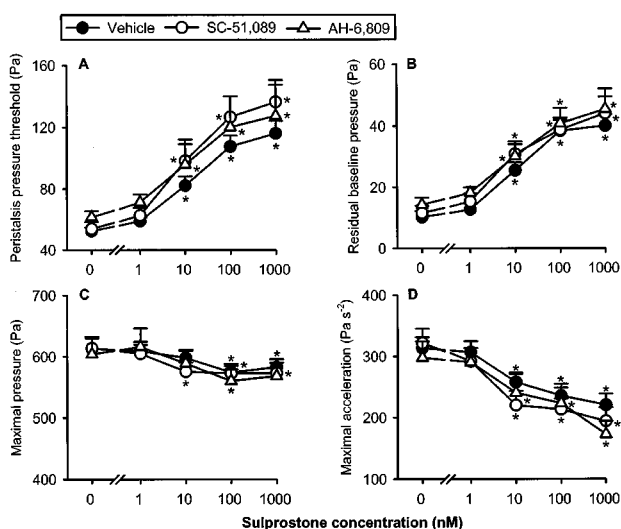
#### Peristaltic motor effects of $\text{PGE}_1$ in the presence of yohimbine

The  $\alpha_2$ -adrenoceptor antagonist yohimbine (0.3  $\mu\text{M}$ ) was devoid of any influence on baseline peristalsis (Table 1) and failed to alter the peristaltic motor changes elicited by 1–1000 nM  $\text{PGE}_1$  ( $n=14$ , data not shown).

**Table 1** Effect of SC-51,089, AH-6,809, SQ-29,548, tomelukast and yohimbine on the peristaltic pressure threshold (PPT)

Treatment	n	PPT before treatment (Pa)	PPT after treatment (Pa)
Vehicle	147	71 ± 2	68 ± 2
SC-51,089 (1 μM)	23	72 ± 5	76 ± 6
AH-6,809 (10 μM)	22	52 ± 3	59 ± 3**
SQ-29,548 (10 μM)	15	64 ± 5	67 ± 6
Tomelukast (1 μM)	7	62 ± 10	66 ± 11
Yohimbine (0.3 μM)	29	72 ± 5	75 ± 5

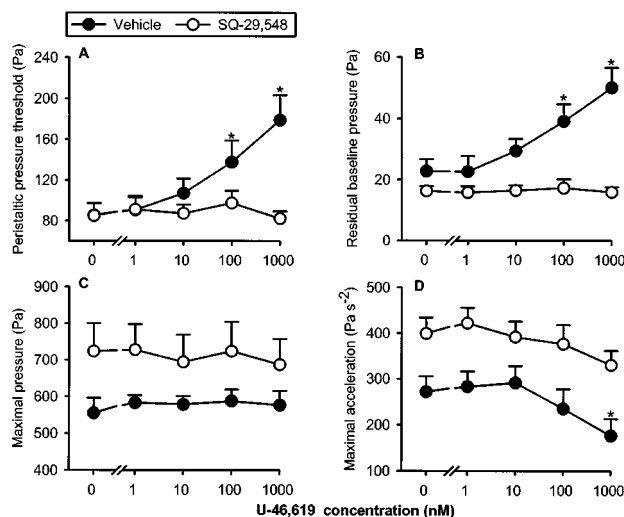
PPT was determined immediately before and 15 min after administration of the drugs or their vehicle to the organ bath. The data given are means ± s.e.mean. None of the drugs except AH-6,809 (\*\* $P < 0.01$ , paired  $t$ -test) caused any significant alteration of PPT.



**Figure 4** Concentration-dependent effect of sulprostone to alter the peristaltic pressure threshold (A), residual baseline pressure (B), maximal pressure (C) and maximal acceleration (D) of the peristaltic waves as assessed in the presence of vehicle ( $n = 16$ ), SC-51,089 ( $n = 9$ ) and AH-6809 ( $n = 7$ ). The concentration–response curves were recorded in a cumulative manner at 15 min intervals. The values represent means + s.e.mean. \* $P < 0.05$  versus parameters recorded immediately before addition of sulprostone as indicated by '0' (ANOVA for repeated measures followed by Dunnett's test).

#### Peristaltic motor effects of U-46,619 in the absence and presence of SQ-29,548 and yohimbine

The TP receptor agonist U-46,619 caused distinct changes in the peristalsis parameters under study (Figures 1D and 5). Thus, PPT and the residual baseline pressure were enhanced by U-46,619 (1–1000 nM) whereas the maximal acceleration of the peristaltic waves was reduced. In contrast, the maximal pressure of the peristaltic waves was not significantly altered by U-46,619 (Figure 5) although in some experiments this parameter of peristalsis was attenuated (Figure 1D). The action of U-46,619 to alter peristaltic motility was suppressed by the TP receptor antagonist SQ-29,548 (10 μM). Thus, the U-46,619-induced increase in PPT and residual baseline pressure and decrease in the maximal acceleration of the



**Figure 5** Concentration-dependent effect of U-46,619 to alter the peristaltic pressure threshold (A), residual baseline pressure (B), maximal pressure (C) and maximal acceleration (D) of the peristaltic waves as assessed in the presence of vehicle ( $n = 6$ ) and SQ-29,548 ( $n = 6$ ). The concentration–response curves were recorded in a cumulative manner at 15 min intervals. The values represent means + s.e.mean. \* $P < 0.05$  versus parameters recorded immediately before addition of U-46,619 as indicated by '0' (ANOVA for repeated measures followed by Dunnett's test).

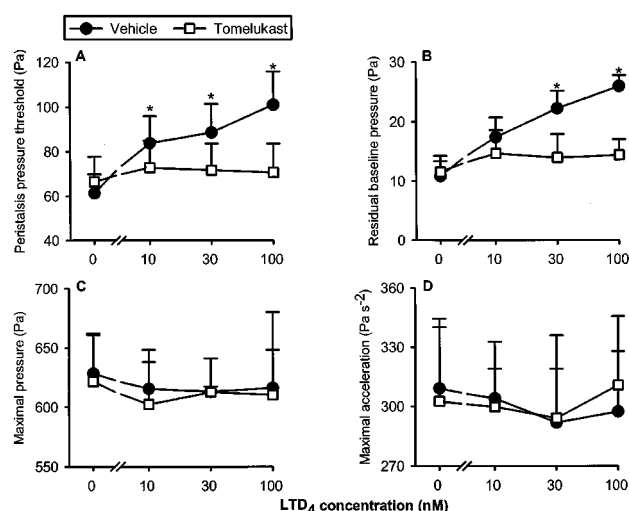
peristaltic waves were no longer seen when SQ-29,548 was present in the bath (Figure 5). However, baseline peristalsis was not influenced by SQ-29,548 (Table 1). The  $\alpha_2$ -adrenoceptor antagonist yohimbine (0.3 μM) failed to modify the peristaltic motor effects of U-46,619 ( $n = 7$ , data not shown).

#### Peristaltic motor effects of LTD<sub>4</sub> in the absence and presence of tomelukast

LTD<sub>4</sub> (10–100 nM) enhanced PPT and the residual baseline pressure in a concentration-dependent manner, although the magnitude of these peristaltic motor changes was minor (Figure 6). The maximal pressure and the maximal acceleration of the peristaltic waves were not modified by LTD<sub>4</sub>. Exposure of the intestinal segments to the cysLT<sub>1</sub> receptor antagonist tomelukast (10 μM) failed to alter baseline peristalsis to any significant degree as deduced from a lack of effect on PPT (Table 1) and the other parameters of peristalsis under study (data not shown). Tomelukast, however, prevented the peristaltic motor alterations caused by LTD<sub>4</sub> (Figure 6).

## Discussion

The effects of prostanoid receptor agonists and LTD<sub>4</sub> on propulsive peristalsis in segments from the guinea-pig small intestine were examined with a method that had previously been employed to analyse drug actions on this clinically relevant motor pattern of the gut (Heinemann *et al.*, 1999; Shahbazian *et al.*, 2001). Elicited by mechanical stimulation of the mucosa or distension of the intestinal wall, peristalsis



**Figure 6** Concentration-dependent effect of leukotriene D<sub>4</sub> (LTD<sub>4</sub>) to alter the peristaltic pressure threshold (A), residual baseline pressure (B), maximal pressure (C) and maximal acceleration (D) of the peristaltic waves as assessed in the presence of vehicle ( $n=7$ ) and tomelukast ( $n=7$ ). The concentration-response curves were recorded in a cumulative manner at 15 min intervals. The values represent means  $\pm$  s.e.mean. \* $P<0.05$  versus parameters recorded immediately before addition of LTD<sub>4</sub> as indicated by '0' (ANOVA for repeated measures followed by Dunnett's test).

involves ascending excitatory, descending inhibitory and descending excitatory reflexes within the enteric nervous system (Costa *et al.*, 2000). These reflexes result in an aborally moving pattern of temporally and spatially coordinated contractions and relaxations of the circular muscle. The focus of our *in vitro* set-up was to refine the analysis of drug-induced peristaltic motor alterations by recording and calculating four parameters of peristalsis. Changes in PPT reflect modifications in the sensitivity of the peristaltic nerve-muscle circuitry to distension, whereas alterations in the maximal pressure (amplitude), maximal acceleration and/or residual baseline pressure of the peristaltic waves indicate modifications of peristaltic motor performance. In this way, it was possible to differentiate between four categories of drug-induced peristaltic motor alterations: a decrease in distension sensitivity and peristaltic performance (sulprostone and U-46,619); a decrease in distension sensitivity without a major change in peristaltic performance (PGD<sub>2</sub> and LTD<sub>4</sub>); a decrease in peristaltic performance without a major change in distension sensitivity (PGE<sub>1</sub>, PGE<sub>2</sub> and iloprost); and a decrease in peristaltic performance associated with an increase in distension sensitivity (butaprost).

In view of this heterogeneity of prostanoid and LTD<sub>4</sub> actions on intestinal peristalsis it was the major aim of this study to define the particular patterns of peristaltic motor effects elicited by selective DP, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, IP, TP and LT receptor activation. This goal was addressed by the use of receptor-selective agonists and antagonists. We did, however, not resort to the comparative study of agonist potencies, because the relative potency of the test compounds in our *in vitro* preparation is determined both by their receptor affinity and pharmacokinetic behaviour. This is because compounds administered into the organ bath need to penetrate the serosa and longitudinal muscle before they reach the myenteric

plexus and circular muscle. In addition, it need to be taken into account that the peristalsis-modifying effect of the test compounds reflects their net action on multiple receptors at multiple sites of the nerve-muscle system subserving peristalsis.

In a first step, the DP receptor agonist BW-245C (Town *et al.*, 1983), the EP<sub>1</sub>/EP<sub>3</sub> receptor agonist sulprostone (Racké *et al.*, 1995; Narumiya *et al.*, 1999; Walch *et al.*, 2001; Kim *et al.*, 2002), the EP<sub>2</sub> receptor agonist butaprost (Lawrence *et al.*, 1992; Narumiya *et al.*, 1999), the EP<sub>1</sub>/IP receptor agonist iloprost (Dong *et al.*, 1986; Lawrence *et al.*, 1992) and the TP receptor agonist U-46,619 (Eglen & Whiting, 1988; Narumiya *et al.*, 1999) were characterized in their concentration-dependent actions on intestinal peristalsis. This agonist-directed identification of receptors was complemented by the use of effective, yet selective concentrations of the EP<sub>1</sub> receptor antagonist SC-51,089 (1  $\mu$ M; Hallinan *et al.*, 1993), the DP/EP<sub>1</sub>/EP<sub>2</sub> receptor antagonist AH-6,809 (30  $\mu$ M; Eglen & Whiting, 1988; Walch *et al.*, 2001), the TP receptor antagonist SQ-29,548 (10  $\mu$ M; Ogletree *et al.*, 1985) and the cysLT<sub>1</sub> receptor antagonist tomelukast (10  $\mu$ M; Fleisch *et al.*, 1985).

The first conclusion derived from the current study is that EP<sub>2</sub> receptor activation by the selective agonist butaprost (Lawrence *et al.*, 1992; Narumiya *et al.*, 1999) attenuates peristaltic performance in parallel with an increase in distension sensitivity, i.e., a decrease in PPT. Since EP<sub>2</sub> receptors have been suggested to occur on sensory and other neurons of the enteric nerve plexuses (Lawrence *et al.*, 1992; Dekkers *et al.*, 1997) it is tempting to postulate that butaprost enhances distension sensitivity by an action on enteric neurons. In contrast, the butaprost-induced attenuation of peristaltic performance is likely to be mediated by EP<sub>2</sub> receptors on intestinal muscle, given that muscular EP<sub>2</sub> receptors have been reported to bring about relaxation (Botella *et al.*, 1993). Another important conclusion deduced from this study is that EP<sub>3</sub> receptor activation results in a decrease in distension sensitivity and peristaltic performance. Our inference is based on the observation that the EP<sub>1</sub> receptor antagonists SC-51,809 and AH-6809 failed to inhibit the peristaltic motor response to the EP<sub>1</sub>/EP<sub>3</sub> receptor agonist sulprostone. Consequently, the sulprostone-evoked decrease in distension sensitivity and peristaltic performance is most probably mediated by EP<sub>3</sub> receptors. Such a pattern of peristaltic motor modification was impossible to predict from the reported effects of sulprostone on intestinal nerve and muscle which bear EP<sub>3</sub> receptors (Lawrence *et al.*, 1992; Botella *et al.*, 1993; Morimoto *et al.*, 1997; Northey *et al.*, 2000; Okada *et al.*, 2000). This is because peristaltic motility involves excitatory and inhibitory motor reflexes (Costa *et al.*, 2000) and, as a consequence, the peristaltic motor alterations caused by the test compounds cannot be categorized as actions mediated by contraction- or relaxation-promoting prostanoid receptors (Coleman *et al.*, 1994; Narumiya *et al.*, 1999). Because any drug that blocks nerve activity eliminates peristalsis (Johnson *et al.*, 1996; Holzer *et al.*, 1998), it was not possible to sort out in our preparation whether the test compounds influence propulsive motility by a neural or muscular site of action.

The inability of the EP<sub>1</sub> receptor antagonists SC-51,089 and AH-6809 to inhibit the peristaltic motor effects of sulprostone, PGE<sub>1</sub>, and, expectedly, butaprost, is important

to consider with respect to the role of EP<sub>1</sub> receptors in intestinal peristalsis. From our data it would appear that EP<sub>1</sub> receptors, which are abundantly present in the guinea-pig ileum (Eglen & Whiting, 1988; Lawrence *et al.*, 1992), have no bearing on propulsive motility. If a role of EP<sub>1</sub> receptors in peristaltic motility of the guinea-pig small intestine is negated, the peristaltic motor response to the EP<sub>1</sub>/IP receptor agonist iloprost (Dong *et al.*, 1986; Lawrence *et al.*, 1992) must be regarded as being due solely to IP receptor activation. Thus, we conclude that IP receptor activation leads to a decrease in peristaltic performance without any change in distension sensitivity. This pattern of peristaltic motor modification is in keeping with the ability of IP receptor agonists to relax intestinal muscle by a direct action on myocytes (Lawrence *et al.*, 1992).

The effects of PGE<sub>1</sub> and PGE<sub>2</sub> to decrease peristaltic performance without changing distension sensitivity are in line with the reported action of PGE<sub>1</sub> to inhibit peristalsis-like contractions in the guinea-pig ileum (Radmanovic, 1972) and of the PGE<sub>2</sub> analogue enprostil to impair antroduodenal motility in man (Hausken *et al.*, 1991). A comparison with the motor responses to butaprost and sulprostone suggests that both EP<sub>2</sub> and EP<sub>3</sub> receptors contribute to the net effects of PGE<sub>1</sub> and PGE<sub>2</sub> on propulsive motility. Since PGA<sub>2</sub> was without major influence on peristalsis, we assume that PGE<sub>2</sub> modifies peristaltic motility *per se* rather than through an action of its dehydration metabolite PGA<sub>2</sub>. Major differences in the induction, recording, analysis and interpretation of peristaltic motility explain why our finding of an inhibitory action of PGE<sub>1</sub> and PGE<sub>2</sub> on propulsive peristalsis is at variance with reports that PGs of the E and F series induce or stimulate peristalsis-like motility in the guinea-pig isolated ileum and colon (Ishizawa & Miyazaki, 1975; Eley *et al.*, 1977; Fontaine *et al.*, 1997; Sanger & Watt, 1978; Grbovic & Radmanovic, 1987). The effect of PGD<sub>2</sub> to lower distension sensitivity without impairing peristaltic performance differed markedly from the motor actions of PGE<sub>1</sub> and PGE<sub>2</sub>. It can be ruled out, however, that the motor response to PGD<sub>2</sub> is brought about by DP receptors because only low levels of DP receptors occur in this small intestine (Eglen & Whiting, 1988; Narumiya *et al.*, 1999), the DP receptor agonist BW-245C (Town *et al.*, 1983) was without any appreciable influence on peristalsis and the DP/EP<sub>1</sub>/EP<sub>2</sub> receptor antagonist AH-6809 (Eglen & Whiting, 1988; Walch *et al.*, 2001) failed to prevent the peristaltic motor effect of PGD<sub>2</sub>. Concentrations of this weak DP antagonist higher than 30 µM could not be employed because of solubility limitations.

Characteristic modifications of peristaltic motility were not only induced by PG receptor agonists but also by the TP receptor agonist U-46,619 (Eglen & Whiting, 1988; Narumiya *et al.*, 1999) and LTD<sub>4</sub>. The decrease in distension sensitivity and peristaltic performance caused by U-46,619 was pre-

vented by SQ-29,548, which indicates that its action was indeed mediated by TP receptors. cysLT<sub>1</sub> receptors appear to be responsible for the ability of LTD<sub>4</sub> to decrease distension sensitivity without attenuating peristaltic performance, since the motor response to LTD<sub>4</sub> was prevented by the cysLT<sub>1</sub> receptor antagonist tomelukast (Fleisch *et al.*, 1985). Our results are in overall agreement with other reports that functional TP and cysLT<sub>1</sub> receptors are present in the gut (Sanger & Bennett, 1980; Gardiner *et al.*, 1990; Metters, 1995; Sametz *et al.*, 2000).

The α<sub>2</sub>-adrenoceptor antagonist yohimbine (0.3 µM; Marcoli *et al.*, 1985) was included in this study because it had previously been proposed that PGE<sub>1</sub> inhibits peristalsis in the guinea-pig ileum *via* activation of sympathetic neurons (Radmanovic, 1972), and the inhibitory motor action of sympathetic nerve stimulation is primarily brought about by α<sub>2</sub>-adrenoceptors on myenteric neurons (Marcoli *et al.*, 1985). In addition, prostanoids can modulate the release of noradrenaline from sympathetic nerve fibres in the gut (Racké *et al.*, 1995). In this study we failed, however, to reveal any contribution of α<sub>2</sub>-adrenoceptors to the peristaltic motor effects of PGE<sub>1</sub> as well as of U-46,619.

The inability of SC-51,089, SQ-29,548 and tomelukast to alter peristaltic motor activity at effective concentrations indicates that endogenous ligands at EP<sub>1</sub>, TP and cysLT<sub>1</sub> receptors do not participate in the physiological control of peristalsis. Whether the effect of AH-6,809 to lower distension sensitivity and peristaltic performance points to a physiological role of DP and/or EP<sub>2</sub> receptors in peristaltic motor regulation or reflects a non-specific action awaits to be elucidated. It seems conceivable that exacerbated production of prostanoids and LTs under pathological conditions modulates peristaltic motility as deduced from the high activity of PGD<sub>2</sub>, PGE<sub>1</sub>, PGE<sub>2</sub>, butaprost, sulprostone, iloprost, U-46,619 and LTD<sub>4</sub> to modify peristaltic performance and/or intestinal sensitivity to distension. This conjecture is consistent with a permissive action of prostaglandins in the fine tuning of peristaltic motility as deduced from the peristaltic motor effects of cyclo-oxygenase inhibitors (Shahbazian *et al.*, 2001). However, the magnitude of the peristaltic motor modifications brought about by prostanoid and LT receptor agonists is fairly small. If our data can be extrapolated to the human gut, it would appear that the ability of PGs to induce diarrhoea, which is their major effect in the intestine, is due to their prosecretory (Bunce & Spraggs, 1990; Beubler & Schuligoi, 2000) but not peristaltic motor action.

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