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European Journal of Pharmacology 539 (2006) 108–115
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Role of bradykinin B_2 receptors in the modulation of the peristaltic reflex of the guinea pig isolated ileum

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Received 14 January 2006; received in revised form 30 March 2006; accepted 3 April 2006
Available online 6 April 2006

Abstract

Bradykinin is well known to have a biphasic action to contract and relax gastrointestinal tissue. However, no studies have investigated the potential action of bradykinin to affect the peristaltic reflex. In the present study, serosally applied bradykinin (1–1000 nM) and the bradykinin B_2 receptor agonist, kallidin (1–1000 nM), had inhibitory actions and increased the pressure threshold for peristalsis (maximum changes seen at 1000 nM were ~60 Pa), as did morphine (IC_{50}=22.3±4.8 nM; maximum increase in the pressure threshold was ~130 Pa). Conversely, the B_1 kinin receptor agonist, [des-Arg^9]-bradykinin (1–1000 nM), had no effect ($P>0.05$). Two potent B_2 receptor antagonists, FR173657 (1 and 100 nM) and icatibant (10 nM), significantly antagonized the inhibitory action of serosally applied bradykinin on peristalsis ($P<0.01$), whilst the B_1 receptor antagonist, Lys-[des-Arg^9, Leu^8]-bradykinin (100 nM) was inactive ($P>0.05$). In comparison, 5-hydroxytryptamine (1–1000 nM) facilitated peristalsis (EC_{50}=37.7±23.0 nM; maximum reduction of the pressure threshold for peristalsis was ~76 Pa), as did FR173657 at 100 nM (reducing the pressure threshold for peristalsis by ~15 Pa; $P<0.05$) but icatibant at 10 nM was inactive ($P>0.05$). The results indicate that bradykinin B_2 receptors mediate an inhibition of peristalsis in the guinea pig isolated ileum.

Keywords: Bradykinin; B_2 receptor; Peristalsis; Ileum; FR173657; Icatibant (Hoe 140)

1. Introduction

The peristaltic reflex consists of regular and coordinated contractions of both longitudinal and circular smooth muscle and can be influenced by extrinsic nerves (Costa and Furness, 1976; Furness et al., 1994, 1999; Goyal and Hirano, 1996). The distension of the gastrointestinal tract by increases in intraluminal pressure represents a key mechanism to facilitate peristalsis, characterized by an initial preparatory phase involving longitudinal shortening, and an emptying phase, involving an advancing circular muscle contraction to propel luminal contents in the oro-anal direction (Trendelenberg, 1917). This sensory–motor arc involves mediators from enterochromaffin cells (e.g. 5-hydroxytryptamine (5-HT)), tension-sensitive neurones, chemosensitive neurones, and other sensory systems in the mucosal/submucosal side, and the myenteric plexus acting as a relay to modulate transmitter release (e.g. acetylcholine) to the smooth muscles (De Ponti et al., 1991; Olsson and Holmgren, 2001).

Bradykinin is an algesic autacoid nonapeptide that is well known to be involved in inflammation (Couture et al., 2001; Regoli et al., 1997). The actions of bradykinin are mediated through interaction with bradykinin B_1 and B_2 receptors, which are differentiated pharmacologically by the rank orders of potency of selected agonists and antagonists. B_1 receptors are preferentially activated by [des-Arg^9]-bradykinin and [des-Arg^{10}]-kallidin and specifically antagonized by Lys-[des-Arg^9, Leu^8]-bradykinin, whereas bradykinin and kallidin (Lys-bradykinin) are selective B_2 agonists and icatibant (Hoe 140) and FR173657 are selective antagonists (see Hall, 1997; Marceau and Regoli, 2004; Regoli et al., 1998, 1997). Several in vitro studies have shown that bradykinin and its analogues have actions on the gastrointestinal tract. In the ileum, bradykinin can release acetylcholine (Yau et al., 1986), but the predominant action is to directly contract longitudinal muscle, although an initial relaxatory response may predominate in pre-contracted tissues (Hall and Bonta, 1973). Biphasic

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doi:10.1016/j.ejphar.2006.04.002

responses are also seen on circular muscle preparations, with a relaxant action probably mediated via the generation of prostanoids (Calixto and Medeiros, 1991; Medeiros and Calixto, 1993). These observations, and in vivo/in situ studies in upper and lower gut, showing an ability of bradykinin to inhibit motility (Fasth et al., 1975; Holzer, 1992; Murrell and Deller, 1967; Pytkowski, 1979), probably involve B_2 receptors in the majority of species, although B_1 receptors may be induced following inflammatory stimuli (Marceau and Regoli, 2004; Zuzack et al., 1996).

The involvement of various substances on the peristaltic reflex is one of the leading topics in gastroenterological research. However, there have been no studies to address if bradykinin can modulate peristalsis, although the gastrointestinal tract (particularly the ileum) has been used to estimate the potency of bradykinin receptor antagonists (see above for references). In the present study, therefore, we set out to investigate if bradykinin can influence an ongoing peristaltic reflex and to characterize the identity of the bradykinin receptors involved. This was done using a range of bradykinin agonists and antagonists, in comparison with the facilitatory actions of 5-HT (Costall et al., 1993; Craig and Clarke, 1991) and the inhibitory actions of morphine (Heinemann and Holzer, 1999; Waterman et al., 1992).

2. Methods

2.1. Animals

Male Dunkin-Hartley guinea pigs (bred at the Chinese University of Hong Kong) weighing 0.5–1 kg were housed in groups of 2–3 and were maintained on a 12 h/12 h light/dark cycle at 21 ± 1.5 °C, with a relative humidity of 40–60%. They had free access to food (guinea pig pellet diet GPHK3, Glen Forrest Stockfeeders, Australia) and water. All experiments were conducted under license from the Government of the Hong Kong SAR and endorsement of the Animal Ethics Experimentation Committee, The Chinese University of Hong Kong.

2.2. Measuring peristalsis

Animals were fasted overnight and then killed by cervical dislocation and exsanguination. A 40 cm segment of ileum was taken 5 cm proximal to the ileocaecal junction transferred to a beaker containing Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.18 mM KH_2PO_4, 25.0 mM $NaHCO_3$, 21.0 mM glucose) at room temperature and gassed with 95% O_2 and 5% CO_2. 3 μM captopril was added to the Krebs–Henseleit solution in all experiments except those involving 5-HT and morphine, to prevent the metabolism of kinins by angiotensin I-converting enzyme (see Rizzi et al., 1997). The mesentery was removed and the lumen flushed of its contents taking care to minimize distension. A segment of ileum, about 7 cm in length, was cannulated at the oral and aboral ends and secured horizontally in a custom made water-jacketed Perspex bath, similar to that described by Costall et al. (1993). The ileum was compressed using forceps at the site just proximal to the aboral cannula to effect a localized destruction of sensory fibres to prevent the spontaneous contraction of the tissue. There were 4 baths in our system, each with a 40 cm^3 organ chamber. The glass oral and aboral cannulae (outer diameter 6.0 mm and internal diameter 2.8 mm) were moveable and entered the bath through opposite walls. The bath also had an outlet on the floor to drain the contents.

The outflow tube at the aboral end of the tissue had two outlets, one below the level of the bath and the other about 3 cm above the level of the fluid in the bath. The latter tube was bent into a J-shape, with a maximum height of 4 cm above the intraluminal fluid level. The intraluminal pressure of the ileum was measured from the oral side by a pressure transducer (P23XL, Spectramed, Statham) linked to a Maclab 4/Macintosh PowerMac computer running Chart v.3 software (AD Instruments Pty. Ltd., New South Wales, Australia) (sampling rate: 40 per min). Krebs–Henseleit solution was introduced into the lumen of the ileum using a peristaltic pump (Gilson Medical Electronics, France). The tissues were allowed to equilibrate for 30 min before peristalsis was induced. During this period, the lumen was continuously flushed with Krebs–Henseleit solution at a rate of 0.5 ml/min with the outlet below the bath open. The fluids in the baths were changed at least twice during the equilibration period. Thereafter, the lower outlet was closed and the fluid exiting the ileum was directed to the open J-tube. The perfusion rate was then increased to 1–2 ml/min to initiate peristaltic activity. Peristalsis was allowed to proceed for 30 min prior to exposure to agonists. All Krebs–Henseleit solutions were continuously gassed with 95% O_2 and 5% CO_2 and were maintained at 37 °C.

A crossover design was used in the experiments to randomize the treatments across the four baths according to a Latin square. In some experiments, antagonists were added to the Krebs–Henseleit solution in the reservoir and were equilibrated for 30 min before the initiation of peristalsis, and were present for the subsequent duration of the experiment. Non-cumulative concentration–response curves to drugs were constructed. To avoid tissue desensitization, the ileal segments were only challenged with no more than three concentrations of the drugs using three equilibration cycles. Thus, a six-point concentration–response curve was obtained from two ileal segments from the same animal. Application of drugs to the serosal side was done after obtaining regular peristalsis of fairly equal intervals in all four tissues. The volume of the drug administered did not exceed 1% of the bath volume and effects were observed for 10 min. To study the actions of drugs on the mucosal side, the agonists were administered to a 30 ml reservoir of Krebs–Henseleit solution. The actions of drugs were observed over a 10 min period. The washing out process and the equilibration cycle were identical to those in serosal side studies.

2.3. Data analysis

The intraluminal pressure threshold for eliciting peristaltic waves was used to quantify the effects of drugs on peristaltic

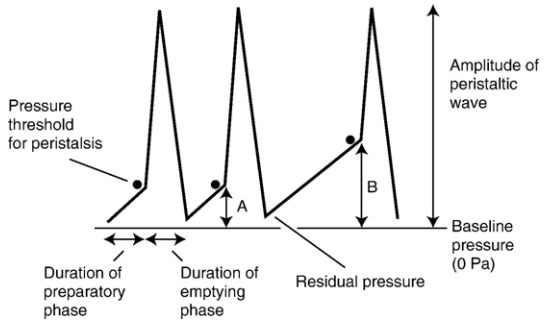

Fig. 1. Schematic diagram of a typical tracing obtained during peristalsis experiments. The change in pressure threshold for peristalsis was calculated as (B − A).

activity (see Fig. 1 for details). A facilitatory response was defined as any decrease in the pressure threshold, while an inhibitory effect was defined as an increase in the threshold, and/or abolition of peristalsis (Costall et al., 1993). The pressure threshold for three consecutive peristaltic contractions was averaged to determine the control values recorded immediately before administration of a drug in any one tissue. In the case of a facilitatory or an inhibitory effect, the same procedure was applied to calculate the lowest or highest values of drug-induced changes in the pressure threshold, respectively. Where applicable, log concentration–response curves were fitted using the non-linear iterative curve-fitting programme, Kaleidagraph (Synergy Software, PCS Inc., Reading, USA). In cases where the IC_{50} value could not be determined, the concentration at which the pressure threshold for peristalsis increased by 40 Pa was taken to compare the relative inhibitory potency of the agonists. Data represent mean ± S.E.M. Statistical significance was assessed by unpaired Student's *t*-test (Statsview, Abacus Concepts, Inc., Berkeley, USA), or one-way analysis of variance followed by pre-planned contrasts of specified means (SuperANOVA, Abacus Concepts, Inc., Berkeley, USA), as appropriate. The latter procedure is very efficient for comparing a limited subset of possible contrasts. This is more useful for testing hypotheses about data that are more specific than the hypotheses automatically tested for by each term in the ANOVA model (Gagnon et al., 1989). A probability value $P<0.05$ was regarded as significant.

2.4. Drugs

Bradykinin tfa salt (Research Biochemicals, USA), [des-Arg^9]-bradykinin acetate (Sigma, USA), Lys-bradykinin acetate (Sigma, USA), Lys-[des-Arg^9, Leu^8]-bradykinin (Bachem, Switzerland), Captopril (Fluka, Switzerland), 5-hydroxytryptamine creatinine sulphate (Sigma, USA), icatibant (Phoenix, USA) and morphine sulphate (Macfarlan and Smith, UK) were dissolved in distilled water and then diluted in Krebs–Henseleit solution. FR173657 ([(*E*)-3-(6-acetamido-3-pyridyl)-*N*-(*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl)acrylamide]) (Fujisawa, Japan) was dissolved in a minimum quantity of dimethylsulphoxide and then diluted in Krebs–Henseleit solution.

3. Results

3.1. General observations

The pressure threshold for peristalsis varied in the tissues obtained both from the same animal and also from different animals. The mean threshold of 61 segments taken from 16 animals was 56 ± 20 Pa.

3.2. Effect of 5-HT

The serosal application of 5-HT (1–1000 nM) caused a concentration-dependent facilitation of peristalsis, with an EC_{50} value of 37.7 ± 23.0 nM ($n=5$; Fig. 2B). The facilitation was characterized by a decrease in the pressure threshold for peristalsis, with a maximum reduction of 76.4 ± 16.0 Pa. The action of 5-HT developed within 1 min and lasted for 1.5–3.0 min (Fig. 2A). The stimulatory effect was accompanied by a decrease in the interval between peristaltic strokes. However, the latter parameter was not quantified, since it has been shown that the concentration–response curves to 5-HT for a reduction of the pressure threshold and the interval between the peristaltic strokes are indistinguishable (Costall et al., 1993). The maximum facilitatory action of 5-HT occurred at 100 nM and 1000 nM. In some tissues at maximal stimulation, the

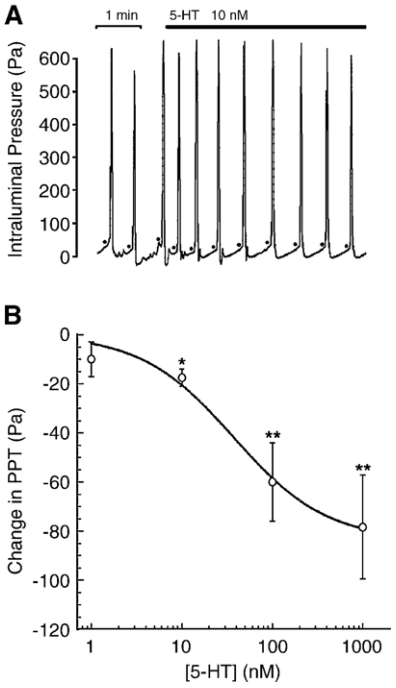

Fig. 2. (A) A representative intraluminal pressure tracing showing a facilitation of peristalsis induced by 5-hydroxytryptamine (5-HT) 10 nM. The decrease in the pressure threshold for peristalsis occurred within 1 min and lasted for approximately 2 min. (B) The facilitatory action of 5-HT on the peristaltic reflex. 5-HT was applied serosally to the tissues. Values are the mean ± S.E.M. of 5 determinations of the pressure threshold for peristalsis (PPT). Significant differences relative to the respective control PPT values prior to 5-HT addition are indicated as $*P<0.05$, $**P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). The PPT prior to drug addition was 66.2 ± 5.5 Pa (10 tissues).

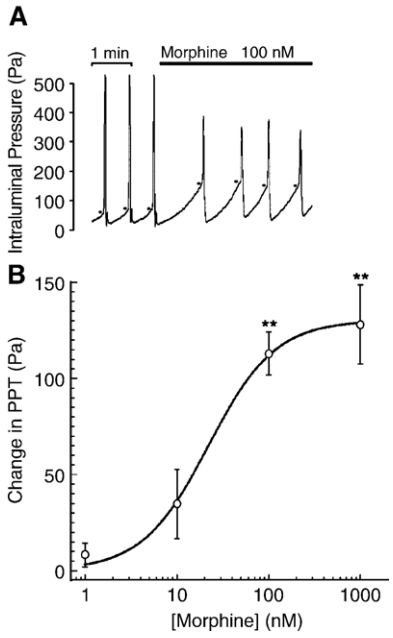

Fig. 3. (A) A representative intraluminal pressure tracing showing an inhibition of peristalsis induced by morphine 100 nM. (B) The inhibitory action of morphine on the peristaltic reflex. Morphine was applied serosally to the tissues. Values are the mean±S.E.M. of 4 determinations of the pressure threshold for peristalsis (PPT). Significant differences relative to the respective control PPT values prior to the addition of morphine are indicated as $*P<0.05$, $**P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). The PPT prior to drug addition was 67.1±7.2 Pa (8 tissues).

contraction of the circular muscle at the oral side had started before the previous contraction reached the aboral side. When this occurred, the pressure threshold of peristalsis was taken as 0 Pa. In no case did 5-HT at concentrations below 1000 nM exert any inhibitory effect on the peristaltic reflex. However, at a concentration of 1000 nM, the response to 5-HT was complex. An initial facilitatory effect was usually observed, followed by slight inhibition. The inhibitory effect of 5-HT developed slowly over 2–5 min and was characterized by an increase in the peristaltic threshold.

3.3. Effect of morphine

Morphine (1–1000 nM) increased the pressure threshold of peristalsis in a concentration-related manner with an IC_{50} value of 22.3±4.8 nM ($n=8$; Fig. 3B). The maximum increase in the threshold pressure for peristalsis was 130±17 Pa and was observed at 1000 nM. Morphine appeared to reduce the frequency and amplitude of the peristaltic waves and increased the residual pressure in the ileum (Fig. 3A). The inhibitory effect of morphine was rapid in onset, affecting the first peristaltic wave following its administration, and lasted for the duration of the observation period (i.e. 10 min). The effect of morphine was reversible on washing the tissue. At a concentration of 1000 nM, morphine abolished peristalsis. In some tissues, the disruption of the regular pattern of peristalsis was depicted by non-propulsive spasms of the circular muscle, which were interrupted by brief periods of coordinated peristalsis.

3.4. Effect of bradykinin receptor agonists

Bradykinin and Lys-bradykinin (1–1000 nM) increased the pressure threshold of peristalsis in a concentration-related manner (Fig. 4A). The action of bradykinin, particularly at high concentrations (100–1000 nM), was time-dependent: immediately after the administration, there was a rapid increase in the pressure threshold followed by sustained contraction of the circular muscle, which lasted about 1 min (Fig. 5A,B). The regular pattern of peristalsis gradually returned in all tissues but there was an increase in the residual pressure, the pressure threshold and the frequency of the peristaltic waves. The amplitude of the peristaltic waves remained unaffected until almost the end of the observation period (roughly 7–9 min after dosing) at which a fall in amplitude was usually observed with a concurrent drop in frequency (Fig. 5A,B). Complete abolition of peristalsis was seen in 1 out of 5 tissues (data not shown). Small inhibitory responses were observed even at the lowest concentration of bradykinin (1 nM) and this was characterized by an increase in the pressure threshold for peristalsis. The

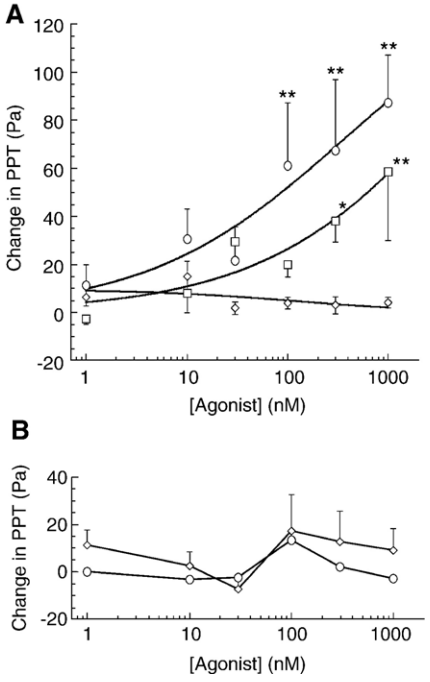

Fig. 4. (A) The action of inhibitory action of serosally applied bradykinin (○), kallidin (□) and [des-Arg^9]-bradykinin (◇) on peristalsis. Values are the mean± S.E.M. of 4–5 determinations of the pressure threshold for peristalsis (PPT). Significant differences relative to the respective control PPT values prior to agonist addition are indicated as $*P<0.05$, $**P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). The PPT prior to drug addition was 58.8±2.4 Pa (28 tissues). (B) Lack of effect of mucosally applied bradykinin (○) and [des-Arg^9]-bradykinin (◇) to modify peristalsis. Values are the mean±S.E.M. of 2–3 determinations. None of agonists showed significant differences relative to the respective control PPT values prior to the agonist addition ($P>0.05$; repeated measures 1 within 1 between one-way ANOVA) (10 tissues).

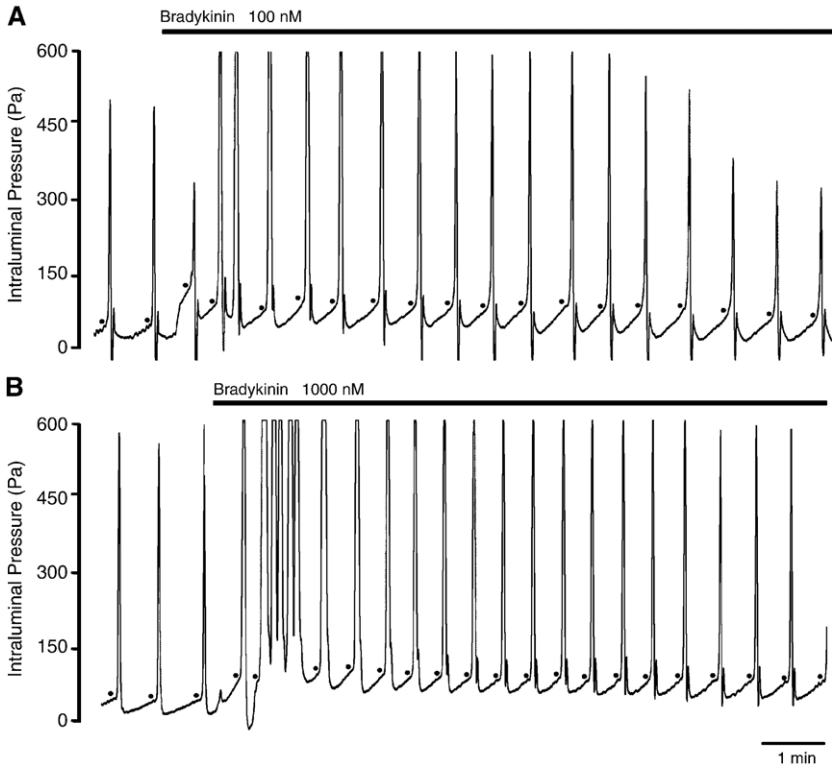

Fig. 5. Representative traces showing the inhibitory action of action of bradykinin on the peristaltic reflex. (A) The inhibitory action of bradykinin 100 nM on peristalsis was characterized as a rapid increase in pressure threshold for peristalsis (PPT) immediately after the addition of the drug and a rise of the residual intraluminal pressure. An increase in frequency of the peristaltic wave was also observed, which lasted approximately 8–10 min, followed by a decrease in the frequency and amplitude of the peristaltic stroke. (B) Bradykinin 1000 nM produced non-propulsive spasms of the circular muscle immediately after a rapid increase in PPT. Peristalsis recovered with an increased frequency and residual pressure.

action of Lys-bradykinin was similar to that of bradykinin, but the threshold concentration that caused an observable inhibition of peristalsis was 10 nM (Fig. 4A). Kallidin also increased the residual pressure in the system. However, none of the agonists produced a maximum response in the tissues (at 1000 nM both agonists increased the pressure threshold by ~60 Pa), even at the highest concentrations used. Therefore, the concentration at which the pressure threshold for peristalsis increased by 40 Pa was taken to compare the relative potency of the agonists. The concentration of bradykinin producing a 40 Pa increase in pressure threshold was approximately 43 nM ($n=5$; calculated from regression analysis of mean data). Kallidin was approximately 8 times less potent than bradykinin with 360 nM being required to increase the pressure threshold for peristalsis by 40 Pa ($n=4$). The slopes of the log concentration–response curves to bradykinin and kallidin were 3.65±3.30 and 0.92±0.43, respectively, which were not significantly different ($P>0.05$). The B_1 kinin receptor agonist, [des-Arg^9]-bradykinin, did not modify significantly the pressure threshold for peristalsis ($n=5$). However, analysis of the log concentration–response curve revealed a slope of 7.68±5.28 that was statistically different from the slope of the concentration–response curve to bradykinin ($P<0.05$; Fig. 4A).

The actions of bradykinin (1–1000 nM; $n=2$) and [des-Arg^9]-bradykinin (1–1000 nM; $n=3$) on peristalsis applied on the mucosal side were briefly investigated. However, both agonists were ineffective to modulate peristalsis (Fig. 4B).

3.5. Effect of kinin receptor antagonists

FR173657 (1 nM), icatibant (10 nM), and Lys-[des-Arg^9, Leu^8]-bradykinin (100 nM) failed to affect peristalsis significantly ($P>0.05$; Table 1). However, FR173657 at 100 nM reduced significantly the threshold for peristalsis by approximately 15 Pa ($P<0.05$; Table 1).

FR173657 (1 and 100 nM) antagonized the inhibitory action of bradykinin on peristalsis, producing a rightward displacement of the log concentration–response curve (Fig. 6). There were significant differences in the change in pressure threshold

Table 1
Effect of bradykinin receptor antagonists on the pressure threshold for peristalsis

Antagonist	Concentration tested (nM)	Pressure threshold for peristalsis (Pa)	
		Without antagonist	With antagonist
FR173657	1	64.2±4.1 (6)	54.8±4.2(5)
FR173657	100		48.6±3.4 (3)*
Icatibant (Hoe 140)	10	63.1±3.6 (7)	66.2±6.4 (3)
Lys-[des-Arg^9, Leu^8]-bradykinin	100		55.7±3.4 (4)

Numbers in parenthesis show the n number of the particular experiment. Significant differences relative to controls are indicated as *$P<0.05$ (unpaired Student's t-test). Measurements were made prior to the administration of bradykinin.

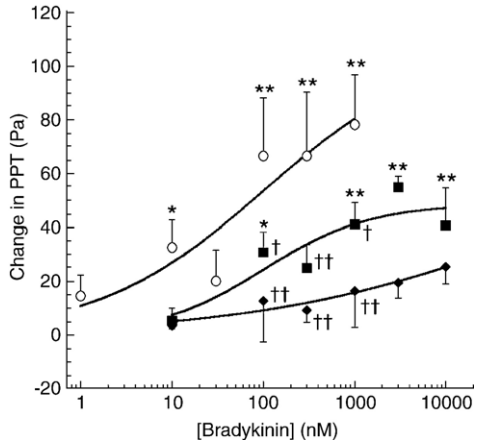

Fig. 6. The effect of FR173657 on the inhibitory action of bradykinin on peristalsis. The figure shows the log concentration–response curves to serosally applied bradykinin to increase pressure threshold for peristalsis (PPT) in the absence (○) and the presence of FR173657 (■, 1 nM or ◆, 100 nM). Values are the mean±S.E.M. of 3–6 determinations. Significant differences relative to the respective control PPT values are indicated as $^{*}P<0.05$,$^{**}P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). Significant difference relative to the change in PPT induced by bradykinin is indicated as $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). The PPT prior to drug addition was 57.5±2.5 Pa (28 tissues).

for peristalsis in the presence of antagonists relative to the change induced by bradykinin alone ($P<0.05$; Fig. 6). The concentration of bradykinin producing a 40 Pa increase in pressure threshold was approximately 34 and 780 nM in the absence and presence of FR173657 (1 nM), respectively. The slope of the curves in the absence and presence of FR173657 (1 nM) were not significant different (control slope, 3.06±2.72; FR173657 slope, 0.98±0.35; $P>0.05$). A higher concentration of FR173657 (100 nM) shifted the log concentration–response curve further to the right and bradykinin was not able to increase the pressure threshold for peristalsis by 40 Pa.

The other B_2 kinin receptor antagonist, icatibant (Hoe 140), also antagonized the inhibitory action of bradykinin on peristalsis. This was characterized by a right displacement of the log concentration–response curve for bradykinin. The resultant curve, with a slope of 6.04±6.82, did not reach an increase in the pressure threshold for peristalsis of 40 Pa in the presence of icatibant (10 nM) (Fig. 7). In the control curve, the concentration of bradykinin producing a 40 Pa increase in the pressure threshold for peristalsis was approximately 44 nM with a slope of 4.40±2.69. The slopes of curves in the absence and presence of icatibant (10 nM) were not significantly different ($P>0.05$).

Lys-[des-Arg^9, Leu^8]-bradykinin (100 nM) also produced a slight rightward shift of the log concentration–response curve of bradykinin (Fig. 7). The resultant concentration of bradykinin required to achieve a 40 Pa increase in the pressure threshold for peristalsis was approximately 200 nM with a slope of 4.81±4.07, which was not significantly different from the control curve ($P>0.05$). Further statistical analysis showed no significant difference in the change in pressure threshold for peristalsis in the presence of Lys-[des-Arg^9, Leu^8]-bradykinin relative to the change induced by bradykinin alone ($P>0.05$; Fig. 7).

4. Discussion

The major finding of the present study was that serosally applied bradykinin and kallidin raised the threshold for peristalsis in a concentration-related manner and that the action of bradykinin was antagonized by the B_2 receptor antagonists icatibant (Hall, 1997) and FR173657 (Asano et al., 1997). Additionally, the B_1 receptor agonist [des-Arg^9]-bradykinin was ineffective to modulate peristalsis and the B_1 receptor antagonist Lys-[des-Arg^9, Leu^8]-bradykinin (Hall, 1997) only had a marginal non-significant action to affect the action of bradykinin on peristalsis. Taken together, it appears that bradykinin is capable of inhibiting peristalsis via an action on B_2 receptors in guinea pig ileum.

It is important to note that our system was capable of detecting both facilitatory and inhibitory actions of drugs on the peristaltic reflex. In our study, 5-HT had an EC_{50} value of 37.7±23.0 nM to lower the threshold for peristalsis, and the facilitatory action lasted 1–3 min. This is in good agreement with the EC_{50} value of 43.7±6.0 and the duration of action of approximately 1.8 min that was reported by Costall et al. (1993). In the same study, they showed the facilitatory action is mediated by 5-HT$_4$ receptors, but we did not confirm this since we were primarily interested in demonstrating our system could detect a drug-induced lowering of the pressure threshold for peristalsis. However, in our study, none of the bradykinin receptor agonists were capable of lowering the pressure threshold. The inhibitory action of 5-HT that we observed at

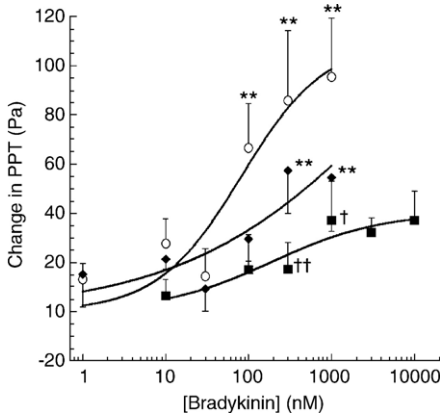

Fig. 7. The effect of icatibant (Hoe 140) and Lys-[des-Arg^9, Leu^8]-BK on the inhibitory action of bradykinin on peristalsis. The figure shows the log concentration–response curve to serosally applied bradykinin to increase pressure threshold for peristalsis (PPT) in the absence (○) and the presence of icatibant (■, 10 nM) or Lys-[des-Arg^9, Leu^8]-bradykinin (◆, 100 nM). Values are the mean±S.E.M. of 3–7 determinations. Significant differences relative to the respective control PPT values are indicated as $^{*}P<0.05$, $^{**}P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). Significant difference relative to the change in PPT induced by bradykinin is indicated as $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ (repeated measures 1 within 1 between one-way ANOVA with pre-planned contrasts of specified means). The PPT prior to drug addition was 62.0±2.6 Pa (28 tissues).

high concentrations probably relates to an activation of 5-HT_7 receptors (Tuladhar et al., 2003).

Our system was particularly sensitive to the actions of morphine to inhibit peristalsis. An elevation of the pressure threshold was seen at 1 μM, which is 10 times lower than the concentrations reported by Heinemann and Holzer (1999). The mechanism is thought to involve an action at μ- and κ-opioid receptors to depress excitatory neurons that innervate the circular muscle (Waterman et al., 1992). A generalized depression of neuronal activity may explain the increase in residual pressure that we observed. It is possible that the contraction of the circular muscle is less powerful to empty the ileal contents (Bartho and Holzer, 1995) in the presence of morphine.

In our study, the action of bradykinin and kallidin to increase the pressure threshold for peristalsis was evident at low (1–10 nM) concentrations, but mid–high (100–1000 nM) concentrations appeared to cause a concurrent contraction of circular muscle. This was expected, since in simpler guinea pig isolated ileum preparations, bradykinin and kalladin contract circular smooth muscle with EC_{50} values of 21 and 92 nM, respectively (Calixto and Medeiros, 1991). Although bradykinin also contracts longitudinal muscle (Hall and Bonta, 1973), we were not able to quantify it with our recording system. Bradykinin is known to elicit biphasic responses of circular muscle, which is characterized by a rapid contractile phasic action, followed by a slow relaxation (Calixto and Medeiros, 1991). It is possible that these factors explain why we were unable to observe maximal effects (i.e. ED_{100} values) of bradykinin and kallidin in the tissues, and the complexity of the responses may account for the elevated residual pressure in the ileum following B_2 receptor stimulation.

The inhibitory effect of bradykinin on peristalsis was almost abolished by FR173657 at 100 nM and by icatibant at 10 nM. The shift of the control concentration–response curve was not parallel in the presence of increasing concentrations of FR173657, suggesting it may be acting non-competitively. Certainly, FR173657 is reported to be non-competitive in guinea pig ileum and pig coronary artery, but is competitive in rabbit jugular vein and human umbilical vein (Rizzi et al., 1997). However, the dynamics of our recording system may complicate an interpretation of the nature of antagonism because FR173657 (100 nM) alone produced a small reduction of the pressure threshold for peristalsis.

There are two possibilities to explain the reduction of the pressure threshold for peristalsis seen with FR173657: (i) there is a tonic activation of B_2 receptors that normally inhibits peristalsis or (ii) FR173657 has non-specific actions to affect the emptying phase. FR173657 is a potent, non-peptide B_2 kinin receptor antagonist with a pK_B value of 9.2 in guinea pig isolated ileum and is without effect on responses to noradrenaline, 5-HT, endothelin-1, angiotensin II, substance P, acetylcholine and histamine (Rizzi et al., 1997). However, icatibant which is also a potent and selective B_2 kinin receptor antagonist, with a pA_2 value of 8.9 in guinea pig isolated ileum (Regoli et al., 1993), did not by itself affect peristalsis, leaving us to favour the latter explanation.

We used Lys-[des-Arg^9, Leu^8]-bradykinin as a classical peptide B_1 kinin receptor antagonist, exhibiting high potency, with pA_2 values ranging between 7.0 and 8.4 (Regoli et al., 1998). Although there was a tendency for a rightward displacement of the log concentration–response of bradykinin in the presence of Lys-[des-Arg^9, Leu^8]-bradykinin (100 nM), there was no significant difference in the change of the pressure threshold for peristalsis relative to that induced by bradykinin at the corresponding concentration. Therefore, we conclude that Lys-[des-Arg^9, Leu^8]-bradykinin is relatively ineffective to antagonize the inhibitory action of bradykinin on peristalsis, providing further evidence to exclude an involvement of B_1 receptors in the inhibitory action of bradykinin.

Studies with 5-HT have shown that 50 times higher concentrations are required on the mucosal, compared to the serosal side, to affect the pressure threshold of peristalsis (Tuladhar et al., 1997). However, in our study, the mucosal administration of bradykinin and [des-Arg^9]-bradykinin failed to affect the pressure threshold for peristalsis. It is possible that higher concentrations of kinins are required to affect peristalsis from the mucosal side, but it is also possible that a tissue barrier prevents kinins from traversing the lumen to the serosal side (Gershon and Tamir, 1981).

In summary, the present study indicates that kinins have inhibitory actions on the peristaltic reflex via an activation of B_2 receptors. Further studies should be pursued to evaluate the use of B_2 receptor antagonists to modify disorders of peristalsis in conditions where an elevated level of kinins is suspected.

Acknowledgement

We thank Dr. B.R. Tuladhar for advice on the methodological aspects of the studies, Prof. R.L. Jones for useful discussions and for advice on the statistical handling of the data, and Dr. M. Asano (Fujusawa Pharmaceutical Co. Ltd.) for the generous gift of FR173657.

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