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Regulatory Peptides 73 (1998) 113–117

REGULATORY PEPTIDES

Kinin receptors mediating the effect of bradykinin on gastric acid secretion

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Received 22 July 1997; received in revised form 3 November 1997; accepted 6 November 1997

Abstract

Kinins, and bradykinin in particular, can affect electrolyte transport in different segments of the intestine, thus being able to stimulate chloride secretion. Since the stomach is the main chloride secretory unit in the gastrointestinal tract, we have investigated the effect of bradykinin on acid secretion in the isolated frog (*Rana catesbeiana*) gastric mucosa. Bradykinin (2×10^{-8} to 2×10^{-6} M) and des-Arg^9-bradykinin (2×10^{-9} to 2×10^{-7} M) were able to stimulate acid secretion in a dose-dependent manner. The bradykinin (2×10^{-7} M) and des-Arg^9-bradykinin (2×10^{-8} M)-induced acid secretion was unaffected by Thi^{5,8},D-Phe^7-bradykinin (2×10^{-7} to 2×10^{-5} M), a B_2-kinin receptor antagonist. Interestingly, the B_1-kinin receptor antagonist, des-Arg^9-(Leu^8)-bradykinin (2×10^{-7} to 2×10^{-5} M) blocked both bradykinin- and des-Arg^9-bradykinin-stimulated acid secretion. Although the kininase I inhibitor, D-L-mercapto-methyl-3-guanidino-ethyl-propanoic acid (2×10^{-6} and 2×10^{-5} M) had no effect on des-Arg^9-bradykinin-induced acid secretion, it inhibited the response to bradykinin. We conclude that bradykinin requires, at least in part, hydrolysis to des-Arg^9-bradykinin to increase gastric acid secretion and that its effect is mediated by B_1-kinin receptors. © 1998 Elsevier Science B.V.

Keywords: B_1-kinin receptor; Kinin antagonists; Kinin analogues; des-Arg^9-BK; Gastric mucosa; Carboxypeptidase inhibition

1. Introduction

Kinins and kinin analogues interact with specific receptors present in several organs and tissues [1], leading to a variety of effects, which depend on the target site of action [2]. At least two types of kinin receptor have already been characterized. They were named B_1 and B_2 receptors and differ in terms of their affinities for kinins and kinin analogues [3]. While the B_2 receptor has a high affinity for intact kinins such as bradykinin (BK) and lysylbradykinin (LBK), the B_1 receptor shows a higher affinity for the kinin metabolites des-Arg^9-BK and des-Arg^{10}-LBK [3,4]. B_2 receptors are responsible for most known physiological effects of BK, mediating responses such as vasodilation [5–7], contraction of the rat uterus [8] and contraction of the rabbit mucosa-free urinary bladder [9]. B_1 receptors are mainly activated by kinin metabolites. These receptors were first identified in rabbit aorta [3] and have the property of being induced in vitro or following injury in vivo [10]. It has been well established that kinins also influence gastrointestinal function since BK induces contraction and relaxation of different segments of intestine [11–14]. BK also promotes an increase in transmural potential difference across rat jejunum and colon in vivo and in vitro, due to chloride secretion [15]. A similar observation was made on the rat descending colon, where BK increased chloride secretion, with no effect on sodium or potassium transport [16]. As is the case for most organs and tissues, most of the gastrointestinal effects of kinins are mediated by B_2 receptors. Thus, these receptors mediate BK effects in the guinea pig ileum and colon [11,12,17], rat duodenum [13] and water movement across everted sacs of the rat jejunum [18]. Although BK increases chloride secretion in various segments of the gastrointestinal tract [15,19], little is known about the

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PII S0167-0115(97)01071-9

effects of kinins on gastric chloride secretion. In this study, we investigated the effect of BK on acid secretion by the isolated frog gastric mucosa.

2. Materials and methods

Frogs (*Rana catesbeiana*) weighing 70–80 g were obtained from a dealer and kept in a tank at room temperature. The experiments were performed on isolated gastric mucosa as previously described [20]. The frog was pithed and the stomach was removed through a transversal incision in the abdomen. A slit was made in the muscularis with a razor blade, and the muscularis was cut with scissors along the greater curvature. The muscularis was stripped from the mucosa and discarded. The stump of the duodenum was slipped and tied over the end of a blunt hypodermic needle attached to a syringe filled with Ringer's solution. The mucosa was washed out and tied at the cardic sphincter. The mucosa was filled with Ringer's solution (4.00 ± 0.15 ml, $n = 136$) and tied at the pyloric sphincter. The preparation was placed in a vessel containing 18 ml of Ringer's solution. The preparation was incubated in a water bath at 26°C for 30 min under an O_2 pressure of 2000 mmHg, with shaking. The period of secretion was counted from the time the preparation was placed under an O_2 pressure of 2000 mmHg. The pH of the Ringer's solution was adjusted to 7.4 before the experiment and the final composition of the solution, in mM, was: NaCl, 92.0; KCl, 8.0; $CaCl_2$, 1.3; $MgSO_4$, 1.2; KH_2PO_4, 0.9; NaH_2PO_4, 12.5 and glucose, 11.0. To avoid peptide degradation, a pool of protease inhibitors, consisting of *para*-hydroxy-mercuri-phenyl sulphonic acid (2 μg/ml) (Sigma), bacitracin (2 μg/ml) (Sigma) and sodium benaziprilat (2 μg/ml) (Ciba Geigy) was added to the Ringer's solution. After the incubation period (30 min), the mucosa was removed from the vessel, blotted and its content emptied into a small beaker.

2.1. Acid secretion

Gastric acid secretion was evaluated by titration of the solution bathing the mucosal surface of gastric mucosa with 0.01 M NaOH to pH 7.4 (original pH of Ringer's solution). Briefly, 0.01 M NaOH in a buret (capacity of 1 ml) was dropped into the beaker containing the fluid bathing the mucosa under continuous monitoring until the pH reached 7.4. The results are given in microequivalents of acid secreted per gram dry weight per 30 min of incubation (μEq/g/30 min). The dry weight was obtained after the mucosa was placed in an oven at 105°C for approximately 12 h. Since gastric mucosa isolated from frogs presents a basal acid secretion, the results are expressed as variations of the acid secretion from control values and as relative increases in the acid secretion produced by BK or des-Arg^9-BK in the absence and in the presence of kinin receptor antagonists and carboxypeptidase N inhibitor. BK, des-Arg^9-BK, the kinin receptor antagonists ((Thi^{5,8},D-Phe^7)-BK and des-Arg^9-(Leu^8)-BK) (Sigma, St. Louis, MO, USA) and the carboxypeptidase N inhibitor, D-L-mercapto-methyl-3-guanidino-ethyl-propanoic acid (Mergetpa; Calbiochem–Behring, San Diego, CA, USA) were dissolved in Ringer's solution and added to both the serosal and luminal surfaces of the mucosa at the doses indicated in Section 3. In all experiments performed in the presence of receptor antagonists or carboxypeptidase N inhibitor, the mucosae were previously incubated for 5 min in Ringer's solution containing the antagonists or the inhibitor.

2.2. Statistical analysis

The data are expressed as mean±SEM and statistical significance of differences was determined by the independent Student's *t*-test.

3. Results

Fig. 1 shows that BK (A) and des-Arg^9-BK (B), at concentrations ranging from 2×10^{-9} to 2×10^{-6} M, increased gastric acid secretion in a dose-dependent manner. At BK and des-Arg^9-BK concentrations of 2×10^{-7} and 2×10^{-8} M, the relative acid secretion increased from 1.0 ± 0.09 (basal secretion = 79.9 ± 7.0 μEq/g/30 min, $n = 9$) to 1.70 ± 0.10 ($n = 5$) and 1.50 ± 0.12 ($n = 10$), respectively. The increase in the acid secretion induced by des-Arg^9-BK was not statistically different from that induced by BK ($P > 0.05$). To investigate the type of kinin receptors involved in BK-induced acid secretion, the mucosae were incubated in Ringer's solution containing BK and antagonists of B_1 or B_2-kinin receptors. In the presence of (Thi^{5,8},D-Phe^7)-BK, a B_2-kinin receptor antagonist, the acid secretion produced by BK was not significantly affected (Fig. 2A). In contrast, des-Arg^9-(Leu^8)-BK, a B_1-kinin receptor antagonist, blocked the increase in acid secretion produced by BK (Fig. 2A). This antagonist (2.0×10^{-5} M) reduced the BK-induced increase in acid secretion from 1.00 ± 0.09 ($n = 30$) to 0.25 ± 0.01 ($n = 5$). At a lower concentration (2.0×10^{-6} M), des-Arg^9-(Leu^8)-BK had no significant effect on BK-induced acid secretion. Neither (Thi^{5,8},D-Phe^7)-BK nor des-Arg^9-(Leu^8)-BK per se (at the highest concentration tested in this study) had an effect on gastric acid secretion. Acid secretion in the mucosae incubated with (Thi^{5,8},D-Phe^7)-BK (2.0×10^{-6} M) and des-Arg^9-(Leu^8)-BK (2.0×10^{-6} M) changed from 1.00 ± 0.03 (basal acid secretion, $n = 5$) to 1.03 ± 0.01 ($n = 3$) and 1.06 ± 0.08 ($n = 5$), respectively. In order to further investigate if the B_1-kinin receptor accounts for the bradykinin effect, gastric mucosae were incubated with des-Arg^9-BK (B_1-kinin agonist) in the presence of B_1- and B_2-kinin antagonists. Fig. 2B shows the effect of blockage of B_1 and B_2-kinin

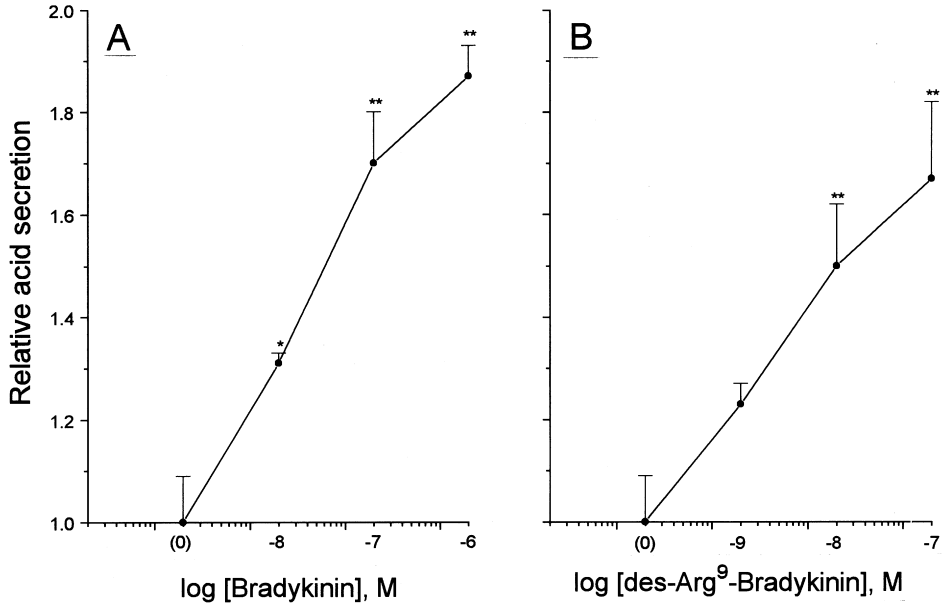

Fig. 1. Effect of BK (A) and des-Arg^9-BK (B) on gastric acid secretion. The isolated frog mucosae were incubated for 30 min at 26°C under an O_2 pressure of 2000 mmHg. The incubation medium consisted of Ringer solution containing increasing concentrations of BK ($n = 4$–9) and des-Arg^9-BK ($n = 5$–10) added to both the serosal and luminal surfaces of the mucosae. At the end of the incubation, the mucosae were emptied and the contents titrated to pH 7.4 with 0.01 M NaOH. The results (mean±SEM) are expressed as the changes in acid secretion from control value (basal secretion, 79.9±7.0 μEq/g/30 min, $n = 9$). *$P < 0.05$ and **$P < 0.01$, independent t-test.

receptors on des-Arg^9-BK-induced gastric acid secretion. (Thi^{5,8},D-Phe^7)-BK, at two different concentrations, did not affect the acid secretion elicited by des-Arg^9-BK. However, des-Arg^9-(Leu^8)-BK blocked the increase in the acid secretion induced by des-Arg^9-BK (2.0×10^{-8} M). The relative increase in acid secretion was reduced from 1.00±0.11 ($n = 23$) to 0.39±0.03 and 0.11±0.003 in the mucosae incubated with des-Arg^9-BK (2×10^{-8} M) in the presence of 2.0×10^{-7} ($n = 5$) and 2.0×10^{-6} M ($n = 5$) des-Arg^9-(Leu^8)-BK, respectively. To determine if BK requires enzymatic hydrolysis to des-Arg^9-BK by kininase I-like enzymes, mucosae were incubated with BK or des-Arg^9-BK in the presence of Mergetpa, a carboxypeptidase N inhibitor. Like the kinin receptor antagonists, Mergetpa alone did not affect gastric acid secretion. It was observed that while this inhibitor did not significantly alter the acid secretion induced by des-Arg^9-BK, it was effective in blocking the BK effect (Fig. 3). The relative increase in acid secretion in the mucosa incubated with BK (2×10^{-7} M) was reduced from 1.00±0.14 ($n = 11$) to 0.39±0.09 and 0.23±0.03 in the presence of 2.0×10^{-6} ($n = 5$) and 2.0×10^{-5} M ($n = 4$) Mergetpa, respectively. Taken together, the data indicate that BK required enzymatic hydrolysis to des-Arg^9-BK to stimulate acid secretion by isolated frog gastric mucosa.

4. Discussion

Although it is recognized that kinins affect gastrointestinal function, little is known about the actions of kinins on gastric secretion. All tested substances were added to both surfaces of the gastric mucosa since previous studies from our laboratory have shown that BK increases H^+ secretion indistinctly when added to the serosal or mucosal surface of the mucosa, or to both (data not shown). A similar observation was reported by Crocker and Willavoys [18], who showed that BK stimulates or inhibits sodium and water movement in the rat jejunum, depending on the control level of transport. The inhibitory effect was observed when BK was added to the serosal, mucosal or both surfaces of the intestine. In this study, we have demonstrated for the first time the presence of B_1-kinin receptors in the frog gastric mucosa, since the B_1 agonist, des-Arg^9-BK, induced an increase in the acid secretion that was antagonized by the specific B_1-kinin receptor antagonist, des-Arg^9-[Leu^8]-BK. In addition, BK requires, at least in part, hydrolysis to des-Arg^9-BK by a kininase I-like enzyme to be effective in stimulating acid secretion in the gastric mucosa. This conclusion arose from the observation that the BK-induced acid secretion is either antagonized by des-Arg^9-[Leu^8]-BK or inhibited by Mergetpa, a selective kininase I inhibitor. This inhibitor had no effect on des-Arg^9-BK-induced acid secretion. On the other hand, the isolated frog gastric mucosa seems to have no B_2 receptors, since the B_2 receptor antagonist (Thi^{5,8},D-Phe^7)-BK had no effect on BK-induced acid secretion in the gastric mucosa. (Thi^{5,8},D-Phe^7)-BK is a potent B_2-kinin receptor antagonist [21] that is able to antagonize the effects of BK on different B_2 receptor systems, such as guinea pig ileum, rabbit jugular vein, dog carotid artery

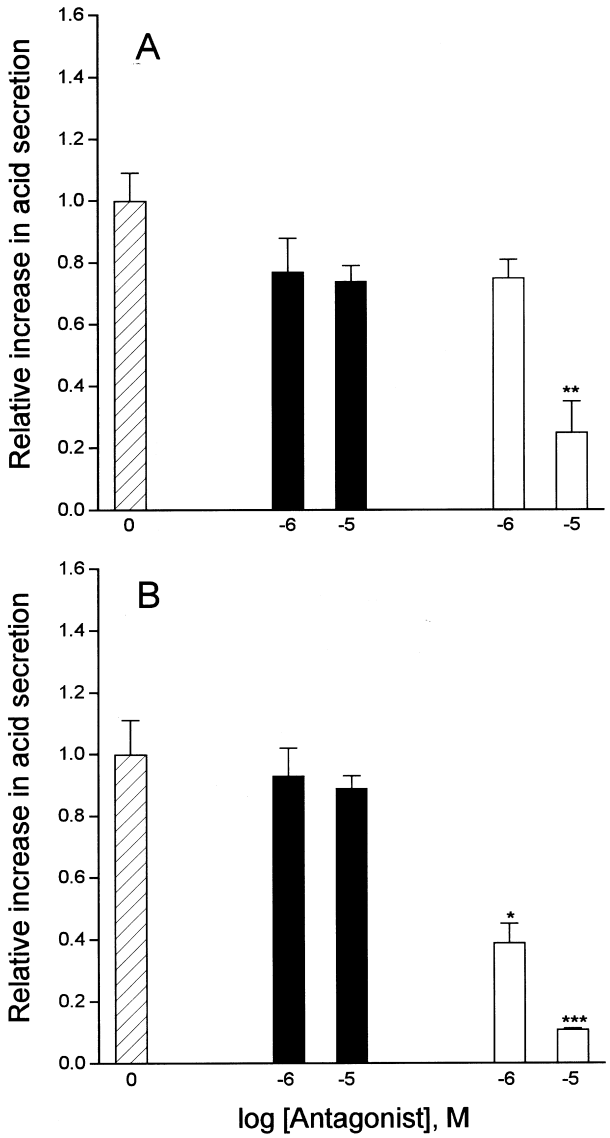

Fig. 2. Effect of B_1 and B_2-kinin receptor antagonists on BK- and des-Arg^9-BK-induced gastric acid secretion. The mucosae were incubated with BK (A; 2×10^{-7} M, $n = 30$) and des-Arg^9-BK (B; 2×10^{-8} M, $n = 23$) alone (diagonal bars) or in the presence of (Thi^{5,8},D-Phe^7)-BK ($n = 5$ and 7) (filled bars) and des-Arg^9-(Leu^8)-BK ($n = 5$) (open bars). The experimental procedure was as described in the legend to Fig. 1. The results (mean±SEM) are expressed as relative increases in the acid secretion produced by BK and des-Arg^9-BK in the absence and presence of antagonists. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to the agonists in the absence of antagonists.

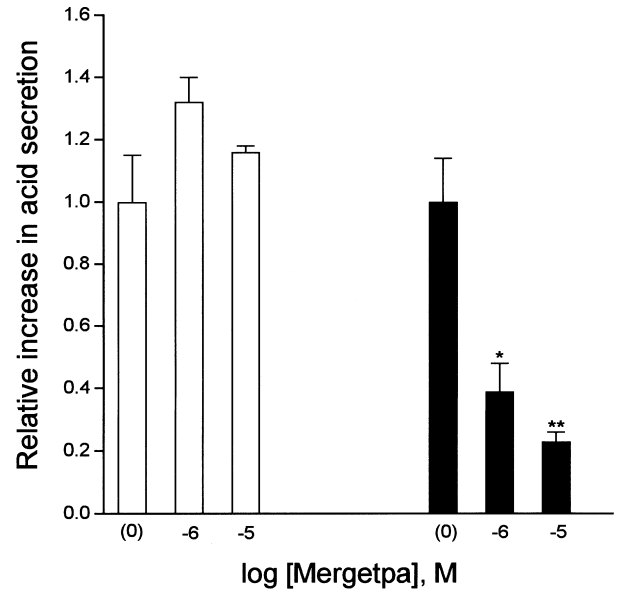

Fig. 3. Effect of kininase I inhibition on BK- and des-Arg^9-BK-induced acid secretion. The incubation was performed with des-Arg^9-BK (0.02×10^{-6} M, $n = 16$) (open bars) and BK (2×10^{-7} M, $n = 11$) (filled bars) alone or in the presence of two different concentrations of Mergetpa ($n = 4$–14). The experimental procedure and results (mean±SEM) are as described in Figs. 1 and 2, respectively. $*P < 0.05$ and $**P < 0.01$ compared to BK alone.

and dog urinary bladder [22]. Although it has been described that this B_2 antagonist can act as an agonist in causing effects such as hind-paw edema and degranulation of isolated peritoneal mast cells [23], in our preparation, it had no effect on its own on acid secretion. B_2 receptors are present in most organs and seem to mediate most kinin effects. For example, BK and LBK produced a biphasic response (contraction followed by relaxation) of the circular muscle of the guinea pig ileum by B_2 receptor activation [11]. B_2 receptors also mediate kinin effects such as contraction and relaxation of isolated rat duodenum [13,14], and augmentation in short circuit current in the canine proximal colon [24]. Surprisingly, our results show that des-Arg^9-(Leu^8)-BK inhibited the increase in acid secretion elicited by both BK and des-Arg^9-BK. It has been shown that BK and des-Arg^9-BK produce relaxations followed by contractions of the stomach fundus and that removal of the mucosal layer abolishes both responses to des-Arg^9-BK and only the relaxant response to BK [25,26]. B_1 receptors were first described in rabbit aorta [3], and can be found in some organs and tissues, such as the rat stomach fundus [25] and isolated perfused rat kidney [27]. In order to confirm that BK is cleaved to des-Arg^9-BK, the acid secretion elicited by BK or des-Arg^9-BK was investigated in the presence of Mergetpa. Although the inhibitor had no effect on the des-Arg^9-BK-induced acid secretion, it promptly blocked the similar effect of BK. Our results indicate that BK is cleaved to des-Arg^9-BK, which may be, at least in part, responsible for the observed increase in gastric acid secretion elicited by BK, and that the effect of des-Arg^9-BK may be mediated by B_1-kinin receptors present in the frog gastric mucosa.

Acknowledgements

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas gerais

(FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Pró-Reitoria de Pesquisa/UFMG.

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