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ORIGINAL ARTICLE

Basic Study

Neurophysiological mechanisms of bradykinin-evoked mucosal chloride secretion in guinea pig small intestine

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Abstract

AIM: To investigate the mechanism for bradykinin (BK) to stimulate intestinal secretomotor neurons and intestinal chloride secretion.

METHODS: Muscle-stripped guinea pig ileal preparations were mounted in Ussing flux chambers for the recording of short-circuit current (*Isc*). Basal *Isc* and *Isc* stimulated by BK when preincubated with the BK receptors antagonist and other chemicals were recorded using the Ussing chamber system. Prostaglandin E2 (PGE_2) production in the intestine was determined by enzyme immunologic assay (EIA).

RESULTS: Application of BK or B2 receptor (B2R) agonist significantly increased the baseline *Isc* compared to the control. B2R antagonist, tetrodotoxin and scopolamine (blockade of muscarinic receptors) significantly suppressed the increase in *Isc* evoked by BK. The BK-evoked *Isc* was suppressed by cyclooxygenase (COX)-1 or COX-2 specific inhibitor as well as nonselective COX inhibitors. Preincubation of submucosa/mucosa preparations with BK for 10 min significantly increased PGE_2 production and this was abolished by the COX-1 and COX-2 inhibitors.

The BK-evoked *Isc* was suppressed by nonselective EP receptors and EP4 receptor antagonists, but selective EP1 receptor antagonist did not have a significant effect on the BK-evoked *Isc*. Inhibitors of PLC, PKC, calmodulin or CaMK Ⅱ failed to suppress BK-induced PGE_2 production.

CONCLUSION: The results suggest that BK stimulates neurogenic chloride secretion in the guinea pig ileum by activating B2R, through COX increasing PGE_2 production. The post-receptor transduction cascade includes activation of PLC, PKC, CaMK, IP_3 and MAPK.

Key words: Brandykinin; Ussing chamber; Brandykinin receptor; Cyclooxygenase; Prostaglandin E; Chloride secretion

Core tip: Bradykinin (BK) can stimulate intestinal chloride secretion and the firing of intestinal secretomotor neurons in the small intestine, but the mechanism is not well understood. In this study, muscle-stripped guinea pig ileal preparations were mounted in Ussing flux chambers for the recording of short-circuit current (*Isc*). BK agonist and BK antagonist were added to check the *Isc* change. Inhibitors of the signal transductors were pre-incubated with the tissue for 10 min before evoking with BK, and the *Isc* change was recorded. The change of prostaglandin E2 (PGE_2) secretion was detected by ELISA after treatment with BK for 3 h. Results suggest that BK stimulates neurogenic chloride secretion in the guinea pig ileum by activating B_2 receptors on secretomotor neurons, activating cyclooxygenase-1, and stimulating PGE_2 production. The post-receptor transduction cascade includes activation of PLC, PKC, CaMK, IP_3, and MAPK.

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INTRODUCTION

Bradykinin (BK) is a nonapeptide that belongs to a group of structurally related 9-11 amino acid peptides (kinins), which are produced by kallikrein-mediated enzymatic cleavage of kininogen at the site of tissue injury and inflammation[1]. BK is formed in plasma and tissues in response to infection, tissue trauma, or inflammatory alterations, such as an increase in vascular permeability, edema formation, and pain. BK is widely distributed in the central and peripheral nervous systems, including the enteric nervous system[2,3]. Two subtypes of BK receptors, namely, BK receptor type 1 (B1R) and BK receptor type 2 (B2R), are identified based on their amino acid sequence and pharmacological properties[4,5]. BK receptors belong to the family of G-protein-coupled receptors with seven transmembrane helices. BK and kallidin are ligands for the constitutively expressed B2R, whereas *des*-Arg^9-BK (in rodents) and *des*-Arg^{10}-kallidin (in human) are ligands for the inducible B1R[6,7].

Previously, we demonstrated that B2R is expressed on a majority of the ganglion cells in the myenteric and submucosal plexuses in the small intestine of guinea pigs[8-10]. Exposing neurons in the guinea pig small intestinal myenteric or submucosal plexus to BK *in vitro* evokes slow activation of depolarization of the membrane potential and enhanced excitability characterized by increased firing frequency during intraneuronal injection of depolarizing current pulses in both AH- and S-type neurons and the appearance of anodal break excitation at the offset of hyperpolarizing current pulses in AH neurons[8,9]. The results suggested that BK acts *via* B2R on myenteric and submucosal neurons to stimulate the formation of prostaglandins. The eletrophysiologic data recorded using "sharp" microelectrodes suggested that BK might act in the enteric nervous system as a paracrine mediator to alter neural control of secretory and motility functions at the organ level.

This work aimed to investigate how the involvement of BK as an excitatory neuromodulator on submucosal secretomotor neurons at the cellular neurophysiological level translates to the physiology of intestinal secretion at the level of the integrated system[11,12].

MATERIALS AND METHODS

Tissue preparation

The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12 h/12 h light/dark, 50% humidity, *ad libitum* access to food and water) for two weeks prior to experimentation. Adult male Hartley-strain guinea pigs (300-350 g) were stunned by a sharp blow to the head and exsanguinated from the cervical vessels according to a protocol approved by Weifang Medical University Laboratory Animal Care and Use Committee. The tissue preparations were essentially conducted as described[13,14]. Briefly, segments of the small intestine were removed, flushed with ice-cold Krebs solution, and opened along the mesenteric border. The "muscle-stripped" preparations were obtained by removing the longitudinal and circular muscle layers together with the myenteric plexus by microdissection. The submucosal plexus remained intact with the mucosa. About 4-6 of the flat-sheet preparations were obtained from the ileum of each animal for mounting in Ussing flux chambers. The Krebs solution was composed of 120, 6, 2.5, 1.2, 1.35, 14.4, and 11.5 mM of NaCl, KCl, $CaCl_2$, $MgCl_2$, NaH_2PO_4, $NaHCO_3$, and glucose,

respectively.

Ussing flux chambers

Ussing flux chambers were equipped with a pair of Ag/AgCl electrodes *via* Krebs-agar bridges connected to Calomel half-cells for the measurement of transmural potential difference (PD). A second pair of electrodes was connected to an automated voltage clamp apparatus, which compensated for the solution resistance between the PD-sensing bridges. The flat-sheet preparations were mounted between halves of Ussing chambers, which had a total cross-sectional area of 0.64 cm^2. The tissues were bathed on both sides with 10 mL of Krebs solution and maintained at 37 ℃ by circulation from a temperature-controlled water bath. The necessary current to change the transepithelial PD by 2.5 mV was used to monitor tissue conductance and calculated according to the Ohm's law, as a determinant of tissue viability. Short-circuit current (*Isc*) was monitored by a voltage-clamp apparatus (DVC-1000, World Precision Instruments, Sarasota, FL). The mounted "muscle-stripped" preparations were balanced at 37 ℃ and bubbled with 95% O_2/5% CO_2 for 30 min before the preparations were treated with BK. Pharmacological agents were applied by addition to the bathing solution. BK (10 nM) was added to the serosal compartment of the chamber. To test the effect of other pharmacological agents on BK-evoked chloride secretion, pharmacological agents were added 10 min before the preparations were stimulated by BK (10 nM). The changes in *Isc* were calculated as ⊿*Isc*, and the effects of pharmacological agents were normalized to the cross-sectional area of the preparations. Each inhibitor was pre-incubated with the tissue for 10 min, and the *Isc* showed no change.

Prostaglandin E2 release

For these studies, all utensils and solutions were sterilized in 75% ethanol or autoclaved. The submucosa/mucosa preparations were prepared by carefully removing the longitudinal and circular muscle layers, as well as the myenteric plexus. The weight of each piece of preparations was recorded. The preparations were incubated in 2 mL of Dulbecco's modified Eagle's medium containing 100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B, and glucose (12 mM) in a humidified 5% CO_2 incubator at 37 ℃. Preparations were pre-incubated for 30 min at 37 ℃ in the presence of FR122047, NS398, U73122, BisI, calmodulin inhibitor W7, or KT5720. Subsequently, 100 nM BK was added to each culture well, and a further 10 min or 3 h incubation was performed. The supernatant of each well was collected for prostaglandin E_2 (PGE_2) enzyme immunologic assay (EIA) (Cayman chemical, Ann Arbor, Michigan 48108 United States). EIA was preformed following the protocol provided by the manufacturer. Three independent experiments were performed, and the data were analyzed with Sigma plot software. Preparations without any treatment were used as controls.

Chemicals

BK acetate, B1R agonist BK fragment 1-8 acetate hydrate, B2R agonist [Hyp3]-BK, B2R antagonist HOE-140, tetrodotoxin (TTX), N-nitro-L-arginine methyl ester (L-NAME), cyclooxygenase (COX)-2 inhibitor NS398, COX-1 inhibitor FR122047, nonspecific COX inhibitors indomethacin and piroxicam, selective IP_3 receptor antagonist 2-APB, MAPK inhibitor PD98059, and tyrosine protein kinase inhibitor genistein were purchased from Sigma (St. Louis, MO). KT5720, W7, and nonselective VIP receptor antagonist VIP_{6-28} (human, bovine, porcine, rat) were obtained from BACHEM Bioscience, Inc., King of Prussia. U73122 (1-[6-[[(17β)-3-methoxyestra-1,3,5(100-trien-17-yl] amino]hexyl]-^{1}H-pyrrole-2,5-dione), bisindolylmaleimide I (2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide, BisI), and KN-62 were purchased from Tocris (Ellisville, MO). Stock solutions were prepared in Krebs solution or deionized H_2O, except for piroxicam, U73122, and KN-62, which were solubilized in DMSO and stored at -20 ℃. AH6809, SC-19220, and GW627368X were from Cayman Chemical (Ann Arbor, MI). The volume added to 10 mL of the bath solutions did not exceed 10 μL. Pharmacological agents were applied by addition to the Krebs' bathing solution.

Statistical analysis

Data are presented as mean ± SE. Student's *t*-test was used for the statistical analysis of significance of differences in the means with $P < 0.05$ considered significant. The statistical methods of this study were reviewed by Xiangyun Li from the Department of Statistics, Weifang Medical University.

RESULTS

BK-evoked increase in Isc is mediated by B2R

Baseline *Isc* and tissue conductance for submucosal/mucosal preparations from the guinea pig ileum were similar to those described previously (Fang *et al*[14], 2006). The addition of BK to the bathing solution on the submucosal side of the preparations evoked a rapid increase in *Isc*, and BK (10 nM), applied to the submucosal side of the submucosa/mucosa preparations, increased baseline *Isc* by 54.3 ± 5.4 μA/cm^2 (Figure 1). The B1R agonist, BK fragment 1-8 (1 μM), failed to elicit any changes in baseline *Isc*. The B2R agonist, [Hyp^3]-BK (1 μM), increased baseline *Isc* by 42.1 ± 5.7 μA/cm^2. Pre-incubation with the B2R antagonist, HOE-140 (1 μM), for 10 min significantly suppressed BK-evoked increase in *Isc* from 54.3 ± 5.4 to -1.1 ± 1.1 μA/cm^2 ($P < 0.01$). The results suggest that BK-evoked increase in *Isc* is mediated through B2R, but not B1R.

BK-evoked increase in Isc is mediated mainly by the enteric nervous system and by an increase of acetylcholine release

Stimulation of *Isc* by BK (10 nM) was significantly decreased in the presence of 1 μM of TTX from 57.8 ±

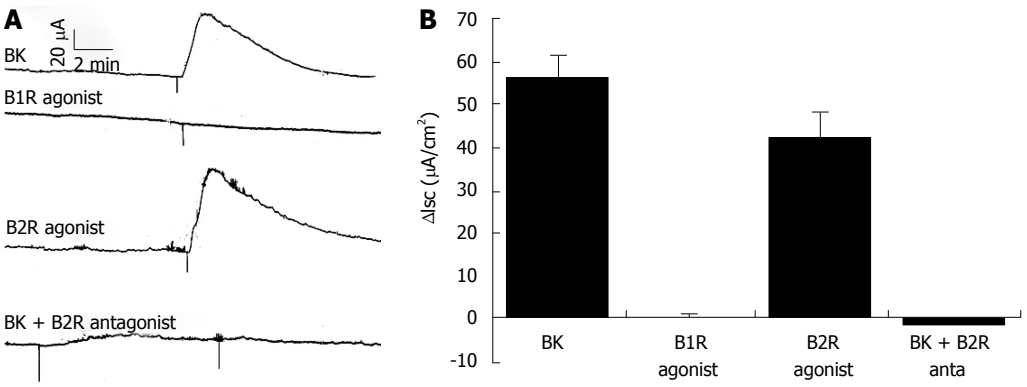

Figure 1 Bradykinin-evoked increase in short-circuit current is mediated through B2R but not B1R. A: Application of BK (10 nM) to the serosal side of the ileal preparation of guinea pigs ($P < 0.001$); B: Quantitative data showing the effect of B1 and B2 receptor agonists on baseline *Isc* and the effect of B2 receptor antagonist on BK-evoked *Isc*. Values are expressed as mean ± SE; $n = 6$-12 animals. BK: Bradykinin; *Isc*: Increase in short-circuit current.

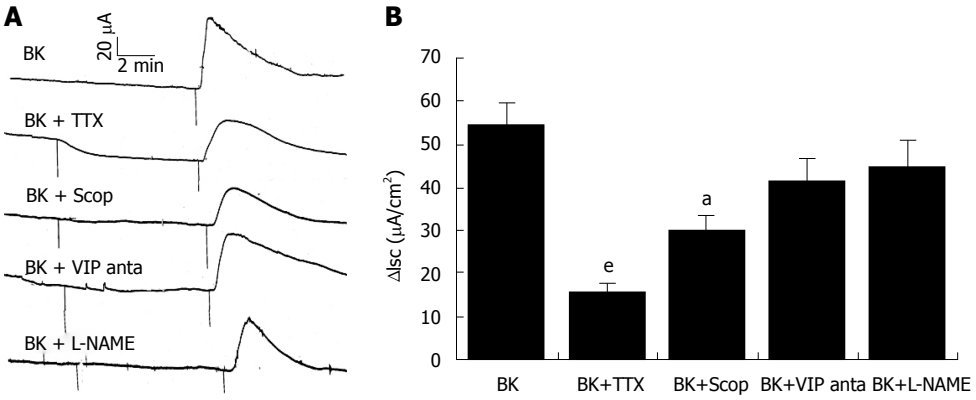

Figure 2 Bradykinin-evoked increase in short-circuit current is mediated mainly by the enteric nervous system and by an increase of acetylcholine release. A: Effect of tetrodotoxin (TTX; 1 μM), a muscarinic receptor antagonist, scopolamine (Scop; 1 μM), a VIP receptor antagonist (VIP 6-28; 1 μM) or an inhibitor of nitric oxide synthase, L-NAME (100 μM), on BK-evoked *Isc*; B: Quantitative data showing the effect of TTX, scopolamine, VIP antagonist and L-NAME on BK-evoked response in *Isc*. The vertical axis represents the changes of *Isc*. Values are expressed as mean ± SE, $n = 6$ animals. $^{a}P < 0.01$, $^{e}P < 0.001$ (*vs* BK alone). TTX: Tetrodotoxin; Scop: Scopolamine; VIP: Vasoactive intestinal peptide; BK: Bradykinin; *Isc*: Increases in short-circuit current.

7.1 μA/cm² to 15.6 ± 1.9 μA/cm² ($n = 6$; $P < 0.01$), which suggested that BK-evoked secretion was largely mediated by activation of the submucosal secretomotor neurons (Figure 2). The nonselective muscarinic acetylcholine antagonist, scopolamine; the vasoactive intestinal peptide (VIP) receptor antagonist, VIP_{6-28}; and the nitric-oxide synthase inhibitor, L-NAME; were used as pharmacological tools to test the kind of enteric neurons involved in BK-evoked secretory responses in the small intestine of guinea pigs. Pretreatment with scopolamine (1 μM) significantly suppressed the 10 nM BK-evoked increase of *Isc* from 54.3 ± 5.4 μA/cm² to 30.2 ± 9.4 μA/cm² ($n = 6$; $P < 0.01$). In the presence of VIP_{6-28} (1 μM) or L-NAME (100 μM), BK (10 nM) increased the baseline *Isc* to 41.5 ± 5.1 μA/cm² ($n = 6$; $P > 0.05$) or 44.6 ± 6.0 μA/cm² ($n = 6$; $P > 0.05$), respectively; neither showed a significant effect on 10 nM BK-evoked increase of *Isc*.

BK-evoked increase in Isc is mediated by the stimulation of prostaglandin release

The specific COX-2 inhibitor NS398 (10 μM), specific COX-1 inhibitor FR122047 (1 μM), and nonspecific COX inhibitors indomethacin (1 μM) and piroxicam (1 μM) were used to test a hypothesis that BK-evoked increase in *Isc* is mediated by prostaglandins, and the results are shown in Figure 3. Pretreatment with NS398 (10 μM) and FR122047 (1 μM) suppressed the 10 nM BK-evoked increases in *Isc* from 59.69 ± 8.45 μA/cm² to 22.8 ± 5.9 μA/cm² ($n = 6$; $P < 0.01$) and 25.3 ± 6.6 μA/cm² ($n = 6$; $P < 0.01$), respectively. The nonspecific COX inhibitors, indomethacin or piroxicam abolished BK-evoked increase in *Isc* ($n = 6$; $P < 0.01$). Suppression of BK-evoked secretory responses by the COX inhibitors suggested that the responses to BK occurred secondary to the release of prostaglandins and their excitatory action on submucosal secretomotor neurons or directly on intestinal epithelial cells.

Involvement of prostaglandins in BK-evoked secretory responses was further supported by the finding that BK (10 nM) stimulated the synthesis and release of PGE_2 from the submucosal preparations of the guinea pig ileum when compared with the preparations that were not treated with BK (Figure 4). Both FR122047 (1

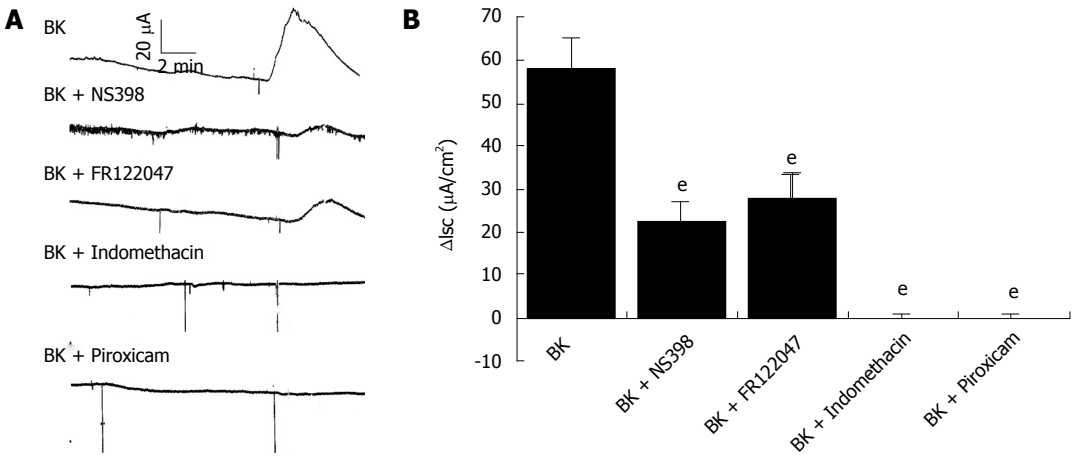

Figure 3 Cyclooxygenase inhibitors suppress bradykinin-evoked increase in short-circuit current. A: Pretreatment with the COX-2 inhibitor NS398, COX1 inhibitor FR122047 (1 μM), non-specific COX inhibitors indomethacin or piroxicam suppressed BK-evoked *Isc*; B: Quantitative data showing the effect of COX inhibitors on BK-evoked increase in *Isc*. Values are expressed as mean ± SE, n = 6 animals. $^{e}P < 0.01$ *vs* BK alone. COX: Cyclooxygenase; BK: Bradykinin; *Isc*: Increase in short-circuit current.

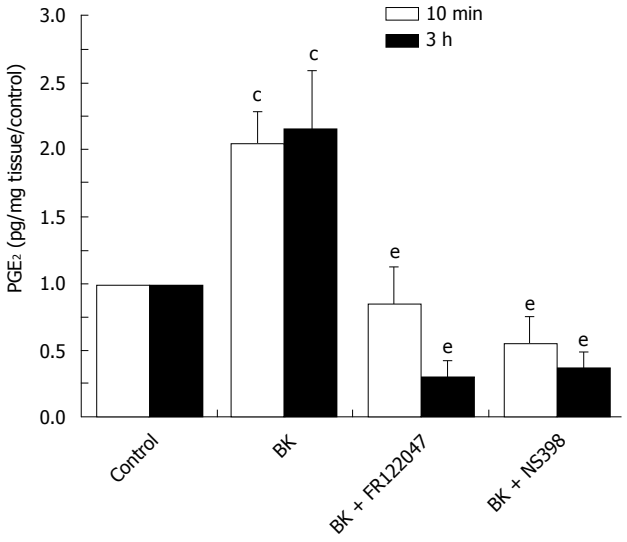

Figure 4 Bradykinin-induced prostaglandin E2 production in submucosal preparations is mediated by both COX-1 and COX-2. Preincubation of submucosal preparations with BK (10 nM) for 10 min or 3 h significantly increased PGE2 production and this was suppressed by the COX-1 inhibitor FR122047 (1 μM) or the COX-2 inhibitor NS398 (10 μM). Values are expressed as mean ± SE, n = 3 independent samples, each assayed in duplicate. $^{c}P < 0.01$ BK *vs* control; $^{e}P < 0.01$ pretreated with agents *vs* BK alone. BK: Bradykinin; PGE2: Prostaglandin E2.

μM) and NS398 (10 μM) suppressed BK-evoked PGE_2 production after incubation with BK for 10 min and 3 h, respectively (Figure 4).

Furthermore, BK-evoked increase in *Isc* was significantly decreased by pretreatment with prostaglandin EP receptor antagonists (Figure 5). Pretreatment with EP1, EP2, EP3-Ⅲ, and DP1 receptor antagonist AH6809 (10 μM) reduced BK-evoked ⊿*Isc* from 69.5 ± 8.3 μA/cm^2 to 24.2 ± 5.5 μA/cm^2 (n = 6, $P < 0.01$), and pretreatment with GW627368X (a potent and selective competitive antagonist of the EP4 receptor, 10 μM) suppressed the ⊿*Isc* from 69.5 ± 8.3 μA/cm^2 to 21.8 ± 5.1 μA/cm^2 (n = 6, $P < 0.01$) (Figure 5). Meanwhile, pretreatment with SC-19220 (a selective EP1 receptor antagonist, 10 μM) did not significantly affect the ⊿*Isc* evoked by BK (n = 6, P = 0.13). All of the above information suggests that BK-evoked increase in ⊿*Isc* was mediated by the stimulation of PGE_2 release.

Signal transduction mechanisms of BK-evoked increase in Isc

Our earlier studies on the mechanisms of post-receptor signal transduction using electrophysiological methods suggested that BK caused excitation of submucosal secretomotor neurons by activation of B2R on submucosal neurons, stimulation of a signal transduction pathway involving phospholipase C (PLC), elevation of intraneuronal IP_3, and elevation of free cytosolic Ca^{2+}[9]. These findings suggested that stimulation of PLC and intracellular IP_3 would also underlie a component of the stimulatory action of BK on ⊿*Isc* at the tissue level. We followed the earlier electrophysiological studies at the cellular level by using the same pharmacological tools to test the hypothesis at the tissue level in Ussing chamber studies.

U73122 was used to inhibit PLC activity. Exposure to 10 μM U73122 for 10 min prior to the application of 10 nM BK resulted in the suppression of BK-evoked responses from 57.3 ± 6.2 μA/cm^2 for BK alone to 9.4 ± 4.3 μA/cm^2 (n = 6; $P < 0.01$) (Figure 6). Pre-exposure of the preparations to the vehicle (DMSO) alone had no significant effect on BK-evoked responses (data not shown). The protein kinase C (PKC) inhibitor bisindolylmaleimide Ⅰ (Bis Ⅰ, 10 μM) decreased the BK-evoked responses to 6.77 ± 5.13 μA/cm^2 (n = 6-12; $P < 0.01$).

Results of our previous work on the cellular neurophysiology of secretomotor neurons suggested that stimulation of calmodulin-dependent protein kinase is involved in B2R-mediated excitation of secretomotor neurons[9]. We used W-7, a membrane permeable calmodulin inhibitor, and KN-62, a selective calmodulin-dependent protein kinase inhibitor, as pharmacological

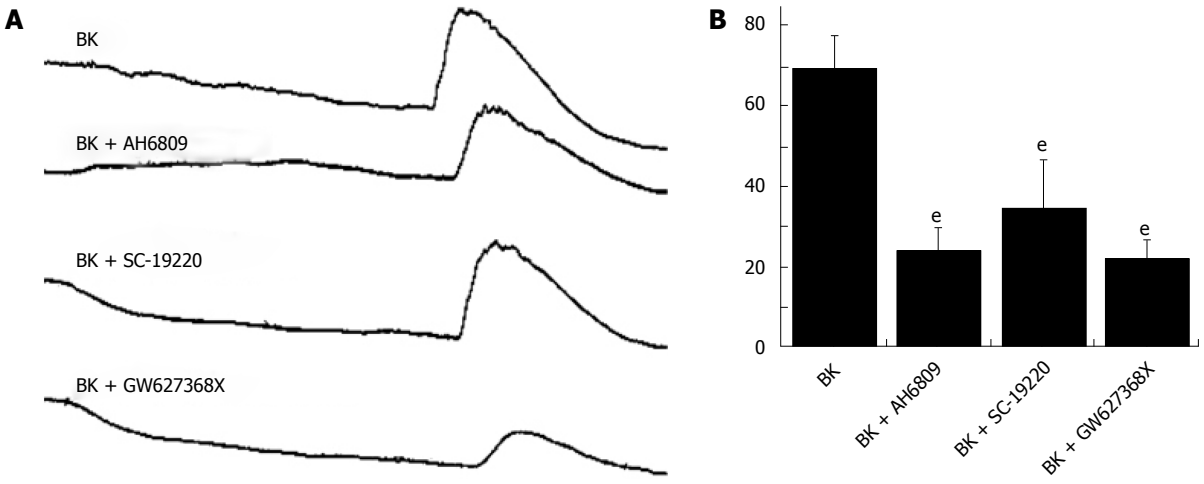

Figure 5 Bradykinin-evoked increase in short-circuit current is mainly mediated by the prostaglandin E2 receptors. A: Pretreatment with EP receptors antagonist AH6809, SC-19220 (a selective antagonist of EP1 receptor), or GW627368X (a selective antagonist of EP4 receptor) suppressed BK-evoked *Isc* ($P < 0.01$); B: Quantitative data showing the effect of AH6809, Sc-19220 and GW627368X on BK-evoked response in *Isc*. The vertical axis represents the changes of *Isc*. Values are expressed as mean ± SE, n = 6-12 animals. $^{e}P < 0.01$ (compared to BK alone). BK: Bradykinin; PGE_2: Prostaglandin E2; *Isc*: Increase in short-circuit current.

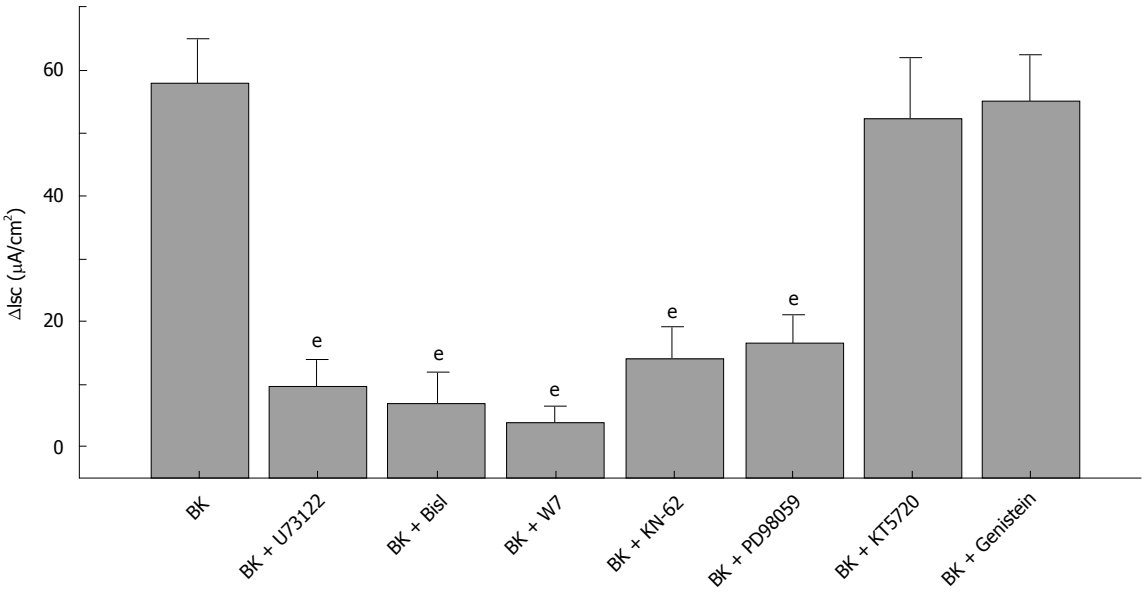

Figure 6 Post-receptor signal transduction for bradykinin-evoked increase in baseline increase in short-circuit current. The BK-evoked *Isc* was suppressed in the presence of the PLC inhibitor U73122, the PKC inhibitor Bis-Ⅰ, the calmodulin inhibitor W7, the calmdulin kinase Ⅱ inhibitor KN-62 (10 μM), or the MAPK pathway inhibitor PD 98059 ($^{e}P < 0.01$ *vs* BK alone). Inhibitor of PKA (KT5720 1 μM) or inhibitor of tyrosine protein kinase (genistein 10 μM) did not have a significant effect on BK-evoked increase in *Isc*. *Isc*: Increase in short-circuit current; BK: Bradykinin; PLC: Phospholipase C; PKC: Protein kinase C; Bis-Ⅰ: Bisindolymaleimide Ⅰ.

tools to test the hypothesis that stimulation of *Isc* by BK involved post-receptor activation of calmodulin-dependent protein kinase. Pretreatment with W-7 (50 μM) and KN-62 (10 μM) for 10 min reduced the responses to 10 nM BK from 57.3 ± 6.2 μA/cm^2 to 3.6 ± 2.5 μA/cm^2 and 14.1 ± 4.9 μA/cm^2 (n = 6; $P < 0.01$), respectively (Figure 6). Meanwhile, pretreatment with 2-APB (10 μM), an IP_3 inhibitor, reduced the response to 3.2 ± 4.0 μA/cm^2 (n = 6; $P < 0.01$). The MAPK pathway inhibitor PD98059 was used to test the downstream signal cascade of PKC. Pre-incubation with PD98059 (10 μM) for 10 min prior to the BK (10 nM) exposure significantly reduced BK-evoked increase of *Isc* to 16.7 ± 4.1 μA/cm^2 (n = 6; $P < 0.01$) (Figure 6).

The protein kinase A (PKA) inhibitor, KT5720, was used to address the question of whether the cAMP/PKA signaling pathway is involved in B2R-evoked secretory response. No suppression of BK-evoked increase of *Isc* occurred in the 6 preparations that were incubated for 10 min in 10 μM KT5720 (Figure 6). The tyrosine protein kinase inhibitor, genistein, was used to verify whether tyrosine protein kinase plays a role in BK-evoked secretory response. The results showed that pretreatment with genistein (10 μM) for 10 min failed to block BK-evoked *Isc* (Figure 6).

To test whether the similar signal transduction mechanisms are also involved in BK-induced PGE_2 production, the submucosa/mucosa preparations were

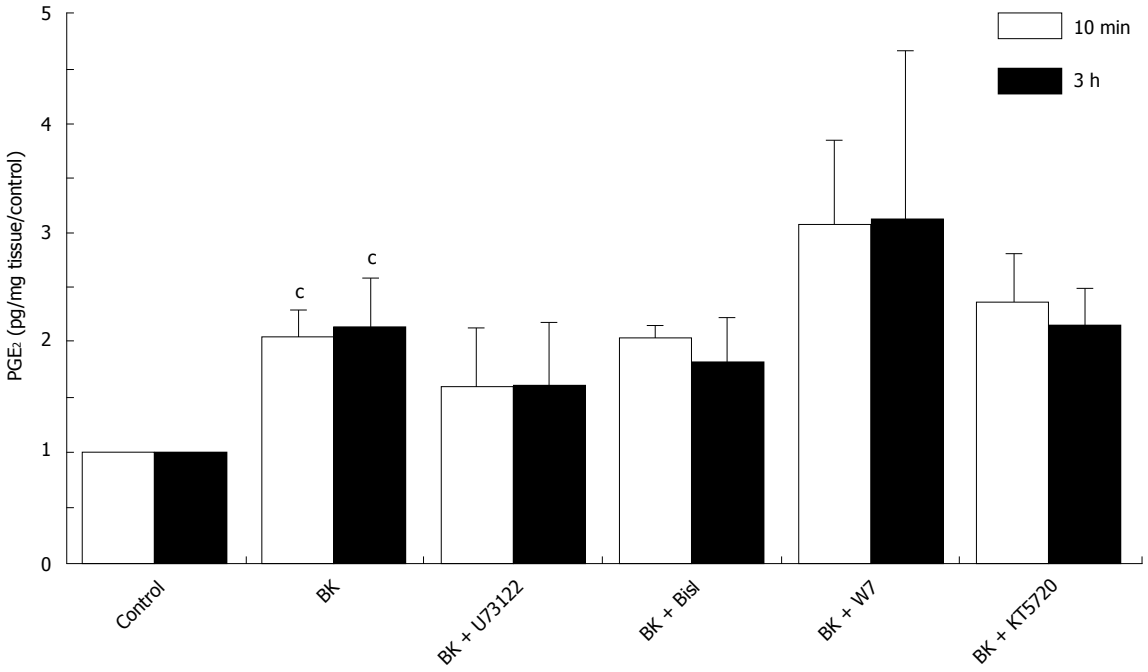

Figure 7 Inhibitors of PLC (U73122; 10 μM), protein kinase C (Bisl; 10 μM), calmodulin (W7; 50 μM), or PKA (KT5720; 1 μM) fail to suppress bradykinin-induced PGE2 production. Values are expressed as mean ± SE, *n* = 3 independent samples, each assayed in duplicate. $^{c}P < 0.01$ BK *vs* control. BK: Bradykinin; PGE_2: Prostaglandin E2.

pretreated with the inhibitors of PLC (U73122), PKC (Bis Ⅰ), CaM (W7), or PKA (KT5720) for 30 min before exposure to BK (10 nM). These inhibitors did not significantly affect BK-induced PGE_2 production (Figure 7).

DISCUSSION

BK stimulates Cl^- secretion by the guinea pig and rabbit ileum[15], rabbit colon[16], rat colon[17], human colon, monolayers of human HCA-7 cells[18,19], and T-84 cells[20]. However, the detailed mechanism of the action of kinins on intestinal secretion is not fully understood, and results are controversial. Diener *et al*[21] have found evidence for the involvement of enteric cholinergic nerves in BK-stimulated secretory response in the rat descending colon. However, also using descending rat colon, another group[22] did not find significant inhibition of BK-induced secretion in the presence of TTX (1 μM) and atropine (1 μM). Furthermore, Manning *et al*[23] reported that BK-induced Cl^- secretion by the guinea pig ileum was not TTX-sensitive and thought to occur by a direct action in the mucosa. The reasons for these differences are unknown but could be caused by the degree that the submucosal plexus has been stripped away from the mucosa.

By using guinea pig ileum submucosa/mucosa preparations, we found that BK-induced intestinal secretion was mainly mediated by the enteric nervous system. The neurotoxin TTX inhibited responses to BK by 73%, hence suggesting that the enteric nervous system plays a pivotal role. Secretomotor neurons in the submucosal plexus are the final common motor pathways from the integrative networks of the enteric nervous system to the intestinal secretory glands. They transmit the signals for autonomic minute-to-minute regulation of mucosal secretion and liquidity of the intestinal contents in concert with submucosal vasodilation and increased blood flow in support of the stimulated secretion[24-27]. Enhanced mucosal secretion, after elevation of excitability in secretomotor neurons, increases the liquidity of the luminal contents and might lead to neurogenic secretory diarrhea. Our previous studies found the expression of B2R mRNA and protein in submucosal neurons. BK increased excitatory in both AH- and S-type neurons in the submucosal plexus[9,10]. The S-type neurons are primarily secretomotor neurons that receive excitatory synaptic input from their AH-type neighbors[24]. Although both cholinergic and VIPergic secretomotor neurons are excited by BK, only the cholinergic muscarinic receptor antagonist, scopolamine, attenuated the secretory responses to BK. The VIP receptor antagonist, VIP_{6-28}, had no significant effect. BK stimulated *Isc* in a TTX- and scopolamine-sensitive manner and therefore correlated with the slow excitatory action of BK on cholinergic secretomotor neurons, which we found at the cellular level. However, direct effects on epithelial cells cannot be ruled out, which is evidenced by the inability of TTX to abolish BK-stimulated secretory responses, and BK stimulates ion transport in cultured monolayers of epithelial cells[28]. The presence of B_2 receptors on intestinal epithelial cells[29] also suggests a potential direct action of BK on epithelial cells.

BK-stimulated intestinal Cl^- secretion depends on the formation of prostaglandins. Inhibition of prostaglandin formation by indomethacin or piroxicam or blocking

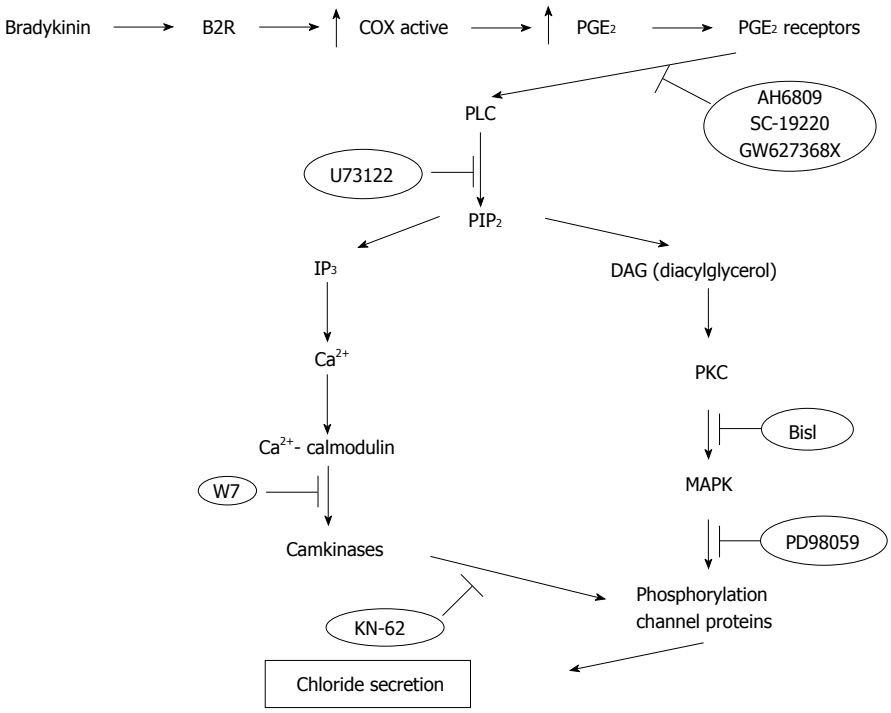

Figure 8 Overview of metabotropic signal transduction cascade for bradykinin evoked chloride secretion in the submucosal plexus of the guinea pig small intestine. Bradykinin activates B2R receptor, G protein, phospholipase C, PIP2 hydrolysis, IP3 and DAG generation, Ca^{2+}-Camodulin, PKC and CaMkinases, cation channel open, and chloride secretion. Termination of chloride secretion is postulated to result from activation of the intraneutonal phosphatase, calcineurin. The blocking agents for each of the steps are included in the figure. PLC: Phospholipase C; PKC: Protein kinase C; PGE_2: Prostaglandin E2.

the prostaglandin receptors suppresses the secretory responses that are evoked by B2R activation in the small intestine of guinea pigs. This observation is in agreement with previous studies in the rat, rabbit, and human intestine[28] where products of the COX pathway were implicated in BK-stimulated ion transport. However, the isoforms of COX that are involved in the process are not clear. COX enzymes have two isoforms, namely, COX-1 and COX-2. COX-1 is constitutively expressed throughout the gastrointestinal tract. COX-2 was initially considered an inducible form that has been highly expressed at sites of inflammation, but subsequently constitutive expression of COX-2 in normal digestive tract has been demonstrated[5,30]. Both COX-1 and COX-2 inhibitors significantly suppressed BK-induced secretory response in the small intestine of guinea pigs, thus suggesting that the constitutive COX-1, as well as constitutive COX-2, is involved in the production of prostaglandins, which is further supported by the observation that BK-stimulated PGE_2 production from submucosa/mucosa preparations was greatly suppressed by COX-1 and COX-2 inhibitors. Several cellular elements of the intestine can produce prostaglandins upon activation of B2R. Our studies and those from others[29] suggest that enteric neurons, fibroblasts, and enterocytes are likely the sources.

We found that the same pharmacological agents, which suppress BK-evoked slow excitatory responses in secretomotor neurons by selective inhibition of individual steps in the PLC-IP_3-Ca^{2+}-PKC signal transduction pathway, also suppressed the BK-evoked increase in *Isc*. These inhibitory effects of the events in the PLC-PKC signal transduction cascade on BK-evoked stimulation of *Isc* are consistent with the inhibition of intracellular post-B2R signaling in the secretomotor neurons. Nevertheless, the agents that suppressed post-B2R signal transduction in secretomotor neurons might act also to suppress the signal transduction in enterocytes. By contrast, the agents that suppressed BK-evoked increase in *Isc* did not significantly affect BK-induced PGE_2 production in the submucosa/mucosa preparations, thereby suggesting that the PLC-IP_3-Ca^{2+}-PKC signal transduction pathway is activated after prostaglandins are released.

In conclusion, the data presented here are consistent with the possibility that BK stimulates secretory responses in the intestine *via* activating B_2 receptors in submucosal secretomotor neurons and in epithelial cells, as shown in Figure 8. Activation of B2R causes the release of prostaglandins through both COX-1 and COX-2 dependent pathways. Once released, prostaglandins excite submucosal cholinergic secretomotor neurons and indirectly stimulate Cl^- secretion. Prostaglandins may also act directly in epithelial cells to stimulate ion transport. PLC, IP_3, Ca^{2+}, PKC, and MAP kinase all play a role in the signal transduction of BK-stimulated secretory responses.

COMMENTS

Background

Bradykinin (BK) is widely distributed in the central and peripheral nervous

systems, including the enteric nervous system. BK can stimulate intestinal chloride secretion and the firing of intestinal secretomotor neurons in the small intestine, but the mechanism is not well understood.

Research frontiers

The present work aimed to investigate how the involvement of BK as an excitatory neuromodulator on submucosal secretomotor neurons at the cellular neurophysiological level translates to the physiology of intestinal secretion at the level of the integrated system.

Innovations and breakthroughs

This study demonstrates that BK stimulates intestinal chloride secretion responses *via* activating B2 receptors in submucosal secretomotor neurons and in epithelial cells. Activation of B2 receptors causes the release of prostaglandins through both cyclooxygenase (COX)-1 and COX-2 dependent pathways. Once released, prostaglandins excite submucosal cholinergic secretomotor neurons and indirectly stimulate Cl^- secretion. Prostaglandins may also act directly in epithelial cells to stimulate ion transport. Phospholipase C (PLC), IP_3, Ca^{2+}, protein kinase C (PKC), and MAP kinase (MAPK) all play a role in the signal transduction of BK-stimulated secretory responses.

Applications

The authors studied the mechanism of how BK stimulates the intestinal chloride secretion. The results suggest that BK stimulates neurogenic chloride secretion in the guinea pig ileum by activating B2 receptors on secretomotor neurons, activating COX, and stimulating prostaglandin E2 (PGE_2) production. The post-receptor transduction cascade includes the activation of PLC, PKC, CaMK, IP_3, and MAPK. This study gives a better understanding of the BK-evoked secretion pathway.

Peer-review

In this manuscript, the authors reported the BK-evoked mucosal secretion in the guinea pig small intestine. By using a list of inhibitors, antagonists, and agonists, the authors concluded that BK-evoked chloride secretion depends on the engagement on the B2 receptor, the activation of COX, production of PGE_2, and downstream signaling cascade. Understanding the mechanism may provide a valuable insight into the regulation of mucosal secretion and other intestinal functions. The manuscript is well-organized, and conclusions were precisely based on obtained data.

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