Bradykinin BK₂ receptors contribute to reflex cardiovascular responses during brief abdominal ischemia

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Chahal, Premjit S., Stephen V. Rendig, and John C. Longhurst. Bradykinin BK₂ receptors contribute to reflex cardiovascular responses during brief abdominal ischemia. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H308–H313, 1998.—Ischemically sensitive visceral sympathetic nerve fibers, which are thought to represent the afferent limb of a strong cardiovascular pressor reflex, can be stimulated by exogenously applied bradykinin (BK). During ischemia, BK also is known to be produced locally and to serve as an endogenous stimulus for activation of ischemically sensitive nerve endings. It is unclear, however, whether ischemically induced BK production is sufficient to elicit a reflex cardiovascular response. Accordingly, femoral arterial and venous catheters were positioned in anesthetized cats, and the superior mesenteric and celiac arteries were isolated for placement of snare occluders. After dual occlusion of these arteries (20 min), one of two chemically dissimilar specific kinin B₂ (BK₂) receptor antagonists, HOE-140 (30–40 µg/kg iv, n = 8) or NPC-17731 (30–40 µg/kg iv, n = 11), was administered and dual occlusion was repeated. The reflex rise of mean arterial blood pressure (BP) of 16 ± 3.7% was significantly (P < 0.05) reduced by HOE-140 to 8.4 ± 2.0%. NPC-17731 similarly attenuated the reflex BP increment from 13 ± 1.2 to 6.2 ± 1.6% (P < 0.05). In a separate set of control animals the first and second periods of ischemia induced reflex BP increments that did not differ significantly (16 ± 2.7 and 16 ± 5.7%, respectively). Qualitatively similar decrements of the BP response were produced by the BK₂ receptor antagonists in two additional groups in which blood flow to the superior mesenteric and celiac arteries was diverted to a venous reservoir to eliminate the initial transient (mechanically induced) rise in BP associated with artery ligation that is known not to be associated with the reflex response. These results indicate that the stimulation of BK₂ receptors on visceral afferent nerves by BK is responsible, at least in part, for the reflex cardiovascular response during visceral ischemia.

METHODS

Preparation. Studies were performed in cats of either sex (1.9–6.0 kg). Anesthesia was initially induced by an injection of ketamine (40–50 mg/kg im) followed by a bolus injection of a-chloralose (50 mg/kg iv). Subsequent injections of a-chloralose were administered as needed to maintain a sufficient anesthetic state, as determined by response to paw pinch, eye reflexes, and abnormal respiration patterns. Auffed endotracheal tube was inserted to artificially ventilate the animals (Harvard pump, model 661, Ealing, S. Natwick, MA). Inspired gas was enriched with 100% oxygen, while arterial blood gases and pH were monitored frequently (model ABL3, Radiometer, Copenhagen, Denmark) and maintained within physiological limits (pH = 7.35–7.45, PaO₂ = 28–35 mmHg, PaCO₂ < 100 mmHg) by adjusting rate or depth of ventilation and/or administering sodium bicarbonate (1 M). A rectal probe was used to measure body temperature, which was maintained between 36.5 and 37.5°C by heating pads and heat lamps. This study was conducted in compliance with the “Guiding Principles in the Care and Use of Animals” endorsed by the American Physiological Society.

The two animal models have been discussed previously (7). Briefly, in group 1, catheters were placed in the left femoral...
vene to administer drugs and fluids and in the left femoral artery to record arterial blood pressure (Statham P23 ID, Gould, Valley View, OH). A midline abdominal incision was performed to expose the viscera, and the proximal portions of the celiac and superior mesenteric arteries were isolated carefully to minimize fiber damage to the surrounding nerve plexi. Loop snares were placed loosely around each artery such that flow through the vessels was not compromised. The inferior mesenteric artery was located and ligated to minimize collateral flow into the ischemic area. Several cats underwent a unilateral pneumothorax to alleviate respiratory influences on the systemic arterial pressure. Abdominal organs were covered and kept moist by a gauze sponge saturated with warm Ringer solution. Abdominal ischemia was induced for 20 min by constricting the loop snare on the superior mesenteric and celiac arteries. On release of occlusion sites, flow through collapsed vessels was confirmed by depression in systemic arterial blood pressure and/or visual inspection.

Group 2 was studied to verify the results from group 1, utilizing a more invasive preparation that eliminated possible blood volume shifts associated with arterial ligation. With the use of extracorporeal autoperfusion blood circuits and diversion of blood flow from the visceral region to a venous return reservoir, the initial transient rise in blood pressure associated with onset of occlusion of the blood vessels could be eliminated, as we demonstrated previously (7). In group 2 the left femoral artery was cannulated for the administration of fluids and drugs. A catheter was placed in the left carotid artery to record arterial blood pressure (Statham P23 ID). After heparinization (3,000 U iv), celiac and superior mesenteric arteries were cannulated carefully to minimize nerve fiber damage; both catheters were connected to a roller pump (Masterflex model 7523-00, Cole-Parmer, Vernon Hills, IL) that received blood input from a catheter in the left femoral artery. A stopcock was placed distal to the roller pump to divert blood flow during the ischemic period to a reservoir connected to a second roller pump. A heat lamp was utilized to maintain temperature of the blood circulating through the external arterial roller pump circuit. The inferior mesenteric artery was ligated to limit collateral flow to the ischemic region. A midline thoracotomy was performed to expose the inferior vena cava, and a catheter was directed caudally to receive blood from the splanchnic region and lower extremities. Venous return through this catheter was allowed to drain into a reservoir. Blood in the reservoir was returned through a catheter in the left external jugular vein by a second roller pump (Masterflex model 7565, Cole-Parmer). Blood flow through the venous circuit was held constant throughout the ischemic period.

Protocols. After surgery the animals were allowed to recover for at least 30 min until blood pressure was stable and blood gases were within the normal range. Abdominal ischemia was induced for 20 min unless the reflex-induced increase in blood pressure attained a constant plateau for several minutes, in which case blood flow was restored to the ischemic region. Animals were given a bolus injection of NPC-17731 [30–40 µg/kg (22–29 nmol/kg) iv; Scios Nova, Mountain View, CA; n = 11 and 4 for groups 1 and 2, respectively] or HOE-140 [Icatibant; 30–40 µg/kg (23–31 nmol/kg) iv; Hoechst-Roussel Pharmaceuticals, Somerville, NJ; n = 8 and 4 for groups 1 and 2, respectively]. After injection, each animal was allowed to equilibrate for at least 30 min, at which time a second period of ischemia was induced. BK (1–10 µg/ml, 0.9–9 µM) and capsaicin (2–200 µg/ml, 6.5–650 µM) were applied to the surface of the gallbladder at the conclusion of the experiment to confirm BK2 receptor blockade and the reflex responsiveness of the preparation, respectively.

To differentiate between drug effect and time-related variations in response, several additional animals (n = 8 and 4 for groups 1 and 2, respectively) were utilized to determine the repeatability of these protocols over the same time frame. In this experimental group, the first period of ischemia was followed by a second period of ischemia without drug intervention. Another set of animals (group 1, n = 3) was treated identically, except celiac and superior mesenteric ganglionectomies were performed after the initial period of ischemia. In this procedure the nerve trunks radiating from the ganglia were severed by a small vessel cautery (Fine Science Tools, Foster City, CA) or, for larger nerves, were tied with surgical silk at two points and incised between the two ties with iris scissors. The lack of a response to BK (1–10 µg/ml) applied to the gallbladder was the criterion for successful ganglionectomy. After the second period of ischemia, capsaicin was injected intravenously to confirm that the preparation was still able to manifest a reflex response.

To test the efficacy of BK2 receptor antagonist blockade, the pressor response to BK (10 µg/ml) applied topically to the gallbladder was tested on a subset of animals before (n = 6) and after administration of HOE-140 (n = 6) or NPC-17731 (n = 6).

Data analysis. Only animals with stable blood pressures and reflex responses of ≥10 mmHg to combined occlusion of the celiac and superior mesenteric arteries were used. Furthermore, animals in which the difference between control absolute blood pressures between the first and second ischemic periods exceeded 25 mmHg were excluded (n = 3).

Values are means ± SE. The Kolmogorov-Smirnov test was used to check the assumption of a normally distributed population, which was confirmed in all groups. Paired data were analyzed by Student's paired t-test. The statistical software package SigmaStat (Jandel Scientific, San Rafael, CA) was utilized for these analyses. The level of statistical significance was chosen as P < 0.05.

RESULTS

Group 1. Occlusion of the celiac and superior mesenteric arteries produced an initial rise in blood pressure followed by a gradual decline in pressure during the next several minutes (Fig. 1A). After a variable time period, a gradual secondary increase in pressure became evident. This secondary response, which has been demonstrated to be reflex in nature (7), was analyzed.

![Fig. 1: A: chart recording of arterial blood pressure response to brief occlusion of superior mesenteric and celiac arteries. B: ischemic period was repeated after administration of HOE-140, a bradykinin (BK2) receptor antagonist. BK2 receptor blockade reduced reflex rise in arterial blood pressure by 75% in this animal.](image-url)
The blood pressure response was measured as the difference between the blood pressure nadir, which was typically attained several minutes after occlusion, and the peak secondary blood pressure response. In the repeatability group, the first and second combined occlusions of the superior mesenteric and celiac arteries resulted in similar changes in mean arterial blood pressure of 16 ± 6.2 and 16 ± 5.7% (P > 0.05), respectively (Fig. 2). Administration of the BK_2 receptor antagonists NPC-17731 and HOE-140 produced significant attenuation of the reflex rise of blood pressure in group 1 (Fig. 1B). For instance, NPC-17731 (n = 11) reduced the ischemia-induced increment of mesenteric arterial pressure from an average of 13 ± 1.2 to 6.2 ± 1.6% (P < 0.05; Fig. 3), whereas HOE-140 significantly attenuated the reflex blood pressure increase from 16 ± 3.7 to 8.4 ± 2.0% (P < 0.05; Figs. 1 and 3). There was no significant change in the control preischemia blood pressure before vs. after NPC-17731 or HOE-140 (Fig. 3). In the group of animals subjected to ganglionectomy after the first combination occlusion, the blood pressure response was virtually eliminated (Fig. 2). The blood pressure increase during the first occlusion (27 ± 5.8%) was reduced to 0.7 ± 4.0% during the second occlusion.

Group 2. The protocol to minimize blood volume shifts resulted in blood pressure patterns different from those of group 1 (Fig. 4). In this preparation, on induction of ischemia, no initial or minimal initial rise in blood pressure was observed (Fig. 4A). After a variable time a gradual rise in blood pressure was noted. Because the induction of ischemia in this preparation typically did not result in a transient blood pressure rise and subsequent nadir, the blood pressure response was measured from preocclusion control to peak blood pressure response. The reflex augmentation of the mean arterial pressure during the first and second ischemic periods was similar (36 ± 5.6 and 40 ± 2.2%, respectively, P > 0.05). The animals receiving NPC-17731 or HOE-140 displayed similar significant attenuation of the rise in blood pressure during ischemia (Fig. 4B). The reflex rise in blood pressure during the first ischemic period was 57 ± 15%, which was significantly attenuated to 20 ± 8.1% after administration of NPC-17731 (Fig. 4, Table 1). The reflex pressor increase of 43 ± 6.1% during the first ischemic period was reduced to 17 ± 8.3% by HOE-140. Neither antago-

Table 1. Effect of BK_2 antagonists on arterial blood pressure response to abdominal ischemia in group 2

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<th>Ischemia I</th>
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<td></td>
<td>Control</td>
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<tr>
<td>HOE 140</td>
<td>92 ± 8.4</td>
<td>40 ± 8.2</td>
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<td>NPC 17731</td>
<td>86 ± 12</td>
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Values are arterial blood pressures (means ± SE) in mmHg; n = 4. After 1st ischemic period, HOE-140 (30–40 µg/kg iv) or NPC-17731 (30–40 µg/kg iv) was administered, and ischemia was repeated. *P < 0.05 compared with ischemia I.
nism significantly altered the control preischemia blood pressure (Fig. 4, Table 1).

Administration of a BK$_2$ receptor antagonist reduced the reflex rise in blood pressure induced by BK application to the gallbladder from $33 \pm 4.7$ to $10 \pm 3.3\% (P < 0.05)$. The application of capsaicin produced a pressor response in all animals tested.

**DISCUSSION**

This is the first study to show that endogenously produced BK is involved in cardiovascular reflexes evoked by abdominal visceral ischemia. Significant attenuation of the cardiovascular reflex was demonstrated for two disparate kinin B$_2$ receptor antagonists, NPC-17731 and HOE-140. Conversely, reflex responses were shown to be repeatable for both control groups 1 and 2 in the absence of the BK$_2$ receptor antagonists. Because celiac and superior mesenteric ganglionectomies eliminated the cardiovascular reflex responses elicited by visceral ischemia, the origin of the reflex pathway was confirmed. Thus endogenous BK, through a B$_2$ receptor mechanism, is capable of stimulating a sufficient number of abdominal visceral afferent endings to reflexly activate the cardiovascular system.

Ischemia alters the interstitial environment and can result in localized acidosis, increased proteolytic activity, and hypoxia, conditions that promote activation of the kinin system (11). Two major pathways are involved in the production of BK and Lys-BK (kallidin); the latter peptide can stimulate BK$_2$ receptors and can be converted to BK (32). In plasma, BK is produced by activated kallikrein; in tissues, activated kallikrein liberates kallidin. In addition, immune cells (e.g., mast cells and basophils) associated with inflammation can produce kinins by the release of cellular proteases, independent of kallikrein activity (32). Although the relative role of tissue or plasma BK or kallidin in the present study is unknown, kallikreins are present in tissues throughout the gastrointestinal tract (4). Furthermore, we previously reported that brief (5 min) abdominal visceral ischemia results in a three- to fourfold increase in portal venous BK (21). It is clear therefore that BK release is evoked by ischemia and contributes to the ischemia-induced pressor reflex, although the exact source of this liberated BK has not been determined.

Afferent nerve endings of C-fibers are fine, unmyelinated fibers located in the interstitial space (18). Their proximity to cells that contain sequestered tissue kallikrein, including the acinar and β-cells of the pancreas and mucous cells of the colon (1), likely is an important factor in determining the concentration of BK at the nerve terminal. No information is available regarding the actual concentration of mediators at receptor sites in an acidic environment. We have reported that protons donated by lactic acid, in particular, contribute to the activation of ischemically sensitive visceral afferents (27). In cutaneous nociceptive afferent fibers, a positive interaction between acidic pH and inflammatory mediators, including BK, was reported with regard to the magnitude and prevalence of nociceptor activation (31). In addition, kinin activity is protected against destruction by kininases in an acidic environment (11). Our previous data show that the extent of acidosis during regional visceral ischemia and the magnitude of the cardiovascular response are correlated; i.e., occlusion of the superior mesenteric artery results in a significantly larger fall in pH in its downstream organs and a significantly greater reflex pressor response than celiac artery occlusion (25). The combined results of the present and past studies (25, 27, 31) suggest that the greater reflex response from the ischemic superior mesenteric vascular bed than from the celiac vascular distribution may be related to enhanced efficacy of liberated BK in a more acidic environment.

BK is unusual in its ability to stimulate many different cell types and activate a number of intracellular messenger systems, which lead directly and indirectly to production of inositol 1,4,5-trisphosphate and diacylglycerol and increased intracellular levels of protein kinase C, arachidonic acid products, superoxide free radicals, intracellular calcium, adenosine 3',5'-cyclic monophosphate, and guanosine 3',5'-cyclic monophosphate (3). These actions lead to many of the clinical manifestations of inflammation, including local pain, hyperemia, increased vascular permeability, and edema (11). The mechanism for stimulation of afferent nociceptive nerve endings, based largely on in vitro studies of hybridoma cell lines, has been proposed to involve a sequence of steps beginning with activation of phospholipase C → diacylglycerol → protein kinase C → potassium channel phosphorylation → membrane depolarization → action potential generation (3). Furthermore, our previous data suggest that activation of phospholipase A$_2$ and the production of arachidonic acid metabolites via the cyclooxygenase or lipoxygenase pathway sensitizes afferent nerve endings (15, 17, 29) and also may play a role in the production of oxygen-containing free radicals (3). In this latter regard, we have shown that reactive oxygen species, particularly H$_2$O$_2$ and ·OH, activate ischemically sensitive afferent nerve fibers during visceral ischemia and reperfusion (28). Further studies are necessary to determine the subcellular mechanism(s) responsible for the effect of BK on visceral afferent nerve terminals.

We previously showed that blockade of BK$_2$ receptors in abdominal organs with HOE-140 or NPC-17731 significantly attenuates the activation of afferent C-fibers during ischemia (21). Furthermore, BK$_1$ receptors are not involved in this response, because ischemically sensitive C-fibers that responded to BK were not activated by a specific BK$_1$ receptor agonist (21). We also observed increased abdominal visceral afferent activity during ischemia after administration of captopril, an inhibitor of the BK degradation enzyme kini-
The combination of present and past findings supports our overall hypothesis that endogenous BK released during abdominal visceral ischemia is responsible, at least in part, for stimulation of BK2 receptors on afferent nerve fibers to induce reflex cardiovascular responses.

It was important in the present study to elicit reproducible reflex responses to visceral abdominal ischemia. With this thought in mind, we chose to study two types of preparations. Group 1 was utilized initially to establish a clear reflex response while minimizing surgically induced trauma to the preparation. However, the reflex responses were obscured partially in group 1 by the large initial hemodynamic changes at the onset of arterial ligation and by antagonist-induced changes in the subsequent blood pressure nadir. The more invasive preparation, group 2, however, demonstrated clear reflex responses due to elimination of several hemodynamic factors induced by a systemic ischemic period. In previous studies performed in our laboratory (7), we postulated that two mechanisms may contribute to the initial increase in systemic arterial pressure during abdominal ischemia. The first is a mechanical effect resulting from shunting more blood from a high- (mesenteric) to a low-compliance (hindlimb) arterial circuit. Second, the initial increase in blood pressure may be related to a sudden elevation of cardiac preload by short circuiting the long time constant mesenteric circulation during ischemia and placing its arterial inflow into the inferior vena cava while simultaneously allowing passive drainage from the mesenteric venous compartment. By utilizing two autoperfusion circuits to maintain a constant venous return to the inferior vena cava and to induce regional abdominal ischemia, we eliminated these direct hemodynamic effects.

The control blood pressures in group 2 animals were less than those in group 1. This likely was the result of greater blood loss associated with the more invasive surgery and the use of a venous return reservoir to maintain an ex vivo blood reserve. Conversely, the reflex increase in blood pressure was greater in group 2, which may have been related to the diminished resting blood pressure allowing a greater potential for blood pressure elevation (33). Furthermore, measurement of the blood pressure increment from nadir to peak response in group 1 may have underestimated the magnitude of the blood pressure increment. In particular, the true prereflex control blood pressure was partly obscured by the declining blood pressure that followed the initial occlusion-induced transient blood pressure elevation.

The blood pressure response to abdominal ischemia was reduced by an average of 53% by two specific BK2 receptor antagonists in the present study. Interestingly, we recently reported that NPC-17731 reduced the discharge frequency of ischemically sensitive afferent nerve fibers by 46% during abdominal visceral ischemia (21). The similar magnitude of these two related effects suggests that changes in the firing frequencies of this subgroup of C-fibers can alter arterial blood pressure in a proportional fashion. Conversely, the inability of BK2 receptor blockade to fully eliminate the reflex pressor response to abdominal ischemia suggests that other mediators are involved in the stimulation of splanchnic afferent nerves during visceral ischemia.

In addition to BK, we previously postulated that several other factors contribute to the activation of ischemically sensitive afferent fibers. These include reactive oxygen species such as \( \cdot \)OH (28), arachidonic acid products (15, 17), hypoxia (in an indirect fashion) (6), and decreased pH through production of lactic acid (27). Other potential stimuli include histamine (30) and serotonin (12). In this regard, we recently reported significant increases in histamine and serotonin concentrations in portal venous plasma and intestinal lymph during brief abdominal visceral ischemia (5). It therefore seems likely that the stimulation of afferent nociceptive fibers is a multifactorial process, which may ensure that nerve endings are activated during an ischemic event regardless of possible tachyphylaxis to one or more mediators (31).

Two potential limitations of the present study should be addressed. First, it is possible that inhibition of the ischemia-induced pressor response by a BK antagonist was related to a nonspecific action of the agent. This explanation appears unlikely, however, because a second, structurally dissimilar BK antagonist produced a similar degree of inhibition.

A second potential limitation was the period of ischemia of up to 20 min, which was longer than the shorter (5–10 min) durations used in previous studies of ischemia-induced BK production (21) and activation of ischemically sensitive C-fibers (15, 17). In this regard, although afferent activity can occur within the first few minutes of ischemia, we recently reported that spatial and temporal summation of sympathetic afferent activity in response to visceral ischemia actually continues to increase beyond 5–10 min and reaches a maximum by 17–20 min (23). Therefore, the rationale for the longer time period in the current study was to allow full development of the cardiovascular response by maintaining the ischemic period for up to 20 min.

In summary, two dissimilar BK2 receptor antagonists, HOE-140 and NPC-17731, reduced the magnitude of the visceral ischemia-induced pressor reflex by ~50%. Control studies verified the repeatability of the pressor response, and the reflex nature of the response was confirmed by combined superior mesenteric and celiac ganglionectomies. These findings support our hypothesis that endogenous BK produced during abdominal ischemia plays a major role in the activation of afferent sympathetic nerve fibers to elicit reflex cardiovascular responses.

Perspectives. A reflex cardiovascular sympathetic response to abdominal visceral ischemia may serve to provide compensatory flow to the ischemic region through collateral blood vessels to maintain local perfusion. The reflexly increased systemic arterial blood pressure, coupled with diminished blood pressure distal to the site of occlusion, would produce an augmented blood pressure differential that would promote blood flow through open collateral vessels to the ischemic...
region. Although this mechanism may provide a degree of nutrient and oxygen supplementation to the compromised tissue, it also is possible that the cellular injury produced by reperfusion (i.e., ischemia-reperfusion injury) is exacerbated by augmented arterial blood pressure and by elevated sympathetic stimulation in general. We believe that an increased sympathetic outflow response to local occlusive ischemia, as utilized in the present study, may be one important aspect of a generalized cardiovascular response to ischemia, whether it is induced by hemorrhagic shock, septic shock, embolic occlusion of a normal or stenotic blood vessel, or, in the clinical setting, arterial cross clamping during surgical repair or organ replacement. The degree to which the cardiovascular response is beneficial or detrimental would depend on a variety of factors, including the specific organ(s) at risk, duration and level of ischemia, body temperature, and tissue metabolic status.

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REFERENCES