Bradykinin and thromboxane A₂ reciprocally interact to synergistically stimulate cardiac spinal afferents during myocardial ischemia

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Fu LW, Longhurst JC. Bradykinin and thromboxane A₂ reciprocally interact to synergistically stimulate cardiac spinal afferents during myocardial ischemia. Am J Physiol Heart Circ Physiol 298: H235–H244, 2010. First published November 6, 2009; doi:10.1152/ajpheart.00782.2009.—Myocardial ischemia is a complex process leading to the simultaneous release of a number of mediators, including thromboxane A₂ (TxA₂) and bradykinin (BK), that activate cardiac spinal afferents. The present study tested the hypothesis that TxA₂ and BK reciprocally interact to excite ischemically sensitive cardiac afferents. Nerve activity of single cardiac afferent units was recorded from the left sympathetic chain or rami communicantes (T₃–T₅) of anesthetized cats. Fifty-two ischemically sensitive afferents (conduction velocity = 0.27–3.35 m/s, 7 Aδ-fibers and 45 C-fibers) were identified. Repeated injections (1 μg) of BK into the left atrium (LA) 4 min after the administration of U-46619 (5 μg into the LA), a TxA₂ mimic, induced a significantly larger cardiac afferent response than the first response to BK (0.61 ± 0.14 to 1.95 ± 0.29 vs. 0.66 ± 0.09 to 2.75 ± 0.34 impulses/s, first injection vs. second injection, n = 8). Conversely, blockade of TxA₂ receptors with BM-13,177 (30 mg/kg iv) attenuated the responses of eight other afferents to BK (1 μg into the LA) by 45%. In contrast, repeated BK (1 μg into the LA) induced consistent discharge activity in six separate afferents. We then observed that the coadministration of U-46619 (5 μg) and BK (1 μg into the LA) together caused a total response that was significantly higher than the predicted response by the simple addition of the individual responses. BK (1 μg) facilitated eight cardiac afferent responses to U-46619 (5 μg into the LA) by 64%. In contrast, repeated U-46619 (5 μg into the LA) without intervening BK stimulation evoked consistent responses in seven other ischemically sensitive afferents. Finally, inhibition of cyclooxygenase with indomethacin (5 mg/kg iv) eliminated the potentiating effects of BK on the cardiac afferent response to U-46619 (5 μg into the LA) but did not alter the afferent response to U-46619. These data suggest that BK and TxA₂ reciprocally interact to stimulate ischemically sensitive cardiac afferent endings leading to synergistic afferent responses and that the BK sensitization effect is mediated by cyclooxygenase products.

sympathetic afferents; U-46619; indomethacin

WHEN PATIENTS DEVELOP ischemic heart disease, their spinal afferent nerve endings are exposed to multiple ischemic metabolites that activate or sensitize these afferents leading to angina pectoris and excitatory cardiac-cardiovascular reflex responses (23, 24, 26). We and others have demonstrated that a number of ischemic metabolites, including thromboxane A₂ (TxA₂), serotonin (5-hydroxytryptamine), histamine, lactic acid (protons), ROS, and bradykinin (BK), in an interactive and multifactorial fashion, stimulate cardiac spinal afferents during ischemia and reperfusion (2, 7, 9, 10, 24, 33, 40, 42). However, despite an almost complete inhibition of stimulation by any of the receptor agonists, the antagonists, which are quite specific for the receptors, never fully block afferent activity during ischemia. This observation suggests that interactions between mediators may occur. In this regard, myocardial ischemia is a complex process that leads to the simultaneous release of a number of mediators that may act alone or through interactive mechanisms to evoke the final response. However, interactions between these mediators during the activation of cardiac spinal afferents that respond to myocardial ischemia remains poorly defined since very few studies have examined this possibility (9, 29).

TxA₂ is one of several ischemic metabolites released from activated platelets during myocardial ischemia. There are a number of studies demonstrating that TxA₂ is a strong vasoconstrictor agent and induces platelet aggregation (1, 13, 31). We recently demonstrated that TxA₂ evokes excitatory cardiac-cardiovascular reflex responses by stimulating cardiac spinal afferents with receptive endings in the left ventricular myocardium (11, 12). There are no studies of interactions between TxA₂ and other ischemic mediators.

The potent nociceptive peptide BK, through the stimulation of kinin B₂ receptors, activates ischemically sensitive cardiac spinal afferents and causes reflex cardiovascular excitation (16, 29, 30, 41). Recently, we observed that BK sensitizes cardiac spinal afferents responding to histamine (9). However, there is no information on interactions between BK and TxA₂ during the activation of ischemically sensitive cardiac afferents.

There are several reasons suggesting that BK might interact with TxA₂ to activate cardiac spinal afferents during ischemia. First, both TxA₂ and BK are released virtually simultaneously during myocardial ischemia (18, 25, 35). Second, we and others have demonstrated that both endogenous TxA₂ and BK independently contribute to the activation of cardiac spinal afferents during myocardial ischemia (12, 41). Third, BK stimulates phospholipase A₂, which, in turn, generates arachidonic acid, the principal substrate for PGE₁ and PGE₂ as well as TxA₂ formation (37). Once formed, these cyclooxygenase (COX) products diffuse out of the cell and are available to activate/sensitize cardiac spinal afferents through their individual receptor mechanisms (12, 41). Hence, there is a strong possibility that BK interacts with TxA₂ to activate and/or sensitize ischemically sensitive cardiac sympathetic afferents to the action of the other metabolite.

The purpose of the present study, therefore, was to evaluate the interactions between BK and TxA₂ in their actions on ischemically sensitive cardiac spinal afferents. We hypothesized that BK and TxA₂ reciprocally enhance the response of...
ischemically sensitive cardiac spinal afferents to the action of the other metabolite. Furthermore, we postulated that the interaction between BK and TXA2 is mediated through a COX mechanism. A preliminary report of this work has been previously presented (6).

METHODS

Surgical Preparation

Adult cats of either sex (2.3–4.2 kg) were anesthetized by an intramuscular injection of ketamine (20–30 mg/kg, Phoenix Scientific, St. Joseph, MO) followed by an intravenous injection of \( \alpha \)-chloralose (40–50 mg/kg) through the femoral vein. Additional injections of \( \alpha \)-chloralose (5–10 mg/kg iv) were given as necessary to maintain an adequate depth of anesthesia, which was assessed by observing the absence of a conjunctival reflex. The trachea of each animal was intubated, and respiration was maintained artificially (model 661, Harvard pump, Ealing, South Natick, MA). Cats were ventilated by air supplemented with 100% \( \text{O}_2 \) through the respirator. A femoral vein and artery were cannulated for the administration of drugs and fluids and for the measurement of blood pressure, respectively. Arterial blood pressure was measured by a pressure transducer (Statham P 23 ID, Gould) connected to the femoral arterial catheter. Arterial blood gases were assessed frequently by a blood gas analyzer (Radiometer ABL-5, Copenhagen, Denmark) and maintained within physiological limits (\( \text{PO}_2 > 100 \text{ mmHg}, \text{PCO}_2 = 28–35 \text{ mmHg}, \text{pH} \ 7.35–7.45 \)) by adjusting the respirator rate and tidal volume or by intravenously administering 2–3 ml of 1 M \( \text{NaHCO}_3 \) [8.4% (wt/vol)]. A polyethylene-90 catheter was introduced into the left atrium (LA) through the left atrial appendage for the intracardiac injection of solutions. Body temperature was monitored by a rectal thermometer and was maintained at 36–38°C with a circulating water heating pad and a heat lamp. At the end of the experiment, animals were deeply anesthetized by administering an additional dose of \( \alpha \)-chloralose (50 mg/kg iv) and were then euthanized by the administration of a solution of saturated potassium chloride. The surgical and experimental protocols used in this study were approved by the Animal Use and Care Committee of the University of California (Irvine, CA). The study conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.”

Cardiac Spinal Afferent Recording

Single-unit activity of cardiac afferents was recorded as previously described (8, 9). In brief, a midline sternotomy was performed, and the first to seventh left ribs and the left lung were removed. The left paravertebral sympathetic chain was isolated, draped over a Plexiglas platform, and then covered with warm mineral oil. Small nerve filaments were dissected gently from the chain and rami communicantes between \( T_2 \) and \( T_3 \) under an operating microscope (Zeiss), and the rostral ends were placed across one pole of the recording electrode. The other pole of the recording electrode was grounded with a cotton thread to the animal. The recording electrode was attached to a high-impedance probe (model HIP511, Grass Instruments, Quincy, MA). Action potentials of afferents were amplified (\( \times 50,000 \)) and bandpass filtered (100–3,000 Hz) through an alternating current amplifier (model P511 Preamplifier, Grass) and processed through an audioamplifier (AM8B, Audiomonitor, Grass) and an oscilloscope (model 2201, Tektronix, Beaverton, OR). Nerve activity and blood pressure signals were recorded on a Pentium computer using data acquisition and analysis software (Spike2), which sampled signals at 10,000 Hz through an analog-to-digital converter (CED micro 1401 mkII, Cambridge, UK) for online and offline quantitative analyses. Discharge frequency was quantified using a software window discriminator, and a histogram was generated for each afferent. Accurate counting of the impulse activity of each afferent was verified by comparing the constructed histogram with the original neurogram.

The receptive field of each afferent was located by mechanical stimulation of the heart. This included constricting the thoracic aorta as well as gently probing the heart with a cotton swab. The location of the afferent nerve ending was confirmed further by placing a stimulating electrode directly on the surface of the myocardium to evoke the afferent’s action potential. Briefly, while the afferent fiber activity was recorded, the epicardium was mapped gradually from the apex to the base of the heart using a bipolar stimulating electrode to search for the location of nerve endings electrically (6–12 V, 0.5 ms, 1-Hz stimuli). Once an action potential was evoked, the precise location of the receptive field was measured by gradually moving the stimulating electrode around the initially identified spot using the minimal intensity of stimulation. The conduction velocity (CV) of each afferent fiber was calculated by dividing conduction distance by conduction time. Conduction distance was estimated by measuring the length of a wet thread between the receptive field and the recording electrode along the supposed afferent pathway (8, 9). Unmyelinated C-fibers and finely myelinated A\( \delta \)-fibers were classified as those with CVs of \(< 2.5 \) and 2.5–30 m/s, respectively. In the present study, each afferent had a single receptive field that could be located precisely in the ventricles. Myocardial ischemia was induced by the complete occlusion of the appropriate coronary artery supplying the regional receptive field of the cardiac afferent nerve with a thread placed around the vessel. Ischemia was confirmed by observing a regional change in the color of the myocardium, which has been closely correlated with the production of lactic acid as indicated by a reduction in tissue \( \text{pH} \) (33). Afferents were considered to be ischemically sensitive if their discharge activity during 3–5 min of myocardial ischemia increased at least twofold above baseline activity (8, 9).

Experimental Protocols

Influence of TXA2 on the response of ischemically sensitive afferents to BK

Three groups of animals were used in this protocol. In the first group, eight cats were instrumented to record the activity of eight single cardiac afferents. After an afferent innervating the heart had been identified, its response to brief myocardial ischemia was recorded. If the afferent responded to ischemia, we injected BK (1 \( \mu \)g) into the LA and recorded the discharge activity of the cardiac afferent as previously described (12). After recovery of the response, the stable TXA2 mimetic U-46619 (5 \( \mu \)g into the LA) was administered. These doses of BK and U-46619 were selected for two reasons: 1) our previous work has shown that they are capable of activating ischemically sensitive cardiac afferents consistently without tachyphylaxis, if recovery periods of at least 20 min are maintained during repeated stimulation with the same agonist (9, 12); and 2) in a pilot study, we examined the influences of two doses of 2.5 and 5 \( \mu \)g U-46619 on the cardiac afferent response to BK. We observed that 2.5 \( \mu \)g U-46619 (into the LA) enhanced two of the three afferent responses to BK (1 \( \mu \)g into the LA), whereas 5 \( \mu \)g of the TXA2 mimetic augmented all three afferent responses to BK. Thus, for the remainder of this protocol in the present study, we selected 5 \( \mu \)g U-46619. Repeated stimulation with BK (1 \( \mu \)g into the LA) was conducted 4 min after the administration of U-46619 and 25 min after the first application of BK. Thirty minutes later, the response of the afferent to a concomitant LA injection of U-46619 (5 \( \mu \)g) + BK (1 \( \mu \)g) was evaluated. BK (Sigma) was dissolved in 0.9% NaCl and was prepared fresh daily. Two milligrams of U-46619 were dissolved in 0.4 ml of 100% ethanol to achieve an initial concentration of 5 mg/ml as a stock solution, which was stored in a \(-70^\circ \text{C} \) freezer. A working solution of 50 \( \mu \)g/ml was made by first removing 20 \( \mu \)l from the U-46619 stock solution and adding 1,980 \( \mu \)l of 0.9% saline to obtain the final concentration.
In the second group \((n = 10)\), after identification of an ischemically sensitive cardiac afferent, BK \((1 \mu g)\) was injected into the LA, and the response was recorded. Fifteen minutes later, 30 mg/kg BM-13,177, a specific \(T_XA_2\) receptor antagonist \((31)\), was administered intravenously. We have shown that this dose of BM-13,177 effectively blocks the action of \(T_XA_2\) on cardiac sympathetic afferents and the associated reflexes \((11, 12)\). BM-13,177 (Hoffmann-La Roche, Nutley, NJ) was dissolved in 1 ml of 8.4% NaHCO\(_3\) and then diluted as needed with 0.9% saline to a concentration of 30 mg/ml. Repeated stimulation with BK \((1 \mu g)\) into the LA) was conducted 15 min after the administration of BM-13,177.

The third group of time-control animals \((n = 6)\) was used to determine the afferent response to repeated stimulation with BK. After identification of an ischemically sensitive unit, each animal in this group was treated identically except that ethanol \((2\%, 0.2 \text{ ml into the LA})\) was used in the place of U-46619 (Fig. 1).

Effects of BK on responses of ischemically sensitive cardiac afferents to U-46619. We examined the effect of BK on the U-46619-evoked discharge activity of nine cardiac afferents. After identification of an ischemically sensitive fiber, the response of the afferent to U-46619 (5 \(\mu g\) into the LA) was evaluated (Fig. 1). After recovery of the response, BK \((1 \mu g)\) into the LA) was administered. Repeated stimulation with U-46619 (5 \(\mu g\) into the LA) was conducted 4 min after BK and 25 min after the first application of U-46619. Thirty minutes later, the response to a LA injection of U-46619 (5 \(\mu g\) + BK \((1 \mu g)\)) was examined. Since these responses were similar to those obtained with U-46619 + BK in the first protocol, the data were combined.

To determine the consistency of the afferent response to U-46619, we studied seven other ischemically sensitive cardiac afferents as time controls (Fig. 1). After identification of an ischemically sensitive unit, each animal in this group was treated identically with the exception that BK was replaced with saline \((0.2 \text{ ml into the LA})\).

Response of the BK-\(T_XA_2\) interaction to COX blockade. In seven other animals, we examined the influence of COX inhibition with indomethacin \((5 \text{ mg/kg iv})\) on the interaction between BK and U-46619 in seven ischemically sensitive cardiac afferents. This dose of indomethacin effectively abolishes visceral afferent responses to prostaglandins \((22)\). Indomethacin (Sigma) was dissolved in 8.4% sodium bicarbonate solution and diluted by 0.9% NaCl to a concentration of 10 mg/ml. After identification of an ischemically sensitive unit, the afferent response to U-46619 (5 \(\mu g\) into the LA) was evaluated (Fig. 1). Indomethacin was then administered intravenously. We repeated the stimulation with U-46619 30 min after its initial application and 15 min after treatment with indomethacin. U-46619 (5 \(\mu g\) into the LA) was injected for a third time 4 min after treatment with BK \((1 \mu g\) into the LA), which was 25 min after the second administration of U-46619.

An identical procedure was conducted in a time-control group of five animals with the exception that saline \((1 \text{ ml iv})\) was substituted for indomethacin (Fig. 1).

Data Analysis

The discharge activity of cardiac spinal afferents (expressed in impulses/s) was averaged during 3–5 min of preischemia and 5 min of ischemia. We measured the response of cardiac afferent nerve endings

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**Fig. 1.** Location of the receptive fields of ischemically sensitive cardiac afferents on the epicardial surface of the left and right ventricles. Receptive fields of the afferents included in study are as follows: □, A-fiber afferents \((n = 7)\); and •, C-fiber afferents \((n = 45)\).

**Fig. 2.** A: bar graph showing impulse activity of 8 cardiac sympathetic afferents to an injection of bradykinin \((BK; 1 \mu g)\) into the left atrium \((LA)\) before and 4 min after treatment with the thromboxane \(A_2\) \((T_XA_2)\) mimetic U-46619 \((5 \mu g)\) into the LA). B: reproducibility of the responses of 6 cardiac afferents to the repeated administration of BK. Values are means ± SE. *\(P < 0.05\) compared with control; #\(P < 0.05\), after U-46619 vs. before U-46619.
to U-46619, BK, and U-46619 + BK by averaging the discharge rates of the afferents during the entire period of response, defined as the time during which sustained activity exceeded baseline activity by 20% (7, 8). Baseline activity was determined over the 1- to 2-min period immediately preceding the chemical injection or the 3- to 5-min period immediately preceding ischemia. We also determined the duration and latency of each response, defined as the time when activity was increased by 20% above baseline and the time from the injection of the agonist to when we observed a 20% increase in activity of the afferent. Finally, we evaluated the total response of Table 1.

Table 1. Duration and total response of cardiac spinal afferents to chemical stimulation with BK and the TxA2 mimetic U-46619

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Duration, s</th>
<th>Total Responses, impulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First stimulus</td>
<td>Second stimulus</td>
</tr>
<tr>
<td>BK + U-46619</td>
<td>8</td>
<td>56±8</td>
<td>65±9</td>
</tr>
<tr>
<td>BK + vehicle</td>
<td>6</td>
<td>53±6</td>
<td>51±7</td>
</tr>
<tr>
<td>BK + BM-13,177</td>
<td>8</td>
<td>57±7</td>
<td>53±6</td>
</tr>
<tr>
<td>U-46619 + BK</td>
<td>8</td>
<td>58±6</td>
<td>75±7*</td>
</tr>
<tr>
<td>U-46619 + saline</td>
<td>7</td>
<td>55±8</td>
<td>54±9</td>
</tr>
<tr>
<td>U-46619 + Indo + BK</td>
<td>7</td>
<td>56±8</td>
<td>55±9</td>
</tr>
<tr>
<td>U-46619 + saline + BK</td>
<td>5</td>
<td>59±7</td>
<td>57±8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of afferents. BK, bradykinin; Indo, indomethacin. BK + U-46619, BK stimulation before and after the addition of U-46619. The same format is used with other mediator combinations. U-46619 + Indo + BK, U-46619 stimulation before, after Indo, and after Indo + BK. *P < 0.05 compared with the first stimulus.
afferent to chemical stimulation by counting all spikes that occurred during the entire period of response when activity exceeded baseline discharge rates by ≥20% to allow a comparison of the responses to stimulation of the individual mediators with the combined response.

Data are expressed as means ± SE. The Kolmogorov-Smirnov test was used to determine if the data were distributed normally. Normally distributed data in all protocols were compared with one-way repeated-measures ANOVA followed by a post hoc Tukey’s test. Non-normally distributed data in all protocols were compared with Friedman repeated-measures ANOVA on ranks followed by a post hoc Mann-Whitney’s test. All statistical calculations were performed with Sigmapstat software (Jandel Scientific Software, San Rafael, CA). Values were considered to be significantly different when \( P < 0.05 \).

RESULTS

Profile of Cardiac Spinal Afferents

We recorded activities of 52 ischemically sensitive cardiac afferents in the present study. The CV for these afferents ranged from 0.27 to 3.35 m/s. Eight-seven percent (45 fibers) of these afferents were classified as C-fibers (CV = 0.74 ± 0.06 m/s), whereas 13% (7 afferents) were classified as A\( \delta \)-fibers (CV = 2.81 ± 0.15 m/s). The endings of most (96%) afferents were located in either the anterior \(( n = 17)\) or posterior \(( n = 33)\) walls of the left ventricle (Fig. 1). Two afferents were located on the posterior wall of the right ventricle. We observed no obvious association between CV and the responsiveness of the afferents to chemical stimulation or ischemia.

Effect of TxA2 on the Response of Afferents to BK Stimulation

We observed that the activity of eight cardiac afferents (1 A\( \delta \)-fiber, CV = 3.35 m/s; 7 C-fibers, CV = 0.78 ± 0.21 m/s) was increased from 0.58 ± 0.19 to 2.57 ± 0.38 impulses/s during brief myocardial ischemia. U-46619 (5 \( \mu \)g into the LA) and BK (1 \( \mu \)g into the LA) stimulated all eight afferents, significantly increasing their activity from 0.58 ± 0.27 to 1.46 ± 0.35 impulses/s and from 0.61 ± 0.14 to 1.95 ± 0.29 impulses/s. Compared with the initial response to BK, the eight ischemically sensitive afferents responded more robustly to BK after U-46619 (1.95 ± 0.29 vs. 2.75 ± 0.34 impulses/s, initial vs. repeat, \( P < 0.05 \)), indicating that U-46619 enhanced the response of these afferents to BK by 48% (Fig. 2A). Also, we observed that U-46619 did not alter the response duration but enhanced the total response of the afferents to BK (Table 1). In contrast, the response of the six other ischemically sensitive cardiac afferents (1 A\( \delta \)-fiber, CV = 2.61 m/s; 5 C-fibers, CV = 0.66 ± 0.12 m/s) to repeated BK was consistent during the same time frame in the absence of U-46619 (Fig. 2B and Table 1).

Figure 3 shows an example of the response of two ischemically sensitive afferents (larger spike, A\( \delta \)-fiber, CV = 3.13 m/s, anterior wall, Fig. 3A; and smaller spike, C-fiber, CV = 0.73 m/s, posterior wall, Fig. 3B) innervating the left ventricle to BK (1 \( \mu \)g into the LA) before (Fig. 3, I) and after (Fig. 3, 2) blockade of the TxA2 receptor with BM-13,177 (30 mg/kg iv). As shown in Fig. 3, the discharge activity of the two responsive cardiac afferents simultaneously increased from 0.71 to 2.83 impulses/s and from 0.53 to 1.92 impulses/s during ischemia. Blockade of TxA2 receptors attenuated the response of two afferents to BK by 41% (Fig. 3, A2 and B2) compared with the initial BK response (Fig. 3, A1 and B1). Similarly, the response of 8 of 10 cardiac afferents (2 A\( \delta \)-fibers, CV = 2.71 and 3.13; 6 C-fibers, CV = 0.62 ± 0.11 m/s) to repeated BK was reduced by 45% after BM-13,177 compared with the initial BK response (Fig. 4). In addition, the total response, but...
not the response duration, of these afferents to repeated BK was attenuated after BM-13,177 (Table 1). We also observed that the responses of two other C-fiber cardiac afferents (CV = 0.43 and 1.26 m/s) to the second BK stimulation after BM-13,177 was unchanged (0.31 to 1.79 vs. 0.1 to 1.85 impulses/s and 0.35 to 2.86 vs. 0.43 to 2.77 impulses/s, initial vs. repeated BK for each afferent).

Influence of BK on U-46619-Evoked Activity

In this protocol, we observed that the activity of nine cardiac afferents (1 Aβ-fiber, CV = 3.15 m/s; 8 C-fibers, CV = 0.76 ± 0.21 m/s) was increased from 0.42 ± 0.25 to 2.16 ± 0.35 impulses/s during brief myocardial ischemia. U-46619 (5 µg into the LA) and BK (1 µg into the LA) stimulated all nine afferents. Compared with the initial response to U-46619, BK augmented the responses of eight of nine ischemically sensitive cardiac afferents to repeated U-46619 (1.58 ± 0.29 vs. 2.60 ± 0.36 impulses/s, initial vs. repeated, P < 0.05; Fig. 5A), resulting in BK potentiating the afferent response to U-46619 by 66% (Fig. 5). BK also enhanced both the response duration and total response of these afferents to U-46619 (Table 1). Only one of nine afferent responses to repeated U-46619 was unchanged after BK. In the control group, we observed that in the absence of BK, the response of the seven other cardiac afferents (1 Aβ-fiber, CV = 3.05 m/s; 6 C-fibers, CV = 0.72 ± 0.19 m/s) to repeated U-46619 was consistent during the same time period (Fig. 5B and Table 1).

Response of Cardiac Afferents to U-46619, BK, and U-46619 + BK

We observed that the discharge activity response of 15 ischemically sensitive cardiac afferents to coadministered BK and U-46619 (3.67 ± 0.21 impulses/s) was significantly higher than the responses to either BK (1.91 ± 0.16 impulses/s) or U-46619 (1.51 ± 0.14 impulses/s) alone (Fig. 6A and Table 2). The duration of response of the afferents during simultaneous stimulation with BK + U-46619 was significantly longer than either BK or U-46619 alone as well as the simple addition of the individual responses (121 ± 4 s; Table 2). Although the average activities of the combined response did not exceed that predicted by simple addition of the individual responses (Fig. 6A), the total response to BK + U-46619 given in combination (768 ± 110 impulses) was significantly higher than either BK (113 ± 9 impulses/s) or U-46619 (492 ± 112 impulses/s) alone (Fig. 6A and Table 2).
impulses) or U-46619 (89 ± 8 impulses) alone as well as that predicted by simple addition (202 ± 15 impulses; Fig. 6B). Figure 6C shows the summed 2-s discharge activity of all 15 cardiac afferents during the injection of U-46619 (C1), BK (C2), or BK + U-46619 (C3). Similar to the changes in the total response, we observed that the summed afferent activity response to BK + U-46619 was dramatically higher than the response to either BK or U-46619.

An example of the response of a C-fiber cardiac afferent (CV = 0.4 m/s, innervating the posterior left ventricle) to BK, U-46619, and BK + U-46619 is shown in Fig. 7. The discharge activity of this afferent was increased from 0.12 to 1.14 impulses/s during myocardial ischemia (Fig. 7A). Similar to observations in our previous studies (9, 12), both BK and U-46619 stimulated this afferent. Simultaneous stimulation with BK + U-46619 led to facilitation in this afferent activity compared with BK and U-46619 alone (3.95 vs. 2.13 and 1.47 impulses/s, respectively). The total response (497 impulses) of this afferent to the coadministration of BK + U-46619 was dramatically higher than either BK (126 impulses) or U-46619.
(88 impulses) alone as well as the predicted total response by simple addition (214 impulses; Fig. 7, B–D).

### Effect of COX Blockade on the U-46619-BK Interaction

In this protocol, the initial administration of U-46619 (5 μg into the LA) increased the activity of seven ischemically sensitive cardiac afferents (1 Aδ-fiber, CV = 2.71 m/s; 6 C-fibers, CV = 0.96 ± 0.19 m/s) from 0.55 ± 0.09 to 1.77 ± 0.23 impulses/s. The responses of the seven afferents to a second application of U-46619 after indomethacin (5 mg/kg iv) was unchanged (1.77 ± 0.23 vs. 1.80 ± 0.25 impulses/s, first vs. second U-46619 response, P > 0.05; Fig. 8A). Furthermore, the response to the TxA2 mimetic 4 min after BK (1 μg into the LA) in the presence of indomethacin was similar to the second U-46619 response (1.80 ± 0.25 vs. 1.65 ± 0.26 impulses/s, second vs. third U-46619 response, P > 0.05; Fig. 8A). Similarly, the response duration and total response of these afferents to repeated U-46619 were unchanged when we compared before versus after treatment with indomethacin and BK (Table 1). Thus, indomethacin eliminated the potentiating effects of BK on the afferent response to U-46619 but did not alter the afferent response to U-46619. In a control group of afferents (5 C-fibers, CV = 0.71 ± 0.26 m/s), we observed consistent responses to repeated U-46619 in the absence of indomethacin and an enhanced response to a third U-46619 stimulation after BK (Fig. 8B and Table 1), similar to our earlier protocol (Fig. 5A and Table 1).

### DISCUSSION

Four novel findings were made in the present study. First, we found that TxA2 facilitated the BK-evoked discharge activity of ischemically sensitive cardiac spinal afferents since the administration of U-46619, a TxA2 mimetic, enhanced the cardiac afferent response to BK while blockade of TxA2 receptors with BM-13,177 attenuated the BK response. Second, BK potentiated the response of ischemically sensitive afferents to TxA2, an interaction that requires an intact COX pathway. In this respect, BK increased the afferent response to U-46619, a response that was eliminated by COX blockade with indomethacin. Third, simultaneous stimulation with BK and U-46619 evoked a synergistic afferent response since the total response of these afferents to the coadministration of BK and TxA2 was significantly higher than the predicted total response by simple addition of the individual response to each mediator. Finally, we observed that the stimulation of TxA2 on cardiac afferents was not mediated by the COX pathway. Thus, these data strongly suggest that BK and TxA2 reciprocally facilitate the responses of ischemically sensitive cardiac afferents to the action of the other metabolite. Furthermore, BK enhances the response of afferents to TxA2 through a COX-dependent mechanism.

Although there have been no prior investigations of interactions between TxA2 and BK on sensory afferent fibers, several observations suggest that reciprocal effects may occur and that the COX system may be involved. First, we have shown that endogenously produced TxA2 and BK stimulate cardiac spinal afferents during ischemia through receptor-specific mechanisms located on the cardiac sensory nerve endings (11, 12, 41). Second, during ischemia, both TxA2 and BK are appear to be released quickly and simultaneously since our previous studies and investigations by others have shown that blockade of TxA2 receptors with BM-13,177 and BK2 receptors with HOE-140 attenuate the early activation of afferents (5, 12, 41). Third, a number of COX products, including PGE2, PGI2, and PGH2, sensitize cardiac and other (visceral and somatic) sensory nerve fibers in vitro to thermal and mechanical stimulation (5, 12, 41). Thus, we felt that it was reasonable to develop the working hypothesis that TxA2 enhances the cardiac afferent response to BK.

The ability of BK to sensitize the afferent response to TxA2 does not appear to be unique since we have found that BK also sensitizes cardiac spinal afferents to the action of histamine through a COX mechanism during myocardial ischemia (10). Others observed that BK also sensitizes somatic and testicular sensory nerve fibers in vivo to thermal and mechanical stimulation, although they have not determined if this sensitization is mediated by the activation of the COX pathway (19, 21). Sensitization refers to an increase in the magnitude of response, sometimes accompanied by an increase in spontaneous activity and/or a decrease in the response threshold (14, 28).

Myocardial ischemia causes virtually the simultaneous release of BK and TxA2 (5, 17, 18). After release, through a BK2 receptor mechanism coupled to G proteins, BK stimulates
phospholipase A2, which, in turn, produces arachidonic acid (20), the major substrate for the COX system, which leads to formation of TXA2. Thus, involvement of the COX system in the BK-TXA2 interaction seems rational.

The mechanism of the interaction between BK and TXA2 on ischemically sensitive cardiac spinal afferents has not been previously evaluated. Both intracellular PKC signaling and the COX pathway potentially are involved. We recently documented that, through direct activation of TXA2 receptors coupled with the phospholipase C-PKC pathway, TXA2 excites cardiac sympathetic afferents during ischemia (12). We and others also have shown that BK stimulates visceral spinal afferents originating from the heart and abdominal visceral organs as well as cutaneous nociceptors responding to heat stimulation through a PKC mechanism (4, 15, 39, 41). BK also activates abdominal visceral spinal afferents and polymodal somatic C-fiber afferents responsive to thermal stimulation through a COX mechanism (34, 36). In the previous study (41), we demonstrated that inhibition of COX with indomethacin attenuates the cardiac spinal afferent response to BK. Since we were able to demonstrate that indomethacin eliminates BK-related facilitation of TXA2’s action on cardiac spinal afferents in the present study, we have been able to provide additional evidence implicating the COX system in the synergistic interaction between BK and TXA2.

In addition to the reciprocal interactions between BK and TXA2, we also evaluated the response of ischemically sensitive cardiac spinal afferents to simultaneous stimulation with BK + TXA2 since when patients or intact animals develop myocardial ischemia, their cardiac spinal afferents are exposed to multiple ischemic mediators that activate, sensitize, or desensitize these afferents, leading to angina pectoris (23, 24). The processes underlying activation/sensitization of the cardiac spinal afferent nerve endings are highly complex. They involve synergistic or suprasadditive, additive, subadditive, and subtractive actions of a variety of ischemic mediators (3, 28). The synergistic actions (effects), a term that is preferred to “more than additive” in this case, describes the afferent response to coadministered mediators that far exceeds the theoretical response that would be observed by adding the responses to individual mediators. In the present study, we did not observe average or peak activity responses to the combined administration of the two mediators that were greater than that predicted by simple addition of the two individual stimuli. However, we observed something quite different when we assessed the total responses. In this regard, the total response to U-46619 was 89 ± 8 impulses, whereas that to BK was 113 ± 9 impulses. The theoretical total response formed by simple addition of the individual responses would be expected to be 202 ± 14 impulses. However, the actual total response to codistributed BK + U-46619 was 768 ± 110 impulses, which clearly represents a synergistic action between the two mediators. In considering what would be important with respect to information received by the central nervous system, average, peak, or total afferent discharge activity, we believe that the total afferent response over time would be most effective in stimulating heightened effector or affective responses, such as angina. Of note, however, this observation is in contrast to what we previously observed to be a less than additive (i.e., subadditive) afferent response to simultaneous stimulation with BK and histamine, because although BK sensitizes afferents to histamine, histamine desensitizes afferents to BK (9). As such, our data show that interactions between the actions of multiple chemical mediators, released simultaneously during myocardial ischemia, on cardiac spinal afferents are very complex.

We found it interesting that indomethacin was unable to attenuate the response of cardiac afferents to the application of U-46619. A previous study (32) has demonstrated that exogenous U-46619 can activate platelets to release TXA2 and other prostaglandins. Thus, we initially speculated that inhibition of COX with indomethacin would decrease the response of afferent to TXA2. However, our data refute this working hypothesis and appear to be consistent with our previous finding that TXA2 stimulates cardiac spinal afferents directly through activation of TXA2 receptors located on cardiac spinal sensory nerves rather than indirectly through a COX mechanism (12). Our data further suggest that TXA2, unlike BK, does not activate the COX system. Thus, while BK enhances response of TXA2 through a COX mechanism, the converse does not appear to be true.

In conclusion, the present study provides the first evidence to demonstrate that BK sensitizes ischemically sensitive cardiac spinal afferents to the action of TXA2 and that such sensitization requires an intact COX pathway. Furthermore, TXA2 potentiates the response of most cardiac afferents to BK, an action that is COX independent. Thus, when released simultaneously during ischemia, BK and TXA2 reciprocally interact to excite cardiac spinal afferents leading to synergistic afferent responses. This information extends our understanding of the highly complicated processes underlying the chemical activation and sensitization of cardiac spinal afferent responses during myocardial ischemia.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

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