Role of cAMP in activation of ischemically sensitive abdominal visceral afferents

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Guo, Zhi-Ling, and John C. Longhurst. Role of cAMP in activation of ischemically sensitive abdominal visceral afferents. Am. J. Physiol. Heart Circ. Physiol. 278: H843–H852, 2000.—A number of metabolites produced during abdominal ischemia can stimulate and/or sensitize visceral afferents. The precise mechanisms whereby these metabolites act are uncertain. Other studies have shown that the adenylate cyclase-cAMP system may be involved in the activation of sensory neurons. Therefore, we hypothesized that cAMP contributes to the activation of ischemically sensitive abdominal visceral afferents. Single-unit activity of visceral C fibers was recorded from the right thoracic sympathtic chain in anesthetized cats before and after ischemia. Forty-six percent of ischemically sensitive visceral C fibers responded to intra-arterial injection of 8-bromo-cAMP (0.35–1.0 mg/kg), an analog of cAMP, with responses during ischemia increasing from 0.50 ± 0.06 to 0.84 ± 0.08 impulses/s (P < 0.05, n = 11 C fibers). Conversely, an inhibitor of adenylate cyclase, 2',5'-dideoxyadenosine (DDA; 0.1 mg/kg iv), attenuated ischemia-induced increase in activity of afferents from 0.66 ± 0.10 to 0.34 ± 0.09 impulses/s (P < 0.05; n = 8). Furthermore, whereas exogenous PGE2 (3–4 µg/kg iv) augmented the ischemia-induced increase in activity of afferents (P < 0.05, n = 10), treatment with DDA (0.1 mg/kg iv) substantially reduced the increase in discharge activity of afferents during ischemia, which was augmented by PGE2 (1.45 ± 0.24 vs. 0.70 ± 0.09 impulses/s, −DDA vs. +DDA; P < 0.05) in six fibers. A control group (n = 4), however, demonstrated similar increases in the activity of afferents with repeated administration of PGE2. These data suggest that cAMP contributes to the activation of abdominal visceral afferents during ischemia, particularly to the action of PGs on activation and/or sensitization of these endings.

sympathetic afferents; nociception; cat; adenosine 3',5'-cyclic monophosphate; prostaglandins

ABDOMINAL ISCHEMIA is a particularly strong stimulus for abdominal visceral afferents, which reflexly excite the cardiovascular system (22). A number of metabolites produced during abdominal ischemia, including lactic acid, reactive oxygen species, PGs, bradykinin (BK), histamine, and serotonin, among others, activate and/or sensitize responsive afferents (8, 9, 23, 24). There is little information about the intracellular mechanism(s) by which these metabolites activate these sensory nerve endings. Currently, our laboratory is concerned with elucidating mechanisms responsible for signal transduction in C fiber afferents. Recent findings have shown that the phosphatidylinositol system is one of the intracellular signaling pathways that contributes to this process during ischemia (11). We believe, however, that other intracellular signal transduction processes likely play a role in the activation of ischemically sensitive abdominal visceral C fibers, because our previous results showed that the inhibition of protein kinase C (PKC) does not completely block the activation of visceral C fibers during abdominal ischemia (11).

Other investigators have demonstrated that the adenylate cyclase-cAMP system is involved in the activation of sensory afferent systems (1, 25, 29–31). In this regard, Taiwo and colleagues (30, 31) showed that cAMP serves as a second messenger in the hyperalgesia of the rat hindpaw. Ricci et al. (29) documented a role for cAMP in sensory-neural communication at the vestibular end organ. Various chemical mediators excite receptors in sensory nerve endings, which then activate adenylate cyclase through guanine nucleotide-binding proteins (G proteins). Activation of adenylate cyclase degrades ATP and generates cAMP. Elevation of intracellular cAMP modulates the responses of neurons by activating protein kinase A, which then phosphorylates cellular components, including membrane-bound receptors, ion channels, and enzymes (3). These processes likely play an important role in the signal transduction of sensory nerve endings (1, 3, 25, 29–32).

PGs, such as PGE2, may mediate the activity of sensory nerves by activating the adenylate cyclase-cAMP system, which increases intracellular cAMP (1, 15). There is some evidence that PGE2 can sensitize mechanosensitive C fibers innervating the dorsum of hindpaw in rats (1, 34), and that the PGE2-related action on these C fibers can be blocked by inhibitors of adenylate cyclase and cAMP-dependent protein kinases (34). However, previous work by Wang et al. (34) raised some concern because of the absence of original raw data and the substantial variability of the influence of PGs on mechanical threshold and discharge activity during mechanical stimulation. Thus the physiological or pathophysiological relevance of this work on pain is uncertain. With respect to chemoafferent afferents, Nerdrum et al. (26) have shown that PGE1 significantly increases the responses of sympathetic cardiac afferents to BK, which can evoke a pressor reflex. These investigators speculated that cAMP may...
be responsible for the effects of PGE1. Previous data by Longhurst and colleagues (23, 24) also indicated that PGs, particularly PGE2, produced during abdominal ischemia stimulate and/or sensitize visceral afferents (23, 24). The contribution of cAMP to this process, however, is unknown. The aim of the present study was to examine the role of cAMP in activation of ischemically sensitive abdominal visceral C fiber afferents. We hypothesized that cAMP contributed to this ischemia-induced activation, especially to PG-mediated activation of sensory nerve endings during ischemia. A preliminary report of this work has been presented (12).

**METHODS**

Adult cats (2.3–3.4 kg) of either sex were induced with ketamine (20–30 mg/kg im) and anesthesia was maintained with α-chloralose (30–40 mg/kg iv). Respiration was maintained artificially (Harvard pump, model 661; Ealing, South Natick, MA). Arterial blood gases were analyzed frequently (model ABL-3; Radiometer, Westlake, OH) and were maintained within physiological limits (PO2, 100–150 mmHg; PCO2, 28–35 mmHg; pH 7.35–7.45). A femoral vein and artery were cannulated for administration of fluids and drugs and for measuring arterial pressure (Statham P23 ID), respectively. Body temperature was maintained between 36 and 38°C. Surgical and experimental protocols used in this study were approved by the Animal Use and Care Committee at the University of California, Irvine.

**Visceral Afferent Recording**

We have described this method in detail previously (8, 11). Briefly, we removed the third through eleventh right ribs and the middle and lower lobes of the right lung after a midline sternotomy. We placed an inflatable occlusion cuff around the thoracic aorta through the femoral artery catheter. 8-Bromo-cAMP (8-BrCAMP) is a stable, membrane-permeable analog of cAMP (4, 14). It is capable of attenuating responses of saphenous nerves to mechanical stimuli in streptozotocin-diabetic rats (1). DDA was dissolved using 10% DMSO in 0.9% saline to make a stock solution (10 mg/ml). A second stock solution (1 mg/ml in 1% DMSO) and the final solution for administration were made using 0.9% saline. PGE2 was stored at −10°C in an absolute ethanol stock solution (10 mg/ml). It was diluted to a concentration of 10 μM with 0.2 M phosphate buffer solution. Fresh solution was prepared daily.

**Experimental Protocols**

Effect of 8-BrCAMP on C fiber afferent activity induced by ischemia. After 30 min of reperfusion. If the afferent did not respond to 8-BrCAMP or if the afferent activity returned to normal levels after activation induced by 8-BrCAMP, a second period of ischemia was induced to observe the response of the afferent to ischemia after administration of 8-BrCAMP. In a separate group of ischemically sensitive C fibers, an area of ischemia was marked with a laser. Effect of inhibition of adenylate cyclase on PGE2-mediated activation of afferents during abdominal ischemia. Ten ischemically sensitive C fiber afferents were identified during 7 min of abdominal ischemia followed by 5 min of reperfusion. If the afferent was ischemically sensitive, a second period of ischemia was repeated 30–40 min later in the presence of DDA (0.1 mg/kg iv; n = 8) to inhibit adenylate cyclase. DDA is a cell-permeable adenylate cyclase inhibitor (17). Intradermal injection of DDA attenuates responses of saphenous nerves to mechanical stimuli in streptozotocin-diabetic rats (1). DDA was given minutes before repeated ischemia. To demonstrate the reproducibility (n = 6), C fiber afferent activity in a control group was recorded in response to the appropriate volume/vehicle control for 8-BrCAMP (0.9% saline) during two repeated periods of ischemia.

**Drugs and Solutions**

8-Bromo-cAMP (8-BrCAMP) was purchased commercially from Calbiochem-Novabiochem (La Jolla, CA), and 2′,5′-dideoxyadenosine (DDA) and PGE2 were from Sigma Chemical (St. Louis, MO). 8-BrCAMP was dissolved in 0.9% saline to a concentration of 10 mg/ml and was diluted further with 0.9% saline. DDA was dissolved using 10% DMSO in 0.9% saline to make a stock solution (10 mg/ml). A second stock solution (1 mg/ml in 1% DMSO) and the final solution for administration were made using 0.9% saline. PGE2 was stored at −10°C in an absolute ethanol stock solution (10 mg/ml). It was diluted to a concentration of 10 μM with 0.2 M phosphate buffer solution. Fresh solution was prepared daily.
mg/kg iv) in six cats. The vehicle for DDA (1 ml of 0.1% DMSO) was examined using an identical protocol in another four animals.

In four separate cats, two bouts of ischemia were induced 30–40 min apart as a time control. The vehicle for PGE$_2$ (1 ml of 0.1% ethanol in 0.2 M phosphate buffer solution) was administered before the second exposure to ischemia.

Data Analysis

Peak discharge rate of ischemically sensitive afferents was averaged over 60 s during 5 min of control and 7 min of ischemia when the greatest number of spikes occurred. Afferents were considered to be ischemically sensitive if the increase in discharge activity during 7 min of abdominal ischemia was sustained at least twofold above baseline. The increase in afferent activity induced by 8-BrcAMP was measured by averaging the discharge rate during the entire response period (15–66 s). The latency of response to ischemia or 8-BrcAMP was measured from the time of arterial occlusion or injection to the time a sustained increase in activity occurred at least 10% over baseline.

Statistical Analysis

Data are expressed as means ± SE. The increase in impulse frequency of ischemically sensitive C fibers induced by ischemia or 8-BrcAMP was compared among interventions in each group using a one-way, repeated-measures ANOVA with a Tukey post hoc test. If data were not normally distributed, as determined by the Kolmogorov-Smirnov test, they were compared using the Friedman repeated-measures ANOVA on ranks, followed by a Dunnett’s post hoc test. The onset latency of discharge activity was compared using a one-way, repeated-measures ANOVA or the Friedman repeated-measures ANOVA on ranks, if data were not normally distributed. All statistical calculations were performed with commercially available software (Jandel Scientific Software, San Rafael, CA). Values were considered significantly different when P < 0.05.

RESULTS

Effect of analog of cAMP on activity of ischemically sensitive C fibers. Thirty minutes after the identification of ischemically sensitive abdominal visceral C fibers, administration of 8-BrCAMP (0.35–1.0 mg/kg ia) transiently (15–66 s) increased discharge activity of visceral afferents from 0.06 ± 0.03 to 0.51 ± 0.06 impulses/s after an onset latency of 93 ± 3 s in 5 of the 11 ischemically sensitive C fibers (CV = 0.56 ± 0.08 m/s, n = 5 vs. CV = 0.55 ± 0.04 m/s, n = 11). Thus 46% of ischemically sensitive C fibers responded directly to injection of 8-BrCAMP. Conversely, discharge activity was unchanged from control period during all vehicle challenges (0.9% saline).

Abdominal ischemia increased activity of C fiber afferents from 0.06 ± 0.02 to 0.50 ± 0.06 impulses/s (P < 0.05) after an onset latency of 193 ± 32 s (n = 11; Fig. 1B). After administration of 8-BrCAMP, the ischemia caused a larger increase (P < 0.05, Fig. 1B) in activity of all afferents from baseline 0.06 ± 0.02 to 0.84 ± 0.08 impulses/s (P < 0.05), with a shorter latency of response (140 ± 31 s). Inflation of the aortic occlusion cuff to induce ischemia reduced (P < 0.05) femoral mean arterial pressure similarly in the ab-
ents (CV = 0.63 ± 0.08 m/s) from 0.10 ± 0.04 to 0.66 ± 0.10 impulses/s (P < 0.05), following a latency of 236 ± 33 s (n = 8). Inhibition of adenylyl cyclase (DDA; 0.1 mg/kg iv), however, decreased (P < 0.05; Fig. 3B) the ischemia-induced augmentation of discharge activity (0.09 ± 0.02 to 0.34 ± 0.09 impulses/s, P < 0.05) after a latency of 266 ± 44 s, compared with the first period of ischemia. Inflation of the aortic occlusion cuff to induce ischemia reduced (P < 0.05) femoral mean arterial pressure similarly before (89 ± 10 to 16 ± 2 mmHg) and after inhibition of cAMP (88 ± 11 to 16 ± 1 mmHg). An original tracing of an ischemically sensitive C fiber (CV = 0.54 m/s) innervating the duodenum is shown in Fig. 4.

In six C fibers (CV = 0.62 ± 0.07 m/s), as a time control protocol, we examined the responses of afferents to repeated ischemia without inhibition of adenylyl cyclase. Comparing the first and second periods of ischemia, we observed similar increases in afferent activity from baseline (0.05 ± 0.02 to 0.60 ± 0.08 impulses/s, and 0.06 ± 0.02 to 0.58 ± 0.10 impulses/s; Fig. 3A), comparable decreases in femoral arterial pressure (89 ± 11 to 16 ± 1 mmHg, and 89 ± 12 ± 15 ± 2 mmHg), and equivalent onset latencies (150 ± 28 s and 148 ± 24 s). Afferents studied in this protocol were located in the duodenum, mesentery, pancreas, or porta hepatitis (Table 1).

Effect of inhibition of adenylyl cyclase on PGE2-mediated activation of afferents during abdominal ischemia. In 10 cats, ischemia caused an increase in discharge activity of C fiber afferents (CV = 0.57 ± 0.04 m/s) from 0.07 ± 0.02 to 0.59 ± 0.09 impulses/s (P < 0.05), after a latency of 145 ± 26 s (n = 10). Administration of PGE2 (10 µg; 3–4 µg/kg ia) augmented (P < 0.05) the ischemia-induced increase in discharge activity (0.09 ± 0.02 to 1.27 ± 0.19 impulses/s, P < 0.05; Fig. 5B) and shortened the latency to 105 ± 15 s. Arterial occlusion reduced (P < 0.05) mean femoral arterial pressure similarly before (89 ± 10 to 16 ± 2 mmHg) and after administration of PGE2 (89 ± 11 to 15 ± 2 mmHg).

In six C fibers (CV = 0.61 ± 0.06 m/s), we noted that treatment with DDA (0.1 mg/kg iv) attenuated (P < 0.05) the increase in discharge rate of afferents during ischemia caused by the second injection of PGE2 (0.07 ± 0.02 to 1.45 ± 0.24 vs. 0.12 ± 0.08 to 0.70 ± 0.09 impulses/s, −DDA vs. +DDA; Fig. 6B) and prolonged the

Table 1. Location of abdominal visceral C fiber afferents

<table>
<thead>
<tr>
<th>Organ</th>
<th>Saline Ischemia</th>
<th>8-BrCAMP Ischemia</th>
<th>DMSO Ischemia</th>
<th>DDA Ischemia</th>
<th>PBS Ischemia</th>
<th>Repeat PGE2 Ischemia</th>
<th>DDA + PGE2 Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mesentery</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Porta hepatitis</td>
<td>6</td>
<td>11</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>35</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are numbers of afferent endings. DDA, 2',5'-dideoxyadenosine; PGE2, prostaglandin E2.
ischemia (0.11 impulses/s; Fig. 6). Femoral mean arterial pressure was reduced similarly before (90 ± 10 to 15 ± 2 mmHg) and after DDA (90 ± 11 to 15 ± 1 mmHg). An original tracing of an ischemically sensitive C fiber (CV = 0.58 m/s) innervating the gallbladder is shown in Fig. 7.

Repeated injection of PGE$_2$ (10 µg ia) induced similar increases in activity of afferents during ischemia (0.14 ± 0.04 to 1.02 ± 0.31 vs. 0.10 ± 0.06 to 1.16 ± 0.44 impulses/s; Fig. 6A), when the vehicle for DDA was used in four other C fibers (CV = 0.51 ± 0.05 m/s). Decreases in femoral arterial pressure (89 ± 12 to 16 ± 1 mmHg, and 89 ± 12 to 15 ± 2 mmHg) and onset latencies also were equivalent (135 ± 26 s and 142 ± 44 s).

The responses to repeated ischemia with the vehicle for PGE$_2$ were examined in four separate C fibers (CV = 0.80 ± 0.11 m/s). Compared with the first bout of ischemia (0.14 ± 0.06 to 0.62 ± 0.13 impulses/s), we observed similar increases in afferent activity during ischemia (0.11 ± 0.05 to 0.55 ± 0.05 impulses/s; Fig. 5A). Comparable decreases in femoral arterial pressure (90 ± 10 to 15 ± 2 mmHg, and 89 ± 11 to 16 ± 1 mmHg) also occurred, and onset latencies were unchanged (166 ± 31 s and 172 ± 42 s). Afferents studied in this protocol were located in the duodenum, gallbladder, mesentery, pancreas, or porta hepatis (Table 1).

**DISCUSSION**

The present study demonstrates, for the first time, a role for cAMP in activation of ischemically sensitive abdominal visceral C fiber afferents. Findings in this study support our hypothesis that cAMP contributes to the activation of these afferents. In this regard, we observed that a stable, membrane-permeable analog of cAMP stimulated approximately half of ischemically sensitive abdominal visceral C fiber afferents and sensitized all of these afferents to ischemia. Inhibition of adenylate cyclase, which reduced production of cAMP, attenuated the response of the abdominal visceral afferent fibers to ischemia. Also, we found that inhibition of adenylate cyclase reduced PGE$_2$-mediated activation of abdominal visceral afferents during ischemia. Taken together, these results suggest that the adenylate cyclase/cAMP system, perhaps in part stimulated by PGE$_2$, is involved in the signaling mechanisms underlying activation of ischemically sensitive abdominal visceral afferents.

CAMP is an important intracellular messenger that mediates the process of cellular signal transduction. When adenylate cyclase is activated by stimulating receptors that bind to G protein, the intracellular concentration of cAMP rises. Once elevated, cAMP activates protein kinase A, which phosphorylates cellular proteins, including receptors, ion channels, and both cytosolic and nuclear proteins, and then causes a number of neuronal responses (3, 25). Through this process, cAMP can regulate a variety of sensory neuron functions, including neuronal excitability and neurotransmitter release (3, 15, 18, 30). Previous investigations have suggested that cAMP is involved in signal transduction of some mechanosensory afferents (1, 30, 31). For example, analogs of cAMP or agents that elevate cAMP induce mechanical hyperalgesia in the hindpaws of rats (1, 30) and enhance the responses to heat in frog skin (25). Elevation of cAMP levels also produces an increase in spontaneous afferent nerve firing and a concomitant decrease in the transepithelial potential at the vestibular end organ (29). PGE$_2$ mediates mechanical hyperalgesia in the skin of rat (1, 34). PGE$_2$-induced hyperalgesia can be blocked by inhibitors of adenylate cyclase and CAMP-dependent protein kinase (34). PGE$_2$ also significantly increases the content of CAMP-like immunoreactive substance (iCAMP) in the sensory neuron cultures and pretreatment of these sensory neurons with an inhibitor of adenylate cyclase (9-tetrahydro-2-furyl adenine) reduces PGE$_2$-induced increases in CAMP (15). These data suggest that the action of PGs on sensory nerves likely is mediated through the activation of the adenylate cyclase-cAMP system, which leads to an increase in intracellular CAMP. Finally, our previous results have demonstrated that PGs produced during abdominal ischemia can activate and/or sensitize ischemically...
sensitive abdominal visceral afferents (23, 24). On the basis of these previous studies, we hypothesized in the present study that cAMP would contribute to the activation of ischemically sensitive abdominal visceral afferents.

To evaluate the role of cAMP in the activation of visceral afferents, we first determined whether cAMP was involved in stimulating and/or sensitizing afferents by using an exogenous analog of cAMP. 8-BrcAMP is a stable, membrane-permeable analog of cAMP (4, 14) that induces peripheral hyperalgesia when injected intradermally (1, 30). In the present investigation, we observed that 8-BrcAMP directly stimulated approximately half of the C fibers that responded to ischemia and increased the discharge rate of these afferents during ischemia by 68%. Together with our inability to alter afferent activity in the time and/or vehicle control group, these data suggest that cAMP contributes to the activation of ischemically sensitive abdominal visceral C fibers.

Limited studies on primary afferents in vivo show that analogs of cAMP (e.g., 8-BrcAMP) sensitize sensory afferents innervating skin and induce hyperalgesia during mechanical stimulation (30, 31). However, previous studies only observed the threshold and discharge activity during mechanical stimulation. Our data provide the first evidence that cAMP contributes to the activation of chemo-sensitive visceral C fibers during abdominal ischemia. These results are consistent with others documenting that cAMP increases excitability of central and peripheral neurons (6, 16, 18, 20, 21), for example, baroreceptor neurons (21). Potential mechanisms underlying the effects of cAMP likely are related to its effect on depolarization of excitable membranes. In this regard, there are several possible explanations for its mechanisms of action. The first is inhibition of outward potassium currents. Voltage-dependent potassium channels are the primary regulator of membrane excitability. Elevation of cAMP levels inhibits potassium currents in dorsal root ganglion neurons (2, 7). Activation of the cAMP cascade also reduces the steep voltage-dependent transient potassium current and the slow-activating potassium currents and increases excitability of Aplysia sensory neurons (10). Furthermore, the cAMP cascade contributes to PGE$_2$-induced inhibition of potassium currents in sensory neurons of dorsal root ganglion (7). In addition, cAMP inhibits calcium-activated potassium current in the aortic baroreceptor neurons of rats (21). Modulation of calcium-activated potassium channels commonly affects action potential duration, spike frequency adaptation, and resting membrane potential (21).

The second explanation is activation of the TTX-resistant sodium channel. Contribution of this sodium current to neuronal excitability may be relevant in long trains of action potentials in these cells (5). Activation of the cAMP cascade increases excitability of neonatal rat dorsal root ganglion neurons by modulation of the TTX-resistant sodium current, such that the current threshold for discharge is reduced, and the magnitude of the peak current is increased (6). A TTX-resistant sodium current has been identified in unmyelinated C fibers of the human sural nerve (27). This current mediates PGE$_2$-induced peripheral hyperalgesia in rats (19).
Finally, modulation of the hyperpolarization-activated current ($I_h$) is the third explanation. $I_h$ is a hyperpolarization-activated nonselective cation current that has been described in the pacemaker cells of the heart and in neurons. It contributes to the generation of spontaneous action potentials (18). Increases in the levels of intracellular cAMP enhance the current of peripheral and central neurons, for example, in sympathetic neurons (33). cAMP modulates $I_h$ in primary afferent neurons of guinea pigs by shifting the voltage dependence and increasing amplitude (18). Augmentation of $I_h$ may lead directly to sensitization and/or excitation of primary afferent neurons (18). As demonstrated by these data, cAMP has been shown to be capable of increasing excitability of sensory neurons, possibly through modulation of ion currents and by initiating membrane depolarization.

Because activation of adenylate cyclase leads to the production of cAMP, we assessed whether the decrease in the production of cAMP by inhibiting activation of adenylate cyclase with DDA would reduce the response of afferents to ischemia. As a membrane-permeable inhibitor of adenylate cyclase, DDA is capable of inhibiting adenylate cyclase and decreasing the intracellular concentration of cAMP (17). Intradermal injection of DDA decreases hyperalgesia in the skin of rats (1, 34). We observed that ischemia-induced increases in afferent activity were attenuated by DDA. These findings were strengthened by control studies demonstrating that increases in discharge activity were similar during repetitive abdominal ischemia. Therefore, the attenuated response to ischemia during inhibition of adenylate cyclase cannot simply be attributed to a time and/or vehicle effect. Thus we believe that cAMP contributes to the activation of visceral afferents during abdominal ischemia.

Previously, we (8, 9, 23, 24) demonstrated that abdominal ischemia leads to the production of several substances, including bradykinin, reactive oxygen species, lactic acid, cyclooxygenase and lipoxygenase products, serotonin and histamine, and that each substance individually contributes to the activation and/or the
sensitization of ischemically sensitive abdominal visceral afferent nerve endings. The precise mechanism(s) by which the transduction process(es) of these products occurs, however, is unclear. In the present study, we chose PGE2 as a physiological way to stimulate adenylate cyclase and to determine the importance of cAMP in activation of visceral afferent C fibers during ischemia. Our reasons for this choice were as follows: 1) abundant evidence from others demonstrating that PGE2 commonly sensitizes nociceptors, mainly through stimulation of the adenylate cyclase-cAMP system and elevation of intracellular cAMP (3, 15); 2) our previous observation that PGE2 specifically sensitizes ischemically sensitive abdominal visceral afferents (23, 24); and 3) evidence that PGE2 is produced and is present in intestinal interstitial fluid where afferent nerve endings are located during abdominal ischemia (28). We observed that the ischemia-induced increases in the activity of afferents was augmented by PGE2. Inhibition of adenylate cyclase with DDA attenuated the PGE2-related increase in impulse activity. Control experiments verified that the inhibitory effect of DDA on PGE2-mediated activation of ischemically sensitive abdominal visceral C fibers was not due to a time and/or vehicle effect, reduced fiber responsiveness, and/or tachyphylaxis to PGE2 and ischemia. Taken together, these data further support our hypothesis that cAMP is involved in the activation of ischemically sensitive abdominal visceral C fiber afferents and, in particular, contributes to PGE2-mediated activation of these afferents.

Concern could be raised that, although DDA attenuated the PGE2-mediated increase in the discharge rate of visceral afferents during ischemia, it did not reduce the activity of these afferents below that observed during control ischemia without exogenous PGE2 in the same group (Fig. 6B). There are at least two possible explanations for this result. First, administration of exogenous PGE2 presumably increased the regional concentration of PGE2 during ischemia more than ischemia alone. The higher concentration of PGE2 may have blunted the influence of DDA. Second, although available evidence demonstrates that PGE2 activates sensory nerves mainly through one of the subtypes of prostanoid receptors (i.e., EP2), which stimulates production of cAMP (3, 34), we cannot exclude a role for other receptor subtypes (e.g., EP1, EP3, and/or EP4), which could be involved in the activation of visceral afferents during ischemia through other intracellular messengers, such as calcium (3). Exogenous PGE2 could activate afferents through these alternative pathways to a greater extent than that occurring with endogenous PGE2 during ischemia. Although we cannot fully explain this result, the data in the present study do provide evidence that DDA is capable of reducing PGE2-related activation of visceral afferents.
during ischemia. This result supports our working hypothesis.

Results from our previous studies show that PKC contributes to the activation of afferents during ischemia (11). Inhibition of PKC attenuates the enhanced activity of afferents during ischemia even in the presence of cyclooxygenase blockade. The role of PKC in the activation of these afferents is not dependent on PGs (13). Thus the phosphatidylinositol system is an intracellular signaling pathway that is independently involved in activation of visceral afferents during abdominal ischemia (11, 13). Data from the present study demonstrate that cAMP contributes to the activation of visceral afferents during ischemia, particularly during PGE₂-related sensitization of these afferents. In combination, these data suggest that both the phosphatidylinositol and the adenylyl cyclase-cAMP systems contribute to intracellular signaling mechanisms underlying the activation of abdominal sensory nerve system during ischemia.

In summary, abdominal ischemia induces profound cardiovascular reflex responses to support blood pressure and improve collateral blood flow during ischemia (22). Whereas our laboratory has demonstrated that many metabolites produced during ischemia contribute to activation of visceral afferents, which form the afferent limb of this reflex, the precise mechanisms regarding their transduction processes have not been clearly identified. Our previous studies (11, 13) have shown that the phosphatidylinositol system is involved independently in the signal transduction process when abdominal visceral C fibers are stimulated during ischemia. The present study provides new information showing an important role of cAMP in the activation of ischemically sensitive abdominal visceral C fibers. In particular, these data demonstrate that cAMP likely contributes to PGE₂-mediated activation of these afferents. In light of our previous work, the present results suggest that both the phosphatidylinositol and adenylyl cyclase-cAMP systems are involved in intracellular signal transduction during the activation of abdominal visceral afferents with ischemia.

We cannot exclude a role for other signaling mechanisms in the ischemia-induced activation of afferents. For example, it is possible that the nitric oxide/cGMP system also could contribute to this process. Furthermore, we do not know the exact mechanisms by which calcium mediates the intracellular signal transduction of sensory afferents during ischemia. These areas will require future investigation.

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REFERENCES


