Reflex cardiovascular response to brief abdominal visceral ischemia is mediated in part by prostaglandins

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ABDOMINAL VISCERAL ISCHEMIA induced by occlusion of the celiac and superior mesenteric arteries evokes a reflex cardiovascular response that produces highly reproducible increases in systemic arterial pressure in the cat (3, 10, 29). The reflex response also is characterized by increases in heart rate, left ventricular contractility, and systemic vascular resistance (10). Although the reflex induced by abdominal ischemia has been well defined, the possible mechanisms of afferent activation and reflex induction during an ischemic period in vivo have yet to be fully determined. A recent study in our laboratory (3) suggests that endogenous bradykinin produced during abdominal ischemia acts on B2 receptors and contributes to the cardiovascular reflex response. However, blockade of B2 receptors reduced the reflex pressor response by ∼50%, suggesting that other mediators likely contribute to the stimulation of ischemically sensitive afferent nerve endings.

Ischemically sensitive visceral sympathetic Aδ fibers (mechanosensitive) and C fibers (chemosensitive) constitute the afferent limb of the reflex cardiovascular response (17). Both groups of fibers display increased firing rates after application of various ischemically derived mediators, including prostaglandins (18, 36), bradykinin (19), and histamine (7, 37). Prostaglandins, a diverse group of lipid products derived from the cyclooxygenase pathway, mediate a wide array of physiological responses, including the inflammatory response and nociception (5, 8), vasodilatation of resistance vessels (4), and extravasation of plasma (16).

Several prostaglandins (i.e., PGE$_2$, PGF$_2\alpha$, PGI$_2$) injected intra-arterially can augment the firing frequency of ischemically sensitive Aδ fibers and C fibers located in abdominal visceral organs (18). However, prostaglandins are unlikely to directly cause the reflex pressor response because application of prostaglandins to the serosal surface of several visceral organs does not evoke a pressor response (36). Rather, prostaglandins appear to sensitize ischemically sensitive afferent fibers located in abdominal visceral organs (18). However, prostaglandins are unlikely to directly cause the reflex pressor response because application of prostaglandins to the serosal surface of several visceral organs does not evoke a pressor response (36). Rather, prostaglandins appear to sensitize ischemically sensitive afferent fibers located in abdominal visceral organs (18).

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METHODS

Preparation. Studies were performed in cats of either sex (1.6–6.0 kg). Ketamine (40–50 mg/kg im) and α-chloralose (50 mg/kg iv) were administered to induce a surgical plane of anesthesia. Subsequent injections of α-chloralose were administered as needed to maintain anesthesia, as determined by abnormal respiration and absence of withdrawal response to paw pinch. Auffed endotracheal tube was inserted to artificially ventilate the animals (Harvard pump, model 661; Ealing, South Natick, MA). Inspired gas was enriched with 100% oxygen, while arterial blood gases and pH were monitored continuously (Radiometer ABL3; Copenhagen, Denmark) and maintained within physiological limits (pH 7.35–7.45, PCO2 28–35 mmHg, Po2 > 100 mmHg) by adjusting the 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 a heating pad and heat lamp. This study was conducted in compliance with the Guiding Principles in the Care and Use of Animals, endorsed by the American Physiological Society, and was approved by the Institutional Animal Care and Use Committee at the University of California.

The two experimental preparations utilized in this study have been discussed previously (10, 29). Briefly, in groups 1 and 2, catheters were placed in the left femoral vein for administration of drugs and fluid and in the left femoral artery to allow measurement of arterial blood pressure (Statham P231D; Gould, Valley View, OH). After a midline abdominal incision to expose the viscera, the proximal portion of the celiac and superior mesenteric arteries was isolated carefully to minimize fiber damage to the surrounding nerve plexi. Loop snare of surgical silk were placed around each artery such that flow through the vessels was not compromised. The inferior mesenteric artery was ligated to minimize collateral flow into the ischemic area. Several cats received a unilateral pneumothorax to alleviate respiratory influences on systemic arterial pressure. A gauze sponge saturated with warm Ringer solution was applied over the abdominal viscera to prevent desiccation. The loop snare around the superior mesenteric and celiac arteries were occluded simultaneously for a maximum of 20 min to produce regional abdominal ischemia. On release of the occlusion sites, flow through the collapsed vessels was confirmed by depression in systemic arterial blood pressure.

Because the reflex blood pressure responses, particularly in the indomethacin group, were confounded by an elevated baseline (nadir blood pressure), another protocol was utilized to study a third group. In group 3, we used a more invasive preparation that eliminated blood volume shifts associated with ligation of the celiac and superior mesenteric arteries. As demonstrated previously (10), the transient initial rise in blood pressure associated with onset of occlusion of the blood vessels could be eliminated by using extracorporeal autoperfusion blood circuits and diverting blood from the visceral region to a venous return reservoir during occlusion. Briefly, in group 3 the left femoral vein was cannulated for the administration of fluids and drugs. A catheter was placed in the left carotid artery to record arterial blood pressure (Statham P231D). After heparinization (3,000 U iv), celiac and superior mesenteric arteries were cannulated carefully to minimize damage to the surrounding nerve fiber plexus; 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 to a reservoir during the ischemic period. 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, into which a catheter was directed caudally to receive blood from the splanchic 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). A heat lamp was utilized to maintain temperature of the blood circulating through the external roller pump circuit. Blood flow into the jugular vein was held constant throughout the ischemic period to maintain a constant venous return.

Protocols. After surgery, the animals were allowed to recover for a minimum of 30 min until blood pressure was stable and blood gases were within the normal range. Abdominal ischemia was induced for 15–20 min; blood flow was restored to the ischemic region when the reflex rise in blood pressure reached a stable plateau but no later than 20 min after ligation of the vessel (groups 1 and 2) or diversion of blood flow (group 3). After the first ischemia period, animals were administered indomethacin (10–20 mg/kg iv; Sigma Chemical, St. Louis, MO; group 1, n = 5 and group 3, n = 4) or acetylsalicylic acid (50 mg/kg iv; Sigma Chemical; group 2, n = 8). Two animals were given acetylsalicylic acid intraarterially to compare effectiveness with intravenous administration (n = 6). The inhibitory effect by intra-arterial administration was similar to that observed with intravenous infusion, and the results therefore were combined into a single acetylsalicylic acid group (n = 8). Drugs were infused over 5–10 min, and animals were allowed to equilibrate for a minimum of 20 min. Dextran (6%) was administered intravenously as necessary to maintain arterial blood pressure. When blood pressure was stable, a second ischemic period was induced. To test for effectiveness of cyclooxygenase blockade, several animals were administered arachidonic acid (1 mg iv, n = 3). The inhibitory effects of acetylsalicylic acid (n = 6) and indomethacin (n = 5) on the pressor response to arachidonic acid were assessed after the second ischemia period.

To differentiate between a drug effect and a time-related change in pressor response, blood was collected from control animals (group 1, n = 8 and group 2, n = 4) were utilized to substantiate repeatability of the pressor response over the same time frame as the experimental protocols (3). Time-control group 1 used the same protocol as experimental groups 1 and 2, whereas time-control group 2 was similar to experimental group 3. Historical time-control experiments were utilized to minimize the number of animals required for the study. In these controls, the first period of ischemia was followed after a minimum of 20 min by a second period of ischemia without drug intervention.

Analysis. Only animals in which the reflex pressor response to abdominal ischemia was >15 mmHg were included in the study. Animals were excluded if blood pressure could not be stabilized (n = 6), if the second control blood pressure preocclusion was increased >25 mmHg from the first control preocclusion (n = 2), or if there was no nadir or blood pressure plateau before the secondary (reflex) rise in blood pressure (n = 2). This latter situation did not allow differentiation between the initial (mechanical) and secondary (reflex) increases in blood pressure. One animal was excluded in which the blood pressure response (>40 mmHg decrease in blood pressure) to a test dose of arachidonic acid indicated inadequate cyclooxygenase blockade.

Data are presented as means ± SE. The Kolmogorov-Smirnov test was used to check the assumption of a normally
RESULTS

In the first protocol, combined occlusion of the celiac and superior mesenteric arteries typically produced an initial sharp rise in blood pressure followed by a gradual decline to a nadir over the next several minutes (Fig. 1). After a variable time period, a secondary gradual increase in pressure became evident. It was this secondary response in arterial blood pressure, which has been determined to be reflex in nature (10), that was measured for purposes of this study.

**Group 1.** Administration of the prostaglandin inhibitor indomethacin attenuated the reflex rise of blood pressure (Fig. 2) by 39% from 31 ± 7 to 19 ± 5 mmHg (P < 0.05). The nadir blood pressure during the second occlusion (149 ± 3 mmHg) was significantly (P < 0.05) greater than during the first occlusion (129 ± 4 mmHg).

**Group 2.** Acetylsalicylic acid reduced the ischemia-induced reflex rise in blood pressure by 46% (28 ± 3 to 15 ± 4 mmHg, P < 0.05; Fig. 2). Like group 1, the nadir blood pressure during the second occlusion (172 ± 7 mmHg) was significantly greater than during the first occlusion (155 ± 9 mmHg).

**Group 3.** In this preparation, arterial ligation did not result in a rapid, transient rise in arterial blood pressure. However, a reflex rise in blood pressure was still apparent after several minutes. The reflex pressor response during the initial period of ischemia (28 ± 6 mmHg) was significantly (P < 0.05) reduced (43%) by indomethacin during the second period of ischemia (16 ± 4 mmHg; Fig. 3).

**Time-control studies.** In historical time-control animals for groups 1 and 2, changes in blood pressure during the first and second ischemia periods were similar (24 ± 4 and 22 ± 6 mmHg, respectively, P > 0.05). For the more invasive preparation used for group 3, blood pressure responses during the first and second ischemia periods also were similar (32 ± 6 and 31 ± 6 mmHg, respectively, P > 0.05; Fig. 3) (3).

**Cyclooxygenase blockade.** The depressor response to arachidonic acid (−27 ± 5 mmHg) was significantly attenuated by both indomethacin (−9 ± 2 mmHg) and acetylsalicylic acid (−10 ± 2 mmHg).

DISCUSSION

This is the first study to demonstrate that endogenous prostaglandins contribute significantly to the cardiovascular reflex response evoked by brief abdominal ischemia. The nonspecific prostaglandin synthesis inhibitors indomethacin and acetylsalicylic acid attenuated the reflex pressor response induced by 20-min occlusion of the celiac and superior mesenteric arteries.

Results from this study as well as previous studies (3, 10) have confirmed the repeatability of the cardiovascular reflex to mesenteric ischemia in this cat model. Furthermore, we have demonstrated previously (3) that celiac and superior mesenteric ganglionectomies completely eliminate the secondary cardiovascular response, thus confirming the reflex nature of the increase in blood pressure.

As discussed previously (3, 10), the protocol for groups 1 and 2 was utilized to minimize surgically induced trauma as well as to reduce visceral manipulations, which could lead to prostaglandin production. However, the nadir blood pressure that preceded the reflex rise in blood pressure was significantly elevated during the second occlusion. Because this augmented pressure may have contributed to the smaller pressor response after cyclooxygenase blockade, a more invasive protocol was utilized to prevent the shifts in blood volume associated with the first protocol and therefore minimize the brief pressor effect of arterial ligation that precedes the reflex response (10) and that could interfere with interpretation of the magnitude of the reflex response. In the present study, indomethacin lowered the pressor response in four of five animals studied with the first protocol, but this effect did not attain statistical significance due to variability of the magnitude of the pressor response. Results from the second protocol (group 3) confirm that indomethacin, like acetylsalicylic acid, can significantly reduce the pressor response caused by brief abdominal visceral ischemia. Only indomethacin was used for the animals in the more invasive second protocol because the magnitude of the reduced reflex hypertension response was similar in group 1 (indomethacin) and group 2 (acetylsalicylic acid) and because these two agents have been shown to produce similar reductions of the discharge response of afferent fibers to abdominal ischemia (20).

Group 3 animals had higher reflex arterial pressures than did groups 1 and 2. Control blood pressures also were smaller in group 3, likely due to the extent of the surgical preparation and the loss of blood volume during the procedure. This lower control pressure may have allowed a greater potential for the increase of blood pressure in group 3 (39). In addition, the transient, nonreflex rise in blood pressure that accompanied vessel ligation in groups 1 and 2 may have resulted in underestimation of the reflex blood pressure response by artificially elevating the nadir that preceded the secondary, reflex increase in blood pressure.

Ischemically sensitive visceral afferent C and Aδ nerve fibers, which comprise the afferent limb of the reflex pressor response, can be directly stimulated by the application of PGE2, PGI2, or PGF2α (18). However, the proportion of fibers that respond to any one prostaglandin varies by fiber type and ranges from 0 to 50% (18). This contrasts with the response to bradykinin which, for example, stimulates 80% of ischemically sensitive C fibers and 90% of Aδ fibers (18). Although prostaglandins can directly stimulate afferent nerve endings, this action may lack sufficient intensity or may involve an insufficient number of fibers, to elicit a cardiovascular effect. For example, application of PGE2, PGI2, or PGF2α to the serosal surface of the gallbladder, stomach, or jejunum generally fails to evoke a pressor response (36). Nevertheless, cyclooxygenase blockade significantly inhibits ischemia-induced activation of ischemically sensitive afferent fibers, most likely by eliminating the sensitizing effect of prostaglandins (20).

Prostaglandins have long been implicated in the manifestations of noception and the inflammatory response (5). In these processes, the primary role of prostaglandins has been to sensitize, rather than directly stimulate, afferent nerve endings. Sensitization results in enhanced responsiveness or reduced threshold of stimulation to an algesic substance such as bradykinin. For example, application of prostaglandins to the stomach 1) augments the cardiovascular response to bradykinin applied to the stomach, 2) restores the pressor response to bradykinin after its inhibition by cyclooxygenase blockade, and 3) partially restores the bradykinin-induced cardiovascular response after the development of tachyphylaxis to bradykinin (36).

Prostaglandins and bradykinin are released in several pathological settings, including abdominal visceral ischemia (27, 30), myocardial ischemia (21), and burns (31). Prostaglandin-induced sensitization of afferent nerves or of hemodynamic responses to thermal, mechanical, or chemical stimuli has been reported in several tissues, including skin (11), skeletal muscle (23, 35), kidney (14), heart (24, 34), lung (14), and abdominal visceral organs (6, 18, 36). It is of interest that prostaglandins can enhance responsiveness to several chemical stimuli, including substance P (13), capsaicin (13, 14), and bradykinin (25, 36, 38). The mechanism(s) by which prostaglandins produce sensitization apparently is related to a reduction of the threshold of activation by augmentation of intracellular cAMP and subsequent modulation of the voltage-sensitive Na+ channel and of a nonselective cation channel (2).

PGE2 is found throughout the gastrointestinal tract (1, 12), and we have reported elevation of PGE2 levels in intestinal lymph during brief abdominal visceral ischemia (30). Detection of augmented PGE2 in lymph suggests increased concentrations at the afferent nerve endings, which are located in the interstitium (22). The separate findings of 1) elevated PGE2 release during brief abdominal ischemia (30), 2) stimulation of ischemically sensitive visceral afferents by PGE2 and PGI2 (18), and 3) sensitization of the hemodynamic response to exogenous bradykinin (36) all point to a role for prostaglandins in visceral ischemia, but it has been unknown whether endogenous prostaglandin production during brief abdominal ischemia is sufficient to elicit a cardiovascular response. The present study indicates that endogenously produced prostaglandins are capable of contributing significantly to visceral-cardiac reflexes.

Although a significant (45%) attenuation of the cardiovascular reflex response in blood pressure by cyclooxygenase inhibitors was observed in the present study, moderate reflex blood pressure changes during brief
abdominal ischemia were still evident. The inability of cyclooxygenase inhibition to entirely eliminate the reflex response in blood pressure supports the potentiating effect of prostaglandins and suggests a possible role of other mediators. Evidence to date has demonstrated that bradykinin, acting on B2 receptors, is involved in the manifestation of the cardiovascular pressor response (3, 27), but other mediators such as histamine (7, 37) and serotonin (15) also have been shown to cause significant activation of visceral afferents. In addition, reactive oxygen species such as hydroxyl radical (33), lactic acid (32), and other arachidonic acid products (26) have been implicated as possible mediators. Interestingly, a prior study reported an ~40% decrease in activation of ischimically sensitive Aδ- and C-fiber afferents with the cyclooxygenase inhibitors indomethacin and aspirin (20). The similar magnitude of reduction ofafferent discharge frequency and pressor response presumably reflects the role of these fibers in the reflex response.

A potential limitation of the present study should be addressed. The decreased ischemia-induced pressor response by prostaglandin inhibition may have been related to a nonspecific action of either agent. However, this possibility seems unlikely because the use of structurally dissimilar cyclooxygenase inhibitors similarly attenuated the cardiovascular reflex response.

In summary, two dissimilar nonspecific cyclooxygenase inhibitors, indomethacin and acetylsalicylic acid, reduced the magnitude of visceral ischemia-induced pressor reflexes by 45%. Control studies verified the repeatability and the reflex nature of the pressor response (3). These findings support our hypothesis that prostaglandins produced during brief abdominal ischemia can play a significant role in the activation ofafferent sympathetic nerve fibers to elicit reflex cardiovascular responses.

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