Endogenous histamine stimulates ischemically sensitive abdominal visceral afferents through H₁ receptors

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Fu, Liang-Wu, Hui-Lin Pan, and John C. Longhurst. Endogenous histamine stimulates ischemically sensitive abdominal visceral afferents through H₁ receptors. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2726–H2737, 1997.—Abdominal ischemia stimulates sympathetic visceral afferents to reflexly activate the cardiovascular system. We have shown previously that topical application of histamine (HA) to the gastric wall causes reflex cardiovascular responses and have documented increased histamine concentrations in intestinal lymph and portal venous plasma during brief abdominal ischemia. In the present study, we hypothesized that histamine produced during ischemia activates ischemically sensitive C-fiber afferents by stimulation of H₁ receptors. Nerve activity of single-unit abdominal visceral C-fiber afferents was recorded from the right thoracic sympathetic chain of anesthetized cats. Injection of histamine (25 µg/kg ia) significantly increased activity of nine ischemically sensitive C fibers from 0.09 ± 0.06 to 1.11 ± 0.20 imp/s. An H₂-receptor agonist, 2-(3-chlorophenyl)histamine (250 µg/kg ia), also increased activity of these afferents from 0.11 ± 0.04 to 0.64 ± 0.18 imp/s (P < 0.05). Furthermore, an H₁-receptor antagonist (pyrilamine, 0.2 mg/kg iv) significantly attenuated the increased activity in 11 other C fibers from 0.91 ± 0.16 to 0.35 ± 0.06 imp/s (ischemia vs. pyrilamine + ischemia) and eliminated the response of 9 separate ischemically sensitive afferents to histamine. Conversely, both the H₂-receptor agonist dimaprit (500 µg/kg ia) and the H₃-receptor agonist (R)-α-methylhistamine (250 µg/kg ia) did not significantly alter the activity of these nine afferents. In nine separate cats treated with indomethacin (5 mg/kg iv), pyrilamine (0.2 mg/kg iv) further significantly attenuated the increased activity in seven of nine C fibers during ischemia, and indomethacin (5 mg/kg iv) attenuated the response of eight other afferents to histamine. These data suggest that during mesenteric ischemia endogenous histamine contributes to the activation of afferents through direct stimulation of histamine H₁ receptors and that histamine's stimulating effect on these afferents is dependent partially on production of prostaglandins.

CHEMICAL OR MECHANICAL stimulation of abdominal visceral sympathetic Aδ- and C-fiber afferents reflexly excites the cardiovascular system (14). Recent studies indicate that mesenteric ischemia is a strong stimulus of abdominal visceral afferent nerve endings, leading to reflex responses that can increase blood pressure by >50 mmHg (22).

The specific metabolic stimuli responsible for activating ischemically sensitive visceral afferents are not fully known. We previously have demonstrated that local tissue hypoxia does not directly stimulate these afferents during ischemia (6). However, hypoxia and ischemia lead to metabolic changes and the production of a number of chemicals that potentially could stimulate these afferent nerve endings. For instance, our laboratory has provided evidence demonstrating that lactic acid, bradykinin, prostaglandins, (8R,15S)-dihydroxy-(5Z,9E,11E,13E)-eicosatetraenoic acid [(8R,15S)-diHETE], and reactive oxygen species can activate or sensitize these visceral afferents during ischemia (14, 15, 20). Conversely, other metabolic products such as leukotriene B₄ (LTB₄) modulate the activity of these nerve endings (20). We believe that other ischemic metabolites responsible for activation of abdominal visceral afferents during ischemia may be present, because inhibition of any of the previously investigated chemicals generally only partially reduces the response of most afferents to ischemia.

Recently, we have begun to consider the possibility that histamine contributes to activation of these nerve endings during ischemia. In this regard, almost all mammalian tissues contain histamine in amounts ranging from <1 to >100 µg/g, with mast cells being the predominant storage site (7). The concentration of histamine is particularly high in tissues that contain large numbers of mast cells, including skin, mesenteric endothelium, and intestinal mucosa (7, 10). A previous investigation indicated that prolonged myocardial ischemia promotes the production and release of histamine in coronary sinus plasma (28). Boros et al. (1) found an increase of histamine levels in plasma only during early reperfusion after prolonged (60 min) mesenteric ischemia. Using a 10-min period of abdominal ischemia, we have demonstrated the increased histamine in intestinal lymph (4), which reflects concentration in the interstitial compartment, where most nerve endings exist (17). We have also shown that application of histamine to the gastric serosa initiates excitatory reflex cardiovascular responses in a manner like ischemia (25). However, although our previous studies have shown that application of histamine to the gastric wall stimulates bradykinin-sensitive abdominal visceral afferents (14), they do not provide direct evidence regarding the role of endogenously produced histamine during brief ischemia with regard to activation of ischemically sensitive abdominal visceral afferents.

Specific histamine receptor antagonists and agonists that compete effectively with histamine for H₁, H₂, and H₃ receptors have become available recently (9, 26). Histamine H₁ and H₂ receptors are known to exist on preganglionic axons or terminals in sympathetic ganglia of the rat (27). We have observed that the H₁ receptor plays a role in the reflex excitatory cardiovascular responses induced by application of histamine to the gastric wall (25). However, the role of H₁ receptors...
responding to endogenously produced histamine in activation of abdominal visceral C-fiber afferents during ischemia has not been examined.

Our previous data indicate that prostaglandins can sensitize abdominal visceral afferents and enhance their responses to abdominal ischemia (15). Although the effect of prostaglandins on histamine-induced activation of ischemically sensitive abdominal visceral afferents is uncertain, prostaglandins potentially could sensitize these afferents to the action of histamine. In this regard, our previous studies (25) have demonstrated that exogenously applied histamine reflexly evokes cardiovascular responses, an effect that is attenuated by cyclooxygenase blockade and restored by application of prostaglandin E2.

Therefore, the general aim of this study was to determine whether endogenously produced histamine stimulates ischemically sensitive abdominal visceral afferents during brief ischemia. We hypothesized that 1) histamine, through an H1-receptor mechanism, contributes to stimulation of abdominal visceral afferents during mesenteric ischemia, and 2) the stimulating effect of histamine on ischemically sensitive abdominal visceral afferents depends on production of prostaglandins. A preliminary report of a portion of this research has been published (5).

METHODS

Surgical Preparation

Experiments were performed on 70 fasted adult cats of either sex (3.2 ± 0.6 kg). All protocols used in this study were approved by the Animal Use and Care Committee at the University of California at Davis. The studies conformed to the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.” Anesthesia was induced with ketamine (20–30 mg/kg im) and maintained with α-chloralose (40–50 mg/kg iv). Additional injections of α-chloralose (5–10 mg/kg iv) were given as needed to maintain an adequate depth of anesthesia. The trachea was intubated and respiration was maintained artificially (Harvard ventilator, model 661, Ealing, South Natick, MA). The cat was ventilated with 100% O2 through the respirator. A femoral vein was cannulated for administration of drugs and fluid. A femoral arterial catheter was positioned with its tip in the descending thoracic aorta for measurement of pressure and administration of drugs. Systemic arterial blood pressure was measured by a pressure transducer (Statham P23 ID) connected to the femoral arterial catheter. We frequently assessed arterial blood gases with a blood gas analyzer (Radiometer ABL-3, Copenhagen, Denmark) and maintained them within physiological limits (P O2 > 100 mmHg, P CO2 = 28–35 mmHg, pH = 7.35–7.45) by adjusting the respirator rate or tidal volume or by administering 1 M NaHCO3 intravenously. 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.

Afferent Recording

We previously have described the surgical preparation used for recording single-unit activity of abdominal sympathetic C-fiber afferents (6, 15). In brief, a midline sternotomy was performed. The 3rd through 11th right ribs and the middle and caudal lobes of the right lung were removed. Both phrenic nerves were isolated and cut. An inflatable occlusion cuff was placed around the descending thoracic aorta just above the diaphragm. Fascia overlaying the right paravertebral sympathetic chain was removed. The chain then was draped over a Plexiglas platform and was covered with warm mineral oil. Small nerve filaments were dissected gently from the chain or rami between T8 and T10 with the use of an operating microscope (Zeiss, Oberkochen, Germany), and the caudal end was placed across a recording electrode. One pole of the recording electrode was grounded with cotton thread to the animal. The recording electrode was attached to a high-impedance probe (Grass Instruments, model H1P511, Quincy, MA); the signal was amplified (Grass, model P511 preamplifier) and processed through an audiomixer (Grass, AM88, Audiomonitor) and an oscilloscope (Tektronix, model 2201, Beaverton, OR) and then recorded on a chart recorder (Gould, TA 4008B, Cleveland, OH). The neurogram also was fed into an IBM-compatible Pentium computer through an analog-to-digital interface card (R.C. Electronics, Santa Barbara, CA) for subsequent off-line analysis. The discharge frequency of afferents was analyzed using data acquisition and analysis software (EGAA, version 3.02, R.C. Electronics).

We exposed abdominal visceral organs through a ventral midline incision. Receptive fields of afferents were located precisely using a fine-tipped glass rod and a stimulating electrode to evoke the afferent’s action potential. We determined conduction distance by measuring the interval from stimulation to the recording of the afferent’s action potential. Conduction distance was estimated with a thread placed from the receptive fields along the supposed afferent pathway through the prevertebral ganglion along the course of the major splanchnic nerve to the sympathetic chain and the recording electrode. C fibers were classified as those with a conduction velocity (CV) < 2.5 m/s. Fibers included in this study had a range of CVs from 0.28 to 1.50 m/s; each had a single receptive field that could be located precisely. Afferents were considered to be ischemically sensitive if their discharge activity during 10 min of abdominal ischemia was increased at least twofold above baseline activity (6). We closed the abdominal incision with towel clamps and covered the viscera with warm saline-soaked gauze to prevent fluid and heat losses.

Experimental Protocols

Effect of histamine, 2-(3-chlorophenyl)histamine, dimaprit, and (R)-α-methylhistamine on afferent discharge activity. This protocol consisted of nine cats subjected to 5–10 min of abdominal ischemia followed by 2–3 min of reperfusion. After an ischemically sensitive unit was identified, we opened the abdomen, located the receptive field of the ending, and measured the CV, as noted previously. Warm, moist gauze sponges were placed over the viscera, and the abdomen was closed with towel clamps. We then tested the effect of intra-arterial injection of histamine (25 µg/kg, Sigma), dimaprit (500 µg/kg, H2-receptor agonist; Sigma), (R)-α-methylhistamine [(R)-α-MA; 250 µg/kg, H3-receptor agonist; Research Biochemicals, Natick, MA], or 2-(3-chlorophenyl) histamine (CPH; 250 µg/kg, H1-receptor agonist; Institute für Pharmakologie, Freie Universität Berlin, Berlin, Germany) on the discharge activity of the afferent endings. Histamine, dimaprit, (R)-α-MA, and CPH were dissolved in 0.9% NaCl and were prepared fresh daily. Histamine, dimaprit, (R)-α-MA, and CPH were injected randomly, maintaining at least 20 min of recovery between injections. The dose of histamine chosen was previously shown to induce maximal reflex cardiovascular responses (25). A pilot study also demonstrated that these doses of histamine and CPH induced significant activation of ische-
cally sensitive abdominal visceral afferents. The afferent activity was recorded as we have described previously (6).

Effect of pyrilamine on the response of afferents to ischemia and histamine. Afferent responses to repeated 10-min periods of ischemia were studied in seven cats. After identification of C fibers, we subjected each afferent to 10 min of ischemia followed by 2–3 min of reperfusion. If the afferent was sensitive to ischemia, we repeated the 10-min period of ischemia and reperfusion 35–45 min after the first period of ischemia to determine whether the response of afferent to ischemia was reproducible.

The effect of H$_1$-receptor antagonism with pyrilamine (200 µg/kg iv) on the afferent’s response to 10 min of ischemia was studied in 10 separate animals. Pyrilamine (Sigma) was dissolved in 0.9% NaCl to a concentration of 2 mg/ml and was prepared fresh daily. A preliminary study demonstrated that this dose of pyrilamine effectively abolished (by 91 ± 15%) the response of afferents to histamine (25 µg/kg ia). We repeated ischemia 35–45 min after the first period and at least 10–15 min after treatment with pyrilamine. In the occasional circumstance in which afferent activity was suppressed completely, we mechanically manipulated the receptive field or stimulated it with an electrode to establish viability of the nerve ending.

In six separate cats, we examined the effect of repeated intra-arterial injection of histamine (25 µg/kg) on ischemically sensitive visceral afferent discharge activity. After identification of an ischemically sensitive unit, 25 µg/kg of histamine were injected into the descending thoracic aorta and the afferent activity was recorded. We subsequently repeated injection of histamine 20–30 min after the initial treatment.

In eight other cats, we determined the effect of H$_1$-receptor blockade with pyrilamine (200 µg/kg iv) on the response of nine ischemically sensitive C fibers to histamine. After identification of an ischemically sensitive unit, we injected histamine (25 µg/kg) into the descending thoracic aorta while recording afferent activity. We repeated the injection of histamine (25 µg/kg ia) 20–30 min after its initial injection and at least 10–15 min after treatment with pyrilamine. After treatment with pyrilamine, we administered bradykinin (10 µg) into the descending thoracic aorta to establish responsiveness of the afferent ending.

Effect of indomethacin + pyrilamine on the response of afferents to ischemia. In this protocol, visceral afferents were subjected to three 10-min periods of abdominal ischemia followed by 2–3 min of reperfusion for recovery periods of 35–45 min intervals.

TIME CONTROL. In this group of cats (n = 8), after identification of a C fiber, we subjected each afferent to 10 min of ischemia followed by 2–3 min of reperfusion. If the afferent was sensitive to ischemia, we then repeated 10 min of ischemia and reperfusion 35–45 min after the first or second period of ischemia, including at least 15 min after injection of an equal volume of a vehicle (2–3 ml of 0.9% NaCl + 100 mM of NaHCO$_3$) to determine the reproducibility of the afferent’s response.

INDOMETHACIN + PYRILAMINE. This group of cats (n = 9) was subjected to 10 min of abdominal ischemia followed by 2–3 min of reperfusion. Indomethacin (Sigma) was dissolved each day in NaHCO$_3$ (100 mM) and was diluted by 0.9% NaCl to a concentration of 10 mg/ml. Indomethacin (5 mg/kg) was injected into a femoral vein after identification of an ischemically sensitive unit. This dose effectively inhibits cyclooxygenase activity in cats (15). Subsequently, we repeated 10 min of ischemia and reperfusion 35–45 min after the first ischemia, including at least 15 min after administration of indomethacin. Pyrilamine (0.2 mg/kg iv) was administered 25–30 min after the second period of ischemia. We repeated 10 min of ischemia and reperfusion 15 min after injection of pyrilamine.

PYRILAMINE + INDOMETHACIN. In this group (n = 5), cats were subjected to 10 min of abdominal ischemia followed by 2–3 min of reperfusion. Pyrilamine (0.2 mg/kg) was injected into a femoral vein after identification of an ischemically sensitive afferent. Subsequently, we repeated 10 min of ischemia and reperfusion 35–45 min after the first ischemia, including at least 15 min after administration of pyrilamine. Indomethacin (5 mg/kg iv) was administered 25–30 min after the second period of ischemia. We repeated 10 min of ischemia and reperfusion 15 min after injection of indomethacin.

Effect of indomethacin on the response of afferents to histamine stimulation. In eight additional animals, we examined the effect of inhibition of cyclooxygenase activity with indomethacin (5 mg/kg iv) on the response of ischemically sensitive afferent nerve endings to histamine. We previously have shown that this dose of indomethacin effectively inhibits the response of visceral afferents to stimulation with endogenous prostaglandins (15). Animals were subjected to 5–10 min of abdominal ischemia followed by 2–3 min of reperfusion. After identification of an ischemically sensitive unit, histamine (25 µg/kg) was injected into the descending thoracic aorta. Thirty minutes after initial injection of histamine, including at least 15 min after intravenous administration of indomethacin, we repeated intra-arterial injection of histamine (25 µg/kg).

Data Analysis

Peak discharge activity of ischemically sensitive afferents was measured over 60 s during 3–5 min of control and 10 min of ischemia, respectively, when the greatest number of spikes occurred (6, 20). We measured the response of afferent nerve endings to histamine, dimaprit, (R)-α-MAP, or CPH by averaging discharge rates of the afferent during the entire period of response. We assessed the latency of afferent response to histamine, dimaprit, (R)-α-MAP, or CPH from the time of arterial occlusion or intra-arterial injection of the chemicals to the point when sustained discharge activity of afferents exceeded a 50% increase over baseline activity. If an afferent did not respond to ischemia after treatment with drugs, an onset latency equal in length to the maximum period of observation was assigned.

Data are expressed as means ± SE. The effects of repeated injection of histamine, pyrilamine, indomethacin, plus pyrilamine, or pyrilamine plus indomethacin and of repeat ischemia on the responses of the afferents were compared using a one-way repeated-measures analysis of variance (ANOVA) with a post hoc Bonferroni t-test. If the data were not normally distributed, as determined by the Kolmogorov-Smirnov test, they were compared with the Friedman repeated-measures ANOVA on ranks with a Dunnnett’s t-test. We compared the effects of histamine, CPH, dimaprit, and (R)-α-MA on the afferent discharge activity with a Student’s paired t-test. A Student’s paired t-test also was used to compare the effects of pyrilamine or indomethacin on histamine-induced increases in discharge activity of the afferents. We used the Wilcoxon signed rank test to compare the data, if the data were not normally distributed. All statistical calculations were performed with SigmaStat software (Jandel Scientific Software, San Rafael, CA). Values were considered to be significantly different when P < 0.05.
RESULTS

Effects of Ischemia, Histamine, (R)-α-MA, Dimaprit, and CPH on Afferent Activity

Figure 1 is an original tracing of an ischemically sensitive C fiber innervating the bile duct with a CV of 0.97 m/s during ischemia (Fig. 1A) and during injection of histamine, dimaprit, CPH, or (R)-α-MA (Fig. 1, B-E, respectively). Inflation of the aortic occlusion cuff decreased mean arterial pressure from 96 to 10 mmHg. During the 10 min of ischemia, impulse frequency increased from 0 to 0.92 impulses/s. After release of aortic occlusion, the frequency of discharge of nerve ending quickly decreased to control levels. Injection of histamine (Fig. 1B) resulted in an immediate burst of afferent activity after 7 s. Injection of CPH also resulted in a burst of discharge activity of the afferent within 8 s (Fig. 1D). In contrast, injection of dimaprit (Fig. 1C) and (R)-α-MA (Fig. 1E) did not alter the impulse activity of this afferent compared with the control period.

The effect of histamine, dimaprit, (R)-α-MA, and CPH treatment on the entire group of nine ischemically sensitive C fibers (CV = 0.83 ± 0.10 m/s) is summarized in Fig. 2. The locations of these nine afferent endings are shown in Table 1 and included the mesentery.
pancreas, porta hepatis, bile duct, and gallbladder. Inflation of the aortic occlusion cuff decreased mean arterial pressure from 94 ± 12 to 11 ± 2 mmHg (P < 0.05). We showed previously that this degree of arterial occlusion is associated with a significant increase in portal venous blood and mesenteric lymph lactate concentration within 5 min (14, 15), and a significant decrease in portal venous blood and tissue Po2 (6, 14). Injection of histamine (25 µg/kg) into the descending thoracic aorta stimulated each of nine fibers, significantly increasing impulse activity from 0.09 ± 0.06 to 1.10 ± 0.20 impulses/s (P < 0.05) after an onset latency of 4.6 ± 0.6 s. Intra-arterial injection of CPH (250 µg/kg) into the descending thoracic aorta stimulated each of nine fibers, significantly increasing impulse activity from 0.09 ± 0.06 to 1.10 ± 0.20 impulses/s (P < 0.05) after an onset latency of 4.6 ± 0.6 s. Intra-arterial injection of CPH (250 µg/kg) stimulated eight of nine C fibers (89%), significantly increasing their discharge activity from 0.11 ± 0.04 to 0.64 ± 0.18 impulses/s (P < 0.05) after an onset latency of 3.7 ± 0.5 s. In contrast, injection of dimaprit (500 µg/kg ia) stimulated only one of nine fibers, and thus overall this agonist did not significantly alter impulse activity of the group of fibers (0.09 ± 0.05 before vs. 0.08 ± 0.04 impulses/s after); injection of (R)-α-MA (250 µg/kg ia) did not stimulate any of nine fibers tested (0.09 ± 0.05 before vs. 0.11 ± 0.05 impulses/s after).

**Table 1. Location of ischemically sensitive abdominal visceral afferent nerve endings**

<table>
<thead>
<tr>
<th>Location</th>
<th>HA, CPH, dimaprit</th>
<th>Repeat ischemia</th>
<th>PA</th>
<th>Repeat HA</th>
<th>PA on HA</th>
<th>TriPLICATE ischemia</th>
<th>IM + PA</th>
<th>PA + IM</th>
<th>IM on HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mesentery</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Porta hepatatis</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Bile duct</td>
<td>3</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gallbladder</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>5</td>
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</table>

Values reflect nos. of afferent endings. HA, histamine; CPH, 2-(3-chlorophenyl)histamine; (R)-α-MA, (R)-α-methylhistamine; PA, pyrilamine; IM, indomethacin. For description of protocols, see METHODS.
C fibers during ischemia (Fig. 5B) and increased the onset latency (299 ± 34 s, P < 0.05).

Effect of Pyrilamine on the Response of Afferents to Histamine

We investigated the afferent responses of six other ischemically sensitive afferents (CV = 0.82 ± 0.15 m/s) to repeated intra-arterial injection of histamine (25 µg/kg). These afferent endings were located in the duodenum, porta hepatis, bile duct, or gallbladder (Table 1). All afferents had a single receptive field. Repeat injection of histamine induced similar increases in discharge activity with similar onset latencies (3 ± 0.7 s vs. 2.5 ± 0.4 s, initial vs. repeat, P > 0.05, Fig. 6A).

The effect of H1-receptor blockade with pyrilamine on nine ischemically sensitive afferents (CV = 0.84 ± 0.10 m/s) was examined (2 C fibers were recorded in 1 of 8 cats; Fig. 6B). Histamine (25 µg/kg) injected intra-arterially significantly increased the discharge activity of all nine C fibers after an average onset latency of 5 ± 1 s. Pyrilamine (200 µg/kg iv) did not alter the mean arterial pressure (93 ± 9 before vs. 92 ± 8 mmHg after) but virtually eliminated the responses of afferents to histamine (Fig. 6B). Each of the nine afferents still responded to intra-arterial injection of 10 µg of bradykinin (0.06 ± 0.03 to 1.01 ± 0.20 impulses/s, P < 0.05) after pyrilamine. These C-fiber afferent endings were located in the mesentery, pancreas, porta hepatis, bile duct, or gallbladder (Table 1).

Effect of Indomethacin + Pyrilamine on the Response of Afferents to Ischemia

In this protocol, a single receptive field was found for all C-fiber endings. Figure 7A summarizes the responses of eight C-fiber afferents (CV = 0.41 ± 0.03 m/s) to abdominal ischemia that was reproduced three times. We observed similar increases in discharge activity of these afferents during each period of ischemia. We also found a similar latency of responses of these afferents for the three ischemic periods (10 min) consisting of 156 ± 18, 176 ± 21, and 158 ± 29 s (initial vs. second vs. third ischemia, P > 0.05). Distal arterial pressure was reduced similarly during each period of ischemia (initial 93 ± 12 to 14 ± 2 vs. second 94 ± 11 to 15 ± 1 vs. third 91 ± 12 to 13 ± 2 mmHg, P > 0.05). These C-fiber endings were located in the duodenum, mesentery, pancreas, porta hepatis, bile duct, or gallbladder (Table 1).

Figure 7B summarizes the effect of treatment with pyrilamine or pyrilamine and indomethacin (n = 5) on the impulse activity of ischemically sensitive C-fiber afferents (CV = 0.41 ± 0.02 m/s) located in the duodenum, pancreas, or porta hepatis (Table 1). Inflation of the aortic occlusion cuff significantly decreased mean arterial pressure from 91 ± 8 to 15 ± 1 mmHg (P < 0.05). Ten minutes of ischemia significantly increased baseline activity of these afferents from 0.12 ± 0.04 to 1.67 ± 0.24 impulses/s after an average onset latency of 146 ± 44 s. We observed no significant difference in distal arterial pressure during ischemia before vs. after treatment with indomethacin and pyrilamine in five cats (initial 15 ± 1 vs. second 14 ± 2 vs. third ischemia 13 ± 1 mmHg, respectively). H1-receptor blockade with pyrilamine (0.2 mg/kg) attenuated the increased discharge activity during ischemia in five C-fiber afferents to 55 ± 4.4% (P < 0.01) of the activity observed during the first period of ischemia. In the presence of pyrilamine, indomethacin (5 mg/kg) further reduced the increased discharge activity during the third period of ischemia in five afferents to 28 ± 4.3% (P < 0.01, value of 1st ischemia = 100%) compared with the second period of ischemia. Both interventions significantly increased the onset latency (second 286 ± 28 s; third 323 ± 38 s, respectively).

Figure 7C summarizes the effect of treatment with indomethacin or indomethacin and pyrilamine on the impulse activity of nine ischemically sensitive C-fiber afferents (CV = 0.40 ± 0.03 m/s) located in the bile duct, gallbladder, duodenum, or porta hepatis (Table 1). Inflation of the aortic occlusion cuff significantly decreased mean arterial pressure from 89 ± 7 to 16 ± 2 mmHg (9.5 ± 1 mmHg after, P < 0.05).
mmHg (P < 0.05). Ten minutes of ischemia significantly increased baseline activity of these afferents from 0.15 ± 0.04 to 1.66 ± 0.22 impulses/s after an onset latency of 151 ± 28 s. We observed no significant difference in distal arterial pressure during ischemia in comparing pyrilamine to treatment with indomethacin plus pyrilamine in nine cats (16 ± 2 vs. 14 ± 2 vs. 15 ± 1 mmHg, initial vs. second vs. third ischemia, respectively). However, cyclooxygenase blockade with indomethacin reduced the increase in discharge rate during ischemia in nine C-fiber afferents to 57 ± 4.3% (P < 0.01) compared with the first period of ischemia. In the presence of indomethacin (5 mg/kg), treatment with pyrilamine, an H₁-receptor antagonist, further attenuated the increase in discharge rate during the third period of ischemia in seven of nine afferents to 31 ± 12% (P < 0.05, value of 1st ischemia = 100%) compared with the second period of ischemia. Both interventions significantly increased the onset latency (second 252 ± 27 s, third 265 ± 32 s, respectively). It should be noted that the increased discharge rate in two of nine afferents during the third period of ischemia was not reduced further (1.14 ± 0.42 vs. 1.33 ± 0.50 impulses/s, P > 0.05) compared with the second period of ischemia.

Effect of Indomethacin on Afferent Response to Histamine

Figure 8 summarizes the effect of indomethacin on the discharge activity of eight ischemically sensitive afferents (CV = 0.63 ± 0.08 m/s) during intra-arterial injection of histamine (25 µg/kg). We found that indomethacin (5 mg/kg iv) significantly attenuated the responses of these afferents to exogenous histamine but did not alter their latency (5 ± 6 vs. 6 ± 1 s, initial vs. repeat, respectively). These afferent endings were located in the duodenum, mesentery, pancreas, porta hepatitis, or gallbladder (Table 1).

DISCUSSION

The results of this study provide the first evidence that endogenous histamine activates abdominal visceral C-fiber afferents during brief mesenteric ischemia. We found that histamine H₂ receptors, but not H₂ or H₃ receptors, were responsible for histamine-induced activation of ischemically sensitive abdominal visceral afferent nerve endings. In this regard, a histamine H₂-receptor agonist, but not H₁ or H₃-receptor agonists, activated ischemically sensitive abdominal visceral afferent endings. Furthermore, the response of abdominal visceral afferents to ischemia or exogenous histamine was attenuated significantly after treatment with pyrilamine, a histamine H₁-receptor antagonist. Finally, we found that inhibition of endogenous prostaglandin production with indomethacin partially reduced the response of ischemically sensitive C-fiber afferents to histamine. Thus the data from the present study strongly suggest that histamine produced during ischemia contributes to activation of ischemically sensi-
tive abdominal visceral C-fiber afferents through stimulation of histamine H1 receptors. Moreover, in a manner like bradykinin (14), histamine activates visceral C-fiber afferent endings partially through a cyclooxygenase-dependent mechanism.

Abdominal ischemia activates visceral sympathetic afferents and evokes reflex excitation of the cardiovascular system (22). Visceral ischemia and reperfusion lead to metabolic changes that result in the production of a number of substances, including lactic acid, prostaglandins, LTB4, bradykinin, and reactive oxygen species (14, 15). These metabolites each individually stimulate, sensitize, or modulate the activity of ischemically sensitive abdominal visceral afferent endings (14, 15, 20). The concentration of histamine is particularly high in tissues such as skin, mesenteric endothelium, and intestinal mucosa, all of which contain large numbers of mast cells, the chief site of histamine storage (7, 10).

Previously, prolonged myocardial ischemia (>30 min) has been shown by others to increase histamine levels in coronary sinus plasma (28). Of more direct relevance to the present study, we have shown that histamine levels are significantly increased in both portal venous plasma and intestinal lymph during brief 10-min periods of abdominal ischemia (4). We hypothesized in the present investigation that increase of histamine concentration in the local tissue likely contributes to activation of ischemically sensitive C-fiber afferents in our model of abdominal ischemia.

Histamine binds to three distinct receptor subtypes: H1, H2, and H3. Through activation of H1 and H2 receptors, histamine acts on a large variety of excitable cell types, including smooth muscle, neurons, endocrine cells, and cells of the immune system (9, 21). Moreover, H3 receptors have been described on sensory neurons in the dorsal root ganglion (18). Previously, topical application of histamine to the serosal surface of the stomach was demonstrated to elicit cardiovascular responses that were blocked by an H1-receptor antagonist (25). Other investigators (19) have shown that...
intravenous perfusion of histamine induced cardiovascular responses that could be abolished by both H₁ and H₂-receptor antagonists. Although H₃ receptors are located on the presynaptic nerve endings, and probably on cell bodies of neurons and mast cells, where they function as autoreceptors to regulate the synthesis and release of histamine and other transmitters (21), the direct role of H₃ receptors in activating ischemically sensitive visceral afferent endings is unknown. In the present study, injection of both the H₂-receptor agonist dimaprit and the H₃-receptor agonist (R)-α-MA into the mesenteric arterial circulation failed to stimulate ischemically sensitive C-fiber afferents, which were responsive to histamine and CPH, a specific H₁-receptor agonist. Moreover, the response of these afferents to histamine was abolished completely by blockade of histamine H₁ receptor with pyrilamine. These results are consistent with our previous finding that histamine elicits cardiovascular reflexes from the stomach through activation of H₁, but not H₂, receptors (25). Sampson and Vidruk (24) also have reported that stimulation of visceral airway rapidly adapting receptors of vagal afferents by histamine is mediated by histamine H₁ receptors. Finally, the increase in impulse activity of abdominal visceral C-fiber afferents during ischemia was significantly attenuated by pyrilamine, a histamine H₁-receptor antagonist. Therefore, we have provided consistent neurophysiological data to document that the stimulating effect of endogenous histamine during ischemia on abdominal visceral C-fiber afferents is mediated by activation of H₁ receptors, which we speculate are located either on afferent nerve endings or in surrounding tissues (23).

Most of the available evidence indicates that the effects of histamine mediated by H₁ receptors are linked to diacylglycerol (DAG) and/or d-myo-inositol-1,4,5-trisphosphate [Ins(1,4,5)P₃] production (2, 23). Data presented in the present study suggest that the signal transduction in stimulation of endogenous histamine on ischemically sensitive abdominal visceral afferents through activation of H₁ receptors likely involves several underlying mechanisms. We found that, in the presence of indomethacin, pyrilamine further significantly attenuated the increased activity in seven of nine C-fiber afferents during ischemia (Fig. 7C). At least two important secondary messenger pathways are responsible for the actions of histamine. The first

![Fig. 7](http://ajpheart.physiology.org/)
pathway is linked to the phosphatidylinositol pathway, which, through the liberation of the second messengers DAG and/or Ins(1,4,5)P₃, leads to intracellular calcium ion release. The second pathway is linked to the release of arachidonic acid and its various metabolites (7). In this latter role, histamine, through stimulation of H₁ receptors, activates phospholipases A₂ or C (12, 27) to catalyze the release of arachidonic acid. Depending on the enzymes present in the tissue, the metabolic products of arachidonic acid in the abdominal visceral region include prostaglandins, 5-lipoxygenase products such as LTB₄ and 15-lipoxygenase products such as (8R,15S)-diHETE. Evidence from our previous studies indicated that intestinal tissue is capable of forming LTB₄ and (8R,15S)-diHETE from arachidonic acid (16). LTB₄ reduces the responses of afferents to ischemia, whereas (8R,15S)-diHETE sensitizes the response of afferents to ischemia (20).

In addition to the 5- and 15-lipoxygenase pathways, another major pathway of arachidonic acid metabolism through cyclooxygenase leads to generation of prostaglandins, particularly prostaglandin E₂ (12). Previous studies have revealed that prostaglandins sensitize abdominal visceral afferents to ischemia (14, 15). Our findings with indomethacin in the present study (Fig. 7C) confirm the results of our previous investigation (15). Histamine evokes local prostaglandin formation by increasing the activation of phospholipases A₂ or C (12, 27) to catalyze the release of arachidonic acid, which increases the pool of substrates available for prostaglandin synthesis. We wondered whether all or part of the action of histamine on afferents during ischemia might be dependent on production of prostaglandin. We found that, following inhibition of cyclooxygenase with indomethacin, blockade of H₁ receptors with pyrilamine did not attenuate the increased discharge activity in two of nine C-fiber afferents during ischemia. This finding implies that the stimulating effect of endogenously produced histamine during ischemia is dependent on an intact cyclooxygenase system for only 22% of abdominal visceral C-fiber afferents. Similar observations were made when we examined the response of afferents to intra-arterial injection of histamine before and after treatment with indomethacin. In this regard, we found that inhibition of the synthesis of prostaglandins with indomethacin eliminated 65% of the histamine-induced excitation of ischemically sensitive visceral C-fiber afferents. This observation suggests that the increased production of prostaglandins is necessary for part of the action of histamine on ischemically sensitive visceral afferents. These results, however, do not allow us to determine clearly whether prostaglandins directly activate or sensitize afferent endings in response to histamine. On the basis of our previous studies (14, 15, 20), we believe that it is most likely that ischemia increases the production of prostaglandins that, in turn, sensitize the ischemically sensitive afferent endings to respond to histamine. Additionally, it is likely that, in addition to prostaglandins, histamine directly stimulates these afferent endings. Therefore, activation of some abdominal visceral afferents may result from direct stimulation by histamine or sensitization by (8R,15S)-diHETE. All of these factors potentially could account for the "residual" afferent response to histamine.

Evidence from our previous studies indicate that prostaglandin E₂/prostaglandin I₂ and bradykinin levels in portal venous plasma and/or intestinal lymph increase during abdominal ischemia (14) and that prostaglandins enhance the response of ischemically sensitive abdominal visceral afferents to bradykinin (14). Prostaglandins likely sensitize visceral afferent nerve endings to the action of other endogenous mediators including bradykinin (14) and histamine, which directly stimulate these visceral afferents during ischemia. In this latter regard, indomethacin further attenuated the increased activity of abdominal visceral afferents during ischemia even after a blockade of histamine H₁ receptors with pyrilamine (Fig. 7B). These data imply that after blockade of histamine H₁ receptors, production of prostaglandins still occurs and the prostaglandins can continue to play a role in activation of these afferents during ischemia.

An important consideration arising from our study relates to the concentration of histamine used in the present study. Howland and Spector (10) have found that, in the cat, the concentration of histamine in the blood vessels of mesenteric artery and mesenteric vein is 9.3 and 5.8 µg/g, respectively. Evidence from our previous investigation demonstrates that the concentration of histamine increases to 6.4 nmol/ml (i.e., ~2 µg/ml) in portal venous plasma or mesenteric lymph during brief abdominal ischemia and reperfusion (4). Probably the concentrations present at the sites of release were considerably higher. Furthermore, histamine probably was diluted during the process of diffusion into the interstitium and with dilution in the arterial blood. In this regard, we previously showed that aortic flow in cats is 200–230 ml/min (11). We used ~5 s (equivalent to 18–20 ml of blood) for injection of 1 ml of histamine (~82 µg/ml, i.e., 25 µg/kg × 3.2 kg body wt = 82 µg) into the thoracic descending aorta in each cat in the present study. Thus the concentration of histamine in celiac or superior mesenteric arterial blood was 4.1–4.5 µg/ml because the histamine concentration was diluted with aortic blood during the period of injection [i.e., 82 µg/(18–20 ml) = 4.1–4.5 µg/ml]. We believe therefore that the concentration of histamine arriving at afferent endings is well within the pathophysiological range present during abdominal ischemia and reperfusion.

The specific metabolic stimuli underlying activation of ischemically sensitive abdominal visceral afferents have not been fully elucidated and most likely are multifactorial. Our laboratory previously has shown that lactic acid, bradykinin, prostaglandins, (8R,15S)-diHETE, and reactive oxygen species can sensitize or directly activate these nerve endings (14, 15, 20), whereas LTB₄ reduces the response of these afferents to ischemia (20). In the present study, ischemically sensitive abdominal visceral afferents were shown to respond to histamine. None of these metabolites, includ-
ing histamine, appear to be solely responsible for abdominal visceral afferents during ischemia. Thus, although lactic acid, bradykinin, prostaglandins, (8R,15S)-diHETE, reactive oxygen species, and histamine can stimulate or sensitize these afferent endings during ischemia and reperfusion, we suspect that still other metabolic factors may play a role in this multifactorial response. For instance, because serotonin [5-hydroxytryptamine (5-HT)] levels in portal venous plasma and mesenteric lymph are increased during brief abdominal ischemia and early reperfusion (4) and exogenous 5-HT has been shown to stimulate abdominal visceral afferents (14), this is one of several other metabolic factors that will require future study.

In conclusion, the results of this study show that histamine and CPH (H₁-receptor agonist), but not dimaprit (H₂-receptor agonist) or (R)-α-MA (H₃-receptor agonist), stimulate ischemically sensitive abdominal visceral afferents in cats. Furthermore, blockade of H₁ receptors with pyrilamine (H₁-receptor antagonist) or pyrilamine virtually eliminates the response of these afferents to ischemia, and pyrilamine virtually eliminates the response of ischemically sensitive C-fiber afferents to exogenous histamine. Finally, inhibition of cyclooxygenase enzyme activity with indomethacin attenuates the response of ischemically sensitive C-fiber afferents through an action on histamine H₁ receptors. The stimulating effect of histamine appears, for the most part, to require an intact cyclooxygenase system.

Perspectives

Ischemia and reperfusion of abdominal visceral organs are capable of causing very strong cardiovascular reflex responses, as evidenced by increases in arterial blood pressure of 25–50 mmHg (22). The importance of this reflex likely is to increase blood pressure and thereby to enhance collateral blood flow into an ischemic mesenteric region as well as to assist in maintaining cardiac function, which can be depressed during shock (13). The vasoconstrictor reflex appears to be manifested not only during ischemia but also during reperfusion, when it assists in maintaining arterial blood pressure at a time when it otherwise would decrease as a result of metabolic vasodilation. The afferent limb of this reflex primarily comprises ischemically sensitive C-fiber afferents, which have been shown to respond during abdominal ischemia and reperfusion (6, 14). Previously, we reported that the concentration of histamine in intestinal lymph and portal venous plasma is significantly increased even during relatively brief abdominal ischemia and reperfusion (4). Moreover, our laboratory has shown that application of histamine to an abdominal organ evokes a reflex cardiovascular response (25). The present investigation demonstrates that endogenous histamine produced during brief abdominal ischemia contributes significantly to the activation of ischemically sensitive C-fiber afferents by stimulation of H₁ receptors. Histamine is released not only during ischemia but also during other conditions such as the carcinoid and mastocytosis syndromes (3, 8). Thus evidence presented in this study as well as in earlier investigations indicates that histamine is important in mediating the reflex autonomic responses during abdominal ischemia and reperfusion and possibly during other pathological conditions involving the abdominal visceral region.

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