Histamine Contributes to Ischemia-Related Activation of Cardiac Spinal Afferents: Role of H1 Receptors and PKC

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1Departments of Medicine and 2Departments of Physiology and Biophysics and Pharmacology, and Center of Biomedical Engineering, University of California, Irvine, California; and 3Fachbereich Pharmazie, Institut für Pharmazie, Freie Universität Berlin, Berlin, Germany

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Fu, Liang-Wu, Walter Schunack, and John C. Longhurst. Histamine contributes to ischemia-related activation of cardiac spinal afferents: role of H1 receptors and PKC. J Neurophysiol 93: 713–722, 2005; doi:10.1152/jn.00528.2004. Myocardial ischemia activates cardiac spinal afferents that transmit the nociceptive information leading to chest pain and elicit excitatory cardiovascular reflexes. Previous studies have shown that histamine is increased in coronary sinus blood during myocardial ischemia and that this autacoid stimulates abdominal visceral afferents. The present investigation evaluated the role of endogenous histamine in stimulation of ischemically sensitive cardiac afferents. Nerve activity of single-unit cardiac afferents was recorded from the left sympathetic chain or rami communicans (T2–T5) in anesthetized cats. Sixty-four cardiac afferents were identified. Injection (5–30 μg/kg) of histamine into the left atrium (LA) stimulated 7 ischemically sensitive cardiac afferents resulting in a significant increase in their activity in a dose-dependent manner. Also, LA injection of histamine (10 μg/kg) stimulated 7 of 8 ischemically insensitive cardiac spinal afferents. Administrations of 2-(3-chlorophenyl)histamine (250 μg/kg, LA), a specific H1 receptor agonist and histamine (10 μg/kg, LA), stimulated 9 other ischemically sensitive cardiac afferents (0.48 ± 0.10 to 1.40 ± 0.20 imp/s). In contrast, dimaprit (500 μg/kg, LA), an H2 receptor agonist, stimulated only one of the 9 afferents and thus did not alter their overall activity (0.40 ± 0.09 to 0.54 ± 0.09 imp/s). (R)-α-Methyl-histamine (500 μg/kg, LA), an H3 receptor agonist, did not stimulate any of the 9 afferents. Pyrilamine (300 μg/kg, iv), a selective H1 receptor antagonist, attenuated the activity of 8 afferents during 5 min of ischemia from 3.32 ± 0.38 to 1.87 ± 0.28 imp/s and abolished the response of 9 other cardiac afferents to histamine. Finally, administration of PKC-(19–36) (30 μg/kg, iv), a selective inhibitor of protein kinase C, attenuated the response of 8 cardiac afferents to histamine by 32%. These data indicate that endogenous histamine contributes to activation of cardiac sympathetic afferents during myocardial ischemia through H1 receptors and that the action of histamine on these cardiac afferents is partially dependent on the intracellular PKC pathway.

INTRODUCTION

Myocardial ischemia can cause cardiovascular reflex responses as well as chest pain. It is generally accepted that cardiac spinal (sympathetic) afferents are the primary pathways transmitting nociceptive information from the heart to the brain to elicit the perception of cardiac pain and to initiate excitatory cardiovascular reflexes, including transient hypertension and tachycardia (Foreman 1999; Malliani 1990; Meller and Gebhart 1992; White 1957). The central nervous mechanisms of cardiac–cardiovascular reflexes and cardiac pain have been studied extensively (Chandler et al. 2000; Foreman 1999; Qin et al. 2003). In contrast, the peripheral sensory signaling mechanisms leading to activation of cardiac spinal afferents remains unclear. However, previous studies from this laboratory and the others have implicated that cardiac spinal afferents can be classified into ischemically sensitive and insensitive afferents (Fu and Longhurst 2002a; Uchida and Murao 1974). A number of ischemic metabolites including 5-hydroxytryptamine (5-HT), lactic acid (protons), bradykinin, prostaglandins, reactive oxygen species (ROS) (Fu and Longhurst 2002b; Huang et al. 1995a; Pan et al. 1999; Tjen-A-Looi et al. 1998), but not adenosine (Pan and Longhurst 1995), either directly stimulate or sensitize cardiac spinal afferents during ischemia and reperfusion. However, inhibition of any of these stimuli does not fully eliminate the response of these afferents to ischemia. We thus believe that other ischemic metabolites likely contribute to activation of these afferents during myocardial ischemia.

For over a decade histamine has been considered to be one of the contributors for activation of cardiac afferents during ischemia (Meller and Gebhart 1992). In this regard, experimental studies have documented that myocardial ischemia promotes the production and release of histamine in coronary sinus plasma. Clinically, increased histamine has been found in patients with ischemic heart diseases (Kounis and Zavras 1991). Coronary histamine comes mostly from cardiac mast cells (Frangogiannis et al. 1998) and activated platelets (Masini et al. 1998; Nakahodo et al. 1994). Previous studies have shown that myocardial ischemia is capable of activating platelets and promoting the degranulation of mast cells, which, in turn, leads to an increase in coronary histamine concentration (Frangogiannis et al. 1998). Additionally, histamine has been shown to be capable of stimulating somatic and abdominal visceral afferents (Fu et al. 1997; Herbert et al. 2001). However, there is no information on the role of increased coronary histamine in activation of these cardiac spinal afferents during myocardial ischemia.

Histamine binds to 4 distinct receptor subtypes: H1, H2, H3, and H4. Through activation of the 4 receptor subtypes, histamine acts on a large variety of excitable cell types, including smooth muscle, neurons, endocrine cells, and cells of the immune system (Hill et al. 1997; Repka-Ramirez 2003). Stimulation of H1 receptors increases coronary vascular resistance, whereas stimulation of H2 receptors slightly decreases coro-
nary resistance (Nakane and Chiba 1987). Moreover, H₁ receptors have been described on sensory neurons in the dorsal root ganglion (Ninkovic and Hunt 1985). In addition, sympa-
thetic nerve endings in the heart contain H₂ receptors that can modulate adrenergic responses by inhibiting norepinephrine release (Imamura et al. 1995). H₂ receptors are responsible for the histamine-induced postsynaptic excitation of submucous plexus neurons in the guinea pig (Tokimasa and Akasu 1989). We have demonstrated that, through an H₁ receptor mecha-
nism, histamine activates abdominal sympathetic afferents during ischemia (Fu et al. 1997) and evokes stomach–cardiovas-
cular excitatory reflex responses (Stebbins et al. 1991). How-
ever, the role of histamine receptors in activation of cardiac spinal afferents during myocardial ischemia is unknown.

The cellular phospholipase C (PLC) and protein kinase C (PKC) pathway plays a pivotal role in the processes concerned with intracellular signaling of sensory neurons in mammals (Bevan 1996). Through stimulation of H₁ receptors, histamine leads to activation of PLC, which results in the hydrolysis of phosphatidyl-inositol-4,5-bisphosphate to form inositol-1,4,5-
trisphosphate (IP₃) and 1,2-diacylglycerol (DAG) (Bevan 1996). IP₃ mobilizes calcium from intracellular stores, whereas DAG activates PKC that leads to phosphorylation and in-
creased activity of certain cation-permeable ion channels in-
volved in control of peripheral afferent nerve fiber activity (Dray and Urban 1996). For instance, Ahlgren and Levin (1994) reported that inhibition of PKC decreases hyperalgesia and C-fiber hyperexcitability. Others observed that the excitatory action of histamine on peripheral afferent fibers is medi-
ated by H₁ receptors positively coupled to the PLC–PKC pathway (Nicolson et al. 2002). Together, these studies suggest that the PLC–PKC pathway potentially plays a role in his-
tamine-mediated activation of ischemically sensitive cardiac afferents.

The present study was designed to test the hypotheses that 1) endogenous histamine, through an H₁ receptor mechanism, stimulates ischemically sensitive cardiac spinal afferents; and 2) stimulation of ischemically sensitive cardiac afferents by histamine is dependent on activation of the PKC pathway.

Portions of these data were previously presented in abstract form (Fu et al. 2003).

M E T H O D S

Surgical preparation

Adult cats of either sex (2.7 ± 0.6 kg, mean ± SD) were anes-
thesized by intramuscular injection of ketamine (20–30 mg/kg, Phoenix Scientific, St Joseph, MO) followed by intravenous (iv) injection of α-chloralose (40–50 mg/kg). Additional injections of α-chloralose (5–10 mg/kg, iv) were given as necessary to maintain an adequate depth of anesthesia that was assessed by observing the absence of a conjunctival reflex. The animal’s trachea was intubated and respira-
tion was maintained artificially (Harvard pump, model 661, Ealing, South Natick, MA). The cat was ventilated by air supplemented with 100% O₂ through the respirator. The femoral vein and artery were can-
nulated for administration of drugs and fluid and measurement of blood pressure, respectively. Another catheter (PE 90) was introduced into the left atrium through the left atrial appendage for intracardiac injection of solutions. Arterial blood pressure was measured by a pressure transducer (Statham P 23 ID, Gould) connected to the femoral arterial catheter. Arterial blood gases were frequently as-
sessed by a blood gas analyzer (Radiometer ABL-5, Copenhagen, Denmark) and maintained within physiological limits (pO₂ > 100 mmHg, pCO₂ = 28–35 mmHg, pH 7.35–7.45) by adjusting the respirator rate or tidal volume, or by intravenously administering 2–3 ml of 1 M of NaHCO₃ (8.4% [wt/vol]). Body temperature was monitored by a rectal thermistor and maintained at 36–38°C with a circulating water heating pad and a heat lamp. At the end of the experiment, the animals were killed by administration of a solution of saturated potassium chloride into the femoral vein under deep anes-
thesia that was ensured by giving an additional dose of α-chloralose (50 mg/kg). Surgical and experimental protocols used in this study were approved by the Animal Use and Care Committee at the University of California at Irvine. The studies conformed to American Physiological Society’s “Guiding Principles in the Care and Use of Animals.”

Cardiac spinal afferent recording

Single-unit activity of cardiac afferents was recorded as described previously (Fu and Longhurst 2002b; Tjen-A-Looi et al. 1998). In brief, a midline sternotomy was performed and the first-seventh left ribs and the left lung were removed. The left paravertebral sympa-
thetic chain was isolated, then draped over a Plexiglas platform and covered with warm mineral oil. Small nerve filaments were dissected gently from the chain and rami communicates between T₂ and T₃ under an operating microscope (Zeiss, Oberkochen, Germany) and the rostral ends were placed across one pole of the recording electrode. The other pole of the recording electrode was grounded with a cotton thread to the animal. The recording electrode was attached to a high-impedence probe (model HIPS11, Grass Instruments, Quincy, MA). The action potential of the afferent was amplified (model P511 Preampilifier, Grass) and processed through an audioamplifier (AMBB, Audiomonitor, Grass) and an oscilloscope (model 2201, Tektronix, Beaverton, OR). Nerve activity and blood pressure were recorded simultaneously on a chart recorder (K2G, Astro-Med, West Warwick, RI). Afferent activity also was simultaneously fed into a Pentium chip–equipped computer through an A/D interface card (R.C. Elec-
tronics, Santa Barbara, CA) for subsequent off-line analysis. The discharge frequency of afferents was analyzed with data acquisition and analysis software (EGAA, version 3.02, R.C. Electronics) and a histogram was created for each afferent. Accurate counting of the impulse activity of each afferent was verified by comparing the constructed histogram with the original neurogram.

The receptive field of each afferent was located by mechanical stimulation of the heart. This included constricting the thoracic aorta as well as gently probing the heart with a cotton swab. The location of the afferent nerve ending was confirmed further by placing a stimulating electrode directly on the surface of the myocardium to evoke the afferent’s action potential. Conduction velocity (CV) of each afferent fiber was calculated by dividing conduction distance by conduction time. The conduction time was determined by measuring the time interval from electrical stimulation and the evoked afferent’s action potential. The conduction distance was estimated by measuring the length of a wet thread between the receptive field and the recording electrode (Fu and Longhurst 2002b; Tjen-A-Looi et al. 1998). C- and Aβ-fiber afferents were classified as those with CV values of <2.5 and 2.5–50 m/s, respectively. In the present study, each afferent had a single receptive field that could be located precisely in the ventricles.

Myocardial ischemia was induced by complete occlusion of the appropriate coronary artery supplying the regional receptive field of the cardiac afferent nerve with a thread placed around the vessel. Ischemia was confirmed by observing a regional change in the color of the myocardium, which was closely correlated to the production of lactic acid, as indicated by a reduction in tissue pH (Pan et al. 1999). Afferents were considered to be ischemically sensitive if their dis-
charge activity during 3–5 min of myocardial ischemia was increased at least 2-fold above baseline activity (Fu and Longhurst 2002a,b).
Experimental protocols

RESPONSES OF ISCHEMICALLY SENSITIVE CARDIAC SPINAL AFFERENTS TO HISTAMINE. This protocol examined the effect of different doses of histamine (5, 10, and 30 μg/kg) on discharge activity of ischemically sensitive cardiac spinal afferents (n = 7). After the location of the receptive field of an afferent fiber in the ventricles was established, the response of the cardiac afferent was identified during 3–5 min of myocardial ischemia. If the afferent did not respond to ischemia we conducted the next protocol. If the afferent responded to ischemia a minimum 30-min recovery period was used to reestablish baseline activity of the afferent. Subsequently, we injected histamine into the left atrium (LA) while activity of the afferent was recorded, as we described previously (Fu and Longhurst 2002a). Various doses of histamine were injected randomly into the LA, maintaining ≥15 min of recovery between injections to avoid tachyphylaxis. On the day of each experiment, histamine (Sigma) was dissolved in 0.9% NaCl (w/vol) and diluted to a concentration of 200 μg/ml.

RESPONSES OF ISCHEMICALLY INSENSITIVE CARDIAC AFFERENTS TO HISTAMINE AND BK. In the ischemically insensitive afferent group (n = 8), histamine (10 μg/kg) was injected into the LA 30–40 min after recording the activity of cardiac afferent during 5 min of ischemia and 2–3 min of reperfusion. We also injected bradykinin (BK, 3 μg, LA) to determine responsiveness of these afferents.

EFFECT OF SELECTIVE HISTAMINE RECEPTOR AGONISTS ON AFFERENTS DISCHARGE ACTIVITY. In this protocol, the effect of histamine (10 μg/kg), 2-(3-chlorophenyl)histamine (CPH) (250 μg/kg, H1 receptor agonist), dimaprit (500 μg/kg, H2 receptor agonist), or (R)-α-methyl-histamine [(R)-α-MA] (500 μg/kg, H3 receptor agonist) on the discharge activity of 9 cardiac afferents was investigated. Histamine, CPH (Institut für Pharmazie, Freie Universität Berlin), dimaprit (Sigma), and (R)-α-MA (Sigma) were dissolved in 0.9% NaCl and were prepared fresh daily. After identification of an ischemically sensitive cardiac afferent unit, histamine, CPH, dimaprit, or (R)-α-MA was injected in a random fashion into the LA, maintaining ≥15 min of recovery between injections to avoid tachyphylaxis. Afferent activity was recorded as described previously (Fu and Longhurst 2002a).

INFLUENCE OF PYRILAMINE ON RESPONSE OF AFFERENTS TO HISTAMINE. In 9 ischemically sensitive cardiac C-fiber afferents, we examined the effect of H1 receptor blockade with pyrilamine (300 μg/kg, iv) on the response of these afferents to histamine. Pyrilamine (Sigma) was dissolved in 0.9% NaCl to a concentration of 2 mg/ml and was prepared fresh daily. A previous study demonstrated that this dose of pyrilamine effectively abolishes the response of abdominal visceral afferents to histamine (Fu et al. 1997). After identification of an ischemically sensitive unit, we injected histamine (10 μg/kg) into the LA while recording afferent activity. We repeated application of histamine (10 μg/kg, LA) 25–30 min after its initial injection, including ≥15 min after treatment with pyrilamine. LA injection of BK (3 μg) was conducted to establish the responsiveness of the afferent after the second injection of histamine.

To determine repeatability of the afferent response to histamine, 8 additional cats were studied as a time control group. After identification of an ischemically sensitive unit, each animal in this group was treated identically except that saline (0.5–1 ml, iv) was used in place of pyrilamine.

INFLUENCE OF PKC-(19–36) ON RESPONSE OF ISCHEMICALLY SENSITIVE AFFERENTS TO HISTAMINE. In 8 other cats, we examined the response of 8 ischemically sensitive cardiac afferents to histamine in the absence and presence of selective inhibition of PKC with PKC-(19–36) (30 μg/kg, iv). PKC-(19–36) (Sigma-RBI) was dissolved in distilled water to a concentration of 300 μg/ml and was further diluted to a final concentration using 0.9% NaCl. After identification of an ischemically sensitive unit, we injected histamine (10 μg/kg) into the LA while recording afferent activity. PKC-(19–36) then was administered intravenously. Repeated injection of histamine was conducted 25–30 min after the first injection of histamine and ≥15 min after treatment with PKC-(19–36). The dose of PKC-(19–36) chosen was previously shown to reduce the response of ischemically sensitive abdominal visceral afferents to BK (Guo et al. 1998). The control group used in the histamine–pyrilamine protocol served as a time control for comparison.

EFFECT OF PYRILAMINE ON RESPONSE OF AFFERENTS TO ISCHEMIA. In this protocol, cardiac afferents were subjected to two 5-min periods of myocardial ischemia separated by 4 min of reperfusion and 25–30 min of recovery. These intervals induce reproducible responses from cardiac afferents without damaging or sensitizing nerve endings (Fu and Longhurst 2002a). The effect of H1 receptor antagonism with pyrilamine (300 μg/kg, iv) on the afferent’s response to 5 min of ischemia was studied in 8 separate animals. After locating the receptive field of a cardiac afferent, 5 min of regional myocardial ischemia was induced while discharge activity of the afferent was measured. If the afferent responded to ischemia, we repeated ischemia 25–30 min after the first period of ischemia and ≥15 min after treatment with pyrilamine. In the occasional circumstance where afferent activity was suppressed completely, we injected BK (3 μg) into the LA to establish responsiveness of the afferent.

To differentiate between variations in afferent response related to drug effect and time-related effects, 7 other cardiac spinal afferents were investigated to determine repeatability of the afferent response to ischemia. In this protocol, after identification, each afferent fiber was treated identically but was not subjected to pyrilamine.

Data analysis

Discharge activity of cardiac spinal afferents was expressed in imp/s and was averaged during 3 to 5 min of preischemia and 5 min of ischemia. We measured the responses of cardiac afferent nerve endings to histamine, CPH, dimaprit, or (R)-α-MA by averaging discharge rates of the afferent during the entire period of response, defined as the time during which sustained activity exceeded baseline activity by 20%. Baseline activity was determined over the 3- to 5-min period immediately preceding ischemia.

Data are expressed as means ± SE. The effects of repeated injection of histamine, pyrilamine, PKC-(19–36), and repeat ischemia on the responses of the afferents were compared using a one-way repeated-measures ANOVA with Tukey’s post hoc 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 Dunnett’s post hoc test. We compared the effect of histamine, CPH, dimaprit, and (R)-α-MA on the afferent discharge activity using a Student’s paired t-test. A Student’s paired t-test also was used to compare the effects of pyrilamine or PKC-(19–36) on histamine-induced increases in discharge activity of the afferents. Alternatively, we used the Wilcoxon signed-rank test to compare the paired data, if the data were not normally distributed. All statistical calculations were performed with Sigmasstat software (Jandel Scientific Software, San Rafael, CA). Values were considered to be significantly different when P < 0.05.

RESULTS

Effect of histamine on activity of ischemically sensitive afferents

The influence of exogenous histamine on 7 cardiac spinal afferents (CV = 0.75 ± 0.25 m/s) is summarized in Fig. 1. The activity of this group of afferents was increased from 0.70 ± 0.12 to 2.95 ± 0.38 imp/s by brief myocardial ischemia. In a pilot study, we observed that LA injection of 1 μg/kg of
Similar to ischemically sensitive fibers, injection of histamine of afferents (0.29 g/kg) into the left atrium (LA) did not alter the discharge activity of this group of fibers (0.40 ± 0.09 to 0.54 ± 0.09 imp/s). Injection of CPH (250 μg/kg) into the LA also stimulated all 9 C-fibers, significantly increasing their discharge activity from 0.48 ± 0.10 to 1.40 ± 0.20 imp/s. In contrast, application of dimaprit (500 μg/kg, LA) also stimulated only one of the 9 fibers, and thus did not significantly alter impulse activity of this group of fibers (0.40 ± 0.09 to 0.54 ± 0.09 imp/s).

Responses of ischemically insensitive afferents to histamine

The responses of 8 cardiac afferents (2 Aβ, CV = 2.81 and 3.26; 6 C-fibers, CV = 0.95 ± 0.28 m/s) to brief ischemia, histamine, and BK were recorded in 8 cats. Myocardial ischemia of 5 min did not alter the discharge activity of these cardiac spinal afferents in a dose-dependent manner (Fig. 1). Injection of the same volume of saline (vehicle) did not alter the activity of these afferents. The location of each of the 7 afferent nerve endings is shown in Fig. 9.

Selective histamine receptor agonists

The influence of histamine, CPH, dimaprit, and (R)-α-MA on discharge activity of 9 ischemically sensitive cardiac afferents (1 Aδ, CV = 4.09 m/s; 8 C-fibers, CV = 0.72 ± 0.21 m/s) is summarized in Fig. 3. The locations of the 9 afferent nerve endings are shown in Fig. 9. Injection of histamine (10 μg/kg) into the left atrium stimulated all 9 fiber afferents, significantly increasing their discharge activity from 0.43 ± 0.09 to 1.53 ± 0.22 imp/s. Injection of CPH (250 μg/kg) into the LA also stimulated all 9 C-fibers, significantly increasing their discharge activity from 0.48 ± 0.10 to 1.40 ± 0.20 imp/s. In contrast, application of dimaprit (500 μg/kg, LA) also stimulated only one of the 9 fibers, and thus did not significantly alter impulse activity of this group of fibers (0.40 ± 0.09 to 0.54 ± 0.09 imp/s). Injection of (R)-α-MA (500 μg/kg, LA) also did not alter activity of the 9 fibers tested (0.40 ± 0.11 to 0.44 ± 0.11 imp/s). A recovery period of ≈ 15 min was used between injections to avoid tachyphylaxis.

Effect of H1 receptor blockade on response to histamine

Figure 4 displays the response of an ischemically sensitive cardiac C-fiber afferent (CV = 1.06 m/s) to histamine before and after treatment with pyrilamine, a selective H1 receptor antagonist. Brief ischemia increased discharge activity of this cardiac C-fiber afferent (0.69 to 2.16 imp/s). After releasing coronary arterial occlusion, discharge activity of this fiber gradually returned to control levels within 5 min. Injection of histamine (Fig. 4A) resulted in an immediate burst of afferent activity. This response was eliminated after blockade of H1 receptors with pyrilamine (300 μg/kg, iv; Fig. 4B).

The effect of H3 receptor blockade with pyrilamine on the response of 9 ischemically sensitive afferents (1 Aδ, CV = 3.88 m/s; 8 C-fibers, CV = 0.69 ± 0.22 m/s) to histamine is shown in Fig. 5A. Histamine (10 μg/kg, LA) stimulated all 9 afferents, leading to a significant increase in their discharge activity. Pyrilamine (300 μg/kg, iv) virtually eliminated the afferent responses to histamine. Conversely, each of the 9 afferents still responded to injection of 5 μg of BK after pyrilamine (Fig. 5A). In 8 other animals, 8 ischemically sensitive afferents (CV = 0.76 ± 0.22 m/s) responded consistently to brief ischemia, histamine, and BK. The locations of all 8 ischemically insensitive afferent nerve endings are shown in Fig. 9.
The responses of 15 (7 afferents for saline control and 8 afferents for pyrilamine treatment) cardiac afferents to brief myocardial ischemia are displayed in Fig. 8. Ischemia for a period of 5 min significantly increased the discharge activity of the 15 afferents. In the presence of saline solution 7 cardiac afferents (7 C-fibers, CV = 0.61 ± 0.25 m/s) responded consistently to 5-min periods of repeated myocardial ischemia (Fig. 8A). Conversely, after blockade of H<sub>1</sub> receptors with pyrilamine the ischemia-induced increase in the activity of 8 other cardiac afferents (1 Aδ, CV = 3.14 m/s; 7 C-fibers, CV = 0.75 ± 0.28 m/s) was significantly attenuated (3.32 ± 0.38 to 1.87 ± 0.28 imp/s), compared with the initial period of ischemia (Fig. 8B). The repeated ischemia was induced 25–30 min after the first period of ischemia and ≥15 min after treatment with saline or pyrilamine. The locations of the 15 afferent nerve endings are provided in Fig. 9.

Figure 8C shows neurohistograms displaying summed 5-s discharge activity during 5 min of ischemia in the 8 cardiac afferents before and after blockade of H<sub>1</sub> receptor with pyrilamine. Similar to the changes in mean discharge activity, summed impulse activity during the entire 5-min period of ischemia was attenuated by 42% after pyrilamine.

**Influence of inhibition of PKC on responses of ischemically sensitive cardiac afferents to histamine**

The influence of PKC inhibition with the selective PKC inhibitor, PKC-(19–36), on the response of 8 ischemically sensitive afferents (1 Aδ, CV = 3.5 m/s; 7 C-fibers, CV = 0.83 ± 0.22 m/s) to histamine is shown in Fig. 6A. Histamine (10 μg/kg, LA) stimulated all 8 cardiac afferents, leading to a significant increase in discharge activity of these afferents (0.48 ± 0.13 to 1.56 ± 0.17 imp/s). The responses of the afferents to histamine were attenuated (1.56 ± 0.17 to 1.07 ± 0.19 imp/s) by PKC-(19–36) (30 μg/kg, iv). The locations of these afferents are shown in Fig. 9. In contrast, 8 other cardiac afferents responded consistently to repeated histamine in the absence of PKC-(19–36) (Fig. 5B). The neurograms in Fig. 6B show the response of a C-fiber afferent to histamine before and after treatment with PKC-(19–36). The response of this afferent to histamine was attenuated 63% by PKC-(19–36).

**Influence of H<sub>1</sub> receptor blockade on cardiac sympathetic afferent response to myocardial ischemia**

Representative tracings of a cardiac C-fiber afferent that responded to myocardial ischemia in the absence and presence of pyrilamine are shown in Fig. 7. Ischemia increased discharge activity of this afferent from 0.01 to 3.16 imp/s (Fig. 7A). Antagonism of H<sub>1</sub> receptors with pyrilamine (300 μg/kg, iv) attenuated the ischemia-induced increase in discharge activity of this afferent (3.16 to 1.33 imp/s) by 58% (Fig. 7B).
A

B

FIG. 6. **A**: bar graph summarizing changes in activity of 8 ischemically sensitive cardiac afferents before and after treatment with protein kinase C (PKC)-(19–36), a selective PKC inhibitor. **B**: original representative tracing showing response of an ischemically sensitive cardiac afferent (CV = 2.32 m/sec) innervating posterior wall of left ventricle to injection of histamine (10 μg/kg) into the LA before (B1–B3) and after (B4–B6) administration of 30 μg/kg of PKC-(19–36). Neurograms B1–B6 represent the discharge activity of this afferent before (B1 and B4), during (B2 and B5), and after injection (B3 and B6) of histamine. Columns and error bars represent means ± SE, respectively. *P < 0.05 compared with control, †P < 0.05 post-PKC-(19–36) vs. pre-PKC-(19–36).

**DISCUSSION**

The present study demonstrates, for the first time, the contribution of endogenous histamine to activation of cardiac spinal afferents during myocardial ischemia through stimulation of histamine H1 receptors. Initially, we observed that LA injections of histamine and CPH, a selective H1 receptor agonist, stimulated ischemically sensitive cardiac spinal afferents, whereas injections of dimaprit, a selective H2 receptor agonist, and (R)α-MA, a selective H3 receptor agonist, did not significantly increase the discharge activity of these afferents. Administration of PKC-(19–36), a selective inhibitor of protein kinase C, attenuated the responses of ischemically sensitive afferents to histamine. Furthermore, the response to histamine was blocked by pyrilamine, a selective H1 receptor antagonist. Finally, pyrilamine attenuated activity of cardiac afferents during myocardial ischemia. In addition, we observed that histamine stimulated ischemically insensitive cardiac afferents. These data thus demonstrate that endogenous histamine contributes to activation of cardiac afferents during myocardial ischemia through excitation of H1 receptors and that histamine stimulates these cardiac afferents partially through the PKC cellular pathway.

For over a decade histamine generally has been thought to contribute to activation of cardiac spinal afferents during myocardial ischemia through stimulation of histamine H1 receptors. Initially, we observed that LA injections of histamine and CPH, a selective H1 receptor agonist, stimulated ischemically sensitive cardiac spinal afferents, whereas injections of dimaprit, a selective H2 receptor agonist, and (R)α-MA, a selective H3 receptor agonist, did not significantly increase the discharge activity of these afferents. Administration of PKC-(19–36), a selective inhibitor of protein kinase C, attenuated the responses of ischemically sensitive afferents to histamine. Furthermore, the response to histamine was blocked by pyrilamine, a selective H1 receptor antagonist. Finally, pyrilamine attenuated activity of cardiac afferents during myocardial ischemia. In addition, we observed that histamine stimulated ischemically insensitive cardiac afferents. These data thus demonstrate that endogenous histamine contributes to activation of cardiac afferents during myocardial ischemia through excitation of H1 receptors and that histamine stimulates these cardiac afferents partially through the PKC cellular pathway.

For over a decade histamine generally has been thought to contribute to activation of cardiac spinal afferents during myocardial ischemia (Meller and Gebhart 1992). Previously, Sakata et al. (1996) observed that the coronary histamine is elevated shortly before coronary spasm in patients with variant angina. Coronary artery occlusion for a period of 30 min elevates coronary sinus histamine in dogs (Wolff and Levi 1988). Platelets and mast cells serve as the major cellular sources of coronary histamine. Most cardiac mast cells are found in the outer layer of the adventitia of coronary arteries (Stary 1990). The degree of mast cell degranulation increases in the coronary adventitia after plaque rupture and in spastic atherosclerotic coronary segments (Laine et al. 1999). Myocardial ischemia/reperfusion and hypoxia also degranulate cardiac mast cells, leading to the release of histamine (Frangogiannis et al. 1998; Laine et al. 1999; Masini et al. 1987). In addition, adventitial mast cells are in close association with sensory nerve fibers in atherosclerotic coronary arteries (Laine et al. 2000). Platelets, the other source of histamine, circulate in the blood stream as small, anucleate, disk-shaped cells. Previous studies have shown that brief myocardial ischemia and hypoxia activate platelets, which then release histamine (Flores et al. 1994; Fu and Longhurst 2002b; Nakahodo et al. 1994). Thrombin and collagen also stimulate platelets to release histamine (Masini et al. 1998; Saxena et al. 1989). Taken together, these studies demonstrate that myocardial ischemia induces activation of platelets and degranulation of mast cells, which then leads to an increase in histamine concentration in coronary circulation and myocardial tissue. Thus it is not surprising that investigators have observed that chest pain in patients with variant angina triggered by coronary spasm can be provoked by histamine (Ginsburg et al. 1981; Kounis and Zavras 1991).

Histamine also is recognized as a mediator of sensory nerve activation in other somatic and visceral regions. For example, Kopfell et al. (2001) observed that application of histamine on isolated rat skin-nerve preparation increases discharge activity of polymodal C-fibers. Data obtained from the articular nerve and knee joint of cats indicate that histamine stimulates both group III and IV afferents (Herbert et al. 2001). We also have
shown that histamine stimulates ischemically sensitive abdominal sympathetic afferents (Fu et al. 1997). However, the influence of histamine on ischemically sensitive cardiac spinal afferents has not been previously investigated. The present investigation demonstrates that exogenous and endogenous histamine stimulates ischemically sensitive cardiac sympathetic afferents in a dose-dependent manner. Taken together these data indicate that histamine plays a role in activating cardiac spinal afferents during myocardial ischemia and the subsequent cardiovascular reflex responses.

Histamine may act through a PKC intracellular mechanism to stimulate ischemically sensitive cardiac sympathetic afferents. First, previous data have shown that PKC plays a pivotal role in processes underlying activation and sensitization of sensory neurons in mammals (Bevan 1996; Guo et al. 1998). Experimental studies documented that PKC contributes to ischemia-mediated activation of abdominal visceral afferents (Guo et al. 1998), and demonstrated that inhibition of PKC with PKC-(19–36) decreases C-fiber hyperexcitability and hyperalgesia in diabetic rats (Ahlgen et al. 1994). Second, histamine activates phospholipase C (PLC) through stimulation of H1 receptors (Hill et al. 1997; Nicolson et al. 2002); PLC cleaves target membrane lipids to produce diacylglycerol and inositol-1,4,5-triphosphate. Inositol triphosphate mobilizes calcium from intracellular stores, whereas DAG activates protein kinase C leading to phosphorylation of cellular components and an increase in activity of specific cation-permeable ion channels involved in regulation of the activity of peripheral nerve fibers (Bevan 1996). However, the interaction between histamine and PKC in activation of cardiac sympathetic afferents has not been studied previously. The present study has demonstrated that PKC-(19–36) attenuated the responses of cardiac spinal afferents to histamine. In agreement with our findings, Nicolson et al. (2002) demonstrated that the excitatory action of histamine on peripheral afferent fibers appears to be mediated by H1 receptors positively coupled to the PLC–PKC pathway. Therefore these data indicate that histamine stimulates ischemically sensitive cardiac spinal afferents in part through the PLC–PKC intracellular pathway.

The physiologic action of histamine is mediated by a group of histamine receptors. Four distinct histamine receptor subtypes including H1, H2, H3, and H4 have been identified in biological tissues and in cells (Hill et al. 1997; Repka-Ramirez 2003). Three types of histamine receptors have been implicated in regulation of neuronal function. For example, stimulation of the H3 receptor inhibits the release of neuropeptides including tachykinins or calcitonin gene-related peptide from sensory C-fibers innervating the heart and airways (Ichinose et al. 1990; Imamura et al. 1996). Sympathetic nerve endings in the heart also contain H3 receptors capable of modulating adrenergic responses by inhibiting the release of norepinephrine (Imamura et al. 1995). H2 receptors mediate histamine-induced postsynaptic excitation of submucous plexus neurons in guinea pigs (Tokimasa and Akasu 1989). H1 receptor mRNA is expressed in unmyelinated sensory neurons of guinea pigs (Kashiba et al. 1999). H1 receptors also have been identified on...
sensory neurons in the dorsal root ganglion (Ninkovic et al. 1985). Neurophysiological studies have shown that application of histamine to primary afferent fibers in mammals induces action potentials through an H1 receptor mechanism (Koda et al. 1996). Previous studies in our laboratory have demonstrated that, through activation of H1 receptor, histamine activates abdominal sympathetic afferents during ischemia (Fu et al. 1997) and evoke cardiovascular excitatory reflex responses (Stebbins et al. 1991). However, the absence of information about the roles of endogenous histamine and histamine receptor subtypes in activation of cardiac spinal afferents during ischemia provided a strong rationale to investigate the role of this mediator during ischemia. The present study demonstrates that ischemically sensitive cardiac spinal afferents respond to CPH, a selective H1 receptor agonist, but not to selective H2 or H3 receptor agonists. Conversely, the increase in discharge activity of cardiac spinal afferents during ischemia was significantly reduced by pyrilamine, a selective H1 receptor antagonist. Thus endogenous histamine, through an H1 receptor mechanism, but not through H2 or H3 receptors, activates ischemically sensitive cardiac spinal afferents.

Cardiac sympathetic afferents can be classified as ischemically sensitive or ischemically insensitive. We and others have demonstrated that ischemic metabolites may or may not selec-
Bradykinin, H2O2, and histamine stimulate both ischemically evoked by chemo-stimulation of cardiac afferents by bradykinin (Fu and Longhurst 2002b; Huang et al. 1995a; Tjen-A-Looi et al. 1998). For example, serotonin selectively stimulates ischemically sensitive cardiac spinal afferents (Fu and Longhurst 2002b; Huang et al. 1995a; Tjen-A-Looi et al. 1998). In the present investigation, we evaluated the relative responsiveness of these 2 groups of afferents to histamine. We found that histamine is not selective and stimulates both groups of ventricular afferents. These data suggest that histamine has a broad stimulus profile, similar to that of bradykinin and reactive oxygen species.

One of the important questions concerns the roles of ischemically sensitive and insensitive cardiac spinal afferents in cardiac cardiovascular reflexes and cardiac nociception. It is generally accepted that ischemically sensitive cardiac spinal afferents function as nociceptors that are responding to the noxious stimulus such as myocardial ischemia and play an important role in cardiac–cardiovascular reflexes and cardiac pain perception (Fu and Longhurst 2002b; Lombardi et al. 1984; Meller and Gebhart 1992; Pan and Chen 2002). In contrast, the role of ischemically insensitive cardiac afferents remains unclear. However, previous studies have demonstrated that powerful excitatory cardiac cardiovascular reflexes are evoked by chemo-stimulation of cardiac afferents by bradykinin and H2O2 (Huang et al. 1995b; Nerdrum et al. 1986). Bradykinin, H2O2, and histamine stimulate both ischemically sensitive and insensitive cardiac spinal afferents (Fu and Longhurst 2002b; Huang et al. 1995a; Pan and Chen 2002). Taken together, these studies implicate that ischemically insensitive cardiac afferents might play a role in cardiac–cardiovascular reflexes attributed to chemo-stimulation, possibly in situations such as allergic reactions or diseases like carcinoid syndrome (Graver et al. 1986; Gustafsen et al. 1988) when high concentrations of chemical mediators are produced and released into the circulatory system.

Another important concern is with respect to the characteristics of sensitivity of cardiac spinal afferents, which function similarly to abdominal spinal afferents. Previous studies have demonstrated that cardiac spinal afferents have a chemosensitivity different from that of abdominal spinal afferents. For example, Stahl et al. (1992) observed that H2O2 stimulates only ischemically sensitive, but not ischemically insensitive, abdominal spinal afferents. In contrast, Huang et al. (1995a) demonstrated that H2O2 stimulates both ischemically sensitive and insensitive cardiac sympathetic afferents. Moreover, previous studies have documented that abdominal spinal afferents are more dependent on the action of prostaglandins than cardiac spinal afferents during ischemia (Pan et al. 1994; Tjen-A-Looi et al. 1998). Thus it is not possible to extrapolate directly from studies of abdominal visceral afferents to cardiac sympathetic afferents.

In conclusion, the present study has demonstrated that histamine stimulates both ischemically sensitive and insensitive cardiac spinal afferents. The action of histamine on ischemically sensitive cardiac afferents is mediated through an H1 receptor mechanism, but not by H2 or H3 receptors. Inhibition of PKC activity attenuates the response of these afferents to histamine. These data broaden our previous studies by demonstrating that endogenous histamine stimulates the ischemically sensitive cardiac spinal afferents through H1 receptors coupled to the PLC–PKC intracellular pathway. Future studies should be directed at elucidating the responses of ischemically sensitive and insensitive cardiac afferents to physiological mechanical stimulation and cardiac–cardiovascular reflexes to histamine.

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