Sensing Tissue Ischemia
Another New Function for Capsaicin Receptors?

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Background—Chest pain is a hallmark of myocardial ischemia, but its underlying signaling mechanisms remain poorly understood. The capsaicin receptor, vanilloid receptor-1 (VR₁), is an important cation channel present on primary nociceptive neurons. We have shown that the VR₁ is expressed on sensory nerve endings of the heart. In the present study, we determined the role of VR₁s in activation of cardiac spinal afferent nerves caused by myocardial ischemia.

Methods and Results—Single-unit activity of cardiac afferents was recorded from the sympathetic chain of anesthetized ferrets. Cardiac afferents responded to 5 minutes of regional myocardial ischemia and topical application of 10 μg/mL bradykinin in a reproducible manner. Topical application of a specific VR₁ antagonist, iodoresiniferatoxin (50 μmol/L), to the receptive field of afferents produced a large attenuation of the firing activity of cardiac afferents caused by myocardial ischemia. Iodoresiniferatoxin also significantly reduced the afferent response to bradykinin applied to the receptive field. Furthermore, treatment with a VR₁ channel blocker, ruthenium red (200 μmol/L), had a similar inhibitory effect on the afferent responses to myocardial ischemia and bradykinin.

Conclusions—This study provides the first functional evidence that ischemic stimulation of cardiac spinal afferent nerves is mediated through VR₁s. The VR₁ on the cardiac sensory nerve may function as a molecular sensor to detect tissue ischemia and activate cardiac nociceptors. (Circulation. 2004;110:1826-1831.)

Key Words: pain • coronary disease • nervous system • angina

Chest pain is a distinct symptom and warning sign associated with myocardial ischemia and infarction in humans. The sensory signals triggering the chest pain are conducted through cardiac spinal (sympathetic) afferents, primarily thinly myelinated Aδ- and unmyelinated C-fibers, that project to the dorsal horn of the upper thoracic spinal cord.1–4 Myocardial ischemia produces an array of chemical mediators that activate or sensitize nociceptors to elicit pain.4–6 However, the signaling mechanisms involved in detection of myocardial ischemia and activation of cardiac spinal afferent nerves remain poorly understood.

The capsaicin receptor, also known as the vanilloid receptor-1 (VR₁), or TRPV1 channel, is an ion channel mainly expressed on nociceptors and serves as the molecular target for capsaicin, the main pungent ingredient in chili peppers.7,8 This VR₁ cation channel can be activated by noxious heat, capsaicin, and other chemicals such as anandamide and lipoxygenase products.8–11 Activation of the VR₁ is essential for noception elicited by heat and capsaicin.12 Because the VR₁ is mainly located on small-sized dorsal root ganglion neurons, it is considered an important sensor for somatic nociception. We recently have shown that the VR₁ is present on the sensory nerve endings that innervate the surface of the heart.13 Although the cardiac VR₁-containing afferent nerves are essential in initiating cardiogenic sympathetically-mediated reflexes,13 little is known about the physiological function of VR₁s in the heart. In this study, we determined the potential role of VR₁s in the activation of cardiac spinal afferent nerves during myocardial ischemia.

Methods

General Surgical Preparations
Experiments were conducted on adult male ferrets (Marshall Farms, North Rose, NY) weighing between 1.2 and 1.5 kg. The procedures and protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and conformed to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Because the chest cavity of rats is too small to allow us to access the sympathetic chain for single-unit recordings, we used ferrets for this electrophysiology study. Ferrets were initially anesthetized with 2% to 3% halothane in O₂. The trachea was cannulated, and ferrets were mechanically ventilated with an animal ventilator (Columbus Instruments). Expired CO₂ concentration was monitored with a CO₂ analyzer (Capstar 100, CWE, Inc) and maintained at 4% to 5% throughout the experiment. The left carotid artery was cannulated, and the arterial blood pressure was measured with a pressure transducer (PT300, Grass Instruments). The arterial pressure was recorded on a PowerLab data acquisition system (model 4SP), displayed, and stored on a Pentium computer. Heart rate, calculated beat to beat, was counted by triggering from the blood pressure pulse. The left jugular vein was cannulated for intravenous injection of drugs. Halothane was discontinued after α-chloralose (50 mg/kg...
IV) and sodium phenobarbital (20 to 40 mg/kg IV) were administered. Supplemental doses of sodium phenobarbital were given to maintain adequate depth of anesthesia. Body temperature was maintained at 37°C to 38°C with a heating lamp. A midline sternotomy was performed, and the first to seventh left ribs and the upper lobe of the left lung were removed. The fascia overlying the left paravertebral sympathetic chain from T2 to T6 was removed. The isolated sympathetic chain was then laid on a microplate and covered with warm mineral oil. The ferret was euthanized with an overdose of sodium phenobarbital (100 mg/kg IV) at the end of the experiment.

**Single-Unit Recording of Cardiac Spinal Afferents**

Small nerve filaments were teased gently from the chain between T2 and T5 under a surgical microscope. The rostral cut end of the nerve was placed across a recording electrode connected to a high-impedance probe. The nerve filaments were dissected gradually until single-unit activity of a cardiac afferent fiber was isolated, as we described previously. The action potential of the afferent was amplified (×50 000) and bandpass filtered (100 to 1000 Hz) through an AC amplifier (PS11, Grass Instruments). Afferent activity was recorded in a Pentium computer installed with data acquisition and analysis software (DataWave Technology) for online and offline quantitative analysis. Discharge frequency was quantified by using a software window discriminator, and a histogram was generated for each afferent. Accurate counting of the afferent discharge frequency was verified for each afferent by comparing the constructed histogram with the raw tracing saved in the computer. When the nerve fiber was on the recording electrode, the epicardium was mapped gradually from the apex to the base of the heart with a stimulating electrode to electrically (5 to 10 V, 0.25 ms, and 0.5 Hz) search for gradually from the apex to the base of the heart with a stimulating electrode to electrically (5 to 10 V, 0.25 ms, and 0.5 Hz) search for receptive field of cardiac ventricular afferents with a thread placed around the vessel. Under an operating microscope, ligatures were placed around the proximal coronary artery. On occlusion of the coronary artery, the ischemic region was verified visually by cyanosis. Conduction time of the cardiac afferent was determined by measuring the time interval from the signal of electrical stimulation to recording of the evoked afferent’s action potential displayed on an oscilloscope. Conduction distance was estimated from the receptive field along the course of the inferior cardiac nerve to the left stellate ganglion and to the recording electrode down the course of the sympathetic chain. C- and A6-fiber afferents were classified as those with a conduction velocity <2.5 and 2.5 to 30 m/s, respectively.

The responses of a cardiac afferent to 5 minutes of myocardial ischemia and topical application of bradykinin (10 μg/mL, Sigma Chemical) were examined before and after topical application of a selective VR, blocker, iodoresiniferatoxin (iodo-RTX; LC Laboratories) or ruthenium red (Sigma). In some animals, capsaicin (10 μg/mL, Sigma) was also topically applied to the receptive fields of afferents to assess the effective concentration of VR, antagonists. To select the appropriate concentrations of VR, antagonists to fully block the VR₈, we examined the effect of topical application of 10 to 100 μmol/L iodo-RTX and 30 to 600 μmol/L ruthenium red on the blood pressure response induced by 10 μg/mL capsaicin in a preliminary study. Capsaicin applied to the anterior surface of the heart significantly increased the mean arterial blood pressure from 74±7 to 104±12 mm Hg (n=7). Both iodo-RTX and ruthenium red at a concentration of 50 and 200 μmol/L, respectively, completely blocked the pressor response evoked by capsaicin.

**Data Analysis**

Values are presented as mean±SEM. The discharge activity of afferents was averaged during a 2- to 5-minute control period, 5 minutes of myocardial ischemia, and 2 minutes of reperfusion. Afferents were considered to be ischemia-sensitive when their discharge frequency during 5 minutes of myocardial ischemia was increased by at least 50% above baseline activity. The response of afferents to bradykinin or capsaicin was measured by averaging the discharge rate during the entire period of responses. Comparisons between control and experimental interventions were made by either a paired Student t test or repeated-measures ANOVA with Dunnett post hoc test. Differences were considered to be statistically significant when P<0.05.

**Results**

A total of 45 ferrets was used in this study. Among 49 afferent fibers studied, the discharge activity of 17 fibers (conduction velocity, 0.74±0.16 m/s) did not increase significantly during 5 minutes of myocardial ischemia, and they were not studied further. The receptive fields of 32 ischemia-sensitive afferents were all C-fibers and located either on the anterior (n=17; conduction velocity, 0.54±0.11 m/s) or posterior (n=15; conduction velocity, 0.48±0.13 m/s) wall of the left ventricle. The blood pressure and heart rate were 77±6 mm Hg and 167±10 bpm, respectively, during control. During ischemia, the blood pressure and heart rate were 75±8 mm Hg and 165±12 bpm, respectively, at the time of the maximal afferent response (typically 2 to 4 minutes after ligation of the coronary artery).

**Effect of Iodo-RTX on Afferent Response to Myocardial Ischemia**

We first examined the role of VR₈ in ischemia-induced activation of cardiac afferents by using a highly specific VR₈ antagonist, iodo-RTX. Iodo-RTX (50 μmol/L dissolved in dimethyl sulfoxide and 2-hydroxypropyl-β-cyclodextrin, ≈30 μL) was topically applied to the afferent receptive field on the epicardium because the VR₈ is located on the surface of the heart. In 10 cardiac afferent fibers studied, the initial 5 minutes of myocardial ischemia led to a large increase in discharge activity (Figures 1 and 2). The afferent nerves were allowed to recover for 15 to 20 minutes after their initial response to ischemia. The response of these afferent nerves to a subsequent 5 minutes of ischemia was reduced substantially by iodo-RTX treatment (Figures 1 and 2). This concentration of iodo-RTX had no significant effect on the baseline activity of afferent nerves. Topical application of 10 μg/mL capsaicin failed to stimulate 6 cardiac afferent nerves examined (from 0.54±0.16 to 0.56±0.17 Hz, P>0.05) in the presence of 50 μmol/L iodo-RTX. In the absence of iodo-RTX, topical application of 10 μg/mL capsaicin significantly increased the firing activity of 5 ischemia-sensitive afferents (from 0.47±0.11 to 2.14±0.16 Hz, P<0.05). In another 6 ischemia-sensitive afferents, the response of cardiac afferent nerves to a repeated 5 minutes of ischemia was not significantly altered by application of the iodo-RTX vehicle (10 μL dimethyl sulfoxide in 140 μL 2-hydroxypropyl-β-cyclodextrin) to the receptive field, compared with that during the initial period of ischemia (Figure 2).
Effect of Iodo-RTX on Afferent Response to Bradykinin

We next determined whether the effect of bradykinin on cardiac spinal afferents is mediated by VR1s. Bradykinin is an endogenous metabolite produced during myocardial ischemia and can stimulate cardiac spinal afferents. Bradykinin was dissolved in normal saline because this vehicle has no effect on cardiac afferents. In 11 ischemia-sensitive afferent fibers examined, the firing activity evoked by topical application of 10 μg/mL bradykinin was significantly reduced 5 minutes after application of iodo-RTX to the receptive field (Figure 3). In another 8 afferent nerves, repeated application of 10 μg/mL bradykinin, separated by an interval of 15 to 20 minutes, caused a large and reproducible increase in the firing activity of afferents in the presence of the iodo-RTX vehicle (Figure 3).

Effect of Ruthenium Red on Afferent Responses to Myocardial Ischemia and Bradykinin

To further determine the role of VR1 channels in the activation of cardiac afferent nerves by ischemia and bradykinin, we used a selective VR1 channel blocker, ruthenium red (200 μmol/L dissolved in normal saline). Ruthenium red is a selective, noncompetitive blocker for VR1 channels. Topical application of ruthenium red (30 μL) to the receptive field of 10 ischemia-sensitive afferent nerves substantially reduced the firing activity of these fibers during 5 minutes of ischemia (Figures 4 and 5). In 8 separate afferent nerves studied, topical application of 10 μg/mL capsaicin failed to activate these afferents (from 0.71±0.13 to 0.72±0.15 Hz, P<0.05) in the presence of 200 μmol/L ruthenium red. Also, we tested the response of 10 ischemia-sensitive afferent fibers to 10 μg/mL bradykinin applied to the receptive field before and 5 minutes after treatment with ruthenium red. Ruthenium red significantly decreased the bradykinin-induced firing activity of these afferent fibers compared with their initial response to bradykinin (Figure 5). The inhibitory effects of ruthenium red on the responses of cardiac afferents to both ischemia and bradykinin were comparable to those of iodo-RTX.

Discussion

Sensory signals triggering chest pain during myocardial ischemia are conveyed by thinly myelinated Aδ- and unmyelinated C-fibers that travel in cardiac spinal afferents. Myocardial ischemia produces various metabolites, including bradykinin and protons, which can stimulate cardiac spinal afferent nerves. However, the signaling mechanisms involved in ischemic stimulation of cardiac nociceptors are still not clear. The VR1 may serve as a polymodal detector of pain-producing chemical and physical stimuli. We recently have demonstrated that VR1-expressing afferent nerves are widely distributed on the epicardial surface of the ventricle. Although capsaicin-sensitive afferents are essential for the excitatory cardiac-sympathetic reflex elicited by bradykinin, little is known about the physiological function of VR1s present on the cardiac afferent nerves. Specifically, the potential role of VR1s in ischemic stimulation of cardiac spinal afferents has not been studied previously. In this study, we used a highly specific VR1 antagonist, iodo-RTX, which is
much more potent than another VR1 antagonist, capsazepine.\(^{14}\) We found that iodo-RTX treatment produced a large reduction in the afferent response to myocardial ischemia. Furthermore, this finding is supported by our data showing the similar inhibitory effect of ruthenium red, a structurally different blocker for the VR1 channel,\(^{16,17}\) on ischemia-elicited cardiac afferent activity. These data suggest that stimulation of cardiac spinal afferent nerves by ischemia is largely through activation of VR1s. Therefore, this study provides the first in vivo functional evidence that the VR1 on the sensory nerve endings of the heart likely functions as a transduction molecule responsible for sensing tissue ischemia and stimulating cardiac nociceptors.

Bradykinin is considered an important ischemic metabolite that activates cardiac afferent nerves through kinin B2 receptors.\(^{3,6}\) The signaling pathways responsible for the stimulating effect of bradykinin on cardiac afferent nerves are not fully known. Bradykinin can interact with VR1s in cultured dorsal root ganglia and HEK293 cells.\(^{18,19}\) In this regard, bradykinin may activate VR1s through protein kinase C\(^{20}\) and 12-lipoxygenase products.\(^{9,18}\) Also, bradykinin increases heat-induced inward currents, and this effect is blocked by the VR1 antagonist capsazepine and a protein kinase C inhibitor.\(^{19}\) The capsaicin-sensitive afferent nerves are essential for the cardiogenic sympathetic reflex elicited by bradykinin,\(^{13}\) suggesting that both kinin B2 receptors and VR1s are probably expressed on the same sensory nerve endings in the heart. Although the bradykinin-evoked cardiac-sympathetic reflex is not significantly reduced by iodo-RTX,\(^{13}\) we found in this study that the direct excitatory effect of bradykinin on ischemia-sensitive cardiac afferents was significantly attenuated by both iodo-RTX and ruthenium red. It should be noted that bradykinin stimulates both ischemia-sensitive and ischemia-insensitive cardiac afferents.\(^{3}\) Nevertheless, the signaling mechanisms for the action of bradykinin are probably dissimilar for ischemia-sensitive and ischemia-insensitive afferent nerves. It is possible that the sympathetic reflex elicited by epicardial bradykinin is the result of stimulation of both ischemia-sensitive and ischemia-insensitive cardiac afferents.\(^{13}\) On the other hand, we specifically studied the role of VR1s in the effect of bradykinin on the single-unit activity of ischemia-sensitive afferent nerves in this study. Alternatively, we cannot exclude the possibility that block of the VR1 with iodo-RTX may decrease the generalized ability of cardiac spinal afferents to discharge action potentials in response to all stimuli. Data from the present study suggest that the VR1 contributes to the stimulatory effect of bradykinin on ischemia-sensitive cardiac afferent nerves.

In the present study performed in ferrets, topical application of capsaicin to the epicardium caused activation of cardiac spinal afferents and the excitatory cardiovascular reflexes that are comparable to those in rats and dogs.\(^{13,21}\) Furthermore, topical treatment with VR1 antagonists significantly attenuated the afferent response to induced ischemia in ferrets. This evidence strongly suggests that VR1s on sensory nerve endings are present on the surface of the heart in different animal species. There are 2 possible reasons why the pressor response during ischemia was not observed in the

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**Figure 3.** A. Original tracings showing response of a cardiac afferent (conduction velocity = 0.38 m/s) located on anterior wall of left ventricle to epicardial application of bradykinin (BK, 10 μg/mL) before and after treatment with iodo-RTX (50 μmol/L). Bradykinin was applied at time point indicated by arrow. B, Effect of topical treatment with iodo-RTX (50 μmol/L) on mean response of 11 cardiac afferents to 10 μg/mL bradykinin. C, Mean firing activity of 7 cardiac afferents in response to bradykinin before and after application of iodo-RTX vehicle. Data are presented as mean±SEM. *P<0.05 vs control; †P<0.05 vs initial afferent response to bradykinin.

**Figure 4.** Response of a cardiac afferent to 5 minutes of myocardial ischemia before (A) and after (B) treatment with ruthenium red (200 μmol/L). Histograms show firing activity of cardiac C-fiber (conduction velocity = 0.46 m/s) innervating posterior wall of left ventricle during 2 minutes of control, 5 minutes of ischemia, and 2 minutes of reperfusion before and 5 minutes after topical treatment with ruthenium red. Original neurograms labeled 1 through 4 are raw tracings of afferent activity at times indicated above histograms. Abbreviations are as defined in text.
ruthenium red (RR, 200 μmol/L). In addition to bradykinin, there are other possible candidates that could activate or sensitize VR1 s. These include several lipoxygenase products, protons, free radicals, protein kinase C, and anandamide. Protons are capable of modulating the activity of a number of receptors and ion channels expressed on primary afferent nociceptors, including acid-sensitive channels and VR1 s. Also, the response of VR1 channels to capsaicin or noxious heat is potentiated by extracellular protons within a pH range encountered during tissue acidosis. We have shown that 5 minutes of myocardial ischemia can reduce the extracellular pH to ≈7.0. At this tissue pH level, protons are less likely to activate the VR1 directly but could sensitize the response of VR1 s to other ischemic metabolites. Thus, the VR1 channel on the cardiac sensory nerve endings could integrate and respond to multiple ischemic metabolites. The roles of putative endogenous VR1 ligands and their interactions in activation of cardiac VR1 s during ischemia should be further investigated.

Silent or painless myocardial ischemia and infarction is a significant clinical problem and has been the subject of many studies. Although clinical studies have suggested that diabetic neuropathy and destruction of nociceptive afferent nerve endings by infarction are the possible explanations in many patients, the reasons for silent ischemia are still not fully known. It should be recognized that unlike somatic tissues, visceral organs such as the heart are innervated with far fewer nociceptive afferent nerve fibers. Also, the projection of cardiac afferent nerves to the thoracic spinal cord is very diffuse. Thus, lack of adequate cardiac afferent innervation and the diffuse central projection of cardiac afferents in general may contribute to silent ischemia in many patients with coronary artery disease. Furthermore, our recent histological study and the present electrophysiological experiments provide strong evidence that cardiac nociceptive afferents expressing VR1 s are located on the epicardial surface of the heart. Consequently, the location of myocardial ischemia and infarction is likely another important factor for the lack of perception of ischemic cardiac pain. In this regard, if the ischemia does not involve the epicardium (ie, nontransmural and localized endocardial infarction), it is less likely that VR1 s on cardiac nociceptive afferents would be activated. This possibility should be further determined in clinical studies.

In summary, this study provides important functional evidence demonstrating a new physiological function of VR1 s for sensing myocardial ischemia. Also, we found that VR1 s contribute importantly to the action of bradykinin on ischemia-sensitive cardiac afferent nerves. Because cardiac spinal afferent nerves are essential for perception of chest pain, the cardiac VR1 s may function as a transduction molecule in the sensory detection of tissue ischemia. Therefore, these new findings are important to our understanding of the sensory mechanisms of cardiac pain caused by myocardial ischemia. Blocking of cardiac VR1 s may be an alternative intervention for treatment of refractory ischemic chest pain that cannot be relieved by conventional therapies, such as nitroglycerin and β-adrenergic receptor blockers.

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References

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