Myocardial Ischemia Recruits Mechanically Insensitive Cardiac Sympathetic Afferents in Cats

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Pan, Hui-Lin and Shao-Rui Chen. Myocardial ischemia recruits mechanically insensitive cardiac sympathetic afferents in cats. J Neurophysiol 87: 660–668, 2002; 10.1152/jn.00506.2001. Chest pain caused by myocardial ischemia is mediated by cardiac sympathetic afferents. Although silent nociceptors exist in somatic structures and some visceral organs, their presence in the heart remains uncertain. The present study examined the presence and the functional characteristics of mechanically insensitive cardiac sympathetic afferents using an electrical search technique. Single-unit activity of afferents innervating the left ventricle was recorded from the sympathetic chain in anesthetized cats. Cardiac afferents were identified initially with a stimulating electrode placed on the surface of the heart. Responses of cardiac afferents to mechanical stimuli, 5 min of myocardial ischemia, and topical application of bradykinin (1–10 μg/ml) and lactic acid (10–50 μg/ml) were then determined. Ischemia activated all 38 mechanically insensitive afferents and 17 of 25 mechanically sensitive afferents. The mechanically sensitive afferents typically were spontaneously active and had a smaller receptive field and a slightly faster conduction velocity. On the other hand, the mechanically insensitive afferents were slow conducting C fibers and had a large electrical receptive field on the epicardium. The response of 38 mechanically insensitive afferents to ischemia [2.83 ± 0.14 (SD) imp/s] was significantly greater than that of 17 mechanically sensitive afferents (from 0.41 ± 0.05 to 0.74 ± 0.15 imp/s). The mechanically insensitive afferents also exhibited a greater response to topical application of bradykinin or lactic acid in a concentration-dependent manner. This study provides important new evidence that the heart is innervated by silent sympathetic afferents, which are activated profoundly by myocardial ischemia. These data also suggest that the mechanically insensitive sympathetic afferents may function as cardiac nociceptors.

INTRODUCTION

Patients with myocardial ischemia typically experience chest pain (angina pectoris) (Cervero 1994; Perez-Gomez et al. 1979; White 1957; White et al. 1933). Sympathetic and vagal nerves innervating the heart contain not only autonomic efferent axons but also afferent fibers that transmit sensory signals generated by cardiac sensory receptors (Baker et al. 1980; Pal et al. 1989; Pan and Longhurst 1995; White 1957). Cardiac primary afferents running in the sympathetic nerves, especially finely myelinated Aδ- and unmyelinated C-fiber afferents, are considered to be the essential pathways for transmission of cardiac nociception to the CNS during myocardial ischemia (Cervero 1994; Foreman 1999; Meller and Gebhart 1992; Pan et al. 1999). Furthermore, activation of cardiac sympathetic afferents (i.e., cardiac spinal afferents) during ischemia is known to initiate cardiovascular reflexes, which lead to hemodynamic alterations and arrhythmias (Malliani et al. 1983; Webb et al. 1972). There is substantial evidence demonstrating that myocardial ischemia excites a subgroup of cardiac sympathetic afferents, namely, ischemically sensitive afferents, which transmit nociceptive information to the CNS to elicit cardiac nociception (Pal et al. 1989; Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). The central mechanisms of cardiac pain have been studied extensively (Ammons et al. 1985; Blair et al. 1982, 1984; Foreman 1999). However, it remains unclear about the encoding mechanisms by cardiac sensory receptors in eliciting nociception during myocardial ischemia.

An important issue related to afferent mechanisms of cardiac pain is the presence of silent afferents. Silent afferents (i.e., afferents exhibiting no spontaneous discharge activity and unresponsive to physiological stimuli) were described first in the knee joint of the cat and have been reported in the skin and pelvic visceral organs (Habler et al. 1990; Meyer and Campbell 1988; Schaible and Schmidt 1988). These silent afferents are considered to function as important nociceptors because they do not respond to physiological stimuli (Cervero 1994; Habler et al. 1990; Michaelis et al. 1996; Pan and Longhurst 1996). Studies of cardiac nociceptors have lagged behind other visceral afferents due to the technical difficulties of single-unit recording of sympathetic afferents from the beating heart. Although recruitment of silent afferent fibers during myocardial ischemia has not been appreciated, we have shown that only a subgroup of cardiac sympathetic afferents is sensitive to ischemia (Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). It is important to recognize that many cardiac sympathetic C-fiber afferents do not respond to ischemia (Pal et al. 1989; Pan and Longhurst 1995; Pan et al. 1999; Uchida and Murao 1974). Such differential responses of cardiac sympathetic afferents to myocardial ischemia strongly suggest that the heart is likely innervated by functionally heterogenous afferent nerves. There is still no substantial evidence demonstrating that the heart is indeed innervated by mechanically insensitive nociceptors. This issue is particularly important
because, unlike abdominal viscera, pain likely is the only sensory experience from the heart (Cervero 1994; Meller and Gebhart 1992; White et al. 1933). Therefore in the present study, we used an electrical search technique to examine specifically the presence and possible functional properties of mechanically insensitive cardiac sympathetic afferents.

**Methods**

**Anesthesia and surgical preparation**

Adult cats of either sex were anesthetized initially with ketamine (20–30 mg/kg im), and anesthesia was maintained with α-chloralose (50–60 mg/kg iv). Supplemental doses of α-chloralose (5–10 mg/kg) were given as necessary to maintain adequate depth of anesthesia, assessed by lack of nociceptive reflexes and fluctuation of blood pressure and heart rate. A femoral artery and vein were cannulated for measurement of blood pressure and administration of fluids and drugs, respectively. The trachea was intubated and respiration maintained artificially with an animal ventilator (model C1V-101, Columbus Instruments, Columbus, OH). The left carotid artery was cannulated with a PE-60 catheter, which was passed retrogradely into the left ventricle for monitoring the left ventricular pressure. Arterial blood pressure was measured with a pressure transducer (PT300, Grass Instruments, Quincy, MA). Arterial blood gases were analyzed with a radiometer blood gas analyzer and maintained within physiological limits (PO₂ >100 mmHg; PCO₂, 32–38 mmHg, pH 7.35–7.45) throughout the experiment. When necessary, arterial PO₂ was increased by enriching the inspired O₂ supply; pH was corrected by administering NaHCO₃ (1 M iv) and/or adjusting ventilation. Body temperature was maintained in the range of 36–38°C with a circulating-water heating pad and heat lamps. The experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals (US Public Health Service). Animals were killed at the end of experiments by an intravenous injection of overdose of pentobarbital sodium.

**Recording of cardiac sympathetic afferents**

A midline sternotomy was performed, and the first to seventh left ribs and the upper lobe of the left lung were removed. An occlusion cuff was placed around the descending thoracic aorta for cardiac distension (Pan and Longhurst 1995; Pan et al. 1999). The fascia overlaying the left paravertebral sympathetic chain from T₂ to T₆ was removed. An occlusion cuff was placed around the descending thoracic aorta for cardiac ischemia (Pan and Longhurst 1995; Pan et al. 1999). Therefore in the present study, the presence and possible functional properties of mechanically insensitive cardiac sympathetic afferents.

**Experimental protocols**

The nerve fibers of the sympathetic chain and rami communicantes were dissected sequentially into small filaments. The nerve filament then was placed on the recording electrode individually. When the nerve fiber was on the recording electrode, the epicardium was mapped gradually from the apex to the base of the heart using a bipolar stimulating electrode to electrically (5–10 V, 0.25–0.5 ms, and 0.5 Hz) search for the nerve endings of cardiac afferents. The stimulating electrode was connected to an isolation unit and a stimulator (S48, Grass Instruments). Once the action potential of an afferent fiber was evoked and isolated by further dissection, the conduction velocity and the size of the electrical receptive field were measured by gradually moving the stimulating electrode around the spot identified initially at a minimal stimulation intensity. Next, the afferent response to mechanical stimulation was tested to determine the mechanosensitivity of the afferent. Mechanical stimulation of cardiac afferents was performed by cardiac distension and application of a series of calibrated von Frey filaments (Stoelting, Wood Dale, IL) placed onto the receptive field of afferents, as we described previously (Pan et al. 1995).

Cardiac distension was performed by an increase in the left ventricular pressure to ~250 mmHg induced by a brief occlusion of the descending thoracic aorta (Pan and Longhurst 1995; Pan et al. 1999). To test the response of afferents to a more localized mechanical stimulus, a series of von Frey filaments (0.5–5.52 g) was applied perpendicular to the receptive field of the afferent with sufficient force to bend the filaments for 5–6 s. In the absence of a response, the filament of higher force was applied. In the presence of a response, the filament of next lower force was applied. The stimulus producing a 50% likelihood of response was determined using the “up-down” calculating method as described previously (Pan et al. 1995, 1998). Each trial was repeated two to three times at ~2–3 min intervals, and the mean value was used as the threshold force to produce afferent activation. Subsequently, the responses of a cardiac afferent to 5 min of myocardial ischemia, topical application of bradykinin (1, 5, and 10 μg/ml, Sigma Chemicals, St. Louis, MO) and lactic acid (10, 20, and 50 μg/ml, Sigma Chemicals) using a pledget were tested. Myocardial ischemia was induced by constricting the coronary vessel supplying the receptive field of cardiac ventricular afferents with a thread placed around the vessel. Under an operating microscope, ligatures were placed around the proximal left anterior descending or left circumflex coronary arteries with care taken not to disturb nerve fibers that course along the vessel. Placement of ligatures was performed after an afferent fiber with a receptive field was precisely located in the left ventricle. The ischemic region was verified visually by cyanosis on occlusion of the coronary artery. We have demonstrated that this procedure can be performed without injuring the afferent nerves because they do not strictly follow the large epicardial arteries (Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). Bradykinin and lactic acid were chosen because they are produced endogenously during ischemia (Pan et al. 1999, 2000) and can induce painful reactions when injected in animals (Guzman et al. 1962). Each substance applied to the epicardial surface was dissolved in 0.9% NaCl because this vehicle has no effect on cardiac afferents (Pan and Longhurst 1995; Pan et al. 1999). The receptive fields of the afferents were washed with normal saline after application of each chemical. Sufficient time (15–25 min) was allowed between applications to prevent tachyphylaxis (Pan and Longhurst 1995; Tjen-A-Looi et al. 1998).

In a few animals (n = 4), mechanical insensitive afferents were first identified accidentally during ischemia when the response of afferents with spontaneous discharges to ischemia was tested. In this case, the location of the receptive field of silent afferents in the ischemic area was searched with a stimulating electrode as described in the preceding text. After the size of the electrical receptive field and the conduction velocity of the afferent were measured, the responses of silent afferents to mechanical stimulation, 5 min of myocardial ischemia,
and exogenous bradykinin/lactic acid were determined. Additionally, we randomly recorded 25 mechanically sensitive cardiac afferents to compare their properties with those of silent cardiac afferents.

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. The 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 (Kuo et al. 1984; Pan and Longhurst 1995; Pan et al. 1999). C- and Aδ-fiber afferents were classified as those with a conduction velocity <2.5 and 2.5–30 m/s, respectively.

Data analysis

The discharge activity of afferents was averaged during a 5-min control period and 5 min of myocardial ischemia, respectively (Pan and Longhurst 1995; Pan et al. 1999). Afferents were considered to be ischemically sensitive if their discharge frequency during 5 min of myocardial ischemia was increased and sustained ≥50% above baseline activity. The response of afferents to bradykinin, lactic acid, or mechanical stimuli was measured by averaging the discharge rate during the entire period of response (Pan and Longhurst 1995; Pan et al. 1999). Comparisons between control and experimental interventions were made by either a paired Student’s t-test or repeated-measures ANOVA with Dunnett’s post hoc test. Differences were considered to be statistically significant when P < 0.05.

RESULTS

Only one afferent per animal was chosen to study in most cases. In four animals, two afferents were studied simultaneously because the amplitude and polarity of the action potential of two afferents were clearly distinguishable. The mean arterial blood pressure in all 59 animals studied was 87 ± 16 mmHg when the cardiac afferents were identified and remained constant during the experiments. The heart rate in these animals was 134 ± 12 beats/min before myocardial ischemia was induced. The overall success rate of recording single-unit activity of cardiac sympathetic afferents was 72% during the course of this study. Eight cats died of sustained ventricular fibrillation during myocardial ischemia or while searching the cardiac afferents by electrical stimulation.

A total of 38 mechanically insensitive and 25 mechanically sensitive cardiac sympathetic afferents were studied. The location and functional properties of these afferent nerve endings are shown in Table 1. The conduction velocity of 38 mechanically insensitive cardiac afferents was significantly slower than that of 25 mechanically sensitive cardiac afferents (0.52 ± 0.06 vs. 0.86 ± 0.10 m/s, P < 0.05). The size of the electrical receptive field of 38 mechanically insensitive cardiac afferents was larger than that of 25 mechanically sensitive cardiac afferents (27.2 ± 5.6 vs. 11.4 ± 3.2 mm², P < 0.05).

Mechanosensitivity and background discharges

Among 25 mechanically sensitive cardiac sympathetic afferents, 19 had background activity ranging from 0.3 to 2.2 imp/s (0.41 ± 0.05 imp/s). Both cardiac distension (threshold pressure = 157 ± 18 mmHg) and application of von Frey filaments (threshold force = 18.5 ± 3.9 g) to the receptive field of afferents increased significantly the discharge activity of these 25 afferents. Cardiac distension, at a level of 220–230 mmHg, stimulated these 25 afferents from 0.34 ± 0.03 to 0.68 ± 0.14 imp/s (P < 0.05). For those remaining six afferents without background activity, cardiac distension (threshold pressure = 162 ± 22 mmHg) and application of von Frey filaments (threshold force = 22.4 ± 4.3 g) to the receptive field activated these afferents. The threshold pressure and force required to activate these 6 afferents did not differ significantly from those 19 afferents with spontaneous activity. For 38 mechanically insensitive cardiac sympathetic afferents, neither maximal cardiac distension (252 ± 11 mmHg) nor application of von Frey filaments (35.2 g) stimulated these afferents. All of these 38 mechanically insensitive afferents had no background activity during the control period for ≥45 min.

Response to myocardial ischemia

All 38 mechanically insensitive afferents were activated (2.83 ± 0.14 imp/s) by 5 min of myocardial ischemia following a latency of 12.6 ± 2.5 s. Figure 1 is a representative tracing showing the responses of one mechanically insensitive to cardiac distension, application of von Frey filaments (5, 15, and 35.2 g), and 5 min of myocardial ischemia. On the other hand, only 17 of 25 (68%) mechanically sensitive afferents responded to 5 min of myocardial ischemia (from 0.38 ± 0.04 to 0.74 ± 0.15 imp/s, P < 0.05). The remaining eight mechanically sensitive afferents did not respond to 5 min of myocardial ischemia (from 0.49 ± 0.06 during control to 0.46 ± 0.07 imp/s during ischemia, P > 0.05). Figure 2 is the histogram showing the differential responses of one mechanically sensitive (F1, Fig 2A) and one mechanically insensitive (F2, Fig 2B) afferent to cardiac distension and 5 min of myocardial ischemia. These two afferents were recorded simultaneously, and the afferent nerve endings were both located in the region perfused by the left anterior descending artery (Fig. 2C). The responses of 38 mechanically insensitive afferents to 5 min of myocardial ischemia was significantly greater than that of 17 mechanically sensitive afferents (Fig. 3). Additionally, we tested the response of 14 mechanically insensitive afferents to cardiac distension (220–230 mmHg) 2 min after myocardial ischemia. We observed that all 14 afferents displayed a transient and weak response to cardiac distension (0.11 ± 0.02 imp/s) and such mechanosensitivity disappeared with 5 min following ischemia. None of the mechanically

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* P < 0.05 compared to mechanically sensitive afferents.
Insensitive afferents exhibited spontaneous discharge activity 2 min after reperfusion.

**Response to bradykinin/lactic acid**

Figure 4 summarizes the responses of mechanically sensitive and insensitive cardiac afferents to bradykinin and lactic acid topically applied to the receptive field of afferents. All the afferents tested responded to bradykinin and lactic acid. Bradykinin and lactic acid stimulated significantly both mechanically sensitive and mechanically insensitive afferents in a dose-dependent fashion. Compared to those mechanically sensitive afferents, the mechanically insensitive afferents exhibited a greater response to these two agents (Fig. 4).

**DISCUSSION**

In the present study, we used an electrical search technique to determine the existence and possible functions of mechanically insensitive sympathetic afferents innervating the heart. We found that a population of cardiac sympathetic afferents identified by electrical stimulation was unresponsive to mechanical stimulation but was activated vigorously during myocardial ischemia. Furthermore, compared with afferents sensitive to mechanical stimulation, the mechanically insensitive cardiac afferents had a slower conduction velocity, a larger electrical receptive field, and a greater response to myocardial ischemia and ischemic metabolites such as bradykinin and lactic acid. Thus our study provides substantial evidence that mechanically insensitive sympathetic afferents are present on the heart. These mechanically insensitive afferents are recruited during myocardial ischemia and likely play a role in the perception of cardiac pain.

Chest pain is one of the hallmarks of myocardial ischemia (Meller and Gebhart 1992; Perez-Gomez et al. 1979; White 1957). Although the mechanisms of chest pain caused by myocardial ischemia are complex and remain to be delineated, cardiac sympathetic afferent nerves are the essential pathways for initiation of this type of nociception. In this regard, removal of both stellate ganglia and excision of the first to the fifth thoracic sympathetic ganglia relieves cardiac pain in patients with ischemic heart disease (Meller and Gebhart 1992; White 1957). Occlusion of coronary arteries also produces severe pain and pseudoadverse reactions in dogs and cats that can be abolished by thoracic sympathectomy but not vagotomy (Brown 1967; White et al. 1933). Increased production of certain metabolites during myocardial ischemia has been proposed to contribute to excitation of primary cardiac sympathetic afferents (Abe et al. 1998; Pal et al. 1989; Pan and Longhurst 1995; Pan et al. 1999). It has been demonstrated that bradykinin and lactic acid contribute to activation of ischemically sensitive cardiac sympathetic afferents (Abe et al. 1998; Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). In human studies, Schaefer et al. (1996) found that intracoronary injection of bradykinin could not mimic angina pectoris, while Gasparone et al. (1999) have recently demonstrated that intracoronary infusion of bradykinin elicits cardiac pain similar to that experienced by the patients during ischemic episodes. Although some studies suggest that adenosine stimulates cardiac sympathetic afferents (Gnecchi-Ruscone et al. 1995; Huang et al. 1995), we have found that endogenously produced adenosine is not responsible for activation of cardiac sympathetic afferents during ischemia (Pan and Longhurst 1995).

One of the important questions of visceral sensory physiology concerns the peripheral encoding mechanisms used by visceral sensory receptors to discriminate different sensory modalities (Cervero 1994; Cervero and Janig 1992; Malliani 1993). There are two fundamentally different views regarding how cardiac sensory receptors encode nociceptive information (Cervero 1994; Cervero and Janig 1992; Malliani 1993; Mal...
liani and Lombardi 1982). One interpretation is based on the existence of nociceptors specifically activated by myocardial ischemia. The alternative view proposes a functionally homogenous population of nonspecific receptors in the viscera, and nociception is encoded simply in the discharge intensity of afferents. In recent years, accumulating evidence on the existence of silent afferents in some abdominal viscera provides strong support for the specificity theory (Cervero 1994; Habler et al. 1993; Pan and Longhurst 1996). However, there is no convincing evidence for the existence of mechanically insensitive cardiac sympathetic afferents. The presence of specific cardiac nociceptors is implied by the findings of Baker et al. (1980), who reported that a small population of receptors was not sensitive to light touch but was sensitive to bradykinin.

**FIG. 2.** Histograms showing the discharge activity of one mechanically sensitive (F1) and one mechanically insensitive (F2) afferent recorded simultaneously during 5 min of control and 5 min of myocardial ischemia. The nerve endings of both afferents were located in the region perfused by the left anterior descending coronary artery. The conduction velocity of the afferents was 0.86 (F1) and 0.25 (F2) m/s. A and B are representative tracings taken from original recordings as indicated in F1 and F2 (F2 afferent had a more negative polarity and amplitude that can be discriminated from the F1 afferent). Note that the afferent (F2) insensitive to cardiac distension had no background activity (A) and was activated only during myocardial ischemia (B). C: a ventral view of the heart showing the location and size of the electrical receptive field (circled areas pointed to by the arrows) of 2 afferents.
Unfortunately, the response of these cardiac afferents to ischemia was not examined, and thus their potential function cannot be determined. Also, these reported silent afferents either have a low background discharge or have a weak response to mechanical stimulation (Baker et al. 1980). It has been argued that these afferents are not nociceptors because nociceptors should not possess any background activity (Malliani and Lombardi 1982; Malliani et al. 1983). Huang et al. (1996) reported that five silent cardiac afferent neurons respond to ischemia, but the detailed mechanosensitivity of these neurons and the methods for the precise location of the nerve endings of these silent neurons are not described. Furthermore, the existence of specific cardiac nociceptors is not supported by a previous study in which Lombardi et al. failed to find any silent afferents by electrical stimulation of the left inferior cardiac nerve (Lombardi et al. 1981). The potential problem with this study is that the inferior cardiac nerve is not the only nerve containing cardiac sympathetic afferents. Also, because only a small portion of the nerve was studied, the authors did not determine whether the electrical stimuli applied to the nerve trunk were sufficient to activate all afferent fibers inside the inferior cardiac nerve (Lombardi et al. 1981). In this regard,
we recently have found that electrical stimulation of afferent fibers located in the center of the nerve requires a much higher intensity (Pan et al. 1996).

Failure to document the presence of mechanically insensitive afferent nerves innervating the heart could be due to, at least in part, the search techniques used for identifying cardiac afferents in previous studies. Maneuvers such as mechanical probing or increasing the left ventricular pressure are often used to search for cardiac afferents (Baker et al. 1980; Casati et al. 1979; Pal et al. 1989; Pan and Longhurst 1995; Uchida and Murao 1974). As a result, a biased sample of the cardiac afferent population (i.e., mechanosensitive afferents) may be studied. Bradykinin is capable of stimulating silent afferents and has been used in previous studies as a tool to identify the location of cardiac afferents (Baker et al. 1980). Because bradykinin can sensitize afferents, which may change the intrinsic functional properties and the responsiveness, it is not suitable to be used as an initial search stimulus. Electrical stimulation of the receptive field of afferents has been utilized to search the silent nociceptive afferents in the skin (Meyer and Campbell 1988). In the present study, we adopted an electrical search technique used by Meyer et al. to identify mechanically insensitive cutaneous afferents (Meyer and Campbell 1988). We have observed that direct electrical stimulation of the receptive field of afferents is the most accurate and reliable means to locate afferent nerve endings on the beating heart (Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). Furthermore, this mapping technique does not sensitize or alter the intrinsic response of afferents to subsequently applied stimuli (Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). Using this technique, we found that many cardiac sympathetic afferents had no background activity and did not respond to mechanical stimuli imposed by cardiac distension and application of von Frey filaments. These mechanically insensitive afferents, however, were activated vigorously during myocardial ischemia. Therefore these data strongly suggest that silent sympathetic afferents indeed exist on the heart. We have shown repeatedly that cardiac sympathetic afferents are activated within a few min after complete occlusion of the coronary artery (Pan and Longhurst 1995; Pan et al. 1999; Tjen-A-Looi et al. 1998). This is likely due to a much higher basal metabolic rate of the myocardium, which may lead to a rapid accumulation of ischemic metabolites following complete occlusion of the coronary artery. This current study has provided new evidence for the potential function of silent cardiac afferents, which have not been clearly documented in previous studies.

We have shown previously that gastrointestinal sympathetic afferents sensitive to ischemia likely function as visceral nociceptors due to their unique capability to encode ischemia and noxious distension (Pan and Longhurst 1996). We and others also have demonstrated that similar to abdominal afferents, only a subgroup of cardiac sympathetic afferents responds to ischemia (Pal et al. 1989; Pan and Longhurst 1995; Pan et al. 1999; Uchida and Murao 1974). Many cardiac sympathetic C-fiber afferents, however, are not responsive to ischemia (Pal et al. 1989; Pan and Longhurst 1995; Pan et al. 1999). This differential response to myocardial ischemia implies that sympathetic afferents innervating the heart are not functionally homogenous in encoding nociceptive stimuli. As we demonstrated in the present study, manual manipulation of the heart or cardiac distension is unlikely an adequate mechanical stimulus for many ischemically sensitive cardiac afferents. Additionally, we found that mechanically insensitive cardiac afferents had a very slow conduction velocity and a large electrical receptive field. The electrical receptive field may reflect the size of arborization of the afferent nerve endings on the heart, which appears to be much larger than that of cutaneous afferents (Meyer and Campbell 1988). The functional properties of these cardiac mechanically insensitive afferents are consistent with the nociceptive function of visceral afferents located in other organs (Habler et al. 1990; Pan and Longhurst 1996). Our data suggest that recruitment of silent cardiac nociceptors could play an important role in the cardiac pain by providing high-order neurons with discriminative information about the location, intensity, and duration of the ischemic stimulus. Therefore activation of these silent nociceptors may generate an additional source of nociceptive input to the CNS during myocardial ischemia (Chandler et al. 1998).

It has been well documented that mechanical stimulation of the heart cannot evoke any sensation of discomfort (Cervero 1994; Ness and Gebhart 1990; White 1957). Thus mechanical stimulation is not an adequate stimulus for cardiac nociceptors. The most commonly held view is that cardiac pain is produced by myocardial ischemia. Because chemical/metabolic factors are mainly responsible for activation of ischemically sensitive cardiac afferents (Pan et al. 1999; Tjen-A-Looi et al. 1998), we further determined the chemosensitivity of mechanically sensitive and insensitive cardiac afferents. Two well-known ischemic metabolites, bradykinin and lactic acid, were used because they both contribute to stimulation of cardiac sympathetic afferents during ischemia (Pan et al. 1999; Tjen-A-Looi et al. 1998). We observed that epicardial application of bradykinin and lactic acid activated mechanically insensitive cardiac afferents. This finding is consistent with our previous findings that these two ischemic metabolites play an important role in activation of cardiac sympathetic afferents during myocardial ischemia (Pan et al. 1999; Tjen-A-Looi et al. 1998). The fact that these mechanically insensitive cardiac afferents respond to both ischemia and ischemic metabolites, bradykinin and lactic acid, applied to their receptive fields suggests that these afferents likely function as cardiac nociceptors. We found that although mechanically insensitive afferents exhibited a greater response to these two chemicals at a given concentration, mechanically sensitive afferents also responded to these two chemicals in the concentration range tested in this study. Lack of specificity of the effect of exogenous bradykinin on ischemically sensitive and insensitive afferents has been observed in the gastrointestinal tract (Pan and Longhurst 1996). Unlike myocardial ischemia, inflammatory processes in the myocardium such as myocarditis do not evoke typical cardiac pain. The reasons for this discrepancy are not clear at the present time.

Limitations of the study

The mechanically insensitive cardiac afferents we studied are consistent with two key features of silent visceral afferents (Cervero 1994): lack of spontaneous activity and normally displaying no mechanosensitivity. Our speculation that silent cardiac afferents may function as nociceptors is based on their preferential and vigorous responses to myocardial ischemia.
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The putative nociceptive function of silent cardiac afferents remains to be further validated in the future studies. Also, it is important to acknowledge that we did not fully explore all the aspect of silent cardiac afferents. For example, sensitization is an important feature of visceral nociceptors. We observed that some silent cardiac afferents displayed a transient response to cardiac distension following ischemia, suggesting that these afferents are sensitized during ischemia. Further studies are warranted to determine changes of the properties of receptive fields and responses of these afferents to other ischemic metabolites. Additionally, it also needs to be determined to what extent cardiac pain can be exclusively attributed to activation of silent cardiac afferents during myocardial ischemia.

In summary, the present study provides definitive evidence that the heart is innervated by a population of silent sympathetic afferents that are insensitive to mechanical stimulation. These silent cardiac afferents could represent a novel population of cardiac sensory receptors and may function as nociceptors (Cervero 1995; Cervero and Janig 1992; Michaelis et al. 1996). These findings are important prerequisites for the understanding of the sensory encoding mechanisms of cardiac pain in patients with myocardial ischemia.

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