Afferent pathways for cardiac-somatic motor reflexes in rats

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Referred pain of angina pectoris is a classic symptom of cardiac disease. The convergence-projection theory of referred pain, which proposes that cardiac and somatic afferent fibers have synaptic connections on the same spinothalamic tract (STT) cells, is accepted as the primary mechanism for explaining cardiac referred pain (11, 27). The theory is based on the concept that the normal source of nociceptive input to these STT neurons arises from overlying injured somatic structures, including muscle. However, ischemic episodes associated with coronary artery disease activate visceral nociceptive afferents from the heart and excite the same neurons that receive somatic inputs. Because pain perception from somatic structures has already been “learned,” the nociceptive input originating from the viscera is referred to overlying somatic dermatomes. However, if nociceptive chemical stimuli are injected into thoracic muscles of patients who have experienced angina pectoris, then this nociceptive stimulus generates angina-like referred pain that is indistinguishable from angina of cardiac origin (15). This leads to a refinement of the original idea of referred pain to the concept that nociceptive stimulation of cardiac receptors activates reflexes that excite muscle in somatic structures overlying the heart. Cardiac pain, therefore, may include a strong muscular component that is not based on the STT convergence mechanism.

The muscular component of cardiac pain was investigated using a rodent model in which chemical activation of cardiac afferents was used to generate spasm-like electromyographic (EMG) activities (14). The objectives of this study were to utilize this model to identify the afferent pathways of the cardiac motor reflexes and to investigate the functions of the various afferent pathway(s) as related to cardiac nociception.

Sympathetic cardiac afferents (SCA) and vagal cardiac afferents (VCA) are two chemically sensitive pathways that transmit nociceptive information (4, 17, 23). Electrophysiological studies (2) show that these pathways cause opposing effects on thoracic STT cells. In general, activation of SCA or VCA excites or suppresses STT cell activity, respectively. On the basis of these observations, we hypothesized that chemical stimulation would generate spinotrapezius EMG activities via SCA and activation of VCA would inhibit the chemically evoked EMG activities. Conversely, transection of SCA or VCA would be expected to produce effects opposite to those obtained by stimulation.

Results showed that electrical excitation of SCA produced spinotrapezius EMG activities similar to that evoked by chemical stimulation of cardiac afferents: activities were reduced or eliminated by transection of these afferents. In contrast, excitation of VCA inhibited the chemically evoked EMG activities. This model suggests that SCA activation might generate muscle hyperalgesia, whereas VCA might counterbalance overstimulation by the SCA.
METHODS AND MATERIALS

Surgery

Experiments were performed in male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 275–500 g. The experiments were approved by the Institutional Animal Care and Use Committee and followed guidelines of the American Physiological Society and International Association for the Study of Pain. Animals were anesthetized initially with pentobarbital sodium (45–55 mg/kg ip). The carotid artery was cannulated to record blood pressure, and the jugular vein was cannulated to supply a continuous infusion of pentobarbital sodium (10–15 mg·kg⁻¹·h⁻¹). After tracheotomy, a small tube was inserted into the trachea, and the animal was artificially ventilated (55–60 strokes/min, 5.0–6.0 ml tidal volume). Arterial blood pressure and pupil diameters were monitored to determine the level of anesthesia. Core body temperature of the animal was maintained between 36 and 38°C.

A silicone rubber catheter (0.020 ID, 0.037 OD, 14–16 cm long) with multiple small holes at the distal 2 cm was inserted into the lobes of the thymus and into the pericardial sac (10). Bipolar hook electrodes (0.050 ID, 0.071 OD, single stainless steel wire insulated with Teflon, except for 0.5 mm at the tip) were inserted bilaterally into the spinotrapezius muscles to measure EMG activities. Electrodes were inserted at an angle of ~30° and a depth of 1.5–1.7 mm at the junction between the latissimus dorsi and rhomboid thoracic muscles.

The left stellate ganglion and sympathetic chain were exposed through the second left intercostal space after a high midline thoracotomy. Bipolar platinum hook electrodes were placed on the chain just caudal to the ganglion. To isolate the cervical vagus nerves, the cervical vagosympathetic trunk was exposed in the neck. Bipolar platinum hook electrodes were placed on each cervical vagus nerve and fixed in position with dental impression material (Reprosil).

EMG signals were amplified by Gould Universal preamplifiers and a Neuro-Corder (model DR-890, Neuro Data Instrument) and then band-pass filtered at 100–3,000 Hz. Processed signals were collected by a CED 1401 data acquisition system sampling at 5,000 Hz and analyzed using Spike 2 software (Cambridge Electronics Design).

Experimental Protocols

Three different techniques were used to study the afferent pathways: chemical stimulation of different cardiac afferents, electrical stimulation, and transection of cardiac afferents. Results generated from the three techniques were used to complement each other, and conclusions were drawn based on all three experiments. The experimental protocol specific to each technique includes the general protocol, which uses a chemical stimulus to evoke EMG activities.

General protocol. Experiments were conducted with the animal in the prone position. The experimental protocol was as follows. 1) Baseline EMG was recorded for 45–60 s without cardiac stimulation. 2) Warm saline (0.2 ml) was infused into the pericardial sac and then withdrawn after 60–65 s. 3) The solution containing the chemical stimulus (0.2 ml) was infused into the pericardial sac and withdrawn after 60–65 s. 4) After the chemical infusion, warm saline was infused and then withdrawn to remove any remaining chemicals within the sac. 5) Pat pads covering the latissimus dorsi muscle were pinched with forceps. Pinching the fat pad activates the somatomotor reflex between the fat pad and the spinotrapezius. This was used to test the viability and the responsive-

ness of the spinotrapezius. A recovery period of 40–45 min elapsed between each protocol to avoid chemical tachyphylaxis.

Chemical differentiation protocol. The general protocol was performed with two different chemical stimuli to activate the cardiac afferent fibers. Cardiac afferent fibers were activated chemically with 10⁻⁵ M bradykinin solution or a nonspecific algogenic chemical mixture containing bradykinin, serotonin, prostaglandin E₂, histamine, and adenosine. Drugs were dissolved in normal saline to 10⁻³ M stock solution and diluted with additional saline to 10⁻⁵ M (except for adenosine, which was diluted to 10⁻³ M) to form the algogenic mixture.

Many studies have demonstrated that chemicals in the algogenic mixture show preferential activation of SCA or VCA. For example, bradykinin preferentially activates SCA over VCA to affect STT cell activity (1, 5) and cardiovascular reflexes (22), while adenosine (8), serotonin (3, 24), and prostaglandin E₂ (13) show preference toward VCA over SCA in similar types of cardiovascular reflex studies. Bradykinin, therefore, provides greater SCA input than the algogenic mixture, while the algogenic mixture introduces stronger VCA input than bradykinin. In the chemical differentiation experiments, roles of SCA and VCA were inferred based on these differences.

Electrical stimulation protocols. Left sympathetic chain. Before electrical stimulation, the general protocol with the algogenic mixture was performed to identify EMG-positive loci on the spinotrapezius. After recovery from the chemical stimulus, the left sympathetic chain was stimulated from 2.0 to 5.0 V at 5 Hz and 0.2 ms (n = 7; Grass S88 stimulator). Parameters of the stimulus were determined on the basis of observation of an evoked motor unit potential (MUP) after each stimulus artifact.

Cervical vagus nerves. Electrical stimulation of vagal afferents (VAS) consisted of two protocols. VAS-1 was direct electrical stimulation of the left or right cervical vagus nerve during baseline activities, without any intrapericardial infusion of saline or chemicals. In VAS-2, the general protocol utilizing the algogenic mixture to obtain the chemically evoked EMG response was conducted first. After recovery, the same general protocol was repeated, but in this case, one vagus nerve was electrically stimulated 20 s before the chemical infusion for a total stimulation time of 45 s. Stimulus parameters of VAS-1 and VAS-2 were 5.0 Hz and 0.2 ms, with stimulus intensity varying between 2.0 and 5.0 V. These parameters were based on other VAS studies that showed facilitation or inhibition of spinal neuron activity (1, 2, 26). The same group of animals was tested with both protocols (n = 7).

Nerve transection protocols. Left sympathetic chain. Before the nerve was transected, the general protocol with the algogenic mixture was conducted to obtain the chemically evoked EMG response (n = 15 animals). A recovery period of 60 min followed the transection. The general protocol was repeated 60 (P-60) and 120 (P-120) min after the transection to obtain the chemically evoked response with reduced sympathetic innervation.

Cervical vagus nerves. The general protocol with the algogenic mixture was performed to obtain a chemically evoked response with intact vagus nerves. Then bilateral vagal transection was performed (n = 16 animals). The same general protocol was repeated 60 min (P-60) after elimination of vagal input.
Pharmacological Blockade of the Cardiac Efferents

Before the blocking agents were administered, the general protocol with the algogenic mixture was conducted to characterize the EMG responses (control). A bolus injection (0.5–0.6 ml) of hexamethonium bromide (10 mg/kg; Sigma Chemical, St. Louis) or atropine sulfate (0.5 mg/kg; Radix Labs) was administered via the jugular vein over 60 s \((n = 6\) animals). The general protocol with the algogenic mixture was performed 10–15 min after an injection of the antagonist to determine effects on chemically evoked EMG activity. After ganglionic or parasympathetic blockade, heart rate and blood pressure were monitored to ascertain efficacy of each antagonist.

Data Analysis

Raw EMG activities were analyzed with the CED-Spike 2 data acquisition system. Determination of an EMG-positive response included an initial screening of the integrated EMG calculated as the cumulative sum of the moving average (50-ms intervals) of the full-wave-rectified raw signal during the chemical infusion/withdrawal period \((~60 \text{ s})\). An increase of activity >10% of baseline activity was considered a positive response.

After the identification of a positive response with integrated EMG, raw EMG activities of a positive response were reanalyzed to obtain the total number of MUP discharges (TMUP) via rate histograms \((\text{bin} = 1 \text{ s})\). Onset and termination of an evoked response were determined when discharge rate increased above or declined below 5 impulses/s, respectively. Signals of EMG, electrocardiogram, and stimulus artifacts were identified with Spike 2 software.

With the use of rate histograms, evoked motor responses were further characterized as follows: 1) latency of response, 2) peak discharge rate, 3) time from onset to peak discharge rate, and 4) duration of response.

Values are means ± SE. Effects of the chemical stimulation by bradykinin or algogenic mixture, electrical stimulation, nerve transection, and pharmacological blockade of autonomic efferent activities on the evoked EMG response \((\text{TMUP})\) were analyzed by ANOVA with repeated measures. Data of left sympathetic chain transection experiments were analyzed by ANOVA with repeated measures followed by Tukey’s comparison test. Statistical significance was established as \(P < 0.05\).

### RESULTS

**Chemical Differentiation**

The general protocol utilizing the algogenic mixture or bradykinin was conducted in 53 and 49 animals, respectively. Intrapericardial infusion of the algogenic mixture or bradykinin evoked increased EMG activities \((\text{TMUP})\) in the spinotrapezius muscles. Rates of positive responses to the algogenic mixture or bradykinin infusion were 71.6% \((38\) of 53 animals) and 67.3% \((33\) of 49 animals), respectively. Because EMG electrodes were implanted bilaterally, 76 \((\text{from 38 animals})\) and 66 \((\text{from 33 animals})\) spinotrapezius muscles were monitored during infusion of the algogenic mixture and bradykinin, respectively. Of these spinotrapezius muscles, infusion of the algogenic mixture or bradykinin evoked positive responses in 53 of 76 and 46 of 66 spinotrapezius muscles \((\text{EMG-positive spinotrapezius})\), respectively, because some animals responded unilaterally and others bilaterally. The algogenic mixture evoked unilateral and bilateral responses in 23 and 15 animals, respectively. Bradykinin evoked unilateral and bilateral responses in 20 and 13 animals, respectively.

Characteristics of the evoked response, namely, latency of response, peak discharge rate, time from onset to peak discharge rate, duration of response, and TMUP between the algogenic mixture and bradykinin, were compared \((\text{Fig. 1, Table 1})\). Analyses showed significant differences in every characteristic, except time from onset to peak discharge rate, of the evoked response between the two stimuli. Preferential activation of left or right spinotrapezius by the algogenic mixture or bradykinin was not observed.

**Neural Afferent Differentiation**

**Sympathetic cardiac afferents.** Electrical stimulation of the left sympathetic chain evoked EMG activities in every animal tested \((n = 7; \text{Fig. 2})\). Increased EMG activities were detected in all the left spinotrapezius preparations \((n = 7)\), but only two of seven right spinotrapezius preparations.

Transections of sympathetic afferents were performed in 15 animals, and the effects were determined from 22 EMG-positive spinotrapezius responses \((\text{Fig. 3A})\). The evoked responses were not completely abol-

### Table 1. Characteristics of EMG response in spinotrapezius muscles of rat evoked by algogenic mixture and bradykinin

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<thead>
<tr>
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<th>Allogenic Mixture ((n = 53))</th>
<th>Bradykinin ((n = 46))</th>
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<tr>
<td>Latency, s</td>
<td>(10.1 ± 1.1^*)</td>
<td>(7.0 ± 0.4)</td>
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<tr>
<td>Peak response, impulses/s</td>
<td>(33.7 ± 2.3^*)</td>
<td>(51.7 ± 3.6)</td>
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<tr>
<td>Time to peak response, s</td>
<td>(13.8 ± 1.4)</td>
<td>(11.5 ± 0.9)</td>
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<tr>
<td>Duration, s</td>
<td>(118.8 ± 12.2^*)</td>
<td>(64.4 ± 6.7)</td>
</tr>
<tr>
<td>TMUP, impulses</td>
<td>(2,195 ± 307^*)</td>
<td>(1,411 ± 106)</td>
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Values are means ± SE. TMUP, total motor unit potentials. *\(P < 0.05\) with respect to bradykinin \((\text{by repeated ANOVA})\).
ished by the transection; however, the evoked TMUP were significantly reduced at P-60 and P-120.

**Vagal cardiac afferents.** Although electrical stimulation of the left or right vagus nerves did not generate any EMG activities (n = 7 animals; Fig. 4A), VAS inhibited the chemically evoked EMG activities of every animal tested (n = 12 animals; Fig. 4B). In most trials of VAS (16 of 17), stimulating the left or right cervical vagus nerve caused >99% inhibition of the chemically evoked responses during VAS (23 of 25). The effective periods of vagal inhibition were variable. Most evoked responses (14 of 23) returned to >75% of control activities within 40 min after termination of VAS, while 3 of 23 responses recovered in 10–15 s and 6 of 23 responses never returned to >75% of control activities.

Analyses of responses of left and right spinotrapezius evoked by the algogenic mixture showed that EMG activities (TMUP) increased significantly after bilateral vagal transection (Fig. 3B). Peak discharge rate and duration of response of the evoked response also were significantly affected by vagotomy (Fig. 5, Table 2).

Pharmacological blockade of cardiac efferent activity by hexamethonium (n = 6) or atropine (n = 6) did not significantly affect the chemically evoked EMG activities.

**DISCUSSION**

The primary explanation for cardiac pain has been the convergence-projection theory of somatic-cardiac afferent input onto STT cells (27). Work by Kellgren (15) and recent results from our laboratory (14) suggest that muscle contractions produced during cardiac dysfunctions can be another source of cardiac pain. In our
proposed model of muscle-related referred pain, the viscerally evoked muscle contractions increase muscle afferent activity, which converges on neurons also receiving input from cardiac afferent fibers, to generate angina-like referred pain. This is not surprising, since the qualities of angina pectoris (19, 30) are more similar to muscle pain than to cutaneous pain.

The observation of bilateral EMG activities evoked by electrical stimulation of SCA was the primary evidence for a direct excitatory role of SCA in the cardiac motor reflexes. Results of the other SCA experiments provided additional support for the excitatory role of SCA. For example, one of the factors that contributed to the differences between the evoked responses could be the differential chemical activation of cardiac afferents. A significantly higher peak discharge rate was evoked by infusion of bradykinin than by the algogenic mixture (Fig. 1, Table 1). The elevated peak discharge rate could have occurred because bradykinin is a chemical stimulus that preferentially activates SCA (2, 5).

The major decrease in total evoked EMG activities during the subsequent unilateral sympathectomy is also consistent with an excitatory role for SCA.

In the analyses presented above, the excitatory role of SCA in the motor reflexes was initially inferred from the differences of peak discharge rate between the two chemical stimuli and then confirmed by the electrical stimulation and transection experiments. A limitation of the chemical stimulation experiments was that inferences needed to be further verified by other techniques, because one of the stimuli, namely, the algogenic mixture, consisted of several chemicals with a variety of properties that could influence the evoked response and complicate the analyses. The complications were observed during the analyses of TMUP and duration of response. For example, the experiments using bradykinin showed smaller TMUP and shorter duration of response than effects of stimulation by the chemical mixture. These differences complicated our conclusion of the excitatory role of SCA, which is based on the assumption that bradykinin preferentially activates SCA. However, review of the literature suggested that factors such as the sensitizing effect of prostaglandin E2 on activation of sympathetic afferents by bradykinin (28) in the algogenic mixture, which is unrelated to preferential afferent activation, might explain the discrepancy. Possible sensitization of prostaglandin E2 is an important reminder that activation of cardiac afferents during angina pectoris has a multiligand character, where different degrees of sensitization and activation of cardiac afferents could have clinical manifestations that range from intractable angina to silent myocardial ischemia.

In contrast to the SCA experiments, an important observation of VAS was that it did not activate the paraspinal EMG activity, nor did it change ongoing EMG activity, because the muscle remained quiescent with afferent stimulations. However, VAS did produce effects, because evoked EMG activities were suppressed during and after the stimulation. We also noted that vagal transection increased EMG responses compared with the EMG response with the intact vagal pathways. This result, coupled with the observation that the algogenic mixture evoked smaller peak responses than bradykinin (Fig. 1, Table 1), suggested that the vagal afferent signals caused a tonic suppression of the EMG activities, but that effect was only revealed when a major response was evoked with noxious cardiac stimulation. This result might occur because less vagal suppression was elicited by bradykinin than by the algogenic mixture.

### Functional Importance of SCA in the Cardiac Motor Reflexes

Activation of SCA to evoke spinotrapezius contractions could have functional relevance as a type of primitive reflex. For example, the mechanism of SCA in cardiac motor reflexes is similar to contractions of the rectus abdominis generated by electrical stimulation of lower thoracic sympathetic afferents (9), which was suggested by some investigators to be a type of protective reflex. Therefore, tightening the chest wall around the cardiac region during cardiac insult might be another type of primitive reflex used to help protect the heart.

### Functional Importance of VCA in the Cardiac Motor Reflexes

Inhibition of spasmlike muscle contractions by VAS indicated that VCA could play an important antinoci-

### Table 2. Characteristics of EMG response in spinotrapezius muscles of rat before and after bilateral vagotomy

<table>
<thead>
<tr>
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<th>Before Vagotomy</th>
<th>After Vagotomy</th>
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<tbody>
<tr>
<td>Latency, s</td>
<td>8.0 ± 0.9</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td>Peak response, impulses/s</td>
<td>34.0 ± 3.8*</td>
<td>39.9 ± 3.7</td>
</tr>
<tr>
<td>Time to peak response, s</td>
<td>17.6 ± 2.8</td>
<td>20.6 ± 2.8</td>
</tr>
<tr>
<td>Duration, s</td>
<td>97.5 ± 10.2*</td>
<td>150.9 ± 12.0</td>
</tr>
<tr>
<td>TMUP, impulses</td>
<td>2,039 ± 372*</td>
<td>3,408 ± 487</td>
</tr>
</tbody>
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Values are means ± SE; n = 18. EMG, electromyographic. *P < 0.05 with respect to after transection (by repeated ANOVA).

*Fig. 5. EMG activities evoked by algogenic mixture before (●) and after (○) bilateral cervical vagal transection. Time error bars for latency of response, time from onset to peak discharge rate, and duration of response are not shown but are listed in Table 2.*
ceptive role in muscle hyperalgesia. During an ischemic episode, pain of muscle spasms could function as a warning about a dysfunctional heart. However, if vagal attenuation (modulation) of muscle contractions were not present in the cardiac motor reflexes, patients might experience intense pain. Thus, instead of a warning signal, the intense pain could exacerbate symptoms, thereby creating a vicious cycle that resulted in chronic muscle pain in some patients (29). Other studies show that vagal modulation of pain via inhibition of noxious motor reflexes is a generalized phenomenon (7, 25). Consequently, excitation of vagal afferents might be a means to treat clinical pain with a muscular component (see Roles of Cardiac Afferents in Muscle Hyperalgesia).

Another benefit gained from the inhibition of the muscle spasms by VAS might be maintenance of homeostasis. Some investigators have suggested (12) that vagal inhibition of motor reflexes might prevent excessive metabolic demand by the skeletal muscles. Consequently, the workload to the heart is decreased, thereby restoring cardiac function and achieving homeostasis.

Roles of Cardiac Afferents in Muscle Hyperalgesia

We demonstrated in an earlier study that muscle contractions generated by chemical activation of cardiac receptors have characteristics of muscle spasms (14). In accordance with reports of pain relief by sympathectomy (21, 30) and vagal nerve stimulation (6), our investigation showed that SCA might play a critical role in the generation of muscle hyperalgesia, and VCA could have physiological importance in the modulation of cardiac pain. This study showed that hyperexcitation of SCA can generate spasmlike muscle contractions, and decreased activation of VCA can augment muscle contraction. The evoked muscle contractions would provide the necessary nociceptive input to muscle afferents to induce angina-like referred pain. Therefore, the mechanism of strong excitation of SCA and/or decreased input of VCA, in addition to the convergence-projection theory of STT, could potentially explain cardiac referred pain. Furthermore, this mechanism might also be responsible for the increased paraspinal muscle tone found in patients with chronic cardiac diseases (18).

Ultimately, maintenance of cardiac afferent balance could be a useful strategy for treating the muscular component of cardiac pain. In fact, therapeutic successes of VAS for attenuation of cardiac pain, prevention of ischemia episodes (31), and relief of pain during epileptic seizure (16) have been documented. The insights into the muscular mechanism of cardiac pain should improve efficacy of treatments in cardiac patients.

Conclusion

The roles of SCA and VCA in a cardiac-evoked muscle hyperalgesia model were investigated. Results showed that somatic contractions were produced by excitation of SCA, and increased activation of VCA could inhibit the contractions. An imbalance between these two groups of afferents arising from the heart might be responsible for symptoms such as muscle hyperalgesia associated with angina pectoris. Our investigation of the functions of cardiac afferents in cardiac-evoked muscle hyperalgesia provides the first potential mechanistic basis to understand the somatic muscle pain experienced during angina pectoris.

Perspectives

Patients with refractory angina pectoris are unresponsive to conventional treatments such as sympathectomy or dorsal rhizotomy. However, human studies have shown that this pain can be treated effectively with spinal cord stimulation (SCS) (20). The most generally accepted explanation for the effects for SCS on angina is based on the gate control theory. Some investigators have suggested that SCS activates large fibers of the dorsal column, which in turn activate inhibitory interneurons. These inhibitory interneurons modulate spinal neuronal processing of small fiber input resulting from noxious stimulation of the heart (20). On the basis of the results of the present study, an alternative hypothesis is that SCS activates spinal inhibitory interneurons. These inhibitory interneurons suppress the visceromotor referred pain leading to muscle hyperalgesia. Thus SCS may provide a “calming” influence on noxious sympathetic afferent input at the spinal cord level and reestablish the balance between the sympathetic and parasympathetic cardiac afferents. This would alleviate the muscular component of refractory angina pectoris.


