Coronary Heart Disease

Muscle Mechanoreflex and Metaboreflex Responses After Myocardial Infarction in Rats

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Background—During exercise, the sympathetic nervous system is activated and blood pressure and heart rate increase. In heart failure (HF), the muscle metaboreceptor contribution to sympathetic outflow is attenuated and the mechanoreceptor contribution is accentuated. Previous studies suggest that (1) capsaicin stimulates muscle metabosensitive vanilloid receptor subtype 1 (VR1), inducing a neurally mediated pressor response, and (2) activation of ATP-sensitive P2X receptors enhances the pressor response seen when muscle mechanoreceptors are engaged by muscle stretch. Thus, we hypothesized that the pressor response to VR1 stimulation would be smaller and the sensitizing effects of P2X stimulation greater in rats with HF due to chronic myocardial infarction (MI) than in controls.

Methods and Results—Eight to 14 weeks after coronary ligation, rats with infarcts >35% had an increased left ventricular end-diastolic pressure and a marked increase in heart weight. Capsaicin injected into the arterial supply of the hindlimb increased blood pressure by 39% (baseline, 93.9±9.5 mm Hg) in control animals but only by 8% (baseline, 94.8±10.1 mm Hg) in rats with large MIs (P<0.05). P2X receptor stimulation by α,β-methylene ATP enhanced the pressor response to muscle stretch by 42% in control animals and by 72% in rats with large MIs (P<0.05).

Conclusions—Compared with control animals, cardiovascular responses to VR1 stimulation are blunted and P2X-mediated responses are augmented in rats with HF owing to large MIs. (Circulation. 2004;110:3049-3054.)

Key Words: afferent ● reflex ● exercise ● blood pressure ● heart failure

The sympathetic nervous system (SNS) is activated during exercise. This leads to increases in blood pressure, heart rate (HR), myocardial contractility, and vascular tone in inactive beds.1,2 Two basic mechanisms contribute to these exercise responses. The first, termed “central command,” suggests that motor and SNS activations occur in parallel.5–7 The second, termed the “exercise pressor reflex,” suggests that afferents in contracting skeletal muscle are engaged and an autonomic reflex is initiated.8–11 This system responds to metabolic stimulation (ie, “metaboreceptor” stimulation) as well as to mechanical deformation of the muscle afferent receptive field (ie, “mechanoreceptor” stimulation).10 Group IV afferents are thought to be predominantly metabosensitive afferents, and group III afferents are thought to be predominantly mechanically sensitive.12 When these receptors are stimulated, thin-fiber muscle afferent nerves are engaged, cardiovascular nuclei in the brain stem are activated, sympathetic activity increases, and blood pressure rises.13

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The autonomic adjustments to exercise in heart failure (HF) are poorly understood. It has been reported that during static handgrip, the metaboreceptor contribution to muscle SNS activity is diminished, but the overall muscle SNS response in HF is preserved.14 These results suggest that a neural mechanism(s), aside from metaboreceptor engagement, must be activated to a greater extent in HF. Subsequent studies have suggested that in HF, mechanosensitive afferents may contribute to a greater degree in HF subjects than in controls.15–18

Chemically sensitive vanilloid receptor subtype 1 (VR1) appears preferentially on metabolite-sensitive group III and IV sensory neurons.19 These receptors are located on afferents in a variety of tissues and mediate the effects of capsaicin.20 When capsaicin is injected into the pulmonary circulation, it stimulates C-fibers and evokes a pulmonary chemoreflex.21,22 The epicardial application of capsaicin stimulates cardiac VR1 receptors, evoking a sympathoexcitatory reflex.23 When capsaicin is injected into the arterial supply of the dog hindlimb, blood pressure rises by 20%, an effect abolished by sectioning afferent nerves.24 The muscle pressor response is likely to be due to the stimulation of both group III and IV fibers, because capsaicin was found to stimulate 71% of group IV and 26% of group III dog hindlimb muscle afferent fibers.25 Because the metaboreflex is attenuated in HF, we postulated in this report that responses to capsaicin would be reduced in HF.

Recently, it has been demonstrated that ATP accentuates muscle mechanoreceptor responses.26 Specifically, when

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ATP or α,β-methylene ATP is infused into the cat hindlimb, the pressor response evoked by muscle stretch is augmented. Muscle stretch is a potent and specific muscle mechanoreceptor stimulant. This effect of ATP and its analogue are blocked by the P2X receptor antagonist PPADS. Additionally, it has been observed that muscle interstitial ATP rises with contraction and stimulates P2X receptors on thin-fiber muscle afferents. Because the mechanoreflex is augmented in HF, we postulated in this report that P2X-mediated muscle reflexes would be accentuated in rats with HF.

Methods

Animal Procedures

All procedures outlined in this study were approved by the Animal Care Committee of this institution.

Coronary Artery Ligation

Male Sprague-Dawley rats (150 to 180 g) were anesthetized by inhalation of an isoflurane-oxygen mixture (2% to 5% isoflurane in 100% oxygen), intubated, and artificially ventilated. A left thoracotomy between the fourth and fifth ribs was performed, exposing the left ventricular wall. The left coronary artery was ligated. Experiments were performed 8 to 14 weeks after coronary ligation, because altered muscle reflexes in rats are seen as early as 6 weeks after coronary ligation. Age- and body weight–matched rats served as controls.

Transcutaneous echocardiography was performed 1 to 2 weeks before the reflex experiments. The rats were anesthetized by inhalation of an isoflurane-oxygen mixture. The transducer was positioned on the left anterior chest, and left ventricular dimensions were measured.

Experimental Preparation for the Reflex Studies

The rats were anesthetized by inhalation of an isoflurane-oxygen mixture. An endotracheal tube was inserted into the trachea and attached to a ventilator. Polyethylene catheters (PE-50) were inserted into the external jugular vein and common carotid artery for drug administration and measurement of arterial blood pressure, respectively. The femoral arteries and arterial collaterals were isolated in both hindlimbs. The popliteal artery was cannulated with a PE-10 catheter for the injection of drugs into the arterial blood supply of the hindlimb muscles. The femoral and sciatic nerves of both legs were isolated so that they could be sectioned at the end of the study. The animals were artificially ventilated, and respiratory parameters were monitored and maintained within normal ranges, as previously described. Body temperature was maintained between 37.5°C and 38.5°C by a heating pad and heat lamps, and fluid balance was stabilized by a continuous infusion of saline.

In one group of studies, decerebration was performed as previously described. This approach afforded the opportunity to examine the effects of muscle stretch on blood pressure. Once the decerebration was completed, anesthesia was removed from the inhalant mixture. The triceps surae muscle was isolated, and the calcaneal bone of one hindlimb was cut, and the Achilles’ tendon was connected to a force transducer (Grass FT10). The pelvis was stabilized in a spinal unit (Kopf Instruments), and the knee joints were secured by clamping the patellar tendon to a spinal unit.

On completion of each experiment, a polyethylene catheter was inserted into the right carotid artery and was threaded into the left femoral artery for measurement of left ventricular end-diastolic pressure (LVEDP). Animals were humanely killed by intravenous injection of an overdose of sodium pentobarbital (120 mg/kg) followed by 1 to 2 mL of a saturated solution of KCl. The heart was excised, and myocardial infarct (MI) size was estimated.

Experimental Protocol

Study 1: Arterial Injections of Capsaicin in Control and MI Rats

Sixty minutes after surgery, capsaicin (1 μg/kg, Sigma) was injected into the arterial blood supply of the triceps surae muscle of anesthetized rats. The injection volume was 0.1 to 0.15 mL, and the duration of injection was 30 seconds. The same volume of saline was then injected to flush the arterial catheter. After waiting 20 minutes, capsazepine (1 mg/kg; VR1 receptor blocker) was then injected intra-arterially. Capsaicin was given 10 minutes later. The same dose of capsaicin was injected intra-arterially after sectioning of the femoral and sciatic nerves.

Study 2: Muscle Stretch–Induced Pressor Responses in Control and MI Rats

Muscle stretch (0.5 kg tension) was produced manually over ~5 seconds by using a rack and pinion attached to the Achilles’ tendon of the decerebrated rats. Each bout of muscle stretch was maintained for 30 seconds after 0.5 kg of tension was achieved. Muscle stretch was performed 10 minutes after arterial injection of saline and α,β-methylene ATP (10 μg/kg, dissolved in saline; Sigma). The injected volume was 0.1 to 0.15 mL. There was a 30-minute rest period between bouts of muscle stretch. At the end of the experiments, the sciatic nerve was cut and muscle stretch was repeated.

Data Acquisition and Analyses

Mean arterial blood pressure (MAP), heart rate (HR), and developed muscle tension were continuously recorded on an Apple computer that used PowerLab software (AD Instruments). Control values were determined by analyzing at least 30 seconds of the data immediately before the interventions (ie, arterial injections or bouts of muscle stretch). The peak response of each variable was determined by the peak change from control.

Experimental data were analyzed with a 1-way repeated-measures ANOVA. Tukey’s post hoc analyses were used to determine differences between groups. All values were expressed as mean±SE. For all analyses, differences were considered significant at P<0.05. All statistical analyses were performed with SPSS for Windows, version 11.5 (SPSS Inc.).

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<tr>
<th>TABLE 1. General Measurements</th>
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<tr>
<td>Control</td>
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<td>Body weight, g</td>
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<td>Heart weight, g</td>
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<tr>
<td>LVEDP, mm Hg</td>
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<td>Infarct size, %</td>
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Values are mean±SE. Abbreviations are as defined in text.

*P<0.05 vs control.
†P<0.05 vs small-MI group.

<table>
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<th>TABLE 2. Echocardiography Measurements</th>
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<tr>
<td>Control</td>
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<tr>
<td>LVDD, cm</td>
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<tr>
<td>LVSD, cm</td>
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<tr>
<td>Anterior wall thickness, mm</td>
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<tr>
<td>Posterior wall thickness, mm</td>
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Values are mean±SE. LVDD indicates left ventricular end-diastolic dimension; and LVSD, left ventricular systolic dimension. All other abbreviations are as defined in text.

*P<0.05 vs control.
†P<0.05 vs small-MI group.
Results

Rats with Mls $<35\%$ and $>35\%$ were characterized as “small-MI” and “large-MI” groups, respectively. Rats with large Mls had an increase in heart weight (Table 1), an increase in LVEDP (Table 1), and a rise in left ventricular diastolic dimension (Table 2).

Capsaicin-Induced Cardiovascular Responses in Control and MI Rats

Baseline values for MAP and HR before arterial injections of capsaicin are presented in Figure 1. There were no significant differences in basal MAP and HR before drug injections in the control (n=6) and MI rats (small MI n=4 and large MI n=6) groups. Capsaicin induced biphasic alterations in blood pressure, with an initial fall that was followed by a rise in blood pressure. The depressor component of the response was similar in magnitude among the 3 groups (30.7±4.6, 32.6±4.2, and 37.9±3.8 mm Hg in controls and small- and large-MI groups, respectively). However, the pressor portion of the capsaicin response was significantly attenuated in the large-MI group compared with the small-MI group and controls (Figure 1).

In addition, the pressor component (but not the depressor component) of the capsaicin-evoked response (n=5 healthy control rats) was attenuated by capsazepine (37.8±5.9 before vs 12.5±3.5 mm Hg after capsazepine, $P<0.05$) and by sectioning of the afferent nerves (39.8±4.4 before vs 9.5±2.8 mm Hg after sectioning, $P<0.05$). These results are in agreement with a recent previous report from this laboratory\textsuperscript{35} and suggest that the capsaicin-induced pressor response was due to VR1 stimulation and that the effect was mediated by engagement of a reflex pathway.

Finally, the same amount of capsaicin was injected before and after decerebration in 4 healthy control rats. In decerebrated rats, capsaicin did not induce a depressor response but did increase blood pressure by 34.5±5.2 mm Hg, suggesting that a depressor response induced by capsaicin was related to the use of anesthesia.

Effect of ATP Activation of P2X Receptors on the Pressor Response Evoked by Muscle Stretch in Control and MI Rats

Basal MAP and HR obtained before passive muscle stretch are shown in Figure 2. There were no significant differences in baseline values of MAP and HR in the control (n=6) and MI rats (small MI n=8 and large MI n=6). Muscle stretch (0.5 kg of tension) significantly increased arterial blood pressure in the decerebrated rats (Figure 2). The reflex response was significantly greater in large-MI rats than in the other 2 groups (Figure 2). Of note, the peak pressor response evoked by muscle stretch was significantly enhanced after arterial administration of $\alpha,\beta$-methylene ATP in all of the groups (Figure 3). However, the sensitizing effects of the P2X stimulant was greater in large-MI compared with small-MI and control animals (72% vs 48% and 42%, respectively, $P<0.05$; see Figure 3). In a prior report from this laboratory, we demonstrated that the augmented pressor response to muscle stretch seen after $\alpha,\beta$-methylene ATP infusion was antagonized by PPADS, a P2X receptor antagonist.\textsuperscript{26}
Discussion

Previous studies have suggested that in HF, the muscle metaboreceptor contribution to the SNS response to exercise is reduced, whereas the muscle mechanoreceptor contribution is augmented. The underlying mechanisms responsible for these observations are poorly defined. Recent studies have shown that hindlimb contraction as well as passive muscle stretch elicits an increase in MAP in decerebrate rats. Furthermore, it has been shown that the exercise pressor reflex is exaggerated in HF rats. Consistent with these findings, the present study demonstrates that the pressor response to muscle stretch is increased in rats with HF compared with that in control animals. In addition, for the first time, the present study offers data suggesting that (1) cardiovascular responses to stimulation of chemically sensitive VR1 receptors are blunted in the HF rat and (2) P2X receptor stimulation enhances mechanoreceptor responses (ie, responses to muscle stretch) more in MI rats than in control animals.

Muscle Metaboreceptor–Mediated Reflex

A prior report in humans suggested that the muscle metaboreceptor contribution to the sympathetic nerve response to handgrip was reduced in HF patients. Interestingly, nuclear magnetic resonance studies suggested that the reduced SNS responses were not due to less muscle acidosis in the contracting muscle of the HF patients, as the muscle pH was similar in the HF and control groups. Based on these data, it was speculated that muscle metabolite-sensitive afferent responses may be reduced in HF.

Metaboreceptor responses are thought to be due to stimulation of chemically sensitive muscle afferents, which are predominantly thought to be unmyelinated group IV afferents. Of note, unmyelinated thin-fiber afferents are rich in VR1 receptors. Although the endogenous ligand is not known, there are 2 lines of evidence that suggest VR1 receptors may play a role in reflex responses to exercise. First, the activity of these receptors increases as pH falls and temperature rises. These physiologic responses are noted during intense muscle contraction. Second, depletion of VR1-sensitive afferents attenuates cardiac and skeletal muscle–based autonomic reflex responses.

The mechanism for the attenuated VR1 response in HF is unclear. It must be emphasized that much less is known about factors that tend to maintain VR1 receptors in the “closed” state than is known about the factors that tend to maintain these receptors in the open state. Nevertheless, it is interesting to note that muscle temperature tends to be lower in HF than in controls. Tissue temperature is an important determinant of VR1 receptor activity. Whether this factor is sufficient to explain the attenuated VR1 responses in HF will await additional studies.

Muscle Mechanoreceptor–Mediated Reflex

A number of studies support the concept that ATP stimulates and/or sensitizes thin-fiber muscle afferents. First, ATP increases in contracting muscle interstitium, where the free nerve endings of group III and group IV afferents reside. Second, stimulation of ATP-sensitive P2X receptors in the cat hindlimb initiates a reflex that causes blood pressure to rise. Third, ATP enhances mechanoreceptor-mediated cardiovascular responses induced by stimulation of P2X receptors, and the pressor response seen with static hindlimb contraction is attenuated when P2X receptor blockers are administered.

The mechanism for the greater pressor response to α,β-methylene ATP and stretch in rats with large MIs is not clear. In HF patients, circulating adenosine concentrations are elevated and are correlated with disease severity. ATP is degraded into ADP, AMP, and adenosine by ectonucleotidases. Thus, the elevation of plasma adenosine in HF may reflect increased muscle interstitial ATP levels. Greater ATP levels in chronic HF could upregulate P2X receptors on thin afferent nerves and lead to the greater level of mechanoreceptor activity that has been described in HF. It should also be noted that P2X activity increases as temperature falls. Thus, the lower muscle temperature in HF could contribute to the enhanced mechanoreceptor (ie, P2X receptor) activity as well as the reduced metaboreceptor activity described earlier. Finally, it is also possible that metabolic changes in the muscle interstitium can alter the sensitivity of mechanically sensitive afferents. For example, it has been shown that changes in lactate concentrations can alter group III afferent discharge during muscle contraction. Whether an increase in interstitial lactate or H+ as seen in HF can alter P2X receptor activity will need to be determined.

Implications

Heightened P2X activity with mechanoreceptor stimulation in HF may contribute to the greater renal vasoconstriction.
seen early in exercise in HF. 16,18,48 This may allow HF subjects to partially compensate for reduced exercise muscle blood flow.49 However, the impaired VRI response may contribute to the impaired exercise tolerance seen with fatigue.50 Sympathetic tone at high workloads aids in the matching of blood flow and oxygen demand within the metabolically active muscle.51

Conclusions
These data demonstrate that cardiovascular responses to stimulation of metabolite-sensitive VRI receptors are blunted and that P2X receptor–mediated muscle mechanoreceptor responses are augmented in MI animals compared with a control group. These results suggest that alternations in VR1 and P2X receptors on muscle afferent nerves influence the processing of sensory information in this disease and in turn, may alter the magnitude, timing, and distribution of SNS activity during exercise in HF.

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