The Capsaicin-Sensitive Afferent Neuron in Skeletal Muscle Is Abnormal in Heart Failure

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Background—In heart failure, the cardiovascular response to activation of the skeletal muscle exercise pressor reflex (EPR) is exaggerated. Group IV afferent neurons, primarily stimulated by the metabolic by-products of skeletal muscle work, contribute significantly to the EPR. Therefore, it was postulated that alterations in the activity of group IV neurons contribute to the EPR dysfunction manifest in heart failure.

Methods and Results—Group IV afferent fibers were ablated in neonatal Sprague-Dawley rats by subcutaneous administration of capsaicin. In neonatal capsaicin-treated adult animals, selective activation of the EPR, by electrically induced static muscle contraction, recapitulated the exaggerated increases in heart rate and blood pressure observed in rats with dilated cardiomyopathy (DCM). Furthermore, compared with control animals, both neonatal capsaicin-treated and DCM rats displayed a decreased pressor response to the intra-arterial administration of capsaicin within the hindlimb, a maneuver that selectively excites group IV afferent neurons. Moreover, expression of mRNA for the capsaicin receptor TRPv1, a marker of group IV fibers, was downregulated in DCM animals compared with controls.

Conclusions—These findings suggest that EPR dysfunction in heart failure results in part from functional and molecular alterations in group IV fibers. Furthermore, the responsiveness of these metabolically sensitive neurons appears to be blunted in DCM, indicating that their contribution to the EPR may be reduced. This occurs despite an overall exaggeration of the EPR in heart failure. These insights into the basic mechanisms of EPR dysfunction are essential to the development of effective therapeutic strategies aimed at improving exercise capacity in heart failure. (Circulation. 2005;111:2056-2065.)

Key Words: blood pressure ■ cardiomyopathy ■ exercise ■ heart failure ■ reflex

Exercise intolerance is a hallmark of chronic heart failure; however, the cause of this reduction in exercise capacity is unclear. One contributing factor to the development of exercise intolerance may be the generation of abnormal circulatory hemodynamics during exercise. In support of this concept, cardiovascular regulation during exercise is clearly altered in heart failure because the sympathetic, vascular resistance, heart rate (HR), and mean arterial pressure (MAP) responses to physical activity have been shown to be augmented.1-7 These persistent, exaggerated responses to exercise may result in the chronic enhancement of peripheral vasoconstriction in heart failure. Combined with the left ventricular dysfunction that characterizes this disease, an enhanced vasoconstrictor tone likely comprises skeletal muscle perfusion during exercise, thus contributing to the development of muscular fatigue.

Two distinct neural control mechanisms activated by exercise potentially drive the altered circulatory hemodynamics in heart failure: central command and the exercise pressor reflex (EPR). Central command is a mechanism whereby signals from a central cerebral site, responsible for recruiting motor units, activate cardiovascular control areas in the brain stem.8,9 The EPR is a mechanism whereby signals from contracting muscle, propagated via group III (predominately mechanically sensitive) and group IV (predominately metabolically sensitive) skeletal muscle afferent fibers,10,11 elevate MAP and HR via increases in sympathetic nerve activity. Because of the peripheral skeletal myopathy that develops,12-17 it has been suggested that the exaggerated cardiovascular responses to exercise in heart failure are mediated primarily by an overactive EPR.18

In support of this hypothesis, we previously determined that the EPR is exaggerated in rats with dilated cardiomyopathy (DCM).19 Furthermore, this potentiation in the EPR appears to be mediated, at least in part, by mechanically sensitive afferent neurons (predominantly group III afferent fibers). Despite these reports of an exaggerated EPR in heart failure, it has been demonstrated that the contribution of the
metaboreflex (predominantly group IV afferent fibers) to the regulation of muscle sympathetic nerve activity during exercise is diminished in patients with heart failure.20 Additionally, reflex renal vasoconstriction is exaggerated in patients with heart failure compared with normal subjects. The contribution of the muscle metaboreflex to this vasoconstriction is likewise blunted.2 In contrast, others show an exaggeration in metaboreflex function in heart failure.7,19 As a result, the contribution of the metabolically sensitive component of the EPR to the cardiovascular response to exercise in heart failure remains unclear.

In the present study we tested the hypothesis that the contribution of group IV (predominantly metabolically sensitive) afferent neurons to the EPR is blunted in heart failure. Distinct sets of studies were performed to test this hypothesis. First, group IV primary afferent neurons were permanently ablated by administering capsaicin to normal 2-day-old rat pups. On reaching adulthood, the EPR was evaluated in these rats. This experiment tests the effect of selective, group IV afferent neuron withdrawal on the EPR in healthy rats. Second, the MAP and HR responses to hindlimb intra-arterial capsaicin injection were measured in adult capsaicin-treated and cardiomyopathic rats. Capsaicin selectively stimulates group IV afferent neurons in this preparation.21 Finally, to determine the contribution of an abnormal EPR to the development of exercise intolerance, we compared exercise tolerance in cardiomyopathic rats to both sham control rats and cardiomyopathic rats. Capsaicin selectively stimulates group IV afferent neurons in this preparation.21 To determine the effect of destruction of group IV primary afferent neurons on the EPR, we administered capsaicin to neonatal rats. Neonatal capsaicin treatment is a well-established model for the development of exercise intolerance, we compared exercise is diminished in patients with heart failure.20 Addition ally, reflex renal vasoconstriction is exaggerated in patients with heart failure compared with normal subjects. The contribution of the muscle metaboreflex to this vasoconstriction is likewise blunted.2 In contrast, others show an exaggeration in metaboreflex function in heart failure.7,19 As a result, the contribution of the metabolically sensitive component of the EPR to the cardiovascular response to exercise in heart failure remains unclear.

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Methods
Experiments were performed in 99 age-matched male Sprague-Dawley rats (Harlan) divided into the following distinct experimental groups: normal, sham, neonatal vehicle-treated, neonatal capsaicin-treated, and DCM.

Model of DCM
A subset of animals within the weight range of 150 to 175 g underwent thoracic surgery. Anesthesia was induced with isoflurane gas (2% to 5% in 100% oxygen), after which animals were intubated and ventilated. A thoracotomy was performed, exposing the heart, lungs and tibia were removed, weighed, and measured. With the exclusion of thoracotomy, all procedures were performed additionally in age-matched normal rats.

Neonatal Capsaicin Treatment
To determine the effect of destruction of group IV primary afferent neurons on the EPR, we administered capsaicin to neonatal rats. Neonatal capsaicin treatment is a well-established model for the selective and permanent destruction of group IV primary afferent neurons.24 In this experiment, a 50-μg/kg subcutaneous injection of capsaicin (neonatal capsaicin-treated group) or its vehicle (neonatal vehicle-treated group) was performed in 2-day-old neonatal rat pups. Six weeks after injection, male animals were challenged by application of a 0.01% capsaicin solution to the cornea. Rats displaying ≤30 protective eye wipings were considered capsaicin insensitive (ie, neonatal capsaicin-treated). Echocardiograms and physiological testing were performed in adult neonatal capsaicin-treated and neonatal vehicle-treated rats at time points and ages matched to normal, sham, and DCM animals. Neonatal capsaicin-treated and neonatal vehicle-treated rats not designated for physiological experimentation were transcardially perfused with 4% paraformaldehyde. In these animals, dorsal root ganglia (DRG) corresponding to the fourth through sixth lumbar vertebrae (L4 to L6) were harvested and prepared for immunohistochemical staining of the capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1), to confirm the presence or absence of capsaicin-sensitive fibers. Briefly, tissue sections were rinsed in phosphate-buffered saline (pH 7.5), incubated with 5% normal goat serum, and followed by a 24-hour incubation period with polyclonal antisera raised in guinea pig against rat vanilloid receptor 1 (1:1000; Novus Biologicals). Visualization was achieved by tissue incubation in a biotinylated secondary antibody (goat anti–guinea pig IgG 1:600; Jackson Laboratories). Biotin-labeled tissue was further processed with the use of the Vectastain Elite ABC reagents (Vector Laboratories) and developed with a solution of hydrogen peroxide (0.003%) and diaminobenzidine (0.02%).

Acute Physiological Preparations
General Preparations
Rats were initially anesthetized with isoflurane gas and instrumented as previously described.19,25 Briefly, animals were intubated for mechanical ventilation and cannulated with jugular venous and carotid arterial catheters. Blood pressure was recorded by connecting the arterial catheter to a pressure transducer (model DTX plus-DTNIZ, Ohmeda). MAP was obtained by integrating the arterial signal with a time constant of 1 to 4 seconds. HR was derived from the blood pressure pulse wave with the use of a photodetector (Gould Instruments). Subsequently, animals were rendered insentient by precollitical decerebration. After forebrain transection and aspiration, gas anesthesia was discontinued.

Ventral Root Stimulation and Passive Stretch
A laminectomy exposing the lower lumbar portions of the spinal cord (L4 to L6) was performed, and stimulating electrodes were placed around the cut peripheral ends of the L4 and L6 ventral rootlets. The calcaneal bone of the right hindlimb was cut, and the Achilles' tendon was connected to a force transducer (FT-10, Grass Instruments). Electrically induced static contraction of the triceps surae was used to activate both the mechanically and metabolically sensitive components of the EPR (ie, group III and IV afferent fibers, respectively). With the use of constant current stimulation (3 times motor threshold, 0.1 ms pulse duration, 40 Hz), 30-second contractions were produced by excitation of the L4 and L5 ventral roots, with the peak MAP, force development, and HR responses recorded. Preferential activation of mechanically sensitive afferent fibers was achieved by passively stretching the hindlimb muscles with the use of a rack and pinion system (Harvard Apparatus, Inc). Collectively, these procedures cause increases in MAP and HR that have been shown to be due to the selective stimulation of skeletal muscle primary afferent fibers in this rat model.25 Static contraction and passive stretch data from our previous study using DCM rats19 are compared with the results of this investigation in Figure 1.

Intra-Arterial Hindlimb Capsaicin Injections
In these experiments, capsaicin was used to acutely and selectively activate group IV primary afferent neurons in the hindlimb. A catheter was placed in the left common iliac artery, and its tip was advanced to the abdominal aorta. Capsaicin was then injected directly into the arterial supply of the right hindlimb via the right common iliac artery. To limit drug delivery to the hindlimb being tested, a reversible ligature was placed around the common iliac vein emptying the right hindlimb. On injection of drug, the ligature was pulled for 2 minutes to trap the injectate in the leg. Each animal was injected first with saline and/or the vehicle for capsaicin, followed by...
5 increasing doses of capsaicin (0.01 to 1.00 μg/100 μL) with 15 minutes between each injection. In addition, a subset of sham-treated animals received intra-arterial capsaicin together with capsazepine (100 μg/100 μL, Tocris Cookson, Inc), a selective TRPv1 competitive antagonist, to confirm that the effects of the drug were the result of TRPv1 receptor activation. In preliminary studies, we randomized the drug doses and found no diminution or exaggeration of drug effect on the basis of order of drug administration (data not shown).

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction
Sham and DCM animals were anesthetized, and tissue was rapidly harvested to isolate total RNA. Specifically, skeletal muscle (soleus) and DRG (L4 to L6) were extracted and immediately frozen in liquid nitrogen. Total RNA was isolated with the use of the Tripure isolation kit (Boehringer Mannheim). Using spectrophotometry, we determined that the A260/A280 ratios were between 1.9 and 2.0. Four micrograms of total RNA was used in each reverse transcription (RT) reaction (Retro-script, Ambion). Complementary DNA (1 μL) was used as a template for the polymerase chain reaction (PCR) in a 20-μL reaction volume including 100 ng of each primer, 2 mmol/L MgCl₂, Taq buffer, and 1 U of Taq polymerase (GIBCO/BRL). After the PCR, 15 μL of the sample was loaded onto 2% agarose gel. Semiquantitative RT-PCR using RNA isolation from skeletal muscle and DRG was performed under conditions in which the abundance of each amplified cDNA varied linearly with input RNA. The primer pairs used in this study were as follows: forward primer, GAC-ATG-CCA-CCC-AGC-AGG; reverse primer, TCA-ATT-CCC-ACA-CAC-CTC-CC.

Exercise Tolerance
Animals were acclimated to a rodent treadmill (Omni-Pacer; Omnitech Electronics) by running daily for 5 days. During this acclimation period, belt speed was increased from 5 to 15 mpm, and exercise duration was maintained at 10 minutes. On the day of tolerance testing, animals were placed on the treadmill at belt speeds of 15, 20, or 25 mpm at a 20-degree angle and allowed to exercise until fatigue. Run-to-fatigue experiments (15, 20, or 25 mpm) were performed on 3 consecutive days. Fatigue was defined as the point at which an animal failed to keep pace with the treadmill despite constant physical prodding for 1 minute. Time to fatigue was taken as the index of maximal capacity for exercise. All exercise acclimation and testing were performed after echocardiogram and before physiological experimentation.
Exaggerated EPR Observed in DCM

Neonatal Capsaicin Treatment Recapitulates the Exaggerated EPR Observed in DCM

Morphometric, Echocardiographic, and Hemodynamic Characteristics

<table>
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<th></th>
<th>Normal (n=25)</th>
<th>Sham (n=25)</th>
<th>Neonatal Vehicle-Treated (n=17)</th>
<th>DCM (n=20)</th>
<th>Neonatal Capsaicin-Treated (n=12)</th>
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<td>Body weight, g</td>
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<td>400±19</td>
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<td>Lung weight/body wt, mg/g</td>
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<td>7.1±0.4</td>
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<td>Heart weight/tibial length, mg/mm</td>
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<td>418±13</td>
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Values are mean±SEM. LVEDD indicates left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension.

*Significance from normal, sham, neonatal vehicle-treated, and neonatal capsaicin-treated groups (P<0.05).

Results

Morphometric, echocardiographic, and baseline hemodynamic characteristics for each group of rats are presented in the Table. As determined by echocardiography, animals with left anterior descending coronary artery ligation had an average fractional shortening (an index of left ventricular systolic function) of 23±2%, which was significantly less than that measured for normal, sham, neonatal vehicle-treated, or neonatal capsaicin-treated animals (P<0.05). Moreover, left ventricular end-diastolic and end-systolic dimensions were significantly increased in ligated rats, indicating the development of a DCM similar to our previous observations and those of others. It should be noted that in previous studies, we determined that an exaggeration in the EPR presents in rats with a fractional shortening of <35%. Therefore, a fractional shortening of >35% was used to exclude ligated animals from this study. In addition, significant increases in heart weight/body weight, heart weight/tibial length, and lung weight/body weight ratios were observed in DCM animals compared with all other groups. It is important to note that there was no difference in any of these parameters between normal, sham, neonatal vehicle-treated, and neonatal capsaicin-treated animals. Furthermore, no differences in body weight or baseline hemodynamics were observed between any groups.

Neonatal Capsaicin Treatment Recapitulates the Exaggerated EPR Observed in DCM

To determine the effect of destruction of group IV primary afferent neurons on the EPR, we administered capsaicin to neonatal rats. To confirm the destruction of group IV afferent neurons in these animals, we evaluated TRPv1 immunoreactivity. Compared with vehicle-treated animals (Figure 1A), TRPv1 immunoreactivity was virtually abolished in the DRG (location of cell bodies of primary afferent neurons) of adult rats after neonatal capsaicin administration (Figure 1B). In addition, we confirmed that neonatal capsaicin-treated rats were insensitive to capsaicin by delivering dilute capsaicin drops to the surface of the cornea. Neonatal vehicle-treated rats displayed a vigorous wiping and blinking behavior in response to capsaicin drops that lasted approximately 3 minutes. Neonatal capsaicin-treated rats, however, were virtually unresponsive to this treatment (Figure 1C). MAP and HR responses to electrically induced contraction and passive stretch were exaggerated in adult neonatal capsaicin-treated animals compared with normal, sham, or neonatal vehicle-treated rats (Figure 1D). The hemodynamic changes recorded in neonatal capsaicin-treated animals, in response to contraction and stretch, closely resembled those previously obtained in DCM rats.

Blunted Circulatory Responses to Injection of Capsaicin Are Observed in DCM

Figure 2 illustrates the effect of increasing doses of hindlimb intra-arterial capsaicin administration on MAP and HR responses in normal, sham, and DCM rats. First, it was observed that intra-arterial capsaicin injection resulted in a dose-related increase in MAP (Figure 2A) and HR (Figure 2B) from baseline in all groups. When compared with normal and sham-treated animals, however, DCM rats displayed significantly blunted MAP and HR responses to intra-arterial injection of capsaicin (P<0.05). Injection of saline had no significant effect on baseline MAP and HR. Finally, it was observed that the competitive TRPv1 antagonist capsazepine significantly attenuated the capsaicin-induced increase in MAP and HR in sham animals.

Left Ventricular Systolic Function and Response to Capsaicin Injection in DCM Rats

Changes in MAP in response to the hindlimb intra-arterial administration of 0.30 μg/100 μL (Figure 3A) and 1.00...
μg/100 μL (Figure 3B) of capsaicin were plotted against an index of left ventricular systolic function (ie, fractional shortening). Significant correlations \((r=0.65\) and 0.52, respectively) between the pressor response to intra-arterial capsaicin administration and fractional shortening were observed. Specifically, at the doses examined, it was observed that as left ventricular dysfunction was more severe, the pressor response to capsaicin injection was decreased.

TRPV1 mRNA Levels Are Decreased in DCM Rats

In sham-treated animals, a robust expression of TRPV1 mRNA in both the DRG and soleus muscle was observed with the use of RT-PCR analysis (Figure 4). In DCM rats, however, expression of TRPV1 mRNA was decreased in both of these tissues.

Destruction of Capsaicin-Sensitive Afferent Neurons Results in Blunted Responses to Capsaicin Administration

Figure 5 illustrates the effect of destruction of group IV, capsaicin-sensitive afferent neurons on the MAP and HR responses to intra-arterial injection of capsaicin into the hindlimb. As expected, destruction of this group of afferent neurons significantly reduced the MAP (Figure 5A) and HR (Figure 5B) responses to capsaicin administration compared with those elicited in vehicle-treated animals. It is important to note, however, that we observed a modest effect of
neonatal vehicle treatment on the cardiovascular responses to capsaicin injection compared with normal, untreated animals (Figure 2).

Relative Responses to Capsaicin Injection in the Hindlimb

Figure 6 presents the relative (ie, percent) cardiovascular responses to hindlimb intra-arterial capsaicin injection in DCM and neonatal capsaicin-treated rats compared with their respective controls (eg, normal, sham, and neonatal vehicle-treated animals). We observed that, at lower doses, the MAP response in neonatal capsaicin-treated rats was significantly more blunted than in DCM animals (Figure 6A). In contrast, no consistent relationship between DCM animals, neonatal capsaicin-treated animals, and their respective controls was observed with regard to HR (Figure 6B).

Left Ventricular Systolic Function and Exercise Tolerance

DCM rats had a significant reduction in their tolerance to exercise as they fatigued more rapidly than sham-treated animals at low, medium, and high intensities of exercise (Figure 7A). Furthermore, the time to fatigue decreased as left ventricular dysfunction (assessed by fractional shortening) increased (Figure 7B). However, there was no significant correlation between fractional shortening and time to fatigue at any intensity of exercise. Finally, we observed that exercise tolerance was normal in neonatal capsaicin-treated animals compared with vehicle-treated rats (data not shown).

Discussion

Effect of Destruction of Capsaicin-Sensitive Group IV Neurons on the EPR

Capsaicin, administered to the neonate, causes the destruction of unmyelinated primary afferent neurons throughout the lifetime of the animal.24 Myelinated fibers28 as well as unmyelinated efferent fibers are unaffected by neonatal capsaicin treatment.29 We have previously demonstrated that the exercise pressor reflex is exaggerated in rats with DCM.19 In the present study it has been demonstrated that selective ablation of capsaicin-sensitive neurons (predominately group IV afferent fibers commonly associated with the metabolically sensitive component of the EPR) in healthy rats...
resulted in an enhanced pressor response to exercise comparable to that elicited in cardiomyopathic animals. The hearts of these neonatal capsaicin-treated animals were observed to be normal after physiological and morphological examination. Therefore, the abnormal cardiovascular responses to electrically induced contraction and passive stretch were the result of the destruction of the group IV afferent neurons and not the result of left ventricular dysfunction.

These data demonstrate that the removal of group IV afferent neurons results in an exaggerated EPR when one might predict a reduction in EPR activity due to a significant loss of neurons known to activate the reflex. On the basis of these results, it is hypothesized that withdrawal of group IV afferent neurons is sufficient to initiate an exaggerated EPR. In contrast to the group IV afferent neurons, the group III afferent neurons remain largely intact after neonatal capsaicin administration. It is possible that these neurons dysregulate the cardiovascular response to exercise by overcompensating for the loss of the group IV primary afferent fibers. In support of this theory, cardiovascular responses to passive stretch of the muscle, a stimulus that predominately activates group III afferent neurons, were exaggerated in neonatal capsaicin-treated animals in a manner similar to that previously demonstrated in DCM rats. In addition, Middlekauff et al have shown that mechanoreflex control of reflex renal vasoconstriction is exaggerated in patients with heart failure. More recently, it has been demonstrated that muscle mechanoreceptors have a heightened sensitivity to mechanical stimuli in patients with heart failure. It has been suggested that this heightened sensitivity may be due to the presence of muscle metabolites because activation of ATP-sensitive P2X receptors within skeletal muscle enhances the pressor response to muscle stretch in cardiomyopathic rats. Therefore, it is predicted that the augmented cardiovascular response to exercise that occurs in neonatal capsaicin-treated rats is mediated by the remaining group III afferent neurons in skeletal muscle. The data of the present investigation also support the concept that the group III afferent neurons overcompensate for the functional abnormalities in group IV afferent fibers in heart failure.

Responses to Hindlimb Capsaicin Injection Are Blunted in DCM and Neonatal Capsaicin-Treated Rats

In humans, the metaboreflex is often studied by performing regional circulatory occlusion of the contracting limb after exercise, thus trapping the metabolic by-products of work within the muscle. Although invaluable in humans, a mass activation of the metaboreflex does not allow for the controlled manipulation of afferent fiber activity. In animals, several approaches can be taken to circumvent this limitation. One approach is to administer chemical agents at various concentrations into the arterial supply of skeletal muscle. For example, intra-arterial injection of capsaicin into the circulation of the hindlimb is a tool for the selective activation of group IV, capsaicin-sensitive afferent neurons. This approach is particularly attractive because it is well documented that the capsaicin receptor TRPv1 is a marker for group IV afferent neurons. Therefore, stimulation of this receptor activates the neuronal population known to primarily mediate metaboreflex activity. A second approach is to experimentally remove the group IV afferent neurons known to mediate the reflex in vivo. Combining these approaches, we used hindlimb intra-arterial capsaicin administration as a tool to selectively and acutely activate group IV afferent neurons in animals in which a majority of these fibers had been ablated as well as in healthy and cardiomyopathic rats. It must be clarified, however, that this approach does not necessarily mimic the metaboreflex contribution to the EPR but instead represents a graded activation of group IV afferent neurons in skeletal muscle.

As would be expected, a significant reduction in the MAP and HR responses to hindlimb intra-arterial capsaicin administration was observed in rats in which group IV afferent fibers were abolished (ie, neonatal capsaicin-treated rats). Interestingly, the responses to capsaicin were also significantly blunted in the cardiomyopathic rats. Likewise, other preliminary reports have suggested that cardiovascular re-
sponses after activation of the TRPv1 receptor are blunted in rats with myocardial infarction.31 In the present study we observed that the magnitude of this blunted response was dependent on the extent of left ventricular dysfunction and that expression of capsaicin receptor (TRPV1) mRNA was downregulated in DCM animals in both DRG and skeletal muscle. These results are suggestive of a reduction in group IV fibers within these tissues. These studies provide both physiological and molecular support for the concept that abnormalities occur in capsaicin-sensitive group IV afferent neurons in heart failure and that these changes facilitate a blunting of metaboreflex. Furthermore, these abnormalities in metaboreflex function can be recapitulated in normal animals by experimental ablation of group IV afferent fibers.

Whether activation of the TRPv1 receptor contributes to the EPR per se is currently a subject of debate.39,40 For example, the results of this study in the rat and investigations in humans39 support a role for the TRPv1 receptor in the activation of the EPR. In contrast, studies performed in cats40 argue against a contribution of the TRPv1 receptor to EPR activity. These disparities may be species related or due to methodological differences between preparations. Our present data, demonstrating that animals treated with neonatal capsaicin have an abnormal EPR, suggest that TRPv1 activation may be necessary for the normal expression of the EPR-mediated cardiovascular responses to exercise. Moreover, the data support the hypothesis that the abnormal function of the capsaicin-sensitive afferent neuron contributes to the alterations in EPR activity observed in heart failure.

Removal of Capsaicin-Sensitive, Group IV Afferent Neurons Recapitulates the Exaggerated EPR but not the Exercise Intolerance Observed in Heart Failure

Although an exaggeration in the EPR was observed after the destruction of capsaicin-sensitive neurons, neonatal capsaicin-treated animals exercised normally. In contrast, DCM rats displayed both an exaggerated EPR and intolerance to exercise. The lack of exercise intolerance in neonatal capsaicin-treated may have several explanations. First, it is possible that compensatory mechanisms, activated during development, may be present in the neonatal capsaicin-treated rats that attempt to sustain normal tolerance to exercise. Second, the data suggest that although the destruction of group IV afferents is sufficient to evoke changes in the EPR, other factors, such as depressed LV function or abnormalities in skeletal muscle, are needed to produce exercise intolerance. It is also possible that time to fatigue testing is not a sufficiently sensitive method to determine subtle differences in exercise tolerance. Finally, it remains a possibility that exaggerations in EPR function are unrelated to exercise intolerance. Further studies are required to clarify this relationship.

Contribution of an Exaggerated EPR to Exercise Intolerance

It has previously been hypothesized that reductions in pump capacity resulting in poor peripheral blood flow and skeletal muscle atrophy ultimately result in reductions in exercise capacity.6 This theory is termed the muscle hypothesis of exercise intolerance in heart failure. Consistent with this hypothesis, the exaggerations in the EPR that have been demonstrated in the cardiomyopathic rat may result in elevations in sympathetic outflow during exercise that enhance peripheral vasoconstriction. In addition, the resultant increases in vascular resistance and MAP could also elicit decreases in cardiac output in the presence of left ventricular dysfunction. Combined, an enhanced vasoconstrictor tone and reduced cardiac output could diminish blood flow to peripheral skeletal muscle during exercise, thus contributing to the development of muscle fatigue. It should be noted, however, that such a cascade of events appears to be requisite for an exaggerated EPR to substantially reduce tolerance to exercise. This conclusion is supported by the finding that alterations in the EPR alone did not appreciably affect exercise capacity in animals with normal ventricular function (ie, neonatal capsaicin-treated animals).

TRPV1 mRNA Expression Is Downregulated in Heart Failure

We have investigated the mechanism via which the metaboreflex is blunted in heart failure and have observed that the levels of TRPV1 mRNA are significantly decreased in both the DRG and skeletal muscle of cardiomyopathic rats. The cause of the downregulation in TRPV1 mRNA is currently unknown. Because TRPV1 marks the primary afferent neuron, downregulation of its mRNA may reflect the death of group IV afferent fibers. In heart failure, downregulation of TRPV1 mRNA could also result from alterations in the local chemical milieu of the tissue in which the afferent fibers are located. For example, decreased peripheral blood flow, skeletal muscle atrophy, fiber-type transformation, and reduced oxidative capacity have been reported in skeletal muscle after the pathogenesis of heart failure and may contribute to local chemical changes.12–17 Decreases in skeletal muscle pH, which are known to occur during heart failure,12–15,17 may alter the function of the TRPV1. Protons (ie, acids) are considered endogenous mediators of the TRPV1 because they can potentiate,41 directly activate,41 and, at higher concentrations, even block TRPV1 conductance.42 Desensitization of the TRPV1 is also known to occur through complex kinetic processes,43,44 but whether this receptor is desensitized in heart failure remains to be determined.

Left Ventricular Dysfunction and Exercise Tolerance

In humans, there is general agreement that decreases in left ventricular function are significantly correlated with EPR dysfunction.4–7 In our rat model, we have also observed that abnormalities in the EPR are well correlated with reductions in left ventricular function.19 In contrast, exercise tolerance and left ventricular function are not tightly correlated in patients with heart failure.45,46 Although a significant decrease in exercise tolerance in DCM rats exhibiting an altered EPR was established in this study, a significant correlation between left ventricular function and exercise tolerance was not observed. These data indicate that the decerebrate rat model of cardiomyopathy used in this investigation shares characteristics with the human heart failure population. Fur-
thermore, the findings suggest that, in heart failure, quantification of the EPR may serve as a valuable index of exercise capacity.

Nociceptors Versus Ergoreceptors
Primary afferent neurons innervate skin, joints, and skeletal muscle. The fine afferent neurons that are localized in skeletal muscle are capable of responding to both noxious and innocuous stimulation (eg, passive stretch). These fibers are classified as nociceptors and ergoreceptors, respectively. Because of the known polymodal characteristics of nociceptive fibers, it is unclear whether some primary afferent neurons can contribute to both the cardiovascular responses to exercise and the perception of painful stimuli. Patients in whom the EPR is activated either by static hand grip or dynamic exercise do not report the exercise bout to be painful. As a result, it may be assumed that the EPR does not involve nociceptive neurons and is activated only by stimulation of ergoreceptors. In contrast, our data show that the abolition of capsaicin-sensitive fibers, known to be nociceptors, causes a significant abnormality in the EPR in response to both contraction and stretch. These data support the concept that nociceptors contribute to the EPR in normal rats and/or that capsaicin-sensitive afferent neurons are both nociceptors and ergoreceptors.

Model for Exaggeration of EPR in Heart Failure
It is feasible that left ventricular dysfunction, resulting in reduced blood flow and muscle atrophy in hindlimb skeletal muscle, can cause changes that result in the destruction or downregulation of receptors on group IV afferent neurons in skeletal muscle. Our data support the concept that the augmentation in the EPR that was observed in cardiomyopathic rats was initiated by the withdrawal or downregulation of group IV afferent neurons. After alterations in the group IV afferent neuron population, it is predicted that other afferent neurons (presumably group III neurons) undergo plastic changes in both gene and protein expression that dysregulate the cardiovascular responses to exercise. It is hypothesized that these group III neurons overcompensate for the loss (or downregulation) of group IV neurons. It is further postulated that this abnormal control results in the exaggerated EPR that we and others observe in cardiomyopathy. In the neonatal capsaicin-treated animals, a similar augmentation of the EPR was demonstrated. This suggests that we may have uncovered an initiating event leading to the exaggeration of the EPR by experimentally inducing the withdrawal of group IV afferent neurons in an otherwise normal animal. Therefore, in heart failure, it is predicted that the withdrawal or downregulation of the group IV afferent neuron is secondary to or downstream of the development of left ventricular dysfunction and skeletal muscle atrophy. Finally, the data support the concept that the abnormalities that occur in group IV afferent neurons are necessary and sufficient to generate an exaggerated EPR.

Conclusions
The data indicate that destruction of group IV primary afferent fibers in a normal (noncardiomyopathic) rat is sufficient to produce an abnormal EPR similar to that observed in the cardiomyopathic animal. Moreover, the results of the present study indicate that functional and molecular abnormalities occur in group IV primary afferent neurons in the cardiomyopathic rat that affect EPR function. These data also suggest that nociceptive neurons may be important to the maintenance of normal cardiovascular responses to exercise. Finally, these data lend important insights into the development of the exaggerated EPR in heart failure that may prove invaluable in understanding the mechanisms of exercise intolerance in this disease.

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References


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