Skeletal muscle reflex-mediated changes in sympathetic nerve activity are abnormal in spontaneously hypertensive rats

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Submitted 12 November 2010; accepted in final form 5 January 2011

Mizuno M, Murphy MN, Mitchell JH, Smith SA. Skeletal muscle reflex-mediated changes in sympathetic nerve activity are abnormal in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 300: H968–H977, 2011. First published January 7, 2011; doi:10.1152/ajpheart.01145.2010.—In hypertension, the blood pressure response to exercise is exaggerated. We demonstrated previously that this heightened pressor response to physical activity is mediated by an overactive skeletal muscle exercise pressor reflex (EPR), with important contributions from its metaboreflex and mechanoreflex components. However, the mechanisms driving the abnormal blood pressure response to EPR activation are largely unknown. Recent evidence in humans suggests that the muscle metaboreflex partially mediates the enhanced EPR-induced pressor response via abnormally large changes in sympathetic nerve activity (SNA). Whether the muscle mechanoreflex induces similarly exaggerated alterations in SNA in hypertension remains unknown, as does the role of the mechanoreceptors mediating muscle reflex activity. To address these issues, the EPR was selectively activated by electrically inducing hindlimb muscle contraction in decerebrate normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Stimulation of the EPR evoked significantly larger increases in mean arterial pressure (MAP) and renal SNA (RSNA) in SHR compared with WKY (\(\Delta RSNA\) from baseline: 140 ± 11 vs. 48 ± 8\%). The mechanoreflex was stimulated by stretching hindlimb muscle which likewise elicited significantly greater elevations in MAP and RSNA in SHR than WKY (\(\Delta RSNA\) from baseline: 105 ± 11 vs. 35 ± 7\%). Blockade of mechanoreceptors in muscle with gadolinium significantly attenuated the MAP and RSNA responses to contraction and stretch in SHR. These data suggest that 1) the exaggerated pressor response to activation of the EPR and muscle mechanoreflex in hypertension is mediated by abnormally large reflex-induced augmentations in SNA and 2) this accentuated sympathetic responsiveness is evoked, in part, by stimulation of muscle mechanoreceptors.

autonomic nervous system; blood pressure; exercise pressor reflex; mechanoreflex

IN HYPERTENSION, THE CARDIOVASCULAR RESPONSE to exercise is abnormally exaggerated and characterized by accentuated increases in arterial blood pressure (ABP), heart rate (HR), and vascular resistance (2, 15, 19, 41, 45). Since such responses are known to be associated with elevated risks for myocardial ischemia, myocardial infarction, cardiac arrest, and/or stroke during and after physical activity, elucidating the cause of this cardiovascular hyperexcitability is clinically important (14, 26, 38, 39). To this end, our laboratory demonstrated recently that selective activation of the exercise pressor reflex (EPR; a peripheral reflex originating in skeletal muscle) elicits markedly increases in mean arterial pressure (MAP) in hypertensive compared with normotensive rats (49). Additional experimentation in rats and humans has suggested that both the mechanically (i.e., muscle mechanoreflex) and metabolically (i.e., muscle metaboreflex) sensitive components of the reflex contribute significantly to this EPR overactivity (28, 44). These findings provide evidence that the exaggerated pressor response to exercise in hypertension is mediated, in part, by a dysfunctional EPR. However, the mechanism(s) by which the EPR drives these accentuated blood pressure responses remains largely undetermined.

Normally, the EPR evokes circulatory adjustments to exercise primarily by increasing sympathetic nerve activity (SNA) (31, 34, 37). Therefore, it is logical to suggest that the exaggerated EPR-mediated pressor response to exercise in hypertension results from abnormally large reflex-driven accentuations in SNA. Alternatively, it is also possible that the sympathetic response to activation of the EPR is normal, and it is the sensitivity of the vasculature to that is increased in this disease. In support of the former, Delaney et al. (7) demonstrated recently that the muscle SNA response to activation of one component of the EPR, the metaboreflex, was elevated in older hypertensive patients compared with their normotensive counterparts. Whether this is also true of the muscle mechanoreflex remains undetermined, since no studies have measured the SNA response to activation of this component of the EPR in either hypertensive patients or animal models of human hypertension. This is an important point given that the mechanoreflex and metaboreflex are activated by distinctly different stimuli; the former predominantly by the mechanical distortion of skeletal myocytes within muscle and the latter primarily by the metabolic by-products of skeletal muscle work. Likewise, no studies to date have investigated the EPR-mediated SNA response in hypertension. Because EPR function is determined by the concomitant activation of both the mechanoreflex and metaboreflex (18, 34, 35), alterations, or lack thereof, in each individual component of the reflex can potentially affect their overall function. In addition, no attempts have been made to determine any of the possible receptor mechanisms that may contribute to EPR overactivity in this disease.

Therefore, the purpose of this study was to determine the SNA response to activation of the EPR as well as the muscle mechanoreflex in hypertension. In addition, the role of skeletal muscle mechanoreceptors in mediating EPR and mechanoreflex induced changes in blood pressure, and SNA in hypertension was also investigated. The results of these studies may prove beneficial in the identification of novel therapeutic targets (e.g., SNA, skeletal muscle mechanoreceptors) for the treatment of muscle reflex overactivity in hypertension, potentially reducing the risks associated with exercise in this disease.

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MATERIALS AND METHODS

Experiments were performed in age-matched (14–20 wk) male Wistar-Kyoto (WKY; n = 21) and spontaneously hypertensive (SHR; n = 45) rats (Harlan). Animals were housed in standard rodent cages on 12:12-h light-dark cycles and were given food and water ad libitum. The procedures outlined were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas. All studies were conducted in accordance with the US Department of Health and Human Services National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Physiological Preparations

General surgical procedures. As described previously (46), rats were initially anesthetized with isoflurane gas (2–4% in 100% oxygen) and intubated for mechanical ventilation (Model 683; Harvard). Isoflurane levels were increased as needed if a withdrawal reflex to pinching of the hindpaw was displayed, a corneal reflex was present, and/or HR increased spontaneously. Liquid-filled catheters were placed within the right jugular vein for the administration of solutions and both common carotid arteries for the measurement of ABP (MLT0380/D; ADInstruments). Needle electrodes were placed on the back of the animal to obtain electrocardiogram (ECG) recordings. The ECG signal was amplified (Animal Bio Amp; ADInstruments). To stabilize fluid balance and maintain baseline ABP, a continuous infusion of solution (2 ml of 1 M NaHCO₃ and 10 ml of 5% dextrose in 38 ml of Ringer solution) was established via the jugular vein (3–5 ml·h⁻¹·kg⁻¹). To minimize edema, 0.2 mg of dexamethasone was given intramuscularly. Arterial blood gases and body temperature were maintained within normal ranges throughout the experiment. To record renal SNA (RSNA), the renal nerve was exposed and attached to a pair of stainless-steel wire electrodes (Bioflex wire AS633; Cooner Wire) through a left flank incision. The nerve and electrodes were covered with silicone glue (Kwik-Sil; World Precision Instruments, Sarasota, FL) for insulation and fixation. To quantify nerve activity, the preamplified nerve signal was band-pass filtered at 150–1000 Hz (Neuro Amp EX; ADInstruments) and then full-wave rectified and low-pass filtered with a time constant of 33.3 ms. Animals were held in a stereotactic head unit (Kopf Instruments), and a precocilliac decerebration was performed. In this procedure, a bilateral craniotomy was conducted by drilling holes into parietal skull. The bone superior to the central sagittal sinus was removed. The dura mater covering the brain was cut and the cerebrum aspirated. The animal was rendered insentient by sectioning the brain rostral to the superior colliculus and removing the remaining forebrain. To minimize cerebral hemorrhage, small pieces of oxidized regenerated cellulose (Ethicon; Johnson & Johnson) were placed on the internal skull surface, and the cranial cavity was packed with cotton. Immediately following the decerebrate procedure, gas anesthesia was discontinued. A minimum recovery period of 1.25 h was employed after decerebration before any experimental protocol was begun. This allowed sufficient time for the effects of isoflurane anesthesia to completely dissipate and blood pressure to stabilize (25).

Surgical procedures for muscle contraction and stretch. A laminectomy exposing the lower lumbar portions of the spinal cord (L₂–L₆) was performed. The meningial layers surrounding the cord were cut and reflected. The L₄ and L₅ ventral roots were carefully isolated and sectioned. The cut peripheral ends of the roots were placed on bipolar platinum electrodes. The exposed neural tissue was immersed in mineral oil. Steel posts were used to stabilize the pelvis in a customized spinal frame. The right hindlimb was fixed in one position by placing a clamp around the tibial bone. The gastrocnemius and soleus muscles of the right hindlimb were isolated. The calcaneal bone of the right hindlimb was cut and the Achilles’ tendon connected to a force transducer for the measurement of muscle tension (FT-10; Grass Instruments).

Surgical procedures for intra-arterial injections within the hindlimb. To allow the injection of chemicals into the arterial supply of the right leg, the circulation of the hindlimb was surgically isolated. A catheter (PE-10, polyethylene tubing) was placed in the left common iliac artery and its tip advanced to the bifurcation of the abdominal aorta. This procedure allowed the direct injection of chemicals into the right common iliac artery without the arterial circulation of the right hindlimb being occluded. A reversible vascular occluder was placed around the common iliac vein, emptying the right hindlimb. This limited the delivery of chemicals to the right hindlimb, thus preventing entrance into the general circulation.

Experimental Protocols

Stimulation of the EPR. The EPR was stimulated in WKY (n = 14) and SHR (n = 39) animals by contracting the gastrocnemius and soleus muscles of the right hindlimb for 30 s via electrical stimulation of isolated L₄ and L₅ ventral roots. This procedure is known to activate both the mechanically and metabolically sensitive components of the EPR concomitantly (37). Constant current stimulation was used at three times motor threshold (i.e., the minimum current required to produce a muscle twitch) with a pulse duration of 0.1 ms at 40 Hz. These stimulus parameters have been demonstrated to elicit maximal levels of developed tension in this rat model (46). Importantly, these procedures are known to evoke changes in cardiovascular hemodynamics via selective activation of the EPR independent of input from central command, a neural mechanism originating in higher brain centers known to contribute importantly to autonomic regulation during exercise (46). To assess responses to graded activation of the EPR, additional contractions were induced using randomized submaximal stimulus intensities (i.e., 1, 1.5, and 2 times motor threshold, 0.1-ms pulse duration, 40 Hz) in a subset of WKY (n = 9) and SHR (n = 8) animals. Before any contraction, muscles were preloaded with 70–100 g of tension. The neuromuscular blocking agent vecuronium bromide (1 mg ml⁻¹) was administered intravenously, and the ventral roots were stimulated at three, five, and 10 times motor threshold at the conclusion of each experiment. This experimental control was performed to confirm that any changes in MAP, HR, and RSNA in response to stimulation of the EPR were due to muscle contraction rather than the inadvertent activation of sensory afferent fibers during execution of electrical stimulation procedures.

Stimulation of the mechanically sensitive component of the EPR. To selectively activate the mechanically sensitive component of the EPR, the gastrocnemius and soleus muscles of the right hindlimb were passively stretched using a calibrated 9.5-mm rack and pinion system (Harvard Apparatus) in WKY (n = 12) and SHR (n = 37) animals. To evoke a mechanical stimulus similar to that elicited during muscle contraction, care was taken to generate the same pattern of muscle tension developed during maximal static contractions. In a subset of WKY (n = 8) and SHR (n = 6) rats, additional stretches were performed at randomized intensities (i.e., 0.25, 0.5, 0.75, and 1 kg tension developed). Before all maneuvers, muscles were preloaded by stretching to 70–100 g of tension. It should be noted that stretching skeletal muscle in this manner does not increase muscle metabolism and, therefore, is often used to preferentially engage the stretch-sensitive afferent fibers associated with the muscle mechanoreflex (50).

Pharmacological blockade of mechanoreceptors. Although passively stretching muscle is commonly used to assess mechanoreflex function, recent evidence suggests that stretch activates a different population of mechanically sensitive afferent fibers than does contraction, although some overlap exists (11). Therefore, we performed an additional protocol to assess the contribution of the muscle mechanoreflex to EPR overactivity in hypertension during skeletal muscle contraction. The trivalent lanthanide gadolinium was used to antagonize stretch-sensitive mechanoreceptors in the hindlimb skeletal muscle of 13 SHR animals. Gadolinium has been shown to effectively

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block the activity of mechanically sensitive afferent fibers in skeletal muscle (12, 13, 20). As such, it can also be used as a pharmacological technique by which the mechanoreceptor contribution to EPR function can be investigated. To begin, baseline resting tension was set to 70–100 g, and the muscle was contracted using the stimulus parameters described. After a >10-min recovery period, the hindlimb muscles were passively stretched at tensions approximately equivalent to those achieved during contraction. Next, gadolinium (10 mmol/l, 0.25 ml) was administered by injection into the right common iliac artery, which supplies the arterial circulation for the right hindlimb. Upon injection, the right common iliac vein was occluded for 15 min by tightening a reversible ligature trapping gadolinium within the circulation of the right leg. Sixty minutes after injection of gadolinium, contraction and stretch protocols were repeated. This dose of gadolinium and time frame for its action were based on previous studies (12, 48). As a control, this protocol was repeated in 16 SHR rats with the vehicle for gadolinium (i.e., isotonic saline) administered into the right common iliac artery.

At the conclusion of all experiments, an intravenous infusion of hexamethonium bromide (60 mg/kg) was used to abolish SNA signals to confirm that the recorded signals represented renal sympathetic fibers. RSNA background noise was determined over a 30-min period after the incisant decerebrate animal was humanely euthanized by intravenous injection of saturated potassium chloride (4 M, 2 ml/kg). Use of this procedure adheres to the guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. In all animals, the heart and lungs were excised and weighed. Additionally, the tibia was harvested, weighed, and measured.

Data Acquisition

All cardiovascular, RSNA, and contractile force data were acquired, recorded, and analyzed using data acquisition software (LabChart; ADInstruments) for the Powerlab8/30; ADInstruments) at a 1-kHz sampling rate. A pressure transducer connected to the left carotid arterial catheter was used to measure ABP continuously. HR was calculated from the time between successive R-waves in the ECG recording. Hindlimb muscle tension was measured by a force transducer. To analyze sympathetic recordings, full-wave rectified signals of RSNA as well as background noise signals were obtained. The noise signal component, which was defined as the signal recorded postmortem, was subtracted from rectified RSNA. To quantify RSNA responses to muscle contraction and stretch, basal measurements were obtained by taking the mean value of 30 s of baseline data immediately prior to the maneuver. This mean was considered 100% of basal RSNA. Subsequently, relative changes in RSNA (ΔRSNA, %) from this baseline were evaluated every second. Integrated ΔRSNA (∫ΔRSNA, arbitrary units) was calculated by summing ΔRSNA during 30-s contraction or stretch. Data sets of 1-s averages for MAP, HR, RSNA, and hindlimb tension were analyzed. Baseline values for all variables were determined by evaluating 30 s of recorded data before a muscle contraction or stretch. The peak response of each variable was defined as the greatest change from baseline elicited by contraction or stretch. Tension-time index (TTI; kg/s), an index of developed muscle tension during contraction or stretch, was calculated by integrating the developed tension (integrated total tension minus integrated baseline tension prior to the maneuver) during the contraction or stretch period (22, 23).

Statistical Analyses

Data were analyzed using unpaired t-tests (WKY vs. SHR) and two-way repeated-measures ANOVA (developed tension × rat group, drug effect × trial). If significant interaction and main effects were observed with ANOVA, a post hoc Tukey’s test was used to identify differences between specific group means. The strength of the correlative relationship between parameters was assessed using Pearson’s product-moment coefficients. Linear relationships between variables were examined using linear regression analysis. The significance level was set at \( P < 0.05 \). Results are presented as means ± SE.

RESULTS

Characterization of Hypertensive Model

Morphometric characteristics and baseline hemodynamics for WKY and SHR animals are presented in Table 1. The mean body weight of WKY rats was slightly but significantly smaller than SHR animals. Ratios of heart weight to body weight and heart weight to tibial length were significantly greater in SHR compared with WKY rats. The lung weight/body weight ratio was not different between groups. Baseline MAP was significantly higher in SHR than in WKY animals, whereas baseline HR was not different between groups. Baseline signal-to-noise ratios for RSNA were not different between groups.

The RSNA Response to EPR Activation is Augmented in Hypertensive Rats

The blood pressure and sympathetic responses to stimulation of the EPR were exaggerated in hypertensive compared with normotensive rats. An example of this finding is presented in representative tracings from WKY and SHR animals (Fig. 1). At maximal intensities, activation of the EPR during electrically induced static muscle contraction elicited significantly larger elevations in MAP and RSNA in SHR compared with WKY rats (Fig. 2). Notably, despite the similar levels of developed tension between groups, the exaggerated sympathetic response to EPR activation in SHR was observed in both the peak (i.e., ΔRSNA) as well as the integrated (i.e., ∫ΔRSNA) response. The HR response to contraction was not statistically different between SHR (12 ± 1 beats/min) and WKY (9 ± 2 beats/min) rats (\( P = 0.27 \)). Graded contractions at submaximal work intensities evoked sympathetic responses that were positively correlated and linearly related to TTI in both WKY (\( y = 34.6x - 18 \), \( r^2 = 0.73 \), \( P = 0.06 \)) and SHR (\( y = 62x - 16 \), \( r^2 = 0.95 \), \( P < 0.01 \)) animals (Fig. 3A). Linear regression analysis determined that the slope of this relationship was 50 ± 25 in SHR and 35 ± 13 in WKY. However, these differences in slope were not found to be statistically significant (\( P = 0.60 \)). For comparison between groups, data were binned into levels corresponding to 0–25, 26–50, 51–75, and 76–100% of maximal TTI. This analysis determined that the RSNA response to EPR activation was greater in SHR compared with WKY at an intensity level corresponding to

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<th>Table 1. Morphometric characteristics and baseline hemodynamics</th>
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<td><strong>WKY (n = 21)</strong></td>
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Values are means ± SE. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity. *\( P < 0.05 \) compared with WKY rats.
51–75% maximal TTI (Fig. 3B). Neuromuscular blockade with vecuronium bromide completely abolished the RSNA, HR, and MAP responses to electrical stimulation of the L4 and L5 ventral roots.

The RSNA Response to Activation of the Mechanically Sensitive Component of the EPR is Augmented in Hypertensive Rats

The pressor and sympathetic responses to activation of the mechanically sensitive afferent arm of the EPR via passive muscle stretch were enhanced in hypertensive compared with normotensive rats. An example of this finding is presented in representative tracings from WKY and SHR animals (Fig. 4). In agreement with a previous report (28), stretching hindlimb skeletal muscle elicited significantly larger elevations in MAP in SHR compared with WKY rats. HR responses were likewise significantly elevated in SHR compared with WKY, although the changes evoked in both group of animals were relatively modest. In addition, the stretch-evoked increases in RSNA were markedly greater in SHR than in WKY animals (Fig. 5). Similar to muscle contraction, the exaggerated sympathetic responses to passive stretch in SHR were observed in both the peak (i.e., ΔRSNA) as well as the integrated (i.e., ∫ΔRSNA) response. However, these responses were smaller in magnitude and more transient than those elicited by contraction. Grading the stretch intensity demonstrated that the changes in RSNA were positively correlated and linearly related to the amount of tension developed in both WKY (y = 8x + 33, r^2 = 0.71, P = 0.22) and SHR (y = 85x + 26, r^2 = 0.97, P < 0.05) animals (Fig. 6A). Liner regression analyses determined that the slope of this relationship was significantly larger (P < 0.05) in SHR (85 ± 27) than in WKY (9 ± 11). For comparison between groups, data were binned into levels corresponding to 1–25, 26–50, 51–75, and 76–100% of maximal developed tension. The passive stretch-induced increase in RSNA was larger in SHR compared with WKY rats beginning at
Intensity levels corresponding to 26–50% of maximal developed tension (Fig. 6B).

**Blockade of Mechanoreceptors Attenuates the Reflex Response to Muscle Contraction and Stretch in Hypertensive Rats**

In hypertensive rats, the MAP, HR, and RSNA responses to contraction were significantly reduced by pharmacologically antagonizing stretch-sensitive skeletal muscle mechanoreceptors with gadolinium (Fig. 7). The pressor, tachycardic, and sympathetic responses to passive stretch were similarly affected by gadolinium in SHR (Fig. 8). In contrast to gadolinium, saline had no effect on the MAP, HR, and RSNA responses to either muscle contraction or stretch. Although the MAP and RSNA responses to contraction and stretch remained greater in SHR treated with gadolinium compared with untreated WKY (i.e., animals in which mechanoreceptors were not blocked), they were more comparable. For example, after mechanoreceptor blockade, the increases in MAP and RSNA in response to contraction were 18.4 ± 2.7 mmHg and 92.7 ± 10.3%, respectively, in SHR compared with 8.6 ± 2.4 mmHg and 47.7 ± 8.4%, respectively, in untreated WKY. Gadolinium produced a similar effect during stretch, with the MAP and RSNA responses being 18.1 ± 3.5 mmHg and 45.3 ± 6.6%, respectively, in SHR compared with 9.6 ± 2.6 mmHg and 35.0 ± 6.5%, respectively, in untreated WKY.

**DISCUSSION**

In this study, it has been demonstrated for the first time that the sympathetic response to activation of the EPR and mechanoreflex is accentuated in hypertension. These aberrantly large augmentations in SNA likely mediate the exaggerated pressor response evoked by muscle reflex stimulation in this disease. Importantly, this investigation provides evidence that the abnormal EPR and mechanoreflex-induced alterations in SNA and blood pressure in hypertensive rats are mediated, in part, by skeletal muscle mechanoreceptors.

Given that the EPR normally evokes circulatory adjustments to physical activity via increases in SNA (31, 34, 37), the results of this study are not surprising yet not as intuitive as might be expected. For example, we have demonstrated previously that the enhanced changes in MAP during EPR activation in hypertensive rats were completely abolished by ganglionic and sympathetic blockade with hexamethonium and phenolamine, respectively (49). Although these findings suggested that the blood pressure changes in response to activation of the EPR were indeed sympathetically mediated, they did not quantify the magnitude of the SNA response. As a result, it could not be determined in these studies whether the accentuated pressor response in hypertensive rats resulted from EPR-induced exaggerations in SNA or from some other mechanism. It is plausible that the SNA response to stimulation of the EPR is normal in hypertension but that the sensitivity of the vasculature to this autonomic input is increased (51). Although this...
remains a possibility, the results from the present study strongly suggest that the enhanced pressor response to activation of the EPR is mediated by abnormally large augmentations in SNA.

In normal healthy cats and rats, activation of the mechanoreflex during muscle contraction and stretch has been shown to reflexively increase RSNA (20, 24, 32, 52). The results of this investigation extend these findings by providing evidence that the RSNA response mediated by the mechanoreflex is abnormally accentuated in hypertension, contributing significantly to EPR dysfunction in this disease. Close examination of the RSNA response patterns to both static muscle contraction and stretch support this contention. For example, the peak of the exaggerated RSNA response in SHR was most often observed at the onset of muscle contraction. In addition, the accentuated RSNA response to passive stretch, a more pure mechanoreflex stimulus, was abrupt and transient in SHR. Consequently, the integrated RSNA response to muscle stretch was smaller than the response to muscle contraction. These findings are consistent with the known discharge properties of mechanically sensitive group III afferent fibers associated with the mechanoreflex, which are activated immediately at the onset of contraction or stretch (17, 18, 35).

Interestingly, in response to muscle contraction, not only was the peak change in RSNA greater in SHR than in WKY, but so too was the integrated change in RSNA. In fact, the integrated change in RSNA to contraction (which takes into account the full RSNA response) was significantly correlated to TTI (i.e., integrated developed tension). This suggests that, in addition to the mechanoreflex, another muscle input may be contributing to the EPR dysfunction manifest in hypertension. A good candidate for this additional input is the muscle metaboreflex. Group IV afferent fibers associated with the metaboreflex are known to increase their activity 15–20 s following the onset of the muscle contraction (17, 18, 21, 35), a time period that could account for the larger integrated RSNA response demonstrated in SHR. Supporting this concept, recently published data in older hypertensive humans have dem-

Fig. 4. Characteristic cardiovascular and sympathetic responses elicited by stimulation of the mechanically sensitive component of the EPR in representative WKY and SHR animals. Passively stretching hindlimb skeletal muscle evoked increases in ABP as well as raw and normalized RSNA that were larger in hypertensive compared with normotensive rats. Insets: the ordinate is expanded for WKY so that the ABP and RSNA responses to stretch can be seen clearly. Arrows demarcate the onset of passive stretch.
onstrated that activation of the metaboreflex elicits augmented increases in SNA (7, 44). It should be noted that this finding is not universal. It has also been reported in middle-aged hypertensive patients that metaboreflex-mediated alterations in SNA are blunted (43). The reason for the disparate results is not clear but highlights the need for further investigation in this area.

The findings of the present study also provide evidence that skeletal muscle mechanoreceptors mediate, at least in part, the exaggerated sympathetic and pressor responses to EPR and mechanoreflex activation in hypertension. For example, static contraction increased MAP by 36 ± 6% from baseline in SHR before the administration of the mechanoreceptor antagonist gadolinium but only 16 ± 2% after its administration. This gadolinium-induced reduction in MAP in SHR was much larger than that reported previously for normotensive rats (48). For comparison, in the investigation in which healthy animals were studied, static contraction increased MAP by 18% from baseline prior to the administration of gadolinium and 10% from baseline after its administration. Perhaps more importantly, in this study, pharmacological blockade of mechanoreceptors partially corrected the abnormal SNA and pressor responses mediated by the EPR and mechanoreflex in SHR, making them more comparable with those elicited in normotensive WKY. It should be noted that gadolinium has been shown to block several types of mechanoreceptors, including mechanogated potassium channels, L- and T-type calcium channels, and mechanogated cation channels (10, 12). Therefore, the specific type of mechanoreceptor that mediates that the abnormal sympathetic and pressor responses to muscle reflex activation cannot be discerned from this study.

Although the results from the present study strongly suggest that the enhanced pressor response to EPR and mechanoreflex activation is mediated by abnormal increases in SNA, caution is warranted in extending this conclusion to the HR responses mediated by these reflexes. Indeed, the HR response to contraction was not found to be significantly different between WKY and SHR animals in this study. In contrast, the HR response to stretch was demonstrated to be significantly greater in SHR compared with WKY. Despite this difference, the HR response in both groups of animals was small, bringing into question the physiological relevance of the statistical significance.

There are several viable possibilities for the enhanced SNA response to activation of the EPR and mechanoreflex in this study, although no definitive conclusions can be drawn. Alterations could occur at any point in the pressor reflex arc upstream from its sympathetic efferent arm. This includes alterations in the sensitivity and/or density of skeletal muscle receptors, as could be the case with the mechanoreceptors examined in this study. Changes in the excitability or transmission characteristics of skeletal muscle afferent fibers and/or processing of afferent signals in the spinal cord or brain could likewise contribute. To give a few examples, it has been demonstrated that the production of reactive oxygen species in...
exercising muscle is increased in angiotensin II-dependent hypertensive rats (55). Increased oxidative stress may serve to sensitize skeletal muscle receptors and afferent fibers in skeletal muscle. Lending support to this concept, it has been demonstrated recently that elevated reactive oxygen species production in skeletal muscle generates an exaggerated SNA response to mechanoreflex activation in heart failure, a disease state that often develops from prolonged hypertension (22). As another example, sensory information generated by activation of the EPR is known to be processed within the nucleus tractus solitarius of the medulla oblongata (16). The activity of neurons within the medulla that receive and process this information is modulated by the endogenous production of nitric oxide (NO) (29, 47). Since the phosphorylation of NO synthase (the enzyme responsible for generating NO from L-arginine) is decreased by the presence of elevated angiotensin II within the nucleus tractus solitarius of hypertensive rats (3), a reduction in the availability of NO could mediate the exaggerated SNA response to activation of the EPR and mechanoreflex in hypertension. Clearly, further research is needed to definitively determine the causes underlying abnormal sympathetic regulation by muscle reflexes in this disease.

**Functional Implications**

In the present study, it is clear that the sympathetic response to maximal static muscle contraction is significantly larger in hypertensive animals compared with normotensive controls. However, muscle is rarely, if ever, maximally contracted under normal physiological conditions. Therefore, it was also important to determine the alterations in RSNA in response to muscle contraction of submaximal intensity. For example, the integrated sympathetic response to stimulation of the EPR via static muscle contraction was 2.7- and 1.9-fold greater in SHR than WKY at 51–75 and 76–100%, respectively, of maximal TTI. Even at 26–50% of maximal TTI, the RSNA response to muscle contraction was 2.9-fold larger in SHR than in WKY, although absolute differences were not statistically significant at this intensity of exercise. A similar response pattern was produced during stretch experiments. These results suggest that, in hypertension, the EPR and its mechanosensitive component are capable of driving markedly accentuated increases in SNA even at low to moderate work intensities. This is a significant finding when one considers that basal SNA is commonly elevated in hypertension (1, 33, 54) and is associated with the progression of end organ damage (e.g., vascular hypertrophy, atherogenesis) (8, 30). Acute exaggerations in SNA mediated by the EPR during moderate exercise could potentially exacerbate and/or accelerate this damage.

**Methodological Considerations**

Autonomic adjustments regulating the cardiovascular system during exercise are determined by integrating input from the EPR as well as central command (9) and the arterial baroreflex (42). These neural inputs are known to interact within discrete nuclei within the brain stem (4–6). Although the decerebrate procedure utilized in this study does not completely remove all components of the central command pathway, it does eliminate the areas of the cerebral cortex from which central command originates. In addition, because muscle contraction and stretch are induced involuntarily in this rat model, central command pathways would likely not be activated. Therefore, central command activity is unlikely to contribute to the SNA responses reported. With regard to the baroreflex, it has been shown that its sensitivity is often reduced in hypertension (27, 36, 40). It has been demonstrated that the cardiovascular response to activation of the EPR is enhanced in normotensive barodenervated animals, suggesting that the baroreflex normally acts to buffer EPR activity (53). As such, it is possible that the EPR-mediated exaggerations in SNA are partly due to a decrease in the buffering capacity of the baroreflex. However, we established previously that the baroreflex maintains its ability to buffer the EPR in hypertensive rats (49). Therefore, contributions by the baroreflex to the accentuated SNA response to EPR activation would be expected to be minimal.

In this study, mechanoreceptors were pharmacologically antagonized with gadolinium in SHR animals. Gadolinium
experiments were not performed in WKY animals. The rationale for this study design was twofold. 1) Similar experiments utilizing gadolinium have been performed previously in normotensive rats (48), and 2) by virtue of these previous studies, a role for these receptors has been established in healthy rats. Therefore, the focus of the present research was to determine the contribution of these receptors to the abnormal pressor and sympathetic responses mediated by the EPR and mechanoreflex in hypertensive rats. In addition, the concentration of gadolinium used attenuated but did not abolish the sympathetic or pressor responses to muscle contraction and stretch. This finding is consistent with previous reports using this pharmacological agent in rats and cats (12, 20, 48). Therefore, it is likely that some mechanoreceptors were not effectively antagonized by the dose of gadolinium utilized. This possibility should be taken into account when interpreting the results of this study.

Conclusions

In summary, the findings of this study suggest that the exaggerated pressor response to activation of the EPR in hypertension is elicited by abnormally large reflex-induced augmentations in SNA. The evidence further supports the contention that the muscle mechanoreflex contributes significantly to the EPR-evoked accentuation in SNA. Importantly, the data suggest that muscle mechanoreceptors mediate, in part, this enhanced sympathetic and pressor responsiveness to muscle reflex activation in this disease.

ACKNOWLEDGMENTS

We thank Martha Romero and Julius Lamar, Jr. for their expert technical assistance.

GRANTS

This research was supported by grants from the National Heart, Lung, and Blood Institute (HL-088422 to S. A. Smith) and the Lawson & Rogers Lacy Research Fund in Cardiovascular Disease (to J. H. Mitchell). M. Mizuno was supported by a Research Fellowship from the Japan Society for the Promotion of Science for Young Scientists.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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