Muscle mechanoreflex mediates vasoconstriction in inactive limb in rats

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Abstract We determined whether, stimulation of mechanosensitive receptors in active muscle (muscle mechanoreflex activation) induces vasoconstriction in the contralateral non-active muscles. In mid-collicular decerebrated rats (n = 9), we measured the blood flow of the left iliac artery when the right Achilles tendon was stretched by 300 g for 30 seconds (s) to stimulate mechanoreceptors in the triceps surae muscles. The stretch significantly increased the mean arterial pressure (MAP: +20 ± 3%, peak increase from baseline; mean ± SEM) and decreased the vascular conductance (VC) in the left iliac artery (–9 ± 1%, averaged over the stimulation period). After cutting the left sciatic nerve, the stretch did not significantly change the VC (+5 ± 1%), while it significantly increased MAP (+13 ± 3%, peak). In conclusion, the muscle mechanoreflex plays a role in mediating vasoconstriction in the contralateral limb via sympathetic activation.

Keywords: vasoconstriction, mechanoreflex, sympathetic nerve

Introduction

Exercise increases skeletal muscle blood flow up to roughly 100 mL/100 g tissue/min from a resting value of about 5 mL/100g tissue/min[10]. To increase the blood flow to exercising skeletal muscle, vasoconstriction is required in organs other than active skeletal muscles, including inactive limbs[4]. Nevertheless, the underlying mechanisms regulating vasomotor tone in inactive limbs are poorly understood.

During exercise, neural signals from active muscles reflexly increase sympathetic nerve activity (SNA). This sympathoexcitatory mechanism is called the exercise pressor reflex[5]. This reflex is generated by the activation of mechanically (mechanoreflex) and chemically (metaboreflex) sensitive skeletal muscle receptors. The role of the metaboreflex in regulating vasomotor tone within inactive limbs has been examined in human studies. It has been well documented that an occlusion of active limb circulation, following static exercise, increases SNA to inactive muscles[6] and induces vasoconstriction in the inactive forearm and/or calf resistance vessels[7] and renal artery[8]. These findings suggest that activation of the muscle metaboreflex contributes to vasoconstriction in inactive limbs via sympathetic activation.

On the other hand, the contribution of the mechanoreflex to the vasomotor regulation in resting limbs remains unclear. Cui and colleagues[9] demonstrated that SNA, directed to inactive muscles, increases during activation of the mechanoreflex by passive muscle stretch. However, the effect of the increase in SNA on the vasomotor tone of the contralateral unstimulated limbs was not elucidated. Thus, we designed the present experiment to determine the role of the muscle mechanoreflex in regulating vasomotor tone in inactive skeletal muscle. In decerebrated rats, we observed changes in blood flow and vascular conductance (VC) in the inactive hindlimb during stretching of the contralateral hindlimb muscle, which selectively stimulates muscle mechanoreceptors[10], and compared the responses before and after surgical removal of innervation of the muscle.

Materials and methods

Preparation and measurements. All experimental procedures in the present study were approved by the Research Ethics Committee of School of Health and Sport Sciences, Osaka University. Nine Sprague-Dawley male rats (7 weeks of age, weight 250 - 320 g) were anesthetized with a mixture of halothane (4%) and oxygen. The trachea was cannulated, and the lungs were artificially ventilated with a respirator (SN-480-7, Shinano, Japan). The left jugular vein and common carotid artery were cannulated to administer drugs and to measure arterial pressure, respectively. The carotid catheter was attached to a pressure transducer (P23XL-1, Ohmeda, USA). Arterial blood pH was measured by 60 min intervals with a pH meter (B-212, Horiba, Japan), and was maintained...
by infusing sodium bicarbonate solution (8.4%) intravenously or by changing the artificial minute ventilation. Needle electrodes were set on the back of the rat for electrocardiogram (ECG) measurements. The ECG signal was acquired with an amplifier (AB-621G, Nihon-Koden, Japan). Heart rate (HR) was calculated beat to beat with detection of the time between successive R waves in the ECG. Body temperature was maintained adequately with a heating lamp.

The right triceps surae muscles were isolated. The right calcaneal bone was severed and attached to a string. Skin flaps were attached to bars to form a pool filled with mineral oil. All visible nerves, except for those innervating the triceps surae muscles, were cut. A small incision was made in the left leg to mark the left sciatic nerve. An abdominal incision was made at midline. The iliac artery supplies blood to organs other than the leg muscles. Thus, the left internal iliac, inferior epigastric and deep circumflex iliac arteries were ligated. The probe of an electromagnetic flowmeter (MFV2100, 1 mm diameter probe, Nihon-Koden) was placed on the left iliac artery.

The rat was placed in a stereotaxic apparatus (ST-7, Narishige, Japan). Decerebration at the midcollicular level was performed as previously described in detail. Immediately after decerebration, anesthesia was stopped. A recovery period of 2 h was allowed to eliminate the effects of halothane and to stabilize the preparation.

Protocols. The right triceps surae muscles were preload - ed with 20 g of tension in a controlled period. After collecting baseline data, the muscles were passively stretched by 300 g for 30 s. At the beginning of the stretch, the weight was brought down by the experimenter’s hand. This recording was repeated three times. Then, the left sciatic nerves were cut to remove the sympathetic components mainly innervating the inactive left triceps surae muscles. Thirty minutes after cutting, the data were again collected with the same protocol described above (denervated condition). After finishing all protocols, the rat was euthanized by an intravenous overdose of pentobarbital sodium (100 mg/kg).

Statistics. All measured variables were stored on a hard disk through analog-digital conversion (Powerlab/8s, ADInstruments). The values were averaged beat by beat and then averaged over 1 s. The vascular conductance (VC) of the left inactive hindlimb was obtained by dividing the iliac blood flow by the mean arterial pressure (MAP). The time-serial change of variables to activation of the mechanoreflex was tested by repeated measures ANOVA over time. When a significant difference was detected with time, this was further examined by the Dunnett’s post-hoc test against the baseline condition. The peak change was obtained from the point where the maximal change in absolute value was found in 2 s averaged data. Statistical significance was accepted at P < 0.05. These statistical analyses were performed with SAS ver. 8.2 (SAS Institute, USA) at the Computing and Communications Center, Kyushu University. Values are expressed as means ± SEM.

Results

The baseline MAP and HR before stretching the muscles were 85 ± 6 mmHg and 329 ± 23 beats/min (bpm) in the intact, and 85 ± 6 mmHg and 326 ± 30 bpm in the

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**Fig. 1** The responses (Mean ± SE) of mean arterial pressure (MAP; top), iliac blood flow (IBF; middle), and vascular conductance (VC; bottom) to 30 s passive stretch of the triceps surae muscles at 300 g force shown as the filled bars (0 - 30 s) in intact (filled circles) and denervated (open circles) conditions. The stretch significantly increased MAP in both conditions. The stretch significantly decreased IBF and VC in the intact condition, whereas the significant changes were not obtained in the denervated one. *(asterisk): the significant difference compared to the baseline value (P < 0.05).*
denervated condition. The baseline iliac blood flow (IBF) and VC were 2.0 ± 0.3 mL/min and 0.023 ± 0.004 mL/min/mmHg in the intact, and 2.6 ± 0.5 mL/min and 0.031 ± 0.008 mL/min/mmHg in the denervated condition. There were no significant differences in these variables between intact and denervated conditions.

Passive stretch of the triceps surae muscles, with 300 g force, significantly increased the MAP in both conditions (Fig. 1). The increase in MAP lasted longer in the intact condition, while in the denervated condition it returned to the baseline level within 20 s after the onset of the muscle stretch. The stretch significantly decreased IBF and VC in the intact condition (Fig. 1). In the denervated condition, on the other hand, the significant changes in IBF and VC during muscle stretch were not observed (Fig. 1). The stretch did not significantly change the HR in either condition (mean HR during stretch; 320 ± 40 and 333 ± 25 bpm in intact and denervated conditions, respectively).

The peak relative changes in MAP, iliac BF, and VC were assessed in each condition (Fig. 2). The relative change in the MAP at individual peak pressure response was significantly greater in the intact condition than in the denervated one (20 ± 3 % vs. 13 ± 4% increase from baseline). The change in VC at peak response was in the opposite direction, and was significantly lower in the intact condition than in the denervated one (-9 ± 1% vs. 5 ± 1%).

Discussion

We observed that passive stretch of triceps surae muscles decreased the IBF and VC in contralateral non-stimulated limbs. The decreases in the IBF and VC, in the contralateral hindlimb, were abolished by dissecting the sciatic nerve, which includes sympathetic components mainly innervating the triceps surae muscles. These results suggest that activation of the muscle mechanoreflex plays a role in decreasing blood flow and vascular conductance in the resting limb through sympathetic activation.

Roles of the muscle mechanoreflex in cardiovascular regulation have been studied through the use of stretching animal and human muscles. Muscle stretch in anesthetized or decerebrate animals has been previously reported to increase blood pressure, heart rate, and renal, cardiac, and lumbar SNA13-16), and to cause renal vasoconstriction13,15). Passive muscle stretch in humans has also been reported to increase heart rate7) and muscle SNA10). Of note, another study showed that this muscle stretch did not increase muscle SNA in healthy humans18). The present rat study provided information on the significant role of this reflex in mediating vasoconstriction in inactive limbs.

Decreasing the VC in organs, other than exercising muscle, is important for increasing the blood flow to exercising muscle (e.g., the review19)). Also, considering the remarkable increase of the VC in exercising muscle, vasoconstriction is important to maintain adequate MAP, and secure cerebral blood flow. The roles played by either muscle mechanoreflex or metaboreflex in inducing vasoconstriction in the kidneys have been previously reported9,20). The mechanism found in this study, namely mechanoreflex-induced vasoconstriction in inactive limbs, may also contribute to maintaining MAP and securing cerebral flow during exercise.

Nevertheless, it should be noted that the possibility remains of the denervation decreasing baseline vascular tone in innervating muscles, changing the relative contribution of VC responses among tissues, i.e., kidneys, inactive limbs and stimulating muscles.

In conclusion, muscle stretch decreased the IBF and VC in inactive limbs; and eliminating sympathetic components in rats abolished such decreases. We suggest that the muscle mechanoreflex plays a role in mediating vasoconstriction within inactive limbs.

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**Fig. 2** The relative change (Mean ± SE) of peak values in mean arterial pressure (MAP), iliac blood flow (BF (iliac)), and its vascular conductance (VC) in intact and denervated conditions (IC and DC, respectively). *(asterisk)*: the significant difference between both conditions (P < 0.05).
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