Muscle mechanoreflex induces the pressor response by resetting the arterial baroreflex neural arc

Kenta Yamamoto,1 Toru Kawada,1 Atsunori Kamiya,1 Hiroshi Takaki,1 Tadayoshi Miyamoto,1,2 Masaru Sugimachi,1 and Kenji Sunagawa1

1Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Osaka 565-8565; and 2Japan Association for the Advancement of Medical Equipment, Tokyo 113-0033, Japan

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Yamamoto, Kenta, Toru Kawada, Atsunori Kamiya, Hiroshi Takaki, Tadayoshi Miyamoto, Masaru Sugimachi, and Kenji Sunagawa. Muscle mechanoreflex induces the pressor response by resetting the arterial baroreflex neural arc. Am J Physiol Heart Circ Physiol 286: H1382–H1388, 2004.—The effects of the muscle mechanoreflex on baroreflex function have never previously been analyzed over the entire operating range of the arterial baroreflex. In anesthetized, vagotomized, and aortic-denervated rabbits (n = 8), we isolated carotid sinuses and changed intracarotid sinus pressure (CSP) from 40 to 160 mmHg in increments of 20 mmHg every minute while recording renal sympathetic nerve activity (SNA) and arterial pressure (AP). Muscle mechanoreflex was induced by passive muscle stretch (5 kg of tension) of the hindlimb. Muscle stretch shifted the CSP-SNA relationship (neural arc) to a higher SNA, whereas it did not affect the SNA-AP relationship (peripheral arc). SNA was almost doubled [from 63 ± 15 to 118 ± 14 arbitrary units (au), P < 0.05] at the CSP level of 93 ± 8 mmHg, and AP was increased ~50% by muscle stretch. When the baroreflex negative feedback loop was closed, muscle stretch increased SNA from 93 ± 8 to 109 ± 12 mmHg (P < 0.05). In conclusion, the muscle mechanoreflex resets the neural arc to a higher SNA, which moves the operating point towards a higher SNA and AP under baroreflex closed-loop conditions. Analysis of the baroreflex equilibrium diagram indicated that changes in the neural arc induced by the muscle mechanoreflex might compensate for pressure falls resulting from exercise-induced vaso-dilatation.

MATERIALS AND METHODS

The arterial baroreflex plays an important role in stabilization of arterial pressure (AP) during daily activity. The input-output relationship of the arterial baroreflex system between baroreceptor pressure input and the resultant AP approximates a sigmoidal stimulus-response curve (19). This stimulus-response curve is known to reset toward a higher AP during exercise (7, 8, 23, 24, 29, 30, 34, 35). Recently, it was reported that exercise shifted the baroreflex curve for sympathetic nerve activity (SNA) to a higher SNA (5, 26). However, the neural mechanism responsible for the changes in baroreflex during exercise remains unclear. During exercise, AP and SNA are regulated by central command and the exercise pressor reflex (15, 22, 27, 42). The exercise pressor reflex is evoked by afferent inputs from metabolic (muscle metaboreflex)- and mechanical (muscle mechanoreflex)-sensitive skeletal muscle receptors. The muscle mechanoreflex has a dominant role in the exercise pressor reflex (6, 9, 20). We therefore hypothesized that the muscle mechanoreflex would reset the carotid sinus baroreflex control of SNA toward a higher SNA, evoking a pressor response under baroreflex closed-loop conditions. Earlier studies have reported that the muscle mechanoreflex reset the arterial baroreflex for AP and/or heart rate (13, 32). In the present study, we aimed to quantitatively investigate the effect of the muscle mechanoreflex on the carotid sinus baroreflex control of SNA.

Although the neural mechanism involved in resetting the baroreflex may be best analyzed using the baroreflex equilibrium diagram (28, 38), to the best of our knowledge, the effects of the muscle mechanoreflex on baroreflex function have never been assessed using this method. The baroreflex equilibrium diagram consists of the neural and peripheral arcs. The neural arc represents the static input-output relationship between baroreceptor pressure input and SNA, and the peripheral arc represents the relationship between SNA and AP. The intersection of the neural and peripheral arcs defines the operating point of the AP regulation under baroreflex closed-loop conditions (see Theoretical considerations: coupling of neural and peripheral arcs in MATERIALS AND METHODS for details).

To construct the baroreflex equilibrium diagram, we performed an open-loop experiment on the carotid sinus baroreflex in anesthetized rabbits (16–18, 38). The muscle mechanoreflex was induced by passive muscle stretch of the triceps surae muscle. The results of the present study indicate that the muscle mechanoreflex resets the baroreflex neural arc to a higher SNA, moving the operating point toward a higher AP under baroreflex closed-loop conditions.

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equilibrated with AP under physiological conditions, we superimposed the functions of the two arcs and determined the operating point of the system from the intersection of the two arcs. The operating point is defined as the AP and SNA under closed-loop conditions of the feedback system. The validity of this framework has been examined in a previous study (38). Using the baroreflex equilibrium diagram, we aimed to quantify the effects of the muscle mechanoreflex on the carotid sinus baroreflex.

Surgical preparations. Animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. Japanese White rabbits weighing 2.3–3.1 kg were anesthetized via an intravenous injection (2 ml/kg) with a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml) and mechanically ventilated with oxygen-enriched room air. Arterial blood was sampled from the left common carotid artery. The rabbits were slightly hyperventilated to suppress chemoreflexes (arterial PCO$_2$ ranged from 30 to 35 mmHg, arterial PO$_2$ > 300 mmHg). Arterial blood pH was within the physiological range when rabbits were examined at the end of the surgical preparation and also at the end of the experiment. Supplemental anesthetics were administered continuously to maintain an appropriate level of anesthesia (0.3 ml/kg $\cdot$ h$^{-1}$. The body temperature of each animal was maintained at $\pm 38^\circ$C with a heating pad. AP was recorded using a high-fidelity pressure transducer (Millar Instruments; Houston, TX) inserted retrogradely from the right common carotid artery to the aortic arch.

We isolated the bilateral carotid sinuses from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus region. The isolated carotid sinuses were filled with warmed physiological saline via catheters inserted through the common carotid arteries. Intracarotid sinus pressure (CSP) was controlled by a servo-controlled piston pump (model ET-126A, Labworks; Costa Mesa, CA). Bilateral vagal and aortic depressor nerves were sectioned at the neck to eliminate reflexes from the cardiopulmonary region and aortic arch.

We exposed the left renal sympathetic nerve retroperitoneally and attached a pair of stainless steel wire electrodes (Bioflex wire AS633, Cooner Wire) to record SNA. The nerve bundle peripheral to the electrodes was tightly ligated and crushed to eliminate afferent signals from the kidney. The nerve and electrodes were secured with silicone glue (Kwik-Sil, World Precision Instruments; Sarasota, FL). The preamplified nerve signal was band-pass filtered at 150–1,000 Hz. It was then full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz to quantify the nerve activity. Pancuronium bromide (0.1 mg/kg) was administered to prevent contamination of SNA recordings by muscular activity.

With the rabbit in the prone position, the sacrum and the left ankle were clamped with a custom-made apparatus to prevent body trunk and hindlimb movement during muscle stretch. The left triceps surae muscle, Achilles tendon, and calcaneus bone were exposed. The left triceps surae muscle was isolated from surrounding tissue. The Achilles tendon was severed from the calcaneus bone and attached to a force transducer (Load Cell LUR-A-SA1, Kyowa Electronic Instruments; Tokyo, Japan). During muscle stretch, the force transducer was also connected to a 5-kg weight via a pulley, which opposed the Achilles tendon.

Protocols. Fourteen rabbits were used in the present study. Two rabbits were subjected to both protocols 1 and 2 as described below.

In protocol 1, we examined the time course of the SNA response to stepwise muscle stretch ($n = 8$). Before muscle stretch, CSP was adjusted to instantaneous AP, and the operating point was determined from mean AP at steady state. CSP was then fixed at the operating point, and the left triceps surae muscle was stretched at 5 kg for 7 min. In protocol 2a, we obtained the baroreflex equilibrium diagram under both control and muscle stretch conditions ($n = 8$). CSP was first decreased to 40 mmHg. After attainment of a steady state, CSP was then increased from 40 to 160 mmHg in increments of 20 mmHg. Each pressure step was maintained for 60 s. When SNA was completely suppressed and AP had fallen below 50 mmHg at the CSP level of 140 mmHg, the CSP level of 160 mmHg was omitted to prevent deterioration of the animal’s condition. Thus the maximum duration of muscle stretch was 7 min. The order of control and muscle stretch conditions was randomized across the animals. In five of eight animals, the estimation of the baroreflex equilibrium diagram was repeated under both control and muscle stretch conditions after the left tibial nerve was severed.

In protocol 2b, we measured the actual operating point in the same eight animals that were used in protocol 2a to determine the accuracy of the operating point derived from the baroreflex equilibrium diagram. The operating point of the carotid sinus baroreflex was defined as the point where CSP equals AP (38). To obtain the actual operating point, CSP was adjusted to match AP via the servo-controlled system so that the carotid sinus baroreflex was virtually closed. After a steady state was reached, mean AP (and thus CSP) and SNA were taken as the values defining the actual operating point under control conditions. We also performed muscle stretch for 1 min while the carotid sinus baroreflex was virtually closed and obtained mean AP and SNA values defining the actual operating point during the last 10 s of muscle stretch.

Data analysis. We recorded CSP, SNA, and AP at a sampling rate of 200 Hz using a 12-bit analog-to-digital converter. Data were stored on the hard drive of a dedicated laboratory computer system for later analyses.

In protocol 2a, we calculated mean AP and SNA during the last 10 s of each CSP step. Because the absolute magnitude of SNA depended on recording conditions, SNA was presented in arbitrary units (au) so that the minimum and maximum values of SNA data obtained under control conditions were set to zero and 100 au, respectively. For each animal, a four-parameter logistic function analysis was performed on the neural arc (CSP-SNA data pairs) and the peripheral arc (SNA-AP data pairs) as follows:

$$y = \frac{P_1}{1 + \exp[P_2(x - P_3)]} + P_4 \quad (1)$$

where $x$ and $y$ represent the input and the output, respectively; $P_1$ denotes the response range (i.e., the difference between the maximum and minimum values of $y$); $P_2$ is the coefficient of gain; $P_3$ defines the midpoint of the logistic function on the input axis; and $P_4$ represents the minimum value of $y$. The maximum gain ($G_{\text{max}}$) is $P_2/P_3$ at $x = P_3$.

In protocol 2b, we measured the actual operating point by closing the arterial baroreflex negative feedback loop. Mean SNA and AP values were obtained by averaging 10-s data at the steady state under both control and muscle stretch conditions.

Statistical analysis. All data are presented as means ± SD. Differences were considered significant when $P < 0.05$. The effects of muscle stretch on the parameters of the neural and peripheral arcs and operating points were examined using the paired $t$-test. The operating point as determined from the equilibrium diagram (protocol 2a) was compared with the operating point actually measured (protocol 2b) using linear regression analysis.

RESULTS

Figure 1 shows the group-averaged step response of SNA to muscle stretch. These data were collected under baroreflex open-loop conditions where CSP was fixed at an operating point of each animal. CSP was $87 \pm 14$ mmHg across all the animals. Muscle stretch transiently decreased and then increased SNA. The SNA value at 7 min was maintained at $93 \pm 9\%$ of the SNA at 1 min.

Figure 2 shows a typical time series of CSP, SNA, and AP under control conditions (left) and muscle stretch conditions
SNA and AP decreased in response to increments in CSP under both control and muscle stretch conditions. Muscle stretch increased SNA and AP at CSP levels up to 100 mmHg in this animal.

Figure 3 illustrates the baroreflex neural arcs (A) and peripheral arcs (B) derived from the same data employed in Fig. 2. The open and closed circles represent data points obtained under control and muscle stretch conditions, respectively. The thin and thick solid lines indicate the fitted logistic functions under control and muscle stretch conditions, respectively. In the neural arcs, SNA decreased in response to the increments in CSP under both conditions. Muscle stretch increased SNA at CSP levels up to 100 mmHg. In the peripheral arcs, AP positively correlated with SNA. Peripheral arcs obtained under both conditions were nearly identical.

Figure 3C depicts the baroreflex equilibrium diagrams combined from Fig. 3, A and B. The thin and thick solid lines indicate the fitted logistic functions under control and muscle stretch conditions, respectively. Because CSP is equilibrated with AP under physiological conditions, the operating point is determined from the intersection of the neural and peripheral arcs. Muscle stretch moved the operating point toward a higher AP (from point a to point b).

Figure 4A illustrates the role of the baroreflex function during muscle stretch and is derived from representative data from one animal. Muscle stretch almost doubled SNA from 54 to 98 au at the CSP level of the control operating point (point a), resulting in an elevation in AP of ~50%, from 87 to 130 mmHg (point c). When the arterial baroreflex was operative, the increases in SNA and AP recorded during muscle stretch were substantially attenuated (point b).

Figure 4B shows the group-averaged CSP, SNA, and AP obtained from eight animals under control and muscle stretch conditions at the CSP level of 93 ± 8 mmHg (control operating point).
The key new findings of the present study are as follows. Muscle stretch reset the carotid sinus baroreflex neural arc to a higher SNA. In contrast, muscle stretch did not affect the baroreflex peripheral arc. As a result, the operating point determined from the intersection of the neural and peripheral arcs moved toward a higher AP during muscle stretch. These results support the hypothesis that the muscle mechanoreflex induces the pressor response by resetting the arterial baroreflex neural arc.

Interaction between the muscle mechanoreflex and arterial baroreflex. Although Potts and Li (31) showed that elevation of CSP attenuated the pressor response induced by muscle stretch, they did not measure SNA in that study. Our study demonstrated that the muscle mechanoreflex shifted the CSP-SNA curve toward a higher SNA primarily at low and midrange CSP readings (Fig. 3 and Table 1). This was not an outcome of the time-dependent changes in the muscle stretch effect, because the muscle stretch produced a sustained SNA increase for at least 7 min (Fig. 1). These results suggest that the baroreceptor input pressure-dependent pressor response due to muscle stretch is a consequence of the neural interaction between the muscle mechanoreflex and arterial baroreflex.

Miki et al. (26) demonstrated that treadmill exercise shifted the relationship between baroreceptor pressure input and SNA to a higher SNA. The shift in the baroreflex neural arc evoked by the muscle mechanoreflex may contribute to the exercise-induced resetting in the baroreflex control of SNA. Further-

Table 2. Effect of muscle stretch after severing of the nerve supply to the hindlimb muscles on parameters of logistic function analysis for the neural arc

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Muscle Stretch</th>
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<tbody>
<tr>
<td>Neural arc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_1$, au</td>
<td>99.5±0.2</td>
<td>100.8±8.2</td>
</tr>
<tr>
<td>$P_2$, au/mmHg</td>
<td>0.12±0.03</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>$P_3$, mmHg</td>
<td>107±5</td>
<td>110±8</td>
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<tr>
<td>$P_4$, au</td>
<td>0.3±1.6</td>
<td>0.2±3.7</td>
</tr>
<tr>
<td>$G_{\text{max}}$, au/mmHg</td>
<td>−2.9±0.9</td>
<td>−2.8±0.6</td>
</tr>
<tr>
<td>Values at operating point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP, mmHg</td>
<td>93±8</td>
<td>109±12*</td>
</tr>
<tr>
<td>SNA, au</td>
<td>63.1±15.1</td>
<td>81.2±21.0*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 animals.
Fig. 5. Relationship between the operating point estimated from the equilibrium diagram and operating point actually measured in 8 animals. Each animal provided two data points determined under control and muscle stretch conditions (16 data points in total). The operating point estimated by the baroreflex equilibrium diagram matched the value obtained from actual measurements. A: measured vs. estimated AP; B: measured vs. estimated SNA. RMS, root mean square.

more, G$_{\text{max}}$ of the neural arc (the CSP-SNA relationship) was increased by muscle stretch when the data were analyzed using a fitted logistic function (Eq. 1). The present result is consistent with previous studies (14, 26) showing that gain in the arterial baroreflex control of vascular sympathetic outflow was increased during static and dynamic exercise. On the other hand, another study showed no changes in the gain of the carotid sinus baroreflex control of SNA during dynamic arm exercise (5). Whether changes in the neural arc gain depend on the intensity and/or modality of exercise awaits further studies.

Previous studies (1, 40) suggest interactions between the muscle mechanoreflex and the arterial baroreflex in the brain stem. Baroreceptor afferent inputs inhibit neurons in the rostral ventrolateral medulla (RVLM). Electrically induced muscle contraction increases the firing frequency in RVLM neurons (2, 3), suggesting that the baroreflex and muscle mechanoreflex share common central pathways. In addition, the contraction-sensitive muscle afferents inhibit the baroreflex signal transduction through activation of GABA receptors in the nucleus tractus solitarii (33). This neural integration in the brainstem may be involved in the resetting of the arterial baroreflex neural arc induced by the muscle mechanoreflex.

Determination of the operating point of the arterial baroreflex. SNA and AP during muscle stretch may be determined via interactions between the muscle mechanoreflex and the arterial baroreflex. Muscle stretch shifted the neural arc to SNA values approximately double at the CSP level derived from the control operating point, whereas it did not affect the peripheral arc (Table 1 and Figs. 3 and 4). As a result, the operating point derived from the intersection of the two arcs moved toward a higher AP (Fig. 3C). These findings suggest that resetting in the neural arc, rather than in the peripheral arc, is responsible for the increases in SNA and AP during muscle stretch observed under baroreflex closed-loop conditions (21, 41). These data are the first to provide quantitative evidence demonstrating that resetting of the carotid sinus baroreflex (via central resetting of the baroreflex neural arc) is necessary to evoke the sympathoexcitatory and pressor responses associated with activation of mechanosensitive afferents in the closed-loop conditions.

The baroreflex equilibrium diagram is useful for intuitive understanding of the cardiovascular controls (28, 38). Figure 4A illustrates the role of the baroreflex function during muscle stretch. The baseline operating point was determined from the intersection of the neural and peripheral arcs (point a). Muscle stretch reset the neural arc from the thin line to the thick line.

If changes in AP were not sensed by the arterial baroreflex, then muscle stretch nearly doubled SNA, resulting in AP elevation above ~130 mmHg (point c). However, when the arterial baroreflex was operative, the increases in SNA and AP during the muscle stretch were substantially attenuated (point b).

Sato et al. (38) demonstrated that the actual operating point matched the intersection of the neural and peripheral arcs under both control and hemorrhagic conditions. In the present study, the operating point estimated from the equilibrium diagram was in close agreement with that actually measured under both control and muscle stretch conditions (Fig. 5). These results confirm the accuracy of the operating point estimation derived from the equilibrium diagram.

Physiological implications. Dynamic exercise causes only a modest rise in mean AP despite a marked sympathoexcitation (36). This phenomenon is believed to be a consequence of the decreased sympathetic constrictor response due to metabolic vasodilation and sympatholysis in exercising muscles (4, 11, 12, 37). Figure 6 illustrates a putative diagram of the arterial baroreflex control of SNA and AP during the dynamic exercise. The muscle mechanoreflex resets the neural arc to a higher SNA. Vasodilation in exercising muscles presumably shifts the peripheral arc downward. If the neural arc is not reset to a higher SNA during dynamic exercise, AP at the operating point might decrease (point c) relative to that observed under resting conditions (point a). However, by resetting the neural...
arc, AP may be maintained against the downward shift in the peripheral arc (point b). Thus we speculate that resetting of the neural arc induced by the muscle mechanoreflex contributes to maintaining AP despite the downward shift of the peripheral arc due to vasodilation in exercising muscles.

In the present study, we did not examine additional factors that may potentially influence SNA and AP regulation such as central command, muscle metaboreflex, and vasodilation in exercising muscles. Further investigations are required to improve our understanding of the arterial baroreflex control of SNA and AP during exercise.

Limitations. The present study has several limitations. First, we performed the experiment in animals under anesthetized conditions. Although anesthesia was convenient for elimination of the central command, the gain of carotid baroreflex and muscle mechanoreflex might have been estimated differently if the animals had been conscious.

Second, we used passive muscle stretch as the input for the muscle mechanoreflex. The shift in the baroreflex neural arc was abolished by the tibial denervation (Table 2), suggesting that the shift was evoked by the neural afferents from hindlimb muscles. Previous studies (10, 25, 39) have suggested that passive muscle stretch selectively activates muscle mechanoreceptors. Thus we believe that muscle stretch had evoked the muscle mechanoreflex in the present study.

In conclusion, the muscle mechanoreflex resets the arterial baroreflex neural arc to a higher SNA. Consequently, the pressor response is evoked during muscle stretch under baroreflex closed-loop conditions. This shift in the neural arc induced by the muscle mechanoreflex might compensate for AP falls resulting from exercise-induced vasodilatation.

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