Sweating response to passive stretch of the calf muscle during activation of forearm muscle metaboreceptors in heated humans

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Amano T, Ichinose M, Nishiyasu T, Inoue Y, Koga S, Miwa M, Kondo N. Sweating response to passive stretch of the calf muscle during activation of forearm muscle metaboreceptors in heated humans. Am J Physiol Regul Integr Comp Physiol 306: R728–R734, 2014. First published March 5, 2014; doi:10.1152/ajpregu.00515.2013.—Activation of muscle metaboreceptors and mechanoreceptors has been shown to independently influence the sweating response, while their integrative control effects remain unclear. We examined the sweating response when the two muscle receptors are concurrently activated in different limbs, as well as the blood pressure response. In total, 27 young males performed passive calf muscle stretches (muscle mechanoreceptor activation) for 30 s in a semisupine position with and without postisometric handgrip exercise muscle ischemia (PEMI, muscle metaboreceptor activation) at exercise intensities of 35 and 50% of maximum voluntary contraction (MVC) under hot conditions (ambient temperature, 35°C, relative humidity, 50%). Passive calf muscle stretching alone increased the mean sweating rate significantly on the forehead, chest, and thigh (SRmean) and mean arterial blood pressure (MAP), but not the heart rate (HR), from prestretching levels by 0.04 ± 0.01 mg·cm⁻²·min⁻¹, 4.0 ± 1.3 mmHg (P < 0.05), and −1.0 ± 0.5 beats/min (P > 0.05), respectively. The SRmean and MAP during PEMI were significantly higher than those at rest. The passive calf muscle stretch during PEMI increased MAP significantly by 3.4 ± 1.0 and 2.0 ± 0.7 mmHg for 35 and 50% of MVC, respectively (P < 0.05), but not that of SRmean or HR at either exercise intensity. These results suggest that sweating and blood pressure responses to concurrent activation of the two muscle receptors in different limbs differ and that the influence of calf muscle mechanoreceptor activation alone on the sweating response disappears during forearm muscle metaboreceptor activation.

THE SWEATING RESPONSE DURING exercise is controlled not only by core and skin temperatures (thermal factors) but also by exercise-related (nonthermal) factors, such as afferent inputs from working muscles. It is well known that group III muscle afferents are stimulated predominantly by mechanically sensitive muscle mechanoreceptors, whereas group IV muscle afferents are stimulated mainly by chemically sensitive muscle metaboreceptors. Activation of muscle metaboreceptors by postexercise muscle ischemia (PEMI) under normothermic and mildly hot conditions induces sweating (2, 4, 22, 38). Additionally, Kondo et al. (23) reported that activating the muscle mechanoreceptors by passive limb movement for 2 min evoked slight sweating under hot conditions. Furthermore, the muscle mechanoreflex increases the sweating response during passive recovery (passive limb movement using a tandem ergometer) compared with inactive recovery (resting) after cycling (20, 39). These studies suggest that the isolated activation of muscle metaboreceptors and mechanoreceptors can independently affect sweating responses. However, the sweating response is presumably controlled by integrative mechanisms, based on afferent signals from muscle metaboloreceptors and mechanoreceptors because physiological integrative control has been identified with cardiovascular regulation in humans (10, 12). To our knowledge, there is no previous report on the integrative control of the sweating response.

Two types of integration associated with two muscle afferent signals are considered: central and peripheral integrations. The former involves muscle metaboreceptor and mechanoreceptor afferent signals integrated at the level of the hypothalamus or medulla oblongata, while the latter involves integration of the two afferents (e.g., sensitization) at the activating muscle level (1, 30). When muscle mechanoreceptors and metaboreceptors are activated in the same muscle, both central and peripheral integration would induce physiological responses. In contrast, when these receptors are activated in different muscles (e.g., forearm and leg), central integration would primarily control the response. Associated with this, it has been reported that muscle mechanoreceptor activation by passive right calf muscle stretching additively increased mean arterial blood pressure (MAP) during concurrent activation of muscle metaboreceptors induced by PEMI in the same leg (10, 12). Although this additional increase in blood pressure response was the result of both central and peripheral integration of muscle metaboreceptor and mechanoreceptor activations, a similar increase in the response was also seen when the passive calf stretch was performed during activation of muscle metaboreceptors in the opposite leg to the stretch, in which only central integration would control the response (10). Because the sweating response is governed by sympathetic nerves, as well as the blood pressure response, it is assumed that sweating would show similar responses to that of blood pressure during concurrent activation of the muscle mechanoreceptors and metaboreceptors.

While integrated sweating responses due to muscle mechanoreceptors and metaboreceptors would be regulated by both...
central and peripheral mechanisms, we focused on the former in this study. Thus, the passive calf muscle stretch, which is commonly used to activate muscle mechanoreceptors in humans (3, 8, 10, 12, 14, 15), was used with and without forearm PEMI to investigate the effects of the concurrent activation of muscle metaboreceptors and mechanoreceptors on the sweating response due to central integration. We hypothesized that the sweating response would be additionally increased by passive calf stretching during concurrent activation of muscle metaboreceptors in the forearm, as well as by the blood pressure response observed previously (10).

MATERIALS AND METHODS

Ethical approval. Prior to the study, each subject was informed of the study’s purpose and the procedures involved. All subjects provided written informed consent. This study was approved by the Human Subjects Committee of the Graduate School of Human Development and Environment, Kobe University (Kobe, Japan), and conformed to the standards set forth in the latest revision of the Declaration of Helsinki.

Participants. In total, 27 young male subjects (age: 21.6 ± 0.4 years, height: 170.3 ± 1.2 cm, and weight: 63.3 ± 1.7 kg) participated. The volunteers ranged from unfit to participants who engaged in regular exercise, ranging from recreational activity to amateur competitive sports. No subject was receiving medication, and all were nonsmokers.

Experimental protocol. All experiments were conducted in an environmental chamber (SR-3000; Nagano Science, Osaka, Japan) maintained at an ambient temperature of 35°C and relative humidity of 50%, with minimal air movement. We selected these environmental conditions to cause sweat gland activation by increased skin temperature, allowing for an evaluation of the nonthermal control of sweat gland activity. After entering the chamber, maximum voluntary contraction (MVC) and maximal calf muscle stretch were assessed in the semisupine position. Two MVCs of the right forearm flexors were performed using a handgrip dynamometer (TKK5710b; Takei Kiki Kogyo, Niigata, Japan), and the highest result was used to calculate 35 and 50% of MVC. The passive calf muscle stretch was conducted manually by dorsiflexion of the foot (Fig. 1A). The stretch board was adjusted, so that both feet were firmly strapped, and both knees were on the seat (Fig. 1A). An investigator pushed the stretch board to flex the feet in the dorsal direction to an angle just before the point at which the subject reported discomfort; a constant angle was maintained between conditions.

The experimental protocol is illustrated in Fig. 1B. The subjects were seated for at least 50 min until sweating reached a steady state, and baseline data were recorded for 5 min. After a 2-min rest, the subjects performed an isometric handgrip (IH) exercise for 1 min using a visual feedback system to maintain the target force. A cuff placed on the right upper arm was inflated to 250 mmHg at 5 s before beginning the exercise. The cuff remained inflated for an additional 3 min after ending the IH exercise. Both feet were passively dorsiflexed to the predetermined angle for 30 s during the 5th min of the protocol (Fig. 1B). The cuff was deflated 30 s after ending the stretch, and recovery data were recorded for 2 min. IH trials at 35 and 50% of MVC were performed in random order. Passive stretching alone was conducted for 30 s between the two IH exercise trials to allow recovery from muscle and voluntary fatigue after the first handgrip exercise. Resting periods between the IH exercises and stretch alone were set at ≥10 min to allow physiological parameters to return to baseline levels. Given that sudomotor neurons are modulated by respiration (26), we used an auditory signal to keep the respiratory frequency at 15 cycles/min throughout the experiment. All three experiments (stretching alone and stretching with PEMI at 35 and 50% MVC) were conducted on the same day for each subject.

Measurements. In all experiments, the sublingual temperature (Tsub), local skin temperature at 10 sites (forehead, chest, right and left scapula, lateral lumbar, biceps, forearm, thigh, calf, and palm), sweating rate (SR) on the forehead, chest, forearm, and palm, heart rate (HR) arterial blood pressure, and rating of perceived effort (RPE) were recorded.

The Tsub and local skin temperature were measured using a copper-constantan thermocouple. For Tsub, the tip of the thermocouple was covered with silicone and held in the oral cavity. The subject was then instructed to breathe through the nose. Mean skin temperature (Tsk) was calculated using a modified version of the formula reported by Nadel et al. (31): 

\[ T_{sk} = T_{forehead} \times 0.07 + T_{biceps} \times 0.07 + T_{right scapula} \times 0.09 + T_{left scapula} \times 0.09 + T_{chest} \times 0.16 + T_{forearm} \times 0.16 + T_{biceps} \times 0.11 + T_{upper arm} \times 0.09 + T_{lower arm} \times 0.07. \]

The SR on the forehead (center of the forehead), chest (below the clavicle), forearm (center of the left ventral forearm), and palm (between the left thumb and wrist) were measured continuously using the ventilated-capsule method. Because it has been reported that the SR caused by isolated muscle mechanoreflex activation is small (below 0.1 mg·cm⁻²·min⁻¹) (23), we used smaller sweat capsules (1.54 cm²) and greater dry nitrogen gas flow (500 ml/min) to the capsules (nitrogen gas rate/capsule area = 324 ml/cm²) than previous studies [147–248 ml/cm² (13, 25, 28, 39, 45)] to detect small changes in the response. The capsules were carefully attached to the skin with glue. The dry nitrogen gas was flushed for at least 1 h before the experiments to obtain stable values of the dry gas. The humidity of the nitrogen gas flowing out of the capsules was measured with a capacitance hygrometer (HMP50 YANAIHIX, Vaisala, Helsinki, Finland). The Tsub, local skin temperatures, and SR were recorded every second using a data logger (MX100; Yokogawa, Tokyo, Japan).

HR was measured using standard electrocardiogram (ECG) leads, and arterial blood pressure was continuously measured from the left middle finger using the Finometer system (Finapres Medical Systems, Amsterdam, The Netherlands). An electromyogram (EMG) was recorded from the rectus femoris and medial gastrocnemius muscles of the left leg. Prior to application of the surface electrodes, the sites were cleaned with alcohol and dead skin was removed by abrasion. ECG and EMG signals were recorded at a sampling frequency of 1,000 Hz using a Bio-Parameter real-time analysis system (Map1058A ver. 6.0; Nihonsanetsu, Osaka, Japan). Each subject was asked to provide a RPE at the end of the IH exercise (5). A goniometer

Fig. 1. Illustration of the experimental setting (A) and protocol (B).
Responses to IH exercise and PEMI. Table 1 shows the absolute changes in \( T_a \), HR, MAP, \( SR_{\text{mean}} \), and \( SR_{\text{palm}} \) during IH exercise protocols and passive stretch alone. All variables were significantly increased during IH exercise compared with rest at both exercise intensities. The changes in HR, MAP, \( SR_{\text{mean}} \), and \( SR_{\text{palm}} \) during IH exercise at 50% MVC were significantly greater than those at 35% (\( P < 0.05 \), Table 1). The increases in \( T_a \), MAP, \( SR_{\text{mean}} \), and \( SR_{\text{palm}} \) were significantly sustained above resting levels during PEMI (\( P < 0.05 \), Table 1). The changes in MAP, \( SR_{\text{mean}} \), and \( SR_{\text{palm}} \) during PEMI were exercise intensity-dependent (\( P < 0.05 \), Table 1).

Responses to passive calf muscle stretch. Figure 2 shows passive stretch-induced changes in \( \Delta HR \), \( \Delta MAP \), \( \Delta SR_{\text{mean}} \), and \( \Delta SR_{\text{palm}} \). \( \Delta HR \) was decreased significantly by passive stretching during PEMI at 50% MVC, and increased significantly after stretching compared with during stretching under stretch alone and 35% MVC conditions (\( P < 0.05 \), Fig. 2). \( \Delta MAP \) was increased significantly by mechanoreceptor activation during passive stretching and returned to prestretch levels regardless of the existence of muscle metaboreflex activation in the forearm (\( P < 0.05 \), Fig. 2). The magnitude of change in \( \Delta MAP \) during stretching was influenced by the level of metaboreflex activation; the change was significantly greater under the stretch-alone condition than at 35% MVC (\( P < 0.05 \), respectively). We confirmed that a voluntary muscle activation was not occurring during passive stretches because the EMGs on the rectus femoris and medial gastrocnemius were not detected during the stretches in any trial. In addition, \( T_{ar} \) was constant throughout the experiment.

RESULTS

The degree of passive calf muscle stretch was not significantly different among the trials (46 ± 2, 47 ± 2, and 47 ± 2° of stretch board rotation for passive calf muscle stretch alone and with PEMI at 35 and 50% of MVC, respectively). We confirmed that a voluntary muscle activation was not occurring during passive stretches because the EMGs on the rectus femoris and medial gastrocnemius were not detected during the stretches in any trial. In addition, \( T_{ar} \) was constant throughout the experiment.

Fig. 2. Passive stretch-induced changes in heart rate (\( \Delta HR \)), mean arterial blood pressure (\( \Delta MAP \)), mean sweating rate (\( \Delta SR \)) on the forehead, chest, and forearm, and \( \Delta SR \) on the palm with and without postexercise forearm ischemia at 35 and 50% of maximum voluntary contraction. *Significantly different from prestretch (\( P < 0.05 \)). #Significantly different from poststretch (\( P < 0.05 \)). &Significantly different from the stretch-alone condition (\( P < 0.05 \)). †Significantly different from the 35% maximum voluntary contraction (MVC) condition (\( P < 0.05 \)).
The isolated activation of muscle mechanoreceptors in the legs by performing passive stretch alone increased the ΔSRmean, as well as the ΔMAP (P < 0.05, Fig. 2). However, the activation of calf muscle mechanoreceptors during the activation of forearm muscle metaboreceptors did not induce ΔSRmean (P < 0.05, Fig. 2). Passive stretching did not influence Tsk, Tsk, or ΔSRpalm under any conditions (P > 0.05, Fig. 2 and Table 1).

**DISCUSSION**

Subjects performed passive calf muscle stretches during forearm PEMI at 35 and 50% of MVC to investigate the effects of the concurrent activation of muscle mechanoreceptors and metaboreceptors in different limbs on sweating, as well as blood pressure responses. Contrary to our hypothesis, the passive calf stretch during activation of muscle metaboreceptors in the forearm did not induce the sweating response, while the stretch alone (isolated muscle mechanoreceptor activation) did stimulate the sweating response, as evidenced by the significant increases in SR and blood pressure during PEMI (2, 4, 7, 22). Although it has been reported that cuff inflation itself can stimulate muscle mechanoreceptors (29, 44), we reported previously that forearm occlusion (240 mmHg) for 2 min without prior isometric handgrip exercise under hot conditions did not evoke any changes in HR, MAP, or SR (22). This suggests that forearm cuff inflation itself during PEMI did not affect the physiological responses observed in the present study. Thus, it was believed that passive calf muscle stretching during PEMI following IH exercise at 35 and 50% of MVC was adequate to activate the muscle mechanoreceptors in the calf and muscle metaboreceptors in the forearm.

It is considered that the physiological responses to concurrent activation of forearm muscle metaboreceptors and calf muscle mechanoreceptors in the present study were regulated by a central mechanism because the two receptors were activated in different limbs. On the basis of animal studies, it has been suggested that the inputs from muscle mechanoreceptor and metaboreceptor afferents excite sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS), inducing vasomotor sympathetic activation (35, 43). Additionally, an evaluation of brain activity using functional magnetic resonance imaging revealed that similar areas of the medulla were activated in humans during a handgrip exercise and following PEMI, associated with the blood pressure response (37). Thus, it is considered that the blood pressure responses to concurrent activation of muscle mechanoreceptors and metaboreceptors in different limbs were the result of the integration of these two afferents in the NTS and/or RVLM in the medulla oblongata in the present study. Conversely, the sweating efferent signal may have arisen from the hypothalamus, which is a thermoregulatory control center (16, 17, 32). Somatosensory afferent signals connect with the paraventricular nucleus of the hypothalamus through the spinohypothalamic tract or through brain stem activation (34). Thus, it is assumed that the two muscle afferent inputs from different limbs might have been integrated in the hypothalamus or brain stem to induce the sweating response. The different responses between blood pressure and sweating to concurrent activation of muscle metaboreceptors and mechanoreceptors in different limbs might have been integrated in the hypothalamus or through brain stem activation (34). Somatosensory afferent signals connect with the paraventricular nucleus of the hypothalamus through the spinohypothalamic tract or through brain stem activation (34). Thus, it is assumed that the two muscle afferent inputs from different limbs might have been integrated in the hypothalamus or brain stem to induce the sweating response.
the different limbs might be due to the differences in the brain regions where the two muscle afferent inputs are integrated, and/or where the efferent outputs are discharged for inducing these responses.

Two mechanisms may explain the elimination of the calf muscle mechanoreflex-mediated increase in SR during muscle metaboreceptor activation in the forearm. First, the influence of muscle mechanoreceptor activation on SR may be inhibited by muscle metaboreceptor activation in the forearm. It has been reported that muscle sympathetic nerve activity (36) and forearm vasoconstriction (42) during isolated muscle metaboreceptor activation in the forearm (PEMI) are inhibited by the activation of muscle mechanoreceptors in a different limb (contralateral forearm or calf) without changing the heart rate or blood pressure. These studies imply that the simultaneous activation of muscle mechanoreceptors and metaboreceptors in different limbs inhibits afferent sympathetic neural activation. Second, muscle metaboreflex activation may “override” the influence of muscle mechanoreceptor activation on the SR. To distinguish these mechanisms, further study is needed.

It is possible that the calf muscle mechanoreflex-induced SR was too small to detect during forearm PEMI because the SRs of isolated calf muscle mechanoreceptor activation were 36 and 19% of the muscle metaboreflex-induced SR in the present study (calculated from the SR of 0.04, 0.11, and 0.21 mg·cm²·min⁻¹ for isolated passive stretch and PEMI at 35 and 50% MVC, respectively). On the other hand, the effect of isolated passive stretch on MAP was also smaller than that of the activation of muscle metaboreceptors (29 and 17% of the muscle metaboreflex-induced MAP, calculated from an MAP of 4.0, 13.6, and 23.4 mmHg for isolated passive stretch and PEMI at 35 and 50% MVC, respectively), while the stretch effect during PEMI was shown only in MAP, not in sweating. Therefore, the different responses of sweating and blood pressure to passive calf stretching during PEMI could be due to differences in the integrated mechanisms between the two responses, not to the small amount of sweating during passive stretch.

While it has been reported that the isolated activation of muscle mechanoreceptors by passive calf muscle stretching increased HR, as well as blood pressure responses (10, 12, 14, 15, 42), we did not find an increase in HR during the stretch in all conditions; rather, HR decreased significantly during PEMI in the 50% MVC condition. Gladwell et al. (15) reported that passive stretch-induced changes in HR were due to the withdrawal of cardiac vagal tone because the stretch did not increase HR when cardiac vagal activity was inhibited by administration of glycopyrrolate and a light rhythmic handgrip exercise. Associated with this, a high ambient temperature can inhibit cardiac vagal tone even at rest (6). Additionally, the absolute blood pressure during the passive stretch at the 50% MVC condition was markedly higher than under other conditions (Table 1), assuming that the arterial baroreflex may have suppressed the stretch-induced increase in HR, especially in the 50% MVC condition. In contrast, the magnitude of the passive stretch-induced increase in MAP was significantly attenuated under PEMI conditions following exercise at 35% MVC vs. that observed with stretch alone (Fig. 2). This suggests that the increase in MAP induced by passive stretch depended on the level of muscle metaboreflex activation, even if the control mechanisms are not clearly defined.

It is known that mental stress can induce sweating responses even in nonglabrous skin (28, 33), and it was demonstrated recently that both thermal and psychologenic sweating may share a common descending pathway in the brain stem (11). Therefore, the potential influence of mental stress on nonglabrous sweating during passive stretches could not be eliminated in the present study, although the passive calf muscle stretches did not increase the ΔSR_palm, which may be sensitive to mental stress. Additionally, it is possible that the arterial baroreflex activity following the increase in ΔMAP affected the sweating response. However, it has been reported that skin sympathetic nerve activity and SR are not altered by phenylephrine-administered increases in arterial blood pressure during mild heating (46). Furthermore, the SR remained elevated during activation of the muscle metaboreceptors, even after MAP returned to baseline levels, with administration of sodium nitroprusside (38). These results suggest that the arterial baroreflex does not influence the sweating response. However, a possible influence of the arterial baroreflex on the sweating response remained since we did not control for baroreceptor status per se in the present study.

Limitations. Because the changes in ΔSR during passive stretch were much lower than those normally seen during whole body exercise or heating, it is an issue as to whether the change is affected by other factors, such as artificial and natural errors. As mentioned, we used a specific sweat measurement system to detect small changes in sweating responses and conducted the experiments carefully. Additionally, we calculated each 30-s mean value during protocols to eliminate the influence of the natural fluctuations in SR as much as possible, especially during PEMI. Furthermore, if the sweating response was influenced by artificial errors, the responses would not be synchronized among the skin sites. However, the SRs among the skin sites were synchronized in the present study (data not shown), suggesting that the changes in SR during passive stretch were not an artificial error, but the physiological sympathetic response to the stretch.

It seems reasonable to use the right and left legs for stimulating muscle metaboreceptors and mechanoreceptors, respectively, as well as to investigate the effects of the peripheral integration of the two muscle afferents (simultaneous stimulation of muscle mechanoreceptors and metaboreceptors in the same muscle), as has previously been done in cardiovascular research (10, 12). However, we observed that a high-pressure cuff occlusion on the thigh induced a pain-related nonglabrous sweating response, as evidenced by an increase in the sweating rate in the palm in a pilot study. Further studies are necessary to elucidate the precise mechanism(s) of the integrative control of the sweating response caused by muscle metaboreflexes and mechanoreflexes. Nonetheless, this is the first reported study to describe part of the integrative control of the sweating response caused by two muscle afferents focused on a central mechanism.

Perspectives and Significance

It is believed that the SR at a given core temperature (thermal input) during exercise is greater than that at rest (e.g., passive heating) because an integrative nonthermal input would increase the SR during exercise in addition to thermal factors (21). Muscle metaboreceptors and mechanoreceptors

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are known to provide afferent inputs from working muscles, and it has been reported that each reflex can induce sweating independently (20, 23, 39). Prior to the current study, however, it remained unknown how these afferent inputs induce sweating when they are activated simultaneously. We revealed that calf muscle mechanoreceptor activation does not influence the SR during the activation of muscle mechanoreceptors in the forearm.

This result may be important for understanding the integrated regulatory mechanism of sweating during exercise.

In conclusion, the passive calf muscle stretch did not increase the sweating response during PEMI in the forearm, whereas the blood pressure response was significantly increased by the stretch even during concurrent activation of the forearm muscle mechanoreceptors. It appears that the influence of combined activation of muscle mechanoreceptors and mechanoreceptors in different limbs on blood pressure and sweating responses differed and that the influence of muscle mechanoreceptor activation alone on SR disappeared when muscle mechanoreceptors were activated concurrently in a different limb.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: T.A. and N.K. conception and design of research; T.A. and M.M. performed experiments; T.A. analyzed data; T.A., M.I., T.N., and N.K. edited and revised manuscript; Y.I., S.K., and N.K. interpreted results of experiments; T.A. prepared figures; T.A. and M.M. drafted manuscript; T.A., M.I., T.N., Y.I., S.K., M.M., and N.K. approved final version of manuscript.

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