Limb-specific differences in the skin vascular responsiveness to adrenergic agonists

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Submitted 18 January 2011; accepted in final form 21 April 2011

Yamazaki F, Yuge N. Limb-specific differences in the skin vascular responsiveness to adrenergic agonists. J Appl Physiol 111: 170–176, 2011. First published April 28, 2011; doi:10.1152/japplphysiol.00068.2011.—In this study, to test the hypothesis that adrenergic vasoconstrictor responses of the legs are greater compared with the arms in human skin, cutaneous vascular conductance (CVC) in the forearm and calf were compared during the infusion of adrenergic agonists in healthy young volunteers. Under normothermic conditions, norepinephrine (NE, α- and β-agonist, 1 × 10−8 to 1 × 10−2 M), phenylephrine (PHE, α1-agonist, 1 × 10−8 to 1 × 10−2 M), dexmedetomidine (DEX, α2-agonist, 1 × 10−9 to 1 × 10−4 M), and isoproterenol (ISO, β-agonist, 1 × 10−8 to 1 × 10−3 M) were administered by intradermal microdialysis. Skin blood flow (SkBF) was measured by laser-Doppler flowmetry, and the local temperature at SkBF-measuring sites was maintained at 34°C throughout the experiments. CVC was calculated as the ratio of SkBF to blood pressure and expressed relative to the baseline value before drug infusion. The dose of NE at the onset of vasoconstriction and the effective dose (ED50) resulting in 50% of the maximal vasoconstrictor response for NE were lower (P < 0.001) in the calf than forearm. The ED50 for PHE and DEX was also lower (P < 0.05) in the calf than forearm. Increases in CVC in response to ISO were potentially smaller in the calf, but the statistical differences in the responses were dependent on the expressions of CVC. These findings suggest that the cutaneous vasoconstrictor responsiveness to exogenous NE is greater in the legs than in the arms due to a higher α1- and α2-adrenoceptor reactivity, while the β-adrenoceptor function plays a minor role in regional differences in adrenergic vasoconstriction in normothermic humans.

IN HUMANS, peripheral vasoconstrictor responses to upright posture show heterogeneity between the upper and lower limbs (14, 16, 22, 48). The findings from previous studies suggest that the leg vasculature is more responsive to orthostatic stimulation than the arm vasculature. The augmented vasoconstrictor response observed in the human lower-leg vasculature is influenced by sympathetic and local mechanisms (14, 22, 24, 38) and could play an important regulatory role by limiting the degree to which blood accumulates in the legs during orthostatic stress (29, 30).

The vasoconstrictor response to the upright posture is via reflex and nonreflex (e.g., venoarteriolar response) mechanisms. The reflex vasoconstriction is influenced by centrally driven activity in sympathetic vasoconstrictor nerves and postjunctional adrenergic receptor responsiveness. With regard to the central sympathetic outflow, similar increases in muscle sympathetic nerve activity have been shown in the arm and leg during orthostatic stress (14, 31). In addition, similar increases in regional norepinephrine (NE) spillover have been reported in the arm and leg during hypotensive stimulation evoked by an intravenous infusion of sodium nitroprusside (SNP) (15). With regard to adrenergic receptor responsiveness, Pawelczyk and Levine (29) reported a relatively greater vasoconstrictor response to α1-adrenergic receptor stimulation in the calf compared with the forearm. In contrast, the vasodilator response to β-adrenergic receptor stimulation evaluated based on the percent change in maximal vascular conductance did not differ between the arm and leg (29), and the vasodilator response evaluated based on the percent change in baseline blood flow was smaller in the leg (15).

Vasomotor control in the skin could be involved in the maintenance of arterial blood pressure during orthostatic stress, especially in hyperthermia given the large fraction of vascular conductance of the skin relative to the entire vasculature (5, 9, 18, 47, 48). Unchanged skin sympathetic nerve activity in response to baroreceptor unloading has been observed in normothermic and heat-stressed humans (7, 39, 41). However, significant cutaneous vasoconstrictor responses in the forearm and palm at the heart level during orthostatic stress in these thermal conditions (6, 34, 45, 47, 48) suggest participation of sympathetic vasoconstrictor function in the responses without a detectable change in the neural signal. Unfortunately, it is unknown whether sympathetic adrenergic vasoconstrictor responsiveness in the skin shows a regional difference between the upper and lower limbs, since blood flow measured in the previous reports represents a composite of flow in both muscle and skin (15, 22, 38). The contributions of muscle and skin to limb blood flow may be different between arms and legs, so that any regional differences in the response of either vascular bed are not clear. If the adrenergic vasoconstrictor responses in the skin show a heterogeneity between the upper and lower limbs, as suggested in studies examining limb blood flow, then postjunctional adrenoceptor responsiveness may be responsible for the different vasomotor responses.

In the present study, therefore, we examined the cutaneous vasomotor responses evaluated employing skin laser-Doppler flowmetry during the local administration of adrenergic agents by intradermal microdialysis in the arms and legs of humans. These methods permit the characterization of the functional effects of adrenergic-receptor stimulation in skin vascular smooth muscle while minimizing confounding systemic responses. Additionally, the intradermal microdialysis technique can maintain a drug concentration at a similar level in the skin regardless of a different limb volume in the arms and legs. We hypothesized that the skin vasculature shows heterogeneous vasomotor control between the upper and lower limbs, and this is achieved by greater α1- and α2-receptor sensitivities in the
legs relative to the arms without a limb-specific difference in β-receptor responsiveness.

METHODS

Subjects. The experiments were approved by the Ethics Committee of Medical Care and Research of the University of Occupational and Environmental Health. All subjects were fully informed of the methods and risks before written informed consent was obtained. A total of twenty-nine subjects (20 women and 9 men; age range 19–22 yr; part 1, 5 women and 3 men; parts 2–4, 5 women and 2 men) participated in the experiments. All subjects were healthy nonsmokers, nonobese (body mass index: 20.9 ± 0.3 kg/m²), and not taking any medications. The menstrual status of the female subjects was recorded, but their actual measurement values in experiments did not differ from those of the male subjects, and so their results were combined for analysis.

Instrumentation. Each subject had microdialysis fibers placed intradermally on two forearm and two calf skin sites. These fibers consisted of 1 cm of microdialysis membrane (inner diameter: 200 µm, 18-kDa nominal molecular mass cutoff) attached at each end to polyimide tubing. Dead space in the tubing prior to reaching the membrane was ~16 µl. Prior to implantation, the area of the skin was temporarily anesthetized by the application of a cold pack for 5 min. Needles (25 gauge) were inserted intradermally into the arm and leg for ~2.5 cm. The fibers were then fed through the lumen of the needle. Fibers were aligned such that the microdialysis membranes were centered within the dermis. The needles were then removed, leaving the fibers in place. To allow for the effects of insertion trauma, we waited 1.5 h before any protocols began.

Measurements. All measurements were performed with the subjects resting in a supine position. Skin blood flow (SkBF) was monitored continuously with laser-Doppler flow meters (ALF21, Advance, Tokyo, Japan). The blood flow measurements are specific to the skin and are not influenced by blood flow to underlying skeletal muscle (33). The local temperature (∆Tm) of the 6.3-cm² area surrounding the site of SkBF measurement was controlled by a local temperature controller (model 888, Scholer-tec, Osaka, Japan). ∆Tm was maintained at 34°C throughout the experiments with this controller. The mean arterial pressure (MAP) was measured every 10 min employing an automated oscillometric blood pressure device (BP-203i, Colin, Komaki, Japan). The heart rate (HR) was determined from the electrocardiogram.

Experimental protocols. The experiments consisted of four different parts, each conducted under constant environmental conditions (ambient temperature: 26 ± 0.5°C, relative humidity: 60 ± 5%). To decrease tonic adrenergic vasoconstrictor activity, subjects were lightly dressed and covered with a blanket except for the left arm, left lower leg, and head. In each part of experiments, two sites on the ventral aspect of the left forearm and two sites on the lateral aspect of the left calf were prepared with microdialysis fibers, SkBF probes, and holders. Agents delivered by microdialysis were filter-sterilized with 0.2-µm micropore syringe filters (AGC Techno Glass, Funabashi, Japan). A solution containing each concentration of agents in sterile saline was perfused at 4 µl/min.

The protocol began with four sites being perfused with saline for 30–40 min. Following this, in part 1, NE (Sigma), a nonselective α-adrenoceptor agonist, was infused at all sites with increasing concentration in the following order (10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³, and 10⁻² M). Each dose of NE was administered for 10 min. Figure 1 shows a representative trace of SkBF changes throughout the experiment in part 1. After dosing at the final concentration, SNP (Sigma) dissolved in sterile saline at a concentration of 56 mM was administered for 30–40 min via microdialysis at a rate of 4 µl/min, which has previously been shown to elicit maximal SkBF (20, 21). As an additional study in part 1, to confirm whether the SNP dose following the administration of NE at a higher concentration can elicit maximal vasodilation, the SNP dose was performed without pretreatment of NE in six subjects. In part 2, phenylephrine (PHE) (Sigma), an α₁-adrenoceptor agonist, was infused at all sites with increasing concentration in the following order (10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³, and 10⁻² M), with each dose being delivered for 10 min. In part 3, dexmedetomidine (DEX) (Precedex, Marushi), an α₂-adrenoceptor agonist, was infused at all sites with increasing concentration in the following order (10⁻⁵, 10⁻⁴, 10⁻³, 10⁻², and 10⁻¹ M), with each dose being delivered for 10 min. DEX causes more α₂-selective vasoconstriction by the stimulation of receptors present in the vascular smooth muscle than clonidine (25, 37). In part 4, isoproterenol (ISO) (Sigma), a β-adrenoceptor agonist, was infused at all sites with increasing concentration in the following order (10⁻⁶, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ M), with each dose being delivered for 10 min. After dosing at the final concentration, SNP at a concentration of 56 mM was administered for 30–40 min. In parts 1–3, the doses of adrenergic agents were selected following pilot studies in which these doses were found to establish dose-response curves without any systemic effects, shown by the lack of significant changes in blood pressure or HR. In part 4, although the ISO doses could not encompass saturation of vasodilator response, we did not treat the ISO doses at 10⁻² M or more because of the large amount of agent induced hypotension and tachycardia via systemic circulation in pilot study.

Data processing and statistical analysis. The SkBF data were recorded every 1 s by a data logger (UAS-A1, Unique Medical, Tokyo, Japan). The data for SkBF from the two sites in the forearm or calf were averaged, and then the averaged data were used for later analysis. Cutaneous vascular conductance (CVC) was calculated from the ratio of SkBF to MAP and normalized to the baseline control (arbitrary units/100 mmHg), was also analyzed. In part 4, CVC was also expressed as a percentage of the maximal value (%max) during the infusion of SNP. The average CVC during the final 60 s of each dosing of agents was obtained and compared across arm and leg sites and doses using a two-way ANOVA. For all ANOVAs, the Student-Newman-Keuls test was used to determine where significant differences occurred. CVC data were mathematically modeled using nonlinear regression curve fitting (GraphPad Prism 5.0, GraphPad
without the pretreatment of NE, the peak CVC responses did not differ \( P = 0.58 \) between the forearm \((30.3 \pm 1.5 \text{ units/100 mmHg})\) and calf \((28.7 \pm 1.6 \text{ units/100 mmHg})\) sites. In the forearm and calf, the pretreatment of NE significantly decreased the peak CVC responses during the administration of SNP.

In part 2, the administration of PHE decreased \( P < 0.0001 \) CVC at both sites (Fig. 3). ANOVA revealed a significant interaction, suggesting that the vasoconstricting action of PHE was different between the two regions. The reductions of CVC in the calf during the doses of PHE in \(10^{-5} \text{ M}\) and \(10^{-4} \text{ M}\) were larger \( P < 0.001\) than those in the forearm. There was no difference in the CVC responses to the highest doses between sites. Goodness of fit from the nonlinear regression model was high for each site \((R^2 = 0.988 \pm 0.003\) and \(0.992 \pm 0.002\) for forearm and calf sites, respectively). The ED\(_{50}\) of the dose-response curve was lower \( P = 0.04\) for the calf \((-5.0 \pm 0.2 \log \text{ M PHE})\) relative to the forearm \((-4.0 \pm 0.3 \log \text{ M PHE})\) sites.

In part 3, similar to the PHE dose, the administration of DEX decreased \( P < 0.0001\) CVC at both sites (Fig. 4). ANOVA revealed a significant interaction, suggesting that the vasoconstricting action of DEX was different between the two regions. The reductions of CVC in the calf with doses of DEX from \(10^{-6}\) to \(10^{-4} \text{ M}\) were larger \( P < 0.001\) than in the forearm, while there was no difference in the CVC responses to the highest doses at both sites. Goodness of fit from the nonlinear regression model was high for each site \((R^2 = 0.992 \pm 0.003\) and \(0.991 \pm 0.002\) for forearm and calf sites, respectively). Similar to the responses to PHE, the ED\(_{50}\) of the dose-response curve was lower \( P = 0.03\) for the calf \((-6.2 \pm 0.2 \log \text{ M DEX})\) relative to the forearm \((-5.7 \pm 0.1 \log \text{ M DEX})\) sites.

In part 4, the administration of ISO increased \( P < 0.0001\) CVC at the forearm and calf sites. As shown in the left panel in Fig. 5, when CVC was expressed as a percentage change from the baseline value before the infusion of ISO, ANOVA
revealed a significant interaction. This suggests that the vasodilating action of ISO was significantly different between the two sites. Since a saturation response of CVC to ISO was not obtained, the ED50 of the dose-response curve was not calculated. The administration of SNP significantly increased SkBF at both sites; the peak CVC responses in the calf (26.7 ± 1.3 units/100 mmHg) did not differ (P = 0.33) from those in the forearm (32.9 ± 5.8 units/100 mmHg). In the forearm and calf, the SNP-induced CVC responses after the ISO doses did not differ (P > 0.63) from those after the non-NE dose in part 1.

As depicted in the right panel in Fig. 5, when CVC was expressed as a percentage of the maximal value during the infusion of SNP, the CVC responses to ISO did not differ (2-way ANOVA, P = 0.35) between the two sites.

DISCUSSION

The major findings of the current study are as follows. First, exogenous NE administration caused more potent cutaneous vasoconstriction in the calf compared with the forearm but produced comparable peak vasoconstrictor responses in both regions (part 1). Second, the sensitivity of the α1-agonist-induced vasoconstriction in the skin was higher in the calf than in the forearm (part 2). Third, vasoconstrictor sensitivity for the α2-agonist was also higher in the calf than in the forearm (part 3). Finally, β-agonist-induced vasodilator responses were potentially less in the calf than in the forearm (part 4).

The lower ED50 of the exogenous NE to reduce CVC in the calf compared with the forearm suggested a higher sensitivity to adrenergic cutaneous vasoconstriction in the lower limbs. Cutaneous vasomotor responses to physiological stimuli are generally affected by the baseline blood flow before the stimuli (1, 40, 46). It is important to note, therefore, that the different sensitivity in the adrenergic vasoconstrictor response was not due to the baseline levels, because the absolute CVC values before the infusion of adrenergic agents did not differ between the forearm and calf sites in all protocols. Consistent with the finding regarding a higher vasoconstrictor sensitivity in the legs, we recently reported a greater reduction of CVC in the calf compared with the forearm during mild whole body cooling in which the mean skin temperature slowly dropped from 34.5 to 32.4°C (44). A similar reduction in SkBF in the forearm and calf has been observed during greater sympathetic activation induced by marked cold stress, whereby the mean skin temperature rapidly decreased from 35.5 to 26.4°C (49). The comparable cutaneous vasoconstriction in both regions corresponds to the present finding that there was no regionality in the cutaneous vasoconstrictor responses to higher NE concentrations (10⁻³ to 10⁻² M). Thus, when baseline SkBF in the arm and leg are comparable, it is...
thought that the skin vasculature in the legs exhibits a higher physiological sensitivity for adrenergic vasoconstriction without changing the maximal efficacy.

The findings obtained in parts 2 and 3 confirm that postjunctional $\alpha_1$- and $\alpha_2$-adrenoceptors are functional in in vivo human skin vasculature. The $\alpha_2$-adrenoceptor function in the skin vasculature plays a pivotal role in thermoregulation under cool conditions, because cutaneous vasoconstriction in response to direct skin cooling is mediated by the $\alpha_2_c$-adrenoceptor subtype, which translocates from the Golgi compartment to the extracellular membrane in the adrenergic pathways (2, 17). It is thought, however, that the cold-induced translocation in $\alpha_2_c$-adrenoceptors did not occur while adrenergic agents were administered, because the local skin temperature was maintained at 34°C throughout the experiments in this study. Blood vessel preparations in skeletal muscle (26) and skin (10) have identified $\alpha_1$-adrenoceptors primarily in the proximal vasculature and $\alpha_2$-adrenoceptors primarily in the distal vasculature. In prior human studies, the arm and leg exhibited comparable $\alpha_1$- and $\alpha_2$-adrenergic responsiveness (8, 25, 35, 43), when the responsiveness was evaluated based on the changes in blood flow or vascular conductance to infused adrenergic agonists. In this study, the peak CVC responses to the highest DEX concentration were not different from those to the highest PHE concentration in the forearm and calf (Figs. 3 and 4), suggesting that $\alpha_2$-receptor function has a similar efficacy to $\alpha_1$-receptor function for constricting the skin microvasculature in both regions. Furthermore, the present study indicated that $\alpha_1$- and $\alpha_2$-receptor functions in skin vessels exhibit heterogeneity in the arms and legs. The different $\alpha$-adrenergic responsiveness may reflect the regionality in receptor density, the difference in the binding of adrenergic agents to postjunctional receptors, and/or the difference in intracellular mechanisms in smooth muscle, but the detailed mechanism remains unknown.

NO is a substance contributing to vasomotor tone in the skin and may participate as a modulator for greater vasoconstriction in the leg skin, insofar as NO decreases the bioavailability of NE in the rat mesenteric arterial bed (23) and attenuates neurogenic vasoconstrictor responsiveness in the rat hindlimb microvascular bed (11). In addition to the tonic production of NO in skin vessels in normothermia, the stimulation of $\alpha_2$-adrenergic receptors on endothelial cells leads to the release of vasodilatory substances like NO and prostanoids (3, 36). Thus, when $\alpha_2$-adrenergic receptors are activated, the inhibitory effects of NO or other vasodilatory substances on the affinity in $\alpha_2$-adrenergic receptors may be lower in the leg skin.

Skin vessels have functional $\beta$-adrenoceptors in the human forearm (4). Our finding derived from part 4 extended the functional participation of $\beta$-adrenoceptors in leg skin vessels. When CVC was presented as the relative change from the baseline values during saline infusion, the increasing response of CVC to ISO administration was smaller in the calf compared with the forearm, suggesting that the $\beta$-adrenoceptor function contributes to the regionality in cutaneous vasomotor control. However, when CVC was presented as the relative change from the maximal values during the infusion of SNP, the CVC responses to ISO administration did not differ between the two regions. The comparable controlling function in the arms and legs in $\beta$-receptor-mediated vasodilation was in agreement with prior studies examining limb blood flow (15, 30). Based on these findings in conjunction with a lower affinity of NE binding to $\beta_2$-receptors relative to $\alpha_1$- and $\alpha_2$-receptors (19), it is thought that the $\beta$-receptor-mediated modulation in the adrenergic vasoconstrictor responses to NE plays a minor role in the regionally different vasomotor control.

The peak CVC values during the infusion of SNP, an NO donor, after NE doses were significantly smaller in the calf than forearm, while those during the infusion of SNP without pretreatment of NE did not differ between the two sites. These findings suggest 1) a greater role of the $\alpha$-adrenergic function at a given NO concentration in the leg skin and 2) a similarity of endothelium-independent vasodilator function in the arm and leg skin. The SNP-induced vasodilator responses after NE doses relative to after the non-NE dose were 79.5% in the forearm and 44.6% in the calf. The relatively smaller vasodilator response after NE doses suggests that the vasocostrictr action of NE persisted during the endothelium-independent vasodilatation at both sites. Thus it is thought that the peak CVC value during SNP administration after the NE doses is not an appropriate index of maximal vasodilation. However, we speculate that the CVC values during SNP administration after the ISO doses would reach the maximal levels, because the pretreatment of ISO did not affect the peak vasodilator response during SNP administration.

The regional characteristics of $\alpha_1$- and $\beta$-adrenergic-mediated control in SkBF resembled those in whole limb blood flow (15, 29), although it is possible that thermal stress modulates $\alpha_2$-adrenoceptor responsiveness in the skin but not in the muscle vasculature. The present results support, at least in normothermia, the suggestion that the cutaneous circulation constitutes a potentially representative vascular bed to examine the mechanisms of microcirculatory function and dysfunction (13). Because the forearm has been exclusively used as a model for studying vascular function, it has become common to extrapolate the findings obtained from this vascular bed to the rest of the body. However, the heterogeneity between the upper and lower limbs in adrenoceptor-mediated control in both muscle and skin blood flow should be considered in the evaluation of vascular function, because morphophysiological changes in the forearm vasculature resulting from a pathological state or aging do not always parallel those observed in the lower extremities (27, 28, 32).

In the present study, we cannot exclude the possibility that the intradermal administration of DEX stimulated prejunctural $\alpha_2$-adrenergic receptors, and thereby inhibited a release of NE from adrenergic terminals, although all experiments were performed during supine rest under thermally comfortable conditions that inhibited cutaneous vasoconstrictor activity. In normothermia (whole body and local skin temperature of 34°C), the infusion of bretylium that blocks NE release from adrenergic terminals in the skin did not alter the CVC levels at rest (12). The previous finding does not indicate the existence of tonic sympathetic vasoconstrictor activity under such a thermoneutral condition. Thus it is suggested that the administration of the $\alpha_2$-adrenoceptor agonist did not affect the prejunctural vasomotor control under the present experimental conditions. The other potential limitation to the interpretation of the results is that the sensitivity of $\beta$-agonist-induced vasodilation was not evaluated owing to the unsaturated vaso-
dilator responses during ISO administration. In a pilot study, the infusion of ISO at $10^{-2}$ M or more decreased blood pressure and would initiate baroreflex-mediated sympathoexcitation. Therefore, we did not administer ISO at a higher concentration to prevent confounding effects.

In conclusion, the leg skin shows higher sensitivity of the vasconstrictor response to exogenous NE relative to the arm concentration to prevent confounding effects.

ACKNOWLEDGMENTS
We gratefully acknowledge the subjects who participated in this experiment. We thank Dr. Kazuo Takahara for medical and technical supports.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


