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Intradermal administration of endothelin-1 attenuates endothelium-dependent and -independent cutaneous vasodilation via Rho kinase in young adults

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Fujii N, Amano T, Halili L, Louie JC, Zhang SY, McNeely BD, Kenny GP. Intradermal administration of endothelin-1 attenuates endothelium-dependent and -independent cutaneous vasodilation via Rho kinase in young adults. Am J Physiol Regul Integr Comp Physiol 312: R23–R30, 2017. First published November 23, 2016; doi:10.1152/ajpregu.00368.2016.—We recently showed that intradermal administration of endothelin-1 diminished endothelium-dependent and -independent cutaneous vasodilation. We evaluated the hypothesis that Rho kinase may be a mediator of this response. We also sought to evaluate if endothelin-1 increases sweating. In 12 adults (25 ± 6 yr), we measured cutaneous vascular conductance (CVC) and sweating during 1) endothelium-dependent vasodilation induced via administration of incremental doses of methacholine (0.25, 5, 100, and 2,000 mM each for 25 min) and 2) endothelium-independent vasodilation induced via administration of 50 mM sodium nitroprusside (20–25 min). Responses were evaluated at four skin sites treated with either 1) lactated Ringer solution (Control), 2) 400 nM endothelin-1, 3) 3 mM HA-1077 (Rho kinase inhibitor), or 4) endothelin-1+HA-1077. Pharmacological agents were intradermally administered via microdialysis. Relative to the Control site, endothelin-1 attenuated endothelium-dependent vasodilation (CVC at 2.000 mM methacholine, 80 ± 10 vs. 56 ± 15%max, P < 0.01); however, this response was not detected when the Rho kinase inhibitor was simultaneously administered (CVC at 2.000 mM methacholine for Rho kinase inhibitor vs. endothelin-1 + Rho kinase inhibitor sites: 73 ± 9 vs. 72 ± 11%max, P > 0.05). Endothelium-independent vasodilation was attenuated by endothelin-1 compared with the Control site (CVC, 92 ± 13 vs. 70 ± 14%max, P < 0.01). However, in the presence of Rho kinase inhibition, endothelin-1 did not affect endothelium-independent vasodilation (CVC at Rho kinase inhibitor vs. endothelin-1 + Rho kinase inhibitor sites: 81 ± 9 vs. 86 ± 10%max, P > 0.05). There was no between-site difference in sweating throughout (P > 0.05). We show that in young adults, Rho kinase is an important mediator of the endothelin-1-mediated attenuation of endothelium-dependent and -independent cutaneous vasodilation, and that endothelin-1 does not increase sweating.

vascular smooth muscle cell; endothelial cell; microcirculation; ROCK

ENDOTHELIN-1 (ET-1) is known as a potent vasoconstrictor peptide (49) that appears to be associated with vascular dysfunction in the human conduit arteries (8, 9, 14, 47) and cutaneous vasculature (7, 43). It has been shown that ET-1 generally causes cutaneous vasoconstriction via endothelin type A (ETA) receptors, but it can also elicit cutaneous vasodilation through activation of endothelin type B (ETB) receptors (33, 43–46). Studies also suggest that the mechanisms underlying the ET-1 modulation of cutaneous vascular tone differ between males and females (25) and are altered by chronic health conditions such as polycystic ovary syndrome (43, 44). Despite our growing knowledge of the role of ET-1 in the regulation of cutaneous vascular responses in humans, our understanding of the basic physiological mechanisms under in vivo conditions remains incomplete. We recently reported that intradermal administration of ET-1 diminished methacholine-induced (i.e., endothelium-dependent) cutaneous vasodilation and that this response occurred independently of nitric oxide synthase, a major endothelium-derived relaxing factor (22). In this study, we also showed that ET-1 attenuated the sodium nitroprusside-induced (i.e., endothelium-independent) cutaneous vasodilatory response (22). However, the precise mechanism(s) associated with these responses have yet to be delineated. In vitro studies have demonstrated that ET-1 may evoke a vasoconstrictive effect through Rho kinase (30, 36); an enzyme that phosphorylates myosin light chain phosphatase. The activation of this enzyme favors the contraction of vascular smooth muscle cells by increasing Ca2+ sensitivity of myofilaments. Hence, Rho kinase may be involved in mediating the action of ET-1 in human cutaneous circulation.

In addition to cutaneous vessels, human skin possesses eccrine sweat glands that are functionally important in secreting sweat to dissipate heat and therefore regulate body core temperature. Interestingly, both ETA and ETB receptors are localized on the human eccrine sweat gland (33). Activation of both ETA and ETB receptors has been found to increase intracellular calcium ions (39, 42). Given that calcium ion is a key ion contributing to sweat secretion (35, 38), it could be surmised that ET-1 may be involved in mediating the sweating response. In this regard, we recently found that endothelin receptor activation with ET-1 did not modulate cholinergic sweating in normothermic resting humans (22). However, in this study, we also showed that ET-1 induced a concomitant attenuation of endothelium-dependent cutaneous vasodilation thereby causing a decrease in cutaneous blood flow (22). Since a lowering of cutaneous blood flow has been shown to be associated with an attenuation of sweating (48), any increase in sweat rate as a result of ET-1 may have therefore been offset by a concomitant lower cutaneous blood flow. If this is true, we should expect to see an increase in sweat rate with adminis-
tation of ET-1 when a concomitant reduction in cutaneous blood flow associated with ET-1 is restored.

In the present study, we evaluated the hypothesis that Rho kinase underlies the impaired endothelium-dependent and -independent cutaneous vasodilation associated with ET-1 administration in young healthy adults. We also hypothesized that ET-1 increases sweat rate when a concomitant cutaneous vasoconstriction is blocked. The present study provides important clinical implications. Numerous reports demonstrate an increase in the risk of cardiovascular disease in individuals with Type 2 diabetes (29) and hypertension (37), as well as women with polycystic ovary syndrome (13). Since microvascular dysfunction may precede alterations in cardiovascular regulation (3, 10, 24), this elevated risk for cardiovascular disease may be related to the presence of underlying cutaneous vascular dysfunction in these populations that could be due to mechanisms associated with increased ET-1 production and/or altered ET-1 receptor activation (7, 17, 43). The results obtained from the current study may provide important knowledge that could help determine whether the prescription of a Rho kinase inhibitor may be an effective intervention(s) to counteract the detrimental influence of ET-1. As such, this could lead to improvements in microvascular function and a reduced risk for cardiovascular disease in vulnerable populations.

MATERIALS AND METHODS

Ethical approval. The present study conforms to the guidelines set forth by the Declaration of Helsinki. Approval by the University of Ottawa Health Sciences and Science Research Ethics Board was attained. Verbal and written informed consent was obtained from all volunteers before their participation in the study.

Participants. Twelve healthy young adults (6 females and 6 males) participated in one experimental protocol. Age, body mass, and height, expressed as means ± SD, were 25 ± 6 yr, 68.0 ± 14.9 kg, and 1.66 ± 0.08 m, respectively. Body mass was measured using a digital weight scale platform (model CBU150X, Mettler Toledo, Columbus, OH), and height was acquired using an eye-level stadiometer (model 2391, Detecto Scale Company, Webb City, MO). All females were tested during their early follicular phase (within 6 days of starting menstruation) or during the placebo stage if participants were using contraceptives. Thus the potential confounding influences of female hormones on cutaneous vasodilation and sweating responses (5, 28) were minimized.

Experimental design. All participants were requested to refrain from taking over-the-counter medications (e.g., nonsteroidal anti-inflammatory drugs, allergy medication, and vitamins) for >48 h before the experiment. Strenuous physical activity, caffeine, and alcohol consumption were abstained for >12 h, as well as any food for >2 h beforehand. Upon arrival, participants entered the experimental room (~23°C) and rested on a bed in a semirecumbent position during which time four intradermal microdialysis fibers (30 kDa cutoff, 10 mm membrane; MD2000, Bioanalytical Systems, West Lafayette, IN) were inserted in the dorsal side of the dermal layer of skin on the left forearm under aseptic conditions. For the fiber insertion, a 25-gauge needle was initially inserted into the unanesthetized skin and then exited ~2.5 cm away from the entry point. A microdialysis fiber was then threaded through the lumen of the needle and thereafter the needle was withdrawn, leaving the fiber in the skin. Both ends of the fiber were secured with surgical tape. Each insertion was separated by a minimum of 4 cm. Each fiber was connected to the outlet port of a liquid switch (model 110, CMA Microdialysis, Kista, Sweden). All four intradermal microdialysis sites were continuously perfused with lactated Ringer solution (Baxter, Deerfield, IL) at a rate of 4.0 μl/min with a microinfusion pump (model 4004, CMA Microdialysis, Solna, Sweden).

Endothelium-independent and -dependent cutaneous vasodilation were assessed using an established protocol performed in our previous study (22). A schematic of the experimental protocol is presented in Fig. 1. Initially we assessed endothelium-independent cutaneous vasodilation at all four sites by infusing 50 mM sodium nitroprusside (Sigma-Aldrich, St. Louis, MO) at a rate of 4.0 μl/min for 20–25 min (defined as pretreatment). A period of 61 ± 10 min was maintained

![Fig. 1. A schematic timeline of the experimental protocol. “Recovery Period” refers to the time taken for cutaneous vascular conductance (CVC) to subside to baseline values after peak CVC was achieved during pretreatment sodium nitroprusside (SNP) administration. BL, first baseline (BL) where all four sites were perfused with lactated Ringer solution. T-BL, treatment baseline.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00368.2016)
between the completion of fiber insertion and the measurement of peak CVC during the administration of sodium nitroprusside. This time period has been shown to be sufficient to ensure that any potential influence of tissue trauma with the insertion of the fiber has subsided (1). After the increased cutaneous blood flow associated with the initial sodium nitroprusside infusion was completely subsided (average duration of 86 ± 13 min), an initial 10-min baseline (defined as baseline) measurement was acquired during which time all four treatment sites were perfused with lactated Ringer solution. Thereafter, the sites were continuously perfused in a counter-balanced manner with either 1) lactated Ringer solution (Control), 2) 400 nM ET-1 (Sigma-Aldrich), 3) 3 mM HA-1077 (Rho kinase inhibitor; Cayman Chemical, Ann Arbor, MI), or 4) a combination of 400 nM ET-1 and 3 mM HA-1077. Each infusion continued until the second sodium nitroprusside infusion was initiated (see below) without switching infusion sites. The concentration of ET-1 (22) and HA-1077 (11, 32, 41) was based on previous studies employing intradermal microdialysis in the human skin. The infusion of these agents (ET-1 and/or Rho kinase inhibitor) continued for at least 40 min, whereby the last 5 min of this period was defined as the second baseline (defined as treatment-baseline). After the treatment-baseline measurement, all four sites were coinfused with methacholine (Sigma-Aldrich) in a dose-dependent fashion (0.25, 5, 100, and 2,000 mM; each for 25 min) at a rate of 4.0 µl/min. We employed a very high concentration of methacholine (2,000 mM) to ensure a full activation of sweat glands and therefore sweating response. Of note, these concentrations of methacholine were chosen based on our previous work (18). After completion of the last dose of methacholine administration (i.e., 2,000 mM), the second 50 mM sodium nitroprusside infusion was initiated at a rate of 4.0 µl/min for 20–25 min (defined as posttreatment). The purpose of second sodium nitroprusside administration was to evaluate the influence of ET-1 and/or Rho kinase inhibitor on endothelium-independent cutaneous vasodilation.

**Measurements.** Local cutaneous blood flow and sweat rate were continuously measured throughout the experimental trial (21). Local cutaneous red blood cell flux (expressed in perfusion units) was measured as an index of cutaneous blood flow at a sampling rate of 32 Hz with laser Doppler flowmetry (PeriFlux System 5000, Perimed, Stockholm, Sweden). Each integrated (seven laser array) laser Doppler flowmetry probe (model 413, Perimed) was housed in a ventilated sweat capsule, which covered an area of 1.1 cm² and was specially designed for use with intradermal microdialysis probes (34). Each laser Doppler probe was centered over the microdialysis membrane. Each sweat capsule was continuously ventilated with anhydrous air at a rate of 0.2 l/min. Water content of the effluent air from the sweat capsule was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM). Local forearm sweat rate was calculated every 5 s using the difference in water content between influent and effluent air. This difference was multiplied by flow rate and then normalized for the skin surface area under the capsule (mg·min⁻¹·cm⁻²).

Manual auscultation was performed using a mercury column phsygromomanometer (Baumanometer Standby model, WA Baum, Copiague, NY) to obtain blood pressures at 5- to 10-min intervals. Mean arterial pressure was calculated using diastolic arterial pressure plus one-third the difference between systolic and diastolic pressures (i.e., pulse pressure).

**Data analysis.** Cutaneous vascular conductance (CVC) was evaluated as cutaneous red blood cell flux divided by mean arterial pressure. All CVC and sweat rate data used for data analyses were obtained by averaging values over the last 5 min of each stage, with the exception of CVC obtained during the initial sodium nitroprusside administration (i.e., average of peak values over 5 min). CVC was presented as percentage of maximum CVC (%max). Maximal absolute CVC (perfusion units/mmHg) was evaluated from the highest value observed throughout the experiment.

**Statistical analysis.** Software package SPSS 24 for Windows (IBM, Armonk, NY) was used for all statistical analyses. CVC (%max) and sweat rate (mg·min⁻¹·cm⁻²) during the first (i.e., baseline) and second (i.e., treatment-baseline) baseline periods, as well as during administration of methacholine were analyzed using a two-way repeated measures analysis of variance with two factors of treatment site and stage. CVC (%max) during sodium nitroprusside administration was also analyzed by a two-way repeated measures analysis of variance with two factors of treatment site (4 levels: Control, ET-1, Rho kinase inhibitor, and ET-1 + Rho kinase inhibitor) and stage (2 levels: pre- and posttreatment). Maximal absolute CVC (perfusion units/mmHg) was analyzed using a one-way repeated measures analysis of variance with a factor of treatment site. When a main effect or an interaction was observed, post hoc multiple comparisons were conducted using a modified Bonferroni procedure [i.e., Hochberg procedure (23)] where Student’s pairwise t-tests were employed for between-site comparisons. We employed four preplanned between-site comparisons to limit the number of comparisons, thus minimizing.
the possibility of a type 2 error (Control vs. ET-1, Control vs. Rho kinase inhibitor, Control vs. ET-1 + Rho kinase inhibitor, Rho kinase inhibitor vs. ET-1 + Rho kinase inhibitor). The P value of ≤ 0.05 was considered statistical significance. All values are presented as mean ± 95% confidence interval unless otherwise indicated.

RESULTS

CVC during baseline did not differ between sites (all \( P > 0.07 \), see BL in Fig. 2). During treatment-baseline, CVC at the Rho kinase inhibitor site was higher relative to the Control site regardless of the presence or absence of ET-1 (both \( P \leq 0.05 \), see T-BL in Fig. 2B). During methacholine administration, ET-1 attenuated CVC relative to the Control site at 100 and 2,000 mM (both \( P \leq 0.05 \), Fig. 2A), whereas this effect was not detected with a coinfusion of Rho kinase inhibitor (both \( P = 1.00 \), Fig. 2B). The sodium nitroprusside-induced increase in CVC was attenuated by ET-1 (\( P < 0.01 \)); however, this response was not observed with simultaneous Rho kinase inhibition (\( P = 1.00 \)) (Fig. 3). There was no between-site difference in the maximal CVC response (\( P = 0.35 \) for a main effect of treatment site, Table 1). Moreover, sweat rate did not differ between sites throughout the protocol (\( P > 0.54 \) for a main effect of treatment site and stage, Fig. 4).

DISCUSSION

We show for the first time that Rho kinase is an important modulator underlying the ET-1-mediated attenuation of endothelium-dependent and -independent cutaneous vasodilation in healthy young adults. We also show that ET-1 does not affect sweating.

Cutaneous vascular response. In accordance with our previous findings (22), we observed that ET-1 administration attenuated endothelium-dependent (Fig. 2A) and -independent (Fig. 3) cutaneous vasodilation. Importantly, our current findings further our understanding of endothelin-dependent mechanisms by demonstrating that Rho kinase does indeed contribute to these responses. This is clearly evidenced by our results that show the effect of ET-1 on endothelium-dependent and -independent cutaneous vasodilation to be absent with Rho kinase inhibition (Figs. 2B and 3). These attenuations do not appear to be associated with the activation of ETB receptors located on the endothelium, as the activation of this receptor typically leads to cutaneous vasodilation rather than vasoconstriction (43, 44). The lack of involvement of the endothelium is also supported by our previous observation that ET-1 administration attenuated cutaneous vascular responses independently of nitric oxide synthase, which is known as a major endothelium-derived relaxing factor. Therefore, ET-1 appears to activate Rho kinase in vascular smooth muscle cells in the skin, a response that parallels previous findings in rabbit basilar arteries (30, 36), and ultimately the net effect of Rho kinase activation on cutaneous microvasculature is a diminished vasodilation response. While the underlying mechanism(s) associated with these responses cannot be fully elucidated from the present study, it is generally known that the activation of Rho kinase phosphorylates myosin light chain phosphatase, preventing the relaxation of vascular smooth muscle cells and thereby leading to sustained vasoconstriction (27). Moreover, given that ETA receptors have been localized in the cutaneous microvasculature (6), and activation of these receptors causes cutaneous vasoconstriction (33, 45, 46), it is plausible that these receptors are primarily involved in mediating ET-1-induced activation of Rho kinase.

Our results suggest that ET-1-induced activation of Rho kinase influences vascular smooth muscle mechanisms underlying sodium nitroprusside (nitric oxide donor)-induced cutaneous vasodilation (i.e., endothelium-independent cutaneous vasodilation). It has been shown that the sodium nitroprusside-derived increase in exogenous nitric oxide activates soluble guanylyl cyclase in the vascular smooth muscle cells (26). Activated soluble guanylyl cyclase in turn increases cGMP, which lowers \( \text{Ca}^{2+} \) bioavailability, ultimately inducing the relaxation of vascular smooth muscle. Alternatively, cGMP may activate myosin light chain phosphatase (16) thereby initiating vasorelaxation. Based on our findings, it appears that these vasodilator effects associated with nitric oxide and cGMP may be partially offset by ET-1-induced activation of Rho kinase.

In the current study, the Rho kinase inhibitor increased basal CVC (see T-BL in Fig. 2B), which is consistent with previous work (31, 32). Taken together, these findings demonstrate that Rho kinase is involved in the regulation of basal cutaneous vascular tone under normothermic conditions. It should be

Table 1. Maximal absolute cutaneous vascular conductance measured at four forearm skin sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Cutaneous Vascular Conductance, Perfusion Units/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.74 ± 0.38</td>
</tr>
<tr>
<td>ET-1</td>
<td>1.94 ± 0.38</td>
</tr>
<tr>
<td>Rho kinase inhibitor</td>
<td>1.80 ± 0.33</td>
</tr>
<tr>
<td>ET-1 + Rho kinase inhibitor</td>
<td>2.07 ± 0.43</td>
</tr>
</tbody>
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Data are means ± 95% confidence intervals. ET-1, endothelin-1. \( P = 0.35 \) for a main effect of treatment site.

Fig. 3. CVC evaluated during SNP administration before (Pretreatment) and after (Posttreatment) continuous infusion of either 1 lactated Ringer solution (Control), 2 endothelin-1 (ET-1), 3 Rho kinase inhibitor, or 4 a combination of ET-1 + Rho kinase inhibitor. Data are means ± 95% confidence intervals. The posttreatment value was lower with ET-1 in comparison with the Control site (\( P \leq 0.05 \)), but it did not differ between Rho kinase inhibitor vs. ET-1 Rho kinase inhibitor (\( P = 0.41 \)).
noted that this vasodilator effect observed with the Rho kinase inhibitor was still present during the lowest dose of methacholine administration (0.25 mM) but was absent thereafter due to a greater vasodilation induced by ≥5 mM methacholine (Fig. 2B). By contrast, ET-1 had no effect on baseline cutaneous vascular tone (see T-BL in Fig. 2A). Although this response is similar to our previous observations in which we utilized the same concentration of ET-1-infused via intradermal microdialysis (22), other studies have reported marked increases in basal cutaneous vasconstrictor tone with intradermal injection of ET-1 (4, 45, 46). In contrast, ET-1 administered iontophoretically in the skin led to elevated basal cutaneous blood flow (2). These disparate findings certainly raise important questions about the influence of the administration techniques employed to deliver ET-1 on basal cutaneous vascular tone. Indeed, studies show that needle insertion (45) and application of an electric current via iontophoresis (15) can independently increase vasodilator substance(s) (as measured by a marked cutaneous vasodilation), which may alter ET-1-dependent mechanism(s) in the skin. Further studies are required to verify these responses.

There was a methacholine dose-dependent increase in CVC from 0.25 to 100 mM, but the increase in CVC leveled off with the administration of 100 to 2,000 mM methacholine (Fig. 2). These observations indicate that tachyphylaxis occurred during the administration of high doses of methacholine only. In this context, we recently found that K⁺ channels (19), but not nitric oxide and cyclooxygenase (20), may be involved in the tachyphylaxis response. It is important to note that our results demonstrate that ET-1 is capable of attenuating endothelium-dependent cutaneous vasodilation through the activation of Rho kinase in the presence of tachyphylaxis.

Although we are the first to report a functional role of Rho kinase in the ET-1-induced attenuation of cutaneous vasodilatory responses, it has been shown that cutaneous vasoconstriction induced by intradermal administration of angiotensin II is exclusively due to Rho kinase (32). Additionally, local (41) or whole body (11, 31) cooling-induced cutaneous vasoconstriction has in part been associated with Rho kinase. Factors such as aging have also been shown to augment the contribution of Rho kinase (31, 41). Altogether, these findings demonstrate that Rho kinase is important in inducing a cutaneous vasoconstrictive effect; a response observed under many different physiological conditions (e.g., administration of angiotensin II, cooling, aging), including the administration of ET-1.

Sweating. We demonstrated that ET-1 did not modulate sweating before and during methacholine administration (Fig. 4), a response that is consistent with our previous work (22). However, since CVC was lower at the ET-1 site (Fig. 2), this may have indirectly mitigated an effect of ET-1 on the sweating response. Specifically, a reduction in cutaneous blood flow has been associated with a concomitant decrease in sweat rate and vice versa (40, 48). However, despite the fact that reduced CVC had been restored back to the Control level by coadministration of the Rho kinase inhibitor, sweat rate remained unchanged (Fig. 4). Therefore, our results lend the support to the notion that activation of endothelin receptors located on the eccrine sweat glands with ET-1 does not modulate the sweating response under a normothermic resting state.

Limitations. A previous study has suggested that sex-related differences may exist in the endothelin mechanisms governing cutaneous vascular regulation (25). As such, it is possible that there may be a sex-related difference in the effect of ET-1 and/or Rho kinase on endothelium-dependent and -independent cutaneous vasodilation. However, given that we evaluated a mixed group of males and females, our results do not allow us to discuss potential sex-related difference. In regard to our female participants, we tested them during the early follicular phase or placebo phase if taking contraceptives to minimize the influence of female hormones. However, high doses of exogenous female hormones from the use of contraceptives may still play a role in tissues, ultimately modulating peripheral end-organ mechanisms of cutaneous blood flow and sweating even during placebo phase. In addition, endogenous female hormones can increase during the placebo phase, which may also affect the peripheral mechanisms underpinning cutaneous blood flow and sweating. Further studies are required to
determine the influence of contraceptives use on the endothelin-mediated mechanisms underpinning the regulation of cutaneous blood flow and sweating. Moreover, recent work has shown that male sex hormones (i.e., androgen) can modulate the mechanisms underlying the regulation of endothelin in the human skin (43). The present study was not designed to examine the specific influences of androgens. As such, future studies are warranted to evaluate the role of male sex hormones in the influence of ET-1 and/or Rho kinase on endothelium-dependent and -independent cutaneous vasodilation.

We infused sodium nitroprusside before commencing the methacholine infusion protocol (Fig. 1). As a result, cutaneous vasodilation and sweating in response to methacholine may have been modulated by the previous sodium nitroprusside administration. It is important to emphasize that all skin sites had been previously exposed with sodium nitroprusside. Thus any difference in CVC observed during methacholine administration in the present study should be specific to the pharmacological agent employed at the respective sites (i.e., ET-1 and/or Rho kinase inhibitor). Moreover, methacholine infusion might have influenced the subsequent endothelium-independent cutaneous vasodilation evaluated by the sodium nitroprusside administration (Fig. 1). However, this effect, if any, appears to be negligible as CVC measured at the Control site did not differ between the first and second sodium nitroprusside administration periods (i.e., pre- vs. posttreatment in Fig. 3).

It is important to consider that the specificity of HA-1077 was not established in this particular study. Although it is known that HA-1077 demonstrates a strong specificity for both Rho kinase isoforms (ROCK-1 and ROCK-2), it may also inhibit other protein kinases such as protein kinase C (12). Therefore, our results with HA-1077 might reflect an inhibition of Rho kinase and protein kinase C.

Clinical implications. Clinical populations such as individuals with Type 2 diabetes (29) and hypertension (37), as well as women with polycystic ovary syndrome (13), are at greater risk of cardiovascular disease. While the pathogenesis of cardiovascular disease is multifaceted, microvascular dysfunction may underlie the development of critical cardiovascular dysfunction and/or dysregulation (3, 10, 24). In line with clinical population with cutaneous vascular dysfunction, this may be related to increased ET-1 production and/or altered ET-1 receptor mechanisms (7, 17, 43). Taken together, our study findings indicate that improving microvascular vasodilator function (e.g., skin) by prescribing Rho kinase inhibitors as a treatment medication might be an effective strategy to mitigate the risk of cardiovascular disease in vulnerable populations. It is important to note, however, that we administered exogenous ET-1 in relatively healthy young adults. Thus our results may not be representative of the endogenous increases in ET-1 occurring in older adults and those with chronic health disorders (e.g., Type 2 diabetes, chronic hypertension, females with polycystic ovary syndrome, and others). To elucidate a role of endogenous increases in ET-1, further studies employing ET-1 receptor blockers in older adults with and without chronic health disorders are warranted.

**Perspectives and Significance**

We show that Rho kinase is a major contributor responsible for the ET-1-induced attenuation of endothelium-dependent and -independent cutaneous vasodilation in healthy young adults. We also show that ET-1 does not influence sweating.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


