RESEARCH ARTICLE | Cardiovascular and Renal Integration

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

Naoto Fujii,1 Tatsuro Amano,2 Lyra Halili,1 Jeffrey C. Louie,1 Sarah Y. Zhang,1 Brendan D. McNeely,1 and Glen P. Kenny1

1Human and Environmental Physiology Research Unit, University of Ottawa, Ottawa, Canada; and 2Laboratory for Exercise and Environmental Physiology, Faculty of Education, Niigata University, Niigata, Japan

Submitted 29 August 2016; accepted in final form 18 November 2016

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 underpinning these responses 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](http://ajpregu.physiology.org/...)

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.
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 (2,000 mM) to ensure a full activation of sweat glands for use with intradermal microdialysis probes (34). 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 sphygmonanometer (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 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

![Fig. 2. CVC evaluated before and during methacholine administration. The four skin sites were continuously perfused with 1) lactated Ringer solution (Control, A), 2) endothelin-1 (ET-1, A), 3) Rho kinase inhibitor (B), or 4) a combination of ET-1 + Rho kinase inhibitor (B), with the exception of first baseline (BL) where all four sites were perfused with lactated Ringer solution, T-BL, treatment baseline. All four sites were coinfused with methacholine in a dose-dependent fashion (0.25, 5, 100, and 2,000 mM) in the presence of ET-1 and/or the Rho kinase inhibitor. Data are means ± 95% confidence intervals. *Control vs. ET-1 (P < 0.05); †Control vs. Rho kinase inhibitor (P < 0.05); ‡Control vs. ET-1 + Rho kinase inhibitor (P < 0.05). Although CVC was attenuated by ET-1 in relation to the Control site at 100 and 2,000 mM methacholine, these effects were not detected in the presence of Rho kinase inhibitor (both P = 1.00).](http://ajpregu.physiology.org/)
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 $\approx 0.05$ 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 an interaction 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 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

![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 ($P \approx 0.05$), but it did not differ between Rho kinase inhibitor vs. ET-1 Rho kinase inhibitor ($P = 0.41$).](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00368.2016)

<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>
</table>

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

Data are means ± 95% confidence intervals. ET-1, endothelin-1. $P = 0.35$ for a main effect of treatment site.
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 vasoconstrictor tone with intraderal 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 stay longer 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 contraceptive use on the endothe-
lin-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 en-
dothelin 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 cutane-
ous vasodilation.

We infused sodium nitroprusside before commencing the
methacholine infusion protocol (Fig. 1). As a result, cuta-
neous vasodilation and sweating in response to methacho-
line 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 re-
spective sites (i.e., ET-1 and/or Rho kinase inhibitor). More-
over, methacholine infusion might have influenced the sub-
sequent 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 isozymes (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 individ-
uals 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 pathogen-
esis of cardiovascular disease is multifaceted, microvascular
dysfunction may underlie the development of critical car-
diovascular dysfunction and/or dysregulation (3, 10, 24). In
line with this clinical population exhibit cutaneous vascular dys-
function, and this may be related to increased ET-1 produc-
tion 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 prescrib-
ing 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 represen-
tative 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 poly-
cystic 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.

ACKNOWLEDGMENTS

We greatly appreciate all of the volunteers for taking their time to partic-
ipate in this study. We thank My-An Tran for her help in conducting this study.

GRANTS

This study was supported by the Natural Sciences and Engineering Re-
search Council of Canada (NSERC; Discovery Grant, RGPIN-06313-2014
and Discovery Grants Program-Accelerator Supplement, RGPAS-462252-2014;
Funds held by G. P. Kenny). G. P. Kenny is supported by a University of
Ottawa Research Chair Award. N. Fuji is supported by the Human and
Environmental Physiology Research Unit. J. C. Louie was supported by a
Queen Elizabeth II Graduate Scholarship in Science and Technology. S. Y.
Zhang was supported by an NSERC Undergraduate Student Research Award.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

N.F., L.H., and G.P.K. conceived and designed experiments. N.F., T.A.,
interpreted the experimental results. N.F. drafted the manuscript. N.F., T.A.,
L.H., J.C.L., S.Y.Z., B.D.M., and G.P.K. edited and revised the manuscript.
All authors approved the final version of the manuscript. All experiments took
place at the Human and Environmental Physiology Research Unit located at
the University of Ottawa.

REFERENCES

1. Anderson C, Andersson T, Wardell K. Changes in skin circulation after
insertion of a microdialysis probe visualized by laser Doppler perfusion
1747.ep1237630.

2. Andersson SE, Edvinsson ML, Alving K, Edvinsson L. Vasodilator
effect of endothelin in cutaneous microcirculation of heart failure patients.
7843.2005.pto.84.x.

3. Antonios TF, Rattray FM, Singer DR, Markandu ND, Mortimer PS,
MacGregor GA. Rarefaction of skin capillaries in normotensive offspring
doi:10.1136/heart.89.2.175.

4. Brain SD, Thomas G, Crossman DC, Fuller R, Church MK. Endothe-
ilin-1 induces a histamine-dependent flare in vivo, but does not activate

5. Brunet VE, Miner JA, Meendering JR, Kaplan PF, Minson CT,
17β-Estradiol and progesterone independently augment cutaneous thermal
hyperemia but not reactive hyperemia. Microcirculation 18: 347–355,

6. Bull HA, Bunker CB, Terenghi G, Springall DR, Zhao Y, Polak JM,
Dowd PM. Endothelin-1 in human skin: immunolocalization, receptor
binding, mRNA expression, and effects on cutaneous microvascular en-
1747.ep12483000.

King GL, LoGerfo FW, Horton ES, Yeves A. Microvascular and
macrovascular reactivity is reduced in subjects at risk for type 2 diabetes.

8. Cardillo C, Kilcoyne CM, Cannon RO III, Panza JA. Increased activity
of endogenous endothelin in patients with hypercholesterolemia. J Am

of endothelin in the increased vascular tone of patients with essential hyper-
Heat stress and local warming in humans.

Cyclooxygenase inhibition does not alter methacholine-induced cutaneous vasodilation during whole body cooling.

Increased coronary heart disease risk are characterized by an impaired vascular tone in humans.

Impairment of skin microvascular reactivity in hypertension and uraemia.


Rho kinase-mediated local cold-induced cutaneous vasoconstriction is augmented in aged human skin.

Regional relation between skin vascular tone and age-specific associations in 1·25 million people.


Effects of menstrual cycle and physical training on heat loss responses during dynamic exercise at moderate intensity in a temperate environment. Am J Physiol Regul Integr Comp Physiol • doi:10.1152/ajpregu.00368.2016 • www.ajpregu.org