Can intradermal administration of angiotensin II influence human heat loss responses during whole body heat stress?

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Fujii N, Meade RD, Paull G, McGinn R, Foudil-bey I, Akbari P, Kenny GP. Can intradermal administration of angiotensin II influence human heat loss responses during whole body heat stress? J Appl Physiol 118: 1145–1153, 2015. First published March 12, 2015; doi:10.1152/japplphysiol.00025.2015.—It is unclear if angiotensin II, which can increase the production of reactive oxygen species (oxidative stress), modulates heat loss responses of cutaneous blood flow and sweating. We tested the hypothesis that angiotensin II-induced increases in oxidative stress impair cutaneous perfusion and sweating during rest and exercise in the heat. Eleven young (24 ± 4 yr) healthy adults performed two 30-min cycling bouts at a fixed rate of metabolic heat production (400 W) in the heat (35°C). The first and second exercises were followed by a 20- and 40-min recovery. Four microdialysis fibers were placed in the forearm skin for continuous administration of either: 1) lactated Ringer (control), 2) 10 μM angiotensin II, 3) 10 mM ascorbate (an antioxidant), or 4) a combination of 10 μM angiotensin II + 10 mM ascorbate. Cutaneous vascular conductance (CVC; laser-Doppler perfusion units/mean arterial pressure) and sweating (ventilated capsule) were evaluated at each skin site. Compared with control, angiotensin II reduced both CVC and sweating at baseline resting and during each recovery in the heat (all P < 0.05). However, during both exercise bouts, there were no differences in CVC or sweating between the treatment sites (all P > 0.05). When ascorbate was coinfused with angiotensin II, the effect of angiotensin II on sweating was abolished (all P > 0.05); however, its effect on CVC at baseline resting and during each recovery remained intact (all P < 0.05). We show angiotensin II impairs cutaneous perfusion independent of oxidative stress, while it impairs sweating through increasing oxidative stress during exposure to an ambient heat stress before and following exercise.

sudomotor; renin-angiotensin-aldosterone system; AT1 receptor; reactive oxygen species; microcirculation

A recent study by Stewart et al. (44) demonstrated that intradermal administration of angiotensin II via microdialysis attenuated cutaneous blood flow responses during local heating of human forearm skin to 42°C under normothermic conditions. The authors reasoned that this response was mediated through the activation of angiotensin II type 1 (AT1) receptors (44), which has been shown elicit increases in the production of reactive oxygen species (i.e., oxidative stress), such as superoxide (3). This was evidenced by the fact that coinfusion of losartan (AT1 receptor antagonist) or ascorbate (an antioxidant) abolished the effect of angiotensin II (44). An angiotensin II-mediated attenuation of cutaneous blood flow may have important implications for heat loss and thereby the regulation of core body temperature during whole body heat stress (such as exposure to an environmental heat load or exercise in the heat). However, it is important to note that distinct and often separate mechanisms govern the responses to local and whole body heating. In contrast to local skin heating, the elevations in tissue temperature associated with whole body heat stress trigger the release of acetylcholine and other co-transmitters from sympathetic cholinergic nerves (22, 26, 27), subsequently inducing cutaneous active vasodilation (25, 33, 39). Therefore, although the results of the study by Stewart et al. (44) demonstrate that angiotensin II is capable of decreasing cutaneous blood flow, it is entirely unclear if a similar response would be observed during a whole body heat stress.

While Stewart et al. (44) did not examine the effects of angiotensin II on the sweating response, it is noteworthy that the presence of AT1 receptors has been observed on the human eccrine sweat gland (45). Furthermore, a recent correlative study demonstrated that greater levels of oxidative stress (as indicated by elevated concentrations of urinary malondialdehyde) were associated with an attenuated sweating response induced by acetylcholine electrophoresis in humans in vivo (17). Taken together, these observations suggest that elevated levels of angiotensin II, and, therefore, reactive oxygen species, may be capable of modulating the sweating response. A role for angiotensin II in modulating sweating during ambient heat exposure and/or exercise in the heat has yet to be examined. However, given that sweating represents the major avenue of heat loss during whole body heat stress, especially during exercise in the heat (14), an angiotensin II-mediated influence on sweating could have profound implications for core body temperature regulation.

Increases in angiotensin II can occur in an exercise-induced dehydrated condition with levels increasing with higher states of dehydration (9). Additionally, advancing age and/or chronic health conditions (e.g., diabetes, hypertension) can also cause elevations in angiotensin II (6, 10, 46). However, it is currently unclear if angiotensin II is capable of modulating cutaneous blood flow and sweating during whole body heat stress. Therefore, assessing the influence of exogenous angiotensin II on these responses is a necessary first step in understanding how increased levels of endogenous angiotensin II, such as those that occur in the above-mentioned situations, may modulate body temperature regulation. Thus the purpose of this study was to evaluate the influence of intradermal administration of angiotensin II on cutaneous blood flow and sweating during whole body heat stress associated with exposure to an environmental heat load and exercise in the heat. We hypothesized that acute local administration of angiotensin II via intradermal microdialysis would attenuate cutaneous blood flow, which
would be paralleled by a similar reduction in sweating. Furthermore, we surmised that these attenuations would be mediated through increases in oxidative stress associated with increased production of reactive oxygen species.

**MATERIALS AND METHODS**

**Ethical Approval**

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and conformed to the guidelines set forth by the Declaration of Helsinki. Verbal and written, informed consent was obtained from all volunteers before their participation in the study.

**Subjects**

Eleven healthy, habitually active (2–4 days/wk, ≥30 min of structured physical activity per day), young adults (seven men and four women) participated in this study. All subjects had no history of skin disorders, hypertension, heart disease, diabetes, autonomic disorders, cigarette smoking or cystic fibrosis transmembrane conductance regulator mutations, and were not taking prescription medications (with the exception of 3 of 4 women using contraceptives). Two women used orally administered pills, and one used an intrauterine device. All female subjects completed their experimental session during the early follicular phase (within 6 days of the start of menstruation), or during the placebo phase, if using contraceptives, to minimize any effect of female sex hormones on cutaneous vasodilation and sweating (5, 30). The characteristics of subjects, expressed as means ± SD, were as follows: 24 ± 4 yr of age, 71.0 ± 8.1 kg of body mass, 1.71 ± 0.05 m in height, 1.83 ± 0.12 m² of body surface area, and 43.1 ± 4.3 ml O₂·kg⁻¹·min⁻¹ of peak oxygen consumption. The characteristics were all determined during a preliminary session, as described previously (11).

**Experimental Procedures**

Subjects completed one preliminary session and participated in one experimental session on separate days. All subjects abstained from taking over-the-counter medications, including nonsteroidal anti-inflammatory agents, vitamins, and minerals for at least 48 h before each session, as well as alcohol, caffeine, and heavy exercise for at least 12 h before each session. On the day of the experimental session, subjects were instructed to avoid consuming any food 2 h before and throughout the study.

On arrival to the laboratory on the day of the experimental session, the subjects changed into shorts and running shoes (with a sports bra for women), provided a urine sample, and voided the remainder of their bladder. Following a measurement of body mass using a digital weight scale platform (model CBU150X, Mettler, Toledo, OH), subjects were then seated in a semirecumbent position in a thermoneutral room (25°C) and instrumented with four microdialysis fibers (30 kDa cutoff, 10 mm membrane) (MD2000, Bioanalytical Systems, West Lafayette, IN) on the dorsal side of the left forearm in the dermal layer of the skin. A 25-gauge needle was first inserted into the unanesthetized skin using aseptic technique and exited the skin ~2.5 cm from the entrance point. The microdialysis fiber was then threaded through the lumen of the needle, after which the needle was withdrawn, leaving the fiber in place. Microdialysis fibers were secured with surgical tape, and each fiber was separated by at least 4.0 cm. In the present study, ice was not used to numb the skin before the placement of each microdialysis fiber. Icing may offset the confounding effect of microdialysis fiber placement on the cutaneous blood flow response during passive heating at rest (16). However, icing itself may modulate the mechanisms underpinning cutaneous blood flow and sweating responses during exercise in the heat; thus we opted not to use it. Furthermore, it is important to note that, in the present study, a microdialysis fiber was placed at each site, including the control site. Thus any influence pertaining to instrumentation was uniform across all sites, thereby validating between-site comparisons. After the four microdialysis fibers were placed, the subjects moved to a thermal chamber (Can-Trol Environmental Systems, Markham, ON, Canada), regulated to an ambient air temperature of 35°C and a relative humidity of 20%, where they rested for 60–70 min on a semirecumbent cycle ergometer (Corival Recumbent, Lode B.V., Groningen, the Netherlands). During this resting period, each microdialysis fiber was continuously perfused in a counterbalanced manner with the following pharmacological agents: 1) lactated Ringer (control; Baxter, Deerfield, IL); 2) 10 μM angiotensin II (angiotensin II; Sigma-Aldrich, St. Louis, MO); 3) 10 mM ascorbate (ascorbate; Sigma-Aldrich), an antioxidant; or 4) a combination of 10 μM angiotensin II + 10 mM ascorbate (angiotensin II + ascorbate). These concentrations were determined based on previous studies in which intradermal microdialysis was employed in human skin (18, 36, 44, 49). Each drug was administered continuously at a rate of 4.0 μl/min using a microinfusion pump (model 400, CMA Microdialysis, Solna, Sweden). Given that angiotensin II can increase both superoxide and hydrogen peroxide from nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine oxidases, respectively, in the human skin (36), we elected to use ascorbate (i.e., a nonspecific antioxidant) to target all possible reactive oxygen species produced as a result of angiotensin II administration. During the experimental session, at least 90 min were allowed between the placement of microdialysis fibers and the start of baseline measurements to ensure that any traumatic effect associated with the insertion of needle and/or microdialysis probe had subsided (16). The drug perfusion continued for the entire experimental protocol until the maximal cutaneous vasodilation procedure began (see below).

Following the instrumentation period, baseline resting data were collected for 5–8 min. Given that the instrumentation period was performed in the thermal chamber, subjects were exposed to an ambient heat stress (35°C, 20% relative humidity) for a minimum of 60 min before baseline data collection. Thereafter, the subjects performed two 30-min bouts of semirecumbent cycling at a fixed rate of metabolic heat production of 400 W, requiring an external work load of 76 ± 4 W and equivalent to 47 ± 4% of the subjects’ peak oxygen consumption. The first and second bouts of exercise were followed by a 20- and 40-min recovery period, respectively. Two bouts of exercise were employed to determine whether the mechanisms underlying cutaneous blood flow and sweating differ between initial and subsequent exercise bouts. Indeed, there is a rapid increase in both cutaneous blood flow and sweating following the onset of a second relative to first exercise (28), inferring that the mechanisms underlying the heat loss responses may be altered. Furthermore, a 20-min recovery was employed after the first exercise to affirm previous findings demonstrating that 1) there is a marked suppression in the heat loss responses, such that both cutaneous blood flow and sweating return to or near baseline resting levels within 20 min, despite a sustained elevation in core body temperature; and 2) that the response remains intact with successive exercise bouts, despite progressive increases in the level of hyperthermia (28). Following the second exercise bout, an extended 40-min recovery was used to determine whether the attenuation of heat loss responses remained intact as typically observed during a prolonged recovery period. Previous studies showed that, irrespective of the number of subsequent exercise/recovery cycles performed (and level of hyperthermia achieved) (28), the suppression of heat loss responses is maintained with an extended recovery, during which time a gradual decay in core body temperature occurs. A fixed absolute heat load was used in the present study to elicit a similar thermal drive for whole body sweating in all subjects (12). After the second (40-min) recovery period, administration of 50 mM sodium nitroprusside (Sigma-Aldrich) at a rate of 6.0 μl/min commenced and was continued for 20–30 min to obtain maximal cutaneous vasodilation. After confirming that maximal cutaneous vasodilation was...
achieved, as defined by a plateau for at least 2 min, blood pressure was taken via manual auscultation to evaluate maximal cutaneous vascular conductance. Thereafter, body mass was measured, and a final urine sample was collected.

Measurements

Sweat capsules, each covering an area of 3.8 cm², were attached to the skin with adhesive rings and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL) over the center of the semipermeable microdialysis membranes. Dry compressed air was supplied to each capsule at a rate of 1.0 l/min, while the water content of the effluent air from the sweat capsule was measured with a capacitance hygrometer (model HMT333, Vaisala, Helsinki, Finland). Long vinyl tubes were used for connections between the gas tank and the sweat capsule, and between the sweat capsule and the hygrometer, so that internal gas temperature was equilibrated to near room temperature (−35°C) before reaching the sweat capsule (inlet) and the hygrometer (outlet). Local forearm sweat rate was calculated every 5 s from the difference in water content between influent and effluent air, multiplied by the flow rate and normalized for the skin surface area under the capsule (mg·min⁻¹·cm⁻²).

Cutaneous red blood cell flux, which is an index of cutaneous blood flow expressed in perfusion units, was locally measured at a sampling rate of 32 Hz with laser-Doppler flowmetry (PeriFlux System 5000, Perimed, Stockholm, Sweden). Integrated laser-Doppler flowmetry probes with a seven-laser array (model 413, Perimed) were housed in the center of each sweat capsule over each microdialysis fiber, allowing for the simultaneous measurement of both local forearm sweat rate and cutaneous red blood cell flux at the four skin sites. Heart rate was recorded at a sampling rate of 15 s using a heart rate monitor (RS400, Polar Electro, Kempele, Finland). Manual auscultation was performed using a validated mercury column sphygmomanometer (Baumanometer Standby model, WA Baum, Copiague, NY) to obtain systolic and diastolic blood pressures every 5 min. Mean arterial pressure was evaluated as diastolic arterial pressure plus one-third the difference between systolic and diastolic pressures (i.e., pulse pressure). Cutaneous vascular conductance (CVC) was evaluated as cutaneous red blood cell flux divided by mean arterial pressure. CVC data were presented as a percentage of maximum (%max), as evaluated during the maximal cutaneous vasodilation procedure.

As an index of core body temperature, esophageal temperature was measured using a general purpose thermocouple temperature probe (Mallinckrodt Medical, St. Louis, MO), which was attached to a two-way T-shape non-rebreathing valve (model 2700, Hans-Rudolph). Oxygen uptake and respiratory exchange ratio were obtained every 30 s and were used to calculate metabolic rate (37). Metabolic heat production during cycling was estimated from metabolic rate minus external work.

A total solids refractometer (model TS400, Reichter, Depew, NY) was used to evaluate urine specific gravity, which is an index of body fluid status, from the urine samples obtained at the start and end of the experimental protocol.

Data Analysis

Baseline resting values were obtained by averaging measurements performed over 5–8 min. Values at the start of intermittent exercise (time 0) were obtained during the last 5 min before exercise commenced. There was no time gap between baseline resting and time 0. Local forearm sweat rate and CVC, core body and skin temperatures, and heart rate data acquired during the exercise and recovery periods were obtained by averaging measurements made over the last 5 min of each 10-min interval. The blood pressure data acquired during the exercise and recovery periods were obtained by averaging the two measurements made over each 10-min interval. The change in CVC (ΔCVC) relative to the control site was evaluated at the angiotensin II, ascorbate, and angiotensin II + ascorbate sites. Likewise, the change in sweat rate (Δsweat rate) from control was evaluated at each skin site. Local forearm absolute maximal CVC, achieved during sodium nitroprusside administration at the end of the experimental protocol, was determined from averaged CVC data over a minimum of 2 min once a plateau was attained.

Statistical Analysis

Local forearm sweat rate and CVC obtained at baseline resting (following a minimum 60 min of ambient heat exposure) and at time 0 were each analyzed using a one-way repeated-measures ANOVA with the factor of treatment site (four levels: control, angiotensin II, ascorbate, and angiotensin II + ascorbate). Both variables were analyzed during exercise using a two-way repeated-measures ANOVA, with the factors of treatment site (four levels) and time (six levels: at 10, 20, and 30 min during exercises 1 and 2). Similarly, local forearm sweat rate and CVC were analyzed during recovery using a two-way repeated-measures ANOVA with the factors of treatment site (four levels) and time (six levels: recovery 1 at 10 and 20 min, and recovery 2 at 10, 20, 30, and 40 min). Moreover, forearm sweat rate and CVC at each skin site were analyzed with one-way repeated-measures ANOVA with the factor of time to evaluate differences from baseline (four levels: baseline resting, recovery 1 at 20 min, and recovery 2 at 20 and 40 min). Core body and mean skin temperatures, as well as cardiovascular variables (heart rate and mean arterial pressure) were analyzed using a one-way repeated-measures ANOVA with the factor of time (six levels: baseline resting, end of exercises 1 and 2, as well as recovery 1 at 20 min, and recovery 2 at 20 and 40 min). Local forearm absolute maximal CVC (expressed in perfusion units/mmHg) was analyzed with a one-way repeated-measures ANOVA with the factor of treatment site (four levels). When a significant main effect or an interaction was observed, post hoc comparisons were carried out using two-tailed Student’s paired t-tests adjusted for multiple comparisons using the Holm-Bonferroni procedure to maintain a fixed α-level of 0.05. Two-tailed Student’s paired t-tests were used to compare body mass as well as urine specific gravity before and after the trial, along with whether ΔCVC or Δsweat rate at each treatment site was different from control (i.e., 0 values). All data used for parametric statistical analyses were normally distributed. The level of significance for all analyses was set at P ≤ 0.05. All values are expressed as means ± 95% confidence interval, unless otherwise indicated. The confidence intervals were calculated as 1.96 × SE of the mean.

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RESULTS

Hydration Status, Body Temperatures, and Cardiovascular Variables

Body mass decreased by 1.6 ± 0.3% (P < 0.001) from the start of the trial, and urine specific gravity was elevated at the end of the trial (1.018 ± 0.003) relative to baseline values (1.011 ± 0.004) (P = 0.028). Relative to baseline resting, esophageal temperature was elevated during each exercise and recovery period, whereas mean skin temperature was increased during each exercise but returned to baseline levels during the recovery periods (Table 1). Esophageal temperature was higher during the second relative to first (37.56 ± 0.08°C) exercise by 0.07 ± 0.06°C at 40 min (both P < 0.044). Compared with baseline resting, heart rate was elevated during each exercise and recovery, whereas mean arterial pressure was elevated only during each exercise (Table 1).

Local Forearm Cutaneous Vascular Response

Baseline resting. CVC at baseline resting and time 0 was reduced at the angiotensin II and angiotensin II + ascorbate sites compared with the control site, while CVC at the ascorbate site did not differ from that of the control site (Fig. 1A). Furthermore, the effect of angiotensin II administration was consistently observed during baseline resting, such that CVC was reduced by ≥10%max relative to the control site in 9 subjects at the angiotensin II site, and 10 subjects at the angiotensin II + ascorbate site (Fig. 2A).

Exercise. CVC was elevated at 20 and 30 min of exercise relative to the 10-min time point of both the first and second

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 1. Time course changes in cutaneous vascular conductance (A) and sweat rate (B) at baseline resting, during two exercise bouts performed at a fixed rate of metabolic heat production (400 W), and during the postexercise recovery periods. Four skin sites were continuously administered with the following: 1) lactated Ringer (control; ○); 2) 10 µM angiotensin II (▪); 3) 10 mM ascorbate (●), an antioxidant; or 4) a combination of 10 µM angiotensin II + 10 mM ascorbate (△). Values are means ± 95% confidence interval. Each value during exercise and recovery represents the average of the last 5 min of each 10-min interval. Start of intermittent exercise (time 0) indicates resting values 5 min before exercise. Rest. baseline resting; Ex 1, first exercise; Rec 1, first recovery; Ex 2, second exercise; Rec 2, second recovery. *Control significantly different from angiotensin II (P < 0.05). †Control significantly different from angiotensin II + ascorbate (P < 0.05).
exercise bouts, irrespective of treatment site \((P < 0.049)\). However, separate or combined administration of angiotensin II and ascorbate did not influence CVC during either exercise bout compared with control \((P = 0.155\) for main effect of treatment site, Figs. 1A and 3, A and B).

**Recovery.** Upon cessation of each exercise, CVC at all skin sites sharply returned back to baseline resting level at 20 min of recoveries 1 and 2 and remained similar to baseline resting at 40 min of recovery 2 \((P > 0.171\) for main effect of time). Parallel to the observations during baseline resting, CVC during both recovery periods was consistently reduced by \(\pm 10\%\) max compared with the control site at the angiotensin II \((10\) subjects in the first recovery and 8 subjects in the second recovery) and angiotensin II + ascorbate \((10\) subjects for both first and second recoveries) sites, but similar to control at the ascorbate site \((P = 0.171\) for main effect of time).

**Maximal absolute values.** Local forearm absolute maximal CVC did not differ across the four treatment sites \((P = 0.830\) for main effect of treatment site: control, \(1.89 \pm 0.28\) perfusion units/mmHg; angiotensin II, \(1.85 \pm 0.35\) perfusion units/mmHg; ascorbate, \(1.73 \pm 0.30\) perfusion units/mmHg; angiotensin II + ascorbate, \(1.73 \pm 0.27\) perfusion units/mmHg).

**Local Forearm Sweat Rate**

**Baseline resting.** Sweat rate was attenuated at the angiotensin II relative to the control site by ~25\% in 10 of the 11 subjects \((P = 0.011\) for comparison with control, Figs. 1A and D). In contrast, angiotensin II and/or ascorbate did not modulate sweat rate during the first or second exercise bouts at any time point \((P = 0.993\) for main effect of treatment site, Figs. 1B and F). Furthermore, there was no effect of ascorbate on sweat rate compared with the control site \((P = 0.993\) for comparison with control, Figs. 1B and F).

**Exercise.** Sweat rate increased more rapidly during the second exercise bout, such that it was greater at 10 min of the second compared with first exercise at all treatment sites \((P < 0.001\) for main effect of treatment site, Figs. 1B and C). In contrast, angiotensin II and/or ascorbate did not modulate sweat rate during the first or second exercise bouts at any time point \((P = 0.419\) for main effect of treatment site, Figs. 1B and D).

**Recovery.** Immediately after each exercise, at all skin sites, sweat rate rapidly dropped, but remained higher than baseline resting level at 20 min into recoveries 1 and 2 as well as at 40 min of recovery 2 \((P < 0.053\) for main effect of time). Similar to baseline resting, sweat rate during the first and second recoveries was consistently reduced by 15–20\% at the angiotensin II site compared with the control site in 10 of the 11 subjects for both first and second recoveries.
second recoveries; however, the effect of angiotensin II on sweat rate during recovery was abolished when administered in combination with ascorbate (Figs. 1B and 2, E and F). Moreover, ascorbate did not affect sweat rate compared with the control site during either recovery period (Figs. 1B and 2, E and F).

**DISCUSSION**

We show that intradermal administration of angiotensin II modulated cutaneous blood flow and, for the first time, sweating during ambient heat exposure (i.e., 35°C). Furthermore, we show that angiotensin II reduced cutaneous blood flow, irrespective of the presence of ascorbate (Figs. 1A and 2A). This is in contrast to the observation made by Stewart et al. (44), who demonstrated that the angiotensin II-induced attenuation of cutaneous blood flow during local heating was due entirely to oxidative stress-dependent mechanisms. Thus the mechanism by which angiotensin II impairs cutaneous blood flow response during whole body heating at rest appears to differ from that observed during local heating.

While our findings cannot define the mechanism(s) by which angiotensin II modulates cutaneous blood flow, some information may be gleaned from prior studies. Specifically, Stewart et al. (44) demonstrated that angiotensin II can decrease cutaneous blood flow in normothermic conditions (participants resting in an ambient air temperature of 25°C), implicating vasoconstriction in the mechanisms underpinning the angiotensin II-mediated reduction in cutaneous blood flow. In fact, angiotensin II can increase calcium ion (1), activate Rho A (20), as well as upregulate endothelin type A receptor (32) in the smooth muscle cell, all of which result in smooth muscle contraction (i.e., vasoconstriction). In addition, activated angiotensin II receptors located on the prejunctional terminals of sympathetic adrenergic nerves can cause the release of vasoconstrictor transmitters (i.e., noradrenaline) (7). Alternatively, angiotensin II has been shown to attenuate acetylcholine re-

**Cutaneous Vascular Response**

Our work demonstrated that, relative to a control site, angiotensin II reduced cutaneous blood flow at baseline resting, which suggests that angiotensin II is capable of attenuating cutaneous blood flow during ambient heat exposure (i.e., 35°C). Furthermore, we show that angiotensin II reduced cutaneous blood flow, irrespective of the presence of ascorbate (Figs. 1A and 2A). This is in contrast to the observation made by Stewart et al. (44), who demonstrated that the angiotensin II-induced attenuation of cutaneous blood flow during local heating was due entirely to oxidative stress-dependent mechanisms. Thus the mechanism by which angiotensin II impairs cutaneous blood flow response during whole body heating at rest appears to differ from that observed during local heating.

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lease from cholinergic nerves in the left ventricle (23). Thus angiotensin II may reduce cutaneous blood flow by inhibiting acetylcholine release from cutaneous cholinergic nerves, which are important contributors to cutaneous blood flow regulation during heat stress (25, 33, 39). However, our study findings do not permit us to discern the precise mechanism by which angiotensin II modulates cutaneous blood flow.

In contrast to baseline resting, no effect of angiotensin II on cutaneous blood flow was observed during either of the two exercise bouts (Figs. 1A and 3, A and B). Numerous reports suggest that nitric oxide (NO) production, and therefore NO bioavailability, increases during exercise and contributes to the cutaneous blood flow response (11, 35, 47). Given NO can impair angiotensin II-mediated vasoconstriction (2), this increase in NO bioavailability during exercise may counteract the influence of angiotensin II on cutaneous blood flow regulation. Furthermore, since NO also contributes to cutaneous blood flow response during passive heating at rest (4, 8, 24, 34, 48), the influence of angiotensin II on cutaneous blood flow may be diminished even during heat stress at rest once core body temperature and NO achieve high levels. These possibilities need to be tested in the future.

During each of the postexercise periods, cutaneous blood flow returned to values similar to baseline resting levels at each treatment site within ~20 min. This rapid postexercise suppression of cutaneous blood flow, and therefore heat loss, is thought to be mediated by nonthermal factors, such as baroreceptor activation (28). Importantly, we demonstrated that postexercise reduction in cutaneous blood flow was exacerbated with the administration of angiotensin II relative to the control site, despite being similar between sites during exercise. Furthermore, this pattern of response was maintained with the coinfusion of ascorbate (Figs. 1A and 2, B and C). Taken together, our findings indicate that angiotensin II modulates cutaneous blood flow during the postexercise period, but this cannot be explained by elevations in oxidative stress. Moreover, given the similarity in the pattern of response between baseline resting and the successive postexercise recovery periods in the heat, it appears that this angiotensin II-mediated response occurs independent of core body temperature and, therefore, thermal drive.

Sweating Response

To the best of our knowledge, this study is the first to evaluate the effect of angiotensin II on sweat production in humans. Our results indicate that angiotensin II consistently decreased sweat rate compared with the control site during baseline resting, and that this attenuation was eliminated by the simultaneous administration of ascorbate (Figs. 1B and 2D). Thus we show that angiotensin II impairs the sweating response during ambient heat exposure at rest, which is likely mediated by increases in oxidative stress. Our findings are likely explained by an angiotensin II-induced activation of AT1 receptors on the sweat gland (45), which in turn increases the reactive oxygen species, including superoxide and hydrogen peroxide through activation of NADPH and xanthine oxidases, respectively (36). Given ascorbate is a nonselctive antioxidant and may not effectively scavenge superoxide (21), future studies are needed to elucidate what reactive oxygen species underlie our results.

Sweat rate was elevated to a similar level at the end of both exercise bouts, albeit a more rapid increase within the first 10 min was observed during the second exercise relative to the first at all treatment sites. This typical response pattern, termed the priming effect (13), occurs during subsequent exercise bouts and results in a decrease in the amount of heat stored during the subsequent exercise period(s) (28). However, in parallel with our observations for cutaneous blood flow, no effect of angiotensin II on sweat rate was noted during either exercise bout, despite differences in the level of hyperthermia (Figs. 1B and 3, C and D). In parallel with cutaneous blood flow (discussed above), previous reports suggest that NO bioavailability is increased at the level of the sweat gland during exercise (11, 41, 47). Given that NO can bind superoxide (40), the oxidative stress-dependent attenuation in sweating elicited by angiotensin II may be offset during exercise due to the associated elevations in NO.

Consistent with previous reports (28), we observed a rapid decrease in sweating during the first and second recovery periods that was comparable at all treatment sites, irrespective of the sustained elevation in core body temperature. Importantly, acute administration of angiotensin II exacerbated the reduction in sweating during both postexercise recovery periods, which suggests that angiotensin II can modulate sweating during postexercise recovery. Noteworthy, while sweat rate during recovery was higher than that at baseline resting at all treatment sites, the angiotensin II-associated decrease in sweating was consistent, irrespective of core body temperature. Moreover, this reduction in sweat rate was eliminated by the simultaneous administration of ascorbate (Figs. 1B and 2, E and F). Thus angiotensin II suppresses postexercise sweating in a manner that is oxidative stress dependent and that does not depend on the level of hyperthermia.

Perspectives and Significance

The results of the present study have important physiological and clinical implications as elevations in angiotensin II can occur in states of dehydration (9), as well as with advancing age and chronic health conditions (e.g., diabetes, hypertension) (6, 10, 46). Given skin itself can produce angiotensin II, as evidenced by the presence of angiotensinogen, renin, and angiotensin-converting enzyme in the skin (43), local changes in the level of angiotensin II may specifically have an important role in modulating local heat loss responses, thus influencing the rate of heat dissipation. It is also important to note that the aforementioned factors, such as hydration status (38), aging (31, 42), as well as chronic health disorders, including diabetes (29) and hypertension (19), have been shown to impair heat loss responses. Taken together with our results, it is therefore possible that elevated levels of angiotensin II may contribute to the attenuated heat loss responses observed in these situations. However, this study was designed as a first step in determining whether exogenous administration of angiotensin II is capable of modulating the heat loss responses. Therefore, follow-up studies employing an AT1 receptor blocker (e.g., losartan) or other antagonistic approaches (e.g., angiotensin-converting enzyme inhibitors) are clearly warranted to elucidate if physiologically relevant elevations in angiotensin II that can occur with dehydration, aging, chronic disease, and other factors may modulate heat loss responses.
Although we did not observe an influence of angiotensin II on the heat loss responses during exercise in the heat in healthy humans, it is important to evaluate whether this is also true for older adults and in clinical populations. As discussed, increased NO bioavailability may offset the effect of angiotensin II on the heat loss responses during exercise; however, NO bioavailability is reduced by aging (41) and hypertension (18). Thus increased levels of angiotensin II in these populations may influence heat loss during exercise in the heat.

While we tested both men and women in the present study, we are statistically underpowered and thereby unable to perform any meaningful analysis with regards to potential sex differences. Given the recent emphasis on sex-related differences in core body temperature regulation during exercise in the heat, the examination of the underlying mechanisms is becoming increasingly relevant. As such, further studies are required to elucidate any sex-related differences in influence of angiotensin II on cutaneous blood flow and/or sweating responses during exercise-induced heat stress.

Summary

We show that, during exposure to an ambient heat before and following exercise, angiotensin II impairs cutaneous blood flow independent of oxidative stress, whereas it impairs sweating by increasing oxidative stress. In contrast, no effect of angiotensin II on the heat loss responses during exercise; however, NO bioavailability may offset the effect of angiotensin II on the heat loss responses during exercise in the heat.

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