Intradermal angiotensin II administration attenuates the local cutaneous vasodilator heating response

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Stewart JM, Taneja I, Raghunath N, Clarke D, Medow MS. Intradermal angiotensin II administration attenuates the local cutaneous vasodilator heating response. Am J Physiol Heart Circ Physiol 295: H327–H334, 2008. First published May 9, 2008; doi:10.1152/ajpheart.00126.2008.—The vasodilation response to local cutaneous heating is nitric oxide (NO) dependent and blunted in postural tachycardia but reversed by angiotensin II (ANG II) type 1 receptor (AT1R) blockade. We tested the hypothesis that a localized infusion of ANG II attenuates vasodilation to local heating in healthy volunteers. We heated the skin of a calf to 42°C and measured local blood flow to assess the percentage of maximum cutaneous vascular conductance (%CVCmax) in eight healthy volunteers aged 19.5–25.5 years. Initially, two experiments were performed; in one, Ringer solution was perfused in three catheters, the response to heating was measured, 2 μg/l losartan, 10 mM nitro-L-arginine (NLA), or NLA + losartan was added to perfusate, and the heat response was remeasured; in another, 10 μM ANG II was given, the heat response was measured, losartan, NLA, or NLA + losartan was added to ANG II, and the heat response was reassessed. The heat response decreased with ANG II, particularly the plateau phase (47 ± 5 vs. 84 ± 3 %CVCmax). Losartan increased baseline conductance in both experiments (from 8 ± 1 to 20 ± 2 and 12 ± 1 to 24 ± 3). Losartan increased the ANG II response (83 ± 4 vs. 91 ± 6 in Ringer). ANG II decreased both angiotensin and Ringer responses (31 ± 4 vs. 43 ± 3). NLA + losartan blunted the Ringer response (48 ± 2), but the ANG II response (74 ± 5) increased. In a second set of experiments, we used dose responses to ANG II (0.1 nM to 10 μM) with and without NLA + losartan to confirm graded responses. Sodium ascorbate (10 mM) restored the ANG II-blunted heating plateau. NO synthase and AT1R inhibition cause an NO-independent angiotensin-mediated vasodilation with local heating. ANG II mediates the AT1R blunting of local heating, which is not exclusively NO dependent, and is improved by antioxidant supplementation.

The vasodilation response of nonglabrous skin to local heating can be described in terms of distinct phases including an initial thermal peak, a decrease to a nadir, and an increase to a plateau (17, 19). The plateau phase is nitric oxide (NO) dependent (17, 19). It has been used to evaluate NO bioavailability (15, 26, 32). Recently, we have observed that plasma angiotensin II (ANG II) is increased in a subset of patients with postural tachycardia syndrome (POTS) (25, 27) in whom we demonstrated blunted cutaneous microvascular NO-dependent vasodilation to local heat (18). We further demonstrated that ANG II type 1 receptor (AT1R) blockade with losartan corrects the cutaneous NO deficit in these patients (26). This is consistent with ANG II vasoconstrictive capabilities including the ability to produce direct smooth muscle vasoconstriction (30). However, one of the most potent vasoconstrictive actions of ANG II is mediated by AT1R-dependent interactions with NADPH oxidase, resulting in an enhanced production of reactive oxygen species (ROS) (11–13). One such ROS is superoxide, which scavenges and combines with NO to produce peroxynitrite (21). Thus a potent oxidative/nitritative agent is produced while available NO is decreased (33).

The mechanistic explanation offered, in which ANG II itself can account for the changes in the local heating response, was not actually demonstrated in that article (33). Rather, we demonstrated that AT1R blockade improves the NO-deficient heat response (26).

Based on these previous findings in POTS patients, the current study was designed to examine the relation of ANG II as an underlying basic mechanism mediating the reduction of cutaneous NO-dependent vasodilation. We initially proposed to demonstrate that local intradermal ANG II delivered through microdialysis catheters in healthy control subjects replicates the blunting of the cutaneous heat response findings in subjects with POTS, that the AT1R blocker losartan mediates the blunting, and that the nonselective NO synthase (NOS) inhibitor nitro-L-arginine (NLA) produces a similar reduction in the heating response. Therefore, we tested the hypothesis that the localized infusion of ANG II attenuates NO-dependent vasodilation in response to local heating in a healthy volunteer population.

Methods

Subjects

In the first series of experiments, we studied the effects of ANG II, NOS inhibition, and AT1R blockade in eight healthy volunteer subjects aged 19.5–25.5 years, median age 23.7 years (5 male and 3 female). In a second series of experiments, we studied angiotensin dose-heat response, the effects of the antioxidant sodium ascorbate, and the effects of the blockade of angiotensin type 2 receptors (AT2Rs) in four different volunteers with a median age of 24 years (2 male and 2 female). Subjects with a history of orthostatic intolerance were specifically excluded. Only subjects free from cutaneous, systemic, and cardiovascular diseases were eligible. Subjects were not taking any medications and refrained from alcohol and caffeinated beverages for at least 24 h before the study. There were no smokers or trained competitive athletes. Informed consent was obtained, and the Committee for the Protection of Human Subjects (Independent Review Board) of New York Medical College approved all protocols. Female subjects were enrolled without regard to the phase of their menstrual cycle except that none were menstruating during testing procedures.

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General Protocol

First experiments. The experiments were performed on 2 separate days in the same subjects. The order of the experimental days was randomized. During each day, three microdialysis catheters were placed to infuse drugs locally into the intradermal space of the leg. Before the microdialysis catheter insertion, laser-Doppler flow (LDF) was measured over each of the three insertion sites to estimate baseline flows for later use in determining when the area had recovered from the trauma of catheter insertion. Laser probes were removed, and the three microdialysis catheters were inserted. After recovery, LDF was measured while perfusing the catheters with lactated Ringer solution, and values were recorded for 10 min. Following this, LDF was recorded during local heating at each site. A recovery period followed, requiring 30–60 min. Before the effects of the drugs were tested, lactated Ringer was perfused through all catheters, subjects were allowed to recover before the introduction of drugs, which were dissolved in lactated Ringer solution (Ringer).

On one day, excess interstitial ANG II concentration was created in the area around the three catheters by perfusing with ANG II dissolved in lactated Ringer solution. After a 40-min run-in period, local heating and heating recovery were repeated at all sites. After recovery from the first local heating response, we perfused one catheter with the AT1R antagonist losartan + ANG II and remeasured the response to local heating. To separate the contribution of the local heat response during AT1R blockade of ANG II that is due to NO from other ANG II-mediated vasconstrictive effects, we perfused a second catheter with the nonisoform-specific NOS inhibitor NLA + losartan + ANG II and repeated local heating. To evaluate the component of the local heating response when NOS and NO formation were inhibited, we perfused the third catheter with NLA + ANG II and repeated local heating.

On another day, we repeated the same experimental design but excluded the perfusion with ANG II. A portion of these data was obtained during a prior investigation (26). Subsequently, one catheter was perfused with the AT1R antagonist losartan (no ANG II), and local heating was repeated. We perfused a second catheter with the NLA + losartan and repeated local heating. We perfused the third catheter with NLA alone and repeated local heating.

The effects of drugs on the local heat response were compared with baseline determinations obtained during the perfusion of lactated Ringer solution, and therefore washout periods were required. Thereafter, we compared the effects of the addition of losartan, losartan + NLA, and NLA alone, either in Ringer solution or in Ringer solution containing ANG II, thereby testing the effects of the AT1R blockade, NO-independent effects in Ringer, and elevated ANG II environments. Drugs were run-in for 40 min. Recovery periods were also 40 min. Before the end of each experimental day, maximum blood flow and conductance were elicited by perfusing 28 mM sodium nitroprusside through each microdialysis catheter (14).

Second experiments. The second series of experiments were performed on 2 other days in a second group of subjects. Four microdialysis catheters were used. Baseline LDF; catheter insertion with Ringer perfusion, and initial local heating at each site were performed as in the first series of experiments while the catheter was perfused with Ringer solution.

On one day, we studied whether sodium ascorbate would correct the blunting of the local heating response by ANG II and obtained a second ANG II dose-heating response. To accomplish this, we perfused 100 nM ANG II through the first catheter, 0.1 nM ANG II through the second catheter, 1.0 nM ANG II through the third catheter, and 10 nM ANG II through the fourth catheter. After the run-in period, local heating and heating recovery were performed at all sites. After recovery from heating, we perfused 1,000 nM ANG II + 10 mM sodium ascorbate through the first catheter, 100 nM ANG II through the second catheter, 1,000 nM (1 μM) ANG II through the third catheter, and 10,000 nM (10 μM) ANG II through the fourth catheter and repeated local heating.

On the other day, we studied the effects of different doses of ANG II combined with NOS and AT1R inhibition to obtain a second ANG II dose-heating response. To accomplish this, we perfused 1,000 nM ANG II + 10 mM NLA + 2 μg/l losartan through the first catheter, 0.1 nM ANG II + NLA + losartan through the second catheter, 1.0 nM ANG II + NLA + losartan through the third catheter, and 10 nM ANG II + NLA + losartan through the fourth catheter. After the run-in period, local heating and heating recovery were performed at all sites. After recovery, we obtained information concerning the potential participation of the AT1R in this effect by perfusing 1,000 nM ANG II + NLA + losartan + 1 μM PD-123319 (a selective AT1R antagonist) through the first catheter. We also continued to study different doses of ANG II combined with NOS and AT1R inhibition by perfusing 100 nM ANG II + NLA + losartan through the second catheter, 1,000 nM (1 μM) ANG II + NLA + losartan through the third catheter, and 10,000 nM (10 μM) ANG II + NLA + losartan through the fourth catheter, after which we repeated local heating.

Use of Heat-Reheat Assessment

We have performed pilot testing using the heat-and-repeat heat model on numerous occasions. Heat-and-repeat comparisons have been reported in detail elsewhere (27) but show that the difference between the plateau phase of the local heating response during sequential heat-reheat is significantly smaller than the difference between local heating plateaus between catheters placed on the same subject.

Instrumentation

All testing was conducted in a temperature-controlled room (≈25°C) at least 2 h after a light breakfast. Skin temperature was continuously monitored by the LDF probes used to make the skin blood flow measurements. Measurements were made in the left calf. Since all experiments were performed with the subject supine, the leg was at the level of the heart throughout all procedures. Subjects were instrumented in the dermal space of the lateral aspect of the left calf after hair was gently removed from the insertion site. Each site was cooled with an ice pack before catheter insertion to reduce discomfort. Each probe (MD-2000 Linear Microdialysis Probes; Bioanalytical Systems, West Lafayette, IN) has a 10-mm microdialysis membrane section that is placed in the intradermal space using a 25-gauge needle as an introducer. Catheters were randomly designated. The molecular mass cutoff is nominally 30,000 Daltons.

Following placement, all catheters were initially perfused with Ringer solution at 2 μl/min. An integrating LDF probe (Probe 413; Perimed) containing seven individual probe tips (each contains a separate transmitting and receiving fiber) was then placed directly over each microdialysis catheter to measure skin blood flow, designated as LDF. LDF was thereafter recorded until values were similar to those measured over the same area before catheter insertion. The return of LDF to approximately preinsertion values indicated recovery from the trauma of the catheter emplacement and usually occurred by 60–90 min (2). When necessary, longer times were allowed until preinsertion LDF was reached. Baseline untreated LDF was then recorded during local heating and 40 min post-heating recovery.

Drug Infusions

First experiments. Once baseline LDF values were obtained and local heating responses were measured under untreated conditions, subjects received perfusate containing either lactated Ringer solution...
or 10 μM ANG II in all catheters. Local heating was performed after
the run-in period. Following recovery from local heating, 2 μg/l losartan, 2 μg/l losartan + 10 mM NLA, and 10 mM NLA were added to the perfusate containing either 10,000 nM (10 μM) ANG II in Ringer solution or Ringer solution alone. The 10-μM dose of ANG II was chosen on the basis of pilot studies, which showed this to be the minimum concentration yielding a maximum attenuation of the NO-dependent local heating plateau.

First and second experiments. We used NLA instead of 1-nitroarginine methyl ester because the latter can act as a muscarinic receptor antagonist (4, 5). Losartan in 2 μg/l concentration was chosen based on prior human use during microdialysis experiments (10). This dose was also the minimum concentration that produced a significant and unequivocal increase in baseline blood flow and the percentage of maximum cutaneous vascular conductance (%CVCmax) in healthy subjects, indicating vasodilation that was related to AT1R blockade. Furthermore, in pilot studies, we found a dose-dependent increase in baseline vasodilation with increasing doses of losartan, although these increasing doses did not affect the local heating plateau. The 10-mM dose of NLA was chosen on the basis of pilot studies, which showed this to be the minimum concentration yielding a maximum attenuation of the NO-dependent (17, 19) local heating plateau. The dose of ascorbate was based on the work of Holowatz and Kenney (15). The dose of PD-123319 is based on the animal literature (28). A 10-fold increase in the dose had no additional effect.

Local Heating

Once baseline LDF values were obtained, the areas under each laser were gradually heated at 1°C/10 s to 42°C for at least 30 min until a plateau was reached. The area underneath the heating unit is ~3 cm². Heat was turned off to allow for recovery to baseline LDF. Work by Kellogg et al. (17) and Minson et al. (19) indicates that the local heating response is determined by NO. These investigators and others have demonstrated that an initial heating peak vasodilation may be mediated by neurogenic reflexes and neuropeptides (16, 20). This first peak is followed by a nadir and then an NO-dependent plateau, which is blunted by NOS inhibition.

Monitoring

Heart rate was monitored by electrocardiography, and right upper extremity blood pressures were measured by finger plethysmography (Finometer; Amsterdam, The Netherlands) intermittently recalibrated against oscillography in the right arm. Mean arterial pressure (MAP) was obtained by averaging the signal over 5 min. The Finometer MAP was always compared against oscillography using the formula MAP = (systolic arterial pressure + 2 × diastolic arterial pressure)/3 since Finometer blood pressure can wander during active procedures. However, since there were no activities occurring, the Finometer and oscillographic blood pressure were in agreement.

Data and Statistical Analysis

Laser-Doppler skin blood flows were measured in arbitrary perfusion units (pfu). Continuous LDF data were collected at a sampling rate of 200 Hz during experiments. Data from the lasers were multiplexed and interfaced to a personal computer through an analog-to-digital converter (DI-720; Dataq, Milwaukee, WI) using custom data acquisition software. LDF data were converted to units of cutaneous vascular conductance (CVC) and then to digital units (%CVCmax) by dividing by the MAP. CVC measurements were then converted to %CVCmax by dividing CVC by the CVCmax achieved after the administration of 28 mM sodium nitroprusside at the end of experiments. This fraction was converted to a percentile by multiplication by 100. Conductance data are therefore displayed as %CVCmax.

Changes in baseline LDF before and after drugs were compared by two-way ANOVA. Results are shown and reported as means ± SE. Angiotensin dose-heat response and NLA + losartan + angiotensin dose-heat response data were evaluated by ANOVA with repeated measures. Other comparisons were made by repeated-measures ANOVA to look at differences in the local heating response between pre- and postdrug infusion using the particular microdialysis catheter as the within factor. We also compared post-drug responses to ANG II + losartan with responses to ANG II + losartan + NLA and compared postdrug responses to NLA with losartan + NLA using catheters as the between factor and subjects as the within factor. Graphical representations in Fig. 1 comparing data obtained in the absence (denoted as ringer in Table 1 or Fig. 1) or presence (denoted as angiotensin in Table 1) of ANG II were obtained by averaging heat responses over all local heating curves for all subjects. Data for averaging were obtained from individual heating curves before and after drugs. Averaged data ± SE at baseline, first thermal peak, nadir, and plateau are shown in Fig. 2.

Results were calculated using Statistical Package for the Social Sciences software version 11.0. The value for α was <0.05.

RESULTS

Supine Hemodynamic Data

Subject height was 170 ± 3 cm, weight was 70 ± 5 kg, and body mass index was 23.6 ± 1.0 kg/m². Supine heart rate was 64 ± 4 beats/min, systolic blood pressure was 122 ± 4 mmHg, diastolic pressure was 68 ± 2 mmHg, pulse pressure was 55 ± 7 mmHg, and MAP was 108 ± 2 mmHg. The average of the maximum LDF achieved with sodium nitroprusside was 184 ± 8 pfu, and the average resting LDF was 16.6 ± 1.7 pfu.

Table 1 shows results from two-way ANOVA for the first experiments. We compared the first peak %CVCmax response, the nadir in %CVCmax following the first peak, and the plateau phase in %CVCmax before and after drug treatment.

The Effects of ANG II on Predrug (prelosartan and pre-NLA) LDF

As shown in Table 1 and Figs. 1 and 2, ANG II alone significantly decreased predrug baseline %CVCmax compared

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Values are ± SE; n = 8 subjects for Ringer and 8 subjects for Angiotensin groups. %CVCmax, the percentage of maximum cutaneous vascular conductance; NLA, nitro-l-arginine. *P < 0.05, smaller than Ringer experiments. †P < 0.05, significantly different than predrugs %CVCmax.
with that of Ringer (8 ± 1 vs. 12 ± 1, \( P < 0.025 \)), decreased the first thermal peak (43 ± 3 vs. 62 ± 4, \( P < 0.01 \)), decreased the nadir of the heat response (24 ± 3 vs. 41 ± 5, \( P < 0.01 \)), and decreased the NO-sensitive plateau (47 ± 5 vs. 84 ± 3, \( P < 0.001 \)) compared with those of Ringer solution alone.

**Effects of Losartan, NLA, and NLA ± Losartan on Baseline LDF With and Without ANG II**

Baseline laser-Doppler \( \%CVC_{\text{max}} \) data are shown for ANG II and Ringer experiments in Table 1 and Figs. 1 and 2.
%CVC\textsubscript{max} is shown before and after losartan, before and after NLA, and before and after NLA + losartan. Before the administration of drugs, baseline %CVC\textsubscript{max} was significantly decreased by ANG II compared with that of Ringer solution. After losartan was given, baseline %CVC\textsubscript{max} was significantly and comparably increased for both ANG II and Ringer experiments (\(P < 0.001\)). Baseline %CVC\textsubscript{max} increased to a similar level for both ANG II and Ringer after losartan was given. NLA alone did not affect baseline %CVC\textsubscript{max} for either ANG II or Ringer, although prior significant differences between ANG II and Ringer baselines disappeared. The increase in baseline with losartan was blunted by the addition of NLA (\(P < 0.05\)). There was no difference between ANG II and Ringer experiments for the NLA + losartan site.

**Effects of Losartan, NLA, and Losartan ± NLA on the Local Heating Response**

Table 1 and Fig. 2 show %CVC\textsubscript{max} measured at key points along the heating curves averaged over all subjects. Key points include baseline, the first thermal peak, the nadir, and the plateau. There was no effect of additional drug treatments on the CVC\textsubscript{max} for either the Ringer or ANG II experiments (\(P = 0.5\)). Since the experiments were performed during the background perfusion of ANG II, we will refer to the addition of losartan, NLA, or NLA + losartan as additional drugs in the text.

**First Thermal Peak**

Before additional drugs, the %CVC\textsubscript{max} of the first thermal peak was reduced in ANG II experiments compared with Ringer experiments (\(P < 0.01\)). After losartan, the %CVC\textsubscript{max} of the peak was similar for both experiments. After NLA, the %CVC\textsubscript{max} of the first peak was reduced (\(P < 0.05\)) for Ringer experiments and unchanged for ANG II experiments. The addition of NLA to losartan caused an increase in the first thermal peak compared with baseline for ANG II experiments (\(P < 0.01\)) but not for Ringer experiments, in which there was a trend for peak size reduction.

**Nadir**

Before additional drugs, the %CVC\textsubscript{max} of the nadir was reduced in ANG II experiments compared with Ringer experiments (\(P < 0.01\)). After perfusion with losartan, the nadir increased in ANG II experiments to a %CVC\textsubscript{max} that was similar to that in Ringer experiments (\(P < 0.001\)). Ringer experiment nadir was unchanged. After NLA, the %CVC\textsubscript{max} of the nadir was not significantly reduced and was decreased in ANG II experiments compared with Ringer experiments (\(P < 0.05\)). During NLA + losartan perfusion, the nadir was increased for ANG II experiments but not for Ringer experiments.

**Plateau**

Before additional drugs, the plateau %CVC\textsubscript{max} was markedly reduced for ANG II experiments compared with Ringer experiments (\(P < 0.001\)). After losartan, the plateau was unchanged in Ringer experiments but markedly increased for ANG II experiments. The plateau decreased in both experiments after NLA (\(P < 0.001\)) but was significantly lower during ANG II experiments. In Ringer experiments, the addition of losartan to NLA (losartan + NLA) caused a small increase in plateau %CVC\textsubscript{max} compared with that of NLA alone (\(P = 0.5\)) and a large decrease in %CVC\textsubscript{max} compared with that of losartan alone (\(P < 0.05\)). In ANG II experiments, the addition of losartan to NLA produced a marked increase (\(P < 0.01\)) compared with that of NLA alone and a small decrease in %CVC\textsubscript{max} compared with that of losartan alone, which was not significantly different (\(P = 0.11\)). The plateau during ANG II experiments was significantly increased above Ringer experiment plateau results (\(P < 0.001\)).

These results may be more readily appreciated in Fig. 2 in which the averaged local heat response curves are shown for ANG II and Ringer experiments before and after additional drugs. Losartan alone improves the overall local heat response for ANG II experiments such that the heat response becomes similar to the Ringer experiment response. NLA alone reduces the overall local heat response in both Ringer and ANG II experiments to a similar low level. Thus the response to nonisoform-specific NOS inhibition produces similar heat responses in both ANG II and Ringer experiments. Comparing losartan + NLA with NLA alone in the Ringer experiments shows a small increase in the local heating response, suggesting that there may be a small component due to ANG II that is independent of NOS inhibition during AT\(_1\)R blockade. Comparing losartan + NLA with NLA alone in the ANG II experiment shows a large increase in the local heating response. In combination with the Ringer experiments, these results suggest that NOS and AT\(_1\)R inhibition unmask an NO-independent, ANG II-mediated vasodilation that occurs during local heating.

**ANG II Dose-Heat Responses With and Without NLA ± Losartan**

Figure 3 shows two dose-response curves. Figure 3, top, demonstrates a significant and monotonic decrease in %CVC\textsubscript{max}...
in response to an increasing ANG II concentration in the perfusate. This reaches significance at 1 nM, which is within the physiological values for other interstitial tissues (9, 31). Similarly, Fig. 3, bottom, illustrates a steady increase in local heating vasodilation with an increasing ANG II concentration in the presence of NOS inhibition with NLA and AT$_2$R inhibition with losartan.

Effects of Ascorbate on ANG II Blunting of the Heat Response

Figure 4 demonstrates that although 100 nM ANG II significantly decreases the %CVC$_{\text{max}}$ of the local heating response, this is restored by the addition of sodium ascorbate ($P < 0.025$).

Effects of AT$_2$R Blockade on ANG II Blunting of the Heat Response

We have shown that NOS inhibition + AT$_1$R inhibition causes an angiotensin-mediated vasodilation with local heating. To test whether this was related to an increase in AT$_2$R stimulation, we used PD-123319, a highly selective AT$_2$R antagonist. The results shown in Fig. 5 demonstrate no change in heat plateau, indicating no significant AT$_2$R-mediated effects.

DISCUSSION

Our main findings are as follows.

Intradermal Cutaneous Perfusion With ANG II Blunts the Local Heating Response in a Dose-Dependent Fashion

These observations are depicted in Figs. 1–4. Figure 3, top, shows that healthy volunteers given ANG II have a dose-dependent decrease in the local heating response plateau. This is also associated with smaller decreases in the baseline %CVC$_{\text{max}}$, first thermal peak, and nadir %CVC$_{\text{max}}$. Angiotensin perfusion in control subjects replicates findings in low-flow POTS patients that we have previously observed (26).

Blunting is Improved by the AT$_1$R Blocker Losartan

Losartan increases baseline flow in ANG II and Ringer experiments (Table 1 and Figs. 1 and 2), and this increase is blunted by the addition of NLA. Resting blood flow and conductance are also reduced during ANG II experiments. Once losartan is administered, differences in local cutaneous conductance between ANG II and Ringer experiments disappear.

Both AT$_1$R and ANG II are found within the skin. ANG II appears to be produced by local angiotensinogen, renin, and ANG I-converting enzyme (ACE) systems (24). Our study confirms that ANG II can affect the heat response and that the reduction in the local heating response is AT$_1$R dependent.

Blunting is Improved by the Ascorbate

Prior work indicates that much of the vasoconstrictive response to ANG II is due to increased superoxide formation and NO scavenging through the binding of ANG II to NADPH oxidase-producing peroxynitrite (11–13). Other ROS are also produced. The antioxidant sodium ascorbate restores local heat-mediated vasodilation, which confirms oxidants as important features of the ANG II-mediated blunting of the local heat response. In vitro data suggest that ROS can induce vasoconstriction through Rho kinase-dependent mechanisms (3). Rho kinase has been shown to have an increasingly important role in cold-induced vasoconstriction (29) and connects well with a hypothesis including increased ROS (33). Angiotensin also directly affects smooth muscle function (30) and can potentiate adrenergic nerve activity and facilitate norepinephrine release (6).

NOS Inhibition Reduces the Local Heating Response in the Presence or Absence of ANG II

We have shown that once NLA is given, the local heating response for ANG II and Ringer experiments decreases significantly and becomes similar. NLA decreases heat responses to a common low level, which is less than the predrug response of the ANG II experiments. This suggests, as shown in Figs. 1 and 2, that the elimination of local NO production equalizes the heat response between ANG II and Ringer experiments by attenuating NO-mediated effects. Figure 1 also shows that the NO-mediated plateau phase is not completely blocked by ANG II because the addition of NLA causes a further decrease in the plateau.

The data, however, add credence to our prior observations in low-flow POTS that cutaneous NO is deficient in these patients (27) and that this defect is likely caused by excessive ANG II (26). The present study was initially intended as proof by the
construction that replicated the finding in POTS; thus, the intradermal perfusion of ANG II caused vasoconstriction related to decreased NO, which was improved by AT1R blockade. Perfusion with ANG II was used as a surrogate for POTS pathophysiology. However, there were additional findings.

Local Heating Response is Enhanced by ANG II in a Dose-Dependent Fashion in the Presence of AT1R Blockade and NOS Inhibition

Subsequent NOS inhibition during AT1R blockade revealed novel findings, which are depicted in Figs. 1 and 2. A, top left, C, bottom left, and D, bottom right. Adding losartan + NLA (A vs. D) increases the heating response to ANG II but decreases the local heating response to Ringer alone. The addition of the AT1R blocker losartan to NLA (C vs. D) increases the local heating response in ANG II experiments but does not significantly affect the heat response in Ringer experiments. Fig. 1D indicates that adding ANG II to NLA + losartan causes an increased heating response including increases in baseline, first peak, nadir, and plateau. Thus ANG II serves as a vasodilator during local heating when AT1Rs are blocked and when NO production is inhibited by NLA. The degree of enhancement of the local heating response is dependent on the dose of ANG II (Fig. 3, bottom).

ANG II-Enhanced Local Heating Response is Independent of AT1R Blockade

AT1R blockade with losartan is highly selective, allowing the continued interaction of ANG II with AT1R. To explore the potential contribution of AT1R-mediated vasodilation, we administered PD-123319 together with angiotensin + NLA + losartan. There was no change in the enhanced local heating response.

A Speculation Concerning Mechanism

Remainder of explaining our observations are contributions from ACE2 and ANG-(1-7) or other vasodilator metabolites within the angiotensin cascade (23). This is conjecture. However, ACE2 has assumed increasing importance as an enzyme that removes ANG II by catabolism to ANG-(1-7) (7, 8). Although ANG-(1-7) may exert certain dilator effects through bradykinin production, its principal ligand is the recently discovered Mas receptor (22). Binding produces vasodilation, as well as antiproliferative and antihypertrophic effects. ANG-(1-7) is coming to be viewed as the principal counter regulatory mechanism for ANG II.

Limitations

We will need to study ACE and ANG-(1-7) in control and POTS patients. We now speculate that vasodilation in the presence of NOS and AT1R inhibition is related to ACE2 and ANG-(1-7). Although this is consistent with the literature, it has not yet been demonstrated as a mechanism within human skin and is difficult to accomplish because of the availability of agonists and antagonists and because their molecular size limits delivery through microdialysis catheters. Alternative delivery systems are under consideration.

We studied females without regard to the menstrual cycle. The phase of the menstrual cycle can exert important effects on NO-dependent mechanisms. However, we found directionally consistent and similar results across all subjects.

Endogenous angiotensin was not considered. Tissues other than the kidney produce ANG II, but there are no data from skin. However, data from skeletal muscle microvessels suggest on the order of 100 pmol/l (1). This was similar to our lowest dose of ANG II administered during dose-response measurements but was far less than the amount of exogenous-administered ANG II delivered during other parts of the experiments. Alternatively, we could have sought to eliminate local ANG II production with an ACE inhibitor. However, since ACE is also bradykininase, the use of an ACE inhibitor would have enhanced bradykinin release, thereby altering microvascular conductance.

Microdialysis is invasive and alters the interstitial milieu. The work of Anderson et al. (2) suggests that flow responses return to baseline levels within ~1 h. This environment likely remains changed for some time despite a return of skin blood flow to precatheter insertion values. However, in pilot experiments we measured baseline flows, removed the LDF probes, instrumented the same site with microdialysis catheters, replaced the probes, waited at least 1 h, and repeated the LDF measurements with (on average) similar results.

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REFERENCES


