**Baroreflex Control of the Cutaneous Active Vasodilator System in Humans**

Dean L. Kellogg Jr., John M. Johnson, and W.A. Kosiba

Cutaneous arterioles are controlled by vasoconstrictor and active vasodilator sympathetic nerves. To find out whether the active vasodilator system is under baroreceptor control, laser-Doppler velocimetry and the local iontophoresis of bretylium were combined to allow selective study of the active vasodilator system. Each of six subjects had two forearm sites (0.64 cm²) treated with bretylium to abolish adrenergic vasoconstrictor control. Laser-Doppler velocimetry was monitored at those sites and at two adjacent untreated sites. Subjects underwent 3 minutes of lower-body negative pressure (LBNP) and 3 minutes of cold stress to verify blockade of vasoconstrictor nerves. They were then subjected to whole-body heat stress (water-perfused suits), and the 3 minutes of LBNP was repeated. Finally, subjects were returned to normothermia, and LBNP and cold stress were repeated to verify the persistence of blockade. During the application of LBNP in normothermia, cutaneous vascular conductance (CVC) fell at untreated sites by 22.7±4.7% (p<0.01) but was unaffected at bretylium-treated sites (p>0.20). During cold stress, CVC at untreated sites fell by 30.2±1.7% (p<0.01) and at treated sites rose by 0.7±4.6% (p>0.10). Both control and bretylium-treated sites reflexly vasodilated in response to hyperthermia. With LBNP during hyperthermia, CVC at untreated sites fell by 23.3±7.1% (p<0.05) and at treated sites by 17.9±9.2% (p<0.05) with no significant difference between sites (p>0.10). After return to normothermia, neither LBNP application nor cold stress caused CVC to fall at treated sites (p>0.10). Thus, the vasoconstrictor system was blocked by bretylium treatment throughout the study, whereas the active vasodilator response to heat stress was intact. Because LBNP in hyperthermia induced similar falls in CVC at both sites, we conclude that baroreceptor unloading elicits a withdrawal of active vasodilator tone and that the baroreflex has control of the active vasodilator system. (*Circulation Research* 1990;66:1420–1426)

The participation of the human cutaneous circulation in thermoregulatory and nonthermoregulatory reflexes has been the subject of much research over the last 30 years. Investigations of thermoregulatory reflexes have clearly demonstrated the neural mechanisms responsible for effecting changes in skin blood flow in response to heat and cold stresses.1–4 However, such clarity of understanding has not been achieved for nonthermoregulatory reflexes. Though much evidence from studies measuring skin blood flow supports the conclusion that the cutaneous circulation participates in reflexes of nonthermoregulatory origin,5–14 some doubts have persisted in the literature.15–17 Given that debate, it is not surprising that the neural mechanisms involved are also uncertain.

Despite some skepticism, it does appear that the cutaneous circulation is on the efferent limb of several nonthermoregulatory reflexes, including the baroreceptor reflex. During baroreceptor unloading in normothermic conditions, cutaneous vasoconstriction has been documented.5,7,11,14,18 Further, the cutaneous vascular responses to nonthermoregulatory reflexes can be modified by thermal status. During periods of heat stress, the absolute decrease in cutaneous vascular conductance (CVC) is usually greater than in normothermia; however, the vasoconstriction induced by the baroreflex does not completely overwhelm the vasodilation induced by heat stress.6,8,9. Thus, the baroreflex competes with simultaneous thermoregulatory reflex drives to produce an integrated cutaneous vascular response.6–9

The integration of neural control mechanisms for the cutaneous circulation in nonapical areas (limbs and trunk) is not well understood. Arterioles in these skin areas receive efferent sympathetic active vasodilator innervation in addition to noradrenergic vasoconstrictor nerves.1 Thus, alterations of CVC during
simultaneous thermoregulatory and nonthermoregulatory reflexes could be effected by changes in either one of these dual vasomotor systems or perhaps by simultaneous changes in both. The neurotransmitter of the vasodilator nerves is unknown, although an indirect relation to sudomotor nerve activity is postulated.\textsuperscript{1,19-21} The active vasodilator system is responsible for 85–95% of the cutaneous vasodilatation occurring with heat stress.\textsuperscript{4,8} Because whole-body skin blood flow can achieve levels approaching 8 l/min, or 60% of cardiac output during heat stress,\textsuperscript{8} it is clear that this vasodilator system is important both to thermoregulatory responses and to systemic hemodynamics.

How the dual neural systems that control the non-apical cutaneous circulation interact to produce integrated cutaneous vascular responses to simultaneously occurring thermoregulatory and nonthermoregulatory drives is unknown.\textsuperscript{3,8,9} Are nonthermoregulatory reflexes that exert control over cutaneous arterioles mediated by both efferent neural pathways? Alternatively, are nonthermoregulatory reflexes mediated solely by alterations in vasoconstrictor tone? At which site or sites are simultaneous thermoregulatory and nonthermoregulatory reflexes integrated?

If active vasoconstrictor control could be abolished without altering the vasodilator system, the reflexes responsible for controlling the latter could be directly studied. Recently, a method based on a similar approach by Lindblad and Ekenvall\textsuperscript{22} for such a selective abolition has been accomplished.\textsuperscript{23} Local iontophoresis of bretylium tosylate in combination with laser-Doppler velocimetry (LDV) has been shown to give reliable pharmacological abolition of responses caused by the cutaneous active vasoconstrictor system without significant effect on the vasodilator system.\textsuperscript{23} Bretylium is an antiadrenergic agent that is taken up by adrenergic nerve terminals and blocks the release of norepinephrine.\textsuperscript{24} The combination of bretylium iontophoresis with LDV appears to be an ideal technique to study directly the active vasodilator system without the confounding effects of the active vasoconstrictor system. Hence, the use of this technique could reveal whether the cutaneous vasodilator system is under baroreceptor reflex control.

\textbf{Subjects and Methods}

Experiments were performed in six supine male subjects (ages 26–44). Their average age was 32.5±2.55 years, average height was 179.07±2.30 cm, and average weight was 75.75±3.16 kg. Physical examinations, including 12-lead ECG, showed all subjects to be in good health. The informed consent of each subject was obtained before participation in this institutionally approved study.

Esophageal temperature (T<sub>es</sub>) was monitored as an index of internal temperature with a catheter containing a thermocouple swallowed to left atrial level. Placement of the T<sub>es</sub> catheter was verified by P wave configuration of the ECG as recorded from the catheter tip.\textsuperscript{25} Skin temperature (T<sub>sk</sub>) was recorded as an average from six thermocouples placed at representative sites over the body surface. Subjects wore two sets of impermeable garments with a water-perfused suit between them. T<sub>sk</sub> was controlled by perfusing the tube-lined suit with water of different temperatures.\textsuperscript{20,22} The suits and garments covered the entire body with the exception of the head, arms, and feet. Cold stress was induced by perfusing the suit with cold water to lower T<sub>sk</sub> from 34°–35° C to 30°–32° C. Similarly, heat stress was induced by perfusion with warm water to raise T<sub>sk</sub> to 38°–38.5° C and T<sub>es</sub> by 1°–1.5° C.

Heart rate was continuously recorded from the ECG. LDV was measured at each of four forearm sites (Blood Perfusion Monitor, TSI, St. Paul, Minnesota). Blood pressure was recorded approximately once per minute with an electrophysgmomanometer applied to the arm contralateral to the LDV probes (PE-300, Narco Bio-Systems, Houston, Texas). Systolic and diastolic pressures were ascertained by analysis of Korotkoff sounds superimposed on the cuff pressure tracing.\textsuperscript{28} Mean arterial pressure was computed from the blood pressure measurements as the diastolic pressure plus one third of the pulse pressure. CVC was calculated as LDV (volts)/mean arterial pressure (mm Hg).

In all experiments, iontophoresis was accomplished with Plexiglas chambers (0.64 cm<sup>2</sup>) mounted on the forearm and filled with a 10 mM solution of bretylium tosylate dissolved in propylene glycol. Total current density for iontophoresis procedures was 400 μA/cm<sup>2</sup> for a duration of 10 minutes. Details and experimental verification of this procedure have been reported previously.\textsuperscript{23}

Lower-body negative pressure (LBNP) was accomplished by enclosing the body below the iliac crests in a chamber attached to a vacuum source to reduce the air pressure within the LBNP chamber.\textsuperscript{29} This drop in pressure increases the transmural pressure gradient across lower-body blood vessels. This, in turn, leads to the pooling of blood in the lower body, causing unloading of cardiopulmonary and, possibly, arterial baroreceptors. LBNP was applied at −40 mm Hg (relative to atmospheric pressure) for a duration of 3 minutes.

The protocol followed in these experiments began with the iontophoresis of bretylium at two experimental sites on the right forearm. There were also two adjacent untreated control sites. Subjects were instrumented and placed in a supine position approximately 1.5 hours after the completion of iontophoresis. During this period the antiadrenergic effects of the drug developed.

The entire protocol subsequent to the iontophoresis procedure is shown for one subject in Figure 1. Data collection began with a 5-minute, normothermic control period followed by a 3-minute application of −40 mm Hg LBNP. After 10–12 minutes of recovery, a 3-minute cold stress period was performed, after which the subject was returned to normothermia for a 5-minute period of recovery.
These manipulations served to confirm the presence of vasoconstrictor blockade. A 35–45-minute period of heat stress (Tsk, 38°–38.5° C) was then performed. After Tsk had increased by approximately 0.5°–1° C and LDV measurements stabilized, the 3-minute period of −40 mm Hg LBNP was repeated. After recovery, subjects were cooled to normothermia. This was followed by a final LBNP period and a second cold stress to verify that the cutaneous vasoconstrictor blockade was sustained. In five studies, including the study depicted in Figure 1, the verification LBNP periods and cold stresses were done after the skin at both the treated and untreated sites was locally warmed to 39°C (T10c) because it has been shown that a local temperature of 39°C is optimal for observing reflex cutaneous vasoconstriction.13 Tloc was controlled by a heating element that surrounded the LDV probe and was monitored by a thermocouple placed between the skin surface and the heating element.12 In one study, local warming was not used in conjunction with the verification of blockade. Because bretylium acts presynaptically to block noradrenaline release, cold stresses and LBNP periods were kept brief to avoid α-receptor activation by circulating catecholamines.24

LDV, Tes, Tsk, heart rate, and LBNP levels were recorded by a laboratory computer equipped with an analog-to-digital converter. Each variable was sampled once per second, and average values for each 20-second period were calculated.

For data analysis, responses from the two bretylium-treated and two untreated sites were averaged to yield treated and untreated average responses for each subject. Effects of bretylium were analyzed by paired t test, which compared the responses at control sites with those at the bretylium-treated sites. Percent changes were calculated from a 5-minute control period to the last minute of LBNP or cold stress.

Results

Initial blockade of the vasoconstrictor system by bretylium iontophoresis was shown by the failure of CVC at treated sites to respond to either LBNP or cold stress in the first period of normothermia. Figure 2 shows responses in CVC from both bretylium-treated and untreated sites to the initial period of LBNP for one subject. On the average, CVC fell by 22.7±4.7% at untreated sites (p<0.01), but did not change significantly at sites treated with bretylium (p>0.20) (see Table 1). The results of LBNP application during normothermia are summa-

FIGURE 1. Illustration of entire protocol and results from one subject. Upper panels show cutaneous vascular conductance (CVC) at untreated and bretylium-treated sites as percentages of initial control levels. The bottom panel shows periods of lower-body negative pressure (LBNP) (continuous line), cold stress (black squares at 15 and 102 minutes), whole-body heat stress (open rectangle between 26 and 58 minutes), and local warming of sites of blood flow measurement (shaded rectangle between 70 and 111 minutes). Also shown are mean arterial pressure (MAP) and esophageal temperature (Tes). Note that CVC at bretylium-treated sites is not reduced by cold stress or by LBNP in normothermia but is reduced by LBNP when cutaneous active vasodilator activity has been increased by whole-body heat stress.

FIGURE 2. Example of the cutaneous vascular conductance (CVC) responses in one subject to lower-body negative pressure (LBNP) under normothermic conditions. CVC values have been normalized to the initial 5-minute control period of the study. Note the prompt and sustained decrease in CVC at the untreated site during LBNP. No such CVC fall was present at the bretylium-treated site.
Table 1. Responses in Cutaneous Vascular Conductance to Cold Stress and Lower-Body Negative Pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control site (%)</th>
<th>Bretylium site (%)</th>
</tr>
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<tbody>
<tr>
<td>Initial cold stress</td>
<td>−30.2±1.7*</td>
<td>0.8±4.5†</td>
</tr>
<tr>
<td>LBNP normothermia</td>
<td>−22.7±4.7*</td>
<td>−1.9±2.5†</td>
</tr>
<tr>
<td>LBNP hyperthermia</td>
<td>−23.3±7.1‡</td>
<td>−17.9±9.2‡</td>
</tr>
<tr>
<td>LBNP blockade verification</td>
<td>−14.6±10.6</td>
<td>3.3±3.7</td>
</tr>
<tr>
<td>Cold stress verification</td>
<td>−34.9±10.7§</td>
<td>1.6±1.9†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
Percent changes in cutaneous vascular conductance (CVC) at control (untreated) and bretylium-treated sites in response to cold stress, lower-body negative pressure (LBNP) in normothermia and hyperthermia, and cold stress and LBNP after hyperthermia (verification). Note that vasoconstrictor responses at the bretylium-treated sites were successfully blocked except in response to LBNP during hyperthermia.

*p<0.01 reduction from rest.
†p<0.01 treated vs. untreated sites.
‡p<0.05 reduction from rest.

Rized in Figure 3. As also shown by Table 1, similar findings were made for the initial period of cold stress. Bretylium-treated sites again showed no significant change in CVC (p>0.30), whereas CVC at untreated sites fell significantly (p<0.01). Paired comparisons revealed a highly significant difference in responses between sites (p<0.005 for both cold stress and LBNP). Heart rate rose slightly, but significantly, during the initial period of LBNP (60.9±3.7 beats/min control to 65.9±3.6 beats/min LBNP, p<0.05). Mean arterial pressure was not significantly affected by LBNP.

Heat stress caused significant vasodilation at all sites, regardless of treatment. At control sites, CVC rose by an average of 497±82% (p<0.001) and at sites treated with bretylium by 361±57% (p<0.005). There was no significant difference in the degree of vasodilation between untreated and bretylium-treated sites (p>0.10). The internal temperature at which vasodilation began was not different between untreated and bretylium-treated sites (36.97±0.07°C and 37.12±0.10°C, respectively, p>0.05 between sites). The failure of bretylium iontophoresis to alter these vasodilator parameters, despite blockade of vasoconstriction, is consistent with earlier findings.23

The period of LBNP during hyperthermia was associated with a significant reduction in CVC at both untreated sites and at sites treated by bretylium iontophoresis. Figure 4 shows responses from one subject to the period of LBNP in hyperthermia. The results of LBNP application during hyperthermia are summarized in Figure 5 and Table 1. At untreated sites, CVC fell by 23.3±7.1% (p<0.05), while at treated sites this fall averaged 17.9±9.2% (p<0.05). Comparison of responses between sites revealed no significant difference (p>0.10). Also, during the second period of LBNP, heart rate rose from 79.7±3.9 to 92.0±6.8 beats/min (p<0.05). There was no significant change in mean arterial pressure.

After heat stress, subjects were returned to normothermia, and LBNP was repeated to verify that vasoconstrictor blockade had persisted through the
of heat stress. This conclusion is also supported by the lack of responses in CVC at treated sites during the initial LBNP application in normothermia. Again, a reflex known to be mediated by increased vasoconstrictor tone was abolished by bretylium iontophoresis. Why LBNP application after heat stress caused no significant falls at either treated or untreated sites is unclear. Nevertheless, it is safe to conclude that bretylium iontophoresis had blocked the vasoconstrictor system for the duration of these studies. Thus, responses in CVC recorded from bretylium-treated sites were due exclusively to the active vasodilator system. However, had bretylium also affected vasodilator responses?

Two pieces of evidence support the conclusion that the active vasodilator system was unaltered by the drug. First, the internal temperature thresholds for the onset of cutaneous vasodilation were not different between treated and untreated sites. Second, there was no significant difference in the extent of vasodilation induced by heat stress between sites. Both observations are in keeping with an earlier study from this laboratory.23 This leads to the conclusion that the responses in CVC at bretylium-treated sites were entirely due to an intact vasodilator system. Thus, baroreceptor unloading directly reduced active vasodilator tone and thereby effected vasoconstriction.

What of the active vasoconstrictor system? Did it play a role in the baroreflex-mediated vasoconstriction during heat stress? Untreated sites, with both vasomotor systems intact, showed CVC reductions that were statistically identical to those at treated sites during LBNP application in hyperthermia. Because the treated sites (with only vasodilator control) and the untreated sites (with dual control) showed statistically identical responses, all of the decrease in CVC may have been due to vasodilator withdrawal. However, we cannot rule out some minor participation by the vasoconstrictor system. Nonetheless, the integrated cutaneous vascular response to simultaneous thermoregulatory and baroreceptor reflexes is due largely to reduction in active vasodilator tone. What can be concluded about the site of this integration?

Before this study, some authors had speculated about the mechanisms and sites of integration of thermoregulatory and nonthermoregulatory reflexes.3,4,8,21 One proposal, based on the assumption that vasodilator systems do not participate in baroreflexes,30 was that integration occurred at the cutaneous arterioles through enhanced vasoconstrictor tone.3,4,8 According to this view, heat stress induced increases in vasodilator tone. This high vasodilator activity would compete with baroreceptor-mediated increases in active vasoconstrictor tone at the cutaneous arterioles. The arterioles would summate these competing neural tones. The outcome of the summation would be an active cutaneous vasoconstriction tempered by the vasodilator system. The arterioles would therefore be the site of integration in this scheme.

Discussion

The major result of this study was the clear demonstration of baroreceptor reflex control of the active vasodilator system in skin and hence the cutaneous circulation. This conclusion is based on reductions in CVC observed during LBNP in hyperthermia in the presence of selective cutaneous vasoconstrictor blockade. Was such blockade present?

Vasoconstrictor blockade by bretylium was demonstrated both by cold stress and by LBNP in normothermia. Both of these manipulations activate the vasoconstrictor system and thus elicit falls in CVC.5,7,11,14,21 Because bretylium treatment abolished the responses in CVC attendant to cold stress both before and after heat stress, vasoconstrictor blockade was present at the initiation and conclusion of the study, including the intervening period

study. In the five studies in which local temperature was raised to 39°C, LBNP application for verification of blockade elicited no statistically significant alterations in CVC at either treated or untreated sites (p>0.10 for both sites) (see Table 1). However, when cold stress verification was performed, CVC at untreated sites fell by 34.9±10.8% (p<0.05), whereas at treated sites CVC was unchanged (p>0.30). These responses were significantly different between sites (p<0.05), indicating vasoconstrictor blockade was still present at treated sites.

Figure 5. Identity plot summarizing data for lower-body negative pressure (LBNP) application during heat stress. Note the clustering of points along the line of identity, unlike that in normothermia (compare with Figure 2). Overall, during LBNP application in hyperthermia, cutaneous vascular conductance (CVC) at untreated sites fell by 23.30±7.12% (p<0.05) and at bretylium-treated sites by 17.90±9.21% (p<0.05). The treated and untreated responses were not significantly different from each other (p>0.10), indicating that bretylium treatment did not alter the vasoconstriction induced by baroreceptor unloading during heat stress. Vasoconstriction must have occurred because of withdrawal of active vasodilator tone.
The results of the present study indicate that, instead, an alternative mechanism of direct vasodilator withdrawal is responsible for integration of the baroreflex and thermoregulatory reflexes. This implies that, during heart stress, integration must have occurred within the nervous system, proximal to the cutaneous arterioles. Further, because untreated sites and those with local vasoconstrictor blockade responded quite similarly to baroreceptor unloading during hyperthermia, one can rule out a major role for the vasoconstrictor system in that setting.

While the results of the direct approach of LDV combined with bretylium iontophoresis are unquestionably clear, it is reasonable to ask why these results were not obtained from studies in which sweat rate was used as an index of cutaneous vasodilator system activity. Solack et al\textsuperscript{21} found a reduction in the rate of sweat rate during baroreceptor unloading in heat-stressed humans; however, sweat rate, per se, did not decline. In the present study, direct reductions in active vasodilator tone were found with baroreceptor unloading in hyperthermia. It appears that the assumption that sweat rate is a reliable index of active vasodilator tone during brief vasoconstrictor reflexes in hyperthermia is invalid. One may speculate that sweat rate does not respond quickly to nonthermoregulatory reflexes because of a long half-life of the sudomotor neurotransmitter or its effects at sweat glands. Alternatively, completely different efferent nerves could be responsible for active vasodilation and for sweating. The latter alternative could also explain the ability of atropine to block sweating, but to leave active vasodilation unaltered or only temporally delayed.\textsuperscript{20,31}

It is interesting that studies with sympathetic nerve recordings have not found evidence for baroreflex control of either active vasodilation or vasoconstriction in the cutaneous vasculature.\textsuperscript{32–34} This disparity of findings has not gone unrecognized. It has been proposed that the complexity of cutaneous nerves, containing separate afferent, active vasoconstrictor, and sudomotor fibers, may be the cause of this discrepancy.\textsuperscript{34} If separate vasodilator efferents are also present, skin sympathetic nerve studies would be even more difficult to interpret. Further refinement and combination of single fiber sympathetic nerve recordings with functional measurements such as LDV may help corroborate conclusions based on measurements of skin blood flow.

Brody\textsuperscript{30} concluded that the active cholinergic vasodilator system in skeletal muscle is unaffected by baroreceptor loading. This was based on studies in which atropinization, sufficient to block the effects of acetylcholine, had no effect on the reflex vasodilation of the skeletal muscle vasculature in response to increased blood pressure. This proposal is at variance with the finding by Ito and Feigl\textsuperscript{35} of active parasympathetic vasodilation in dog coronary arteries during baroreceptor loading. Participation by histamine release in the vasodilator response to increased baroreceptor activity has been noted.\textsuperscript{30} That response was secondary to reduced release of norepinephrine and is not similar, in that respect, to the participation by the cutaneous active vasodilator system in the baroreceptor reflex as identified here. The finding that the cutaneous active vasodilator system responds to baroreceptor unloading indicates that vasodilator systems can participate in baroreceptor reflexes. If baroreceptor loading during hyperthermia elicits a cutaneous vasodilation by enhancing active vasodilator tone, then baroreceptor reflexes could buffer blood pressure challenges over all thermoregulatory conditions. This question requires further experimental work.

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