Nonnoradrenergic mechanism of reflex cutaneous vasoconstriction in men

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Stephens, Dan P., Ken Aoki, Wojciech A. Kosiba, and John M. Johnson. Nonnoradrenergic mechanism of reflex cutaneous vasoconstriction in men. Am J Physiol Heart Circ Physiol 280: H1496–H1504, 2001.—We tested for a nonnoradrenergic mechanism of reflex cutaneous vasoconstriction with whole body progressive cooling in seven men. Forearm sites (<1 cm²) were pretreated with: 1) yohimbine (Yoh; 5 mM id) to antagonize α-adrenergic receptors, 2) Yoh plus propranolol (5 mM Yoh-1 mM PR id) to block α- and β-adrenergic receptors, 3) iontophoretic application of bretylium tosylate (BT) to block all sympathetic vasoconstrictor nerve effects, or 4) intradermal saline. Skin blood flow was measured by laser Doppler flowmetry and arterial pressure by finger photoplethysmography; cutaneous vascular conductance (CVC) was indexed as the ratio of the two. Whole body skin temperature (Tsk) was controlled at 34°C (water-perfused suit) for 10 min and then lowered to 31°C over 15 min. During cooling, vasoconstriction was blocked at BT sites (P > 0.05). CVC at saline sites fell significantly beginning at Tsk of 33.4 ± 0.1°C (P < 0.05). CVC at Yoh-PR sites was significantly reduced beginning at Tsk of 33.0 ± 0.1°C (P < 0.05). After cooling, iontophoretic application of norepinephrine (NE) confirmed blockade of adrenergic receptors by Yoh-PR. Because the effects of NE were blocked at sites showing significant reflex vasoconstriction, a nonnoradrenergic mechanism in human skin is indicated, probably via a sympathetic cotransmitter.

skin blood flow; sympathetic nervous system; cold stress; yohimbine; propranolol; β-adrenergic receptors; cotransmitter

REFLEX CONTROL OF SKIN BLOOD FLOW (SkBF) is accomplished through two branches of the sympathetic nervous system: a noradrenergic vasoconstrictor system and an active vasodilator system of unknown neurotransmitter (15). The vasoconstrictor system mediates both the subtle reflex alterations in SkBF that occur during periods of normothermia as well as the more dramatic reductions in blood flow that occur during a hypothermic challenge (31). Reflex vasoconstriction that occurs during whole body cooling is known to occur through sympathetic vasoconstrictor nerves and can be completely abolished by presynaptic blockade with bretylium tosylate (10, 18) which demonstrates the noradrenergic nature of these nerves.

Perivascular sympathetic nerves are known to secrete multiple neurotransmitters (1, 2, 24, 35). Norepinephrine (NE) is considered the primary neurotransmitter of sympathetic noradrenergic vasoconstrictor neurons and has been shown to be colocalized in perivascular nerves with the cotransmitters neuropeptide Y (NPY), galanin, and ATP (42). Furthermore, sympathetic cotransmitters such as NPY and ATP have been shown to participate in vasoconstriction in rat (11, 12), pig (23), and rabbit (25, 29) models.

The role of nonnoradrenergic cotransmitters in humans has not been clearly established, but recent data are suggestive of a participation in reflex vasoconstriction. For example, hypertensive humans exhibit a nonnoradrenergic mechanism of vasoconstriction in the forearm during simulated hemorrhage (8, 17, 39). In vitro biopsies from nasal mucosa exhibit nonnoradrenergic vasoconstriction in response to NPY application (6) as do subcutaneous resistance arteries (26) and saphenous vein preparations (36). The results from these studies indicate that nonnoradrenergic mechanisms of vasoconstriction can occur in humans. To date, the moment-to-moment control of the cutaneous circulation has been attributed to subtle alterations of noradrenergic vasoconstrictor nerve activity releasing NE, which acts through both α₁- and α₂-adrenergic receptors to cause vasoconstriction. However, the role of nonnoradrenergic mechanisms in reflex control of SkBF is unknown.

Thus the aim of this study was to test whether a nonnoradrenergic component of reflex cutaneous vasoconstriction participates in the regulation of SkBF in response to whole body cooling. During the course of these experiments we made observations consistent with NE acting through cutaneous β-adrenergic receptors to contribute a vasodilatory component to SkBF. Therefore, we also tested whether β-adrenergic receptors modulate the reflex vasoconstriction caused by whole body cooling. Our approach was to antagonize pharmacologically the effects of NE at α- and β-adrenergic receptors both separately and in combination and
observe the effects of such blockade on reflex cutaneous vasoconstriction.

METHODS

The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved this study. The subjects were men who provided voluntary written consent before participating in the experiments. All subjects were nonsmokers and in good health. They did not take any medications (including nonprescription medications) nor did they consume caffeine for 12 h before the beginning of the study. Female reproductive hormones have been shown to regulate the putative mediators of a nornoradrenergic mechanism of vasoconstriction (28, 30). We also tested for nonnoradrenergic vasoconstrictor function in women during two phases of the menstrual cycle. These findings will be reported in a separate communication.

Measurements. Each subject participated in one experiment. SkBF was monitored by laser Doppler flowmetry (Moor MBF3D or Vasamedics LaserFlo) on the dorsal forearm (13, 27, 37). Laser Doppler flow probes were housed in custom-made devices that allow the local temperature (TLOC) of the area surrounding the site of blood flow measurement (~12 cm²) to be controlled independently of whole body skin temperature (TSK). Mean arterial pressure (MAP) was measured continuously at the finger by photoplethysmography (Finapres; Ohmeda, WI). Cutaneous vascular conductance (CVC) was indexed from the ratio of SkBF to MAP. TSK was continuously at the finger by photoplethysmography (Finapres; Ohmeda, WI). Cutaneous vascular conductance (CVC) was indexed from the ratio of SkBF to MAP. TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32). TSK was controlled with a water-perfused suit that covered the entire body except for the head, hands, feet, and forearm (3, 32).

Rationale. The protocols were designed to observe changes in CVC at sites where: 1) the vasoconstrictor effects of NE were blocked, 2) the vasodilator effects of NE were blocked, 3) all postsynaptic vasomotor effects of NE were blocked, 4) all transmitter release from vasoconstrictor nerves was blocked presynaptically, and 5) vasoconstrictor control was intact. A persistent vasoconstriction during whole body cooling at sites unresponsive to NE would suggest participation by a mechanism other than NE–α-adrenergic receptor-mediated vasoconstriction. Pretreatment with the presynaptic noradrenergic antagonist bretylium abolishes reflex vasoconstriction during whole body cooling (18, 32), thus reflex cutaneous vasoconstriction is mediated by the sympathetic noradrenergic nerves. Taken together (persistent vasoconstriction at sites unresponsive to exogenous NE and noradrenergic origin of vasoconstriction) such results would indicate a role for a vasoconstricting transmitter coreleased from sympathetic nerves. The term cotransmitter is used here to describe a mechanism of vasoconstriction that appears to originate from noradrenergic nerves but is not mediated by NE. We do not demonstrate corelease or vesicular colocalization in this study.

Part I. One hour before data collection began, each of 10 subjects was administered 50-μl injections of 5 mM id yohimbine (Sigma) and saline at separate sites on the dorsal forearm. These solutions were sterilized by microfiltration (0.2 μm, Acrodisk; Pall, MI). Intradermal injections were administered through a 27-gauge needle advanced ~1 cm within the skin. Bretylium tosylate (ICN; Aurora, OH) was applied to a third site (0.6 cm²) by iontophoresis (250 μA, 10 min) (18). At the end of the study, blockade of the yohimbine-treated site was tested by iontophoretic application of exogenous NE (Sigma) (20 μA, 10 min). Frequently the application of NE to yohimbine-treated sites was associated with a vasodilatation: this lead to the design of part II.

Part II. Each of seven subjects was administered 50 μl id injections of a solution of 5 mM yohimbine-1 mM propranolol (Sigma). Six of these subjects participated in part I. A second site was treated by intradermal injection with saline as a vehicle control. A third site was treated with a 5 mM id yohimbine injection and a fourth site was treated by injection with 1 mM id propranolol. These solutions were sterilized by microfiltration. One subject required a higher dose of yohimbine (7.5 mM) to completely block NE-induced vasoconstriction.

Part III. In three subjects, the α-adrenergic receptor antagonist idazoxan (Sigma) was substituted for yohimbine (38). The pretreatment was essentially the same as described in part I with ±10 mM idazoxan and ±10 mM propranolol being applied by intradermal injection. The aim of these studies was to confirm that any persistent vasoconstriction observed at yohimbine + propranolol-treated sites was not unique to yohimbine treatment.

Protocol. The protocols for parts I, II, and III were essentially identical. During the hour after the intradermal injections subjects were instrumented as described above. TLOC at the site of blood flow measurement was held at 34°C throughout all studies. Whole body TSK was slowly reduced from 34°C to 31°C. After whole body cooling, blockade of yohimbine-containing, yohimbine + propranolol-, and idazoxan + propranolol-treated sites was tested by iontophoretic application of exogenous NE (20 μA to 0.6 cm², 10 min). If blood flow was reduced to less than 90% of baseline by min 8 of NE application, the sites were considered not blocked and those data were not included. By min 8 of iontophoretic application of NE in part II, CVC at the saline-treated site was reduced to 62.8 ± 7.9% of baseline. This value of CVC is similar to that observed at the coldest temperatures achieved during whole body cooling, 66.5 ± 6.7% of baseline. Exogenous NE was also applied to a saline site at the same current and duration to demonstrate the efficacy of the NE. Adequacy of the β-adrenergic blockade at the propranolol-treated sites was tested by the iontophoretic application of isoprotenerol (Sigma) (20 μA, 10 min). Isoprotenerol was applied to control sites at the same current and duration to demonstrate its efficacy.

Data analysis. Laser Doppler blood flow values, MAP, TSK, and TLOC were sampled once per second (LabView, National Instruments), averaged into 20-s samples, and stored in a laboratory computer. Data were further compiled into 1-min averages and normalized to the average of the 3-min period immediately preceding either whole body cooling, NE iontophoresis, or isoprotenerol iontophoresis. Data for CVC from bretylium-, yohimbine-, yohimbine + propranolol-, idazoxan + propranolol-, propranolol-, and saline-treated sites were analyzed relative to TSK during the whole body cooling period. First, to detect a reduction in CVC from the control period, data from each site were analyzed independently by a one-way ANOVA with repeated measures and a Dunnett’s post hoc analysis when a significant difference was detected. To test whether responses differed according to α- or β-adrenergic blockade, values for CVC between the yohimbine + propranolol and saline-, idazoxan + propranolol and saline-, or propranolol and saline-treated sites were compared by a two-way ANOVA with repeated measures and a Bonferroni posttest when a significant difference was detected. To test
for the adequacy of the blockade, CVC data collected during the application of NE to yohimbine-, yohimbine + propranolol-, idazoxan + propranolol-, and saline-treated sites were each analyzed by a one-way ANOVA and a Dunnett’s post hoc analysis when a significant difference was detected. To test whether the dose of exogenous NE applied by iontophoresis after the study caused a similar vasoconstriction as that recruited by whole body cooling, we compared by two-way ANOVA the CVC data from saline-treated sites during whole body cooling with CVC data from the same saline-treated sites during application of NE. The level for significance was set at \( P < 0.05 \). All data are reported as means ± SE.

RESULTS

Part I. Figure 1A shows the average response in CVC from bretylium-, yohimbine-, and saline-treated sites during whole body cooling as a function of \( T_{SK} \). During whole body cooling, \( T_{SK} \) was reduced from 34.5 ± 0.4 to 30.6 ± 0.2°C. The onset of whole body cooling induced a progressive decrease in CVC at saline-treated sites, first reaching statistical significance at a mean whole body \( T_{SK} \) of 33.9 ± 0.3°C (CVC = 83.9 ± 3.5% of baseline; \( P < 0.05 \)) and remaining significantly reduced from baseline for the remainder of the cooling protocol. Vasoconstriction at yohimbine-treated sites did not achieve statistical significance until whole body \( T_{SK} \) reached 30.6 ± 0.3°C (CVC = 82.6 ± 8.8% of baseline; \( P < 0.05 \)). CVC at the bretylium-treated sites was unchanged during whole body cooling (\( P > 0.05 \)). At the lowest \( T_{SK} \) analyzed (30.6 ± 0.3°C), CVC was reduced to 50.1% of baseline at the saline-treated sites (\( P < 0.05 \)) and 82.6% of baseline at the yohimbine-treated sites (\( P < 0.05 \)).

After whole body cooling, \( \alpha \)-adrenergic receptor blockade at the yohimbine sites was tested by iontophoresis of NE over the site of blood flow measurement. Figure 1B shows the response to exogenous NE at both yohimbine- and saline-treated sites. CVC at yohimbine-treated sites did not change significantly from the preiontophoresis control period at any time during or after NE iontophoresis (\( min \ 8 \) of iontophoresis CVC = 120.5 ± 11.5% of baseline; \( P > 0.05 \)). CVC at saline-treated sites was significantly reduced by \( min \ 5 \) of NE application and beyond (\( min \ 5 \) of iontophoresis CVC = 79.4 ± 6.8% of baseline; \( P < 0.05 \)) and reached 59.1 ± 5.5% of baseline at \( min \ 8 \) of iontophoresis. The application of exogenous NE to the saline-treated site caused a reduction in CVC (59.1 ± 5.5% of baseline) that was not significantly different from the reduction caused by whole body cooling (CVC = 57.1 ± 6.3% of baseline) at the same site (\( P > 0.05 \)).

Figure 2 shows the individual data from the yohimbine-treated sites from part I during whole body cooling. Note that although 5 of 10 subjects showed significant vasoconstriction during whole body cooling, 3 subjects had little or no change in CVC and two subjects showed vasodilation. It was this observation that suggested to us that at yohimbine-treated sites, NE released during cooling or exogenous NE might be stimulating \( \beta \)-adrenergic receptors and causing (in some subjects) a vasodilation that masked the vasoconstricting effects of the hypothesized cotransmitter. For this reason part II was instituted in which \( \alpha \)- and \( \beta \)-adrenergic blockade were combined to eliminate all vasomotor effects of NE.

Part II. Figure 3A shows the average response in CVC from seven men during whole body cooling at saline- and yohimbine + propranolol-treated sites. During whole body cooling, \( T_{SK} \) was decreased from 34.0 ± 0.02 to 31.3 ± 0.02°C. One-minute averages of CVC data corresponding to 0.3°C decreases in \( T_{SK} \) from 34 to 31.3°C are shown. Values for CVC at saline-treated sites were significantly reduced from baseline at \( T_{SK} \) of 33.4 ± 0.01°C (CVC = 83 ± 7.5% of baseline) and remained reduced throughout the whole body cooling. CVC at sites treated with the combined blockade of
yohimbine + propranolol was temporarily significantly reduced from baseline at $T_{SK}$ of 33.0 ± 0.01°C (CVC = 88.8 ± 5.3% of baseline; $P < 0.05$) and was significantly reduced from baseline at $T_{SK}$ of 32.4 ± 0.01°C (CVC = 87.4 ± 5.8% of baseline; $P < 0.05$) until the end of whole body cooling. At the end of whole body cooling $T_{SK}$ was 31.4 ± 0.02°C. CVC at saline-treated sites was reduced to 66.5 ± 6.7% of baseline ($P < 0.01$) and at yohimbine + propranolol-treated sites to 86.1 ± 5.5% of baseline ($P < 0.01$). Two-way ANOVA detected a significant difference in the degree of reduction in CVC between the yohimbine + propranolol- and saline-treated sites during whole body cooling. A Bonferroni posttest indicated that CVC at yohimbine + propranolol-treated sites was significantly different from that at saline-treated sites at $T_{SK}$ of 31.7 ± 0.01°C and at $T_{SK}$ of 31.4 ± 0.02°C ($P < 0.05$; see Fig. 3).

Figure 3B illustrates the average CVC in response to application of exogenous NE by iontophoresis at the same saline- and yohimbine + propranolol-treated sites as in Fig. 3A. CVC at saline-treated sites was significantly reduced from min 5 of iontophoresis (min 5 of CVC = 79.0 ± 10.4% of baseline) and remained reduced throughout the application of NE (min 8 of CVC = 62.8 ± 7.8% of baseline; $P < 0.05$). Sites treated with yohimbine + propranolol did not show any vasomotor response during application of exogenous NE (CVC at min 8 = 98.2 ± 4.8% of baseline; $P > 0.05$). CVC data at sites treated with saline during whole body cooling (CVC = 66.5 ± 6.7% of baseline) and application of exogenous NE (62.8 ± 7.8% of baseline) were not significantly different ($P = 0.99$), suggesting the test of adrenergic receptor antagonism causes the same degree of vasoconstriction as does reflex activation of the noradrenergic nerves at control sites. These data suggest the test of adrenergic receptor antagonism is similar in intensity with regard to vasomotor response as the degree of vasoconstriction caused by whole body cooling.

Figure 4 shows the individual responses at the yohimbine + propranolol-treated sites during whole body cooling. Note that six of the seven subjects vasoconstricted during whole body cooling and one subject slightly vasodilated. Five of these subjects participated in part I. The subject showing the apparent vasodilator response in Fig. 4 did not participate in part I. One of the two subjects showing a clear vasodilator response to whole body cooling in part I had a vasoconstrictor response in part II.

Figure 5A shows the average response in CVC from saline ($n = 7$) and propranolol ($n = 7$)-treated sites...
during whole body cooling. CVC at saline-treated sites was significantly reduced from baseline at T_SK of 32.0 ± 0.01°C (CVC = 75 ± 8.8% of baseline) and remained reduced until the end of whole body cooling (T_SK of 31.4°C, CVC = 70.5 ± 7.8% of baseline). CVC at propranolol-treated sites was significantly reduced from baseline at T_SK of 33.0 ± 0.01°C (CVC = 75 ± 8.3% of baseline) and remained reduced throughout cooling (T_SK of 31.4 ± 0.01, CVC = 61.4 ± 7.8% of baseline). Although there was a tendency for propranolol-treated sites to show a greater reduction in CVC, two-way ANOVA did not detect a significant difference between the saline- and propranolol-treated sites during whole body cooling.

After whole body cooling, exogenous application of isoproterenol at propranolol-treated sites did not cause a significant increase in CVC (at 10 min postiontophoresis CVC = 110.1 ± 14.9% of baseline; P > 0.05) (Fig. 5B). CVC at control sites was significantly increased after iontophoresis of isoproterenol (10 min postiontophoresis CVC = 270.2 ± 56.7% of baseline; P < 0.05).

Figure 6A shows the average response in CVC from three men at sites pretreated with idazoxan + propranolol and saline. T_SK was reduced from 34.0 ± 0.04 to 31.3 ± 0.01°C. CVC at idazoxan-treated sites significantly differed from baseline at T_SK of 33.7 ± 0.01°C (CVC = 85.6 ± 0.4% of baseline) and remained reduced through the end of whole body cooling (CVC = 65.1 ± 9% of baseline). CVC at saline-treated sites was significantly reduced from baseline at T_SK of 33.0 ± 0.1°C (CVC = 74 ± 4.2% of baseline) and remained so through the end of whole body cooling (CVC = 49.8 ± 8.6% of baseline). Two-way ANOVA did not detect a significant difference in responses between idazoxan + propranolol- and saline-treated sites (P = 0.2). One-way ANOVA did not detect a significant change in CVC at idazoxan + propranolol-treated sites during application of exogenous NE (P = 0.28). CVC at saline-treated sites was significantly reduced from baseline at mins 9 and 10 of iontophoresis (min 9 of CVC = 67.1 ± 2.9% of baseline; P < 0.05). Figure 6B shows the response at idazoxan + propranolol- and saline-treated sites during iontophoresis of NE.

DISCUSSION

The major new finding from this study is that in addition to the primary vasoconstricting neurotransmitter NE, there is a nonnoradrenergic mechanism of vasoconstriction that is involved in the reflex response during whole body cooling. CVC at saline-treated sites was significantly reduced from baseline at T_SK of 32.0 ± 0.01°C (CVC = 75 ± 8.8% of baseline) and remained reduced until the end of whole body cooling (T_SK of 31.4°C, CVC = 70.5 ± 7.8% of baseline). CVC at propranolol-treated sites was significantly reduced from baseline at T_SK of 33.0 ± 0.01°C (CVC = 75 ± 8.3% of baseline) and remained reduced throughout cooling (T_SK of 31.4 ± 0.01, CVC = 61.4 ± 7.8% of baseline). Although there was a tendency for propranolol-treated sites to show a greater reduction in CVC, two-way ANOVA did not detect a significant difference between the saline- and propranolol-treated sites during whole body cooling.

After whole body cooling, exogenous application of isoproterenol at propranolol-treated sites did not cause a significant increase in CVC (at 10 min postiontophoresis CVC = 110.1 ± 14.9% of baseline; P > 0.05) (Fig. 5B). CVC at control sites was significantly increased after iontophoresis of isoproterenol (10 min postiontophoresis CVC = 270.2 ± 56.7% of baseline; P < 0.05).

Figure 6A shows the average response in CVC from three men at sites pretreated with idazoxan + propranolol and saline. T_SK was reduced from 34.0 ± 0.04 to 31.3 ± 0.01°C. CVC at idazoxan-treated sites significantly differed from baseline at T_SK of 33.7 ± 0.01°C (CVC = 85.6 ± 0.4% of baseline) and remained reduced through the end of whole body cooling (CVC = 65.1 ± 9% of baseline). CVC at saline-treated sites was significantly reduced from baseline at T_SK of 33.0 ± 0.1°C (CVC = 74 ± 4.2% of baseline) and remained so through the end of whole body cooling (CVC = 49.8 ± 8.6% of baseline). Two-way ANOVA did not detect a significant difference in responses between idazoxan + propranolol- and saline-treated sites (P = 0.2). One-way ANOVA did not detect a significant change in CVC at idazoxan + propranolol-treated sites during application of exogenous NE (P = 0.28). CVC at saline-treated sites was significantly reduced from baseline at mins 9 and 10 of iontophoresis (min 9 of CVC = 67.1 ± 2.9% of baseline; P < 0.05). Figure 6B shows the response at idazoxan + propranolol- and saline-treated sites during iontophoresis of NE.
of the cutaneous circulation to whole body cooling in humans. This conclusion is drawn from the observations that sites pretreated with yohimbine + propranolol vasoconstricted during whole body cooling, and importantly these same sites failed to vasoconstrict in response to the subsequent direct application of exogenous NE. To conclude that the antagonism of the adrenergic receptors during whole body cooling is complete, the blockade must be tested with a stimulus that is at least as strong as that recruited during whole body cooling. The vascular response at saline-treated sites to direct application of exogenous NE was compared with the vascular response at those same sites during whole body cooling and found not to be significantly different. Thus the dose of NE applied to test the antagonism of adrenergic receptors caused a vasoconstriction similar in magnitude to the vasoconstriction that occurred during reflex activation of the noradrenergic vasoconstrictor nerves.

Bretylium, a drug that inhibits noradrenergic nerve transmission, abolished reflex vasoconstriction in response to whole body cooling in keeping with our prior experience (16, 31, 34). Absence of vasoconstriction at bretylium-treated sites during whole body cooling (part I) strongly suggests that the mechanism for vasoconstriction observed at saline, yohimbine (part I), propranolol, yohimbine + propranolol (part II), and idazoxan + propranolol (part III)-treated sites must be of sympathetic noradrenergic origin. Furthermore, control sites pretreated with saline vasoconstricted during both whole body cooling and exogenous NE application. We reasoned that if sites pretreated with yohimbine + propranolol failed to vasoconstrict or vasodilate to exogenous NE at the end of the study, then those same sites would not have responded to endogenous NE earlier during whole body cooling. Vasoconstriction at saline-treated sites in response to exogenous NE confirmed the efficacy of the exogenous NE. Therefore, vasoconstriction observed at yohimbine + propranolol-treated sites is likely mediated by noradrenergic nerves but not by NE. On the average the nonnoradrenergic mechanism accounted for ∼40% of the vasoconstriction at the lowest whole body TSK.

Although generally considered an α₂-noradrenergic receptor antagonist, at high enough levels yohimbine can function as a nonspecific α-adrenergic receptor antagonist, blocking both α₁- and α₂-adrenergic receptors (9). In preliminary experiments we successfully blocked phenylephrine-induced vasoconstriction with the dose of yohimbine used in this study. Thus any vasoconstriction observed at yohimbine + propranolol-treated sites during whole body cooling must be due to a nonnoradrenergic mechanism. In a limited number of subjects we explored the possibility that the reflex vasoconstriction observed at yohimbine + propranolol-treated sites during whole body cooling was affected by a nonnoradrenergic action of yohimbine. However, we found that the nonnoradrenergic mechanism of reflex cutaneous vasoconstriction observed at the yohimbine + propranolol-treated sites during whole body cooling was also observed at sites pretreated with idazoxan + propranolol (Fig. 6A). Thus yohimbine does not appear to exert nonspecific effects on CVC that might otherwise confound interpretation of data collected during whole body cooling.

In part I, we noted a nonnoradrenergic vasoconstriction at sites pretreated with yohimbine alone (Fig. 1A). However, analysis of these data identified a statistically significant vasoconstriction only at the lowest whole body TSK. Review of the individual data suggested that the average data may have been confounded by vasodilation observed in two subjects during whole body cooling (Fig. 2). Furthermore, exogenous NE also caused a vasodilation at yohimbine-treated sites in this subject pool (Fig. 1B). We were concerned that measured blood flow at yohimbine-treated sites represented a net blood flow response from β-adrenergic-mediated vasodilation and cotrans-
mmitter-mediated vasoconstriction. Therefore, we combined α- and β-adrenergic antagonists in part II and found that vasoconstriction became more consistent during whole body cooling at sites with all vasomotor influences of NE removed (Figs. 3A and 4). This suggests that β-adrenergic-mediated vasoconstriction may have obscured a nonnoradrenergic mechanism of vasoconstriction at sites treated with only yohimbine.

Johnson and colleagues (14) utilized phentolamine as a nonspecific α-adrenergic receptor antagonist to test for noradrenergic cotransmitter participation in the cutaneous vasomotor response to local cooling. However, the time course of antagonism of α-adrenergic receptors by phentolamine was often too short to be assured of an effective blockade throughout studies of 1-h duration or more. Also in preliminary studies we were unable to antagonize vasoconstriction induced by iontophoretic application of phenylephrine or NE with the traditional α1-adrenergic receptor antagonists prazosin, terazosin, or doxazosin. Our findings agree with those previously published that yohimbine would antagonize both α1- and α2-adrenergic receptors for an appropriate length of time (~3 h) (9). The rigid exclusion criteria for acceptance of an adequate blockade caused several studies to be excluded from analysis. The studies that did not meet the criteria for a complete block may contain important data related to α-adrenergic subtype participation in whole body cooling and response to exogenous NE. Clearly more work is needed in the area of noradrenergic pharmacology of the cutaneous vasculature. We sought to find whether a cotransmitter participated in control of SkBF and limited our experiments to those that clearly tested the hypothesis which required complete blockade of the vasomotor effects of NE.

\( T_{\text{LOC}} \) at the sites of blood flow measurement at the bretylium-, yohimbine-, idazoxan-, yohimbine + propranolol-, idazoxan + propranolol-, propranolol-, and saline-treated sites was held constant at 34°C to eliminate any confounding effects of \( T_{\text{LOC}} \) affecting transmitter release or receptor affinity for NE (5, 7, 33, 41). Thus the responses recorded at those sites during whole body cooling are due to reflex effects of sympathetic vasoconstrictor activation and antagonism.

Charkoudian and Johnson (3) demonstrated that progressive decrease of \( T_{\text{SK}} \) causes a reflex vasoconstriction that is mediated solely by the vasoconstrictor nerves and does not have a component of active vasodilator withdrawal. Similarly, Pérgola and colleagues (32) demonstrated that increasing \( T_{\text{SK}} \) caused a passive vasodilation due to vasoconstrictor withdrawal, and that active vasodilator tone was not present at internal temperatures below ~37°C. The design of the cooling protocol in this study followed that of Charkoudian and Johnson (3), so the vasoconstriction observed here is due only to activation of the noradrenergic vasoconstrictor system; i.e., the nonnoradrenergic component is not withdrawal of active vasodilator activity. This is substantiated by the lack of a vasoconstrictor response to whole body cooling at sites pretreated with bretylium.

The data here do not specify the identity of the nonnoradrenergic vasoconstrictor. NPY, ATP, and other neurotransmitters are implicated in other species and/or vascular beds (16, 22, 24, 34) and one or more may be involved in human skin. The identification, however, awaits further studies.

The observation that vasoconstrictor control of cutaneous blood flow has at least three components (α-adrenergic and β-adrenergic receptors and a cotransmitter) led us to test whether α-adrenergic receptors modulate α-adrenergic-mediated cutaneous vasoconstriction. Crandall and colleagues (4) demonstrated the presence of β-adrenergic receptors in the cutaneous circulation but found no role for them in mediating active vasoconstriction. We hypothesized that blockade of β-adrenergic receptors would remove a modulatory vasodilator influence on SkBF leading to a more intense vasoconstriction during reflex activation of the sympathetic vasoconstrictor nerves. Although the average CVC at sites pretreated with β-adrenergic receptor blockade was consistently lower than that at control sites during whole body cooling, no statistically significant difference was detected (Fig. 3A). If β-adrenergic receptors do contribute a modulatory role to vasoconstrictor control of cutaneous circulation, that contribution is small (mean difference between propranolol- and saline-treated sites averaged 10.4 ± 1.6% of baseline).

Kenney and co-workers (20, 21) investigated the relative contributions of α-adrenergic receptors to the control of the cutaneous circulation utilizing systemic administration of α-adrenergic antagonists. They observed that the reflex vasoconstrictor response of the forearm to facial cooling was completely blocked by the systemic administration of the α1-antagonist prazosin (20). Their results suggest no role for nonnoradrenergic cutaneous vasoconstriction during a 90-s period of facial cooling. It is not clear whether a stronger stimulus or one of a greater duration might have revealed a vasoconstrictor component independent of α-adrenergic receptors. Results from a later study by Kenney and colleagues (21), in which systemic yohimbine was used, are difficult to interpret with regard to nonnoradrenergic mechanisms of vasoconstriction because at those concentrations only the α2-adrenergic receptor inhibition by yohimbine would be present.

Finally, although we did not demonstrate colocalization of nonnoradrenergic neurotransmitters with NE, our findings are in keeping with multiple neurotransmitters being released from sympathetic vasoconstrictor nerves in human skin. Our approach was to eliminate the effects of NE to test for other mechanisms of vasoconstriction. Unfortunately this does not permit an evaluation of the two mechanisms working synchronously or of any interaction between the two. Our interpretation that the vasoconstriction not accounted for by NE is a cotransmitter depends on the specificity of bretylium to noradrenergic nerves. Although this is a reasonable assumption, the interpretation could be strengthened by other approaches. These findings are similar to earlier findings support-
ive of colocalized vasodilator neurotransmitters in the sympathetic cutaneous active vasodilator system (19). In that study it was found that presynaptic inhibition of cholinergic nerves by botulinum toxin but not postsynaptic inhibition of muscarinic receptors by atropine effectively abolished cutaneous active vasodilation. Similarly in the present study, presynaptic blockade by bretylium eliminated vasoconstrictor responses, whereas postsynaptic blockade of α-adrenergic receptors was only partially effective. In both cases participation by a noncholinergic nonnoradrenergic cotransmitter is strongly suggested.

In summary, we propose that a nonnoradrenergic mechanism of control has a significant role in reflex vasoconstriction in the cutaneous circulation in humans. This nonnoradrenergic mechanism of control is demonstrated by the persistent reflex vasoconstriction during whole body cooling at sites pretreated with a combination of antagonists that completely block the vasomotor effects of NE. Although some uncertainty exists, β-adrenergic receptors may modulate noradrenergic vasoconstrictor function by providing a small vasodilatory influence. The frequency dependence of cotransmitter release remains in question in this experimental model. Furthermore, the identity of the nonnoradrenergic mechanism awaits further study.

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