Effect of skin temperature on cutaneous vasodilator response to the β-adrenergic agonist isoproterenol

Gary J. Hodges,1,2 Dean L. Kellogg,2,3 and John M. Johnson2

1Department of Kinesiology, Brock University, St. Catharines, Ontario, Canada; 2Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas; and 3Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

Submitted 11 December 2014; accepted in final form 13 February 2015

Hodges GJ, Kellogg DL, Johnson JM. Effect of skin temperature on cutaneous vasodilator response to the β-adrenergic agonist isoproterenol. J Appl Physiol 118: 898–903, 2015; doi:10.1152/japplphysiol.01071.2014.—The vascular response to local skin cooling is dependent in part on a cold-induced translocation of α2C-receptors and an increased α-adrenoreceptor function. To discover whether β-adrenergic function might contribute, we examined whether β-receptor sensitivity to the β-agonist isoproterenol was affected by local skin temperature. In seven healthy volunteers, skin blood flow was measured from the forearm by laser-Doppler flowmetry and blood pressure was measured by finger photoplethysmography. Data were expressed as cutaneous vascular conductance (CVC; laser-Doppler flux/mean arterial blood pressure). Pharmacological agents were administered via intradermal microdialysis. We prepared four skin sites; one site was maintained at a thermoneutral temperature of 34°C (32 ± 10% CVCmax); one site was heated to 39°C (38 ± 11% CVCmax); and two sites were cooled, one to 29°C (22 ± 7% CVCmax) and the other 24°C (16 ± 4% CVCmax). After 20 min at these temperatures to allow stabilization of skin blood flow, isoproterenol was perfused in concentrations of 10, 30, 100, and 300 µM. Each concentration was perfused for 15 min. Relative to the CVC responses to isoproterenol at the thermoneural skin temperature (34°C) (+21 ± 10%max), low skin temperatures reduced (at 29°C) (+17 ± 6%max) or abolished (at 24°C) (+1 ± 5%max) the vasodilator response (+40 ± 9%max) to isoproterenol. These data indicate that β-adrenergic function was influenced by local skin temperature. This finding raises the possibility that a part of the vasoconstrictor response to direct skin cooling could include reduced background β-receptor mediated vasodilation.

local cooling; local heating; β-adrenergic receptors; noradrenaline

A CONSTANT, NORMAL BODY TEMPERATURE is the result of a regulated balance between heat production and heat loss. Controlling skin blood flow manipulates the rate of heat transfer from the core to the surface of the body and its elimination to the environment. Adjustments in perfusion of the skin are largely in response to changes in core, whole body skin, and local skin temperature. The reflex portion of the fluctuations in skin perfusion in response to reductions in whole body skin temperature are achieved primarily through the sympathetic noradrenergic system, relying principally on skin sympathetic nerve activity causing the release of norepinephrine and neuropeptide Y (24, 34–36). Local changes in skin temperature from direct cooling of the skin also stimulate a cutaneous vasoconstriction via both local stimulation of norepinephrine release and inhibition of a nitric oxide-based system (17, 22, 46). Crandall et al. (9) demonstrated the presence of functional β-receptors in the cutaneous vasculature of humans. Although these receptors played no significant role in mediating reflex cutaneous vasodilatation [a cholinergic cotransmitter mechanism (25)], Stephens et al. (34) found that addition of the β-receptor antagonist propranolol revealed a greater reflex cutaneous vasoconstriction to whole body cooling than under conditions of α-receptor antagonism only. This latter finding suggests some inclusion of β-receptors in the net vascular response (i.e., their presence buffers the α-mediated vasoconstriction). Furthermore, Stephens et al. (34) reported that administration of norepinephrine under conditions of α-receptor antagonism frequently resulted in vasodilation, a phenomenon that was blocked by the inclusion of propranolol. This finding suggests a potential for β-receptor function to be an important background factor in cutaneous vascular control.

Previously, we demonstrated that the cutaneous vasoconstrictor response to direct local skin cooling can be abolished under conditions of combined blockade of transmitter release from sympathetic noradrenergic nerve endings and nitric oxide synthase inhibition (17). In vitro and in vivo data suggest that the adrenergic portion of the vascular response to reduced local skin temperature is due primarily to increased α2C-receptor expression. Although that portion of the vasoconstrictor response to reduced tissue temperature is mediated by norepinephrine, data from in vitro and isolated systems data clearly demonstrate that synthesis and release of norepinephrine is impaired by cooling (4, 10, 42). The adrenergic response to local skin cooling has been shown to be dependent on a cold-induced translocation of α2C-receptors from the Golgi apparatus to the plasma membrane via Rho kinase (2, 3, 7, 8, 20), rather than on an increase in norepinephrine release. This finding was demonstrated in the cutaneous circulation of humans by Thompson-Torgerson et al. (38), who reported a marked reduction of the cutaneous vasoconstrictor response to local cooling under conditions of Rho kinase inhibition. It is not known whether a concomitant change occurs in β-receptor function during local skin cooling. Could local tissue cooling also enhance β-receptor function? Contrary to that supposition, we have unpublished observations that β-receptor sensitivity might actually be reduced by local skin cooling. We found that the concentration of the β-receptor agonist isoproterenol required to elicited a given degree of vasodilation was greater at cooler temperatures relative to warmer temperatures. If local cooling reduces β-receptor-mediated vasodilation, that mechanism could contribute to the vasoconstrictor response to cooling. Consequently, we sought

898 8750-7587/15 Copyright © 2015 the American Physiological Society http://www.jap.physiology.org
to resolve the question of whether β-receptor-mediated cutaneous vasodilation was positively or negatively affected by changes in local skin temperature.

METHODS

Participants. The Institutional Review Board of The University of Texas Health Science Center at San Antonio approved this study. All participants were fully informed of the methods and risks before written and verbal consent was obtained. All seven participants (five men and two women, age 28 ± 3 yr) were healthy nonsmokers, nonobese (body mass index 23 ± 2), and not taking any medications. Participants refrained from consuming alcoholic or caffeinated beverages for at least 12 h prior to the study. For women participants, the phase of the menstrual cycle was recorded but not controlled for in these experiments. Previous studies have reported that the vasoconstrictor responses to local cooling are unaffected by female reproductive hormones (6).

Instrumentation. Each participant had four microdialysis probes placed intradermally on the ventral aspect of the left forearm. As described previously (9, 23), these custom-built microdialysis probes consisted of 2 cm of cellulose microdialysis tubing (ID 200 μm, 18 kDa nominal molecular weight cutoff) attached at each end to polyimide tubing, leaving 1 cm of membrane for drug exchange. Before implantation, the area of forearm skin selected for probe insertion was temporarily anesthetized by the application of an ice pack for 5 min (13). A 22-gauge needle was introduced aseptically for ~2.5 cm into the skin before exiting. The microdialysis probe and the connecting tubing were introduced into the skin via the lumen of the needle; the needle was then removed leaving the probe in place. All probes were placed in this manner, and ~1.5 h was allowed for the effects of the insertion trauma to subside. The probes were placed ~3 cm apart.

Measurements. All measurements were performed with participants in the supine position. Skin blood flow was measured from the ventral aspect of the forearm by laser-Doppler flowmetry (Moor Instruments, Axminster, UK) and expressed as laser-Doppler flow (in arbitrary units). Whole-body skin temperature was recorded via the Peltier cooling and heating probe holders (1, 14–16, 22, 46). These probes were placed in the center of the holder to enable placement of the laser-Doppler probe. A thermocouple between the skin surface and the ventral aspect of the forearm used for blood flow measurements. This arrangement allowed independent control of local skin temperature and whole body skin temperature. Local and whole body skin temperatures were initially maintained at 34°C (thermoneutral).

Chemicals. Sodium nitroprusside and isoproterenol (Sigma Aldrich, St. Louis, MO) were dissolved in sterile saline immediately prior to use. Isoproterenol was prepared in four concentrations (10, 30, 100, and 300 μM). Sodium nitroprusside was prepared at 58 mM. Solutions were sterilized using a 0.2-μm syringe filter (Acrodisc; Pall, Port Washington, NY).

Protocols. Protocols were designed to test whether local temperature affects the vasodilator response to the β-agonist isoproterenol (Fig. 1). Following 15 min of baseline measures at a local temperature of 34°C, one site was heated to 39°C, and two sites were cooled, one to 29°C and the other 24°C, while the final site remained at the thermoneutral temperature of 34°C. After 20 min at these temperatures to allow stabilization of skin blood flow, isoproterenol was perfused in increasing concentrations of 10, 30, 100, and 300 μM to each site. Each concentration was perfused for 15 min. All sites were then returned to a saline infusion for 15 min and local skin temperature was returned to 34°C. Sodium nitroprusside (58 mM) was then administered and local temperature was increased to 42°C to attain maximal CVC (26, 27).

Data and statistical analysis. Data for CVC were analyzed for the final 5 min of each section (see Fig. 1). CVC data were expressed as a percentage of maximal as determined by the administration of sodium nitroprusside and local skin warming to 42°C. Statistical analysis was by repeated-measures ANOVA with a Bonferroni post hoc test. Statistical significance was assumed when P < 0.05. Power analysis indicated that a minimum of six participants would be required for a P < 0.05 with 90% power (nQuery; Statistical Solutions, Cork, Ireland). Data are presented as means ± SD.

RESULTS

Figure 2 shows representative results from one participant in the protocol outlined in Fig. 1. Observe the distinct differences in the responses to isoproterenol among sites, in particular how the magnitude of the CVC response is reduced at lower local

<table>
<thead>
<tr>
<th>Microdialysis perfusion</th>
<th>Baseline 15 min</th>
<th>Isoproterenol infusions 60 min</th>
<th>Temp Δ 15 min</th>
<th>SNP 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tskin</td>
<td>Saline</td>
<td>10 μM</td>
<td>30 μM</td>
<td>100μM</td>
</tr>
<tr>
<td>Site 1 Tloc:</td>
<td>34 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 2 Tloc:</td>
<td>34 °C</td>
<td>39 °C</td>
<td>34 °C</td>
<td>42 °C</td>
</tr>
<tr>
<td>Site 3 Tloc:</td>
<td>34 °C</td>
<td>29 °C</td>
<td>34 °C</td>
<td>42 °C</td>
</tr>
<tr>
<td>Site 4 Tloc:</td>
<td>34 °C</td>
<td>24 °C</td>
<td>34 °C</td>
<td>42 °C</td>
</tr>
</tbody>
</table>

Fig. 1. Outline of protocol. Four sites were prepared with microdialysis probes local skin heater/cooler laser-Doppler probe holders, and laser-Doppler probes. Local skin temperature (Tloc) and whole-body skin temperature (Tskin) were initially maintained at 34°C. All probes were perfused with saline for 35 min. After 15 min of baseline data collection, Tloc increased to 39°C at one site and reduced at two sites to 29°C and 24°C, respectively. Perfusate for all sites was then infused every 15 min to 10, 30, 100, and 300 μM isoproterenol. Following these infusions, perfusate was changed to saline and Tloc changed to 34°C at all sites for 15 min. Finally, Tloc was increased to 42°C and 58 mM of sodium nitroprusside (SNP) was infused at all sites to obtain maximal cutaneous vascular conductance.
temperatures. Furthermore, note the slight vasoconstriction toward the end of the administration of 300 μM isoproterenol; this was observed in all participants and is most likely due to isoproterenol saturation of β-adrenergic receptors and having an action via α-adrenergic receptors.

During the local temperature adjustment phase, CVC (group averages) at the warm site increased from 25 ± 8%max to 38 ± 11%max, and CVC decreased at the cool and cold sites from 34 ± 5%max to 22 ± 7%max and 26 ± 2%max to 16 ± 4%max, respectively (all P < 0.05). CVC at the thermoneutral control site (34°C) did not change during this period (32 ± 7%max to 32 ± 10%max; P > 0.05). CVC generally reached a steady state during the 20-min period following the temperature changes. This was always the case for cooled sites. However, at the warmed skin site (39°C), the vasodilation to increasing concentrations of isoproterenol is augmented.

There were main effects for both local temperature and isoproterenol treatment (both P < 0.05). There was also statistically significant interaction between local temperature and treatment (P < 0.05). Figure 3 shows the responses in CVC normalized to maximum averaged from the seven participants. In response to the administration of 10 μM isoproterenol, CVC at the thermoneutral and warm skin sites in
creased to 49 ± 8%max and 61 ± 6%max, respectively (both P < 0.05). At the cool skin site (29°C), CVC averaged 22 ± 7%max before and 25 ± 5%max during perfusion with 10 μM isoproterenol. CVC at the cold site (24°C) was 16 ± 4%max before and 14 ± 4%max during perfusion with isoproterenol. Neither of these latter responses was statistically significant (P > 0.05).

Isoproterenol at 30 μM elicited an increase in CVC from 49 ± 8%max to 78 ± 7%max at the warm (39°C) sites, and from 25 ± 5%max to 39 ± 7%max at the cool (29°C) skin sites (both P < 0.05). CVC at the thermoneutral (34°C) site changed from 10 ± 3% max to 59 ± 10%max (P = 0.06) in response to 30 μM isoproterenol. Finally, CVC at the cold skin sites (24°C) remained statistically unchanged (P > 0.05) at 14 ± 4%max before and 17 ± 4%max during 30 μM isoproterenol perfusion.

Perfusion of 100 μM isoproterenol elicited no further changes of statistical significance in CVC at any of the skin sites relative to the levels observed with 30 μM isoproterenol (all P > 0.05). Interestingly, in response to 300 μM isoproterenol, CVC decreased (P < 0.05) at the warm (70 ± 10%max) and thermoneural (49 ± 8%max) sites relative to the responses achieved from perfusion of 100 μM isoproterenol. CVC at the cool and cold sites was statistically unchanged (P > 0.05), although there was a slight reduction.

DISCUSSION

The major finding of this study was that local skin temperature is an important determinant of the CVC response to the infusion of isoproterenol, suggesting that β-adrenergic function is influenced by local skin temperature. Relative to the CVC responses achieved at the thermoneural skin temperature (34°C), low skin temperatures reduced (at 29°C) or abolished (at 24°C) the vasodilator response to isoproterenol, whereas warm (39°C) skin temperatures enhanced the vasodilator response. However, whether these effects are via changes in β-receptor number or receptor sensitivity remains an open question.

Crandall et al. (9) reported the existence of functional β-adrenergic receptors in human cutaneous circulation. Data consistent with a role for β-adrenergic receptors in cutaneous vascular control comes from studies by Stephens et al. (34), who found that β-adrenergic receptor blockade enhanced the vasoconstrictor responses to whole-body cooling (a reflex vasocostriction) and to directly applied norepinephrine. Accepting the fact that local skin cooling involves vasoconstrictor effects of norepinephrine release (17, 22, 31, 46), the above observations fit with a working model in which locally released norepinephrine also affects β-adrenergic receptors. Hence, the possibility is raised that β-adrenergic function contributes to the vasoconstrictor stimuli stimulated via direct tissue cooling.

We have unpublished observations that the concentration of the β-receptor agonist isoproterenol required to elicit a given degree of vasodilatation was greater at cooler local skin temperatures relative to that required at warmer skin temperatures. Because cutaneous vasoconstriction in response to local cooling appears to be dependent in part on changes in α2C- adrenergic receptor expression via a Rho kinase system (2, 7, 20, 38), we thought it important to resolve the question of whether β-receptor sensitivity was similarly affected by changes in local skin temperature. We found this to be the case, with cooling reducing and warming enhancing the vascular responses to the β-agonist isoproterenol. These data suggest marked changes in the sensitivity of β-receptor function in response to changes in local skin temperature. Herman et al. (12) reported that in frogs, blood pressure responses to isoproterenol infusions were greater at warm ambient temperature compared with cold temperature, indicating β-receptor function was diminished under cool conditions (12), similar to the vascular responses we found in human cutaneous circulation.

Temperature effects on β-receptor responsiveness has been reported in other tissues. Examining isolated canine saphenous veins, Vanhoutte and Shepherd (40) reported that at 29°C, isoproterenol had no vascular effect; by contrast, at 37°C isoproterenol elicited a marked venodilation. In conjunction with electrical stimulation, the authors concluded that under conditions of lower temperatures, higher doses of isoproterenol were required for maximal β-adrenergic stimulation and that increasing the concentration of isoproterenol may result in a partial α-receptor stimulation. Additionally, Venugopal et al. (43) reported that cooling reduced β-receptor relaxation to isoproterenol in guinea pig pulmonary tissues. These data and conclusions are congruent with our current human skin data that β-receptor actions are temperature sensitive. It is worth noting, however, that in earlier in vivo experiments using canine cutaneous veins (45), no significant effect of perfusate temperature on the dilator response to isoproterenol infusions was demonstrated.

At the highest concentration of isoproterenol, we observed a vasoconstriction at all skin temperatures. Vanhoutte and Shepherd (40) previously demonstrated α-adrenergic actions in response to high concentrations of isoproterenol. Indeed, they observed that these α-adrenergic actions were augmented when preparations were cool (27°C). As previously stated, recent work demonstrated that cooling causes an increase in α-receptor expression (2), which might explain why cooling augmented the isoproterenol-induced α-adrenergic stimulation. In the present study, the vasoconstrictor effects of isoproterenol were most apparent at the higher skin temperature (Fig. 3); this may be a product of the degree of constriction already developed by the vascular smooth muscle (i.e., the size of the constrictions caused by 300 μM isoproterenol appear greater due to the increased prior levels of smooth muscle relaxation). Further work examining the effects of local temperature on isoproterenol-mediated vasoconstriction might consider increasing the level of smooth muscle relaxation prior to administration to augment the effects.

Any role of modified β-receptor function in the cutaneous vasoconstrictor response to local skin cooling is dependent on both the temperature-induced changes in receptor sensitivity to norepinephrine and to the rate of norepinephrine release at the vasoconstrictor nerve terminals (4, 11, 33, 39). At neutral tissue temperatures in the region of 34°C, the level of cutaneous vasoconstrictor nerve activity is low, approaching zero as indicated by the lack of effect of blockade of transmitter release from adrenergic nerves (14). Any perturbation that increased norepinephrine release would support cutaneous vasoconstriction and a simultaneous reduction in β-receptor function would enhance vasoconstriction. This will take on greater importance in situations in which the extant level of vasocon-
strictor activity and norepinephrine release at neutral temperatures are affected by age (19) or disease (5, 18).

The current study does not allow us to completely define the mechanism(s) for the temperature-induced alteration of the cutaneous vasodilator response to isoproterenol. Whether the observed differences in vasodilation to isoproterenol at differing local skin temperatures are due to changes in β-receptor number, sensitivity, or the affinity of isoproterenol to β-receptors has yet to be determined. Additionally, the vasoconstriction elicited by high concentrations of isoproterenol may not be due only to isoproterenol binding to α-receptors, because stimulation of β2-receptors has been reported to potentiate the release of catecholamines, which may be acting on α-receptors to produce vasoconstriction (33, 41, 44). Additionally, differences in baseline CVC can affect the degree of response to a vasoactive substance (14). Indeed, normalization of the data to baseline tends to bring the data to similar response patterns, with the important exception that, at the coolest local temperature, there was a complete abolition of the vasodilator response to isoproterenol. That cannot be explained on the basis of the baseline level of CVC against which the β-adrenergic agonist was delivered. Nevertheless, differences in baseline may be one of the contributors to the differences in response among temperatures. Furthermore, it has been shown that epinephrine elicits a different response than isoproterenol in human cardiac tissue (28); consequently, it is important to note that endogenous epinephrine and norepinephrine could produce different responses compared with those observed with (synthetic) isoproterenol.

In summary, we observed that cooling of the skin either markedly reduced (at 29°C) or abolished (at 24°C) the vasodilator response, whereas warming the skin (to 39°C) enhanced the vasodilator response to the β-adrenergic agonist isoproterenol. These data strongly suggest that β-adrenergic function is altered by local skin temperature, which raises the distinct possibility that β-adrenergic receptors may contribute to the vascular responses to changes in local skin temperature.

ACKNOWLEDGMENTS

We thank the study participants for their time and effort.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants R01 HL-059166 to J.M. Johnson and R01 HL-065599 to D.L. Kellogg.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


