HUMAN SKIN CONTAINS TWO POPULATIONS of nerves capable of mediating marked cutaneous vasodilation: sympathetic vasodilator nerves activated by increases in internal temperature and primary afferents sensitive to local heat (1, 6, 10). Sympathetic vasodilator nerves are responsible for 80–90% of the large global increases in skin blood flow seen during hyperthermia in humans (10). The mechanism for this active cutaneous vasodilation is not completely understood, but it appears to involve cholinergic nerve cotransmission (11). Primary sensory afferents cause substantial localized vasodilation when stimulated due to “efferent” release of neuropeptides, predominantly calcitonin gene-related peptide (CGRP) and substance P (6). These afferents are also sensitive to chemical stimulation by capsaicin (1, 6). Our aim in the present study was to investigate whether these two vasodilator nerve types can interact during whole body hyperthermia to alter the reflex cutaneous vasodilator response.

Changes in local control of skin blood flow can modify the reflex vasodilator response at that site via an effect on its sensitivity (17, 18), whereas modifications in central control of skin blood flow generally alter the internal temperature threshold for vasodilation (2, 3, 17). We hypothesized that capsaicin-sensitive afferents locally increase the reflex sensitivity of the cutaneous vasodilator response to hyperthermia without affecting the internal temperature threshold. To address this hypothesis, we used a twofold approach. First, we stimulated activity of heat- and capsaicin-sensitive afferents in a small area of forearm skin using acute topical application of capsaicin cream (6). Second, we inactivated these afferents with chronic topical capsaicin (16). After each intervention, whole body heat stress was performed to activate reflex cutaneous vasodilation. This was followed by local warming of the skin for assessment of the maximal cutaneous vasodilator response. By using this approach, three related questions were addressed. 1) Is the internal temperature threshold for cutaneous vasodilation during whole body hyperthermia altered by acute or chronic local capsaicin? 2) Is the reflex sensitivity of the vasodilator response altered? 3) Is maximal cutaneous vascular conductance during local warming altered?

METHODS

Subjects for this study were eight healthy young adults (age, 26.3 ± 1.3 yr; height, 165.5 ± 3.2 cm; weight, 59.6 ± 2.2 kg) who gave their informed consent before participation in this institutionally approved study. Subjects participated in one or both of two sets of experiments.

Part 1: Acute capsaicin. Seven subjects participated in these experiments (5 women, 2 men). Capsaicin cream (0.025%, Capzasin-P, Chattanooga, TN) was applied acutely...
to an area ~24 cm² on the ventral forearm. The cream was left on for 30 min and then removed, and the arm was thoroughly cleaned. A further 30 min were allowed between the cleaning of the arm and the beginning of the experiment to allow for the return to baseline of any effects due to the physical removal of the capsaicin and cleaning of the arm. This dose and time course were determined in preliminary experiments to cause sufficient stimulation of capsaicin-sensitive afferents to result in an observable local vasodilatation that was simultaneously not so large as to mask further cutaneous vascular responsiveness to whole body heat stress.

For the experiment, the subject lay supine in a water-perfused suit that covered the entire body except head, arms, and feet. Laser-Doppler flow (LDF) probes (Perimed, Stockholm, Sweden) were placed on the ventral aspect of each forearm for the measurement of skin blood flow (as LDF). LDF was measured at the capsaicin-treated site and at an untreated site on the contralateral forearm. Mean arterial pressure (MAP) was measured noninvasively by a Finapres finger blood pressure cuff (Ommeda, Englewood, CO). Internal temperature (measured as oral temperature \(T_{or}\)) was assessed by sublingual thermocouple. The thermocouple was placed in the sublingual sulcus, and the subject was instructed to keep the thermocouple in place throughout the experiment and not to talk or open his or her mouth during the experiment.

Baseline measurements were taken for 10–15 min. Heat stress was then performed by perfusing warm (47–50°C) water through the suit for 30–45 min until an increase in \(T_{or}\) of ~0.5°C was attained (2, 3, 8). After whole body heat stress, the sites around the LDF probes were locally warmed to 42°C for 25–30 min for assessment of maximum cutaneous vasodilation (9, 23). LDF, MAP, and \(T_{or}\) were each sampled at 50 Hz by a computer-based data acquisition system (Biopac, Santa Barbara, CA) and subsequently converted to 20-s averages for further analysis.

**Part 2: Chronic capsaicin.** Subjects \(n = 6\); 3 women, 3 men) applied 0.075% capsaicin cream (Capzasin-HP) to an area ~24 cm² on the ventral forearm twice per day for 7 days. For each application, subjects were instructed to remove the cream from the previous application, clean the arm, and apply new capsaicin cream at the same location (marked with a pen), leaving a thickness of ~2 mm on the forearm. The area was kept covered with a bandage to prevent accidental removal of the capsaicin cream. In this way, the area was continuously exposed to capsaicin over the entire 7-day period. Nolano et al. (16) previously used a similar protocol of capsaicin treatment with the same concentration of topical capsaicin cream and demonstrated that desensitization and epidermal fiber degeneration occurred within ~3 days. For the purposes of this study, desensitization was defined as a cessation of the sensory response to capsaicin application; subjects were instructed to inform the investigators when epidermal fiber degeneration occurred within ~3 days. For the purposes of this study, desensitization was defined as a cessation of the sensory response to capsaicin application; subjects were instructed to inform the investigators when this subjective desensitization occurred. No other sensory testing was performed.

Experiments were performed on the afternoon of the seventh day of capsaicin administration. The day of the experiment, the capsaicin was removed and the arm thoroughly cleaned 30 min before the beginning of the experiment, as in part 1. Whole body heat stress was then performed, followed by local warming of the skin as in part 1.

**Data analysis.** All data are presented as means ± SE. Cutaneous vascular conductance (CVC) was calculated as the ratio of LDF to MAP. Maximum CVC was assessed as the average CVC over the last 3 min of local warming to 42°C. To assess whether acute or chronic capsaicin treatment altered the maximal response to local heating the absolute values of CVC [in arbitrary units (AU)/mmHg] were compared between control and capsaicin-treated sites by paired t-test. For all further analyses, CVC was expressed as a percentage of this maximum value (%max) (2, 3, 17, 18). It is recognized that the use of single-site laser-Doppler measurements can be limiting due to the heterogeneity of the forearm skin vasculature. However, the similarity among sites in maximal CVC argues against the introduction of bias or misinterpretation of overall results due to the use of this normalization procedure. CVC responses were always compared between control and treated sites within the same subject and experiment to control for interindividual variability such as that due to gender or hormone status (2, 3, 21).

The sensitivity of the cutaneous vasodilator response to heat stress was assessed using regression analysis of the CVC-sublingual temperature relationship during the rising phase of the vasodilator response. The slope of the regression line was taken as the sensitivity of the response (2, 3, 22). If a plateau in the vasodilator response occurred toward the end of heat stress, this section was excluded from the calculation of sensitivity. The \(T_{max}\) threshold for the onset of cutaneous vasodilatation was assessed by solving the above regression equation for \(T_{max}\) using the average baseline value of CVC (22). In the case of acute capsaicin treatment, some subjects presented baseline CVCs that drifted slightly downward. In these individuals, baseline CVC was calculated as the average of the 3 min where CVC was at its lowest. Peak CVC during heat stress was identified as the highest CVC value attained during the heat-stress protocol.

Baseline CVC, sensitivities, and thresholds were compared between control and acute capsaicin sites (for part 1) and between control and chronic capsaicin sites (for part 2) by paired t-test. For part 1, the influences of heat stress and acute capsaicin treatment on CVC were assessed by two-way ANOVA with repeated measures, as were the influences of heat stress and chronic capsaicin treatment on CVC in part 2. Statistical significance was accepted for \(P < 0.05\).

**RESULTS**

**Part 1: Acute capsaicin.** All subjects reported local sensations of mild burning, itching, and/or warmth with topical capsaicin application. The sensations were not, however, considered painful by any subject. It should be noted that 10 subjects originally volunteered for participation in this protocol; of these, 3 were light smokers. It appeared that these individuals were much more susceptible to the effects of capsaicin than the others: the initial vasodilator response to capsaicin application was so large in these subjects that no further response was observable (baseline blood flow between 80 and 90% of maximum). This higher sensitivity to the vasodilator effects of capsaicin may have been due to the fact that they were habitual smokers. Although experiments were completed in these subjects, the data are difficult to interpret because any reflex vasodilatation was masked by the large initial response to the capsaicin. Because assessment of the reflex portion of the vasodilatation was therefore not possible for these subjects, all analyses were limited to the data from the remaining seven subjects.

Maximum CVC in response to local warming was not affected by acute capsaicin (control: 2.45 ± 0.15 vs. acute capsaicin: 2.51 ± 0.20 AU/mmHg; \(P > 0.10\)). CVC was therefore expressed as a percentage of max-
imum for further analysis (2, 3, 18, 19). Baseline CVC was significantly higher at acute capsaicin sites (25.34 ± 6.25%max) compared with control sites (10.57 ± 2.42%max; P < 0.05). Whole body heat stress resulted in an increase in T or of 0.50 ± 0.03°C, which was sufficient to cause marked cutaneous vasodilation and sweating in all subjects.

Figure 1 shows the relationship between CVC and T or during heat stress in a representative subject from part 1. Acute capsaicin administration resulted in a localized vasodilation that tended to persist throughout heat stress. Importantly, however, the rise in CVC during heat stress began at the same T or at both sites and the slope of this rise (sensitivity of the response) was also similar between sites. This was true for all subjects. The T or threshold for vasodilation was not different between control and acute capsaicin sites (control: 37.27 ± 0.04 vs. acute capsaicin: 37.46 ± 0.13°C; P > 0.10). The sensitivity of the reflex vasodilator response was also unaffected by acute capsaicin (control: 112.74 ± 36.83 vs. acute capsaicin: 91.35 ± 23.62%max°C; P > 0.10).

Average CVC responses to whole body heat stress at control and acute capsaicin sites are shown in Fig. 2. CVC tended to be higher at acute capsaicin sites throughout heat stress, but this effect was not statistically significant (P = 0.12, ANOVA). There was, however, a statistically significant interaction term (capsaicin × heat stress; P < 0.05). This was likely due to the elevated baseline at capsaicin-treated sites such that further dilation, whereas above that at control sites, represented less of an increase from the elevated baseline at the capsaicin-treated sites. Peak CVC was 37.72 ± 5.41%max at control sites and 44.32 ± 6.42%max at acute capsaicin sites.

**Part 2: Chronic capsaicin.** All subjects completed the 7 days of topical capsaicin administration as instructed. Subjects reported desensitization of the capsaicin-treated site between days 3 and 5 of capsaicin administration.

Maximum CVC was not affected by chronic capsaicin treatment (control: 3.10 ± 0.17 vs. chronic capsaicin: 2.54 ± 0.40 AU/mmHg; P > 0.10). As in part 1, further analysis was conducted using CVC values expressed as a percentage of maximum. Unlike sites treated acutely with capsaicin, baseline blood flow at chronic capsaicin-treated sites was not different from that at control sites (control: 5.49 ± 0.53 vs. chronic capsaicin: 6.22 ± 1.57%max; P > 0.10).

T or increased 0.59 ± 0.06°C during whole body heat stress, which caused marked vasodilation and sweating in all subjects as in part 1. Figure 3 shows the relationship between CVC and T or during heat stress in a representative subject, and Fig. 4 shows average reflex vasodilator responses to heat stress at control and chronic capsaicin sites. As can be seen in these figures, there was no influence of chronic capsaicin administration on the reflex cutaneous vasodilator response. T or threshold for vasodilation was not different between sites (control: 37.09 ± 0.13 vs. chronic capsaicin: 37.04 ± 0.14°C; P > 0.10). The sensitivity of the response was also unaffected by chronic capsaicin (control: 142.45 ± 62.57 vs. chronic capsaicin: 132.12 ± 52.60%max°C; P > 0.10).

**DISCUSSION**

The major new finding of the present study is that heat- and capsaicin-sensitive afferents in human skin do not modulate the reflex cutaneous vasodilator response to hyperthermia. This conclusion is based on
the observations that neither acute capsaicin (stimulation of these afferents) nor chronic capsaicin (inactivation of these afferents) influenced the internal temperature threshold or the reflex sensitivity of the cutaneous vasodilator response to whole body hyperthermia.

Potential limitations. It could be argued that a modulatory effect of capsaicin-sensitive afferents on cutaneous vascular control during hyperthermia was masked in the present study by a nonspecific direct effect of capsaicin on the blood vessels themselves, altering their ability to dilate. The vasodilator responses to local warming argue against this possibility: maximal cutaneous vasodilation was not altered by either acute or chronic capsaicin. This indicates that the blood vessels’ ability to dilate was not compromised by either mode of capsaicin treatment.

Another potential limitation of the present study is the possibility that the chronic capsaicin treatment did not have the desired inhibitory effect on the targeted afferents. On the basis of several observations, we are confident that the chronic capsaicin intervention was effective in locally desensitizing and inactivating capsaicin-sensitive afferents. First, all of our subjects confirmed subjective desensitization after 3–5 days of capsaicin treatment. Second, baseline CVC values were similar between control and treated sites after chronic capsaicin administration; that is, capsaicin no longer caused a localized vasodilation at the desensitized site as it did when administered acutely. Finally, a recent report from Nolano et al. (16) provides histological evidence that chronic topical application of 0.075% capsaicin causes inactivation of capsaicin-sensitive fibers via reversible epidermal nerve fiber degeneration within as few as 3 days.

A role for capsaicin-sensitive afferents in thermoregulatory control during heat stress was demonstrated in rats (4, 7). In this model, systemic capsaicin administration caused cutaneous vasodilation and a decrease in rectal temperature, which was absent after primary afferent desensitization with neonatal capsaicin (4). Furthermore, systemic capsaicin desensitization impairs rats’ ability to thermoregulate in the heat (7). Whether a direct peripheral role exists for capsaicin in this phenomenon is unclear because systemic desensitization could have both central and peripheral effects.

Kurozawa et al. (13) reported that cutaneous vasodilation during cycle exercise in humans was diminished 24 h after a single iontophoretic application of capsaicin to forearm skin. In addition to the different experimental protocols (exercise vs. passive heat stress), a fundamental difference between the two studies is that Kurozawa et al. used a single iontophoretic application of capsaicin 24 h before experiments were performed, presumably to desensitize capsaicin-sensitive afferents. However, it is not clear from the literature in what manner and to what extent this single capsaicin application affects a treated area 24 h later. Although the authors state that depletion of substance P and CGRP from perivascular sensory afferents would be a reasonable explanation for their findings, data were not presented to rule out other nonspecific effects of their mode of capsaicin administration.

Interactions between local and reflex control of skin blood flow were investigated by Pérgola et al. (17), who reported that local cooling of the skin during exercise inhibited reflex cutaneous vasodilation by reducing the sensitivity of the response. Thus skin blood flow at a given internal temperature during exercise was much lower at the cooled site compared with an adjacent site maintained at a neutral temperature. This influence was dependent on local stimulation of neurotransmitter release from adrenergic nerves (17).

Although the present studies involved chemical and not thermal stimulation, our results indicate that, in contrast to the local neural response to cold, local nerves involved in the response to heat do not alter reflex cutaneous vasodilation. In previous studies, whole body heat stress after local warming to 42°C did not cause further vasodilation (8, 22). However, lack of further vasodilation was likely due to minimal remaining vascular tone such that further vasodilation was not possible (8, 22). It is important to note that, in the present study, we were careful to stimulate heat-sensitive afferents in such a way that only a moderate vasodilator response was elicited. If the vasodilation to acute capsaicin stimulation had been similar to that seen in the above studies with local warming (8, 22), it would have been impossible to measure further vasodilator responses to whole body heat stress.

As mentioned above, the local vascular effects of capsaicin in the present study did not include an influence on the vasodilator response to prolonged local warming (that is, maximal CVC). In contrast, shorter (5-min) periods of local warming resulted in vasodilation that was augmented by acute capsaicin (Stephens DP, Charkoudian N, Mueller J, Johnson JM, and Saumet JL, unpublished observations). However, recent evidence indicates that immediate responses to local warming occur via mechanisms that are distinct from those that take place when the warming is prolonged (15).

That acute capsaicin did not augment maximal CVC provides further support for the idea that the vasodilation to prolonged local warming to 42°C results in maximal relaxation of cutaneous vascular smooth mus-
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cle in humans (9, 22). The lack of influence of chronic capsaicin on the maximal vasodilator response indicates that the mechanism for this vasodilation is independent of local activity of capsaicin-sensitive sensory nerves. This local vasodilation was recently shown to be dependent on nitric oxide (12) and independent of sympathetic neural elements (17). As such, our finding is also consistent with a report from Roberts et al. (20), who found that neither acute nor chronic topical capsaicin altered the nitric oxide-dependent local vasodilator response to iontophoresis of acetylcholine in human forearm skin.

We noted in part 1 of the present study that 3 of the original 10 subjects had substantially augmented vasodilator responses to acute topical application of capsaicin. These subjects were all light smokers, suggesting a possible enhancement of capsaicin’s vasodilator influences in this population. The influences of chronic cigarette smoking on peripheral vascular responsiveness to vasodilator stimuli are not clear, as enhancement (19), inhibition (5), and no effect (14) have been reported. Furthermore, to our knowledge, a potential interaction between the vasodilator effects of capsaicin and nicotine metabolites or other components of tobacco smoke in the human skin circulation has not been specifically investigated. This interesting possibility deserves further study.

In summary, we report here that neither acute stimulation nor chronic desensitization of capsaicin-sensitive afferents in the skin affected the functional parameters of the reflex vasodilator response to hyperthermia (threshold and sensitivity). We conclude that these afferents do not have a major role in modifying the reflex cutaneous vasodilator response to hyperthermia in humans.

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