Age-Specific Skin Blood Flow Responses to Acute Capsaicin

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Local skin blood flow (SkBF) responses can be modified by vasoactive neurotransmitters released from sensory nerves, notably, capsaicin-sensitive primary afferents (CSPAs). Age-specific CSPA function was examined in this study by assessing the SkBF response to acute capsaicin (CPZ). Eight young (18–30 years), 8 middle-aged (40–55 years), and 9 older (65–80 years) healthy men participated in the study. Treatment patches of varying CPZ concentration (ethanol and 0.001%–10.0% CPZ) were applied to sites on the ventral forearm. SkBF was measured with a laser Doppler imager. There were significant ($p < .001$) age-specific differences in the SkBF response to CPZ. In the older group, SkBF was reduced at least 2-fold at all concentrations of CPZ compared with the younger group. The middle-aged group showed an intermediate response. SkBF was significantly elevated above that obtained with a vehicle solution when sites were treated with CPZ concentrations $\geq 0.1\%$ in middle-aged and younger skin, whereas a significant elevation in SkBF was only achieved with 10% CPZ in older skin. The elevated SkBF in the young and middle-aged groups at higher concentrations of CPZ was the result of both an increased area of vasodilation and an increased magnitude of vasodilation within the vasodilated areas. In the older group, the increase in cutaneous vascular conductance at the highest concentration of CPZ was entirely the result of an increased area of vasodilation. These results suggest impaired CSPA function in aged skin and age-specific differences in the ability of sensory nerves to modify local inflammatory SkBF responses.

While the primary function of sensory nerves in the skin is to transmit pain signals to the central nervous system and activate protective reflexes, during acute tissue trauma they also preserve homeostasis at the site of injury (1–4). Sensory nerves can modify the local cutaneous microcirculation and initiate neurogenic inflammation through the release of vasoactive neuropeptides (1). This local release of transmitter substances enables sensory nerves to exert a local control over the microvasculature by modifying cutaneous blood flow and vascular permeability. Consequently, sensory nerves are important for the promotion of wound healing and maintenance of tissue integrity in the skin.

Accordingly, the increased incidence and resistance to healing of leg ulcers in elderly persons may be partially explained by local impairment of sensory nerve function in aged skin (3,5). It has been shown that neurogenic vasodilation is markedly reduced in patients with varicose ulcers compared with controls, while other assessments of endothelial and vascular responsiveness remained unchanged (5). Furthermore, older individuals with varicose ulcers respond to chemical stimulation with a reduced neurogenic inflammatory response in their lower limbs, which may be associated with a progressively attenuated axon reflex vasodilator response with age (5). Aging studies in rats have suggested that changes occur at both pre- and post-terminal sites of the peripheral neurovascular apparatus in aged skin (3,4). Because decreases in neurogenic vasodilation associated with aging may have important health implications, gaining a better understanding of the underlying changes in sensory nerve function that affect neurogenic vasodilation in aged skin seems to be warranted.

Capsaicin, the pungent ingredient in a wide variety of hot peppers, is a useful pharmacological tool for investigating sensory nerve function. Application of capsaicin to the skin stimulates a population of capsaicin-sensitive primary afferents (CSPAs), which is characterized by a sensation of warmth, burning pain, cutaneous hypersensitivity, as well as a local vasodilation and flare (2,6–9). Capsaicin-sensitive primary afferents are generally classified as thin, primary afferent neurons, including most C-fiber polymodal nociceptors, some warm-sensitive thermoreceptors, and most type II Aδ-polymodal nociceptors (10,11). The resulting neurogenic vasodilation elicited by CSPA activation can be used as an index of sensory nerve function in the skin.

The purpose of the present study was to evaluate age-specific skin blood flow (SkBF) responses to acute capsaicin in order to assess sensory nerve function in aged skin. It was hypothesized that the SkBF responses to acute capsaicin would be progressively reduced with advancing age, i.e., there would be an impaired CSPA function in aged skin.

METHODS

Subjects
Eight young (18–30 years), 8 middle-aged (40–55 years), and 9 older (65–80 years) healthy men participated in the
study. Health status was determined by a physician-supervised screening at The Pennsylvania State University General Clinical Research Center (GCRC). Subjects were noninstitutionalized and normally active. Exclusionary criteria included: obesity (body mass index $\geq 30$ kg/m$^2$), hypertension (systolic $> 140$ mmHg and/or diastolic $> 90$ mmHg), underweight (body mass index $< 20$ kg/m$^2$), smoking, any medication with the potential to alter cardiovascular or thermoregulatory control or response, allergies to hot peppers, and various dermatological conditions or diseases. Table 1 describes the physical characteristics of the subjects.

Table 1. Physical Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Middle</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Age</td>
<td>24.3 4.2</td>
<td>44.8 6.0*</td>
<td>70.0 3.8*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.3 6.3</td>
<td>176.4 2.9</td>
<td>173.3 4.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.6 12.3</td>
<td>73.9 8.5</td>
<td>82.8 11.1</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg/min)</td>
<td>53.5 13.1</td>
<td>51.9 7.9</td>
<td>26.9 5.6*</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>14.2 7.6</td>
<td>17.7 4.6</td>
<td>26.5 4.8*</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>24.3 3.1</td>
<td>23.7 2.7</td>
<td>27.5 3.2*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
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<td>132.0 13.1</td>
<td>139.1 5.7</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76.5 8.3</td>
<td>81.0 8.9</td>
<td>84.8 2.8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>93.8 6.8</td>
<td>98.0 9.4</td>
<td>102.9 1.9*</td>
</tr>
</tbody>
</table>

Notes: Data are mean $\pm$ SD.

* = Significantly different ($p < 0.05$) from Young.

† = Significantly different ($p < 0.05$) from Middle.

VO$_{2\text{max}}$ = maximal oxygen consumption; MAP = mean arterial pressure.

Preparations

Prior to any applications, an LDI scan was taken within 1 site to serve as a baseline measurement. After the initial scan, a series of acute capsaicin applications proceeded. Each application consisted of saturating the pad of a small bandage (14.5 cm$^2$/2.25 in$^2$) with a 300-ml capsaicin solution (Sigma Chemical Co., St. Louis, MO) and applying it to the skin at randomized sites. Each solution, except for the vehicle, contained a different concentration of capsaicin and inactive alcohol (95% ethanol [EtOH]). Seven different concentrations of capsaicin (0.001%–10%) were used, along with EtOH alone, which served as a vehicle. Thus, a total of 8 application pads were used. Each pad was prepared with solution immediately before application and added successively at 4-minute intervals. The application patches were removed in order after each patch had been applied for 32 minutes. Immediately after removal of each patch, an LDI scan was taken of the respective application site.

Several precautions were made to ensure that acute capsaicin applications were standardized. These included randomizing all application sites and using the same batches of capsaicin solutions for multiple subjects. In addition, capsaicin solutions underwent a uniform mixing procedure and were dispensed onto application patches in precise amounts using a micropipette. Other methods of topical capsaicin application such as painting liquid solutions or applying a cream onto the skin would have introduced mechanical interference with the SkBF response.
low-power laser beam scanned the surface of the skin in a raster pattern. A Doppler shift caused by moving blood in the microvasculature was processed to create a color-coded image of skin perfusion. At the size and distance chosen, a 1600 pixel (40 × 40 pixel) perfusion map was produced for each scan. Each pixel in the map had a unique flux value, proportional to SkBF. For each scan, the principal measurement of interest was the mean flux value. A mean flux value for an individual scan was found by averaging the mean flux values of 5 sampled regions of interest (ROIs) within the scan area. The LDI software calculated mean flux values for each ROI.

All LDI measurements were indexed to percentage of maximal cutaneous vascular conductance (%CVCmax) by dividing mean cutaneous vascular conductance (CVC) by CVCmax. Mean CVC was determined as the ratio of LDI flux to mean arterial pressure (MAP) at the time of the scan (CVC = LDI Flux/MAP). Maximal cutaneous vascular conductance was found by locally heating an area of the opposite arm with warm air to 42°C at a rate of 1°C/5 min and maintaining the local temperature of that site at 42°C for 30 minutes (12,13). Laser Doppler imaging scans were taken at 5-minute intervals, and the scan with the highest mean CVC value was used to determine CVCmax.

The percentage of the area that was vasodilated (%AVD) was also determined for scans taken at 0.001%, 0.01%, 0.1%, 1.0%, and 10.0% capsaicin sites. A threshold value that represented vasodilation (defined in this study as CVC > 25%CVCmax) was subtracted from each scan, and the LDI software calculated the percentage of valid pixels (those still remaining) to yield %AVD. Finally, %CVCmax of just those areas that were identified as being vasodilated also were determined.

**Statistical Analysis**

Comparisons of physical characteristics among the 3 age groups were done using a one-way analysis of variance (ANOVA) (Excel). When significant main effects of age were found, t tests were performed using Bonferroni corrections to determine between which groups the differences existed. Acute capsaicin measurements were compared using an ANOVA for detection of concentration differences, and a two-way ANOVA to probe for age differences among the results (SAS, SAS Institute Inc., Cary, NC). When appropriate, a post hoc analysis for multiple comparisons was performed. The significance level for all statistical tests was set at α = 0.05. Data are presented as mean ± SE.

**RESULTS**

There was a significant effect of age on %CVCmax elicited by acute capsaicin applications as revealed by two-way ANOVA (Figure 1). Dose-response characteristics were observed for the young and middle-aged groups, whereas in the older group, an appreciable increase in %CVCmax was only obtained at the highest concentration of capsaicin tested (10.0%). The SkBF response was significantly greater in the young group compared with the older group at and above a capsaicin concentration of 0.03%. An intermediate response was observed for the middle-aged group at all concentrations of capsaicin tested. However, the only significant difference in %CVCmax between the middle-aged group and the other two groups was at the 1.0% capsaicin sites, where the middle-aged %CVCmax was significantly greater than that of the older group. Percentage of maximal cutaneous vascular conductance was significantly elevated above that obtained with a vehicle solution at capsaicin concentrations ≥0.1% in middle-aged and younger skin.
respectively. In older skin, %CVC\textsubscript{max} was only significantly higher than that caused by the vehicle solution at the site with the highest concentration of capsaicin used (10.0%).

The reduced SkBF response to acute capsaicin application in the older group was the result of both a reduced %AVD and a diminished %CVC\textsubscript{max} of the area that was vasodilated (Figure 2). The increase in %CVC\textsubscript{max} in the older group at the higher capsaicin concentrations was entirely the result of an increased area of vasodilation, since cutaneous vascular conductance in the vasodilated area showed little or no increase. This was contrary to the young and middle-aged groups in which both the area and magnitude of vasodilation increased in response to higher concentrations of capsaicin.

Figure 2. Area of vasodilation caused by acute capsaicin applications. A. A demonstration of the percentage of area that was vasodilated (%AVD) at acute capsaicin application sites at various capsaicin concentrations. B. The lower panel shows the percentage of maximal cutaneous vascular conductance (%CVC\textsubscript{max}) in the areas identified in A. There were significant effects of age on both of these responses *Significantly different than Young (p < .05), †Significantly different than Middle (p < .05). (Note: This figure has fewer x-values than Figure 1, as the %AVD analysis was not performed for every concentration of capsaicin).
The SkBF response of the young group was at least 2-fold that of the older individuals at all concentrations of capsaicin. This difference was greatest at the 10% capsaicin sites, where the %CVC\text{max} of the young group was significantly higher (79.7 vs 32.4 %CVC\text{max}, p < .05) than that of the older group (Figure 3). Similarly, the significant age effect on %AVD and %CVC\text{max} within the vasodilated area was greatest at the 10% capsaicin sites. The substantially smaller increases in %AVD (40% vs 98%, p < .05) and magnitude of vasodilation (44.7 vs 81.1 %CVC\text{max}, p < .05) of the older individuals contributed comparably to the overall decline in SkBF in response to a 10% capsaicin solution. For the middle-aged group, values for both %AVD and %CVC\text{max} within that area were between those for the young and older groups, with significant differences only occurring with the highest concentrations of capsaicin. There were relatively large intra- and interindividual variations in SkBF due to acute capsaicin applications, consistent with what has been reported in the literature (6,7,14,15).

**DISCUSSION**

An assessment of sensory nerve function in the skin of 3 age-groups of healthy men was accomplished using acute capsaicin applications to stimulate CSPAs and evoke a SkBF response. Seven concentrations of capsaicin were applied that caused slight to significant elevations in local SkBF as measured by LDI. Age had a significant effect on the local vasodilation, with the younger individuals having a %CVC\text{max} at least 2 times greater that seen in the older group at all concentrations of capsaicin used. Previous observations of a decreased flare size in older skin following acute capsaicin application suggested that such reductions in SkBF likely occur (6). However, since blood flow measurements were not taken, it was not possible to confirm that the more pronounced erythema in the skin of younger individuals was necessarily increased SkBF. Results from the present study substantiate the assertion of attenuated SkBF in older skin following acute capsaicin application, and demonstrate that visual observations of reduced erythema are associated with decreased SkBF.

Examinations of %AVD and the magnitude of this vasodilation (Figure 2) reveal some possible explanations for the age differences that were observed. First, in Figure 2A, the higher %CVC\text{max} of the young group at the lowest concentrations of capsaicin is primarily the result of a greater %AVD. Specifically, there were more areas of skin that had CVC values above a defined threshold for vasodilation. Since the %CVC\text{max} of these vasodilated regions was nearly identical, it is likely that the larger %AVD in young skin was the result of an increased number of vasodilated vessels within the cutaneous vasculature. Another way of stating this is that %AVD reflects the number of activated neurovascular units. The anatomical properties of the neurovascular unit itself, including the size of the collateral nerve network and distribution of each innervated vessel in the skin, are implicated as contributing to this decline in vasodilated area with increasing age (6,15,16). Additionally, the relatively large intra- and interindividual variations in SkBF responses to acute capsaicin reported here and elsewhere are likely due to regional and individual differences in receptive field area and neurovascular unit arrangement (6,7,14,15).

There appears to be a threshold difference in the activation of these neurovascular units in the middle-aged and...
older individuals. Looking at Figure 2A, the approximate value of %AVD reached with a 0.001% capsaicin solution applied to younger skin is obtained with a 0.1% solution on middle-aged skin and a 10.0% solution on older skin. Furthermore, since the magnitude of SkBF within these vasodilated areas is very similar, the overall %CVC\textsubscript{max} is nearly the same with a 0.001% capsaicin solution in young, a 0.1% capsaicin solution in middle-aged, and a 10.0% capsaicin solution in older individuals, respectively (Figure 1). This observation suggests a higher activation threshold of neurovascular units by acute capsaicin with advancing age. Thus, a higher concentration of capsaicin was necessary to produce a given area of neurogenic vasodilation in the skin of both middle-aged and older individuals.

A variation with age in the number of neurons within the collateral nerve network or the distribution of each innervated vessel would help to provide an explanation. Anatomical studies show no appreciable decline with age in the number of sensory nerve fibers (17). In contrast, a definite loss and disorganization of vessels within the cutaneous microcirculation is obvious in older skin (17–19). Therefore, a reduction in number and/or structural impairments of cutaneous blood vessels in older individuals may contribute to the results obtained in this study. In this scheme, a greater magnitude of stimulation would be required to vasodilate a damaged or sparse vasculature, resulting in the observed threshold shift. Several investigators have attributed the lower SkBF in aged skin during local heating to structural limitations including decreased functional capillary plexus units, damaged or obliterated vessels, and/or attenuated precapillary recruitment (20–23).

Maximal cutaneous vascular conductance is thought to reflect structural limitations in the vasculature of the skin, so that when values are expressed as %CVC\textsubscript{max}, the index essentially controls for these differences among individuals (24). Therefore, the attenuations in %CVC\textsubscript{max} of the older individuals suggest that in addition to structural characteristics, functional decrements are responsible for the reductions in SkBF during acute capsaicin stimulation. It is known that capsaicin causes neurogenic vasodilation in the skin by activation of the vanilloid receptor subtype 1 (VR1) complex on CSPA nerve endings, leading to an exocytotic release of vasoactive neuropeptides (2,25). Most notable among these neurotransmitters are Substance P, which predominantly affects plasma extravasation of the cutaneous microvasculature, and calcitonin gene-related peptide (CGRP), the primary mediator of neurogenic vasodilation in the skin. Accordingly, another interpretation of the threshold shift in middle-aged and older skin is that a diminished release of these neuropeptides from CSPAs occurs at a given concentration of capsaicin, while the cutaneous vessels retain a similar capacity to respond to the vasoactive stimulus.

Aging studies in rats that used electrical stimulation to elicit neurogenic vasodilation have suggested a decrease in neurotransmitter content in the sensory nerves of older rats (3,4). When capsaicin is used to stimulate CSPAs, a receptor-mediated activation takes place; therefore, a loss of receptors in the neuronal membrane or alterations in cellular signaling mechanisms may also account for the diminished responsiveness that has been described. While this distinction is beyond the scope of this discussion, it is interesting to note that while reductions in SkBF are seen in older rats during high-frequency electrical stimulation, with low-frequency stimulation, such decrements do not occur (26). This may imply preservation of CSPA capability, but also suggest limitations in cellular signaling processes with advanced age.

Diminished CSPA activity in the skin has important implications for repair processes associated with wound healing and the maintenance of tissue integrity in elderly skin. While the primary job of CSPAs is to receive and transmit information from the internal and external environment to preserve an organism’s homeostasis, neurogenic inflammation prompted by CSPAs at the site of injury aids in maintaining local homeostasis (1–5). Given the results of the present study, it is not surprising that a resistance to healing of leg ulcers happens with greater incidence in the elderly, as this pathology is associated with a reduced neurogenic inflammatory response (5). For example, the neurogenic axon-reflex flare response to chemical stimulation is markedly reduced in patients with varicose ulcers when compared with controls (5). This implies that age-associated impairments in sensory nerve function likely compromise the promotion of wound healing in the skin of older individuals.

It should be noted that all subjects who participated in this study were men, and nearly all were Caucasian. Thus, caution should be taken when attempting to generalize the results from this study to women and different racial groups. Further study is needed to be able to confidently extend these findings to more diverse groups of individuals. Still, major deviations from the primary implications of this study should not be expected.

In summary, the results from this study demonstrate that cutaneous vasodilation caused by acute capsaicin applications was significantly attenuated in older skin compared with what occurred in the skin of younger individuals. Acute capsaicin stimulates a specific population of sensory nerves (CSPAs) in the skin that possess the capsaicin receptor, eliciting a local release of vasoactive neurotransmitters from their terminal nerve endings. Therefore, this assessment was used to evaluate CSPA function and provides evidence for impaired CSPA activity in aged skin. Results indicate that there was a higher activation threshold for neurovascular units within the skin of middle-aged and older individuals. Although it is speculative, reduced neuropeptide content and/or associated release mechanisms may be responsible for the decreased neurovascular reactivity observed in aged skin. Receptor availability and related signaling pathways also may contribute to the decrement in CSPA activity. These findings document functional changes in CSPAs and their ability to modify cutaneous vascular responses in older skin.

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