Transient receptor potential vanilloid type 1 channels contribute to reflex cutaneous vasodilation in humans

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Wong BJ, Fieger SM. Transient receptor potential vanilloid type 1 channels contribute to reflex cutaneous vasodilation in humans. J Appl Physiol 112: 2037–2042, 2012. First published April 19, 2012; doi:10.1152/japplphysiol.00209.2012.—Mechanisms underlying the cutaneous vasodilation in response to an increase in core temperature remain unresolved. The purpose of this study was to determine a potential contribution of transient receptor potential vanilloid type 1 (TRPV-1) channels to reflex cutaneous vasodilation. Twelve subjects were equipped with four microdialysis fibers on the ventral forearm, and each sample received 1) 90% propylene glycol +10% lactated Ringer (vehicle control); 2) 10 mM L-NMMA; 3) 20 mM capsazepine to inhibit TRPV-1 channels; 4) combined 10 mM L-NMMA + 20 mM capsazepine. Whole body heating was achieved via water-perfused suits sufficient to raise oral temperature at least 0.8°C above baseline. Maximal skin blood flow was achieved by local heating to 43°C and infusion of 28 mM nitroprusside. Systemic arterial pressure (SAP) was measured, and skin blood flow was monitored via laser-Doppler flowmetry (LDF). Cutaneous vascular conductance (CVC) was calculated as LDF/SAP and normalized to maximal vasodilation (%CVCmax). Capsazepine sites were significantly reduced compared with control (50 ± 4%CVCmax vs. 47 ± 5%CVCmax, respectively; P < 0.05). L-NMMA (33 ± 3%CVCmax) and L-NMMA + capsazepine (30 ± 4%CVCmax) sites were attenuated compared with control (P < 0.01) and capsazepine (P < 0.05); however, there was no difference between L-NMMA and combined L-NMMA + capsazepine. These data suggest TRPV-1 channels participate in reflex cutaneous vasodilation and TRPV-1 channels may account for a portion of the NO component. TRPV-1 channels may have a direct neuronal contribution or have an indirect effect via increased arterial blood temperature. Whether the TRPV-1 channels directly or indirectly contribute to reflex cutaneous vasodilation remains uncertain.

Microdialysis; heat stress; nitric oxide

Increases in skin blood flow and sweating represent humans’ primary physiological defense against an increase in core temperature. The initial increase in skin blood flow during hyperthermia is driven primarily by withdrawal of tonic sympathetic vasoconstrictor tone, which results in approximate doubling of resting thermoneutral skin blood flow (8, 23). Further increases in skin blood flow, concomitant with the onset of sweating, are mediated by reflex sympathetic cholinergic nerve activity (8). This reflex cutaneous active vasodilation accounts for 85–95% of the increase in skin blood flow during hyperthermia and can result in nearly 8 liters/min of cardiac output directed to the cutaneous vasculature (24).

The co-transmission theory of cutaneous active vasodilation suggests acetylcholine and one or more unknown vasodilators are co-released from sympathetic cholinergic nerves, where acetylcholine is primarily responsible for the sweat response and the unknown vasodilator(s) are responsible for the reflex cutaneous vasodilation (15). The precise vasodilator(s) released from sympathetic cholinergic nerves remains unresolved; however, several vasodilators have been implicated in reflex cutaneous vasodilation, including vasoactive intestinal polypeptide (VIP) (1, 18), vasodilator prostanooids (20), histamine receptors (33), and neurokinin-1 (NK1) receptors (31). In addition to the aforementioned vasodilators, nitric oxide (NO) has been shown to directly contribute ~30–45% to active vasodilation (14, 25, 28), and several sources of NO have been implicated (17, 26, 31, 33), including neuronal NO synthase (13, 16).

The transient receptor potential vanilloid (TRPV) family of ion channels are widely distributed through the body, including cutaneous sensory nerves and vascular endothelium and are activated by various stimuli, including heat (2, 27). The TRPV-1 isoform has been shown to be activated by heat and capsaicin (2, 19, 27). Although TRPV-1 channels are located predominantly on afferent sensory nerves and Charkoudian et al. (3) found no effect of acute and chronic capsaicin treatment on reflex cutaneous vasodilation, it is possible alternative sources/locations of TRPV-1 channels contribute to reflex cutaneous vasodilation. In this context, TRPV-1 channels have been shown to be located in endothelial cells. Data suggests activation of TRPV-1 channels can modulate vascular tone and contribute to endothelial-dependent vasodilation and temperature regulation (2, 34), and the TRPV family of ion channels, including TRPV-1, have recently been shown to be activated by NO and may enhance NO production (22, 34, 35). The purpose of the present study was to test the hypothesis that inhibition of TRPV-1 channels would attenuate reflex cutaneous vasodilation in humans and that TRPV-1 channel activation would account for a portion of the NO component of reflex cutaneous vasodilation.

Methods

Ethical approval. The Institutional Review Board at Kansas State University approved all protocols for this study. Verbal and written, informed consent were obtained from each subject before participation in the study, and all protocols conformed to the guidelines as set forth by the Declaration of Helsinki.

Subjects. Twelve subjects (eight men, four women; age 19–29 yr) participated in this study. All subjects were healthy, nonobese (BMI range 19–26 kg/m²) nonsmokers, had no history of cardiovascular or metabolic diseases, and were not taking any medications, except for two female subjects who were taking oral contraceptives. Female sex hormones are known to alter the skin blood flow response to whole body heating (4–6, 11); however, phase of menstrual cycle or oral contraceptive use was noted for female subjects but not controlled for in these experiments.
Instrumentation. Subjects were equipped with four microdialysis fibers on the ventral aspect of the left forearm. Microdialysis fibers were placed in the absence of anesthesia; however, ice was used to numb the skin before placement (9). Microdialysis fibers were placed by first placing a 23-gauge needle in the dermal layer of the skin. The fiber was then threaded through the lumen of the needle, the needle was removed from the skin, and the semi-permeable membrane of the fiber was left in the skin. The semi-permeable membranes of the fibers were 10 mm in length with a 55-kDa molecular mass cutoff (CMA 31 Linear Probe; CMA Microdialysis). Placement of microdialysis fibers results in a minor trauma hyperemia that was allowed to subside before commencement of the experimental protocol (~45–90 min). Based on our previous experience, resolution of the trauma response was determined when visible redness around the microdialysis insertion sites subsided and when laser-Doppler flux values were stable (visual inspection) within 10 flux units for at least 10 min. Each microdialysis fiber was perfused with lactated Ringer solution during the trauma resolution period.

Subjects’ blood pressure was measured on a beat-by-beat basis via photoplethysmography (NexfinHD; BMEYE, Amsterdam, The Netherlands) from a cuff placed on a finger on the right (nonexperimental) hand. Blood pressure was also measured via automated brachial auscultation every 10 min, and subjects’ heart rates were recorded from a three-lead electrocardiogram (S/5 Light Monitor; Datex-Ohmeda, GE Healthcare, Madison, WI).

Red blood cell flux measured via laser-Doppler flowmetry (Periflux 5010 laser-Doppler perfusion monitor, Perimed, Jarfalla, Sweden) was used to provide an index of skin blood flow and local heating units (PF5020 local heating units and Periflux 5020 Temperature Unit, Perimed) were placed on the skin directly over each microdialysis membrane. An integrated laser-Doppler probe (Probe 413, Perimed) was placed in the center of each local heating unit to measure red blood cell flux directly over each microdialysis site. The temperature of the local heaters at each of the microdialysis sites was clamped at 33°C during baseline and for the duration of the whole body heating period; the temperature was increased to 43°C at the end of the whole body heating period (see below).

Experimental protocol. Baseline skin blood flow, blood pressure, and oral temperature data were collected for 5–10 min following the trauma resolution period during which time thermoneutral water was pumped through the water-perfused suit. Drug infusion through each microdialysis fiber commenced following baseline measurements. Each site randomly received one of four treatments: 1) 90% propylene glycol (USP grade, Sigma, St. Louis, MO) in 10% lactated Ringer (Baxter Healthcare, Deerfield, IL) served as a vehicle control; 2) 10 mM L-NAME (Tocris Biosciences, Ellisville, MO) to inhibit NO synthase; 3) 20 mM capsazepine (Tocris Biosciences) to selectively inhibit TRPV-1 channels; and 4) combined 10 mM L-NAME + 20 mM capsazepine (final concentrations) to simultaneously inhibit NO synthase and TRPV-1 channels and determine a potential interaction between NO and TRPV-1 channels. Capsazepine is minimally soluble in aqueous solution and thus required use of a 90% propylene glycol in 10% lactated Ringer vehicle. Our laboratory has shown previously this vehicle of 90% propylene glycol in 10% lactated Ringer does not result in vasodilation (i.e., there is no baseline shift), and 20 mM capsazepine significantly attenuates the initial peak phase of cutaneous thermal hyperemia in response to local heating (29). We, and others, have previously shown that a 10 mM concentration of L-NAME adequately inhibits NO synthase in human skin (7, 10, 20, 28, 30–33). All drug treatments were dissolved in 90% propylene glycol and 10% lactated Ringer and were perfused at a constant rate of 2 μl/min with a microinfusion pump (Bee Hive controller and Baby Bee Syringe Pumps, Bioanalytical Systems, West Lafayette, IN).

All drugs were infused for at least 60 min before commencement of whole body heating (10, 28, 29). To initiate whole body heating, subjects were covered with a plastic water-impermeable rain suit to minimize evaporative heat loss, and 50°C water was circulated through the water-perfused suit. Whole body heating continued until subjects’ oral temperatures were raised at least 0.8°C above baseline (~35–50 min in duration), after which subjects’ temperatures were maintained at this level to acquire a stable 10-min plateau in skin blood flow. Once a plateau in skin blood flow was achieved, subjects were cooled by perfusing 33°C water through the suit and removing the plastic rain suit. Maximal cutaneous vasodilation was elicited via infusion of sodium nitroprusside (SNP) at a rate of 2 μl/min and increasing the temperature of the local heating units to 43°C. This temperature increase and dose of SNP have been previously determined effective in eliciting a maximal skin blood flow response (10, 12, 20, 21, 28, 29).

Data collection and analysis. Data were digitized and stored at 100 Hz on a personal computer and were analyzed offline using signal-processing software (Windaq, Dataq Instruments, Akron, OH). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by systemic arterial pressure and normalized to maximal vasodilation (%CVCmax) via SNP infusion and local heating to 43°C.

To calculate CVC, values for systemic arterial pressure from the beat-by-beat (Nexfin) system were used and validated against calculations [systemic arterial pressure (SAP) = 1/3 pulse pressure + diastolic blood pressure] from brachial auscultation measurements. The percent contribution of TRPV-1 channels and NO were calculated using the following equation:

\[
\left(\frac{\%CVC_{\text{max Control}} - \%CVC_{\text{max Treatment}}}{\%CVC_{\text{max Control}}} \right) \times 100
\]

where %CVCmax Treatment is the %CVCmax value at capsazepine, L-NAME, or combined capsazepine + L-NAME sites.

A stable 5-min period of skin blood flow was used for analysis of baseline, whole body heating plateau, and maximal skin blood flow. To determine the magnitude of increase in %CVCmax for a given increase in oral temperature (ΔTor), skin blood flow during the final minute of each 0.1°C increase in oral temperature from baseline (ΔTor, 0.0°C) to the end of heat stress (ΔTor, 0.8°C) was used for analysis.

Since there were only four female subjects who participated in this study, a full statistical analysis with sufficient power to analyze differences between male and female data could not be performed; thus data from all subjects was grouped for statistical analysis. A one-way, repeated-measures ANOVA was used to compare the effect of drug treatment between experimental sites; 2) the effect of drug treatment on the increase in %CVCmax during hyperthermia; 3) the percent contribution of TRPV-1 and NO; and 4) absolute maximal CVC values. A two-way ANOVA with repeated measures was used to compare the effect of drug treatment on the increase in %CVCmax for each 0.1°C increase in oral temperature (drug treatment × %CVCmax × ΔTor). For all ANOVAs, when a significant F value was observed, Tukey’s post hoc analysis was used to determine where significant differences occurred. All statistical analyses were performed using SigmaStat 3.5 (Systat Software, Point Richmond, CA) and SPSS 19 (IBM; Armonk, NY). P values of <0.05 were considered to be significant, and all data presented are means ± SE.

RESULTS

There was no effect of drug administration on baseline CVC between experimental sites. Baseline CVC averaged 12 ± 3%CVCmax at control sites, 11 ± 3%CVCmax at L-NAME sites, 13 ± 4%CVCmax at capsazepine sites, and 13 ±
3%CVCmax at l-NAME + capsazepine sites. Similarly, drug administration had no effect on maximal CVC (Table 1).

The effect of drug treatment on the CVC response to whole body heating is shown in Fig. 1. The data are presented for %CVCmax at each treatment site as a function of increasing oral temperature (%Tmax vs. ΔTtor). At the peak of heat stress (ΔTtor = 0.8°C), the magnitude of the increase in CVC at control sites averaged 67 ± 5%CVCmax. The increase in CVC was attenuated at capsazepine (50 ± 4%CVCmax; P < 0.05), l-NAME (33 ± 3%CVCmax; P < 0.01), and combined l-NAME + capsaizepine (30 ± 4%CVCmax; P < 0.01) compared with control. The increase in CVC at l-NAME and l-NAME + capsazepine sites was attenuated compared with capsazepine (P < 0.05). There was no significant difference in CVC between l-NAME and combined l-NAME + capsazepine sites.

Table 1. Absolute maximal CVC values

<table>
<thead>
<tr>
<th>Treatment Site</th>
<th>Maximal CVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.31 ± 0.33</td>
</tr>
<tr>
<td>l-NAME</td>
<td>2.18 ± 0.20</td>
</tr>
<tr>
<td>Capsazepine</td>
<td>2.40 ± 0.20</td>
</tr>
<tr>
<td>l-NAME + capsazepine</td>
<td>2.34 ± 0.27</td>
</tr>
</tbody>
</table>

Values are means ± SE. There was no statistical difference in maximal cutaneous vascular conductance (CVC; mV/mmHg) between treatment sites. l-NAME: NO synthase inhibition.

Figure 1 further depicts the increase in Ttor required to elicit a significant increase in CVC above baseline. At control sites (thick solid lines), CVC began to increase with a 0.2°C increase in oral temperature (P < 0.05 vs. baseline). The increase in CVC was delayed at capsazepine (thick dashed lines; O; ΔTtor = 0.3°C), l-NAME (thin solid lines; ■; ΔTtor = 0.4°C), and l-NAME + capsazepine (thin dashed lines; □; ΔTtor = 0.4°C). The magnitude of the increase in CVC at l-NAME and l-NAME + capsazepine sites was significantly attenuated compared with control and capsazepine only sites at all increments of ΔTtor ≥ 0.2°C and 0.3°C, respectively.

Figure 2 shows the percent contribution of TRPV-1 channels and NO to reflex cutaneous vasodilation. The contribution of TRPV-1 channels was significantly less than the contribution of NO or combined TRPV-1 channels + NO. Values are means ± SE. *Significant difference vs. %NO and %TRPV-1 + NO (P < 0.01).

DISCUSSION

In support of our hypothesis, the main finding from this study suggests activation of TRPV-1 channels accounts for a portion of reflex cutaneous vasodilation in humans. This was evidenced by an attenuated CVC response at microdialysis sites that received the TRPV-1-specific inhibitor capsazepine...
(Fig. 2). Inasmuch as CVCs at t-NAME and l-NAME + capsazepine were not significantly different from each other, our data further suggest TRPV-1 channel activation may account for a portion of the NO component of reflex cutaneous vasodilation.

The data from the present study suggest TRPV-1 channel activation accounts for $\sim 25\%$ of cutaneous active vasodilation, whereas NO and TRPV-1 channels combined account for $\sim 55\%$ of cutaneous active vasodilation. Taken together, these data suggest TRPV-1 channels directly contribute to cutaneous active vasodilation and may contribute indirectly via NO-dependent mechanisms. These data further suggest the TRPV-1 channels involved in cutaneous active vasodilation are most likely located on the vascular endothelium. Although TRPV-1 channels are most commonly located on afferent sensory nerve terminals, there is currently no experimental data to suggest cutaneous sensory afferent nerves are involved in cutaneous active vasodilation. There are at least two lines of evidence to support a role for endothelial TRPV-1 channels. First, TRPV-1 channels have been shown to be located in vascular endothelial cells and to be involved in regulation of vascular tone and endothelial-dependent vasodilation, and TRPV-1 channels have been shown to have an interaction with NO where TRPV-1 channels can both be activated by as well as enhance the production of NO (22, 34, 35). Second, Charkoudian et al. (3) found that neither acute nor chronic capsaicin, which preferentially activates TRPV-1 channels, had no effect on reflex cutaneous vasodilation. Together, these previous data and data from the present study suggest a role for endothelial TRPV-1 channels to reflex cutaneous vasodilation. It also remains undetermined whether TRPV-1 channels are activated by NO or whether TRPV-1 channel activation enhances NO production. Data from Kellogg et al. (13) suggests neuronal NO synthase is the NO synthase isoform involved in cutaneous active vasodilation and if TRPV-1 channels are of endothelial origin, it would appear TRPV-1 channels may be activated by NO rather than by TRPV-1 channels enhancing NO production.

The methods employed in this study do not allow us to delineate mechanisms by which TRPV-1 channels are activated during heat stress; however, there are at least two possible mechanisms by which TRPV-1 channels may be activated. First, as discussed above, our data suggest the vascular endothelium is the most probable location for the observed TRPV-1 component of cutaneous active vasodilation. If this is indeed the case, it is possible that, as cutaneous blood flow increases, there is an increase in shear stress that activates TRPV-1 channels. A limitation to this proposition is that assessing shear stress in the microvasculature, including the cutaneous circulation, and how shear stress is transmitted from the conduit arteries to microvessels is difficult. This potential mechanism is further limited since data (32, 37) has shown reactive hyperemia is not mediated by NO-dependent mechanisms. If TRPV-1 activation during whole body heating does indeed interact with NO, the data from Wong et al. and Zhao et al. (32, 37) suggest increased shear stress may not be the mechanism by which NO is produced in human skin; however, a shear stress mechanism for TRPV-1 channel activation cannot be ruled out.

Second, it is possible the increase in arterial blood temperature during heat stress activates the TRPV-1 channels. TRPV-1 channels have a reported activation temperature of $\sim 42^\circ\mathrm{C}$ (2, 27, 34), yet neither blood nor skin temperature would be expected to reach $42^\circ\mathrm{C}$ under mild heat stress such as in the present investigation or that experienced under daily conditions and activities. Recent data suggests there is an interaction between NK$_1$ receptors and TRPV-1 channels (36), where activation of NK$_1$ receptors lowers the activation temperature of TRPV-1 channels to $\sim 35$–$37^\circ\mathrm{C}$ and TRPV-1 channels are “sensitized” to heat stimuli (36). Inasmuch as NK$_1$ receptor activation has been shown to contribute to cutaneous active vasodilation (31), it is possible that there is an interaction between NK$_1$ receptor activation and TRPV-1 channel activation such that NK$_1$ receptor activation directly mediates a portion of cutaneous active vasodilation and also sensitizes the TRPV-1 channels, which then also contribute. If TRPV-1 channels are indeed activated by an increase in arterial blood temperature, this would suggest an indirect contribution of TRPV-1 channels to reflex cutaneous vasodilation. This potential mechanism and interaction with NK$_1$ receptors has not been directly tested in human skin and awaits further investigation.

The data from Charkoudian et al. (3) and the data from the present investigation are discordant, yet intriguing. There are at least two possible reasons that may explain the difference in results. First, desensitization of cutaneous sensory nerves via chronic topical capsaicin may not attenuate reflex cutaneous vasodilation if cutaneous sensory nerves are not involved in active vasodilation. To date, there is indirect, but no direct, evidence for the involvement of cutaneous sensory nerves to reflex cutaneous vasodilation (31). Second, it is possible topical capsaicin only affects TRPV-1 channels located on sensory nerve terminals but does not reach endothelial TRPV-1 channels. If cutaneous sensory nerves are not involved in active vasodilation, desensitization of sensory afferent TRPV-1 channels would not be expected to attenuate reflex vasodilation. As mentioned above, the data from the present investigation and those of Charkoudian et al. (3) provide strong evidence to suggest endothelial TRPV-1 channels are involved in reflex cutaneous vasodilation. Studies aimed at investigating a potential role for cutaneous afferent sensory nerves to cutaneous active vasodilation may shed more light on this question.

**Limitations**

At least three limitations to this study should be addressed. First, 20 mM capsazepine was the maximal concentration that could be used. Capsazepine is minimally soluble in aqueous solution, which made it necessary for us to utilize a vehicle of 90% propylene glycol + 10% lactated Ringer. Greater concentrations of capsazepine could be achieved only if dimethyl sulfoxide (DMSO) was added to the vehicle; however, addition of DMSO to the vehicle resulted in vasodilation at rest and thus a baseline shift ($\sim 25\%$ increase in baseline). To minimize the confounding effects of baseline shifts, we used a maximal concentration of capsazepine that did not require addition of DMSO to the vehicle. It is possible more substantial reductions in CVC would have been observed with higher concentrations of capsazepine; thus our data may underestimate the contribution of TRPV-1 channel activation to cutaneous active vasodilation.

Second, it is unclear how capsazepine is affected when it diffuses out of the microdialysis fiber into the aqueous interstitial fluid of the skin. As just discussed, capsazepine is indeed interactive with NO, the data from Wong et al. and Zhao et al. (32, 37) suggest increased shear stress may not be the mechanism by which NO is produced in human skin; however, a shear stress mechanism for TRPV-1 channel activation cannot be ruled out. The methods employed in this study do not allow us to delineate mechanisms by which TRPV-1 channels are activated during heat stress; however, there are at least two possible mechanisms by which TRPV-1 channels may be activated. First, as discussed above, our data suggest the vascular endothelium is the most probable location for the observed TRPV-1 component of cutaneous active vasodilation. If this is indeed the case, it is possible that, as cutaneous blood flow increases, there is an increase in shear stress that activates TRPV-1 channels. A limitation to this proposition is that assessing shear stress in the microvasculature, including the cutaneous circulation, and how shear stress is transmitted from the conduit arteries to microvessels is difficult. This potential mechanism is further limited since data (32, 37) has shown reactive hyperemia is not mediated by NO-dependent mechanisms. If TRPV-1 activation during whole body heating does indeed interact with NO, the data from Wong et al. and Zhao et al. (32, 37) suggest increased shear stress may not be the mechanism by which NO is produced in human skin; however, a shear stress mechanism for TRPV-1 channel activation cannot be ruled out.
minimally soluble in aqueous solution, and it is possible capsazepine precipitates out of solution or the efficacy is reduced by some other means when exposed to the aqueous milieu of the interstitial fluid. If the efficacy of our dose of capsazepine was reduced when exposed to an aqueous environment, the data from the present study may underestimate the contribution of TRPV-1 channels to reflex cutaneous vasodilation.

Third, we used oral temperature measured via thermistor placed in the sublingual sulcus to obtain an index of core temperature. The ambient temperature if the subject talks or breathes through their mouth can influence this method of temperature monitoring. Oral temperature measurements can also be altered by slight changes in the placement of the thermistor in the sublingual sulcus. All subjects were instructed to maintain a consistent placement and to not breathe through their mouth or talk; however, we cannot rule out bias or error of our temperature measurement. A more reliable measurement may be obtained via rectal temperature or ingestible telemetric temperature pill.

In conclusion, the data from this study provide evidence to suggest that TRPV-1 channel activation contributes ~25% to cutaneous active vasodilation and that TRPV-1 channel activation may interact with NO. We propose that TRPV-1 channels located in the vascular endothelium are the most likely source of TRPV-1 channels that contribute to cutaneous active vasodilation; however, more research is required to more clearly determine the precise TRPV-1 channels involved in cutaneous active vasodilation and whether the contribution of TRPV-1 channels is direct or indirect.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.J.W. conception and design of research; B.J.W. and S.M.F. performed experiments; B.J.W. and S.M.F. analyzed data; B.J.W. and S.M.F. interpreted results of experiments; B.J.W. and S.M.F. prepared figures; B.J.W. drafted the manuscript; B.J.W. and S.M.F. edited and revised the manuscript; B.J.W. and S.M.F. approved the final version of the manuscript.

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