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Gustatory Effects of Capsaicin that are Independent of TRPV1 Receptors

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Introduction

In order to choose which foods are palatable and safe to ingest, it is important to integrate information from different modalities, not only chemosensory (taste and olfaction), but also somatosensory (texture, irritation, nociception) (Scott and Verhagen, 2000). Capsaicin, the pungent component from chili peppers, has been shown to alter taste perception (Lawless and Stevens, 1984; Prescott and Stevenson, 1995; Simons *et al.*, 2002) and has traditionally been added to foods to change their palatability. The effects of capsaicin on taste could emerge from the integration throughout the gustatory axis of information originating from capsaicin's direct effects on taste receptor cells (TRCs) (Liu and Simon, 2001; Park *et al.*, 2003; Lyall *et al.*, 2004) and/or from information resulting from the effects of capsaicin on TRPV1 receptors in trigeminal nerve endings in the mouth (Liu and Simon, 1996, 2000). Recent studies have shown that capsaicin can alter neural responses to tastants at the level of the nucleus tractus solitarius (NTS) and that these effects seem to be independent of trigeminal transmission (Simons *et al.*, 2003). Previously, Okuni (1977)showed that chorda tympani fibers can be activated by capsaicin, indicating capsaicin can affect the gustatory pathway and more recently, Lyall *et al.* (2004) presented evidence for a TRPV1 splice variant in TRCs that is responsible for the amiloride-insensitive responses to salts. Here, we found that in dissociated rat TRCs capsaicin inhibits voltage-gated inward and outward currents. Furthermore, we found that in *TRPV1*$^{-/-}$ mice capsaicin can alter taste preference to sucrose. These results demonstrate that the effects of capsaicin in taste perception do not result exclusively from the activation of capsaicin-sensitive receptors, but also through non-specific TRPV1-independent mechanisms.

Materials and Methods

Taste Preference

All procedures were approved by Duke IACUC. *TRPV1*$^{-/-}$ mice—WT and congenic *TRPV1*$^{-/-}$ littermates in the C57/B6J genetic background, males and female, age 3–7 months—were the generous gift from Dr David Julius and were previously described (Caterina *et al.*, 2000).

Mice were water restricted and habituated to drink for 1 h/day (4 days) from two tubes with sippers; one containing water and the other 100 mM sucrose. To test for taste preference (sucrose/water + sucrose) of the *TRPV1*$^{-/-}$ mice in the presence of capsaicin, the solutions consisted of water, 1% DMSO, 100 μM capsaicin and 100 mM sucrose, 1% DMSO, 100 μM capsaicin. As controls, the solutions consisted only of water with 1% DMSO and 100 mM sucrose with 1% DMSO. The relative position of the sucrose and water tubes in the cage (right or left) varied from day to day, and every condition (with or without capsaicin) was tested twice in each side (right or left) and the results for each side averaged. Two independent groups, totaling 13 *TRPV1*$^{-/-}$ mice, were used to study the effects of capsaicin on taste preference.

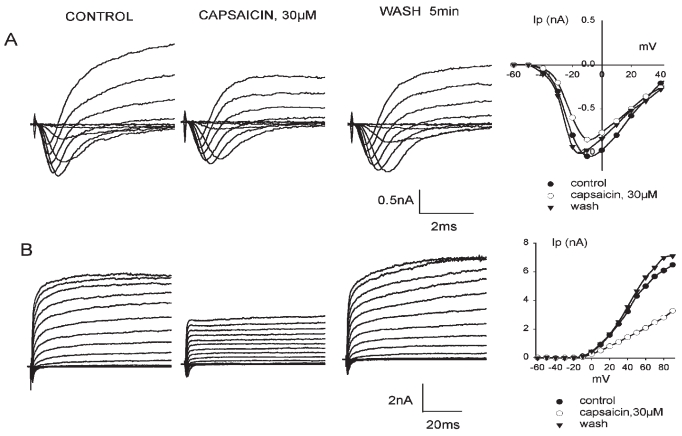

Figure 1 Capsaicin inhibits voltage-gated currents in taste cells from rat circumvallate papillae. Shown are control responses, the responses to 30 μM capsaicin and the responses after a 4 min washout. The peak current–voltage plots under these three conditions are also shown. **(A)** Rapidly activating inward currents. **(B)** Currents from the same cell highlighting the outward currents. Holding potential = 60 mV.

Isolation of TRCs and Electrophysiology

TRCs from rat circumvallate papillae were isolated as previously described (Herness *et al.*, 1997). The isolated taste buds were gently triturated to obtain individual TRCs that were plated into the recording chamber. Whole cell patch clamp experiments were done as described (Liu *et al.*, 2001). In all the TRCs tested, 30 μM capsaicin did not activate an inward current when the cells were held at a potential of –60 mV. The peak current-voltage (Ip-V) relationship was determined using a voltage step protocol in which the voltage was increased in 10 mV step increments from the holding potential of –60mV to test potentials up to 80mV. Peak currents were analyzed using pCLAMP software and plotted against the applied voltage to obtain the Ip-V relations.

Results

Capsaicin inhibits inward and outward currents in isolated taste cells

Figure 1 shows that that the application of 30 μM capsaicin inhibited both the voltage-dependent inward and outward currents. Also shown is that the currents are largely reversible after a 4 min washout. On average, 30 μM capsaicin inhibited the peak inward currents 20 ± 3% (n = 6) and the steady state outward currents 31 ± 4% (n = 6) The inward currents are voltage-gated sodium and

calcium currents and the outward current primarily reflects a delayed rectifier potassium current (Zhao *et al.*, 2002).

Capsaicin alters taste preference in *TRPV1*$^{-/-}$ mice

The results from electrophysiological recordings suggest that capsaicin can affect inward and outward currents in TRCs in a non-specific manner. We therefore tested, using a two-bottle preference test, whether taste preference would be affected by capsaicin in mice that lack TRPV1 receptors. We verified that deletion of TRPV1 *per se* did not affect preference for sucrose as *TRPV1*$^{-/-}$ mice showed the same preference for sucrose as their WT littermates [$t(9) = 1.59$, $P > 0.05$, data not shown]. Additionally, while WT mice refused to drink any solution containing 100 μM capsaicin, *TRPV1*$^{-/-}$ mice consumed the same amount of liquid in the presence or absence of 100 μM capsaicin [$t(12) = 0.28$, $P > 0.05$, $P > 0.05$; Figure 2B]. However, the preference of *TRPV1*$^{-/-}$ mice for 100 mM sucrose was decreased in the presence of 100 μM capsaicin. That is, whereas in the absence of capsaicin *TRPV1*$^{-/-}$ mice displayed a clear preference for 100 mM sucrose [right DMSO $t(12) = 3.41$, $P < 0.05$; left DMSO $t_{(12)} = 2.89$, $P < 0.05$], in the presence of 100 μM capsaicin they failed to show that preference [right capsaicin $t(12) = 0.89$, $P > 0.05$; left capsaicin $t(12) = 1.27$, $P > 0.05$; Figure 2A]. Furthermore, *TRPV1*$^{-/-}$ mice exhibited a preference index that was significantly lower in the presence than in the absence of capsaicin [$t(12) = 2.89$, $P < 0.05$; Figure 2A]. This effect was repeatable (see Materials and methods) and independent of the relative side of placement of the sucrose bottle (right or left), indicating that indeed capsaicin could affect taste preference in *TRPV1*$^{-/-}$ mice.

Discussion

In this study we showed that capsaicin could inhibit voltage-gated inward and outward currents in TRCs in a non-specific manner that is similar to the way it does it does in sensory trigeminal neurons (Liu *et al.*, 2001; Liu and Simon, 2003). These voltage-gated currents have been shown to be important in several aspects of gustatory physiology (Herness and Gilbertson, 1999). We also showed that capsaicin can affect taste perception in *TRPV1*$^{-/-}$ mice. Taken together, these results suggest that the effects of capsaicin may affect taste perception not exclusively via the activation of capsaicin-sensitive receptors, but also via a direct, non-specific and TRPV1-independent effect of capsaicin in TRCs.

Being a non-polar compound, capsaicin can partition into the acyl chain region of the plasma membrane where it can alter its material properties, thereby altering channel (including sodium channel) function (Lundbaek *et al.*, 2004) or even possibly $TRPM_5$, receptors. Similarly it can also partition into the cytoplasm where it can affect the function of GPCRs, including those associated with taste (Peri *et al.*, 2000). Another possibility is that capsaicin can act on alternative receptors in TRCs. A recent study has shown that a TRPV1 variant is expressed in TRCs (Lyall *et al.*, 2004). Although this variant could be responsible for some the effects of capsaicin in salt taste, it could still not explain capsaicin's inhibitory effects on taste preference for sucrose in *TRPV1*$^{-/-}$ mice (Figure 2). In summary, capsaicin can evoke a variety of effects on taste—it can produce a burning sensation, be involved in salt taste and diminish sweet taste. Which of these myriad of effects it will produce will depend on the concentration, time of application and presence of capsaicin-sensitive receptors (Caterina *et al.*, 2000; Jordt *et al.*, 2004).

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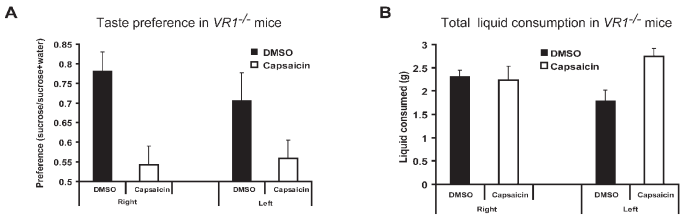

Figure 2 Capsaicin alters taste preference in TRPV1–/– mice. TRPV1–/– mice show a preference for sucrose in a two-bottle preference test (black bars). This preference is attenuated in the presence of capsaicin (white bars). This effect is independent of side placement of the sucrose bottle (right or left). Capsaicin did not affect the overall amount of liquid consumed.

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