

A Novel Vanilloid Receptor-1 (VR-1) Variant Mammalian Salt Taste Receptor

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Key words: capsaicin, chorda tympani, resiniferatoxin, SB-366791, taste receptor cells

Introduction

An amiloride-insensitive (AI) salt taste receptor is the predominant transducer of Na⁺ taste in some mammalian species. Accordingly, the objective of this study was to characterize the AI-salt taste receptor. The AI-salt taste receptor in rat and mouse fungiform taste receptor cells (TRCs) was activated by the vanilloid receptor-1 (VR-1) agonists, resiniferatoxin (RTX), capsaicin (CAP) and elevated temperature (>38°), and was inhibited by the VR-1 antagonist, SB-366791 (Lyall *et al.*, 2004). VR-1 knockout mice demonstrated no functional AI-salt taste receptor and no salt sensitivity to vanilloids and temperature. We conclude that the AI-salt taste receptor is derived from the VR-1 gene.

Materials and methods

All protocols were approved by the IACUC of Virginia Commonwealth University. The AI-salt taste receptor was investigated by RT-PCR, measurement of apical Na⁺ fluxes in polarized fungiform TRCs, and chorda tympani (CT) nerve recordings. The CT responses to salts were recorded in Sprague–Dawley rats, wildtype (WT) mice (C57BL/6J) and homozygous VR-1 knockout (KO; B6.129S4-Trpv1^{TM1jul}) mice in the presence of benzamil (Bz; a more potent and specific blocker of epithelial Na⁺ channels than amiloride), the vanilloids RTX and CAP, the VR-1 antagonist SB-366791, and at elevated temperatures (Lyall *et al.*, 2004).

Results and discussion

Rat CT responses to 100 mM NaCl + 5 μM Bz were modulated by RTX and CAP, and gave bell-shaped concentration–response curves (Figure 1A). RTX, between 0.1 and 1 μM concentration, enhanced the CT responses, above 1 μM RTX the CT responses decreased, and were completely inhibited at 10 μM RTX. CAP gave a maximum activation of the NaCl CT response at 45 μM and completely inhibited the response at 200 μM. Cetylpyridinium chloride (CPC), another modulator of the Bz-insensitive NaCl CT response (DeSimone *et al.*, 2001), gave maximum activation and inhibition at 250 μM and 2 mM, respectively (Figure 1A).

Increasing the temperature to 38° produced a sharp increase in the Bz-insensitive NaCl CT response and gave a maximum enhancement at 42° (Figure 1B, open circles). RTX and temperature produced integrated effects on the CT response. RTX increased the CT response at 23° and shifted the temperature curve to the left in a dose-dependent manner (Figure 1B). Increasing RTX to 10 μM completely inhibited the CT response at all temperatures (Figure 1B, open triangles). SB-366791 (1 μM) completely blocked the CT response at all temperatures (Figure 1C, filled triangles).

CPC also gave bell-shaped concentration–response curves in the presence of 100 mM KCl or 100 mM NH₄Cl (Figure 1D). The CT responses to KCl and NH₄Cl are amiloride- and Bz-insensitive. Unlike the case with NaCl, RTX (10 μM), CAP (200 μM) and CPC

(2 mM), produced ~40% inhibition in the CT response to KCl or NH₄Cl (DeSimone *et al.*, 2001; Lyall *et al.*, 2004).

The CT responses were monitored at –60 mV, 0 and +60 mV lingual voltage-clamp in the presence and absence of RTX (Figure 2A). RTX (0.5 μM) increased the response at each voltage and also the slope of the voltage–response relationship. This indicates that RTX increases the apical conductance which results in increased apical cation flux in TRCs (DeSimone *et al.*, 2001; Lyall *et al.*, 2004). In the presence of RTX (0.25 μM), a change in external pH (pH_o) from 6 (Figure 2B, filled circles) to either 4 (Figure 2B, filled triangles) or 10 (Figure 2B, filled squares) shifted the temperature curve to the right. Adenosine 5'-triphosphate (ATP; 500 μM), a VR-1 agonist, enhanced the CT response and shifted the temperature curve to the left (Figure 2B, open circles). Thus, both ATP and pH_o act to lower the temperature threshold of the CT response.

The above results demonstrate many functional similarities between VR-1 and the Bz-insensitive NaCl CT responses. In a

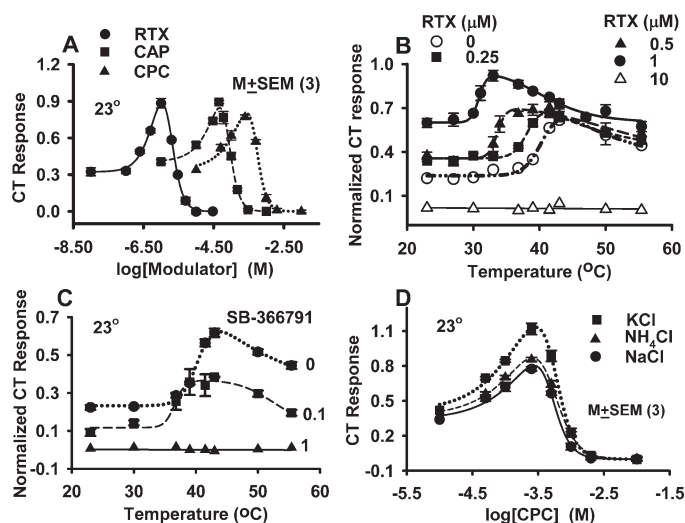


Figure 1 Effect of VR-1 agonists and antagonists on the rat NaCl CT response. (A) Effect of RTX (filled circles), CAP (filled squares), and CPC (filled triangles) on Bz-insensitive NaCl CT responses at 23°. (B) Effect of temperature (23–55.5°) on the CT response to 100 mM NaCl + 5 μM Bz in the presence of RTX (0–10 μM). (C) Effect of 0 (filled circles), 0.1 μM (filled squares) and 1 μM (filled triangles) SB-366791 on the CT response to 100 mM NaCl + 5 μM Bz at 23°. (D) Effect of CPC on the CT response to 100 mM NaCl + 5 μM Bz (filled circles), 100 mM NH₄Cl (filled triangles), and 100 mM KCl (filled squares) at 23°. The CPC-sensitive CT responses to KCl and NH₄Cl were obtained by subtracting the maximum suppression value at 10 mM CPC. Each point = mean ± SEM of the normalized CT response from three animals.

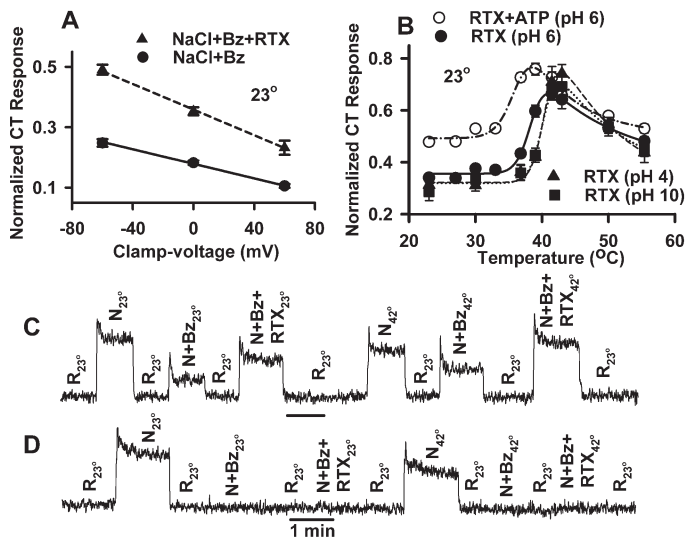


Figure 2 Effect of RTX, temperature, and ATP on the rat NaCl CT response. **(A)** CT responses were recorded during superfusion of the tongue with a rinse solution (10 mM KCl) and with stimulating solutions containing 100 mM NaCl + 5 μ M Bz (filled circles; NaCl + Bz; $n = 8$) or 100 mM NaCl + 5 μ M Bz + 0.5 μ M RTX (filled triangles; NaCl + Bz + RTX; $n = 4$) at 23°. **(B)** Effect of pH_o 4.7 (filled triangles; $n = 3$), 6.0 (filled circles; $n = 9$), 9.7 (filled squares; $n = 6$) and 0.5 mM ATP (open circles; $n = 4$) on the temperature-induced CT response to 100 mM NaCl + 5 μ M Bz + 0.25 μ M RTX. Each point = mean \pm SEM of the normalized CT responses from at least three animals. CT responses in **(C)** wild-type mice (WT) and **(D)** VR-1 knockout mice (KO). Tongues were stimulated with 100 mM NaCl (N_{23° ; N_{42°), 100 mM NaCl + 5 μ M Bz ($N + Bz_{23^\circ}$; $N + Bz_{42^\circ}$), and 100 mM NaCl + 5 μ M Bz + 0.75 μ M RTX ($N + Bz + RTX_{23^\circ}$; $N + Bz + RTX_{42^\circ}$) at either 23° or 42°.

cDNA library from fungiform TRCs, using VR-1 specific sense and antisense primers (Liu and Simon, 2001), we identified a VR-1 mRNA transcript that yielded 100% homology with rVR-1, rVRL-1, rSIC and rVR5'sv (Lyall *et al.*, 2004). In WT mice (Figure 2C), ~25%

of the NaCl CT response was Bz-insensitive at 23°. Its magnitude was enhanced by RTX and by increasing the temperature to 42°. The VR-1 KO mice (Figure 2D) demonstrated no Bz-insensitive NaCl CT response and no sensitivity to RTX and elevated temperature.

In summary, the AI-salt taste receptor is a constitutively active non-selective cation channel. It accounts for all of the AI-CT response to Na⁺ salts and part of the response to K⁺ and NH₄⁺ salts. It is activated by vanilloids and temperature (>38°), and is inhibited by VR-1 antagonists. Vanilloids, external H⁺ and ATP lower the temperature threshold of the channel. This allows for increased salt taste sensitivity without an increase in temperature. VR-1 knockout mice demonstrate no functional AI-salt taste receptor and no salt taste sensitivity to vanilloids and temperature. We conclude that the mammalian non-specific salt taste receptor is a VR-1 variant. Since the entire CT response to NaCl can be accounted for by apical ENaC and VR-1 variant cation channel, it is unlikely that basolateral ENaC has a role in NaCl taste, as previously hypothesized. However, the relative anion permeability of the paracellular pathway and its effect on taste cell membrane potential accounts for the sizable anion effects on salt taste. Moreover, the possibility of a paracellular transduction mechanism that accounts for the part of the K⁺ and NH₄⁺ CT responses that are insensitive to VR-1 antagonists and amiloride cannot be presently excluded.

Acknowledgements

This work was supported by NIDCD grants DC-02422 and DC-00122.

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