Effect of Nicotine on Chorda Tympani Responses to Salty and Sour Stimuli

Vijay Lyall, Tam-Hao T. Phan, Shobha Mummalaneni, Mahdis Mansouri, Gerard L. Heck, Gerd Kobal, and John A. DeSimone

1Department of Physiology, Virginia Commonwealth University; and 2Philip Morris USA, Richmond, Virginia

Submitted 31 March 2007; accepted in final form 29 June 2007


INTRODUCTION

Nicotine is found in tobacco leaves and thus is a component of tobacco products. Nicotine is a gustatory stimulus. It elicits responses in both glossopharyngeal and chorda tympani (CT) taste nerves (Dahl et al. 1997) and in neurons of the nucleus of the solitary tract (Lemon and Smith 2005). Nicotine also elicits responses in single gustatory neurons from the insular cortex in monkeys (Scott et al. 1999). Nicotine is perceived as predominantly bitter by humans but its effects on other taste qualities are presently unknown. Two additional properties of nicotine suggest that it could be a potential modulator of both amiloride- and Bz-sensitive epithelial Na⁺ channel (ENaC) salt taste receptors and sour taste transduction mechanisms in taste receptor fields other than the anterior tongue (Ruiz et al. 2006; Treesukosol et al. 2007). TRPV1 is activated by vanilloids: resiniferatoxin (RTX) and capsaicin (CAP) and by elevated temperatures (>38°C), and is inhibited by the TRPV1 antagonists [capsazepine and N-(3-methoxyphenyl)-4-chlorocinnamide (SB-366791)]. TRPV1 demonstrates many similarities with the cloned TRPV1 (Lyall et al. 2005c). Therefore it is expected that agonists that either activate or sensitize the TRPV1 channel, such as endocannabinoids, lipoxygenase metabolites of arachidonic acid, lipid derivatives, nicotine, ethanol, H⁺, and intracellular second messengers (Davis et al. 2002; Geppetti and Trevisani 2004; Gunthorpe et al. 2002; Liu et al. 2004; Lyall et al. 2004b, 2005a,b,c; Trevisani et al. 2002) will also have similar effects on TRPV1. To test whether nicotine interacts specifically with TRPV1, additional studies were conducted to determine whether nicotine also affected the amiloride- and Bz-sensitive epithelial Na⁺ channels (ENaCs) in the apical membrane of TRCs.

Second, in solution nicotine behaves as a base and has the potential to alter cell pH. Accordingly, we also tested the hypothesis that on entry into TRCs, nicotine alkalizes resting intracellular pH (pHi) and inhibits CT responses to acidic stimuli. This hypothesis is based on the observations that the proximate stimulus for sour taste transduction is an acid-induced decrease in pHi in a subset of taste bud cells, the acid-sensing TRCs (DeSimone and Lyall 2006). PKD2L1, a polycystic-kidney-disease-like ion channel, expressed in a subset of TRCs is most likely involved in pH sensing (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006). In particular, the transduction mechanism for the phasic component of the CT response to acidic stimuli involves a decrease in pHi that results in TRC shrinkage through the change in F-actin to G-actin equilibrium. Cell shrinkage activates a flufenamic acid–sensitive nonspecific cation conductance in the basolateral membrane of TRCs that is responsible for the elicitation of and benzamil (Bz)-insensitive TRPV1 variant salt taste receptor (TRPV1t) (Lyall et al. 2004b, 2005a,b,c; Simon and De Araujo 2005). This hypothesis is based on the observations that in rat and mouse fungiform taste receptor cells (TRCs) TRPV1t is a constitutively active nonselective cation channel present in the apical membrane of TRCs and is derived from the TRPV1 gene. All of the Bz-insensitive CT taste nerve responses to Na⁺ salts and part of the response to K⁺, NH₄⁺, and Ca²⁺ salts are elicited by cation flux through TRPV1t (Lyall et al. 2004b, 2005a,b,c). However, TRPV1 knockout (KO) mice maintain normal salt detection performance, suggesting that there may be other amiloride- and Bz-insensitive salt transduction mechanisms in taste receptor fields other than the anterior tongue.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
EFFECT OF NICOTINE ON SALTY AND SOUR TASTE

the phasic response to acid stimulation (Lyall et al. 2006). Nicotine can potentially modulate one or more steps in this transduction pathway.

The interactions between nicotine and the amiloride-insensitive and amiloride-sensitive salt taste receptors were investigated by measurement of TRC Na\(^{+}\) ([Na\(^{+}\)]\_i) and H\(^{+}\) (pH\textsubscript{i}) activity in polarized rat fungiform TRCs and by the CT taste nerve recordings (Lyall et al. 2005c). The CT responses were monitored in two animal models: a rat model and a TRPV1 KO mouse model (Caterina et al. 2000; Lyall et al. 2004b, 2005a,c). CT responses were recorded while the tongue was stimulated with nicotine alone and in mixtures with mineral salts in the absence and presence of specific agonists (RTX and elevated temperature) and antagonists (SB-366791) of TRPV1t and ENaC in the presence of Bz, a specific ENaC blocker. The interactions between nicotine and the acidic stimuli were investigated by the measurement of TRC pH\textsubscript{i} and cell volume in polarized rat fungiform TRCs and by the CT recordings while stimulating the tongue with HCl (Lyall et al. 2006). The results presented herein support the conclusion that nicotine modulates the CT responses to mineral salts by interacting with both TRPV1t and ENaC and it modulates CT responses to acidic stimuli by inducing intracellular alkalization and cell swelling.

METHODS

In this study we used the combined methodology of in vitro imaging and in vivo neural recordings to investigate the interaction of nicotine with TRPV1t and ENaC in the apical membranes of fungiform TRCs (Simon 2002).

In vitro studies

pH\textsubscript{i} AND CELL VOLUME MEASUREMENTS IN POLARIZED FUNGIFORM TRCS. Simultaneous measurement of cell volume changes and intracellular pH (pH\textsubscript{i}) were made using the pH-sensitive dye 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF, Molecular Probes, Eugene, OR) and recording at both the pH-sensitive and pH-insensitive (isosbestic) wavelengths (Lyall et al. 2006). Changes in pH\textsubscript{i} were monitored as variations in the fluorescence intensity ratio (FIR; F\textsubscript{490}/F\textsubscript{440}) of BCECF (Lyall et al. 2001). At the end of each experiment the changes in TRC pH\textsubscript{i} and cell volume in polarized rat fungiform TRCs and by the CT taste nerve recordings (Lyall et al. 2005c). The CT responses were monitored in two animal models: a rat model and a TRPV1 KO mouse model (Caterina et al. 2000; Lyall et al. 2004b, 2005a,c). CT responses were recorded while the tongue was stimulated with nicotine alone and in mixtures with mineral salts in the absence and presence of specific agonists (RTX and elevated temperature) and antagonists (SB-366791) of TRPV1t and ENaC in the presence of Bz, a specific ENaC blocker. The interactions between nicotine and the acidic stimuli were investigated by the measurement of TRC pH\textsubscript{i} and cell volume in polarized rat fungiform TRCs and by the CT recordings while stimulating the tongue with HCl (Lyall et al. 2006). The results presented herein support the conclusion that nicotine modulates the CT responses to mineral salts by interacting with both TRPV1t and ENaC and it modulates CT responses to acidic stimuli by inducing intracellular alkalization and cell swelling.

SOLUTIONS. Compositions of various solutions used in the in vitro experiments are given in Table 1.

In vivo studies

CT TASTE NERVE RECORDINGS. Animals were housed in the Virginia Commonwealth University animal facility in accordance with institutional guidelines. All in vivo and in vitro animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University. Female Sprague–Dawley rats (150–200 g) were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg) and supplemental pentobarbital (20 mg/kg) was administered as necessary to maintain surgical anesthesia. The animal’s corneal reflex and toe-pinch reflex were used to monitor the depth of surgical anesthesia. Body temperatures were maintained at 37 °C with a Deltaphase Isothermal PAD (Model 39 DP; Braintree Scientific, Braintree, MA). The left CT nerve was exposed laterally as it exited the tympanic bulla and placed onto a 32-gauge platinum/iridium wire electrode. The CT responses were recorded under zero

<table>
<thead>
<tr>
<th>TABLE 1. Composition of solutions used in in vitro experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (M)</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>CaCl\textsubscript{2}</td>
</tr>
<tr>
<td>MgCl\textsubscript{2}</td>
</tr>
<tr>
<td>NaPy</td>
</tr>
<tr>
<td>HEPES*</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>NMDG-Cl</td>
</tr>
<tr>
<td>NMDG-Glu</td>
</tr>
<tr>
<td>CaSO\textsubscript{4}</td>
</tr>
<tr>
<td>MgSO\textsubscript{4}</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>

R, Ringer solution; C, control solution; 0Na, Na\(^{+}\)-free solution; 0Na-0K, Na\(^{+}\)- and K\(^{+}\)-free solution; 0Na-0K-0Cl, Na\(^{+}\)-, K\(^{+}\)-, and Cl\(^{-}\)-free solution; HK, high K\(^{+}\) solutions containing 0.01 M nigericin. In some experiments the control solution pH was adjusted to 6.5. *In some solutions Tris[hydroxymethyl]-aminomethane was used instead of HEPES [4-[(2-hydroxyethyl)piperazine-1-ethanesulfonic acid] and the pH of the solution was adjusted to 9 with 1 N HCl. NaPy, sodium pyruvate.
current-clamp and voltage-clamp conditions referenced to the oral cavity (Lyall et al. 2005a,b).

The anterior lingual surface was stimulated with a rinse solution (R; 0.01 M KC1) and with NaCl solutions (0.01 M KCl + 0.1 M NaCl; N) containing (−)nicotine hydrogen tartrate (Nic; 0–0.1 M). In some experiments both rinse (R) and NaCl solutions (N) with or without nicotine contained, in addition, 0.01 M 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES) or 0.01 M Tris[hydroxymethyl]-ammonomethane (Tris) and were adjusted to different pH values with 1 N NaOH or HCl. CT responses were recorded in the presence of benzamil (Bz; 5 × 10^{-6} M), a specific and potent blocker of the apical ENaC. CT responses were also recorded in the presence of the TRPV1 containing (LY11002) and with NaCl solutions (0.01 M KCl cavity (Lyall et al. 2005a). In vitro studies

RESULTS

In vitro studies

EFFECT OF NICOTINE ON TRC pH AND VOLUME. Nicotine has two pK values; 6.16 and 10.96. Nicotine is a base and on entering the apical membrane.

Accordingly, in a lingual epithelial preparation perfused on both sides with control solution (Table 1; C; pH 7.4) increasing nicotine concentration in the basolateral compartment (BLNic) in a stepwise manner from 0.0005 to 0.01 M produced a dose-dependent increase in TRC pH (Fig. 1A). The relationship between basolateral nicotine concentration and TRC pH was almost linear (pH1; 0.028 [Nic] + 7.24; r^2 = 0.95; n = 8). Essentially similar results were obtained in two additional experiments. The results suggest that at physiological pH, the basolateral membrane is significantly greater than that of the apical membrane.

The membrane permeability of nicotine was strongly dependent on pH. At pH 6.5 (BL_{pH}), basolateral nicotine (0.005 M) produced a significantly smaller increase in TRC pH (Fig. 1C, D: effect of 0.025 M nicotine in apical solution at pH 6.5 or 7.4. D: effect of 0.025 M nicotine in apical solution at pH 6.5 or 9.0. Values are presented as means ± SE of the number of regions of interest (ROIs) within the taste bud.
Nicotine-induced alkalization was accompanied by a decrease in F₄₄₀, indicating cell swelling (Fig. 2A). Cell swelling will cause a decrease in dye concentration inside the cells and a decrease in F₄₄₀. Nicotine-induced increase in pHi and a concomitant decrease in F₄₄₀ were also observed when TRCs were perfused on both sides with 0 Na solution (Table 1; 0Na;

---

**FIG. 2.** Effect of nicotine on TRC volume and the apical Na⁺ flux. A: effect of 0.01 M basolateral nicotine (BLNic) on pHi, and F₄₄₀. Values are presented as means ± SE of n, where n = number of ROIs within the taste bud. B: effect of apical nicotine (APNic) on the benzamil (Bz)-insensitive apical unilateral apical Na⁺ flux in polarized TRCs. A lingual epithelial preparation loaded with 4.4% [1,10-trioxa-7,13-diazacyclo-pentadecane-7,13-diylibis(5-methoxy-6,12-benzo-furanyl]bis-[tetrazakis(acetoxy)] (SBFI) was initially perfused on both sides with 0 Na⁺ Ringer solution (pH 7.4). Temporal changes in F₄₄₀ (F₄₄₀) were monitored while the apical membrane was perfused with control Ringer solution (pH 7.4) with (a–b) and without (b–c) 0.015 M nicotine. Relative changes in [Na⁺]ᵣ are presented as changes in F₄₄₀ relative to apical 0 Na⁺. Values are presented as means ± SE of n, where n = number of ROIs within the taste bud. C: effect of basolateral nicotine (BLNic) on the Bz-sensitive unilateral apical Na⁺ flux in polarized TRCs. A lingual epithelial preparation loaded with Na-free solution (Table 1; 0Na; pH 7.4) was initially perfused on both sides with 0 Na⁺ Ringer solution (pH 7.4). Temporal changes in F₄₄₀ (F₄₄₀) were monitored while the apical membrane was perfused with control Ringer solution containing 0.15 M NaCl + 5 × 10⁻⁶ M Bz + 0.015 M nicotine (Table 1; C; pH 7.4) increased F₄₄₀ (a–b). Subsequently perfusing the control solution with Bz but without nicotine decreased F₄₄₀ (b–c). Nicotine also produced an increase in Bz-sensitive apical Na⁺ flux in isolated taste bud fragments in which the basolateral Na⁺-H⁺ exchanger-1 (NHE-1) was blocked by 10 × 10⁻⁶ M zoniporide (Lyall et al. 2004a) (data not shown). The nicotine-induced increase in the Bz-sensitive apical Na⁺ flux was blocked in the presence of 1 × 10⁻⁶ M SB-366791 (data not shown). Under these conditions no effect of nicotine was observed on the Bz-sensitive unilateral Na⁺ flux (data not shown). These results suggest that nicotine produces a greater unilateral Bz-sensitive apical Na⁺ flux relative to control through the TRPV1 channel (Lyall et al. 2005b).

Figure 2C shows the effect of basolateral nicotine (BLNic) on the Bz-sensitive Na⁺ flux across the apical membrane of TRCs. A lingual epithelial preparation loaded with Na-green was initially perfused on the apical side with Na⁺-free solution (Table 1; 0Na; pH 7.4) and on the basolateral side with control solution (Table 1; C; pH 7.4). Perfusing the apical membrane with control solution containing 0.15 M NaCl + 1 × 10⁻⁶ M SB-366791 reversibly increased F₄₉₀ (a–b–c). SB-366791 inhibits Na⁺ flux through the Bz-insensitive pathway. Thus the increase in F₄₉₀ represents the Na⁺ flux exclusively through ENaCs. Following this the basolateral membrane was perfused with control solution + 0.015 M nicotine (BLNic). In the continuous presence of basolateral nicotine, perfusing the apical membrane with control solution + 1 × 10⁻⁶ M SB-366791 produced a significantly greater increase in F₄₉₀ relative to control (P < 0.01; n = 7; paired). The basolateral membrane of TRCs is readily permeable to nicotine (Fig. 1, A and C) and on entering TRCs it produced intracellular alkalization resulting in the activation of apical ENaC (Lyall et al. 2002b).
Taken together the results support the conclusion that nicotine produces differential effects on ENaC and TRPV1t depending on pH, and the cell membrane at which TRCs are exposed to nicotine.

**In vivo studies**

**EFFECT OF NICOTINE ON THE CT RESPONSE TO 0.01 M KCl.** We first investigated whether CT responses to 0.01 M KCl are modulated by nicotine in a dose-dependent fashion. A rat tongue was initially rinsed with 0.01 M KCl + 0.01 M HEPES (R; pH 6.1) and then with R containing varying concentrations of nicotine (0–0.1 M; pH 6.1). Figure 3A shows that in the presence of 0.01 M KCl (R) nicotine at 0.005, 0.01, and 0.015 M elicited phasic CT nerve response with a rapid onset and decay. However, the rate of return to a steady-state level varied with the nicotine concentration >0.015 M. This is illustrated in the inset above Fig. 3A using an expanded timescale for 0.005 and 0.05 M nicotine. The nicotine-induced variation in the phasic CT response (phasic 0.01 M KCl CT response/0.3 M NH₄Cl CT response) in three animals is shown in Fig. 4A. The increased response seen at 0.025, 0.05, and 0.1 M nicotine is the result of the delayed return to steady-state levels at these concentrations. As seen in Fig. 3A, nicotine presented in R also produced tonic responses, starting at about 0.025 M concentration. The concentration dependence of the tonic response (0.01 M KCl/0.3 M NH₄Cl) in three animals is shown in Fig. 4B. Tonic responses peaked at 0.05 M nicotine.

**EFFECT OF NICOTINE ON THE Bz-INSENSITIVE PART OF THE CT RESPONSE TO 0.1 M NaCl.** Liu et al. (2004) showed that nicotine induced an increase in CA-transactivated currents in cells with heterologously expressed TRPV1c cation channel, indicating that nicotine sensitizes the channel to activation by vanilloids. We hypothesize that nicotine either activates or serves as a modulator of TRPV1t. Figure 3A shows the effect of nicotine on the tonic CT response to 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.01 M HEPES, pH 6.1 (N + Bz). The results show that nicotine enhanced the Bz-insensitive response to NaCl in a dose-dependent manner. The increase in the response to N + Bz + 0.015 M nicotine was 72% greater than the response to N + Bz alone. On the other hand, the response to N + Bz + 0.05 M nicotine was inhibited by 19% relative to N + Bz. In three animals nicotine produced a bell-shaped dose–response relationship on the Bz-insensitive CT response to 0.1 M NaCl (Fig. 4C).

To investigate whether nicotine changes the response to the Bz-insensitive NaCl CT response by modulating the apical membrane conductance in TRCs, we monitored the sensitivity of the NaCl responses to the applied voltage across the receptive field. Figure 3C shows the CT response to 0.1 M NaCl + 5 × 10⁻⁶ M Bz in the absence (R + N + Bz) and presence of 0.015 M nicotine (R + N + Bz + Nic) as a function of the applied voltage across the receptive field at −60, 0, and +60 mV. As reported earlier (Lyall et al. 2004b, 2005a,c), the rat CT responses to 0.1 M NaCl + 5 × 10⁻⁶ M Bz were slightly enhanced at −60 mV and slightly suppressed at +60 mV. In the presence of 0.015 M nicotine (N + Bz + Nic), the same voltages exerted significantly larger effects on the response (Fig. 3C). The changes in the CT response at 0,−60, and +60 mV in the absence and presence of nicotine from three animals are summarized in Fig. 4D. In each case the slope of the relation between CT response and voltage was calculated as described previously (Lyall et al. 2004b). Nicotine (0.015 M) increased the slope from (6.37 ± 0.92) × 10⁻⁴ response units/mV (●) under control conditions to (13.39 ± 1.36) × 10⁻⁴ response units/mV (■), P < 0.05; n = 3; paired). This suggests that in nicotine-sensitive TRCs the increase in the Bz-insensitive NaCl CT response is due to an increase in the apical membrane conductance to Na⁺. This is consistent with the observation that in in vitro experiments nicotine increased the unilateral apical Na⁺ flux in polarized fungiform TRCs (Fig. 2).
MODULATORY EFFECT OF NICOTINE IS BLOCKED BY SB-366791. To determine whether the effects of nicotine on the Bz-insensitive NaCl CT response are exerted through TRPV1t, we obtained the nicotine concentration versus the Bz-insensitive NaCl CT response function in the presence of SB-366791, a selective competitive inhibitor of both TRPV1 (Gunthorpe et al. 2004) and the TRPV1t (Lyall et al. 2004b, 2005a,b,c).

Effect of SB-366791 on the CT response to 0.1 M NaCl. Figure 3B shows a typical CT response to 0.1 M NaCl + 5 × 10^{-6} M Bz (R + N + Bz) obtained in the presence of 0.05 × 10^{-6} M SB-366791. Note that nicotine <0.025 M has no effect on the CT response. Increasing nicotine to 0.05 and 0.1 M produced an increase in the CT response. In contrast, under control conditions (Fig. 3A) at these concentrations the response decreased. The results from three animals are also summarized in Fig. 4C and show that at a concentration as low as 0.05 × 10^{-6} M, SB-366791 shifts the nicotine concentration versus the tonic response function to the right along the nicotine concentration axis. This is consistent with the idea that SB-366791 acts as a competitive inhibitor of nicotine on the TRPV1t. At 0.25 × 10^{-6} M SB-366791 (Fig. 4C; ▲), the rightward shift of the nicotine concentration versus the tonic response function is increased. As reported previously (Lyall et al. 2004b), at this higher concentration of SB-366791, the constitutive activity of the TRPV1t channel is partially blocked.

In the presence of 0.5 × 10^{-6} M SB-366791, the CT response to 0.1 M NaCl + 5 × 10^{-6} M Bz + 0.015 M nicotine (R + N + Bz + Nic) was not different from baseline and there was no sensitivity of the NaCl CT responses to the applied voltage across the receptive field (data not shown). This suggests that SB-366791 not only inhibits the constitutively active Bz-insensitive apical membrane Na^+ conductance but also the nicotine-induced increase in the Na^+ conductance.

Effect of SB-366791 on the CT response to 0.01 M KCl. The effect of SB-366791 was also observed on the tonic response to nicotine when presented with 0.01 M KCl (R). The data shown in Fig. 3B demonstrate that SB-366791 at 0.05 × 10^{-6} M had no effect on the CT response to R + nicotine relative to R alone. The mean data from three animals are summarized in Fig. 4B (○ vs. ●). However, on increasing SB-366791 to 0.25 × 10^{-6} M, the nicotine concentration versus tonic response function was shifted significantly to the right along the nicotine concentration axis (Fig. 4B; ▲ vs. ○ or ●).

EFFECT OF EXTERNAL pH ON THE CT RESPONSE TO NICOTINE IN 0.01 M KCi OR 0.1 M NaCl. We have previously shown that changes in pH^e modulate the effect of vanilloids on TRPV1t (Lyall et al. 2004b, 2005a). We hypothesize that the effect of nicotine on TRPV1t will also be modulated by changes in pH^e. In these experiments we held the nicotine concentration at 0.015 M (the concentration that produces the maximal enhancement of the tonic response in the medium containing 0.01 M KCl + 0.1 M NaCl + 5 × 10^{-6} M Bz). Consistent with previous studies (Lyall et al. 2002b), in the absence of nicotine the Bz-insensitive NaCl CT responses were insensitive to pH^e (Fig. 5A, inset). Nicotine (R + N + Bz + Nic) enhanced the Bz-insensitive NaCl CT response at all pH values relative to R + N + Bz alone and produced a bell-shaped tonic CT response function in the presence of 0.01 M KCl + 0.1 M NaCl + 5 × 10^{-6} M Bz at varying pH^e values (Fig. 5A). The mean pH^e versus CT response profile obtained in three animals is shown in the inset. In the presence of 0.01 M KCl (R), the response to 0.015 M nicotine was not significantly affected by changes in pH^e (inset). The results support the conclusion that the effect of nicotine on TRPV1t in the presence of 0.1 M NaCl is modulated by changes in pH^e and that nicotine enhances the activity of TRPV1t optimally at pH 6–7.

EFFECT OF ELEVATED TEMPERATURE ON THE CT RESPONSE TO NICOTINE IN THE PRESENCE OF 0.1 M NaCl. Next we tested the effect of nicotine on the temperature threshold of the TRPV1t cation channel in the presence of 0.1 M NaCl. CT responses to 0.01 M KCl + 0.1 M NaCl + 5 × 10^{-6} M Bz (R + N + Bz) were monitored in the presence and absence of 0.01 M nicotine (R + N + Bz + Nic), while the temperature of the solutions was varied between 23 and 55.5°C. The tongue was superfused with the rinse solution (Bz; 0.01 M KCl), 0.01 M KCl + 0.1 M NaCl + 5 × 10^{-6} M Bz solution (R + N + Bz + Bz), and R + 0.1 M NaCl + 5 × 10^{-6} M Bz + 0.01 M nicotine solution.
stimulated with 0.01 M KCl while the rat tongues were first rinsed with 0.01 M KCl (R) at 23°C and then at temperatures between 23 and 55.5°C. Rinse solution (R23°), R

M Tris-HCl (R

2005b). An increase in temperature produced a sharp increase in the rate of superfusion (8 ml/min) the phasic component of the NaCl CT response at 23°C (R

Nic) or with 0.01 M KCl

23°) maintained at room temperature (0.1 M NaCl). The CT responses to 0.01 M KCl (R

0.1 M NaCl. The CT responses to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.01 M nicotine (R + N + Bz + Nic) enhanced the CT response at 23°C and at elevated temperatures (Fig. 5B). In three animals the temperature at which nicotine produced maximum increase in the CT responses was 42.2 ± 0.3°C, a value not different from R + N + Bz alone (41.9 ± 0.2°C). This is consistent with the idea that nicotine enhances the CT response to NaCl without affecting the temperature threshold of the TRPV1t cation channel.

NICOTINE–RTX INTERACTIONS WITH THE TRPV1 SALT TASTE RECEPTOR. The Bz-insensitive NaCl CT responses are insensitive to pHₐ and adenosine triphosphate (ATP). However, in the presence of a subthreshold concentration of RTX, a TRPV1 and TRPV1t agonist, the Bz-insensitive NaCl CT responses become sensitive to both pHₐ and ATP (Lyall et al. 2004b, 2005a). Therefore we tested whether a subthreshold concentration (0.25 × 10⁻⁶ M) of RTX modulates the effects of nicotine and elevated temperature on the Bz-insensitive NaCl CT response.

Figure 5C shows that superfusing the tongue with 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.25 × 10⁻⁶ M RTX at 23°C (R + N + Bz + RTX) produced a small increase in the Bz-insensitive NaCl CT response relative to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz (R + N + Bz). Subsequently, superfusing the tongue with 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.25 × 10⁻⁶ M RTX + 0.015 M nicotine at 23°C (R + N + Bz + RTX + Nic) produced a CT response whose magnitude was significantly >0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.015 M nicotine at 23°C (R + N + Bz + Nic). These results suggest that in the presence of a subthreshold concentration of RTX, nicotine produces a greater increase in the CT response relative to either RTX or nicotine alone.

We also monitored the sensitivity of the NaCl CT responses to ±60-mV applied voltage across the receptive field in the presence of RTX alone and RTX + Nic. The applied voltages exerted significantly larger effects on the 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.25 × 10⁻⁶ M RTX, nicotine (R + N + Bz + RTX + Nic) CT response relative to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.015 M nicotine (R + N + Bz + Nic) CT response relative to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.015 M nicotine (R + N + Bz + Nic) (Figs. 3C and 4D). The mean data from three such experiments are also summarized in Fig. 4D. In the presence of 0.015 M nicotine + 0.25 × 10⁻⁶ M RTX, the slope of the response (21.53 ± 2.38) × 10⁻⁴ response units/mV (■) was greater relative to its value (8.09 ± 2.14) × 10⁻⁴ response units/mV in the presence of RTX alone (●; P < 0.05; n = 3; paired). These results suggest that in the presence of 0.25 × 10⁻⁶ M RTX, nicotine produces a greater increase in the apical Bz-insensitive Na⁺ conductance relative to 0 RTX.

Next we tested the effect of RTX and nicotine on the temperature threshold of the TRPV1t cation channel in the presence of 0.1 M NaCl. The CT responses to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz (R + N + Bz) were monitored in the presence and absence of 0.01 M nicotine (R + N + Bz + Nic) and 0.25 × 10⁻⁶ M RTX (R + N + Bz + Nic + RTX), while the temperature of the solution was varied between 23 and 55.5°C (Fig. 5C). The addition of 0.25 × 10⁻⁶ M RTX to 0.01 M nicotine solution enhanced the NaCl CT response and shifted the temperature curve to the left. The mean temperature at which the CT response gave the maxi-
mum response in the presence of 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁵ M Bz + 0.015 M nicotine (R + N + Bz + Nic) and 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + 0.015 M nicotine + 0.25 × 10⁻⁶ M RTX (R + N + Bz + Nic + RTX) was 42.2 ± 0.3 and 39.3 ± 0.1 °C (n = 3; P < 0.05), respectively. The results suggest that nicotine enhances the CT response to NaCl without affecting the temperature threshold of the TRPV1 cation channel. In contrast, a mixture of nicotine and a subthreshold concentration of RTX enhanced the magnitude of the NaCl CT response and shifted the temperature curve to the left. The results further suggest that nicotine and RTX act on different sites on the TRPV1 cation channel.

EFFECT OF NICOTINE ON SALT RESPONSES IN WT AND TRPV1 KO MICE.

To confirm that nicotine produces its effect on the Bz-insensitive NaCl CT responses as a function of apical pH. At pH 6.5 stimulating the tongue with 0.01 M KCl + 0.1 M NaCl (R + N) produced a CT response and a significant part of the CT response was Bz-insensitive (0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁵ M Bz; R + N + Bz). Stimulation of the tongue with 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz + nicotine (Nic; 0–0.015 M) produced a dose-dependent increase in the CT response. The data from three WT mice are summarized in Fig. 6B and suggest that CT responses in WT mice are qualitatively similar to the responses observed in rats (Fig. 3) within the range of 0–0.015 M nicotine concentration.

In contrast, TRPV1 KO mice (Fig. 6C) demonstrated no CT response to 0.01 M KCl + 0.1 M NaCl + 5 × 10⁻⁶ M Bz containing 0.005 M (R + N + Bz + 0.005 M Nic), 0.01 M (R + N + Bz + 0.01 M Nic), or 0.015 M nicotine (R + N + Bz + 0.015 M Nic). Essentially similar results were obtained in two additional KO mice. These results are consistent with the observations that CT salt responses in TRPV1 KO mice are insensitive to TRPV1 agonists (Lyall et al. 2004b, 2005a,b,c).

EFFECT OF NICOTINE-INDUCED INTRACELLULAR ALKALINIZATION AND CELL SWELLING ON THE CT RESPONSES TO NaCl AND HCl. Figure 7A shows the effect of nicotine on the Bz-sensitive and Bz-insensitive NaCl CT responses as a function of apical pH. At pH 6.5 stimulating the tongue with 0.01 M KCl + 0.1 M NaCl + 0.015 M nicotine (R + N + Nic₆.₅) produced a bigger CT response (e–f) relative to 0.01 M KCl + 0.1 M NaCl (R + N₆.₅; a–b). At pH 6.5 the increase in the NaCl CT response could be accounted for by the increase in the Bz-insensitive component of the NaCl CT response [(g–h) > (c–d)]. At pH 6.5 nicotine produced no effect on the Bz-sensitive component of the NaCl CT response [(j–g) = (b–c)]. At pH 9, NaCl (R + N₉.₀) produced a bigger CT response (i–j) relative to pH 6.5 (R + N₆.₅; a–b). The increase in the NaCl CT response could be accounted for by the increase in the Bz-sensitive component of the NaCl CT response [(i–j) > (b–c)]. At pH 9, superfusing the tongue with R + NaCl + 0.015 M nicotine (N + Nic₉.₀), further enhanced the magnitude of the CT response [(m–n) > (i–j)]. The increase in the NaCl CT response could be accounted for by the increase in the Bz-sensitive component of the NaCl CT response [(n–o) > (j–k)]. At pH 9, nicotine produced no effect on the Bz-insensitive component of the NaCl CT response [(o–p) = (k–l)]. In three animals (Fig. 7C), R + NaCl at pH 9 and R + NaCl + 0.015 M nicotine at pH 9 increased the Bz-sensitive NaCl CT response by 23.5 ± 4.5 and 57.6 ± 5.2%, respectively, relative to pH 6.5 (P < 0.01; n = 3). At pH 6.5, nicotine produced no significant increase in the Bz-sensitive NaCl response but specifically increased the Bz-insensitive component of the NaCl CT response (P < 0.01; paired; n = 3; Fig. 7C). These results suggest that at pH 6.5, nicotine specifically affects the Bz-insensitive part of the NaCl CT response and at pH 9, it affects only the Bz-sensitive ENaC component of the response.

As reported earlier (Lyall et al. 2002b), a decrease in pHᵢ of the acid-sensing TRCs is the proximate signal for the elicitation of both the phasic and tonic parts of the CT response. We further tested whether nicotine entry into TRCs and the subsequent decrease in pHᵢ will interfere with the CT response to acidic stimuli. CT responses to HCl were monitored in the presence and absence of nicotine at pH values that facilitate apical nicotine entry into TRCs (Fig. 1D). Figure 7B shows that when the rinse solution (0.01 M KCl + 0.01 M HEPES) was maintained at pH 6 (R₆), stimulating the tongue with 0.02 M HCl (HCl) elicited a fast phasic response (a) that slowly declined to the tonic response level (b). At a rinse pH of 6 (R₆), the magnitude of the phasic response to two 0.02 M HCl
stimulations is represented by (a–b) and (k–l) at the beginning and at the end of the CT record. At a rinse pH of 9 (0.01 M KCl + 0.01 M Tris; R9), the phasic CT response during two stimulations with 0.02 M HCl (c–d and e–f) was smaller relative to at pH 6. In the final step, rinsing the tongue with rinse solution at pH 9 containing 0.015 M nicotine (R9) further inhibited the magnitude of the CT response during two stimulations with 0.02 M HCl (g–h and i–j). In contrast to the phasic response, the magnitude of the tonic part of the CT response to 0.02 M HCl was not altered when the rinse solution was at pH 9 or when the rinse solution contained, in addition, 0.015 M nicotine. Essentially similar results were obtained in two additional experiments. These results suggest that at alkaline pH nicotine specifically attenuates the magnitude of the phasic response to acidic stimulation.

**DISCUSSION**

Results presented in this study support the conclusion that nicotine modulates both the Bz-insensitive (ENaC) and Bzsensitive (TRPV1t) salt taste receptors. Whether nicotine will stimulate TRPV1t or ENaC is largely determined by changes in the apical pH (pHo) or TRC pHi, respectively. Nicotine at 0.015 M had an enhancing effect on TRPV1t (as demonstrated by the increase in the CT response to NaCl + Bz) at all pHo values tested ranging from 3.1 and 9.0. However, the magnitude of the enhancement varied with pHo as a bell-shaped function (Fig. 5A, inset). At pHo values between 6 and 7, nicotine produced maximum enhancement of the apical TRPV1t cation channel. Above pH 7 the CT response declined and at pHo values >8, the CT response was about the same as at pH 3.1. At pH values <8, the permeability of the apical membrane to nicotine is negligible (Fig. 1). This suggests that changes in TRC pH do not play a role in the nicotine-induced activation of TRPV1t. The physiological implication of the interactions of nicotine and RTX with TRPV1t will be discussed in the following text. In contrast, at pH values >8, the permeability of the apical membrane to nicotine is increased (Fig. 1). Its entry into TRCs caused intracellular alkalinization that in turn activated ENaCs (Fig. 7, A and C). At pH values >8, TRPV1t is much less responsive to nicotine than at pH 6–7 (Fig. 5A, inset). Above pH 8, nicotine primarily affects ENaC. The basolateral application of nicotine induced rapid intracellular alkalinization and cell swelling, cellular responses consistent with its properties as a "base" (Fig. 1). The effects of nicotine entry from the apical membrane at alkaline pH on TRC pH, volume, and on salty and sour taste modalities will also be discussed in the following text.

**Modulation of TRPV1t by nicotine**

Results presented herein demonstrate that nicotine modulates CT responses to mineral salts. The effect of nicotine on the CT responses to mineral salts was observed even at low salt concentrations (0.01 M KCl). At low salt concentration increasing nicotine concentration increased the duration of the phasic response (Figs. 3A and 4A) and at higher concentrations elicited both phasic and tonic CT responses (Figs. 3A, inset and 4B). The increase in the duration of the phasic response and the appearance of the tonic response is related to the increase in the unilateral apical cation flux in the presence of nicotine (Fig. 2, B and C). The increase in the magnitude of the CT response to
0.01 M KCl in the presence of nicotine was dose dependent, indicating that the cation flux also varies with nicotine concentration. There is a bell-shaped relationship between nicotine concentration and the CT response to 0.1 M NaCl (Figs. 3A and 4C). In our previous studies, a similar bell-shaped dose–response relation was observed with other TRPV1t agonists, such as RTX, CAP, cetylpyridinium chloride (CPC) (Lyall et al. 2004b), and ethanol (Lyall et al. 2005c). RTX and CAP produced maximum enhancement of the Bz-insensitive NaCl CT response at $1 \times 10^{-6}$ and $40 \times 10^{-6}$ M and completely inhibited the CT response at $10 \times 10^{-6}$ and $200 \times 10^{-6}$ M, respectively (Lyall et al. 2004b, 2005a). By comparison, nicotine is a far less potent modulator of TRPV1t because it caused a maximum enhancement in the Bz-insensitive NaCl CT response at 0.015 M and inhibited the response at 0.1 M by 80% relative to N + Bz alone (Fig. 4C). There are additional important quantitative differences between nicotine and other TRPV1t agonists. Nicotine induced a mean maximum enhancement of the CT response in the presence of 0.1 M NaCl + $5 \times 10^{-6}$ M Bz by 72%. In contrast RTX, CAP, CPC, and ethanol increased the maximum enhancements in the NaCl + Bz CT response of >100%. Second, the maximum suppression of the CT response to 0.1 M NaCl + $5 \times 10^{-6}$ M Bz by nicotine (19%) is less than that of the other agonists, which can suppress the response by as much as 100% (Lyall et al. 2004b, 2005a,c). Nonetheless, nicotine produces a concentration–response function that is qualitatively similar to that of a typical TRPV1t agonist.

Second, in the case of TRPV1, nicotine is not itself an agonist of the receptor, but it significantly sensitizes TRPV1 to the vanilloid, CAP (Liu et al. 2004). Thus nicotine has no effect on TRPV1 unless it is presented with capsaicin. In the case of the TRPV1t, nicotine itself is an agonist (Figs. 3A and 4C). This difference may be explained by the fact that, unlike TRPV1, which requires heat or a chemical agonist to become active, TRPV1t is constitutively active, so that nicotine modulates the receptor ion channel that is already in a conducting state.

Third, TRPV1 is activated by a decrease in pHo (Davis et al. 2002; Geppetti and Trevisani 2004; Gunthorpe et al. 2002). In contrast, in the absence of a TRPV1t agonist, the Bz-insensitive NaCl CT response is not affected by changes in pHo. Similarly, in the absence of an agonist, changes in pHo between 4.7 and 9.7 had no effect on the temperature threshold of the Bz-insensitive NaCl CT response. This indicates that the constitutively active TRPV1t is not affected by changes in pHo (Lyall et al. 2004b, 2005a). However, the CT responses activated by a TRPV1t agonist were strongly dependent on pHo. In our studies, CT response to NaCl + Bz + RTX was bell-shaped with the maximal response around pH 6 with lower responses observed both above and below pH 6 (Lyall et al. 2004b, 2005a). In addition, changing pHo from 6.0 to either 4.7 or 9.7 in the presence of RTX decreased the temperature threshold of the CT response (Lyall et al. 2004b, 2005a). Taken together, the data indicate that the TRPV1t cation channel in TRCs is affected by pHo only in the presence of an agonist (Lyall et al. 2004b, 2005a) such as nicotine (Fig. 5A). At constant pHo, changes in TRC pH do not affect salt responses in the presence of RTX (Lyall et al. 2004b, 2005a). Therefore it is likely that H+ ions bind to an external site on the TRPV1t channel protein and modulate the affinity of nicotine and RTX to their respective intracellular binding sites on the channel protein.

Nicotine enhanced the NaCl CT response without a shift in the temperature threshold of the CT response (Fig. 5B). In this regard the effect of nicotine on TRPV1t is similar to that reported on the cloned TRPV1 receptor. Nicotine did not alter the threshold temperature of heat-activated currents in trigeminal ganglion neurons expressing TRPV1 (Liu et al. 2004).

SB-366791 inhibited the constitutive activity of TRPV1t and caused a rightward shift in the concentration–response to nicotine (Fig. 4C). Topical application of SB-366791 also inhibited RTX-, CAP-, temperature-, and ethanol-induced increases in Bz-insensitive NaCl CT responses (Lyall et al. 2004b, 2005a,c). Similarly, SB-366791 inhibits TRPV1 when activated by H+, elevated temperatures, CAP, and ethanol. SB-366791 produced similar rightward shift in concentration–response to CAP in hTRPV1-expressing cells (Gunthorpe et al. 2004). This suggests that SB-366791 may also act as a competitive antagonist of TRPV1t cation channel. Because at present the detailed structure of TRPV1t is not known, it is not clear how SB-366791 competitively blocks the effects of nicotine, RTX, CAP, ethanol, and temperature on the receptor cation channel. However, based on the effect of SB-366791 on TRPV1 receptor channel two mechanisms of inhibition have been proposed. One possibility is that SB-366791 binds at the CAP-binding site on the TRPV1 receptor and affects the gating mechanism of the cation channel. SB-366791 can work not only as a true competitive antagonist but also as an inhibitor of the agonist properties of RTX, CAP, ethanol, and temperature by an allosteric effect on the cation channel (Gunthorpe et al. 2004). The second possibility is that SB-366791 is a pure allosteric effector of TRPV1t and produces an agonist-independent inhibition of the channel at an independent allosteric site (Gunthorpe et al. 2004). However, the preceding two mechanisms are based on the assumption that all agonists produce effects on TRPV1 receptor by a common mechanism that involves gating of the receptor by shifting the temperature threshold of channel activation to lower temperatures. However, nicotine sensitizes both cloned TRPV1 and TRPV1t without a shift in the temperature threshold of the channel.

In HEK-293 cells expressing TRPV1, CAP increased the outward currents at room temperature, and induced a leftward shift in the voltage-dependent activation curve in a dose-dependent manner (Voets et al. 2004). This suggests that TRPV1 agonists function as gating modifiers. Because the Bz-insensitive NaCl CT response is observed in the absence of any agonists and demonstrates significant voltage sensitivity but not voltage dependence (Fig. 3C), it suggests that TRPV1t is constitutively active at room temperature in the absence of agonists (Figs. 3–6). It follows that TRPV1t is active at resting TRC membrane potential. Nicotine not only increased the Bz-insensitive NaCl CT response but also increased the Bz-insensitive apical Na+ conductance. In addition, making the TRC potential more negative enhanced the CT response to 0.1 M NaCl (Figs. 3C and 4D). Taken together, these results suggest that RTX-, CAP-, ethanol-, and nicotine-induced activation of TRPV1t most likely does not involve a leftward shift in the voltage-dependent activation curve of the channel. It is likely that TRPV1t agonists increase salt responses by stabilizing the channel in the open state, and thereby further poten-
tivating the Bz-insensitive Na\(^+\) flux across the apical membrane of TRCs. This results in the enhancement of the CT responses to mineral salts.

At present the exact mechanism of how nicotine modulates the activity of TRPV1 or TRPV1t is not known. However, several hypotheses have been put forward as possible mechanism of nicotine-induced sensitization of the TRPV1 channel (Liu et al. 2004). These include: nicotine-induced increase in the number of functional channels that increases the probability of the channel to remain in its open state (Hui et al. 2003; Kwak et al. 2000) or alterations of the cell membrane’s mechanical properties that can in turn affect its binding to phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) (Hui et al. 2003; Prescott and Julius 2003).

Nicotine also demonstrated interactions with RTX (Figs. 3C and 4D). In the presence of a subthreshold concentration of RTX (0.25 \(\times\) \(10^{-6}\) M) the nicotine-induced increase in the Bz-insensitive NaCl CT response was greater than that observed with RTX or nicotine alone. This increase in the response is related to the corresponding increase in the Bz-insensitive apical Na\(^+\) conductance in the presence of nicotine and RTX (Fig. 4D). In addition, in the presence of 0.25 \(\times\) \(10^{-6}\) M RTX, nicotine enhanced the CT response at room temperature and at elevated temperatures and shifted the temperature threshold of the channel to the left (Fig. 5C). These results suggest that the binding of RTX either to the intracellular binding site for CAP or to another ligand-independent allosteric site alters the conformation of the channel and sensitizes the channel to further stimulation with nicotine. The specificity of nicotine as a salt taste modulator is further demonstrated by the observation that TRPV1 KO mice, which lack the Bz-insensitive component of the NaCl CT response, also do not respond to nicotine (Fig. 6C), ethanol, RTX, and elevated temperatures (Lyall et al. 2005c). The data support the conclusion that nicotine produces its effect on salt responses by the amiloride- and Bz-insensitive TRPV1t cation channel in fungiform TRCs. However, the exact mechanism of how nicotine modulates the TRPV1t cation channel in TRCs remains to be established.

**Possible consequences of nicotine-induced intracellular alkalinization and cell swelling in TRCs**

Changes in TRC pH\(_i\) and cell volume are involved in modulating salt taste (Lyall et al. 1995, 1999, 2002b) and acid taste transduction (Lyall et al. 2001, 2002a,b, 2006). Consistent with this our data indicate that at pH values >8, nicotine enters TRCs across the apical membrane, causing intracellular alkalinization and activation of ENaC. This results in an increase in Bz-sensitive NaCl CT response (Fig. 7, A and C). The pH of saliva secreted by the unstimulated human parotid gland ranges from 5.45 to 6.06. On stimulation the pH of parotid saliva increases to a maximum of 7.8 (Davenport 1982). It is likely that under these conditions the apical entry of nicotine into TRCs is enhanced. In a subset of TRCs an acid-induced decrease in pH\(_i\) is the proximate signal for sour taste transduction (Lyall et al. 2001, 2002a,b, 2004a). In acid-sensing TRCs, a decrease in pH\(_i\) may be sensed by PKD2/L1 channel (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006). A decrease in TRC pH\(_i\) is responsible for the elicitation of both the phasic and the tonic parts of the CT response to acid stimulation (Lyall et al. 2001, 2002b).

The nicotine-induced intracellular alkalinization is accompanied by cell swelling (Fig. 2A). This results in a significant inhibition of the phasic part of the CT response to acids.

In addition, cell shrinkage activates Bz-sensitive ENaCs (Ji et al. 1998; Lyall et al. 1999, 2005c). Indeed, hypertonic mannitol- or cellobiose-induced TRC shrinkage in vivo increased the Bz-sensitive NaCl CT responses in rats (Lyall et al. 1999). The activity of the rat α-, β-, and γ-subunits of the epithelial Na\(^+\) channel expressed in Xenopus oocytes was not affected by cell swelling or mechanically induced changes of membrane tension (Awadya and Subramanyam 1998). However, in another study (Ji et al. 1998), in hypotonic media, rat α-, β-, and γ-subunits of the epithelial Na\(^+\) channel expressed in Xenopus oocytes demonstrated a decrease in current amplitude through the expressed channel. It is likely that the nicotine-induced cell swelling will inhibit Na\(^+\) flux through apical Bz-sensitive ENaCs and attenuate CT responses to NaCl. Thus in the presence of nicotine, both an increase in TRC pH\(_i\) and cell swelling will affect NaCl CT responses. However, under these conditions there was a net increase in the Bz-sensitive NaCl CT response (Fig. 7, A and C). This indicates that in TRCs the activation of ENaC by intracellular alkalinization is significantly greater than its inhibition by cell swelling.

The results presented in this study demonstrate that nicotine produces specific effects on the CT taste nerve responses to salty and sour taste stimuli. These effects are taste quality specific and occur through specific taste receptors and downstream intracellular effectors. Nicotine effects are dependent on pH\(_i\) and nicotine concentration and are modulated by temperature and the presence of other agonists. In the case of both salty and sour taste, although an increase in pH\(_i\) and cell volume are common intracellular effectors, they inhibit phasic responses to HCl but increase the ENaC activity, resulting in enhanced Bz-sensitive NaCl CT response. In contrast, nicotine is an agonist of TRPV1t and, depending on its concentration, is both an agonist and antagonist of the Bz-insensitive NaCl CT response. These effects of nicotine are modulated by temperature, pH\(_i\), and the presence of other TRPV1t agonists, such as RTX. This is in contrast to the nicotine-induced suppression of all gustatory responses (sucrose, NaCl, citric acid, monosodium glutamate, and quinine) of neurons in the nucleus of the solitary tract. This effect is most likely due to a nicotinic acetylcholine receptor-mediated excitation of trigeminal afferents that inhibit nucleus of the solitary tract units centrally (Simons et al. 2006).

The main points of this study can be integrated into a model of Na\(^+\) transport and salt taste transduction mechanism in the anterior tongue (Fig. 8). In fungiform TRCs Na\(^+\) transport occurs through both cellular and transcellular pathways. Na\(^+\) ions enter TRCs across the apical membrane by at least two pathways. One pathway involves Na\(^+\) entry through the apical amiloride- and Bz-sensitive ENaCs and is responsible for the amiloride- and Bz-sensitive CT component of the NaCl CT response. The second pathway involves TRPV1t, a nonselective cation channel that is permeable not only to Na\(^+\) but also to K\(^+\), NH\(_4\)\(^+\), and Ca\(^{2+}\) ions (Lyall et al. 2004b, 2005a,b). In rat fungiform TRCs it accounts for all of the Bz-insensitive CT responses to Na\(^+\) salts and part of the CT response to K\(^+\), NH\(_4\)\(^+\), and Ca\(^{2+}\) salts (Lyall et al. 2004b, 2005a). TRPV1t is maximally active around pH 6. The entry of Na\(^+\) depolarizes the receptor potential leading to the activation of membrane
Voltage-gated Ca$^{2+}$ channels (VGCC), an increase in [Ca$^{2+}$], and subsequent release of the neurotransmitter. The exit of Na$^+$ from TRCs occurs by the basolateral Na$^+-$K$^+$ ATPase. An additional Na$^+$ transport mechanism involves the basolateral Na$^+$-$H^+$ exchanger isofom 1 (NHE-1) (Vinnikova et al. 2004). The apical Na$^+$-$H^+$ exchanger isofom 3 (NHE-3) seems to be quiescent in TRCs (Vinnikova et al. 2004a). The transcellular transport of Na$^+$, K$^+$, NH$_4^+$, and Ca$^{2+}$ ions also occurs by the paracellular shunt mechanism and is anion dependent (Ye et al. 1991). In the presence of mineral salts nicotine increases the Bz-insensitive apical cation flux in TRCs and elicits CT responses that are similar to salt responses, consisting of both a phasic component and a sustained tonic component. Below 0.015 M nicotine enhanced and above 0.015 M it inhibited CT responses to 0.1 M NaCl. Nicotine produced maximum enhancement of the CT response between pH 6 and 7. Stimulating the tongue with solutions containing RTX and nicotine at elevated temperature increased the sensitivity of the CT response to nicotine. Because the effects of nicotine on mineral salts are blocked by SB-366791 and because TRPV1 KO mice are insensitive to nicotine, ethanol, RTX, and temperature, we conclude that nicotine produces these taste effects by direct action on the Bz-insensitive TRPV1 salt taste receptor. Above pH 8 or when nicotine levels increase in the blood, nicotine permeates the TRC membranes and alkalizes TRCs. The increase in TRC pH$_i$ in turn, increases ENaC activity and the magnitude of the Bz-sensitive NaCl CT response.

An acid-induced decrease in a subset of TRCs is a proximate signal for both the phasic and tonic components of the CT response to acidic stimuli (DeSimone and Lyall 2006). A decrease in pH$_i$ leads to cell shrinkage and the activation of a flufenamic acid-sensitive membrane conductance that gives rise to the phasic CT response to acids. Nicotine increases resting TRC pH$_i$ and volume and thus leads to the inhibition of the phasic component of the CT response to acid stimulation. However, at present it is not clear whether nicotine affects salty or sour taste transduction mechanisms residing in type II (receptor) or type III (presynaptic) cells (Huang et al. 2007). These conclusions are consistent with the observations that in human nonusers of smokeless tobacco, use of smokeless tobacco reduces perceived intensity of salty, sour, and bitter stimuli (Mela 1989). In humans the amiloride-sensitive component contributes about 20% to the taste response to NaCl (DeSimone and Lyall 2006). Thus it is likely that in humans the predominant effect of nicotine on salt taste is by the amiloride-insensitive component of the taste response to NaCl.

**ACKNOWLEDGMENTS**

We thank V. Bickel for help with artwork.

**GRANTS**

This work was supported by National Institute on Deafness and Other Communication Disorders Grants DC-00122 to J. A. DeSimone and DC-005981 to V. Lyall and by a Philip Morris USA grant to J. A. DeSimone.

**REFERENCES**


---

**FIG. 8.** Proposed model for Na$^+$ transport in fungiform TRCs and salt taste transduction in the anterior tongue. ENaC, amiloride-sensitive epithelial Na$^+$ channel; TRPV1, transient receptor potential variant salt taste receptor-1; NHE-1, basolateral Na$^+$-$H^+$ exchanger-1; NHE-3, apical Na$^+$-$H^+$ exchanger-3; Bz, benzamid; Nic, nicotine; ETH, ethanol; RTX, resiniferatoxin; SB-366791, N-(3-methoxyphenyl)-4-chlorocinnamide; [Na]o, external Na$^+$; tight junction: increase (↑) and decrease (↓). See text for details.


