TRPM5-dependent amiloride- and benzamil-insensitive NaCl chorda tympani taste nerve response

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Ren Z, Rhyu M, Phan TT, Mummalaneni S, Murthy KS, Grider JR, DeSimone JA, Lyall V. TRPM5-dependent amiloride-and benzamil-insensitive NaCl chorda tympani taste nerve response. Am J Physiol Gastrointest Liver Physiol 305: G106–G117, 2013. First published May 2, 2013; doi:10.1152/ajpgi.00053.2013.—Transient receptor potential (TRP) subfamily M member 5 (TRPM5) cation channel is involved in sensing sweet, bitter, umami, and fat stimuli, complex-tasting divalent salts, and temperature-induced changes in sweet taste. To investigate if the amiloride- and benzamil (Bz)-insensitive NaCl chorda tympani (CT) taste nerve response is also regulated in part by TRPM5, CT responses to 100 mM NaCl + 5 μM Bz (NaCl + Bz) were monitored in Sprague-Dawley rats, wild-type (WT) mice, and TRP vanilloid subfamily member 1 (TRPV1) and TRPM5 knockout (KO) mice in the presence of resiniferatoxin (RTX), a TRPV1 agonist. In rats, NaCl + Bz + RTX CT responses were also monitored in the presence of triphenylphosphine oxide, a specific TRPM5 blocker, and capsazepine and N-(3-methoxyphenyl)-4-chlorocrocinamid (SB-366791), specific TRPV1 blockers. In rats and WT mice, RTX produced biphastic effects on the NaCl + Bz CT response, enhancing the response at 0.5–1 μM and inhibiting it at >1 μM. The NaCl + Bz + SB-366791 CT response in rats and WT mice and the NaCl + Bz CT response in TRPV1 KO mice were inhibited to baseline level and were RTX-insensitive. In rats, blocking TRPV1 by capsazepine or TRPM5 by triphenylphosphine oxide inhibited the tonic NaCl + Bz CT response and shifted the relationship between RTX concentration and the magnitude of the tonic CT response to higher RTX concentrations. TRPM5 KO mice elicited no constitutive NaCl + Bz tonic CT response. The relationship between RTX concentration and the magnitude of the tonic NaCl + Bz CT response was significantly attenuated and shifted to higher RTX concentrations. The results suggest that pharmacological or genetic alteration of TRPM5 activity modulates the Bz-insensitive NaCl CT response and its modulation by TRPV1 agonists.

PAINS: triphenylphosphine oxide; resiniferatoxin; capsazepine; SB-366791; TRPV1

APPETITIVE AND AVERSIVE NEURAL and behavioral responses to NaCl are most likely transduced by a variety of mechanisms (35). Several studies suggest that in the anterior tongue Na+ from a Na+ salt taste stimulus enters a subset of taste bud cells by at least two types of cation channels located in taste cell apical membranes. One channel type is the Na+-specific epithelial Na+ channel (ENaC), in which Na+ transport is inhibited by amiloride and benzamil (Bz) (1, 3, 8). The second involves Na+ transport through a putative transient receptor potential (TRP) vanilloid subfamily member 1 (TRPV1)-dependent amiloride- and benzamil-insensitive NaCl chorda tympani taste nerve response. In rats, blocking TRPV1 by capsazepine or TRPM5 by triphenylphosphine oxide inhibited the tonic NaCl + Bz CT response and shifted the relationship between RTX concentration and the magnitude of the tonic CT response to higher RTX concentrations. TRPM5 KO mice elicited no constitutive NaCl + Bz tonic CT response. The relationship between RTX concentration and the magnitude of the tonic NaCl + Bz CT response was significantly attenuated and shifted to higher RTX concentrations. The results suggest that pharmacological or genetic alteration of TRPM5 activity modulates the Bz-insensitive NaCl CT response and its modulation by TRPV1 agonists.

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in the presence of capsazepine (CZP) and SB-366791, specific blockers of TRPV1 and the Bz-insensitive NaCl CT response (15, 26, 27). Our results show that the CT response at 100 mM NaCl was significantly smaller in TRPM5 KO than WT mice. However, at high NaCl concentration (300 mM), the CT response was not different between the two genotypes. The difference in the CT response to 100 mM NaCl between WT and TRPM5 KO mice was due to the absence of the Bz-insensitive component of the NaCl CT response in TRPM5 KO mice. There was no difference in the Bz-sensitive component of the NaCl CT response between the two genotypes. The dose-response relationship between RTX concentration and the magnitude of the Bz-insensitive tonic NaCl CT response was significantly attenuated and was shifted to higher RTX concentrations in TRPM5 KO than WT mice. A similar shift in the RTX dose-response relationship was observed when TRPM5 activity was inhibited with TPPO or when TRPV1 activity was inhibited with CZP. Taken together, our results suggest that pharmacological or genetic alteration of TRPM5 activity in TRCs modulates the Bz-insensitive NaCl CT response at low NaCl concentrations in the absence and presence of TRPV1 modulators.

MATERIALS AND METHODS

CT taste nerve recordings. Animals were housed in the Virginia Commonwealth University animal facility in accordance with institutional guidelines. All animal protocols were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Female Sprague-Dawley rats (150–200 g body wt; Charles River, Wilmington, MA) were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg; Sigma-Aldrich, St. Louis, MO), and supplemental pentobarbital sodium (20 mg/kg) was administered as necessary to maintain surgical anesthesia. The animal’s corneal reflex and toe-pin 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. Stimulus solutions maintained at room temperature were injected into a Lucite lingual perfusion channel (3 ml at 1 ml/s) affixed by vacuum to a 28-mm² patch of anterior dorsal lingual surface. CT responses were recorded under zero lingual current clamp and analyzed as described previously (20).

CT responses were also monitored in WT (C57BL/6j) mice and homozygous TRPV1 KO mice (B6.129S4-TRPV1<sup>−/−</sup> Jackson Laboratory, Bar Harbor, ME), TRPM5 KO mice (obtained from Dr. Charles Zucker, University of California, San Diego), and PLCβ2 KO mice (obtained from Dr. Stephen D. Roper, University of Miami). Mice (30–40 g body wt) were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg), and supplemental pentobarbital sodium (10 mg/kg) was administered as necessary to maintain surgical anesthesia. Because our lingual perfusion chamber is too large for the mouse tongue, CT recordings were made in mice while the anterior tongue was stimulated by perfusion of the rinse solution or taste stimuli (3 ml) at the rate of 1 ml/s. The rest of the procedure was the same as described above for rats. At the end of each experiment, animals were humanely killed by an intraperitoneal overdose of pentobarbital sodium (~195 mg/kg body wt for rats and 150 mg/kg body wt for mice).

The rinse solution (R) was 10 mM KCl, and stimulating solutions contained 100 mM NaCl, 100 mM NaCl + 5 mM Bz, a specific blocker of ENaC; 100 mM NaCl + 5 mM Bz + 1 µM SB-366791 (SB), a specific blocker of TRPV1; 100 mM NaCl + 5 µM Bz + RTX (0–10 µM), a specific agonist of TRPV1 (26, 27); and 100 mM NaCl + 5 µM Bz + RTX (0–10 µM) + 10 µM CZP, a blocker of TRPV1. Bz, SB, CZP, and RTX were obtained from Sigma-Aldrich. In some experiments, CT responses were monitored in rats before and after the topical lingual application of TPPO (Fisher Scientific, Pittsburgh, PA), a specific blocker of the TRPM5 cation channel (38). TPPO was directly dissolved in DMSO (Sigma-Aldrich) and applied topically to the dorsal surface of the rat tongue for 20 min at a concentration of 2 mM. After 20 min, the tongue was thoroughly washed with the rinse solution for 10 min before the CT recordings. We previously showed that topical application of DMSO alone does not alter CT responses to taste stimuli (24). Typically, stimulus solutions remained on the tongue for 1 min. Control stimuli consisting of 300 mM NH₄Cl and 300 mM NaCl applied at the beginning and end of the experiment were used to assess preparation stability (31, 32). As in our previous studies, the control responses did not differ by more than 2–5% at the beginning and end of the experiment (11, 48).

The stimulus series used in the CT experiments to generate the relationship between RTX concentration and the magnitude of the NaCl + Bz CT response was as follows: R → 300 mM NH₄Cl → R → 300 mM NaCl → R → 100 mM NaCl → R → (100 mM NaCl + 5 µM Bz) → R → (100 mM NaCl + 5 µM Bz + RTX) → R. The R → (NaCl + Bz + RTX) → R step was repeated for each concentration of RTX between 0 and 10 µM. At the end of the RTX concentration series, the control stimuli were again applied (R → 300 mM NH₄Cl → R → 300 mM NaCl → R). In additional series, the stimulating solutions were 100 mM NaCl + 5 µM Bz + 1 µM SB + RTX or 100 mM NaCl + 5 µM Bz + 10 µM CZP + RTX.

In CT experiments, the tonic (steady-state) part of the NaCl CT responses was quantified. For quantification of the tonic part of a response, the area under the response vs. time curve was taken over the final 30 s of the response. To normalize this result, this area was divided by the area under the 300 mM NH₄Cl response curve over the final 30 s of the tonic response period. In some CT experiments, we also quantified the transient (phasic) part of the NaCl CT response. For quantification of the phasic part of the CT response, the height of the stimulus-induced maximum CT response relative to the baseline response was divided by the mean steady-state (tonic) response to 300 mM NH₄Cl. We also quantified the transient (phasic) response to the application of rinse solution to a tongue already superfused with the rinse solution (rinse artifact). The normalized data are reported as means ± SE of the number of animals (n) or percent change in tonic NaCl CT response relative to the response of NaCl + Bz in the control (6). Student’s t-test was employed to analyze the differences between sets of data. Since we are comparing the normalized CT responses to NaCl + Bz before and after RTX in the same CT preparation, a paired t-test was used to evaluate statistical significance. Comparisons between two strains of mice were made using an unpaired t-test (GraphPad).

For clarity, the points on the graphs of the mean normalized tonic responses vs. the log of the RTX concentration were connected by smooth curves. The curves were generated using a fitting function that models the characteristic biphasic property of the agonists of TRPV1. The biphasic property has been observed with every agonist of TRPV1 thus far examined (6, 11, 20, 26–30, 48). The fitting function was

\[ R = \frac{r + bh(x)}{1 + h(x) + j(x)} \]  

where

\[ h(x) = 10^{\theta(x-a)} \]  

and

\[ j(x) = 10^{\theta(x-d)} + m(x-c) \]

where \( R \) is the response, \( x \) is the log of the RTX concentration (mol/l), and \( a, b, d, m, n \), and \( r \) are parameters chosen by least-squares criteria.
RESULTS

Effect of RTX on the NaCl + Bz CT response in rats. Consistent with previous studies (20, 26, 27, 29–32, 48), RTX, a specific TRPV1 agonist, produced a biphasic effect on the rat CT response to 100 mM NaCl + 5 μM Bz. RTX at 0.5–1 μM enhanced the tonic CT response (Fig. 1, A and B). At >1 μM RTX, the NaCl + Bz CT response was inhibited. At 5 μM RTX, the response was the same as control. At 10 μM RTX, the response was below the control value. As expected, Bz inhibited the NaCl CT response (Fig. 1C; \( P = 0.0001 \) by paired \( t \)-test, \( n = 3 \)). In the presence of Bz + 1 μM SB-366791, the entire NaCl tonic CT response was inhibited to the baseline rinse level (Fig. 1C). RTX at 1 μM increased the NaCl + Bz tonic CT response (Fig. 1C; \( P = 0.0036 \)); however, it did not enhance the CT response above the baseline rinse level to lingual stimulation with NaCl + Bz + SB-366791 (Fig. 1C). These results show that SB-366791 inhibits the constitutive Bz-insensitive NaCl CT response and the RTX-induced enhancement of the NaCl + Bz CT response.

Effect of TPPO on the CT response to quinine. Topical lingual application of 2 mM TPPO, a specific blocker of TRP5 (38), partially inhibited the CT response to 20 mM quinine (Fig. 2; \( P = 0.023 \) by unpaired \( t \)-test, \( n = 3 \)). These results indicate that topical lingual application of 2 mM TPPO is sufficient for the drug to diffuse below the tight junctions to partially inhibit TRP5 localized in the basolateral membrane of a subset of TRCs (19).

Effect of TPPO and CZP on the Bz-insensitive NaCl CT responses in the absence and presence of RTX. As shown in a representative CT trace, after topical lingual application of 2 mM TPPO (Fig. 3B), the mean normalized tonic CT response to 100 mM NaCl + 5 μM Bz increased in the presence of RTX (Fig. 3C). While under control conditions the maximum increase in the Bz-insensitive NaCl CT response was observed at 1 μM RTX (Fig. 3, A and C), the maximum increase in the CT response following TPPO treatment was observed at 3 and 10 μM RTX relative to 0 RTX. \( P \) values at 0.75, 3, and 10 μM RTX were 0.04, 0.011, and 0.009, respectively, relative to 0 RTX. We hypothesize that, after TPPO treatment, higher concentrations of RTX may be needed to inhibit the CT response to the level at 0 RTX. TPPO decreased the magnitude of the NaCl + Bz + RTX tonic CT response and shifted the relationship between the RTX concentration and the magnitude of the tonic CT response to the right on the RTX concentration axis relative to control (Fig. 3C). These results suggest that modulation of the Bz-insensitive NaCl CT response by RTX is partially dependent on TRP5 activity in a subset of TRCs.

To directly test if the inhibition of the putative TRPV1t cation channel results in a shift in the relationship between the agonist concentration and the magnitude of the Bz-insensitive NaCl CT response, further experiments were performed in 100 mM NaCl and 100 mM NaCl + 5 μM Bz solutions containing 10 μM CZP. As shown by the representative CT trace (Fig. 4A) and our earlier studies (26, 27), under control conditions, RTX produced a biphasic effect on the CT response in the presence of NaCl alone or NaCl + Bz. We compared normalized CT responses to NaCl alone or to NaCl+Bz at two RTX concentrations, 0 and 1 μM. As shown in Fig. 4C, the Bz-sensitive component of the NaCl CT response [NaCl-(NaCl + Bz)] and the RTX-induced enhancement of the NaCl and NaCl + Bz CT response were not different from each other. Similarly, we did not observe differences in the Bz-sensitive component of the NaCl CT response and the RTX-induced increase in the CT response in the presence of NaCl or NaCl + Bz at other RTX concentrations (data not shown). In our previous studies (6), modulators of TRPV1/TRPV1t did not alter CT responses in

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Fig. 1. Effect of benzamil (Bz), resiniferatoxin (RTX), and SB-366791 (SB) on the rat NaCl chorda tympani (CT) response. A: representative rat CT response to lingual application of a rinse solution (R; 10 mM KCl), 100 mM NaCl + 5 μM Bz, and 100 mM NaCl + 5 μM Bz + RTX (0.25–10 μM). B: Bz-insensitive NaCl CT responses in each rat normalized to the corresponding CT responses obtained with 300 mM NH4Cl. Values are means ± SE of 3 animals. *\( P = 0.016, 0.007, 0.001, 0.006, \) and 0.0217 at 0.25, 0.50, 1.0, 2.5, and 10 μM RTX, respectively, relative to 0 RTX (paired). C: rat CT responses to lingual application of a rinse solution (10 mM KCl), 100 mM NaCl, 100 mM NaCl + 5 μM Bz, 100 mM NaCl + 5 μM Bz + 1 μM RTX, 100 mM NaCl + 5 μM Bz + 1 μM SB, and 100 mM NaCl + 5 μM Bz + 1 μM SB + 1 μM RTX. In each rat, the Bz-insensitive NaCl CT responses were normalized to the corresponding CT responses obtained with 300 mM NH4Cl. Values are means ± SE of 3 animals. *\( P = 0.0036 \) (paired).
the presence of NaCl + SB-366791, again demonstrating that TRPV1/TRPV1t modulators do not alter the Bz-sensitive component of the NaCl CT response. These results demonstrate that RTX modulates only the Bz-insensitive NaCl CT response.

In the presence of 10 μM CZP, the RTX-induced increase in the CT response to NaCl or NaCl + Bz was attenuated and the relationship between RTX concentration and the magnitude of the CT response was shifted to the right on the RTX concentration axis (Fig. 4B). The mean normalized rat CT responses to NaCl + Bz + RTX in the absence and presence of 10 μM CZP are summarized in Fig. 4D. In the presence of CZP, RTX still produced biphasic effects on the NaCl + Bz tonic CT response. However, the relationship between RTX concentration and the magnitude of the NaCl + Bz tonic CT response is shifted downward and to the right on the RTX concentration axis relative to control (Fig. 4D).

Studies with WT and KO mice. We recorded CT responses to 10 and 20 mM quinine in WT and TRPM5 KO mice. As expected (36), the WT mice responded with a concentration-dependent increase in the CT response to 10 and 20 mM quinine (Fig. 5A). In contrast, TRPM5 KO mice elicited only transient phasic CT responses to 10 and 20 mM quinine. No tonic CT response to 10 and 20 mM quinine above the baseline rinse level was observed in TRPM5 KO mice (Fig. 5, A and B).

In TRPM5 KO mice, the tonic CT response to quinine was eliminated. Furthermore, the phasic CT response to quinine was TRPM5-independent. These studies are consistent with the observation that neural responses to bitter compounds are significantly attenuated in TRPM5 KO mice (7, 50). In contrast to TRPM5 KO mice, even at high TPPO concentrations (2 mM), only partial inhibition of the quinine CT response was observed in rats (Fig. 2). Since TRPM5 is localized in the basolateral membrane of a subset of TRCs (19), significantly higher concentrations of TPPO may have to be applied topically to the tongue for it to diffuse below the tight junctions to completely inhibit the TRPM5 activity and the CT response to quinine to the baseline rinse level. We did not test the effect of TPPO in WT mice. We predict that TPPO will have the same effect in WT mice.

We recorded CT responses to 300 and 100 mM NaCl in WT and TRPM5 KO mice. While the mean normalized tonic CT response to 300 mM NaCl was not different between WT and TRPM5 KO mice (Fig. 5C), the tonic response to 100 mM NaCl was significantly lower in TRPM5 KO than WT mice. To determine if the difference between the CT responses is due to the Bz-sensitive or Bz-insensitive component of the NaCl CT response, neural responses were monitored in the presence of 5 μM Bz, a potent blocker of ENaC. At 100 mM NaCl, the WT mice demonstrated a Bz-sensitive and a Bz-insensitive component of the CT response (Fig. 6A). However, in TRPM5 KO mice, Bz inhibited the NaCl tonic CT response to the baseline rinse level (Figs. 5C and 6B). These results demonstrate that, at low NaCl concentrations (100 mM), TRPM5 KO mice lack the constitutive Bz-insensitive NaCl CT response. In contrast, the normalized Bz-sensitive (ENaC-dependent) component of the NaCl CT response was not different between the TRPM5 KO and WT mice (Fig. 5C).

To further characterize the differences in the Bz-insensitive NaCl CT responses in WT and TRPM5 KO mice in situ, we generated the relationship between RTX concentration and the magnitude of the tonic CT response to 100 mM NaCl + 5 μM Bz in the two mouse strains (Figs. 6 and 7). Consistent with our previous data (20, 26, 27, 29 –32, 48), in WT mice (Figs. 6A), RTX produced a biphasic effect on the NaCl + Bz CT response. Similar to the case with rats (Fig. 1, A and B), in WT mice, 1 μM RTX produced a maximum enhancement of the NaCl + Bz CT response. In contrast to the WT mice (Figs. 6A and 7A), TRPM5 KO mice elicited no Bz-insensitive NaCl CT response (Figs. 6B and 7A), and the relationship between RTX concentration and the magnitude of the NaCl + Bz response was shifted downward and to the right on the RTX concentration axis. As shown in Fig. 7B, the normalized peak NaCl + Bz phasic CT response in TRPM5 KO mice was significantly smaller than its value in WT mice but was not significantly different from the rinse artifact (Fig. 7B). At 1 μM, RTX enhanced the phasic CT response in WT and TRPM5 KO mice; however, the enhancement was significantly smaller in TRPM5 KO than WT mice. These results show that, at 100 mM NaCl, phasic and tonic components of the Bz-insensitive NaCl CT response are diminished in TRPM5 KO mice relative to their values in the WT mice.

Similar to TRPM5 KO mice, TRPV1 KO mice also did not elicit a CT response to 100 mM NaCl + 5 μM Bz. However, in TRPV1 KO mice, the NaCl + Bz + RTX tonic CT response remained at the baseline rinse level over the entire RTX...
concentration range (0–10 μM) (Fig. 7A) (48). In TRPV1 KO mice, the phasic CT response decreased to the rinse artifact level and was not altered in the presence of 1 μM RTX (Fig. 7B). These results show that phasic and tonic components of the Bz-insensitive NaCl CT response are inhibited to the baseline rinse levels in TRPV1 KO mice (31).

Since PLC/β2 and TRPM5 are essential downstream intracellular signaling effectors in sweet, bitter, and umami taste transduction, we next tested if Bz-insensitive NaCl CT responses in the absence and presence of RTX are also dependent on PLC/β2 activity. Unlike TRPV1 and TRPM5 KO mice, PLC/β2 KO mice elicited a Bz-sensitive and Bz-insensitive NaCl CT response. Varying RTX concentrations modulated the NaCl + Bz CT responses in a biphasic manner (Fig. 8A). Similar to rats and WT mice, in PLC/β2 KO mice, 1 μM RTX produced a maximum increase in the NaCl + Bz tonic CT response (Fig. 8B). These studies suggest that the Bz-insensitive NaCl CT response is specifically inhibited by pharmacological blocking or genetic silencing of the TRPM5 cation channel in TRCs but is not affected by genetic elimination of the PLC/β2 enzyme activity.

**DISCUSSION**

**Relationship between TRPV1/1 and TRPM5 and Bz-insensitive NaCl CT responses.** We observed significant differences in the CT response between TRPM5 KO and WT mice at low (100 mM), but not high (300 mM), NaCl concentrations (Fig. 5C). Consistent with these results, Damak et al. (7) also reported significant differences in the CT responses between TRPM5 KO and C57BL/6J control mice at lower (30 and 100 mM), but not higher (300 and 1,000 mM), NaCl concentrations. In our studies, we used TRPM5 KO mice with a partial deletion of TRPM5, such that they retain most of the amino-terminal portion of the gene (50). In contrast, Damak et al. used TRPM5 KO mice null for TRPM5 protein expression. Thus, despite slight differences in the TRPM5 KO mice constructed, in both studies TRPM5 KO mice show significantly
smaller NaCl CT responses at low (100 mM), but not high (300 mM), NaCl concentrations. In contrast, in two other studies, TRPM5 KO mice gave a normal CT response to 100 mM NaCl normalized to the CT response to 100 mM citric acid (50) or to 100 mM NH₄Cl (46) relative to C57BL/6J control mice. Since normalized to the CT response to 100 mM NaCl (50) or NaCl concentrations. In contrast, in two other studies, smaller NaCl CT responses at low (100 mM), but not high (300 mM), NaCl concentrations. In contrast, in two other studies, TRPM5 KO mice gave a normal CT response to 100 mM NaCl normalized to the CT response to 100 mM citric acid (50) or to 100 mM NH₄Cl (46) relative to C57BL/6J control mice. Since the difference in the magnitude of the NaCl CT response between the two genotypes is small (Fig. 5C), the difference in the magnitude of the NaCl CT response in the above-mentioned studies.

In earlier studies (7, 50), in WT and TRPM5 KO mice, amiloride was not used while CT responses to NaCl were recorded. Thus the magnitudes of the Bz-sensitive and Bz-insensitive components of the NaCl CT response were not quantified in the two genotypes in earlier studies (7, 46, 50). Our results using Bz show that the difference in the CT response between TRPM5 KO and WT mice at 100 mM NaCl is due to the absence of the Bz-insensitive component of the NaCl CT response in TRPM5 KO mice (Figs. 5C, 6, and 7). We did not observe any differences in the Bz-sensitive ENaC-dependent component of the NaCl CT response between the two genotypes. These results indicate that TRPM5 KO mice demonstrate a specific decrease in the Bz-insensitive NaCl CT response, while the Bz-sensitive NaCl CT response remains the same as in WT mice. These results suggest that the Bz-sensitive and Bz-insensitive NaCl CT responses not only originate in different taste cells within the taste bud but are also regulated independently of each other (3, 35).

WT and TRPM5 KO mice also showed marked differences in the relationship between RTX concentration and the magnitude of the tonic CT response to NaCl + Bz (Figs. 6 and 7). While in WT mice RTX produced biphasic changes in the NaCl + Bz tonic CT response with a maximum increase in the CT response at 1 μM RTX, in TRPM5 KO mice the RTX...
The constitutive Bz-insensitive NaCl response is blocked by submicromolar concentrations of the TRPV1 blockers SB-366791 and CZP in WT mice (Fig. 1), Sprague-Dawley rats, alcohol-preferring (P) rats, and alcohol-non-preferring (NP) rats (6, 26). In addition, several low-affinity blockers that inhibit the Bz-insensitive NaCl CT response in the micromolar-to-millimolar range include RTX, capsaicin, cetylpyridinium chloride, Maillard reacted peptides (MRPs), N-geranylcyclopropylcarboximide, ethanol, and nicotine (6, 11, 20, 26, 27, 29, 30, 40, 41, 48). Our whole nerve data are supported by the effect of TRPV1 agonists on single CT nerve fibers. In the CT nerve, a subset of the broadly tuned E-type fibers (presumably the amiloride-insensitive fibers) demonstrated enhancement and another subset of E-type fibers showed suppression of the NaCl response when the rat tongue was stimulated with a mixture containing 100 mM NaCl/5 μM Bz (37). It is likely that single units in the CT nerve have variable capsaicin dose-response relationships. In our earlier studies (26), capsaicin produced maximum enhancement and inhibition of the Bz-insensitive NaCl CT response at 40 and 200 μM, respectively. In contrast to the above studies, Breza and Contreras (2) reported that SB-366791 and cetylpyridinium chloride did not alter CT nerve or single-cell responses. The lack of effect of these antagonists may be related to significant differences in the methodology used: 1) in the study of Breza and Contreras, the CT responses were recorded while the tongue was perfused at a significantly slower flow rate (50 ml/s vs. 1
ml/s in our study); 2) the rinse solution was artificial saliva vs. 10 mM KCl in our study; 3) the tongue was superfused with solutions maintained at 35°C vs. room temperature in our study; and 4) a short sampling time of 5 s or 60 s in conjunction with slow flow rate. We previously showed that flow rate and temperature have significant effects on the NaCl CT response in the absence and presence of Bz (26). In our studies, at slow flow rates (~133 µl/s), the phasic component of the CT response to NaCl and HCl was not observed and the tonic CT response reached its maximum value ~2 min after the stimulation onset (25, 26).

The constitutive Bz-insensitive NaCl response is modulated by changes in TRC Ca²⁺ concentration, protein kinase C, calcineurin, and membrane PIP₂ levels (31, 32). It is spontaneously upregulated in P rats relative to NP rats. Exposure of NP rats to oral ethanol in a no-choice paradigm upregulated the Bz-insensitive NaCl CT response relative to that of the naive NP rats (6). Naive P rats and NP rats exposed to ethanol demonstrated enhancement of the NaCl + Bz CT response in the presence of RTX, with the maximum enhancement of the neural response at 1 µM. At low NaCl concentrations (2–32 mM), the amiloride-insensitive NaCl CT response was enhanced in the A/J mouse (5, 34). TRPM5 KO mice also show a deficit in the CT response at low NaCl concentrations only (30 and 100 mM) (7).

Consistent with our previous studies (26, 27), the spontaneous Bz-insensitive NaCl CT response was absent in TRPV1 KO mice (Fig. 1). Unlike WT mice, TRPV1 KO mice did not respond to RTX, ethanol, GalA-MRPs, and nicotine with an increase in the CT response above the baseline rinse level (6). However, in another study, CT responses to a concentration series of NaCl with and without amiloride did not differ between the two genotypes (45).

Fig. 6. Effect of RTX on the NaCl + Bz CT response in WT and TRPM5 KO mice. Representative CT responses of a WT (A) and a TRPM5 KO (B) mouse tongue were recorded during stimulation first with a rinse solution (10 mM KCl) and then with 100 mM NaCl + 5 µM Bz and 100 mM NaCl + 5 µM Bz + RTX (0.25–10 µM).
We previously showed that TRPV1 KO mice elicited CT responses to monosodium glutamate (MSG) + Bz + SB-366791 and MSG + Bz + SB-366791 + IMP that were not different from those observed in WT mice (32). In our earlier studies, 10 μM RTX inhibited the rat Bz-insensitive NaCl CT response to baseline but did not alter CT responses to quinine or sucrose (26). In addition, 1 μM SB-366791 inhibited the rat Bz-insensitive NaCl CT response to baseline but did not alter CT responses to quinine and sucrose and the IMP-induced increase in the CT response to MSG + Bz (32). These results would tend to support the notion that TRPV1 inhibition does not affect TRPM5 activity in type II cells within the taste bud. The results of the present study suggest that inhibition of TRPM5 activity attenuates the putative TRPV1/TRPV1t channel activity and results in the rightward shift of the RTX dose-response curve.

Relationship between neural and behavioral responses in WT and KO mice. The differences in the Bz-insensitive NaCl CT responses are most likely related to the reported behavioral difference between WT and KO mice. In two-bottle 48-h

result in a shift in relationship between the agonist concentration and the magnitude of the Bz-insensitive NaCl CT response. Therefore, the rightward shift in the RTX dose-response curve cannot be due to the changes in the phosphorylation/dephosphorylation state of the putative TRPV1 channel protein or changes in membrane PIP2 levels. However, in our studies, in the presence of a subthreshold concentration of a TRPV1 blocker, CZP (10 μM), the RTX dose-response curve was shifted to the right on the RTX concentration axis and the RTX-induced enhancement of the CT response was significantly blunted relative to control (Fig. 4). It is important to note that this concentration of CZP is significantly less than the CZP concentration (100 μM) that blocks 50% of the enhancement of the NaCl + Bz CT response in the presence of 0.75 μM RTX (26).
preference tests, TRPM5 KO mice were indifferent to NaCl concentrations between 18 and 75 mM, whereas WT mice tended to prefer these concentrations of NaCl (7). Both genotypes avoided NaCl concentrations between 300 and 600 mM, with TRPM5 KO mice showing slightly diminished avoidance relative to WT mice. In brief-access tests, WT mice showed aversion to 100–1,000 mM NaCl, and TRPM5 KO mice showed aversion to ≈200 mM NaCl. The KO mice showed decreased aversion to ≈1,000 mM NaCl relative to the WT mice. This relationship is also observed between different strains of mice. A/J mice, which show spontaneously greater amiloride-insensitive NaCl CT responses than C57BL/6J mice, were also behaviorally different from control mice. C57BL/6J mice preferred 25 mM NaCl over water, whereas A/J mice consumed water and 25 mM NaCl equally. At higher concentrations (75 and 225 mM), both genotypes had identical NaCl concentrations of behavioral differences between WT and KO mice (TRPV1 and TRPM5) may be able to directly modulate the Bz-insensitive NaCl response specifically by interacting with TRPV1t in human taste cells. Further studies are needed to resolve these issues.

In summary, the data presented here suggest that, at low NaCl concentrations, the constitutive Bz-insensitive NaCl CT response and its modulation by putative TRPV1t agonists are dependent on TRPM5 activity but are independent of PLCβ2 activity in a subset of TRCs (49). This pathway seems to be different from the classical bitter, sweet, and umami taste transduction pathway, which shows a strict requirement for PLCβ2 and TRPM5 (50).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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AUTHOR CONTRIBUTIONS

REFERENCES


