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Detection of NaCl and KCl in TRPV1 Knockout Mice

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Abstract

Both amiloride-sensitive and -insensitive mechanisms contribute to NaCl taste transduction. The amiloride-sensitive mechanism relies on the epithelial Na^+ channel ENaC, which is widely expressed on the apical membrane of fungiform taste cells. The amiloride-insensitive mechanism, which predominates in circumvallate and foliate taste buds, was recently reported to involve a variant of the nonselective cation channel TRPV1. We performed 2-bottle preference and threshold experiments with TRPV1 knockout mice and wild-type (C57BL/6J) controls to test for NaCl preference and detection thresholds in the presence and absence of amiloride. Surprisingly, TRPV1 knockout mice not only detected NaCl in the presence of amiloride but they preferred NaCl over water at concentrations avoided by the wild-type mice. NaCl detection thresholds were between 2 and 3 mM for both genotypes. Amiloride increased the detection thresholds of wild-type mice but not knockout mice. The knockout mice also preferred 100 mM KCl compared with wild-type controls, suggesting that TRPV1 receptors may mediate a general aversive response to salts. Analyses of consumption data also revealed that TRPV1 knockout mice ingested more of the NaCl, with and without amiloride, and KCl solutions than the wild-type mice. However, comparisons of preference ratios and consumption volumes indicated that both wild-type and TRPV1 knockout mice avoided citric acid in quite a similar manner, suggesting that TRPV1 receptors do not mediate the detection of citric acid. These data, taken together, suggest that additional mechanisms must contribute to the amiloride-insensitive NaCl response.

Key words: amiloride, behavior, salt, taste transduction, transient receptor potential, vanilloid

Introduction

Salt taste transduction in rodents involves both amiloride-sensitive and -insensitive pathways (Heck et al. 1984; DeSimone and Ferrell 1985; Ninomiya and Funakoshi 1988; Ye et al. 1993; Doolin and Gilbertson 1996; Lindemann 2001). The amiloride-sensitive pathway is blocked by low nanomolar concentrations of the diuretic drug amiloride. This pathway is mediated by the epithelial Na channel ENaC, a highly Na^+-selective cation channel expressed on the apical membrane of a large subset of taste cells in fungiform papillae (Kretz et al. 1999; Lin et al. 1999). In C57BL/6J mice, approximately 80% of the chorda tympani response amplitude to NaCl is amiloride-sensitive, suggesting that this pathway predominates in anterior tongue (Ninomiya et al. 1989; Gannon and Contreras 1995). However, the remaining chorda tympani response and the entire glossopharyngeal response to NaCl is amiloride insensitive, indicating that alternative mechanisms contribute to NaCl transduction (Ninomiya 1998). Recently, a variant of the nonselective cation channel TRPV1 was proposed as the amiloride-insensitive Na^+ taste receptor (Lyall et al. 2004). TRPV1 is a member of the vanilloid class of transient receptor potential (TRP) channels. TRPV1 is widely expressed in dorsal root and trigeminal ganglion neurons, where it mediates thermal nociception and pain sensation (Caterina et al. 2000). Several lines of evidence support a role for TRPV1 as a salt taste receptor (DeSimone et al. 2001; Lyall et al. 2004). First, vanilloid compounds and temperature, activators of TRPV1 in other cell types, modulate the amiloride-insensitive response to NaCl and also the response to other mineral salts. Second, the gene for TRPV1 is expressed in taste buds. Third, TRPV1 knockout mice lack an amiloride-insensitive chorda tympani response to NaCl and show reduced responses to other mineral salts. However, these mice have not been tested behaviorally for responsivity to NaCl or to other salts. One would predict that if TRPV1 is the only amiloride-insensitive Na^+ receptor, then TRPV1 knockout

mice should not be able to detect NaCl when amiloride is present to inhibit ENaC channels.

In this study we utilized both 2-bottle preference tests and detection threshold tests to examine preferences and detection thresholds for NaCl in TRPV1 knockout and wild-type (C57BL/6J) mice. Surprisingly, our 2-bottle preference data show that the TRPV1 knockout mice preferred NaCl compared with wild-type mice, independent of the presence of amiloride. Consequently, detection threshold tests were conducted to determine if NaCl thresholds of TRPV1 knockout mice were similar to those of wild-type mice and, more importantly, to determine if NaCl thresholds were predictably elevated by amiloride to a much greater extent in TRPV1 mice than in wild-type mice. The data for these experiments reveal that detection thresholds for NaCl are actually lower in knockout mice than wild-type mice when amiloride is present. These data suggest that although TRPV1 plays a role in Na^+ detection, it is not the only amiloride-insensitive mechanism for detection of NaCl.

Materials and methods

Two-bottle preference tests

Subjects

A total of 36 TRPV1 knockout mice and 36 age-matched adult wild-type controls (C57BL/6J) were obtained from Jackson Laboratories (Bar Harbor, ME). The TRPV1 strain was originated on a B6;129S background. It was then backcrossed to the C57BL/6 strain for at least five generations (JAX®Mice Database). In view of the strength of the C57Bl/6 background, the C57Bl/6J mice were appropriate as wild-type controls. Both males and females were used. The mice were housed in individual clear plastic cages with food (Teklad Rodent Diet 8640, Harlan Sprague Dawley) and water available ad libitum throughout the experiment. The colony was maintained on 12-h light/dark cycle with the onset of lights at 6 AM. The temperature of the room was kept at 20 °C. Each mouse was tested at approximately the same time of day.

Procedures

The 2-bottle preference test paradigm was used as described earlier (Ruiz et al. 2003). Prior to an experiment, mice were assigned to individual cages and given two 25-ml sipper bottles containing distilled water. These assured mice would become used to drinking from both bottles. Four days of water data were collected to determine and/or establish a 50/50 bottle preference ratio. Mice that preferred one sipper tube to the other were provided new sipper tubes and kept on water until the desired 50/50 ratio was established.

Distilled water was used in the control sipper tubes and as the vehicle for all taste solutions. Nine TRPV1 knockout mice and C57BL/6J mice were tested with the following concentrations of NaCl: 1, 3, 10, 30, 100, 200, and 300 mM. Mice (9 knockout and 9 wild type) assigned to the amiloride condition had 100 μM amiloride present in the control water as well as the NaCl solution. Because TRPV1 knockout mice showed reduced chorda tympani responses to KCl and other mineral salts (Lyall et al. 2004), another 9 knockout and 9 wild-type mice were tested with the following concentrations of KCl: 1, 3, 10, 30, 100, 200, and 300 mM (no amiloride). All mice were naive, that is, they had not been tested previously with taste stimuli and were tested with all concentrations of tastant presented in ascending order.

To determine if the enhanced preference in the knockout mice was specific for salts, naive mice (9 wild type, 9 knockout) were also tested with citric acid, a compound that is aversive to mice at higher concentrations. Mice were presented with citric acid at the following concentrations: 0.03, 0.1, 0.3, 3, 10, and 30 mM (no amiloride).

Testing consisted of presenting each mouse with a test solution in one bottle and water in the other bottle. Each test lasted 48 h. The amount of fluid sampled from each tube was measured every 24 h, and the bottles were switched to the opposing side to control for possible side preferences. After 48 h the quantity consumed was measured, new solution was added, and the bottles were switched to the opposite side. There were no rest periods between the different taste stimuli.

Data analyses

Prior to analyzing the results, the data for each mouse were converted into preference ratios: Preference ratio = (Total stimulus volume ingested in 48 h/Total fluid volume ingested in 48 h) × 100. The preference ratios for both knockout and wild-type mice were then compared with analysis of variance (ANOVA) and simple effects tests (Howell 1997) to determine if there were genotype differences in preference ratios for a particular taste stimulus relative to water. In addition, the total amount of each tastant consumed over each 48-h test period was analyzed to determine if the two genotypes ingested comparable amounts of each stimulus solution.

Detection threshold tests

Subjects

The NaCl detection thresholds of 5 TRPV1 knockout mice and 5 C57BL/6J wild-type mice were tested. All animals began the experiment at 65–70 days of age and were housed in separate cages with food (Purina Lab chow) available ad libitum. The mice were entrained to a 22-h water deprivation schedule that was maintained throughout the experiment. Colony lighting was maintained on a 12-h light/dark cycle with lights on at 7 AM. All testings took place during the light portion of the cycle, and each mouse was tested at the same time each day.

Testing apparatus

The mice were tested with a computer-controlled Knosys Ltd gustometer (Brosvic and Slotnick 1986) consisting of a Plexiglas operant chamber (12.5 × 12.5 × 16.5 cm). A small circular opening was located in one wall, 1 cm in diameter, and centered 3 cm above the floor. This opening gave the mouse access to a drinking spout with its tip positioned flush with the inner surface of the portal. A fan, mounted in the ceiling of the chamber, reduced olfactory cues by forcing air out of the chamber through the opening for the spout. Each taste solution was stored in 1 of 9 3-ml unpressurized syringe barrels, a minimum of 15 cm above the drinking spout. The flow of solution from each syringe barrel was regulated by solenoids, located at least 15 cm from the chamber. All syringe barrels were connected to capillary tubing through which each solution flowed to individual 24-gauge stainless steel tubes within the drinking spout. The tips of these tubes were recessed 2 mm from the end of the spout. Each taste stimulus was presented as a 6-μl aliquot delivered over 0.25 s. Licks were counted when the animal's tongue made contact with the drinking spout. Testing was conducted in 30 ± 5 lx white fluorescent room lighting. To reduce auditory cues, an independent solenoid mounted just above the lick spout was activated simultaneously with the solenoid delivering the taste stimulus. In addition, a room fan, along with a Radio Shack Sleep Machine, generated masking noise (SPL A scale: 80 ± 5 dB) throughout the test period.

Procedures

Threshold methods were similar to those used in previous experiments (Stapleton et al. 2002; Delay et al. 2004, 2006). To initiate a trial during a session, a mouse licked on a variable ratio 20 schedule that resulted in a 3-μl aliquot of water. This served to rinse the tongue and to encourage further licking. Three seconds later, the mouse could begin a second variable ratio 20 schedule which, when completed, resulted in the delivery of the test stimulus. Once the stimulus was delivered, the mouse had 2 s (decision interval) to determine if the stimulus was water or NaCl. If the mouse correctly identified the stimulus as water, the mouse had to lick the spout during the last 0.4 s of the decision interval to receive a 7-μl water reinforcer (correct detection). Upon delivery of NaCl, a correct detection was registered if the mouse did not lick during the last 0.4 s of the decision interval. If the animal failed to correctly respond to the NaCl solution, a weak shock (15–25 mV) was delivered through the lick spout to the animal's tongue. The shock intensity was adjusted for each animal by increasing the intensity until the mouse's licking stopped briefly when shock was applied. Shock was always presented to the lick spout for 2 s following the end of the decision interval of NaCl trials, but the animal only experienced shock if it licked the spout during the shock presentation. A 10-s intertrial interval occurred before the start of the next variable ratio 20. A session was completed after the animal completed 160 trials or 35 min elapsed, whichever came first. During each training session, 5 of the stimulus barrels contained different concentrations of NaCl, 3 contained deionized water for testing, and the ninth barrel contained water for reinforcement. An equal number of water and NaCl trials was presented within each session and the order of the stimulus presentations followed a random sequence established by Latin square ordering. Each day a different concentration sequence was tested, and each concentration was stored in a different syringe barrel to minimize the possibility that the mouse could identify a taste stimulus using the location of the stimulus delivery in the spout. Following the end of a session, the mouse was returned to its home cage where 30 min later it received access to water for an additional 60 min.

Training began with NaCl concentrations ranging from 200 to 500 mM. When the mouse was able to detect the highest concentration at >75 % in 3 consecutive sessions, the range of concentrations was decreased in the next session by replacing the highest concentration with a new low concentration. Once the highest concentration was decreased to 150 mM, training continued for a minimum of 14 days to ensure stable responding. Once data collection began, daily testing included 1 stimulus selected randomly from 0.01, 0.1, 1.0, 2.5, and 5 mM; 2 stimuli selected randomly from 5, 10, 25, and 50 mM; and 2 selected randomly from 75, 100, 150, 200, 300, and 500 mM. After the last test session, the animals were tested in an additional session with water in all tubes and with reinforcement and shock consequences randomly assigned to determine if any animal was using nongustatory cues. To determine if amiloride (a sodium channel blocker) altered the threshold for either mouse genotype, these procedures were then repeated with 100 μM amiloride added to all solutions, including water reinforcer. All concentrations were tested in a minimum of 2 sessions under each amiloride condition.

Results

Two-bottle preference tests

We hypothesized that if both ENaC and TRPV1 contribute to NaCl preference, then TRPV1 knockout mice should show reduced preferences for NaCl when amiloride is absent. With amiloride present, we predicted that the knockout mice would not detect NaCl and would show preference ratios similar to that of water. Further, we predicted that the absence of TRPV1 may also alter preferences for KCl because TRPV1 is a nonselective cation channel and is permeable to K^+.

NaCl preference and consumption without amiloride

In the absence of amiloride, knockout and wild-type mice showed different preference ratios for NaCl (Figure 1, upper panel). There were significant differences related to genotype

[$F(1,16) = 23.86$, $P < 0.05$], concentration [$F(7,112) = 26.79$, $P < 0.001$], and genotype by concentration interaction [$F(7,112) = 7.65$, $P < 0.001$]. In the wild-type mice, the preference for NaCl depended on the concentration of the tastant. Their preference ratios for NaCl decreased at higher concentrations. This also was true for knockout animals (Figure 1, upper panel), but surprisingly, the decrease in their preference ratios did not occur until higher concentrations of NaCl were presented. The preference ratios for the knockout mice were significantly larger at 30, 100, 200, and 300 mM NaCl relative to those of the wild-type controls [simple effects tests, $F(1,16) \geq 8.33$, $P < 0.025$]. The analysis of the NaCl intake data (Figure 1, lower panel) indicated that the volume of NaCl consumed was significantly related to concentration [$F(6,96) = 16.38$, $P < 0.001$] and to the interaction of concentration × genotype [$F(6,96) = 10.23$, $P < 0.001$]. Simple effects tests indicated that the knockout mice ingested significantly larger volumes of the NaCl solutions than wild-type mice when the concentration of NaCl was 100 or 200 mM (P values <0.01).

NaCl preferences and consumption with amiloride

When 100 μM amiloride was added to each NaCl solution, preference ratios for NaCl for both knockout mice and wild-type mice were higher than when amiloride was absent, but knockout mice still preferred NaCl more than wild-type mice [ANOVA, $F(1,16) = 7.28$, $P < 0.001$; Figure 2, upper panel]. As the concentration of NaCl increased, the preference scores of both genotypes also increased significantly [$F(7,112) = 30.37$, $P < 0.001$]. TRPV1 knockout mice had higher preference scores for 30, 100, and 200 mM NaCl solutions than did their wild-type counterparts (P values <0.05). Differences between genotypes were also revealed by the analysis of the NaCl consumption data (Figure 2, lower panel). This ANOVA showed that the knockout mice drank significantly more of the NaCl solutions than the

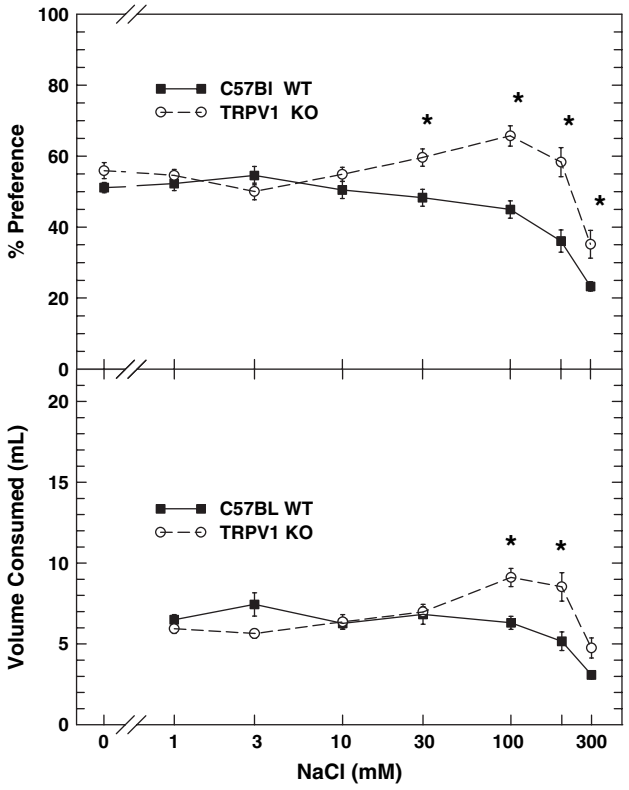

Figure 1 **(Upper panel)** This panel shows the mean (SEM) preference ratios of TRPV1 KO and wild-type (C57Bl/6J) mice for NaCl in the absence of amiloride. Mean preference ratios are reported with standard error bars in this and all subsequent figures. The asterisk represents a significant difference ($P < 0.05$ or less) between knockout (open circles) and wild-type (closed squares) mice in this and all subsequent figures. The 50% level indicates no preference for the taste stimulus versus water. The concentrations of the taste stimuli were presented in ascending order. Note that knockout mice preferred NaCl significantly more than wild-type mice at high concentrations. **(Lower panel)** The mean (SEM) volume consumed for each concentration of NaCl by both genotype mice are shown. The knockout mice drank significantly more (*$P < 0.01$) NaCl at 100 and 200 mM than the wild-type mice.

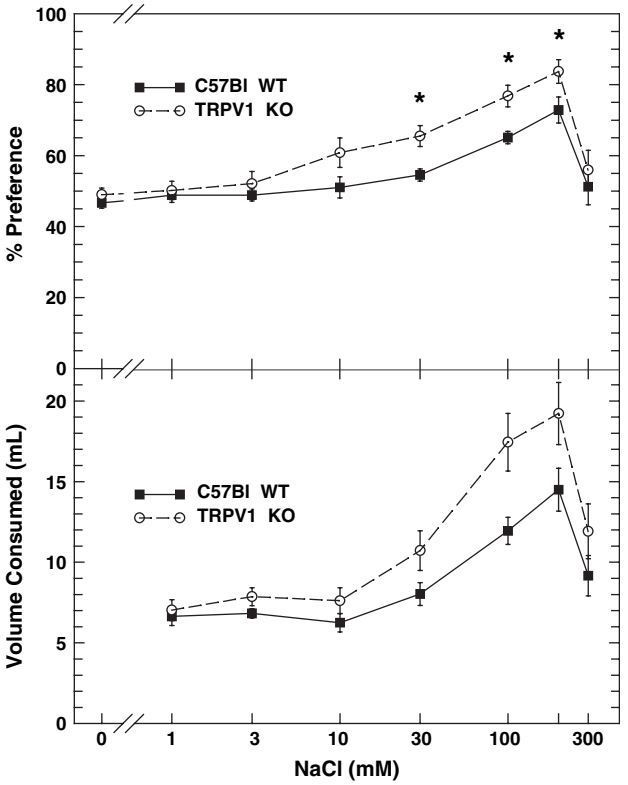

Figure 2 **(Upper panel)** This panel shows the mean (SEM) preference ratios of knockout and wild-type mice for NaCl with 100 μM amiloride added to both taste and control water bottles. Note that amiloride shifted the preference ratios for both knockout and wild-type mice to more positive values, but knockout mice still preferred NaCl significantly more than the wild-type mice. **(Lower panel)** ANOVA results indicated that the TRPV1 knockout mice drank significantly more of NaCl with amiloride than the wild-type (C57Bl/6J) mice. However, because the interaction between genotype and concentration was not significant, this difference between genotypes was not specific to a concentration.

wild-type mice [$F(1,16) = 5.98$, $P < 0.05$] and that both groups of mice increased their intake in a comparable manner as the concentration of NaCl increased, more than doubling their intake over the range of concentrations tested [$F(6,96) = 38.66$, $P < 0.001$]. The interaction between genotype and concentration, however, was not significant.

KCl preference and consumption

Chorda tympani nerve recordings show that in addition to lacking an amiloride-insensitive response to NaCl, TRPV1 knockout mice show reduced chorda tympani responses to KCl and other mineral salts (Lyall et al. 2004). Thus, KCl solutions of varying concentrations were presented to TRPV1 and wild-type mice to test for genotype differences in preference ratios (Figure 3, upper panel). The analyses of these data showed that the concentration of KCl had a significant effect on the preference for KCl [$F(7,112) = 15.86$, $P < 0.001$] and that knockout mice had significantly higher preference scores for the 100 mM KCl solution than the wild-type mice [simple effects test, $P < 0.005$]. The analysis of the KCl consumption data also showed that the knockout mice drank significantly more of the 100 mM solution than the wild-type mice [$F(1,16) = 12.71$, $P < 0.005$; Figure 2, lower panel]. These findings suggest that TRPV1 contributes to KCl as well as NaCl taste.

Citric acid preference and consumption

Because the TRPV1 knockout mice unexpectedly showed preferences for both NaCl and KCl, we then asked if the knockout mice were responding to some general sensory experience or to taste quality–specific sensory features of these substances. Consequently, an additional 9 knockout and 9 wild-type naive mice were tested with citric acid, a compound that is aversive to mice at high concentrations and, in addition, activates TRPV1 in afferent nerve fibers (Leffler et al. 2006). Preference scores of both knockout and wild-type mice for citric acid decreased significantly as the concentration of citric acid increased [$F(6,96) = 196.31$, $P < 0.001$] (Figure 4, upper panel). In contrast to the results obtained with NaCl and KCl, the preference ratios for 3 mM citric acid were significantly lower for the knockout mice than for the control mice ($P < 0.025$). This resulted in a significant

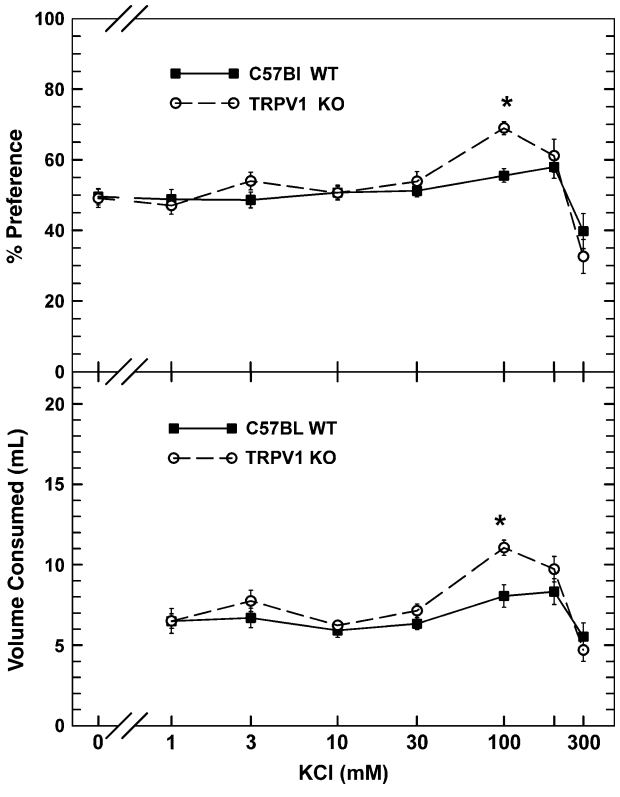

Figure 3 **(Upper panel)** This shows the mean (SEM) reference ratios of knockout and wild-type mice for KCl. Note that knockout mice prefer the 100 mM concentration of KCl significantly more than the wild-type mice, but both genotypes reject KCl at high concentrations. **(Lower panel)** This panel shows the mean (SEM) consumption of each concentration of KCl by the TRPV1 knockout and wild-type mice. Consistent with the preference ratios, the knockout mice drank significantly more of the 100 mM KCl than the wild-type mice.

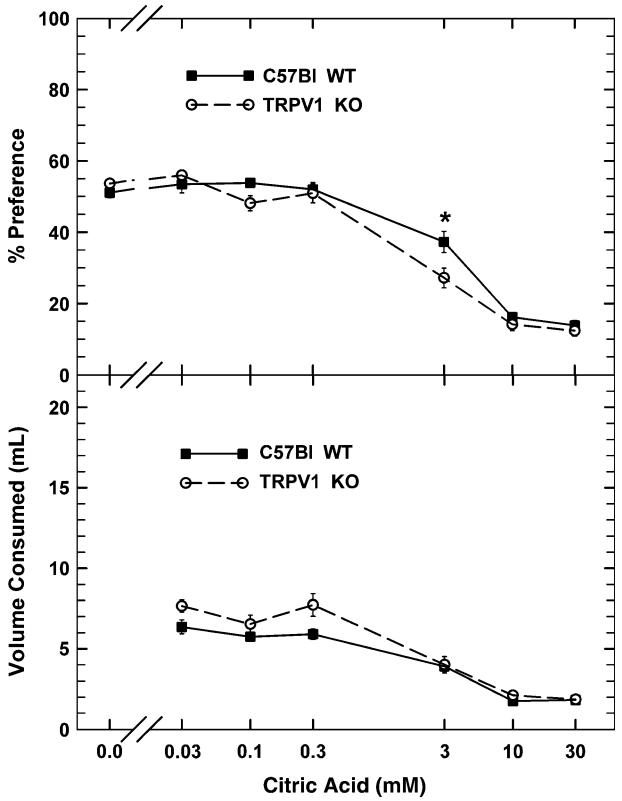

Figure 4 **(Upper panel)** The mean (SEM) preference ratios of knockout and wild-type mice for citric acid are shown. Note that both knockout and wild-type mice reject citric acid at high concentrations but that the knockout mice reject it significantly more than the wild-type mice ($P < 0.025$) at 3 mM. **(Lower panel)** Both genotypes similarly decreased the volume of citric acid consumed as the concentration increased. Thus, the knockout mice behave differently to citric acid than to salts.

concentration by genotype interaction [$F(6,96) = 2.97$, $P < 0.025$], but in the opposite direction from the salts. The analysis of the citric acid consumption data (Figure 4, lower panel) showed that both genotypes significantly decreased their intake of citric acid as the concentration of citric acid increased [$F(5,80) = 98.69$, $P < 0.001$], but the 2 types of mice did not differ significantly. These data suggest that the enhanced preferences for NaCl and KCl are mediated by taste-specific qualities of these substances rather than some type of general sensory afferent sensation.

Detection thresholds

Because wild-type mice often prefer low concentrations of NaCl and avoid high concentrations (Tordoff and Bachmanov 2003), we hypothesized that the enhanced preference for NaCl observed in the knockout mice might be due to a higher detection threshold, resulting in the knockout mice preferring concentrations of NaCl normally avoided by wild-type mice. We also predicted that the addition of amiloride should elevate detection thresholds for both knockout and wild-type mice.

Thresholds, defined as the concentration detected 50% of the time, were determined for each mouse in each amiloride condition. The psychophysical functions with the corresponding thresholds for each taste condition are shown in Figure 5. In the no amiloride condition, the geometric mean NaCl thresholds were 3.07 mM for the wild-type mice and 2.21 mM for the knockout mice. When 100 μM amiloride was present, mean threshold was 21.12 mM for the wild-type mice and 1.36 mM for the knockout mice. An ANOVA comparing the threshold values of wild-type and knockout mice indicated a significant genotype by amiloride interaction [$F(1,8) = 10.97$, $P < 0.05$]. When analyzed further with

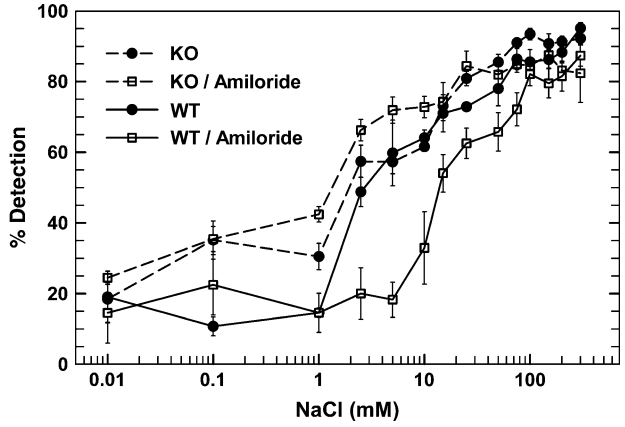

Figure 5 Threshold functions of the wild-type (solid lines) and knockout (dash lines) mice without (filled circles) and with (open squares) 100 μM amiloride in all solutions. When amiloride was not present, NaCl thresholds of wild-type and knockout mice were equivalent. When amiloride was present, however, thresholds of wild-type mice were significantly elevated whereas the thresholds of the knockout mice were unaffected.

T-tests, the only difference between thresholds found was the significantly ($P < 0.05$) elevated thresholds of the wild-type mice when amiloride was present. Thus, wild-type mice and TRPV1 knockout mice appear to have similar detection thresholds for NaCl. The addition of 100 μM amiloride raised the threshold of the wild-type mice but not those of the knockout mice.

Discussion

Important and rather surprising conclusions can be drawn from this study. Hedonic (preference) properties of NaCl and KCl were assessed using 24-h 2-bottle preference testing. We predicted that the absence of TRPV1 receptors would reduce the preference for these salts, and in the presence of amiloride, mice would show little or no interest in either salt at any but the highest concentrations. Surprisingly, these TRPV1 knockout mice actually preferred NaCl and KCl more than the wild-type mice did. We then assessed the sensitivity of these mice for NaCl by measuring detection (absolute) thresholds. One would predict that with fewer receptors available (e.g., by adding amiloride to block Na^+ taste receptor channels), detection thresholds go up (sensitivity decreases). This happened with the wild-type mice but to our surprise, even when amiloride-sensitive channels are blocked by 100 μM amiloride, TRPV1 knockout mice can still detect NaCl concentrations as low as 2–4 mM while the thresholds of the wild-type mice were elevated to over 20 mM (Figure 5). We cannot explain why amiloride elevated the detection threshold for NaCl in the wild-type mice but not in the knockouts. It is possible that the knockout of TRPV1 produced compensatory changes in other proteins involved in salt transduction, such as ENaC, but the 2-bottle preference data argue against an impairment of ENaC in the knockouts because amiloride shifted preferences in both mouse strains.

Obviously these two behavioral measures are assessing quite different sensory capacities that might be affected by the genetic deletion. Detection or absolute threshold experiments measure the sensitivity of an organism for detecting a stimulus by determining the minimum intensity that can be detected (differentiated from water), for example, in 50% of trials. In this study the animal is motivated to make this detection to avoid the shock, even when the animal cannot identify other qualities of the taste stimulus. Detection thresholds do not provide information about the hedonic (preference) value that the mice might attribute to a taste stimulus because hedonic qualities of taste usually require higher concentrations to elicit. On the other hand, a shift in detection threshold can produce a shift in a preference function. However, the differences in preferences for NaCl and KCl in this study do not appear to be related to a shift in detection threshold. For example, the amiloride-induced increased preference of the TRPV1 mice for NaCl compared with wild type was not correlated with an elevated detection

threshold because the knockout mice had similar detection thresholds for NaCl whether amiloride was present or absent. Yet, knockout mice showed an increased preference for NaCl and an increase in consumption compared with wild-type mice when amiloride was present. This adds further support for the suggestion that some form of compensation, such as altering other receptor proteins or possibly the upregulation of another amiloride-insensitive salt receptor, might have occurred in the knockout mice. It is also possible that some form of postingestive effect might have influenced the hedonic characteristics of the salts in the 2-bottle preference studies, although postingestive effects are not likely to affect knockout and wild-type mice differentially.

Our data indicate that the knockout mice showed greater preference for NaCl at concentrations between 30 and 200 mM than wild-type mice. These data suggest that TRPV1 may be responsible for some of the negative qualities associated with NaCl. The results with KCl lend further support to this hypothesis, since KCl is even more aversive than NaCl at high concentrations, yet the knockout mice preferred KCl at concentrations avoided by the wild-type mice. Citric acid preference was not affected by the knockout, suggesting that the TRPV1 expressed in taste cells does not contribute to sour transduction. Importantly, the normal citric acid preference of the knockout mice suggests that TRPV1 expressed in general sensory afferents of the tongue does not contribute to the aversion to NaCl and KCl because they also mediate aversion to acids. Thus, the TRPV1 in taste cells is likely to be a variant of the receptor expressed in trigeminal and dorsal root ganglion afferents, as suggested previously by Lyall et al. (2004).

TRPV1 knockout mice are missing TRPV1 not only from taste buds but also from lingual nerve fibers, which are known to respond to taste stimuli, including NaCl and citric acid at concentrations used in this study (Pittman and Contreras 1998). However, we do not believe that TRPV1 expression in lingual fibers is responsible for the differences observed in this study. TRPV1 expressed in lingual nerve fibers is not normally conductive to Na unless activated by nociceptive stimuli (Tominaga et al. 1998). Further, citric acid has been shown to activate lingual nerve fibers in guinea pigs by a mechanism independent of TRPV1 (Canning et al. 2006). Thus, we believe that the differences in NaCl and KCl preference scores between TRPV1 knockout and wild-type mice are likely due to the TRPV1 variant expressed in taste buds (Lyall et al. 2004). Further, the lack of difference in preference for citric acid between knockout and wild-type mice suggests that the TRPV1 variant in taste buds is specific for salt taste (NaCl and KCl) and is not involved in the detection of citric acid, but this needs further testing.

Although the TRPV1 mice originated on a B6;129S background, they were backcrossed for at least 5 generations into the C57BL/6J background. Hence, it is unlikely that the differences observed between the knockout and the wild-type mice were due to genetic background alone. Instead, these data suggest that the TRPV1 pathway is not the primary pathway involved in detection of amiloride-insensitive NaCl taste. The data obtained by Lyall et al. (2004), showing the absence of an amiloride-insensitive nerve response in TRPV1 knockout mice, focused on the chorda tympani nerve, which is highly sensitive to amiloride. In contrast, responses to NaCl recorded from the glossopharyngeal nerve are completely amiloride insensitive. Thus, the behavioral data reported here suggest that the additional amiloride-insensitive mechanisms are likely expressed in circumvallate and foliate taste buds rather than fungiform, although palatal taste buds cannot be ruled out.

Finally, it is important to note that the amiloride-induced shift in threshold of the C57BL/6J wild-type mice reported by Geran and Spector (2000) and Eylam and Spector (2002) was only partially replicated in this study. In the Eylam and Spector study, 100 μM amiloride shifted NaCl detection thresholds from about 65 mM to well over 500 mM. In contrast, NaCl thresholds for C57BL/6J wild-type mice in this study were measured at 2–4 mM without amiloride and over 20 mM with amiloride. These values are much closer to those measured for rats (Geran and Spector 2000). The most obvious explanations for the discrepancies between this study and the Eylam and Spector study are related to the differences in methods used in each study. Eylam and Spector employed a 2-choice method with a time-out penalty for missing the presence of NaCl. In the current study, mice were required to either continue to lick for water reinforcement or, after presentation of NaCl, to stop licking to avoid shock. It is possible that the shock, even though low intensity, motivates mice to respond better to low concentrations of a substance than methods such as a cued time-out penalty. This possibility needs further exploration, especially as transgenic mice are playing an increasing prominent role in the study of gustatory phenomena.

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