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A/J and C57BL/6J mice differ in chorda tympani responses to NaCl

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Abstract

The molecular mechanisms of sodium taste transduction are not completely understood, especially those responsible for the portion of NaCl's taste in rodents that is not blocked by amiloride. As a prelude to conducting genetic analyses of peripheral NaCl taste responsiveness, we performed multiunit electrophysiological recordings from the chorda tympani (CT) nerve in C57BL/6J (B6) and A/J mice. Mice were anesthetized, the CT was accessed, and taste solutions were flowed over the tongue in order to measure the integrated whole-nerve response. NaCl was delivered before and during application of 100 μ M amiloride. Pre-amiloride responses were significantly larger in A/J than B6 mice for 1–8 mM NaCl. Responses to NaCl were suppressed significantly by amiloride in both strains and to similar degrees. However, the size of the amiloride-insensitive NaCl response component was significantly larger in A/J mice than in B6 mice for NaCl at 2–16 mM. These data help to explain the prior observation that the strains differ in behavioral taste thresholds for NaCl. Specifically, the results suggest that perception of sodium-specific taste by mice depends on the ratio of amiloride-sensitive and -insensitive responses in the CT, rather than on the absolute level of the whole-nerve response to NaCl or on the size of the amiloride-sensitive component alone. Because the B6 and A/J mice differed in the size of their amiloride-insensitive components, they may prove useful in future genetic work designed to characterize the underlying transduction mechanisms.

Keywords

Mouse; Salt; Electrophysiology

1. Introduction

Sodium chloride possesses a unique taste quality that distinguishes it from non-sodium compounds. Humans describe this taste as “salty”, and non-human species also have the ability to distinguish sodium-containing compounds from chemicals that are not labeled as salty by humans, suggesting that these animals perceive a “sodium-specific taste” (McCaughey and Scott, 1998). There is also evidence, however, that sodium chloride does not taste purely salty. For example, a sour side-taste of NaCl has been reported by human subjects (Ossebaard and Smith, 1996).

In rodents, the sodium-specific component of NaCl's taste is thought to be mediated by passage of sodium through amiloride-sensitive epithelial sodium channels (ENaCs) found in taste bud cells. Blockade of this mechanism by amiloride results in the loss of perception of NaCl's unique taste quality (McCaughey and Scott, 1998). Amiloride, however, does not completely block the taste response evoked by NaCl. The insensitive portion is thought to be responsible for the non-sodium-specific taste of NaCl. For example, rats have difficulty distinguishing NaCl mixed in amiloride from KCl (Spector et al., 1996), and electrophysiological evidence indicates that this is due to the two stimuli evoking perceptions with similar taste qualities (St. John and Smith, 2000). Several mechanisms have been proposed to mediate the amiloride-insensitive response to NaCl, including passage of sodium through basolateral ENaCs that are inaccessible to amiloride, due to the tight junctions between taste receptor cells (DeSimone et al., 1993). Another proposed mechanism is a variant of the TRPV1 (or VR-1) channel, which allows sodium as well as other ions to pass (Lyall et al., 2004; Coleman, 2011). However, none of the proposed mechanisms has been definitively proven to be responsible for the amiloride-insensitive component of NaCl taste. The mechanisms for human salt taste are even less clear. Recent findings have linked variation in the genetic sequences that code for ENaC and TRPV1 to variation in suprathreshold ratings of NaCl (Dias et al., 2013), but amiloride has been found to not reduce perception of saltiness in human subjects (Ossebaard and Smith, 1996).

The involvement of ENaC, and possibly TRPV1, does not preclude a role for other proteins in the gustatory transduction of sodium and the preference for NaCl in rodents. One means of discovering such mechanisms will be to take advantage of the wide variation that mouse strains display in their taste responses to sodium. This variation includes differences in taste nerve responses (Ninomiya et al., 1989; Gannon and Contreras, 1995; Ninomiya et al., 1996, 1998; Ohkuri et al., 2006) and in two-bottle preference tests (Tordoff et al., 2007). Another behavioral test has also been developed recently in order to determine the threshold for sodium-specific taste in mice (Ishiwatari and Bachmanov, 2009). Briefly, the method involved giving mice lithium chloride to drink, and later conducting two-bottle tests with a concentration series of NaCl to find the lowest concentration of NaCl that was avoided by the mice, which was considered to be the threshold for sodium-specific taste. Thus, the threshold obtained would presumably be analogous to the recognition threshold for NaCl obtained in human subjects, which is defined as the lowest concentration of NaCl that is perceived as salty. This measure should be distinguished from the detection threshold (i.e., the lowest concentration that can be distinguished from water) and the preference threshold (i.e., the lowest concentration that is preferred over water). Ishiwatari and Bachmanov (2012) found that sodium-specific thresholds ranged from 3 to 30 mM across 13 mouse strains. In addition, the order of threshold values across strains did not correlate well with the rank orders for preference of suprathreshold concentrations of NaCl, which is consistent with other evidence that independent mechanisms mediate sodium's taste quality at different concentrations (Contreras, 1977; Colbert et al., 2004).

Two mouse strains that are especially useful for discovering novel genes, gustatory and otherwise, are the C57BL/6J (B6) and A/J, for which there are available chromosome substitution strains (i.e., consomics), which greatly reduce the time involved in gene discovery through genetic mapping (Singer et al., 2004). These two inbred strains are known to differ in their behavior toward sodium. B6 mice prefer 25 mM NaCl over water, whereas A/J mice do not, though the two strains prefer 75 and 225 mM NaCl to similar extents (Tordoff et al., 2007). Furthermore, B6 and A/J mice were near the extremes of a strain distribution in thresholds for sodium-specific taste (5 and 30 mM, respectively; Ishiwatari and Bachmanov, 2012). Although taste nerve responses to NaCl have been studied in B6 mice (Ninomiya et al., 1989; Gannon and Contreras, 1995; Ninomiya et al., 1996), no such data are available for A/J mice. We measured taste-evoked responses to NaCl and other

stimuli in the chorda tympani nerve (CT) of B6 and A/J mice, in order to characterize strain differences in peripheral gustatory factors before conducting future genetic work. The CT was chosen because of transection data (Spector et al., 1990; Golden et al., 2011) indicating that this nerve is especially important for NaCl's taste in rodents. We also sought to characterize the NaCl sensitivity of B6 mice more thoroughly than in earlier reports by looking at lower concentrations than have been used previously.

2. Methods

2.1. Subjects

Measurements of CT activity were made in 5 male C57BL/6J (B6) and 7 male A/J mice which were purchased from The Jackson Laboratory (Bar Harbor, ME). Procedures were approved by the Animal Care and Use Committees of Ball State University and the Monell Chemical Senses Center. All the mice were kept at 23–26 °C with a 12:12 h light:dark cycle (lights off at 7 PM). Animals were maintained in a room at on standard chow (Harlan Teklad 8604) and deionized water ad libitum.

2.2. Surgery

Each animal was anesthetized with a mixture of ketamine, xylazine and acepromazine (90, 20, and 3 mg/kg, respectively; i.p.), with further doses as necessary. A cannula was placed in the trachea to prevent suffocation, and the animal was placed supine with the head secured in a non-traumatic head holder. The nerve was accessed through the right ear, by puncturing the tympanic membrane and exposing the right CT nerve adjacent to the malleus (Cheal, 1977). An electrode made of platinum/iridium wire was placed on the nerve, and the multiunit signal was amplified, filtered, rectified and integrated with a time constant of 1.0 s.

2.3. Stimuli and delivery

Stimuli, which were mixed in distilled water, included: NaCl at 1, 2, 4, 8, 16, and 32 mM; 100 mM KCl; 500 mM sucrose, 10 mM HCl, and 20 mM quinine. Non-sodium stimuli were applied at commonly used concentrations to make broader comparisons of the strains across representative tastes. We chose to focus on low concentrations of NaCl, given prior behavioral evidence that B6 and A/J mice differ in their behavior toward NaCl only in this range (Tordoff et al., 2007; Ishiwatari and Bachmanov, 2012). We used two strategies to address the possibility that using such low concentrations might yield a small signal-to-noise ratio. First, we applied multiple low concentrations. Second, we typically applied each concentration of NaCl at least twice for each condition (i.e., before and during amiloride). Non-sodium stimuli were occasionally applied twice when deemed necessary. For each mouse, the relative response size across all presentations of a stimulus for a given condition (e.g., pre-amiloride) was then averaged in order to obtain a single relative response size. We also calculated the Pearson product moment correlation between the response sizes of the first and second applications for all 143 instances in which a stimulus was applied twice for an animal. The result was +0.93, indicating that there was a high degree of stability in our preparations.

The stimulus array first was delivered with deionized water rinses between stimuli. The rinse solution was then changed to 100 μ M amiloride, and KCl and the NaCl series were reapplied, with the solutions mixed in 100 μ M amiloride rather than water. In addition, 100 mM NH_4Cl was also applied at regular intervals (usually after every 4–5 non-ammonium stimuli) throughout the entire process to serve as a reference stimulus. Typically, the NH_4Cl applications immediately before and after each taste stimulus were averaged in order to obtain that stimulus' reference value, though there were rare instances in which there was only one NH_4Cl application that was considered to be appropriate for a reference. We chose

NH₄Cl in part because it is the most commonly used reference stimulus for CT recording in rodents, but it was also especially appropriate because B6 and A/J mice have nearly identical preferences for 100 mM NH₄Cl in two-bottle tests with water (Bachmanov et al., 2002).

In order to apply taste solutions, the tongue was extended slightly and placed in a flow chamber. Room-temperature deionized water or 100 μ M amiloride were used as a rinse and stimulus solutions at room temperature were applied by continuous flow with a rate of 0.5 ml/s. Rinse and stimulus were separated by a small air bubble that allowed for a distinct, sharp onset, but that minimized interruption to the flow. This method results in no responses to water as a “stimulus” or to weak concentrations of compounds that are thought to be undetectable (see Fig. 1), and thus our responses to higher concentrations can be taken as purely gustatory in nature, without a tactile component. Each stimulus presentation lasted for 20 s and was followed by at least 60 s of rinse. Concentration series of NaCl were applied in ascending order, but otherwise the order of presentation for different stimuli was random.

2.4. Analysis

Response sizes for stimulus applications were based on the area-under-the-curve of the integrated voltage for 20 s after stimulus onset (evoked) minus the area for 10 s before onset (baseline). Relative response sizes were calculated for each stimulus application based on the size of the NH₄Cl reference. The relative responses to NaCl and KCl prior to amiloride application are described either as “pre-amiloride responses” or as the “overall response” to these stimuli; the relative responses during the amiloride series (i.e., with the compounds mixed in 100 μ M amiloride and with 100 μ M amiloride as rinse) were considered to be the response “during amiloride” or the “amiloride-insensitive portion” of the response. The latter was then subtracted from the former for each mouse to yield an estimate of the “amiloride-sensitive portion” of its response.

Data were analyzed using repeated measures analysis of variance (ANOVA) with strain as a between-subjects factor and amiloride, concentration and/or stimulus as within-subjects factors. Three-way ANOVA was used to analyze responses to NaCl with and without amiloride (effects of strain, amiloride and concentration). Two-way ANOVAs were used to analyze the amiloride-sensitive component of NaCl responses (effects of strain and concentration), responses to KCl (effects of strain and amiloride) and non-sodium stimuli (effects of strain and stimulus). Fisher LSD tests were used to conduct planned comparisons of differences for group means. Threshold responses for NaCl were defined as the lowest concentration for which the strain mean minus a 95% confidence interval was greater than zero. The statistical analyses were performed using the Statistica software package (StatSoft, Inc., Tulsa, OK; <http://www.statsoft.com>). For all tests $p < 0.05$ was considered to be significant.

3. Results

Fig. 1 shows examples of pre- and during-amiloride responses to NaCl in a B6 and an A/J mouse. The effect of 100 μ M amiloride (i.e., the moment when the amiloride rinse first hit the tongue) is also shown, confirming that it did not evoke a taste response by itself. Clear elevation of the activity above baseline was observed at 1 mM NaCl in the A/J mouse and at 2 mM in the B6 mouse, which matches the statistical outcome when all animals were considered. In the A/J strain, the threshold for responding to NaCl was 1 mM both before and during amiloride. In the B6 strain, the comparable thresholds were 2 and 4 mM, respectively.

Overall responses to NaCl were larger in A/J than in B6 mice, and they were suppressed by amiloride in mice from both strains (Fig. 2). Pre-amiloride responses to NaCl were significantly larger in A/J mice compared with B6 mice at 1–8 mM (Fig. 2A). Responses to NaCl during amiloride (i.e., amiloride-insensitive responses) were significantly larger in A/J mice at 2–16 mM (Fig. 2B). Amiloride significantly suppressed responses to all NaCl concentrations in A/J mice and to 2–32 mM NaCl in B6 mice (Fig. 2B). We also examined the size of the amiloride-sensitive component to NaCl's response, by subtracting the size of relative responses to NaCl in amiloride from relative pre-amiloride responses.

B6 and A/J mice did not differ in the size of their amiloride-sensitive components (Fig. 2C). B6 and A/J mice did not differ significantly in KCl responses before or during amiloride. Amiloride slightly but significantly suppressed KCl responses in A/J but not in B6 mice (Fig. 3). The strains also did not differ in responses to sucrose, HCl or quinine (Fig. 4).

4. Discussion

Pre-amiloride responses were significantly larger in A/J than B6 mice for 1–8 mM NaCl, and the former also had a lower threshold for responding to NaCl than did the latter (1 and 2 mM, respectively). Responses to NaCl were suppressed significantly by amiloride in both strains and to similar degrees. However, the size of the amiloride-insensitive NaCl response component was significantly larger in A/J mice than in B6 mice for NaCl at 2–16 mM. The strains did not differ in their responses to non-sodium stimuli.

Our data generally provide a good match with prior behavioral results, including data on two-bottle preferences in B6 and A/J mice. B6 mice preferred 25 mM NaCl over water, whereas A/J mice consumed water and 25 mM NaCl equally, and the strains had nearly identical preferences for the other two concentrations of NaCl that were tested, 75 and 225 mM (Tordoff et al., 2007). The strain differences that we found were also limited to the concentrations that we tested below 25 mM, but not the one higher concentration that we tested (32 mM). Thus, our data suggest that the taste transduction mechanisms activated by NaCl depend in part on its exact concentration, which is consistent with prior studies involving CT transection and sodium deprivation in rats (Contreras, 1977; Colbert et al., 2004).

We used extremely low concentrations of NaCl that had not been used previously in CT recordings from B6 mice, and so we were able to calculate the neural response threshold for this compound, which equaled 2 mM. This is close to a threshold value of 3 mM that was obtained in this strain using a task in which animals were punished for failing to discriminate NaCl from water (i.e., a detection threshold task; Ruiz et al., 2006). This task has not been performed in A/J mice, and so we cannot compare appropriate behavioral data with our neural response threshold of 1 mM in this strain (and the true value may be even lower, in fact, given that our threshold was at the lowest concentration of NaCl that we tested).

Both the B6 and A/J strains, though, have been tested in a generalization task designed to find the threshold for sodium-specific taste (Ishiwatari and Bachmanov, 2009), with values of 5 and 30 mM, respectively (Ishiwatari and Bachmanov, 2012). Our neural data also provide a good match with these behavioral data, bearing in mind the precise nature of the task that was assigned to the animals; that is, the threshold measure did not involve discrimination of NaCl from water, but rather a conditioned taste aversion and generalizations relative to higher concentrations of NaCl that are thought to taste primarily salty to mice. Our results match this generalization assay, then, because the large CT responses to NaCl at 1–16 mM in A/J mice were composed primarily of an amiloride-insensitive component, which should not mediate perceptions of sodium-specific taste in the

animals. In contrast, responses to NaCl were smaller overall in B6 mice, but the amiloride-sensitive component made up a larger portion of the NaCl response than was the case in A/J mice. Thus, our results reinforce the complex nature of NaCl's taste and suggest that the relative contribution of amiloride-sensitive and -insensitive components of neural responses, rather than the overall level of the peripheral input per se, is most relevant to the animals' perceptions.

It appears that there are several means of making rodents relatively insensitive to the sodium-specific taste of NaCl in behavioral tests, with the shared feature being that the taste response is dominated by amiloride-insensitive mechanisms. For example, rats and mice have a reduced ability to discriminate NaCl from KCl when the former is mixed in amiloride (Spector et al., 1996; Eylam and Spector, 2005) or when the CT is transected (St. John et al., 1997), which leaves taste signals partially intact, but carried to a greater degree by amiloride-insensitive nerves such as the glossopharyngeal. Furthermore, Yasumatsu et al. (2003) examined CT responses to NaCl in mice during recovery from nerve crush; they found that the amiloride-insensitive portion of the nerve's response reappeared sooner than did the amiloride-sensitive portion, and recovery of the ability to discriminate NaCl from KCl was delayed until the full recovery of the latter component.

Mouse strains are often categorized as being either sensitive or insensitive to amiloride, based on the drug's ability to reduce the size of the taste responses evoked by NaCl. Our data are consistent with prior findings that CT responses to NaCl are partially suppressed by amiloride in B6 mice (Ninomiya et al., 1989; Gannon and Contreras, 1996). Previous studies, though, did not use concentrations of NaCl below 10 mM, and we found that 2 mM was the lowest concentration at which the response was inhibited in this strain. Presumably, then, sodium at this low concentration passes through ENaCs in the taste buds of B6 mice, resulting in enough of an intracellular change to affect CT fibers. This value may seem low, given that behavioral tests have indicated that B6 mice do not discriminate the salty taste of NaCl until 5 mM (Ishiwatari and Bachmanov, 2012). However, as mentioned earlier, presumably the behavior reflects the complete neural signal, including the amiloride-insensitive component of the CT, and possibly other taste nerves such as the glossopharyngeal as well.

Our experiment provides the first knowledge about CT responses in the A/J mouse strain. We found that they, too, had amiloride-sensitive responses, even at the lowest NaCl concentration that we tested (1 mM). The amiloride sensitivity of B6 and A/J mice differentiates them from the amiloride-insensitive BALB/cCrSlc, DBA/2CrSlc and 129P3/J strains (Ninomiya et al., 1989; Gannon and Contreras, 1995; Ninomiya et al., 1996; Ohkuri et al., 2006). The latter strain had a behavioral threshold for sodium-specific taste of 30 mM, which was identical to the threshold of the A/J strain (Ishiwatari and Bachmanov, 2012). This fact, combined with the current findings for B6 and A/J mice, further reinforces that amiloride sensitivity alone does not predict behavioral thresholds for sodium-specific taste. We should also add that any strain differences described above may depend on prior experience, given that expression levels of lingual sodium channels can be changed in rats by factors such as sodium deprivation or exposure to ethanol (Ye et al., 1993; Coleman et al., 2011). In the case of our data, though, all of the animals had prior experience with only water and standard chow, which would preclude such effects.

Mean responses to 100 mM KCl did not differ between the strains, though the effects of amiloride on KCl responses did. We found that responses were not significantly suppressed in B6 mice, which is consistent with prior work (Gannon and Contreras, 1995). In contrast, KCl responses were suppressed significantly in the A/J strain, though the size of the suppression was rather small, so that the strains still did not differ in their mean response to

KCl mixed in amiloride. In previous studies, KCl responses were found to be suppressed by amiloride in mice from the CBA/J strain, but not from 129P3/J, C57BL/6 or NZB/B1NJ strains (Gannon and Contreras, 1995; Ninomiya et al., 1998). In rats, there are reports of KCl responses being both sensitive (Ninomiya and Funakoshi, 1988; St. John and Smith, 2000) and insensitive (Brand et al., 1985; Lundy and Contreras, 1999; Verhagen et al., 2005) to amiloride application, depending in part on factors such as the particular subclass of neurons examined. The molecular basis for this variation is not known. Our data could be explained by the B6 and A/J strains differing slightly in how potassium ions interact with ENaC channels that are sensitive to amiloride. However, any such strain difference would presumably be specific to potassium ions, given that the effects of amiloride on NaCl responses were similar in the two strains.

Because we measured the afferent activity of peripheral neurons, the underlying cause of any strain differences presumably arises in the periphery (i.e., in fungiform taste buds or in CT fibers themselves). The fact that the strains had similar responses to KCl and HCl, which give larger responses in amiloride-insensitive than -sensitive CT fibers (Ninomiya, 1996), argues against a strain difference in the CT fibers themselves. That is, if the larger NaCl response in A/J mice were due solely to of the neural signal being dominated by amiloride-insensitive CT fibers, then we should have observed larger responses to not only NaCl mixed in amiloride, but also HCl and KCl, in the A/J strain compared to B6 mice. Thus, our results are more consistent with a mechanism located in taste receptor cells, in particular one related to amiloride-insensitive sodium transduction. Further work will be needed to address this possibility more directly. This additional work could include comparisons between the B6 and A/J strains on the function of the TRPV1 receptor that has been proposed to mediate amiloride-insensitive sodium transduction (Lyll et al., 2004; Coleman et al., 2011). Furthermore, novel genes that influence amiloride-insensitive transduction of sodium in mice might be identified by using not only the B6 and A/J strains, but also the currently available consomic strains (Singer et al., 2004) derived from them.

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Fig. 1.

Representative neural recordings from a B6 and an A/J mouse, which show changes in the integrated voltage over time. For each animal, one pre-amiloride (“pre”) and one during-amiloride (“amil”) series of NaCl applications is shown, along with the first and last reference NH_4Cl applications for each series (on the left and right, respectively). When the rinse was switched to 100 μM amiloride, the time at which it contacted the tongue was noted to confirm that it did not induce a taste response on its own (far right of “pre-” condition for each mouse). All stimulus applications are drawn using the same vertical scale for each mouse. Vertical bars indicate the stimulus onset time for a given column.

Fig. 2.

Mean ($\pm$ SEM) chorda tympani responses to a concentration series of NaCl in B6 (solid lines) and A/J (dashed lines) mice before (A) and during (B) application of 100 μM amiloride. Three-way ANOVA detected significant effects of strain ($F[1,9] = 13.1$, $p < 0.01$), amiloride ($F[1,9] = 39.6$, $p < 0.001$), NaCl concentration ($F[5,45] = 141.4$, $p < 0.0001$), strain $\times$ concentration ($F[5,45] = 3.4$, $p < 0.05$) and amiloride $\times$ concentration ($F[5,45] = 3.4$, $p < 0.05$) interactions. Strain $\times$ amiloride ($F[1,9] = 0.01$, $p = 0.9$) and strain $\times$ amiloride $\times$ concentration interactions ($F[5,45] = 1.4$, $p = 0.2$) were not significant. (C) The amiloride-sensitive NaCl component for each strain, which was calculated by subtracting values during amiloride application from those prior to amiloride. Two-way ANOVA detected a significant effect of concentration ($F[5,45] = 16.1$, $p < 0.0001$), but not of strain ($F[1,9] = 0.01$, $p = 0.9$) or strain $\times$ concentration interaction ($F[5,45] = 1.4$, $p = 0.2$). * $p <$

0.05, B6 versus A/J; $^+p < 0.05$, during amiloride versus pre-amiloride within the same strain; LSD tests.

Fig. 3.

Mean ($\pm$ SEM) chorda tympani responses to 100 mM KCl in B6 and A/J mice before (filled bars) and during (open bars) application of 100 μ M amiloride. Two-way ANOVA detected significant effects of amiloride ($F[1,9] = 5.9$, $p < 0.01$) but not strain ($F[1,9] = 1.1$, $p = 0.3$) or strain $\times$ amiloride interaction ($F[1,9] = 1.1$, $p = 0.3$). ⁺ $p < 0.05$, during amiloride versus pre-amiloride within the same strain; LSD tests.

Fig. 4. Mean ($\pm$ SEM) chorda tympani responses to non-sodium stimuli in B6 (filled bars) and A/J (open bars) mice. Two-way ANOVA detected a significant effect of taste stimulus ($F[2,18] = 8.7$, $p < 0.01$), but not strain ($F[1,9] = 0.04$, $p = 0.8$) or strain $\times$ stimulus interaction ($F[2,18] = 0.04$, $p = 0.96$). Suc, 500 mM sucrose; HCl, 10 mM HCl; Q, 20 mM quinine HCl.