Taste discrimination between NaCl and KCl is disrupted by amiloride in inbred mice with amiloride-insensitive chorda tympani nerves

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Eylam, Shachar, and Alan C. Spector. Taste discrimination between NaCl and KCl is disrupted by amiloride in inbred mice with amiloride-insensitive chorda tympani nerves. Am J Physiol Regul Integr Comp Physiol 288: R1361–R1368, 2005; doi:10.1152/ajpregu.00796.2004.—The amiloride-sensitive salt transduction pathway is thought to be critical for the discrimination between sodium and nonsodium salts in rodents. In rats, lingual application of amiloride appears to render NaCl qualitatively indistinguishable from KCl. In this study, we tested four strains of mice for salt discriminability. In one strain (C57BL/6J), chorda tympani nerve (CT) responses to NaCl are attenuated by amiloride, and in the other three strains (BALB/cByJ, 129P3/J, DBA/2J) they are not. Under water-restriction conditions, these mice (7 mice/strain) were trained in a gustometer to lick for water from one reinforcement spout in response to a five-lick presentation of NaCl and to lick from another in response to KCl [salt concentration was varied (0.1–1 M) to render intensity irrelevant]. Mice were then tested with the stimuli dissolved in amiloride (25). However, using an operant conditioning paradigm, we have previously demonstrated that amiloride reduces behaviorally measured sensitivity to NaCl by an order of magnitude in mice from both the B6 and the D2 strains (10). For these same strains, as well as for rats, amiloride is an ineffective conditioned stimulus in taste-aversion learning paradigms, suggesting that the drug is tasteless (11, 23) and that taste masking is not likely the root of amiloride’s attenuating effect on NaCl detectability.

Although the apparent absence of epithelial sodium channels (ENaCs) in the anterior tongue of D2 mice does not affect sensitivity to NaCl nor the effectiveness of amiloride in raising the detection threshold for this salt in this strain relative to the amiloride-sensitive B6 strain, it remains to be seen whether D2 mice, or B6 mice for that matter, can competently discriminate between sodium and nonsodium salts and whether amiloride would compromise such performance. In the detection task, the animal merely needs to report that a stimulus different from water (the vehicle) is present, but in a discrimination task the animal must differentiate between taste compounds, placing a higher premium on the specificity of the respective signals. By virtue of the cation selectivity of the ENaC, this taste receptor channel type would be expected to play a critical role in allowing animals to discriminate sodium from nonsodium salts, as shown behaviorally in rats. Accordingly, we tested three inbred mouse strains with reportedly amiloride-insensitive CTs (BALB, 129, D2) in a NaCl vs. KCl taste-discrimination task and compared their performance with that seen in amiloride-sensitive B6 mice before and after amiloride treatment.

where adulteration of the taste solutions with amiloride reduces the ability of rats to discriminate NaCl from KCl salt to chance levels (21, 33), as well as in taste-aversion conditioning studies, where lingual application with amiloride causes rats to generalize between NaCl and nonsodium salts (18). Moreover, the expression of a sodium appetite, the phenomenon in which sodium-depleted rats specifically seek out and ingest sodium salts, is marked attenuated by the adulteration of the salt solutions with amiloride (2, 4, 15, 24, 29).

In mice, electrophysiological examination of the CT as well as recording from taste cells of various inbred mouse strains indicates that, in some strains [such as C57BL/6 (B6)], CT responsiveness to sodium is markedly suppressed by lingual application of amiloride as in rats, whereas other strains [such as DBA/2 (D2), BALB/c (BALB), and 129P3/J (129)] do not show this suppression (14, 28) or have a reduced number of taste cells that are responsive to lingual application of amiloride (25). However, using an operant conditioning paradigm, we have previously demonstrated that amiloride reduces behaviorally measured sensitivity to NaCl by an order of magnitude in mice from both the B6 and the D2 strains (10).
METHODS

Subjects

Twenty-eight naïve adult male mice from four inbred strains (7 mice/strain); B6, BALB, 129, and D2, from Jackson Laboratory (Bar Harbor, ME) served as subjects. On arrival, the mice were 8 wk old, and their mean body mass (±SE) was 20.3 ± 0.73, 23.3 ± 0.35, 20.8 ± 0.57, and 22.3 ± 0.80 g, respectively. Mice were individually housed in shoebox cages in a colony room kept at a controlled temperature with an automatic lighting cycle (12 h light, 12 h dark). All mice were handled and tested during the light phase. The mice received free access to pellets of laboratory chow (LabDiet 5001, PMI Nutrition International, Brentwood, MO) and purified water (Millipore Elix 10, Billerica, MA). Eight days after arrival, they were put on a restricted water-access schedule in which fluid was only available during the training or testing sessions (see below). Body mass was monitored daily, and mice that dropped below 85% of their hydrated weight received 1 ml of supplemental distilled water after their corresponding session. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Solutions

The training solutions were 0.6 M NaCl and KCl (Fisher Scientific, Atlanta, GA). This concentration of NaCl was chosen because it is well above threshold and can be easily detected by D2 and B6 mice regardless of the amiloride sensitivity of the CT, based on our previous experiments (9, 10). The KCl concentration was isomolar with the NaCl with the assumption that it too can be easily detected, although no behavioral detection experiments were conducted. The testing concentrations were 0.1, 0.3, 0.6, and 1.0 M of both NaCl and KCl. This range was selected to include some clearly detectable concentrations and to render intensity an irrelevant cue. The amiloride hydrochloride (Sigma, St. Louis, MO) concentrations used were 0.1, 1, 3, 10, 30, and 100 μM in a descending order. The highest concentration of amiloride in this range potentely suppresses NaCl responses by the CT in electrophysiological studies of rodent salt taste transduction, notwithstanding the inbred mouse strain exceptions noted above. In fact, the majority of electrophysiological and behavioral research with mice has involved concentrations at this value or lower (e.g., Refs. 9, 10, 25, 28, 34). Lower concentrations of amiloride were added until performance was not significantly different than that on control days (see below).

Apparatus

The gustometer, modified from one designed for use in rats (32), has been described previously (9, 10). Three openings are located on one side wall of the testing chamber. The middle opening is the sample access slot through which the mouse can protrude its tongue to lick the sample spout when available. Two response spouts protrude through the other two openings that are located on either side of the sample access slot. The response spouts can deliver water when licked and, for certain sessions, can be manually retracted and the opening covered. The testing chamber is enclosed in a sound-attenuation box (BRS/LVE, Laurel, MD). The solutions are delivered to the sample spout from reservoirs located outside the sound-attenuation box via Teflon tubing through solenoid valves. A computer controls the opening and closing of the solenoid valves that, in turn, control the delivery of the solutions. White noise is delivered from a speaker inside the sound-attenuation box throughout the session.

Procedure

Trial structure. Each trial began with a presentation of either NaCl or KCl. The trial started when the mouse took at least two licks within 250 ms. Once the mouse took five licks (~1.6 μl/lick) or the allocated time for sampling had ended (2 s), the house lights were turned off and the cue lights above the response spouts were illuminated to signal the decision period. At this time, the mouse had 10 s to approach one of the response spouts and lick from it to receive a water reinforcer. If the mouse made a correct response, it received up to 20 licks of water in 30 s (whichever came first). If the mouse responded incorrectly or did not respond during the allocated time, the mouse received a 30-s timeout, during which the lights were turned off and no fluid was available. There was an intertrial interval of 6 s during which the sample spout was rotated over the funnel, rinsed with distilled water, and dried with pressurized air.

Training schedule. All mice, randomly assigned to one of two gustometers, were tested for 3 days in the spout training phase (Table 1) in 30-min sessions. In this phase, the mice were allowed to lick water freely from a single stationary spout, changed daily (sample, left reinforcement, or right reinforcement spout), in an effort to familiarize the animals with the location of each spout. On the first day of this phase of training, the sample spout was made available by positioning it in front of the access slot while the two response spouts were retracted and their access openings in the wall covered. On day 2 of this phase, one of the two response spouts was made available. On day 3, the other response spout was made available for ad-lib licking while the other spouts were unavailable. The side training phase (Table 1) began next. In this phase, the appropriate response spout was made available along with the sample spout, which delivered either 0.6 M NaCl or 0.6 M KCl (counterbalanced between mice and strains). On each trial, the animal was required to lick the assigned salt stimulus in the sample spout and then lick the available response spout to receive its water reinforcer. The alternation phase (Table 1) followed, in which one stimulus (NaCl or KCl, counterbalanced between mouse strains and individual mice within each strain) was presented repeatedly until a certain criterion number of correct responses were made (not necessarily successive). Once the required number of correct responses was achieved, the other stimulus was presented until the number of required correct responses was reached again. This criterion number of correct responses before the stimulus was shifted was initially 6 and was decreased from 6 to 4, 2, and finally to 1 across sessions. Mice were moved from one criterion to the next when their performance reached 75% correct responses. Mice progressed individually, but the next phase did not start until all mice reached a criterion of 1.

Table 1. Training and testing schedule

<table>
<thead>
<tr>
<th>Phase</th>
<th>Sessions</th>
<th>Stimuli</th>
<th>Limited Hold, (s)</th>
<th>Time Out, (s)</th>
<th>Presentation Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spout training</td>
<td>3</td>
<td>dH2O</td>
<td>none</td>
<td>none</td>
<td>Constant</td>
</tr>
<tr>
<td>Side training</td>
<td>6</td>
<td>0.6 M NaCl or 0.6 M KCl</td>
<td>180</td>
<td>none</td>
<td>Constant</td>
</tr>
<tr>
<td>Alternation</td>
<td>25–45*</td>
<td>0.6 M NaCl and 0.6 M KCl</td>
<td>15</td>
<td>30</td>
<td>Criteria1 (6–1)</td>
</tr>
<tr>
<td>Discrimination training</td>
<td>10–30</td>
<td>0.1, 0.3, 0.6, 1.0 M NaCl and KCl</td>
<td>10</td>
<td>30</td>
<td>Randomized blocks</td>
</tr>
<tr>
<td>Testing</td>
<td>30</td>
<td>0.1, 0.3, 0.6, 1.0 M NaCl and KCl</td>
<td>10</td>
<td>30</td>
<td>Randomized blocks</td>
</tr>
</tbody>
</table>

Limited hold, amount of time the mouse was given to make a response; constant presentation schedule, presentation of the same stimulus throughout the entire session (no randomization). *Due to problems with one of the gustometers, some mice took longer to meet the criterion of performance required to progress to the next phase (80% correct with at least 3 blocks of all stimuli). ΤA stimulus is presented repeatedly until a certain number of correct responses are made (not necessarily successive). This criterion number of responses was decreased from 6 to 4, 2, and finally to 1. Mice were moved from one criterion to the next when their performance reached 75% correct responses. Mice progressed individually, but the next phase did not start until all mice reached a criterion of 1.
performance reached 75% correct responses. The mice progressed individually, but the next phase did not start until all mice reached a criterion of 1. During this phase, the lick circuit of one of the gustometers was found to be faulty and the mice were transferred to another gustometer. Due to this problem, some of the mice took longer to meet the criterion of performance required to progress to the next phase (80% correct with at least 3 blocks of all stimuli). Last, in the discrimination training phase (Table 1), the mice were tested with all four concentrations of both NaCl and KCl in randomized blocks until their performance across sessions stabilized (there was no significant main effect of sessions in the overall performance of all strains across the last 2 wk of training).

Testing schedule. During the testing phase, sessions were included in which all solutions as well as the water reinforcer were mixed with the appropriate concentration of amiloride. This phase also included control days so that stimulus control of behavior could be measured and maintained. The amiloride concentrations were tested in a descending series across sessions on Tuesday and Friday, with Monday, Wednesday, and Thursday providing control days in which amiloride was not mixed in the solutions. The data from Wednesday sessions was not included in the analysis because this was considered a “retraining” session, although performance was typically very good on this day, indicating little loss in stimulus control. The series of descending amiloride concentrations was tested twice, and each amiloride concentration that was tested on a Tuesday in the first round was tested on a Friday in the second round and vice versa. This was done to avoid any test-day bias. Due to technical problems with one gustometer during 1 wk of the testing phase, the amiloride concentrations of this week were repeated for mice in this specific apparatus, and the remainder of their testing was pushed back 1 wk.

Water control test. At the termination of the experiment, all reservoirs were filled with water, one-half were assigned to the NaCl-associated response spout, and one-half were assigned to the KCl-associated response spout. Mice were tested in this manner for two consecutive sessions. This test was done to ensure that the mice were responding according to the chemical nature of the taste cue rather than to any other potential external cues.

Data Analysis

Means were calculated for the overall performance as well as the performance on each salt for the two sessions of the same amiloride concentration (amiloride sessions). Only trials with a response were included. Control days preceding the same amiloride concentrations were also collapsed together, and a mean was calculated for them (control sessions). Means were compared across amiloride concentrations using repeated-measures ANOVA, whereas performance at specific concentrations was compared with chance (50%) as well as with the matching control days using t-tests with and without Bonferroni corrections for multiple comparisons. Performance on control sessions was compared across strains using one-way ANOVA followed by Tukey’s post hoc tests when appropriate.

In addition, a logistic function (Eq. 1) was fit to the amiloride concentration-response data for overall performance and for performance on NaCl trials:

\[
f(x) = \frac{a - d}{1 + 10^{\left(\frac{\log(x) - c}{b}\right)}} + d
\]

where \(x\) represents the amiloride concentration, \(a\) is a constant for each strain (mean asymptotic maximal performance on control sessions), \(b\) is the slope, \(c\) is the amiloride concentration at one-half maximal asymptotic performance, and \(d\) is the asymptotic minimum of performance (constrained to a 0 minimum). These curve parameters were compared across strains using one-way ANOVA. Amiloride dose-response curves could not be fit to the overall performance data for one mouse (D2 strain) and to the data from NaCl trials for seven mice (2 of 7 for B6, 2 of 7 for BALB, 1 of 7 for 129, and 2 of 7 for D2 strain). Even in these cases, however, the mice displayed a trend of improved performance on NaCl trials as the amiloride concentration was lowered, but the variability in their dose-response functions precluded estimation of the parameters in the logistic equation (Eq. 1) above. Thus, statistical analyses involving curve parameters only included data from mice for which curves could be fit.

Finally, the normal approximation of the binomial distribution (1-tailed test) was used to determine any positive deviation of performance from chance on water control test sessions. The conventional \(P\) value of \(\leq 0.05\) was considered significant in all statistical tests.

RESULTS

Overall Performance

Mice initiated many trials during control and amiloride sessions, as can be seen in Table 2. There was no significant difference between the strains in the total number of trials initiated across the collapsed control sessions \([F(3,24) = 1.40; P = 0.27]\). Moreover, there was no significant main effect of strain for the total number of trials taken on amiloride sessions \([F(3,24) = 2.09; P = 0.13]\), but there was a main effect of amiloride concentration \([F(5,120) = 4.41; P = 0.001]\). The strain \(\times\) concentration interaction, however, was not significant \([F(15,120) = 0.62; P = 0.85]\). Thus, in terms of number of trials taken, the strains acted comparably.

Control Sessions

There was no significant difference in performance across control sessions within each strain \([all F(5,30) < 1.63; all P > 0.185; Fig. 1]\). Therefore, for all further analyses, the control sessions were collapsed into one mean for each strain. A one-way ANOVA of the overall performance on collapsed control sessions indicated a significant strain effect \([F(3,24) = 7.17; P = 0.001]\). Post hoc Tukey’s paired comparisons revealed that the overall performance of the B6 mice on control sessions was significantly lower than that of the 129 and D2 mice (both \(P \leq 0.01\)) but not the BALB mice \((P = 0.17)\); there were no significant differences between the latter three strains \((all P > 0.14)\).

Table 2. Total number of trials

<table>
<thead>
<tr>
<th>Strain</th>
<th>B6</th>
<th>BALB</th>
<th>129</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sessions (collapsed)</td>
<td>575.29±32.52</td>
<td>658.00±19.15</td>
<td>596.29±51.24</td>
<td>571.86±22.55</td>
</tr>
<tr>
<td>0.1 μM Amil</td>
<td>107.86±7.03</td>
<td>131.14±5.52</td>
<td>118.29±11.32</td>
<td>114.00±6.45</td>
</tr>
<tr>
<td>1 μM Amil</td>
<td>104.57±5.30</td>
<td>117.43±5.46</td>
<td>103.43±8.87</td>
<td>99.71±5.98</td>
</tr>
<tr>
<td>3 μM Amil</td>
<td>103.57±3.90</td>
<td>115.43±5.76</td>
<td>100.00±11.37</td>
<td>98.43±8.04</td>
</tr>
<tr>
<td>10 μM Amil</td>
<td>106.14±4.01</td>
<td>113.57±4.61</td>
<td>100.00±5.71</td>
<td>109.43±3.37</td>
</tr>
<tr>
<td>30 μM Amil</td>
<td>106.86±4.47</td>
<td>119.43±2.46</td>
<td>110.29±3.45</td>
<td>110.00±3.76</td>
</tr>
<tr>
<td>100 μM Amil</td>
<td>108.86±3.11</td>
<td>116.00±1.98</td>
<td>104.43±7.57</td>
<td>105.86±3.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. B6, C57BL/6J mice; BALB, BALB/cByJ mice; 129, 129P3/J mice; D2, DBA/2J mice; Amil, amiloride.
Amiloride Sessions

Overall performance dropped to chance at 100 μM amiloride and monotonically improved as the adulterating amiloride concentration was lowered (Fig. 1). A one-sample t-test between 50% and performance on each amiloride concentration indicated that both 100 as well as 30 μM amiloride were not significantly different than chance in B6 and 129 mice, whereas only 100 μM amiloride was not different than chance in the BALB and D2 mice. A Bonferroni correction for multiple comparisons eliminated the difference for these latter two strains at the 30 μM concentration and also eliminated the significant difference observed for the B6 and 129 mice at the 10 μM concentration.

A strain × amiloride concentration ANOVA of the data revealed a significant main effect of concentration [F(5,120) = 166.39; P < 0.001] and of strain [F(3,24) = 6.18; P = 0.003] as well as an interaction [F(15,120) = 1.94; P = 0.025]. However, when curves were fit to the amiloride session data for individual mice, there was no significant difference between strains in the c values [B6 mean: 2.26 μM; BALB mean: 3.10 μM; 129 mean: 4.73 μM; D2 mean: 3.08 μM; F(3,23) = 0.79; P = 0.51], nor was there a difference in the slope [B6 mean: 4.39; BALB mean: 4.0; 129 mean: 3.6; D2 mean: 1.89; F(3,23) = 0.30; P = 0.83] or the minimum asymptote [B6 mean: 51.5; BALB mean: 50.7; 129 mean: 46.7; D2 mean: 51.5; F(3,23) = 0.57; P = 0.64]. Thus the strain differences were likely driven by the difference in baseline levels of performance.

Comparison Between Control Sessions and Amiloride Sessions

A paired t-test indicated no significant difference between control sessions and amiloride sessions for the lowest concentration of amiloride tested (0.1 μM) in all mouse strains [all t(6) < 1.67; all P > 0.14]. Overall performance on amiloride sessions did not differ from control sessions for 1 μM in B6 mice as well [t(6) = 1.18; P = 0.28], and the same was true for the BALB mice after a Bonferroni correction for multiple comparisons. The performance in sessions testing all other amiloride concentrations differed from performance on control sessions (all P < 0.05).

Analysis by Salt

Control sessions. There was no significant difference between performance across control days for either NaCl [all F(5,30) < 2.48; all P > 0.05] or KCl [all F(5,30) ≤ 1.77; all P ≥ 0.15] within each strain. When all control sessions were collapsed, no significant difference between the strains was found for performance on NaCl trials [F(3,24) = 2.43; P = 0.09]. There was, however, a significant strain effect for KCl trials [F(3,24) = 5.72; P = 0.004]. Tukey’s honestly significant difference post hoc analysis revealed that the performance of B6 mice on control days was different from that of 129 and D2 mice on KCl trials (both P ≤ 0.014) but did not differ from that of BALB mice (P = 0.13), and the performance of 129, D2, and BALB mice was not different from each other (all P ≥ 0.45). This finding suggests that the difference in overall performance between the strains was driven primarily by responses during KCl trials.

Amiloride Sessions. Performance on NaCl trials was much more affected than that on KCl trials (Fig. 2). A three-way ANOVA of salt × strain × amiloride concentration revealed a significant main effect of salt [F(1,24) = 11.69; P = 0.002], strain [F(3,24) = 6.30; P = 0.003] and of amiloride concentration [F(5,120) = 164.84; P < 0.001], as well as a significant interaction between amiloride concentration and strain [F(15,120) = 1.95; P = 0.025] and between amiloride concentration and salt [F(5,120) = 14.26; P < 0.001] but not between strain and salt [F(3,24) = 1.03; P = 0.40], nor was a triple interaction evident [F(15,120) = 0.72; P = 0.76].

SODIUM CHLORIDE. When analyzed separately, there was a significant main effect of amiloride concentration for NaCl [F(5,120) = 84.17; P < 0.001] on performance but no significant main effect of strain [F(3,24) = 0.35; P = 0.79] or interaction [F(15,120) = 1.04; P = 0.43]. For BALB and 129 mice, performance on NaCl trials dropped significantly below...
The individual fits revealed no significant difference between strains. A Bonferroni correction for multiple comparisons failed to reach statistical significance for all mouse strains [all \( t(6) \leq 1.54; P \geq 0.17 \)]. Performance was significantly higher than chance on 0.1 and 1.0 \( \mu M \) amiloride for all mouse strains [all \( t(6) \geq 6.80; P < 0.001 \)]. For 129 mice, performance was also above chance when the solutions were adulterated with 3 \( \mu M \) amiloride [\( t(6) = 3.63; P = 0.01 \)]; this was no longer the case, however, after a Bonferroni correction for multiple comparisons. At all other amiloride concentrations used, performance on NaCl did not differ from chance. A paired \( t \)-test revealed that at amiloride concentrations [all \( t(6) \geq 2.63; P \leq 0.04 \)] but the lowest one [all \( t(6) \leq 2.24; P \geq 0.07 \)] used, the performance on NaCl trials significantly differed from those on the matching control days in all four strains of mice. A Bonferroni correction for multiple comparisons did not change these results much (see Fig. 2).

A one-way ANOVA for each of the curve parameters from the individual fits revealed no significant difference between the strains in the \( c \) values [B6 mean: 3.83 \( \mu M \); BALB mean: 3.0 \( \mu M \); D2 mean: 6.46 \( \mu M \); \( F(3,17) = 0.46; P = 0.72 \)], nor was there a difference in the slope [B6 mean: 5.06; BALB mean: 0.90; 129 mean: 1.59; D2 mean: 0.95; \( F(3,17) = 1.37; P = 0.29 \)] or the minimum asymptote [B6 mean: 37.9; BALB mean: 24.3; 129 mean: 28.8; D2 mean: 16.9; \( F(3,17) = 1.54; P = 0.24 \)].

Potassium Chloride. When analyzed separately, there was a significant main effect of amiloride concentration for KCl \( [F(5,120) = 18.89; P < 0.001] \) but no significant main effect of strain \( [F(3,24) = 2.34; P = 0.10] \) or interaction \( [F(15,120) = 1.10; P = 0.37] \). For all mouse strains but B6, performance on KCl trials differed from chance at most (but not all) concentrations of amiloride, suggesting that amiloride has much less effect on identification of KCl than on identification of NaCl. The 129 mice performed significantly above chance on KCl trials for all amiloride concentrations [all \( t(6) \geq 2.90; P \leq 0.03 \)], and BALB mice performed significantly above chance on all [all \( t(6) \geq 3.2; P \leq 0.02 \)] but the 3 and 30 \( \mu M \) amiloride concentrations [both \( t(6) \leq 2.36; P \geq 0.06 \)], whereas the D2 mice performed significantly above chance at all [all \( t(6) \geq 3.06; P \leq 0.02 \)] but the 10 and 30 \( \mu M \) concentrations [both \( t(6) \leq 1.96; P \geq 0.10 \)]. It is notable that there was no systematic concentration-dependent effect of amiloride on performance in the 129, BALB, and D2 strains. In contrast, the B6 mice performed significantly above chance only at the two lowest concentrations of amiloride used: 0.1 and 1 \( \mu M \) [both \( t(6) \geq 4.36; P \leq 0.005 \)]. Bonferroni corrections applied to the above statistical tests produced similar outcomes except that in three cases [once in each strain of BALB (10 \( \mu M \)), 129 (30 \( \mu M \)), and D2 (100 \( \mu M \))] differences failed to reach significance. The greater effectiveness of amiloride in the B6 mice compared with the other strains might be related, in part, to the relatively poorer performance of this strain on KCl trials during control sessions.

Performance during amiloride sessions was significantly different than that on the matching control sessions for only a few amiloride concentrations, and these concentrations differed between the strains. For the BALB mice, it was significantly different on 0.1 and 30 \( \mu M \) concentration [both \( t(6) \geq 2.47; P < 0.05 \)]; for 129 mice, it was significantly different on the 3, 10, and 30 \( \mu M \) concentrations [all \( t(6) \geq 2.51; P < 0.05 \]); for D2 mice, it was different on only the two highest amiloride concentrations [both \( t(6) \geq 2.75; P < 0.03 \)]; whereas, for B6 mice, performance on amiloride sessions significantly differed from control sessions at 3, 10, and 100 \( \mu M \) concentrations [all \( t(6) \geq 2.51; P \leq 0.05 \)]. A Bonferroni adjustment eliminated the significance of all of these comparisons.

Correlations. As noted above, high concentrations of amiloride affected performance on NaCl trial much more than it did on KCl trials. The results suggested that amiloride treatment alters the taste quality of NaCl, changing it to one that is similar to KCl. One way that we tested this hypothesis was to examine the relationship of performance between KCl and NaCl trials. The correlation was strong \( (r = -0.80) \), as depicted in the scatter plot in Fig. 3. This result shows that
mice that performed very well on KCl trials performed well below chance on NaCl trials, whereas mice that performed only modestly above chance on KCl trials were only modestly below chance on NaCl trials.

**Water Control Test**

In the water control test, only one animal (from the D2 mouse strain) responded significantly different from chance (50%) out of the 28 mice tested (all P values except one 0.05), with a performance average of 50.93 ± 1.42% for the B6 mice, 48.88 ± 1.34% for the BALB mice, 46.28 ± 1.33% for the 129 mice, and 50.54 ± 3.06% for the D2 mice (Fig. 4). The one mouse that responded significantly above chance nonetheless performed poorly (61.57%), a result that was no longer significant after a Bonferroni correction, and performed well during control testing (80.8% on mean control days). These results confirmed that the mice were not responding to extraneous cues but rather on the basis of the chemical nature of the stimulus. It is interesting to note that B6 mice performed more poorly on KCl control trials relative to the other stains. It would be worthwhile, using other behavioral tasks as well as electrophysiological measures of gustatory nerve activity, to examine whether B6 mice are less sensitive to KCl, especially compared with D2 and 129 mice.

Although we cannot rule out that the difference in the effectiveness of amiloride to disrupt responses in the electrophysiological studies of the CT and in our behavioral experiment may be due to some genotypic idiosyncrasy of the specific substrains used (the strains used in the Japanese electrophysiological studies were DBA/2CrSlc and BALB/cCrSlc); in the case of the 129, the substrain used in the electrophysiological study (14) was the same as used here, yet the result is different. Thus the uniformity of the amiloride disruption of salt discrimination performance in the four different strains tested here weakens the argument that substrain differences between studies can reconcile the discrepancy between the electrophysiological and the behavioral results.

**Fig. 3.** Correlation between individual performance on NaCl trials and performance on KCl trials of mice of all strains collapsed together.

**DISCUSSION**

The apparent absence, or at most limited presence, of an amiloride-sensitive NaCl transduction mechanism in the anterior tongue of BALB, 129, and D2 mice (14, 25, 26, 28) does not preclude these animals from discriminating NaCl from KCl and also does not curtail the effectiveness of amiloride to compromise discrimination performance. Mice from all four strains performed relatively well during baseline test sessions and exhibited amiloride concentration-dependent decrements on salt discrimination performance regardless of the reported amiloride sensitivity or insensitivity of their CT. The results of the water control test confirmed that the mice were not responding to extraneous cues but rather to the chemical nature of the stimuli tested. Moreover, it is likely that the mice were responding on the basis of the taste quality as opposed to the intensity of the stimuli because a broad range of concentrations were used, which generated presumably overlapping sensation magnitudes, making intensity an unreliable predictor of reinforcement. These results are also in concordance with our previous study (10) in which 100 μM amiloride increased the behaviorally measured NaCl detection threshold in B6 as well as in D2 mice, with the latter strain having an amiloride-insensitive CT.

It is interesting to note that B6 mice performed more poorly on KCl control trials relative to the other stains. It would be worthwhile, using other behavioral tasks as well as electrophysiological measures of gustatory nerve activity, to examine whether B6 mice are less sensitive to KCl, especially compared with D2 and 129 mice.

Fig. 4. Individual performance expressed as overall % correct (solid bars) as well as means ± SE (hatched gray bars) on the water control test collapsed across the 2 days of testing for the 4 mouse strains tested.
We cannot entirely dismiss the possibility that the limited amount of amiloride-sensitive sodium channels in the anterior tongue, although not enough to exhibit changes in responsiveness to sodium with lingual amiloride application when measured in the CT nerve, may be enough to allow behavioral discrimination of this cation from potassium. A more parsimonious explanation, however, is that there are ENaCs in taste receptor fields innervated by gustatory nerves other than the CT. The most likely candidate would be the greater superficial petrosal nerve that innervates the palatal taste buds. In rats, the palatal taste receptors decrease their responsiveness to NaCl after amiloride treatment (31), but the posterior tongue taste buds, innervated by the glossopharyngeal nerve, are immune to the effects of the drug (13, 19). To our knowledge, the greater superficial petrosal nerve in the B6, BALB, 129, or D2 strains has not been tested for its amiloride sensitivity.

With respect to the potential existence of functional ENaCs in palatal taste receptor cells, it is possible that, in the B6 strain, the majority of taste ENaCs are found in the anterior tongue. Consistent with this possibility, Yasumatsu et al. (34) found that, after CT nerve crush, B6 mice that have a conditioned taste aversion to NaCl displayed concentration-dependent decreases in lick avoidance that completely overlapped that seen for KCl, and, more to the point, 30 μM amiloride was without effect on these responses. Before CT nerve crush or after regeneration of the CT, the mice showed much more lick avoidance to most of the NaCl concentrations tested relative to KCl, and, importantly, amiloride treatment (30 μM) eliminated the difference in the concentration-response functions seen for the two salts (i.e., amiloride treatment emulated the effect of CT crush). We predict that the effectiveness of amiloride to impair performance on conditioned taste aversion generalization or explicit taste discrimination tasks involving sodium vs. nonsodium salts would be unaffected by CT transection in the BALB, 129, or D2 strains given that the nerve does not display sensitivity to the ENaC blocker in the first place, but this remains to be tested.

Regardless of the potential strain differences in ENaC distribution among the taste receptor fields in the oral cavity suggested by the constellation of behavioral and electrophysiological results, it is clear that this ion channel is a critical transduction component underlying the ability of mice and other rodent species to discriminate between NaCl and KCl. The cation selectivity of the ENaC, at least in rodent species, provides a clear receptor mechanism through which these animals can identify sodium salts. Without the presence of the ENaC or when it is functionally disabled (e.g., amiloride treatment), the poor cation selectivity of the remaining salt transduction pathways leads to a peripheral signal that does not discriminate among many common salts. Providing electrophysiological support for this notion in rats, the peripheral taste fibers that are amiloride sensitive are, in general, narrowly tuned to respond to Na⁺ and Li⁺, whereas those fibers for which responses to NaCl are unaffected by oral amiloride treatment respond broadly to sodium and nonsodium salts and HCl (27). This provides a basis for explaining why amiloride-treated rats conditioned to avoid NaCl generalize the aversion to KCl and other nonsodium salts (18) and why rats trained to discriminate NaCl from KCl treat NaCl as if it were KCl (33) when the stimuli are adulterated with 100 μM amiloride. Here, we found similar behavioral profiles with the mice. That is, the BALB and 129 mice performed significantly (unadjusted P) below chance on NaCl trials when 100 μM amiloride was the solvent, and a similar trend, although failing to reach significance, was observed in the B6 and D2 strains. That means that when 100 μM amiloride was the solvent, the mice were more likely to lick the KCl-associated reinforcement spout than the NaCl-associated reinforcement spout on NaCl trials. Performance on KCl trials, although impaired, was much less affected by amiloride treatment. These results are consistent with the view that amiloride changes the taste quality of NaCl to resemble that of KCl. The variability in the degree to which mice (and strains) licked the KCl-associated reinforcement spout on NaCl trials and the degree to which performance on KCl trials was impaired when 100 μM amiloride was serving as the solvent is linked. In some mice, stimulus control of behavior began to break down during 100 μM amiloride treatment, driving performance on both NaCl and KCl closer toward chance. This is because animals did not receive their expected reinforcer when they licked the KCl-associated spout on NaCl (+100 μM amiloride) trials. Stimulus control in other mice, however, appeared to survive the amiloride adulteration of NaCl as indicated by good performance on KCl trials in the context of below chance performance on NaCl trials during the 100 μM amiloride test sessions. Indeed, the negative correlation between performance on NaCl trials and that on KCl trials when 100 μM amiloride was the solvent was strong (r = −0.80). Thus the behavioral effects of amiloride treatment on salt taste discrimination displayed by the mice tested here correspond well with those seen in rats and suggest that ENaC blockade during NaCl stimulus presentations leads to a peripheral signal that the animal interprets as KCl.

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GRANTS

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REFERENCES


