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NaCl-preferring NZB/B1NJ Mice and NaCl-avoiding CBA/J Mice Have Similar Amiloride Inhibition of Chorda Tympani Responses to NaCl

Yuzo Ninomiya[1], Alexander A. Bachmanov, Akira Yatabe[2] and Gary K. Beauchamp

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA and Departments of [1]Oral Physiology and [2]Pediatric Dentistry, Asahi University School of Dentistry, Hozumi, Motosu, Gifu 501-02, Japan

Correspondence to be sent to: Gary K. Beauchamp, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA

Abstract

Integrated chorda tympani nerve responses to NaCl were studied in two mouse strains, an NaCl-preferring NZB/B1NJ and an NaCl-avoiding CBA/J. The NaCl responses of both strains had similar magnitude and were suppressed by amiloride to a similar extent. This suggests that peripheral gustatory responsiveness to NaCl is not the only mechanism underlying mouse strain variation in NaCl acceptance.

Introduction

Previous studies have demonstrated that a sodium transport blocker, amiloride, suppresses gustatory neural responses to NaCl in several species of mammals (Schiffman *et al.*, 1983; Heck *et al.*, 1984; Hellekant *et al.*, 1988; for review see Halpern, 1998). In mice, there are prominent strain differences in the amiloride sensitivity of the NaCl taste receptor system. That is, chorda tympani responses of C57BL/6 and C3H/He mice to 0.1 M or higher concentrations of NaCl were suppressed by amiloride by ~50% of control, whereas no suppression was observed in BALB/c, DBA/2 and 129/J mice (Ninomiya *et al.*, 1989, 1996; Gannon and Contreras, 1993). Since the former two strains behaviorally avoid 0.1 and 0.15 M NaCl solutions whereas the latter three strains prefer or are indifferent to them (Ninomiya *et al.*, 1989; Beauchamp and Fisher, 1993; Bachmanov *et al.*, 1996), it is possible that behavioral preference/avoidance for NaCl in mice is related to the presence or absence of an amiloride-sensitive NaCl receptor system (Ninomiya *et al.*, 1989, 1996).

Recently, Bachmanov *et al.* (1998) identified two mouse strains that show more pronounced NaCl preference (NZB/B1NJ) and avoidance (CBA/J) than the above-mentioned strains. That is, in 48 h two-bottle preference tests the NZB/B1NJ mice prefer NaCl at 0.0375–0.15 M relative to plain water and are even indifferent to 0.3 M NaCl, a concentration avoided by virtually every other mouse strain tested. In contrast, the CBA/J mice avoided even 0.0375 M NaCl and showed extremely low preference for all concentrations of NaCl above this concentration. These two strains therefore provide a good opportunity to further test the hypothesis that amiloride sensitivity and NaCl preference are related in mice.

Materials and methods

Subjects were 9-week-old male mice of the NZB/B1NJ (NZB, $n = 5$) and CBA/J (CBA, $n = 5$) strains, ranging in weight from 25 to 30 g. They were purchased from The Jackson Laboratory (Bar Harbor, ME), housed in a temperature-controled room at 23°C on a 12 h light:12 h dark cycle, and had free access to tap water and Teklad Rodent Diet 8604.

Mice were anesthetized with an i.p. injection of sodium pentobarbital (40–50 mg/kg body wt) and maintained at a surgical level of anesthesia with supplemental injections of sodium pentobarbital. The trachea was cannulated and the mouse was then fixed in the supine position with a head holder to allow dissection of the chorda tympani nerve. The chorda tympani nerve was exposed at its exit from the lingual nerve and cut near its entrance to the bulla. For whole nerve recording, the entire nerve was placed on a silver wire electrode. An indifferent electrode was placed in nearby tissue. Neural responses resulting from chemical stimulations of the tongue were fed into an amplifier, and monitored on an oscilloscope and audiomonitor. Whole nerve responses were integrated and displayed on a recorder. The time constant of the integrator was 0.5 s.

Solutions used as chemical stimuli were: (i) 0.1 M NH_4Cl; (ii) 0.3 and 1.0 M sucrose; (iii) 0.02 M quinine-HCl; (iv) 0.01 M HCl; (v) 0.01–1.0 M NaCl; (vi) 0.01–1.0 M KCl; (vii) mixtures of 0.01–1.0 M NaCl/0.1 mM amiloride HCl; and

(viii) mixtures of 0.01–1.0 M KCl/0.1 mM amiloride HCl (Sigma Chemical Co., St Louis, MO). These chemicals were dissolved in distilled water. Methods for chemical stimulation were the same as those described in our previous report (Ninomiya *et al.*, 1996). Each stimulus solution was applied to the tongue for 30 s. Intervals between the stimuli were >1 min. During the intervals, the tongue was rinsed with distilled water. The test solutions and distilled water flowed at the same rate (0.5 ml/s). The solutions were typically applied to the tongue in the following sequence: 0.01 M NaCl, 0.01 M KCl, 0.03 M NaCl, 0.03 M KCl, 0.1 M NaCl, 0.1 M KCl, 0.3 M NaCl, 0.3 M KCl, 1.0 M NaCl and 1.0 M KCl; 0.01 M HCl, 0.02 M quinine, 0.3 and 1.0 M sucrose were randomly applied between these stimuli. NH_4Cl was applied as the first stimulus, after every ~10 stimulations, and any time after adjustments in recording equipment were made. To examine amiloride inhibition of NaCl and KCl responses, the tongue was treated with 0.1 mM amiloride HCl for 30 s by running it over the tongue; after that, mixtures of NaCl or KCl with amiloride were applied in the same sequence as in the beginning of the experiment.

In the analysis of whole nerve responses, the magnitude of the integrated response at 20 s after stimulus onset was measured. Relative response magnitude for each stimulus was calculated with the response magnitude to 0.1 M NH_4Cl taken as unity (1.0), and this was used for statistical analysis. The response magnitudes were analyzed using three-way ANOVAs with strain as a between-group factor and NaCl or KCl concentration and presence of amiloride as within-group factors.The amiloride-sensitive components were calculated for each mouse as a difference between responses to a salt solution with and without amiloride. The amiloride-sensitive components were also expressed (in %) relative to responses to corresponding salt concentrations without amiloride. The amiloride-sensitive components were analyzed using two-way ANOVAs with strain as a between-group factor and solution concentration as a within-group factor. Differences between individual means were assessed using planned comparisons. Responses to sucrose, HCl and quinine-HCl were compared using *t*-tests. All statistical tests used a two-tailed criterion for significance at $P < 0.05$.

Results

Integrated responses of the chorda tympani nerve to 0.01–1.0 M NaCl (relative to 0.1 M NH_4Cl) with and without amiloride were similar in the NZB and CBA mice (Figures 1 and 2A); no strain differences were detected by ANOVA (Table 1) or by planned comparisons made for each NaCl concentration. Amiloride significantly suppressed responses to NaCl at 0.03 M or higher concentrations in both strains (Figure 2A). The amiloride suppression of NaCl responses was similar in both strains

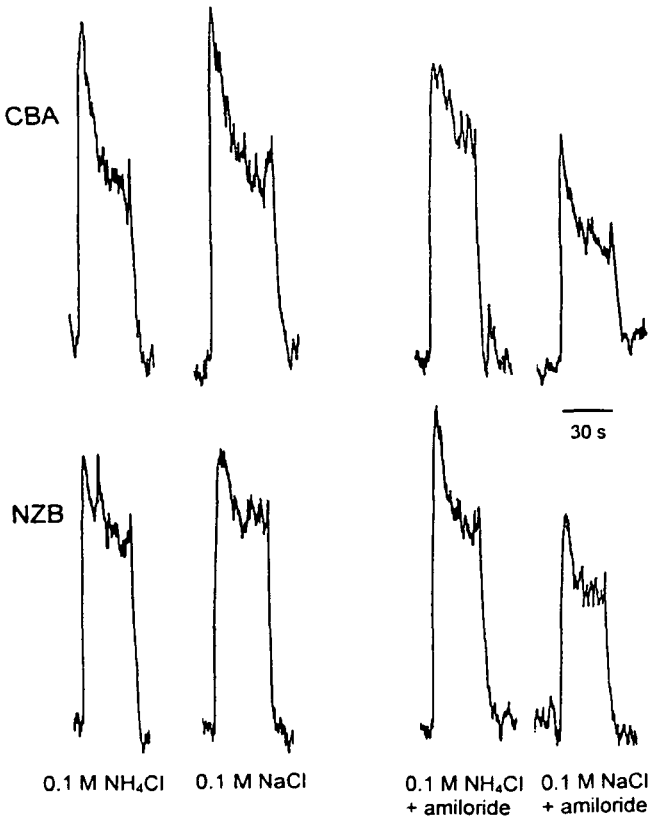

Figure 1 Sample recordings of the integrated chorda tympani responses to 0.1 M NH_4Cl and 0.1 M NaCl, and their mixtures with 0.1 mM amiloride, in NZB and CBA mice.

(no significant interaction between effects of strain and amiloride, Table 1). Consequently, amiloride-sensitive components of responses to NaCl did not differ between the two strains [Figure 2B,C; effect of strain on amiloride- sensitive components expressed in absolute units or in % respectively were $F(1,8) = 0.78$, n.s. and $F(1,8) = 0.95$, n.s.]. Amiloride suppressed 35–54% of the chorda tympani responses to NaCl in both strains (Figure 2C).

Integrated chorda tympani responses to KCl tended to be higher in the CBA mice relative to the NZB mice (Figure 3A), but this difference was not significant (Table 1). In both strains, suppression of the responses to KCl by amiloride was less than the suppression of the responses to NaCl (Figure 3A, cf. Figure 2A). This suppression was statistically significant in the CBA mice but not in the NZB mice (Figure 3A; significant interaction between strain and amiloride effects, Table 1). Consequently, amiloride-sensitive components of responses to KCl expressed in absolute units were higher in the CBA mice than in the NZB mice [Figure 3B, effect of strain, $F(1,8) = 10.9$, $P < 0.02$]. However, there were no significant strain differences in amiloride-sensitive components expressed as a percentage of KCl response [Figure 3C, effect of strain $F(1,8) = 2.25$, n.s.].

The NZB and CBA strains did not differ in responses to

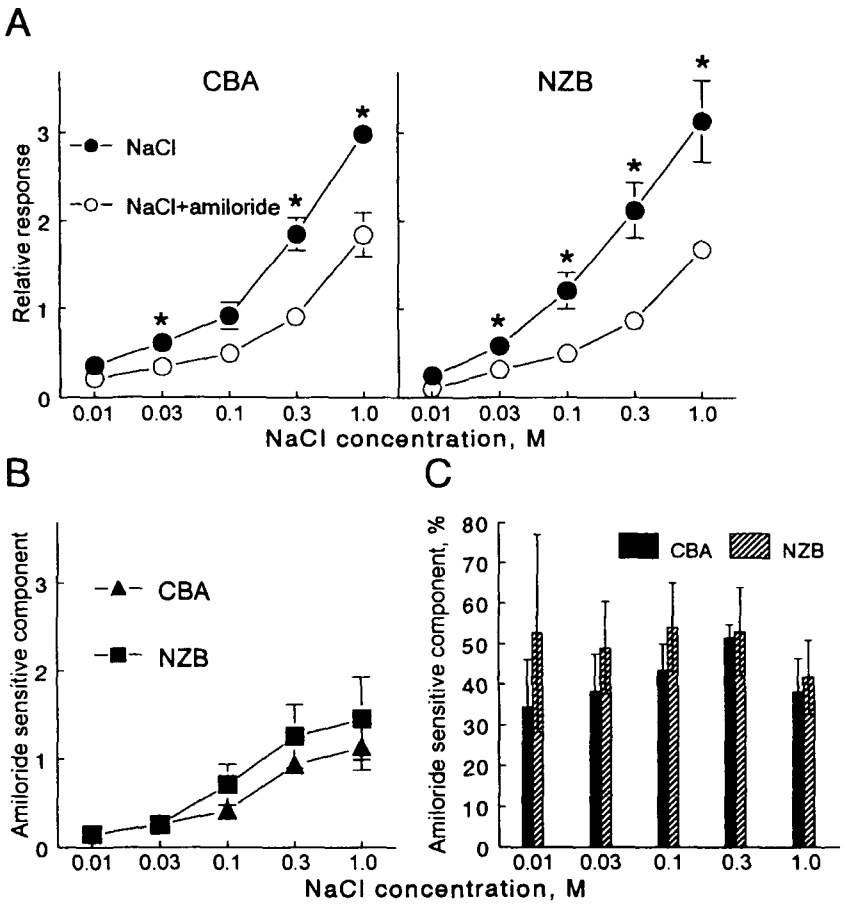

Figure 2 The chorda tympani responses of CBA and NZB mice to NaCl. **(A)** Integrated responses (relative to 0.1 M NH_4Cl) to NaCl and its mixture with 0.1 mM amiloride. *Significant difference between responses to NaCl with and without amiloride ($P < 0.05$, planned comparison tests). **(B, C)** Amiloride-sensitive components of the responses to NaCl expressed in absolute units (B) or relative to responses to pure NaCl, in % (C). Means ± SE are plotted.

sucrose, HCl or quinine-HCl (data not shown). From these results, we conclude that, except for minor variations in responses to KCl, taste responses of the chorda tympani nerve of the NZB mice, including the amiloride inhibition of NaCl responses, do not differ greatly from those of the CBA mice.

Discussion

For reasons described in the introduction, we hypothesized that the amiloride sensitivity in mice may be related to avoidance of osmotically isotonic and/or hypotonic NaCl solutions (Ninomiya *et al.*, 1989, 1996). In this study, using the CBA strain that behaviorally avoids even 0.0375 M NaCl solution (Bachmanov *et al.*, 1998), we found as predicted that these mice possess high amiloride sensitivity, even at 0.03 M NaCl. Thus, in this strain the threshold for behavioral aversion to NaCl is consistent with that for amiloride inhibition of the chorda tympani responses. It is therefore possible that a gustatory signal transduced through the amiloride-sensitive pathway is perceived by the CBA mice as hedonically aversive. However, inconsistent with our hypothesis, the NZB mice, which showed great avidity for NaCl even up to 0.3 M, also showed an amiloride sensitivity of similar magnitude to that of the CBA mice. Therefore, unlike the other NaCl-liking strains so far examined (BALB/c, DBA, 129), relatively high NaCl

Table 1 ANOVA results for the chorda tympani responses to NaCl and KCl with and without amiloride

Effect of:	NaCl	KCl
Strain	$F(1,8) = 0.03$	$F(1,8) = 3.46$
Amiloride	$F(1,8) = 42.6$***	$F(1,8) = 29.1$***
Concentration	$F(4,32) = 167.7$***	$F(4,32) = 214.2$***
Strain × amiloride	$F(1,8) = 0.78$	$F(1,8) = 10.9$*
Strain × concentration	$F(4,32) = 0.61$	$F(4,32) = 1.14$
Amiloride × concentration	$F(4,32) = 13.6$***	$F(4,32) = 2.41$
Strain × amiloride × concentration	$F(4,32) = 0.39$	$F(4,32) = 2.35$

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

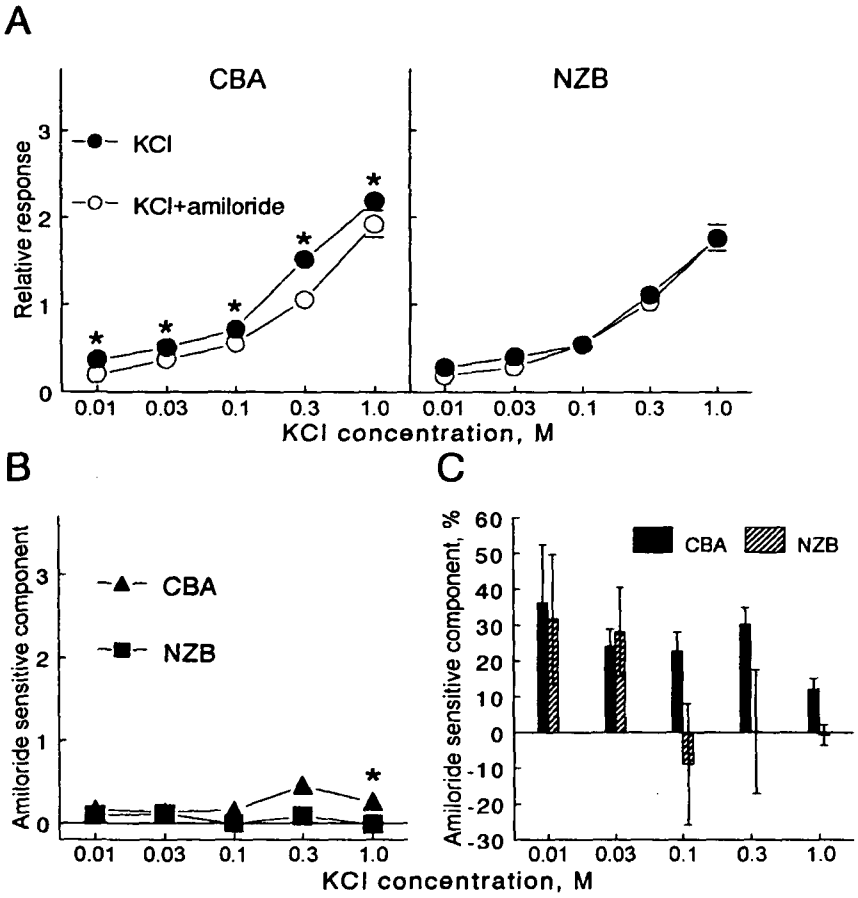

Figure 3 The chorda tympani responses of CBA and NZB mice to KCl. **(A)** Integrated responses (relative to 0.1 M NH_4Cl) to KCl and its mixture with 0.1 mM amiloride. *Significant difference between responses to KCl with and without amiloride ($P < 0.05$, planned comparison tests). **(B, C)** Amiloride-sensitive components of the responses to KCl expressed in absolute units (B) or relative to responses to pure KCl, in % (C). *Significant difference between CBA and NZB mice ($P < 0.05$, planned comparison tests). Means ± SE are plotted.

acceptance by the NZB mice was not coupled with amiloride insensitivity.

Clearly, amiloride sensitivity of peripheral gustatory responses to NaCl may not be the only mechanism underlying the marked differences in NaCl acceptance between the NZB and CBA mice, a result consistent with studies in different rat strains (Formaker and Hill, 1990; Bernstein *et al.*, 1991; Minear *et al.*, 1996). The NZB mice possess several abnormalities that may possibly contribute to their large NaCl consumption: impaired renal sodium conservation (Bachmanov *et al.*, 1997), autoimmune glomerulonephritis (Mellors, 1965; Hicks and Burnet, 1966) and sialadenitis [salivary gland pathology, a model of Sjogren's syndrome in humans (Kessler, 1968; Suzuki *et al.*, 1976; Hayashi, 1995)], and high blood pressure (Svendsen, 1977; Fujisaki, 1983). It is possible that effect of these abnormalities on NaCl acceptance by the NZB mice is stronger than any hypothetical effect of their amiloride sensitivity.

The results of this study suggest that mouse strain differences in NaCl acceptance cannot be explained only by differences in amiloride sensitivity of their peripheral gustatory responses to NaCl. However, further studies are required to determine whether amiloride sensitivity is one of the factors affecting NaCl acceptance or whether correlations between them are fortuitous.

Acknowledgements

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References

Bachmanov, A.A., Tordoff, M.G. and **Beauchamp, G.K.** (1996) *Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice.* Alcohol. Clin. Exp. Res., 20, 201–206.

Bachmanov, A.A., Tordoff, M.G., Ninomiya, Y., Inoue, M., Schlager, G. and **Beauchamp, G.K.** (1997) *Genetic differences in NaCl intake in inbred mouse strains: taste, learning and metabolism.* Abstracts of the XXXIII International Congress of Physiological Sciences (St Petersburg, Russia, 1997), P099.03.

Bachmanov, A.A., Tordoff, M.G. and **Beauchamp, G.K.** (1998) *Voluntary sodium chloride consumption by mice: differences among five inbred strains.* Behav. Genet., 28, 117–124.

Beauchamp, G.K. and **Fisher, A.S.** (1993) *Strain differences in consumption of saline solutions by mice.* Physiol. Behav., 54, 179–184.

Bernstein, I.L., Longley, A. and **Taylor, E.M.** (1991) *Amiloride sensitivity of chorda tympani response to NaCl in Fisher 344 and Wistar rats.* Am. J. Physiol., 261, R329–R333.

Formaker, B.K. and **Hill, D.H.** (1990) *Peripheral taste responses in genetically hypertensive rats.* Physiol. Behav., 47, 1229–1237.

Fujisaki, H. (1983) *Genetic and physiological studies of mouse systolic blood pressure.* Nippon Seir. Zasshi, 45, 701–710 (in Japanese).

Gannon, K. and **Contreras, R.J.** (1993) *Sodium intake linked to amiloride-sensitive gustatory transduction in C57BL/6J and 129/J mice.* Physiol. Behav., 57, 231–239.

Halpern, B.P. (1998) *Amiloride in vertebrate gustatory responses to NaCl.* Neurosci. Biobehav. Rev., in press.

Hayashi, Y. (1995) *Investigations of various animal models for Sjogren's syndrome.* Nippon Rinsho, 53, 2383–2388 (in Japanese).

Heck, G.L., Mierson, S. and **DeSimone, J.A.** (1984) *Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway.* Science, 223, 403–405.

Hellekant, G., DuBois, G.E., Roberts, T.W. and **van der Wel, H.** (1988) *On the gustatory effect of amiloride in the monkey (*Macaca mulatta*).* Chem. Senses, 13, 89–93.

Hicks, J.D. and **Burnet, F.M.** (1966) *Renal lesions in the 'autoimmune' mouse strains NZB and F1 NZB×NZW.* J. Pathol. Bact., 91, 467–477.

Kessler, H.S. (1968) *A laboratory model for Sjogren's syndrome.* Am. J. Pathol., 52, 671–685.

Mellors, R.C. (1965) *Autoimmune disease in NZB/B1 mice. I. Pathology and pathogenesis of a model system of spontaneous glomerulonephritis.* J. Exp. Med., 122, 25–40.

Minear, M.M., Hammack, S.E., Lundy, R.F. and **Contreras, R.J.** (1996) *Amiloride inhibits nerve responses to NaCl and KCl in Sprague–Dawley and Fisher 344 rats.* Physiol. Behav., 60, 507–516.

Ninomiya, Y., Sako, N. and **Funakoshi, M.** (1989) *Strain differences in amiloride inhibition of NaCl responses in mice,* Mus musculus. J. Comp. Physiol., A, 166, 1–5.

Ninomiya, Y., Fukami, Y., Yamazaki, K. and **Beauchamp, G.K.** (1996) *Amiloride inhibition of chorda tympani responses to NaCl and its temperature dependency in mice.* Brain Res., 708, 153–158.

Schiffman, S.S., Lockhead, E. and **Maes, F.W.** (1983) *Amiloride reduced the taste intensity of Na^+ and Li^+ salts and sweeteners.* Proc. Natl Acad. Sci. USA, 80, 6136–6140.

Suzuki, T., Omatsu, H., Arai, T. and **Yamamoto, K.** (1976) *Sjogren's syndrome in New Zealand mice.* Ryumachi, 16, 285–289 (in Japanese).

Svendsen, U.G. (1977) *Spontaneous hypertension and hypertensive vascular disease in the NZB strain of mice.* Acta Pathol. Microbiol. Scand., A, 85, 548–554.

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