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Rearing on basal or high dietary NaCl modifies chorda tympani nerve responses in rats

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Abstract

Two experiments were conducted to determine the influence of dietary NaCl level on the integrated responses of the chorda tympani (CT) nerve to salt stimulation alone and mixed with the sodium-channel blocker, amiloride hydrochloride. Five groups of adult male rats were reared on regular chow containing either basal 0.1%, intermediate 1.0%, or high 3.0% NaCl from conception to postnatal day (PD) 30 or from conception to adulthood. Adult rats reared from conception to adulthood on basal dietary NaCl demonstrated a reduction in the CT nerve response to NaCl due to a decrease in the amiloride sensitive transduction mechanism. However, the CT nerve responses of adult rats reared on basal dietary NaCl to PD30 and then switched to intermediate dietary NaCl were similar to those of rats reared for a lifetime on intermediate dietary NaCl. Similarly, the CT nerve responses to NaCl in animals reared on high dietary NaCl from conception to PD30 and then switched to an intermediate NaCl diet were comparable to animals reared on intermediate and basal dietary NaCl. However, we found that exposure to high dietary NaCl led to a greater amiloride inhibition of NaCl responses. Thus, there is critical association between dietary NaCl level over two different exposure periods and CT nerve responsiveness to NaCl specifically regarding the degree of amiloride inhibition.
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Keywords: Electrophysiology; Taste; Amiloride; Transduction mechanisms

1. Introduction

Little is known about the parameters of sensory stimulation necessary for normal development of the gustatory system. Hill et al. have pursued an important line of research investigating the influence of dietary NaCl restriction on the development of the peripheral and central gustatory system. Restriction of dietary NaCl during an early period of taste development from embryonic day (ED) 3 to at least postnatal day (PD) 28 reduced chorda tympani (CT) nerve responses to NaCl compared to rats reared on intermediate (1.0%) dietary NaCl [15,20,21]. While the reduction in CT neural responsiveness was reversible following repletion of a intermediate NaCl-containing diet [21,32,36], the same NaCl restriction paradigm led to permanent morphological [22,23] and electrophysiological changes [38] in the first central synapse of the gustatory pathway, the nucleus of the solitary tract (NST).

Following a similar approach as Hill et al. of manipulating dietary NaCl early in development, the present set of experiments further defines the parameters of NaCl experience necessary for development of normal CT neural responsiveness to salts. The amount of dietary NaCl was increased from a restricted level of 0.03% to a basal level of 0.1% that supports normal pregnancy without compromising the number of live births and body weight of the offspring [4]. Additionally, we examined the CT responsiveness to salts following implementation of a high level of dietary NaCl (3.0%) during both a short-term selective period of prenatal and early postnatal development and a long-term maintenance of the high dietary NaCl. Prior research has shown that animals reared on basal dietary NaCl consumed less saline and those reared on high dietary

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NaCl more saline compared to animals reared on intermediate dietary NaCl [4,5,7,30]. In these experiments, the animals were reared on either basal or high dietary NaCl from conception to PD30, and then switched to chow containing an intermediate level of 1.0% NaCl from PD30 through behavioral testing that began 2 months later.

Utilizing methodology patterned after work conducted by Hill et al. [20] using dietary NaCl restriction, the goal of this research was to contribute to the understanding of gustatory mechanisms that may be responsible for changes in adult saline intake as consequence of early rearing on a diet containing either basal or high levels of NaCl. In a companion paper, we have examined the influence of early exposure to basal or high dietary NaCl on the central organization of CT neurons projecting to the NST [31]. In the present study, our purpose was to determine the influence of early exposure to basal or high dietary NaCl on CT nerve responsiveness to NaCl stimulation. In this respect, we examined the influence of basal or high dietary NaCl over two time periods: from conception to PD30 and from conception to adulthood. The former restricted the dietary treatment to an early time period when CT nerve responses to NaCl increase progressively in amplitude [16,19] through a gradual increase in the number of functional amiloride-sensitive proteins on taste receptor cells [13,17,37]. This is in keeping with the hypothesis of a sensitive period for salt taste development and its vulnerability to dietary salt experience in influencing sensitivity. The latter time period provided an opportunity to determine whether a lifetime of maintained exposure to basal or high dietary NaCl may be more important in influencing CT nerve responses. In these two experiments and in prior studies, the 1.0% NaCl (intermediate) diet was the control condition because 1.0% NaCl is the concentration found in commercial chow for rodents.

In the present study, we recorded the integrated responses of the CT nerve to NaCl with and without the sodium-channel blocker, amiloride hydrochloride [2]. It has been established that the transduction mechanism for salt taste involves plasma membrane proteins, residing on the apical membranes of taste receptor cells, which act as specific ion channels for sodium [12,13]. Additional evidence indicates that salt taste transduction involves another mechanism(s) (amiloride-insensitive) other than passive diffusion through amiloride-sensitive sodium channels [12]. While the nature of this amiloride-insensitive mechanism is unknown, it has been suggested that it may involve an apical membrane process, as well as a process utilizing a paracellular pathway [10,26,39,40]. With respect to development, the rat CT nerve responds to NaCl stimulation mostly through amiloride-insensitive mechanisms prior to PD21, with amiloride sensitivity appearing gradually thereafter [14,17].

In summary, prior research has provided evidence linking manipulations of dietary NaCl intake of pregnant and lactating rat mothers and their young offspring during early postnatal development with alterations in adult salt intake. It is suspected that at some critical point in development, animals exposed directly or indirectly to either basal or high dietary NaCl may undergo changes in the mechanisms of salt taste transduction that may underlie changes in salt intake. This study is the first comprehensive examination of the amiloride- and amiloride-insensitive components of the adult CT response to various concentrations of NaCl and KCl following early developmental or maintained exposures to basal 0.1% and high 3.0% levels of dietary NaCl. The results from the present study provide compelling evidence that exposure to both basal and high dietary NaCl levels during development can alter the responsiveness of the adult CT nerve to NaCl.

2. General methodology

Experiment 1 examined CT nerve responses to NaCl, KCl, and QHCl with and without amiloride in adult male rats reared on either basal, intermediate, or high dietary NaCl from conception through PD30, and then switched to the intermediate NaCl diet until recording after PD90. Experiment 2 examined CT nerve responses to NaCl, KCl, and QHCl with and without amiloride in adult male rats reared and maintained on either basal, intermediate, or high dietary NaCl from conception through recording after PD90. The intermediate group was the same in Experiments 1 and 2. The research reported herein fully conforms to the current *Guiding Principles for Research Involving Animals* published by the American Physiological Society.

2.1. Subjects

Thirty nulliparous female rats (Sprague–Dawley, CrL: CD(SD)BR, Charles River Breeding Laboratories), 66 days old and nonlittermates, were housed doubly in clear plastic cages in a temperature-controlled room with a 14:10 light–dark cycle. Each rat was given access to deionized water and a standard pelleted test diet (Harlan Teklad, modifications of Sodium-Deficient Diet TD 90228) consisting of either 0.1% (basal), 1.0% (intermediate), or 3.0% (high) NaCl. These NaCl concentrations correspond roughly to typical NaCl intake found in humans [9,28,35]. The basal amount of NaCl approximates the recommended NaCl intake of persons under treatment for high blood pressure (0.5 g/2500 kcal). The intermediate amount approximates the average recommended daily human intake of NaCl (5 g/2500 kcal). The high amount of NaCl approximates the amount of NaCl consumed by persons with a higher than average daily NaCl intake (15 g/2500 kcal).

The females were adapted to their respective NaCl diet for 14 days and then each female was housed with a single adult male. The breeding pairs were not littermates. The

male was removed after 14 days and the females were housed singly thereafter. The females remained on their respective NaCl diet throughout pregnancy and lactation until weaning at PD21. Pups were born as early as 21 days after the initial pairing of males and females. Approximately 24 h after birth, litters were culled to eight pups, retaining as many males as possible per litter. There were no litters with fewer than eight pups.

At PD21, pups were weaned from their mothers and given ad libitum access to deionized water and the same NaCl diet of their mother. Pups remained on their respective NaCl diet for 9 days following weaning. At PD30, male pups were separated into cages of two males per cage. All male offspring in Experiment 1 were then switched to the intermediate NaCl diet from PD30 until testing in adulthood. All male offspring in Experiment 2 were maintained on their respective basal or high NaCl pelleted chow until testing in adulthood. Female offspring were euthanized at PD30. Between 30 and 45 days of age, offspring were identified with a tail tattoo to indicate dietary group and litter number. A potential confound of litter was eliminated through use of only one male offspring per litter as a subject; this resulted in six animals in each dietary NaCl group.

2.2. Neurophysiological recordings

Whole nerve electrophysiological recordings were obtained from the CT branch of the facial nerve of adult male rats at least 90 days old. Only one male from each litter was used for testing. All rats were anaesthetized with urethane (1.5 g/kg body weight) administered in two intraperitoneal injections spaced 15 min apart. Supplementary injections (0.1 ml) of the same urethane dosage were administered whenever pinching of the foot could evoke the flexion withdrawal reflex. Rectal temperature was monitored and maintained at 36–38 °C throughout the experiment using a feedback circuit and electric heating pad. The trachea was cannulated and a small suture was attached to the ventral surface of the tongue. During the preparation, the tongue was kept moist with cotton soaked in physiological saline.

After placement in a nontraumatic head holder, the right CT branch of the facial nerve was exposed using a mandibular approach. The CT nerve was transected proximally where it enters the tympanic bulla and its perineurium removed to the point where the lingual nerve joins the CT nerve. The whole nerve was placed on a tungsten wire electrode and coated with Vaseline to prevent drying. A silver indifferent electrode placed in the underlying muscle tissue near the base of the nerve allowed differential amplification ($\times$10,000) of action potentials. The animal was grounded via the headholder. The whole nerve activity was stored on a video recorder tape for off-line analysis.

2.3. Stimulus presentation

The tongue was slightly extended from the oral cavity and held in place by fixing the ventral tongue suture to the preparation table. Stimuli were presented to the anterior portion of the tongue by computer-controlled stepping motors driving syringes to maintain a constant flow rate of 100 μl/s. Throughout the experiment, six stimulus bottles and a two rinse bottles were connected by polyethylene tubing and luer-lock adapters to input ports on the stimulus mixing platform. An independent valve within the mixing platform controlled the stimulus flow from each of the input ports. These input valves were controlled by a custom Power Macintosh 7100/80 computer program (designed by the technical support staff at Florida State University) that permitted rapid switching and/or mixing between any two stimulus or rinse channels while maintaining a continuous solution flow from the mixing chamber into the delivery faucet. From the delivery faucet, the solution flowed through a Peltier heat exchange device before being presented to the anterior portion of the tongue. The temperature of the solution was maintained at 35 °C throughout the experiment. A suction tube placed underneath the tongue removed the solution as it flowed off the tongue.

2.4. Chemical stimulation

Solutions made from reagent grade chemicals were dissolved in deionized distilled water and kept refrigerated and protected from light when not in use. Chemical stimuli consisted of NaCl (75, 150, 300, 450, 555, 750 mM), KCl (75, 150, 300, 450, 555, 750 mM), and QHCl (3, 15, 30 mM). Each stimulus was presented both with and without 100 μM amiloride hydrochloride mixed in solution. Amiloride hydrochloride (amiloride) is a sodium channel antagonist [2] known to inhibit sodium transduction in taste receptors cells presumably through competitive inhibition of passive sodium channels [25,26]. Between stimulations, distilled deionized water at 35 °C flowed continuously over the tongue thereby eliminating thermal and tactile transients from the taste responses. Baseline neural activity was recorded during the water rinse for at least 30 s preceding each stimulus presentation. Each stimulus was presented for 10 s and was followed by a water rinse ranging from 90 to 120 s depending on the recovery period necessary to return to stable baseline activity. The integrity of each recording session was monitored by bracketed presentations of 750 mM NaCl and 30 mM QHCl.

2.5. Data analysis

All data were analyzed off-line on a PC computer equipped with a GW Instrument 15-μs data acquisition board and custom data analysis software. The recorded amplified responses of the CT nerve were integrated with a time constant of 150 ms using a root mean square

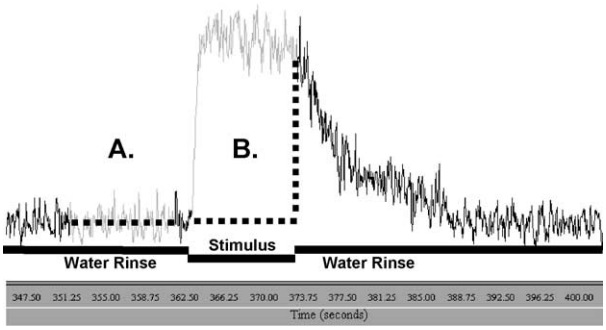

Fig. 1. An example of the integrated (150 ms) CT nerve response. (A) The dotted line represents the mean response during 10s of baseline activity. (B) The AUC bounded by the response to stimulation and prior baseline activity was calculated as the absolute response measure.

calculation. A period of baseline activity (10 s) was selected prior to each stimulus. The average baseline activity was used to calculate the area under the curve (AUC) for the integrated response during each 10-s stimulus (see Fig. 1). To allow across-subject comparisons, responses were normalized and presented as a ratio relative to a standard stimulus of 30 mM QHCl. Quinine, a bitter stimulus, is transduced through a metabotropic transduction mechanism not utilized during salt stimulus transduction [13]. Additionally, amiloride is not known to affect the QHCl transduction or the CT responsiveness to QHCl. Therefore, QHCl appeared to be a good candidate for a standard stimulus to which the data could be normalized.

2.6. Statistical analyses

A two-factor analysis of variance (ANOVA) was used with either group or amiloride as a between-group factor and repeated measures as an analysis of concentration effect within subjects. Tukey HSD post hoc tests were used to determine the source of significant concentration effects. Statistical results with an alpha level of $P < .05$ were reported as significant.

3. Results

3.1. Exposure to basal, intermediate, or high dietary NaCl from conception until PD30 followed by intermediate dietary NaCl until testing in adulthood

3.1.1. QHCl responsiveness

As shown in Fig. 2, the mean response amplitudes of the CT nerve increased [$F(2,51) = 26.301$, $P < .01$] with

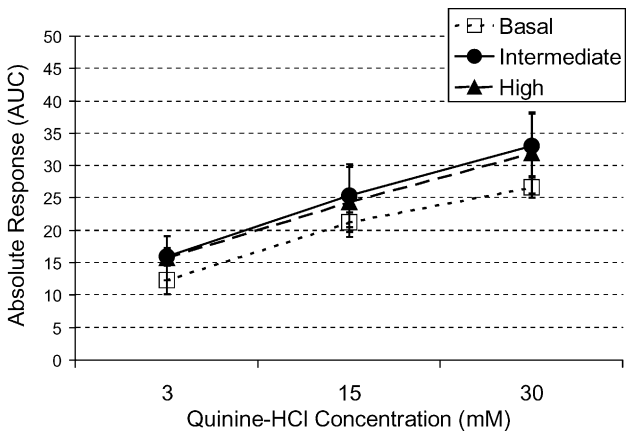

Fig. 2. The mean (±S.E.M.) absolute response of the CT nerve to ascending QHCl concentrations in adult rats reared either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl from conception to postnatal (PD) 30.

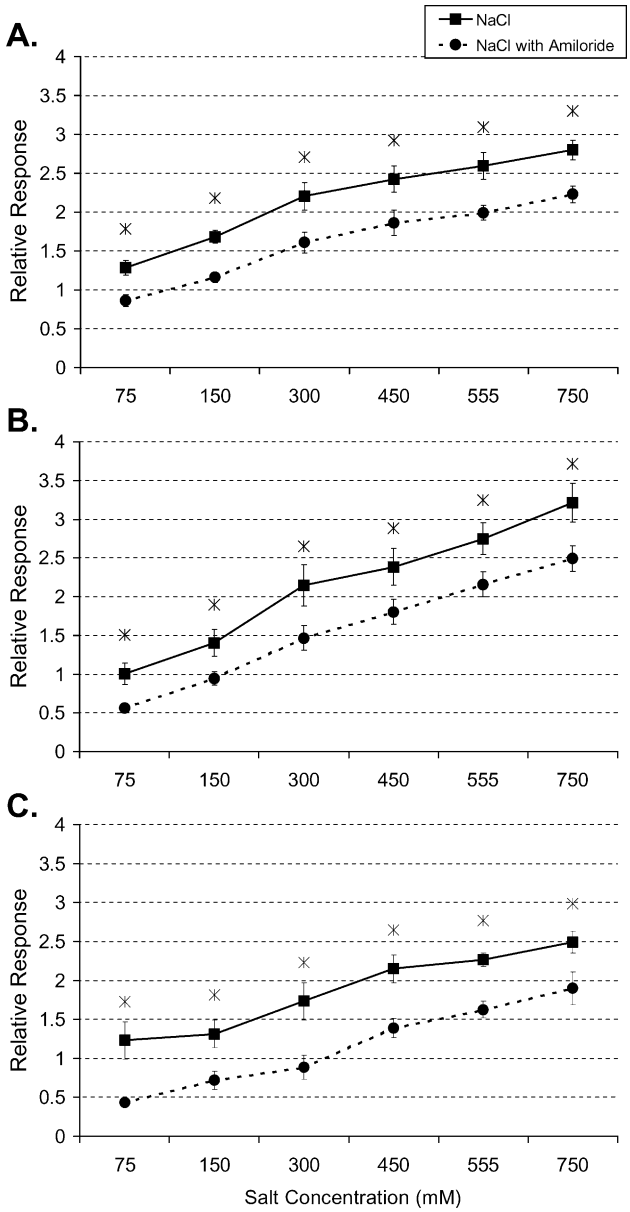

Fig. 3. The mean (±S.E.M.) relative responses of the CT nerve to NaCl alone and mixed with 100 μM amiloride in adult rats reared either on basal (A), intermediate (B), or high (C) dietary NaCl from conception to PD30. Stars represent significant differences ($P < .05$).

increasing concentrations of QHCl. Furthermore, the responses of the CT nerve to QHCl alone or mixed with 100 μM amiloride were similar for the three dietary NaCl groups [$F(2,51) = 0.135$, $P = .874$].

3.1.2. NaCl responsiveness

Fig. 3 shows the mean relative responses of the CT nerve to NaCl alone and mixed with amiloride for each of the three dietary NaCl groups. As can be seen, 100 μM amiloride inhibited CT nerve responses to NaCl for all three groups. This was supported by a significant NaCl concentration effect [$F(2,105) = 59.007$, $P < .01$] as well as a significant amiloride effect [$F(2,105) = 85.489$, $P < .01$] for basal, intermediate, and high NaCl groups. Furthermore, Tukey HSD post hoc tests revealed that amiloride inhibited the CT nerve responses to each NaCl concentration.

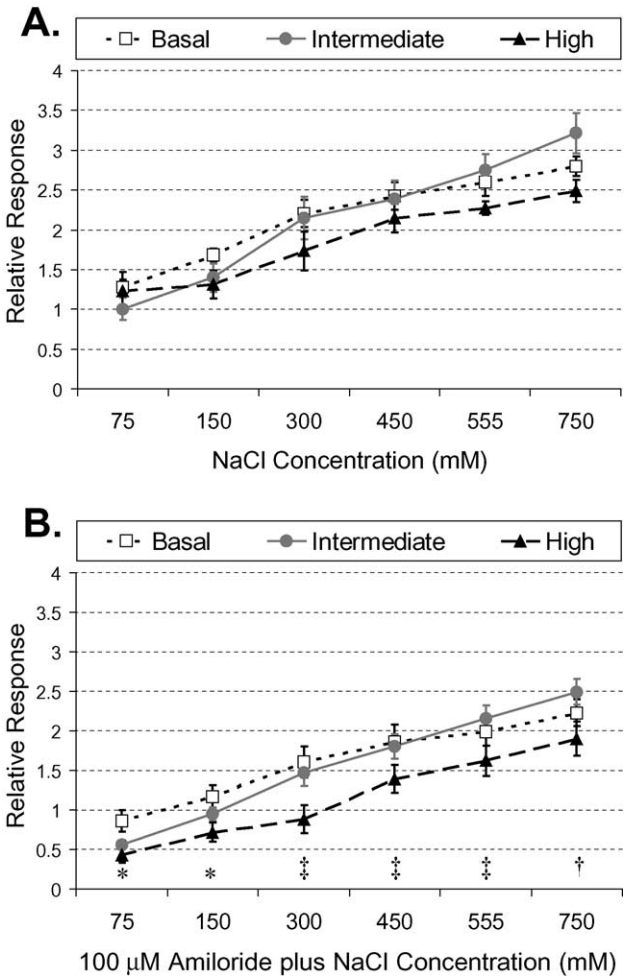

Fig. 4. The mean (±S.E.M.) relative responses to NaCl alone (A) and NaCl mixed with 100 μM amiloride (B) in adult rats reared either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl from conception to PD30. Stars represent significant differences between high and basal groups ($P < .05$). Double crosses represent significant differences between high and both basal and intermediate groups ($P < .05$). Single crosses represent significant differences between high and intermediate groups ($P < .05$).

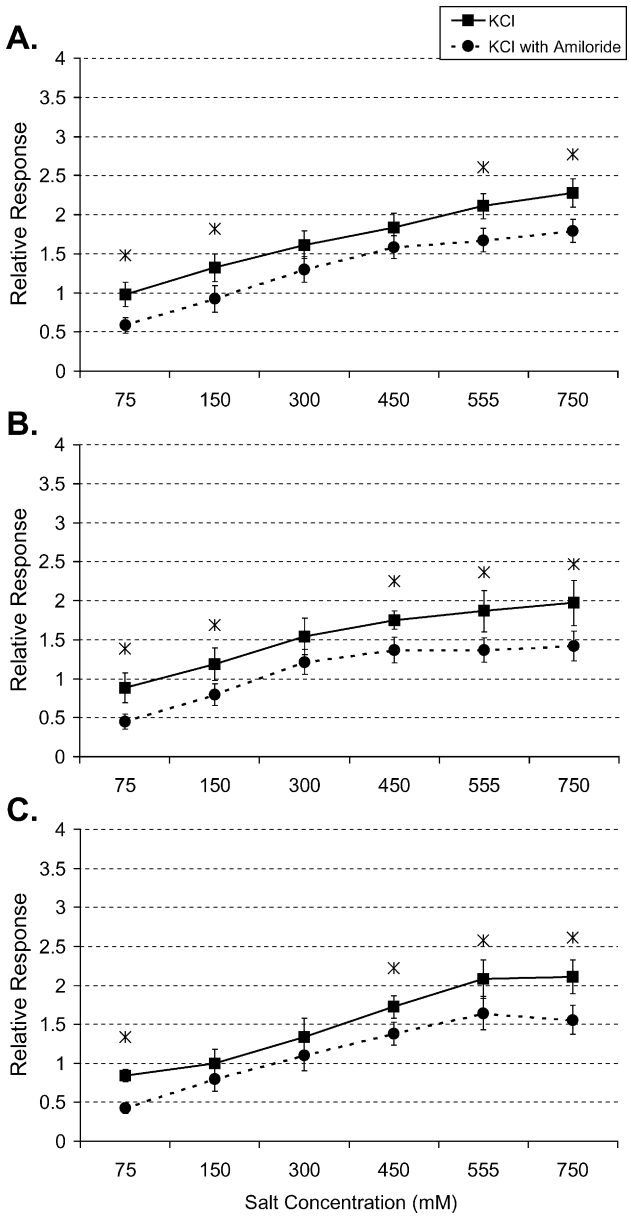

Fig. 5. The mean (±S.E.M.) relative responses of the CT nerve to ascending concentrations of KCl alone and with 100 μM amiloride in adult rats reared either on basal (A), intermediate (B), or high (C) dietary NaCl from conception to PD30. Stars represent significant differences ($P < .05$).

Fig. 4 compares the mean relative responses to NaCl alone and mixed with amiloride across the three dietary NaCl groups. The three groups differed in their CT nerve response functions to NaCl alone [$F(2,105) = 3.432$, $P = .037$]. However, Tukey HSD post hoc tests failed to reveal the source of this main effect (basal vs. intermediate, $P = .992$; intermediate vs. high, $P = .075$; basal vs. high, $P = .057$). The relative response amplitudes of the high NaCl group tended to be smaller than those of the other two groups.

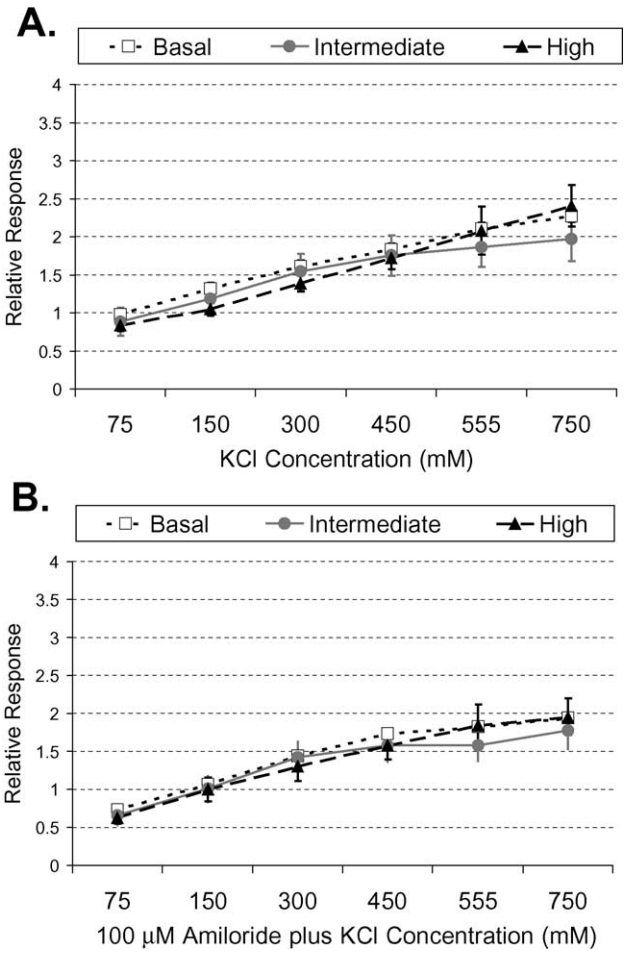

Fig. 6. The mean (±S.E.M.) relative responses to KCl alone (A) and KCl mixed with 100 μM amiloride (B) in adult rats reared either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl from conception to PD30.

The three dietary NaCl groups also differed in their CT nerve response functions to NaCl mixed with amiloride [$F(2,105) = 13.919$, $P < .01$]. The relative response amplitudes of the high NaCl group were significantly smaller than those of the other two groups (all P values $< .05$). Tukey HSD post hoc tests revealed that the source of this difference was from 75 to 555 mM NaCl between the high and basal dietary NaCl groups, and from 300 to 750 mM NaCl between the high and intermediate dietary NaCl groups.

3.1.3. KCl responsiveness

Figs. 5 and 6 are graphs of CT nerve responses to KCl comparable to those described above for NaCl. While the overall pattern of results was similar, the concentration and amiloride effects were typically smaller depending on the concentration of KCl. Fig. 5 shows the mean relative responses of the CT nerve to KCl alone and mixed with amiloride for each of the three dietary NaCl groups. As can be seen, 100 μM amiloride inhibited CT nerve responses to KCl for all three groups. This was supported by a significant KCl concentration effect [$F(2,105) = 44.496$, $P < .01$] as well as a significant amiloride effect [$F(2,105) = 12.458$, $P < .01$] for basal, intermediate, and high NaCl groups. Furthermore, Tukey HSD post hoc tests revealed that amiloride inhibited the CT nerve responses to most KCl concentrations, with the exception of one or two intermediate concentrations.

Fig. 6 compares the three dietary NaCl groups' mean relative responses to KCl alone and mixed with amiloride. In both cases, the responses to KCl alone [$F(2,105) = 0.402$, $P = .943$] and KCl mixed with amiloride [$F(2,105) = 0.860$, $P = .427$] were the same for basal, intermediate, and high NaCl groups.

3.1.4. Amiloride sensitivity

Fig. 7 summarizes differences among the dietary NaCl groups regarding the overall effectiveness of amiloride to inhibit NaCl, KCl, and QHCl. The data were collapsed across stimulus concentration to yield a mean for NaCl, KCl, and QHCl. Shown is the percentage of the overall response that was inhibited by amiloride for each chemical stimulus in each dietary NaCl group. An overall ANOVA of amiloride sensitivity for the three stimuli across groups was significant [$F(2,51) = 14.17$, $P < .001$]. Between-group comparisons of amiloride sensitivity were significant only for responses to NaCl and not for KCl or QHCl responses. Tukey HSD post hoc tests revealed a larger amiloride-sensitive component of the NaCl response in animals reared under the high dietary NaCl condition than that of the intermediate ($P = .013$) and basal dietary NaCl groups ($P = .014$), which were not different from each other.

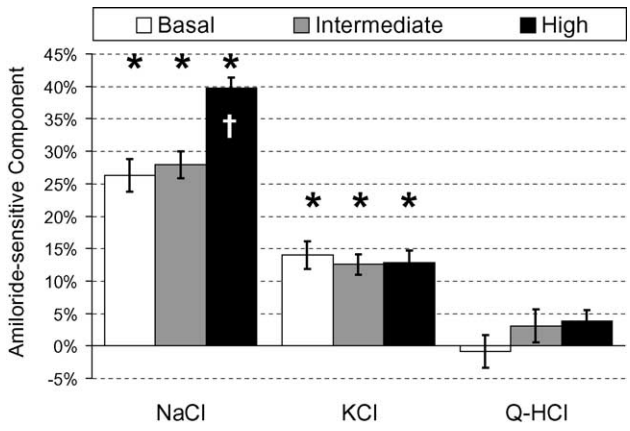

Fig. 7. The mean (±S.E.M.) percent of the CT nerve response inhibited by 100 μM amiloride for adult rats reared either on basal, intermediate, or high dietary NaCl from conception to PD30. The data were collapsed across stimulus concentration to yield a single mean for each stimulus in each dietary salt group. Stars represent significant inhibition by amiloride for each stimulus (NaCl, KCl, and QHCl). The white cross represents significant differences between dietary groups (high>basal ≈ intermediate).

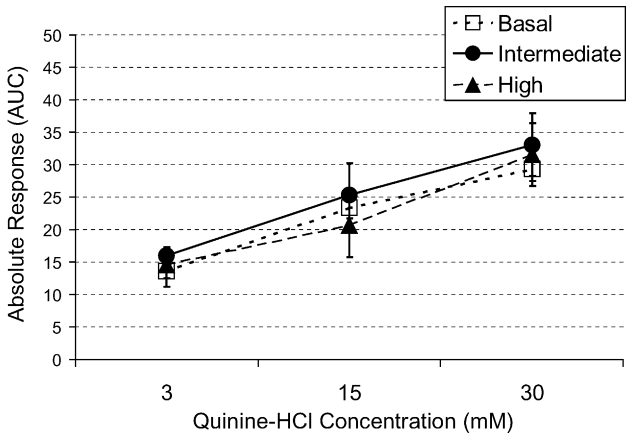

Fig. 8. The mean (±S.E.M.) absolute response of the CT nerve to ascending QHCl concentrations in adult rats reared for a lifetime either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl.

Within-group comparisons using Tukey HSD post hoc tests indicated that for all three dietary NaCl groups, amiloride sensitivity was significantly greater for responses to NaCl than KCl, both of which were greater than QHCl (all P values $<.01$).

3.2. Exposure to basal, intermediate, or high dietary NaCl from conception until testing in adulthood

3.2.1. QHCl responsiveness

As shown in Fig. 8, the mean response amplitudes of the CT nerve increased [$F(2,51)=69.064$, $P<.01$] with increasing concentrations of QHCl. Furthermore, the responses of the CT nerve to QHCl alone or mixed with 100 μM amiloride were similar for the three dietary NaCl groups [$F(2,51)=0.762$, $P=.473$].

3.2.2. NaCl responsiveness

Fig. 9 shows the mean relative responses of the CT nerve to NaCl alone and mixed with amiloride for each of the three dietary NaCl groups. As can be seen, 100 μM amiloride failed to inhibit CT nerve responses to NaCl [$F(2,69)=0.184$, $P=.670$] in basal dietary NaCl animals. However, there was a significant amiloride effect for both the intermediate dietary NaCl group [$F(2,69)=36.156$, $P<.01$] and the high dietary NaCl group [$F(2,69)=6.041$, $P<.05$]. Tukey HSD post hoc tests (all P values $<.05$) revealed that amiloride significantly inhibited CT nerve responses to each NaCl concentration for the intermediate dietary NaCl group (Fig. 9B) and the 75, 555, and 750 mM concentration of NaCl for the high dietary NaCl group (Fig. 9C).

Fig. 10 compares the mean relative responses to NaCl alone and mixed with amiloride for each of the three dietary NaCl groups. As shown in Fig. 10A, the three groups differed in their CT nerve response functions to NaCl alone [$F(2,105)=21.018$, $P<.01$]; there was no group by NaCl concentration interaction effect [$F(2,105)=0.827$, $P=.604$]. Tukey HSD post hoc tests revealed that the NaCl responses of the basal dietary NaCl group were significantly smaller than those of the intermediate and high groups at every NaCl concentration.

Fig. 10B shows the differences in CT nerve response functions to NaCl mixed with amiloride among the three dietary NaCl groups [$F(2,105)=8.641$, $P<.01$]. Tukey HSD post hoc tests (all P values $<.01$) revealed that the relative response amplitudes of 150–750 mM NaCl were significantly smaller for the basal dietary NaCl group than

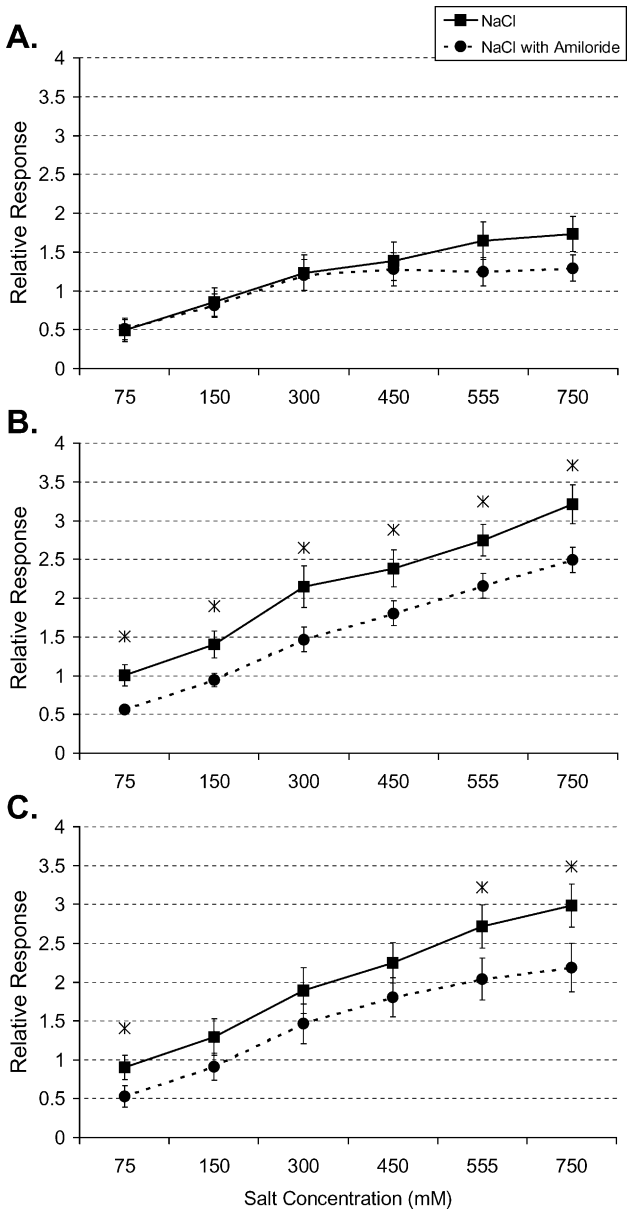

Fig. 9. The mean (±S.E.M.) relative responses of the CT nerve to ascending concentrations of NaCl alone and with 100 μM amiloride in adult rats reared for a lifetime either on basal (A), intermediate (B), or high (C) dietary NaCl. Stars represent significant differences ($P<.05$).

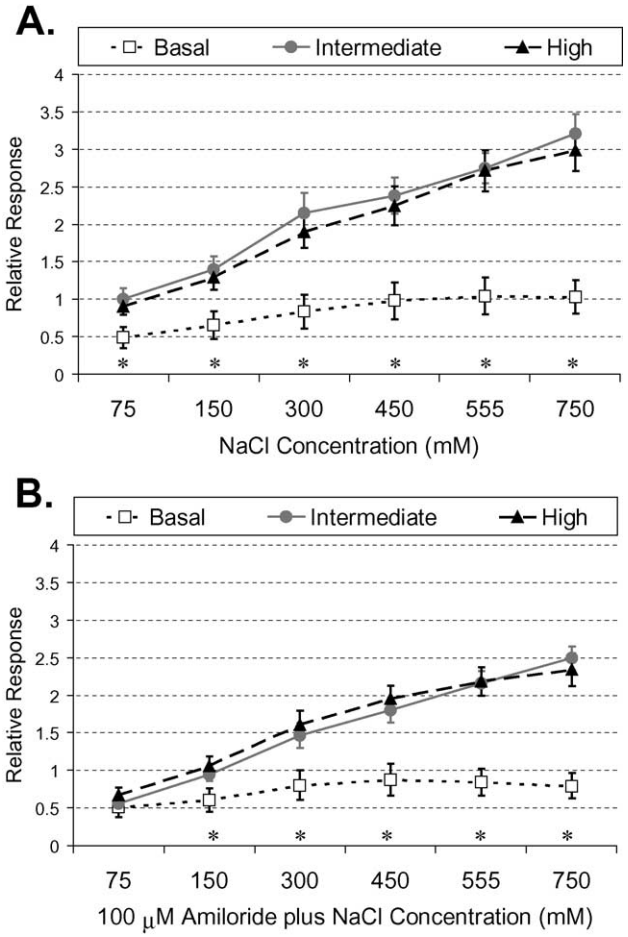

Fig. 10. The mean (±S.E.M.) relative responses to NaCl alone (A) and NaCl mixed with 100 μM amiloride (B) in adult rats reared for a lifetime either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl. Stars represent significant group differences (basal < intermediate ≈ high; $P < .01$).

those of the intermediate and high dietary NaCl groups, which did not differ.

3.2.3. KCl responsiveness

Fig. 11 compared the mean relative responses of the CT nerve to KCl alone and mixed with amiloride for each of the three dietary NaCl groups. As can be seen, 100 μM amiloride failed to inhibit CT nerve responses to KCl [$F(2,69) = 0.247$, $P = .621$] in animals maintained on the basal NaCl diet. However, there was a significant amiloride effect for the intermediate NaCl group [$F(2,69) = 8.666$, $P < .01$] with Tukey HSD post hoc tests revealing amiloride inhibition of CT nerve responses to each concentration, except 300 mM KCl. There was also a significant amiloride effect for high dietary NaCl group [$F(2,69) = 4.041$, $P < .05$] at 75, 150, 555, 750 mM concentrations of KCl (Tukey HSD post hoc tests, $P < .05$).

Fig. 12 compares the mean relative responses of the CT nerve to either KCl alone or KCl mixed with amiloride across each of the three dietary NaCl groups. As can be seen in Fig. 12A, the responses of the dietary groups were similar to KCl alone as there was neither a group effect nor a group by concentration effect. As illustrated in Fig. 12B, the three dietary NaCl groups differed slightly in their CT nerve response functions to KCl mixed with amiloride as confirmed by a significant interaction of group by KCl concentration [$F(2,105) = 23.618$, $P < .01$]. Tukey HSD post hoc tests revealed that the responses to 75 and 150 mM KCl were larger for the basal dietary NaCl group than those of the other two groups, which did not differ.

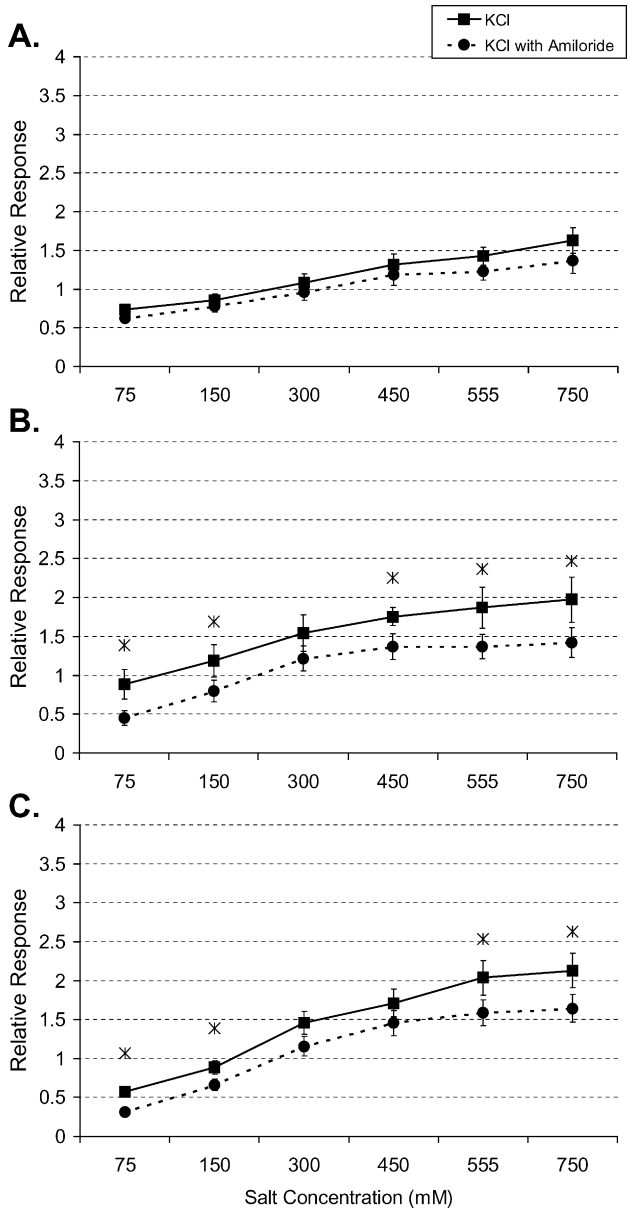

Fig. 11. The mean (±S.E.M.) relative responses of the CT nerve to ascending concentrations of KCl alone and with 100 μM amiloride for adult rats reared for a lifetime either on basal (A), intermediate (B), or high (C) dietary NaCl. Stars represent significant differences ($P < .05$).

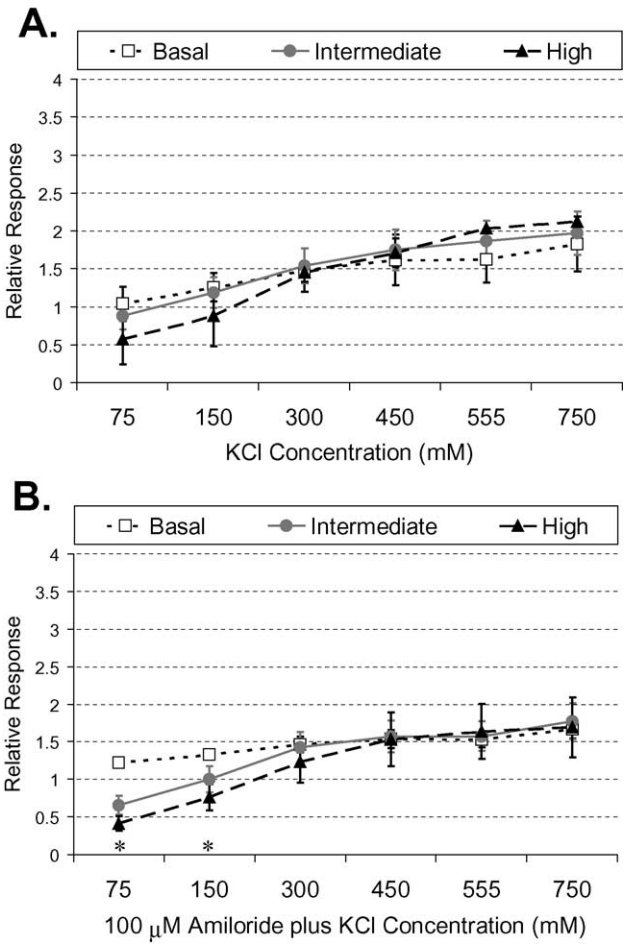

Fig. 12. The mean (±S.E.M.) relative responses to increasing concentrations of KCl alone (A) and KCl mixed with 100 μM amiloride (B) in adult rats reared for a lifetime either on basal (open box, dotted line), intermediate (closed circle, solid line), or high (closed triangle, dashed line) dietary NaCl. Stars represent significant group differences (basal>intermediate ≈ high; $P < .01$).

3.2.4. Amiloride sensitivity

Fig. 13 summarizes differences in overall effectiveness of amiloride to inhibit NaCl, KCl, and QHCl among the three dietary NaCl groups. For each dietary NaCl group, the data were collapsed across concentration to yield a mean percentage of the CT response inhibited by amiloride during stimulation by NaCl, KCl, and QHCl. An overall ANOVA of amiloride sensitivity for the three chemical stimuli across the dietary NaCl groups was significant [$F(2,51) = 8.18$, $P < .001$]. Tukey HSD post hoc tests revealed significant between-group differences in the amiloride-sensitive component of the CT response to NaCl and KCl, but not to QHCl. The amiloride-sensitive component of the CT response to NaCl was less for the basal dietary NaCl group than that of the intermediate ($P < .001$) and high dietary NaCl groups ($P = .009$). Furthermore, the amiloride-sensitive component of the CT response to NaCl for the high dietary NaCl group was significantly less than that of the intermediate group ($P = .012$). The amiloride-sensitive component of the CT response to KCl was much smaller for the basal dietary NaCl group than that of the intermediate ($P < .001$) and high ($P = .004$) dietary NaCl groups, which did not differ from each other. Between-stimulus comparisons within the basal dietary NaCl group indicated no significant difference in the amiloride-sensitive component of the CT response across NaCl, KCl, and QHCl. Within the intermediate dietary NaCl group, the amiloride-sensitive component of the CT response significantly differed between stimuli with QHCl < KCl < NaCl (Tukey HSD post hoc tests, all P values $< .01$). The amiloride-sensitive component of the CT response to NaCl and KCl was similar and significantly larger than QHCl ($P = .001$) for high dietary NaCl group.

3.2.5. Selective prenatal through PD30 exposure compared with maintained exposure to basal or high dietary NaCl

Fig. 14A shows the raw recording record from one basal animal reared under the dietary NaCl exposure paradigm of Experiment 2. Fig. 14B shows the raw recording record from one basal animal reared under the dietary conditions of Experiment 1 in which basal dietary NaCl exposure was switched at PD30 to intermediate dietary NaCl. The raw recording record from the animal maintained on the basal NaCl diet demonstrates the reduction in the amplitude of CT nerve response to NaCl and the relative absence of amiloride inhibition for most NaCl concentrations. This is in contrast to the larger NaCl responses and significant amiloride inhibition illustrated in the raw recording record of the animal switched from exposure to the basal NaCl diet at PD30 to intermediate dietary NaCl (Fig. 14B). Comparing the effectiveness of

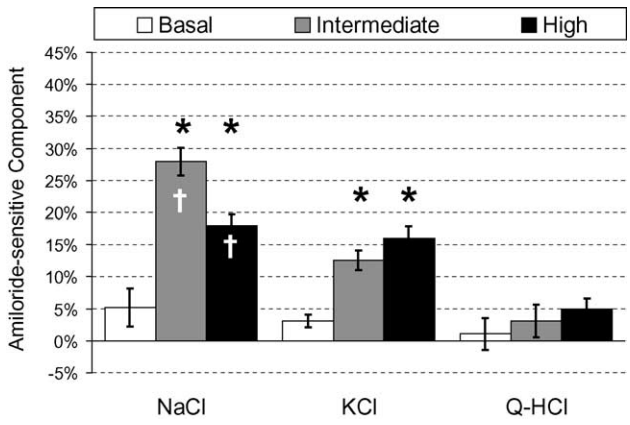

Fig. 13. The mean (±S.E.M.) percent of the CT nerve response inhibited by 100 μM amiloride for adult rats reared for a lifetime either on basal, intermediate, or high dietary NaCl. The data were collapsed across stimulus concentration to yield a single mean for each stimulus in each dietary salt group. Stars represent significant inhibition by amiloride for each stimulus (NaCl, KCl, and QHCl). White crosses represent significant differences between dietary groups (intermediate>high>basal).

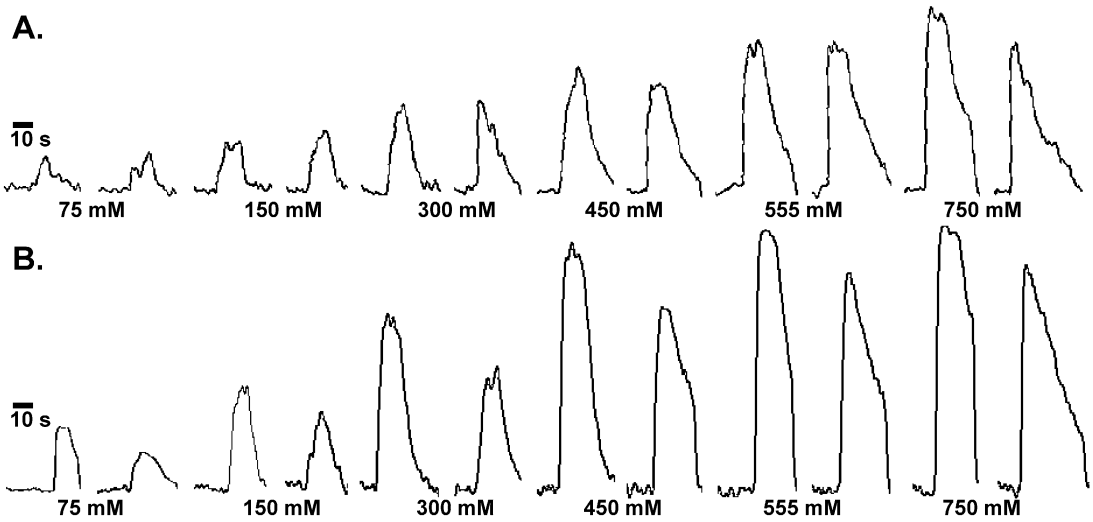

Fig. 14. Typical raw records of CT nerve responses to pairs of increasing concentrations of NaCl alone followed by NaCl mixed with 100 μM amiloride in an adult reared for a lifetime on basal dietary NaCl (A) and a rat reared on basal dietary NaCl to PD30 and then switched to intermediate dietary NaCl (B).

amiloride to inhibit the CT nerve response to NaCl between animals maintained on the basal or high NaCl diet (Fig. 13) vs. animals switched to an intermediate NaCl diet at PD30 (Fig. 7) reveals an increase in amiloride sensitivity for both groups of offspring following implementation of the intermediate NaCl diet at PD30. Approximately 25% of the CT nerve response to NaCl was inhibited by 100 μM amiloride in offspring switched to the intermediate NaCl diet at PD30 as compared to approximately 5% inhibition of the CT nerve response in offspring maintained on the basal NaCl diet. Similarly, adult offspring maintained on the high NaCl diet showed less inhibition (18%) of the CT nerve response to NaCl by 100 μM amiloride than adult offspring switched from the high NaCl diet to the intermediate NaCl diet at PD30, with 40% of the CT nerve response to NaCl inhibited by 100 μM amiloride.

4. Discussion

Recording multifiber activity from the whole CT nerve and integrating the responses during 10 s of chemical stimulation proved to be an excellent means to obtain stimulus concentration–response functions, examine the effectiveness of amiloride as a salt taste transduction antagonist, and determine the influence of dietary NaCl level on salt taste sensitivity. The CT nerve responded to lingual application of a broad range of NaCl, KCl, and QHCl concentrations that reflected its well-established sensitivity to stimulus quality and intensity [6,24]. The salt-sensitive CT nerve responded robustly to NaCl and KCl and modestly to QHCl. In addition, the present results confirm the role of amiloride as a selective antagonist of salt taste transduction as demonstrated by the strong inhibition of NaCl responses, relatively mild inhibition of KCl responses, and no inhibition of the QHCl responses [25,26,29]. Furthermore, as shown in Fig. 14B, the magnitude of amiloride inhibition of NaCl responses was maximal at 75 mM NaCl and declined progressively as concentration increased, consistent with a competitive model of inhibition [12,13]. Most importantly, this research further defined the parameters of dietary NaCl exposure capable of influencing the development of adult peripheral taste sensitivity to salts.

The present study demonstrated that a reduction in dietary NaCl from an intermediate level of 1.0% found in commercial chow to a basal level of 0.1% NaCl from conception to adulthood led to a profound reduction in CT nerve responses to NaCl concentrations (Fig. 10A). Moreover, this sizeable reduction was relatively specific to NaCl solutions as the responses to KCl (Fig. 12A) and QHCl (Fig. 8) were unaffected. In addition to a reduced neural response to NaCl, the magnitude of inhibition by amiloride of NaCl responses was reduced drastically, if not completely eliminated, by a maintained basal NaCl diet (Fig. 9A). These findings suggest that the reduction in CT nerve response to NaCl resulted mostly from a reduction in amiloride sensitivity. Further, these findings are consistent with the notion that maintenance of basal dietary NaCl from early in development until adulthood hinders the development of functional, amiloride-sensitive sodium channels on the apical membranes of taste receptor cells [37,41].

The present findings regarding the ability of a basal NaCl diet to influence development of peripheral salt sensitivity in the CT nerve complement the results obtained by Hill et al. [20] using a lower, NaCl-restricted diet of 0.03%. Thus, the present results with basal dietary NaCl together with prior

research with dietary NaCl restriction demonstrate the critical importance of dietary NaCl level in determining CT nerve responsiveness to salt; dietary NaCl level seems to play a direct role on salt taste development and appears not to act secondarily through any nonspecific debilitating consequence of dietary NaCl restriction [4,5,31].

It would seem that it makes little difference whether animals are reared on a restricted 0.03% or basal 0.1% NaCl-containing diet. Both levels of dietary NaCl have been shown to produce a reduction in CT nerve responsiveness that may be recovered after a suitable period of intermediate dietary NaCl. However, King and Hill [22] demonstrated that dietary NaCl restriction was associated with an enlarged terminal area for CT afferents in the dorsal aspect of the NST. In contrast, we have found recently that rearing rats on basal dietary NaCl from conception to adulthood was without effect on the volume of the CT nerve projection in the NST [31]. Therefore, while a dietary NaCl level of 0.1% can alter CT nerve responsiveness; there is no anatomical consequence to the CT terminal field within the NST.

This is the first study to explore the possible influence of high dietary NaCl on CT nerve responses to NaCl and amiloride inhibition. Rats were reared on a threefold higher dietary NaCl level (3.0%) than fed to animals in the intermediate NaCl group and a 30-fold higher level than fed to basal dietary NaCl animals. The CT nerve responses to NaCl in animals reared on high dietary NaCl from conception to PD30 and then switched to an intermediate NaCl diet were similar to those in animals solely reared on intermediate dietary NaCl. However, we found that selective prenatal and early postnatal exposure to high dietary NaCl led to greater amiloride inhibition of NaCl responses (Fig. 7).

Several previous studies suggest that the CT salt response and in particular the amiloride-sensitive component plays a critical role in salt intake [5,34]. The amiloride-sensitive portion of the CT response is thought to be the receptor mechanism underlying NaCl detection by the peripheral gustatory system [12,13]. Indeed, when amiloride was used in behavioral experiments, it disrupted taste-mediated saline intake in rodents. For example in rats, 100 μM amiloride raised the NaCl detection threshold by approximately 1 $\log_{10}$ unit [11], the unconditioned licking response to NaCl decreased [8], and amiloride resulted in disruptions of a conditioned aversion to NaCl [18], the expression of a depletion-induced NaCl appetite [3,27,33], as well as NaCl–KCl discrimination [34]. Additionally, it has been reported previously that prenatal exposure to a high dietary NaCl diet through PD30 elevates saline intake [5]. It may be that high dietary NaCl influences the amiloride-sensitive component of the CT nerve input during a critical period of gustatory development, which in turn reorganizes central gustatory mechanisms underlying salt intake. In this respect, we recently found that the total NST area occupied by CT afferent fibers was the same for animals reared for a lifetime either on basal, intermediate, or high dietary NaCl [31]. However, the pattern of CT innervation differed such that there was an enlarged dorsal terminal field in rats reared and maintained on high dietary NaCl compared to animals reared and maintained on either basal or intermediate levels of dietary NaCl [31]. Although it is unclear how, the central reorganization of CT afferents in the NST may be related to long-term changes in saline intake due to high dietary NaCl early in development.

A less interesting possibility is that the greater amiloride inhibition of CT nerve responses in animals reared on high dietary NaCl to PD30 and then switched to an intermediate diet is unrelated to the previously demonstrated elevated saline intake [5]. In fact, a permanent effect on the CT nerve response due to selective exposure to a high NaCl diet is surprising in the context that the effect of low dietary NaCl can be reversed [32]. Thus, there is an apparent asymmetry between dietary NaCl restriction and excess; dietary NaCl restriction retards the development of CT responsiveness that can be reversed, while an enhanced dietary NaCl leads to a long-lasting increase in the amiloride-sensitive portion of the CT nerve response. Furthermore, given the decrease in amiloride sensitivity after a lifetime of basal dietary NaCl, it would seem logical that amiloride sensitivity might increase after high dietary NaCl; however, there was a small reduction in the amiloride-sensitive component of the CT nerve response in animals reared for a lifetime on high dietary NaCl.

Despite what may appear to be an apparent asymmetry, it seems clear that CT nerve responsiveness to NaCl adapts to the level of dietary NaCl. When rats were switched from high to intermediate dietary NaCl at PD30, amiloride inhibition of the CT nerve response to NaCl increased from 18% to 40% (see Figs. 7 and 13). Likewise, amiloride inhibition increased from 5% to 25% when switched from basal to intermediate dietary NaCl at PD30. Combined, these results suggest that the level of dietary NaCl may regulate the relative contributions of the amiloride- and amiloride-insensitive components of the CT nerve response. We suspect that Stewart et al. [37] are correct in suggesting that dietary NaCl level influences the salt taste transduction mechanisms of taste receptor stem cells and that it take several weeks encompassing two cycles of cell replacement [1] to witness an effect.

In conclusion, rats were reared and maintained either on basal, intermediate, or high dietary NaCl that spanned a broad 30-fold concentration range, yet permitted normal gross development. The specific NaCl levels were selected on the basis of correlates to human NaCl consumption and prior work demonstrating a relationship with persistent, long-term changes in saline intake. In the present study, there was a critical association between dietary NaCl level over two different exposure periods and the CT nerve responsiveness to NaCl, specifically in regard to the degree of amiloride sensitivity. The time period over which

manipulated dietary NaCl levels led to alterations in CT nerve responsiveness suggests a mechanism that most likely involves taste receptor stem cells and the cycle of cell replacement known to exist in the lingual epithelium. While the specific role that changes in CT nerve responsiveness plays in taste-mediated saline intake remains to be elucidated, it is likely that the alterations demonstrated in the amiloride-sensitive component of the CT nerve response to NaCl have a critical influence on saline intake in adulthood.

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