Chem. Senses 25: 719–727, 2000

Lack of Functional and Morphological Susceptibility of the Greater Superficial Petrosal Nerve to Developmental Dietary Sodium Restriction

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Abstract

Restriction of dietary sodium during gestation has major effects on taste function and anatomy in the offspring. The chorda tympani nerve of offspring that are maintained on sodium-reduced chow throughout life (NaDep) has reduced neurophysiological responses to sodium and altered morphology of its terminal field in the nucleus of the solitary tract. There are many anatomical and physiological similarities between the chorda tympani nerve that innervates taste buds on the anterior tongue and the greater superficial petrosal nerve (GSP) that innervates taste buds on the palate. To determine if the GSP is similarly susceptible to the effects of dietary sodium restriction, the present study examined neurophysiological responses and the terminal field of the GSP in NaDep and control rats. Neurophysiological responses of the GSP to a variety of sodium and non-sodium stimuli did not differ between NaDep and control rats. Furthermore, the volume and shape of the GSP terminal field in the nucleus of the solitary tract did not differ between the groups. Therefore, despite the high degree of functional and anatomical correspondence between the chorda tympani nerve and the GSP, the GSP does not appear to be susceptible to the effects of lifelong dietary sodium restriction.

Introduction

Restriction of maternal dietary sodium during gestation in the rat has considerable effects on the offspring. Specifically, reducing the normal 0.5% NaCl content of rat chow to 0.03% during early post-conception and through weaning results in dramatically reduced neurophysiological responses to sodium and lithium salts in the chorda tympani nerve (Hill *et al.*, 1986; Hill, 1987; Hill and Przekop, 1988). Responses to NaCl are reduced by as much as 60% in sodium-restricted rats. In contrast, taste responses to NH_4Cl and non-salt stimuli are unaffected (Hill *et al.*, 1986; Hill, 1987; Hill and Przekop, 1988), showing the selective deficits in chorda tympani nerve function that are caused by developmental sodium restriction. The selective decrease in sodium-salt-elicited responses reflects the absence of the primary sodium transduction pathway, the amiloride-sensitive sodium channel (Hill, 1987). Moreover, corroborative data on the mechanism responsible for the lack of sodium sensitivity demonstrates that there is a 90% reduction in the density of functional amiloride-sensitive sodium channels in the apical domain of taste receptor cells in developmentally sodium restricted rats compared with control rats (Ye *et al.*, 1993).

In addition to the dietary-induced functional alterations that are present in NaCl-restricted rats, dramatic morphological changes occur in the first central synaptic relay, the nucleus of the solitary tract (NTS). One of the most impressive consequences of early dietary manipulation is alteration in the terminal field of the chorda tympani. Specifically, dietary sodium restriction during pre- and postnatal development produces abnormally distributed, large and irregularly shaped chorda tympani terminal fields (King and Hill, 1991; Walker and Hill, 1995). In fact, a 9 day period of sodium restriction from post-conception day 3 to day 12 produces a permanent alteration in the chorda tympani terminal field (Krimm and Hill, 1997). Thus, a presynaptic morphological alteration at the first central gustatory relay is caused by dietary restriction initiated during the brief interval during which chorda tympani neurons are born (approximately embryonic day 11, or E11) (Altman and Bayer, 1982). Although severe sodium restriction must occur during early embryonic development, expression of the altered effects is delayed until the terminal field normally expands (i.e. during the first three postnatal weeks) (Walker and Hill, 1995).

Recent neurophysiological work has demonstrated that there is another gustatory nerve that carries significant afferent activity in response to sodium salts. The greater superficial petrosal nerve (GSP), which like the chorda tympani nerve is a branch of the facial nerve and has cell somata in the geniculate ganglion, also responds vigorously to application of sodium stimuli to taste receptors (Sollars and Hill, 1998). Moreover, these responses are strongly

suppressed by application of amiloride (Sollars and Hill, 1998). In contrast to the chorda tympani nerve, which innervates fungiform taste buds located on the anterior two-thirds of the tongue, the GSP innervates palatal taste buds. Thus, during an ingestive bout, the chorda tympani and GSP nerves are stimulated simultaneously with stimuli (Nejad, 1986), including salts, and this composite afferent information is sent to the NTS. In fact, both nerves project to extensively overlapping fields in the rostral pole of the NTS (Hamilton and Norgren, 1984).

Since the chorda tympani and the GSP share considerable functional and morphological similarities, it is possible that the GSP also shares the susceptibilities to developmental sodium restriction already seen in the chorda tympani. That is, both nerves may transmit a reduced sodium response centrally and have altered terminal fields. In the current study, we examined the generalizability of developmental sodium restriction effects by recording multifiber responses of the GSP and by labeling its terminal field in developmentally sodium-restricted (NaDep) and control rats.

Materials and methods

Subjects

Adult Sprague–Dawley rats were obtained from Harlan Sprague-Dawley (Dublin, VA). Adult rats (age 47–79 days), obtained directly from Harlan Sprague-Dawley, were maintained on standard rat chow and water, and served as the control group. NaDep rats were bred in the vivarium at the University of Virginia. Breeding pairs of normal Sprague–Dawley rats were housed together for a period of 10 days. For the first 6 days, rats had access to regular chow and water. On day 7, breeding pairs were placed in clean cages and given specially formulated chow containing 0.03% NaCl (ICN Biochemical) and distilled water. Dams remained on the sodium-deficient diet until pups were weaned at postnatal day 28. Pups were weaned to the sodium-deficient diet and remained on the diet until the time of testing between 51 and 64 days of age.

Surgery for neurophysiology

Rats were anesthetized with chloral hydrate (300 mg/kg i.p.). At the time of testing, each rat was placed in a non-traumatic head-holder and body heat was maintained throughout the experiment with a water-circulating heating pad. Once a deep level of anesthesia was obtained, animals were tracheotomized. The hypoglossal nerves were severed to reduce tongue movement.

In order to allow stimulus delivery to the palate, PE 190 tubing that was flanged at approximately the midpoint was placed through the esophagus into the oral cavity. The flange was placed to cover the opening to the esophagus in the oral cavity. Perforated tubing extended the length of the palate so that taste solutions could be applied to the posterior palatine field, the geschmacksstreifen and nasoincisor ducts (Miller, 1977; Miller and Spangler, 1982). The other end of the tubing exited the esophagus in the neck through a small incision and was fitted with polyethylene tubing to accommodate a 10 cc syringe. During electrophysiological recordings, syringes were filled with the appropriate stimulus or rinse and solutions were sent through the tubing to flow into the back of the mouth and drip out of the front. This method allowed stimulation of all areas of the palate.

The tympanic bulla was exposed through the neck incision by retracting the posterior belly of the digastricus. After making a hole in the ventral aspect of the tympanic bulla, the tympanic muscle was cut and retracted, and the cochlea was removed. The GSP was exposed from within the temporal bone. For neurophysiological recordings, the GSP was transected and desheathed.

Neurophysiology

A total of 12 rats (NaDep, three male and three female; control, six female) were used for the neurophysiological recordings. Two rats from each of the experimental and control groups did not survive through the long recording session or had unstable responses through some of the stimulation series. However, most data sets contained all six data points from each experimental condition and no sets of data were obtained from fewer than four rats within each condition. Multifiber neural activity from the whole nerve was amplified, displayed on an oscilloscope and monitored with an audio amplifier. For data analysis, the amplified signal was passed through an integrator with a time constant of 0.5 s (Beidler, 1953; Harper and Knight, 1987), and the summated electrical activity was led to and analyzed with PowerLab Scope software (ADInstruments, Mountain View, CA).

Stimuli and stimulation procedures

Responses were recorded to a variety of salt and non-salt stimuli. Stimulation series of NaCl, NaAc (0.05, 0.1, 0.25 and 0.5 M), NH_4Cl (0.05, 0.1 and 0.25 M), sucrose (0.05, 0.1, 0.25, 0.5 and 1.0 M), 0.01 N HCl and 0.01 M quinine hydrochloride flowed over the palate through the esophageal fistula. All stimuli were mixed in distilled water. Each stimulus was presented for 10 s at an approximate rate of 0.5 ml/s. After an additional 30 s period during which the stimulus remained on the palate, distilled water was applied through the fistula for a rinse period of at least 40 s. After all of the stimulation series were completed, the palate was adapted to 100 μM amiloride hydrochloride for ~ 5 min. Amiloride (100 μM) served as the solvent for NaCl and NaAc (0.05, 0.1, 0.25 and 0.5 M), and as the rinse for these series. Throughout the recording session, the stability of neural activity was monitored by periodic application of 0.5 M NH_4Cl. Recordings were considered stable and included in the analysis only if the preceding and subsequent NH_4Cl responses deviated by <10%.

Terminal field label

Five adult rats from each experimental group (NaDep, two male and three female; control, one male and four female) were used to determine the GSP terminal field volume. The surgical approach for GSP labeling was identical to the surgery described above, except the cochlea was left intact and sodium pentobarbital (Nembutal, 50 mg/kg i.p.) was used as the anesthetic. The GSP was sectioned distal to the geniculate ganglion and dimethyl sulfoxide was applied to the cut nerve for ~15 s. Crystals of 3 kDa biotinylated dextran amine (BDA; Molecular Probes, Inc., Eugene, OR) were applied directly to the nerve. After 15 min, petroleum jelly was placed inside the tympanic bulla to ensure that the GSP did not dry out. Throughout the time required for the dye to transport to the brain, the surgical level of the anesthesia was maintained with doses of Nembutal as needed. Approximately 5 h later, rats were perfused with modified Kreb's solution, followed by 8% paraformaldehyde. The brains were removed and placed in 8% paraformaldehyde overnight. Horizontal 50 μm sections through the NTS were made using a Vibratome. The tissue was processed with a standard diaminobenzidine protocol for visualization of the BDA label.

Data analysis

Neurophysiology

In order to be consistent with earlier reports involving whole-nerve gustatory responses (Hill, 1987; Sollars and Hill, 1998), 0.5 M NH_4Cl was used as the standard measure to which all other solutions were compared. In addition, responses to NH_4Cl showed little change in magnitude following amiloride. Thus, an accurate comparison of response magnitudes could be made for solutions before and after amiloride. Response magnitudes were calculated as the averaged height of the steady-state response that occurred between 20 and 35 s after stimulus onset (Sollars and Hill, 1998) (see Figure 1). Ratios relative to 0.5 M NH_4Cl were calculated from these averages, which provided a basis to compare magnitudes (i.e. relative magnitude) across conditions. For each solution presented in amiloride, suppression ratios were calculated as follows:

$$\text{percent suppression} = [1 - (RM_{after}/RM_{before})] \times 100$$

where RM_{after} is the relative magnitude of a response to a solution presented in amiloride and RM_{before} is the relative magnitude of a response to a solution presented before amiloride. Thus, a percent suppression of 100 indicates a response reduced by amiloride to baseline neural activity and 0% suppression indicates no suppressive effect of amiloride.

Relative magnitudes and percent suppression ratios were compared with Repeated Measures ANOVA. Statistically reliable ANOVA results were followed with post-hoc analysis with Bonferroni or independent t-tests, with the alpha level set at $P < 0.05$, and adjusted to compensate for the number of statistical tests conducted within a series (e.g. $P < 0.05/7$) (Kirk, 1968).

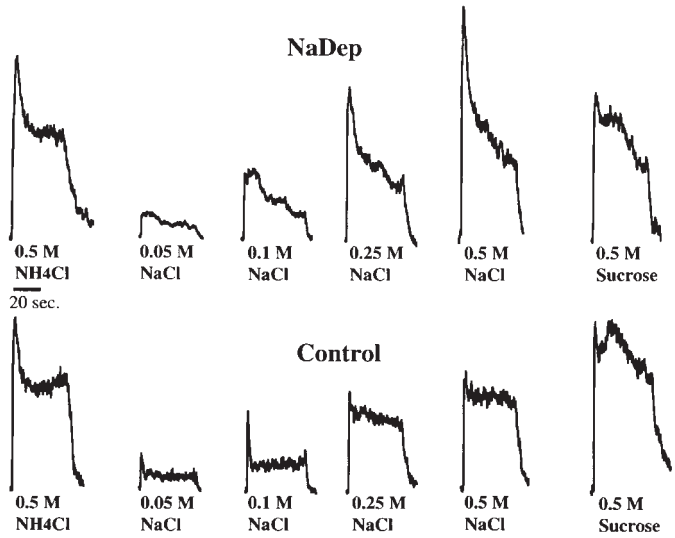

Figure 1 An example of typical GSP integrated neurophysiological responses to stimuli applied to the palates of NaDep and control rats.

Terminal field label

Computer reconstruction of the GSP terminal field label was accomplished using Neurolucida software (MicroBrightField, Inc., Colchester, VT). The volume of label was calculated for each tissue section. The GSP terminal field area was found in an average of 10 sections (~500 μm) of tissue for each rat. In order to examine possible effects of early sodium deprivation on different dorsal–ventral regions of the NTS, field volumes were calculated for the dorsal third, the intermediate third and the ventral third of the NTS in which there was label. The intermediate level was defined as the region in which the solitary tract was most evident. Statistical differences between NaDep and control rats in total volumes and regional volumes of terminal field size were analyzed with independent *t*-tests. The alpha level was set at $P < 0.05$ and adjusted to compensate for the number of *t*-tests conducted (Kirk, 1968).

Results

Sodium responses

The GSP of rats in both the NaDep and control groups showed similarly strong responses to NaCl and NaAc, and similar degrees of suppression by amiloride were also observed (see Figure 2A,C). In addition, the relative responses to NaCl and NaAc increased with increasing concentration in both NaDep and control rats. Furthermore, post-hoc analysis with Bonferroni tests indicated that there were no significant differences between NaDep and control rats in their responses to any of the sodium stimuli ($P > 0.05$

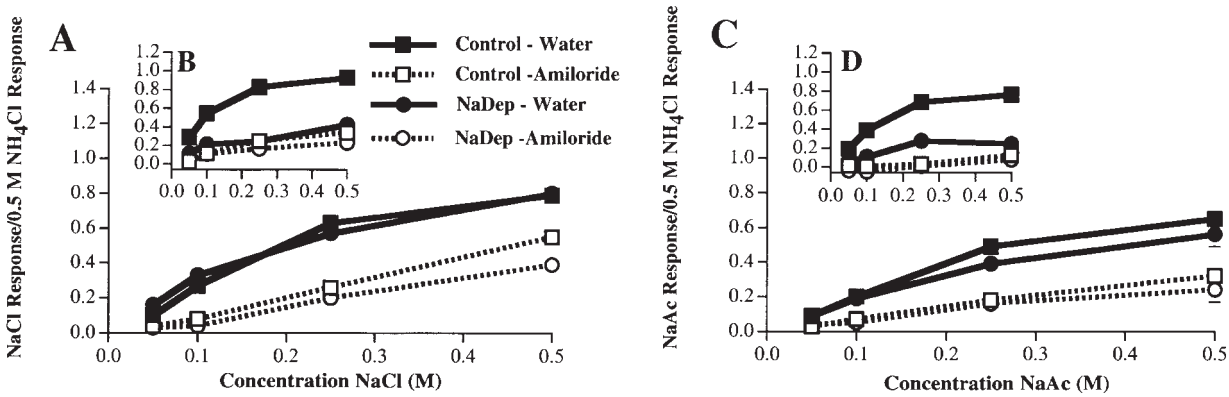

Figure 2 Averaged GSP response ratios (mean ± SE) to **(A)** NaCl and **(C)** NaAc in NaDep and control rats. **Inset:** Response ratios to NaCl **(B)** and NaAc **(D)** from the chorda tympani nerve in one NaDep rat and one control rat. Responses were obtained from solutions mixed in distilled water or 100 μM amiloride. (In A and C, the SE range is 0.01–0.07, so many of the error bars are not detectable) (NaCl and NaAc with water rinse: NaDep, $n = 6$ and control, $n = 6$; NaCl/amiloride rinse: NaDep, $n = 5$ and control, $n = 4$; NaAc/amiloride rinse: NaDep, $n = 4$ and control, $n = 4$).

at each concentration tested). These results are in sharp contrast to the drastically reduced responses of the chorda tympani nerve to sodium stimuli that have previously been noted in NaDep rats (Hill *et al.*, 1986; Hill, 1987). Although this effect on chorda tympani responses has been replicated several times, in order to establish that chorda tympani responses to sodium stimuli were diminished in the group of rats in the current experiment, responses to NaCl and NaAc were recorded from the chorda tympani of one NaDep littermate and one control rat [see the surgical methods of Hill (Hill, 1987)]. Responses from the chorda tympani of the NaDep rat were obtained 1 day after the GSP responses were recorded from its littermate. The results of those recordings can be seen in the insets of Figure 2B,D. As clearly indicated in the figure, chorda tympani responses to both NaCl and NaAc were dramatically diminished relative to those of the control chorda tympani. Taken together, these results demonstrate that developmental sodium deprivation has a strong effect on sodium responses of the chorda tympani, but no effect on the sodium responses of the GSP.

Amiloride reduced the GSP responses to NaCl and NaAc in both NaDep [NaCl: $F(1,9) = 96.04$, $P < 0.0001$; NaAc: $F(1,8) = 28.19$, $P < 0.001$] and control [NaCl: $F(1,8) = 82.63$, $P < 0.0001$; NaAc: $F(1,8) = 99.29$, $P < 0.0001$] rats (see Figure 3). These results are also indicated in Figure 2A,C. Specifically, in NaDep rats, the relative responses to all concentrations of NaCl were significantly reduced by amiloride, and NaAc responses to 0.1, 0.25 and 0.5 M NaAc were significantly lower after amiloride application. The GSP responses of control rats to 0.1, 0.25 and 0.5 M concentrations of both NaCl and NaAc were reduced by amiloride. Thus, not only was the GSP of NaDep and control rats similar in response to sodium before amiloride, but the suppressive effects of amiloride were also similar between the experimental conditions. The percent suppression by amiloride in NaDep rats ranged from 86.8% for 0.1 M NaCl to 52.5% for 0.5 M NaCl. Similar results were obtained for control rats, with the highest degree of suppression occurring with 0.1 M NaCl (83.8%) and the lowest (36.8%) for 0.5 M NaCl.

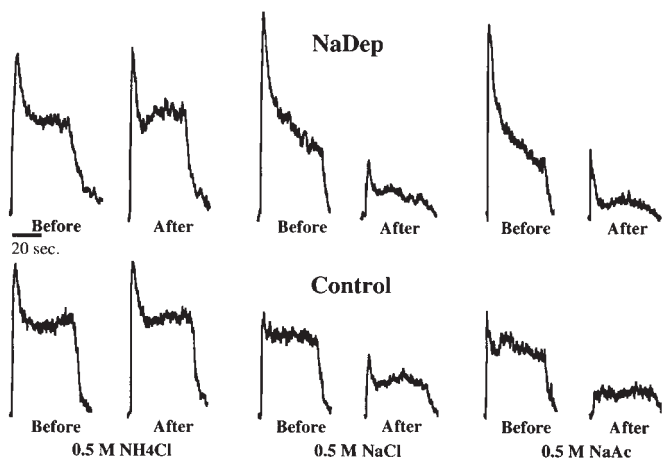

Figure 3 Integrated GSP neural responses to stimuli applied to the palates of NaDep and control rats. Stimuli were mixed either in distilled water (Before) or 100 μM amiloride (After).

Non-sodium stimuli

The only difference in GSP responses between NaDep rats and control rats was in the relative magnitude of response to 0.01 N HCl. As seen in Figure 4A, relative responses to HCl were more than double in NaDep rats compared with controls [$t(7) = 3.0$, $P < 0.02$]. No other stimulus elicited responses that were different between the two groups. Figure 4A shows the relative magnitude of quinine, which is not a very effective stimulus at this concentration in the rat ($P > 0.10$ between experimental groups). The results of GSP responses to NH_4Cl indicate a strong degree of similarity in the relative magnitude of a range of NH_4Cl concentrations [see Figure 4B; $F(1,9) = 2.17$, $P > 0.10$]. There were no

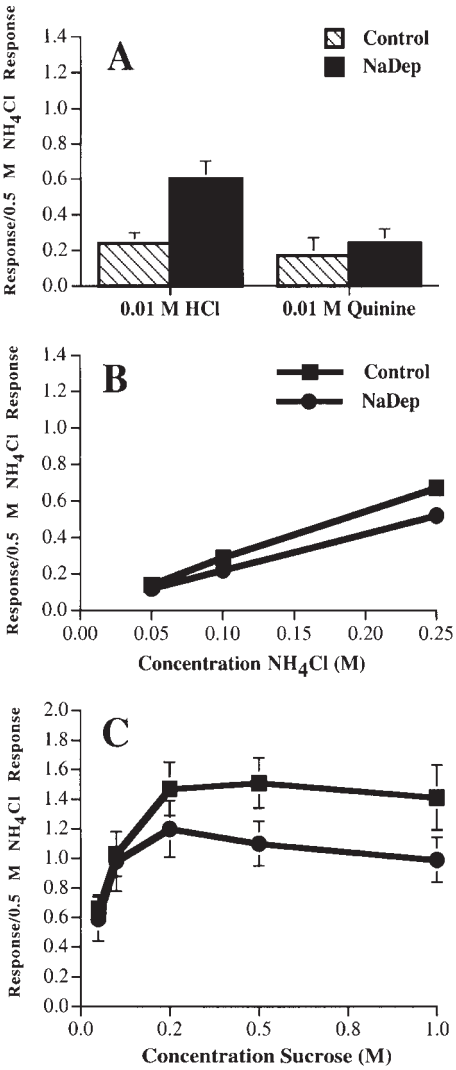

Figure 4 Average (± SE) GSP response ratios of NaDep and control rats calculated for **(A)** HCl (NaDep, $n = 5$; control, $n = 4$) and quinine hydrochloride (NaDep, $n = 5$; control, $n = 4$); **(B)** NH_4Cl ($n = 6$ per group); and **(C)** sucrose ($n = 5$ per group), all presented in distilled water. (The SE for NH_4Cl ranged from 0.02 to 0.05 and cannot be detected on B.)

statistically reliable differences in the sucrose responses between experimental conditions [$F(1,8) = 1.25$, $P > 0.10$; Figure 4C]. In general, sucrose responses of the GSP have a much wider degree of individual variability than any other stimulus tested. The relative responses of control rats to 0.5 M sucrose ranged from 1.0 to 1.9. We have observed that these individual tendencies in sucrose responses remain relatively stable within an individual rat (unpublished observations). However, with the exception of one rat in the NaDep group, individual rat responses tended to cluster, with relative magnitudes between 0.8 and 1.1.

Terminal field volume

Figure 5A,B shows an example of BDA label in the GSP terminal field in sections of dorsal and intermediate zones of a NaDep rat. Figure 5C,D shows the same type of label in comparable planes of section from a control rat. There were no differences in the volumes of terminal field label in the NTS of NaDep rats compared with controls ($P > 0.10$; see Figure 6A). Subdividing the total terminal field volume into three zones indicates that the intermediate zone is substantially larger than the dorsal or ventral zone (Figure 6B). Although there is a slight tendency for the dorsal zone in NaDep rats to be larger, there were no statistically significant differences in any of the volumes between experimental groups ($P > 0.10$). In contrast, previous reports have shown large increases in the volumes of the dorsal zone of rostral NTS when the chorda tympani nerve was labeled in NaDep rats (King and Hill, 1991).

Discussion

Developmental sodium restriction results in GSP responses that are similar to those in control rats. The similarity in sodium responses between the experimental groups is reflected in a comparable degree of sodium response suppression by amiloride: application of amiloride to the palate reduces GSP responses to 0.1 M sodium stimuli by >80% in both groups of rats. In addition to the general lack of susceptibility on the function of the GSP, developmental sodium restriction did not affect central morphological features specific to the GSP. The volume and distribution of the GSP terminal field in the rostral NTS is similar between NaDep and control rats. The failure of developmental sodium restriction to produce neurobiological alterations in the GSP are in sharp contrast to the profound effects that have been noted in another gustatory nerve, the chorda tympani.

Major alterations in chorda tympani function and anatomy occur as a result of developmental sodium restriction. As detailed earlier, chorda tympani nerve responses are drastically and specifically reduced to NaCl with a concomitant lack of response suppression by amiloride (Hill *et al.*, 1986; Hill, 1987; Hill and Przekop, 1988). Additionally, the size of the dorsal zone of the chorda tympani terminal field in the rostral NTS is significantly larger and different in shape in NaDep rats compared with controls (King and Hill, 1991; Walker and Hill, 1995). The contrast between the extensive effects of developmental sodium restriction on the chorda tympani and the absence of an effect on the GSP is especially intriguing given the similarities between the chorda tympani and the GSP. Both nerves in adult control rats respond well to sodium salts and to amiloride, and both nerves have cell somata in the geniculate ganglion that project to extensively overlapping fields in the rostral pole of the NTS. Therefore, it is difficult to attribute the diet-induced functional differences on the basis of distinct sodium response characteristics and to attribute terminal field differences on the basis of gross neuroanatomical characteristics.

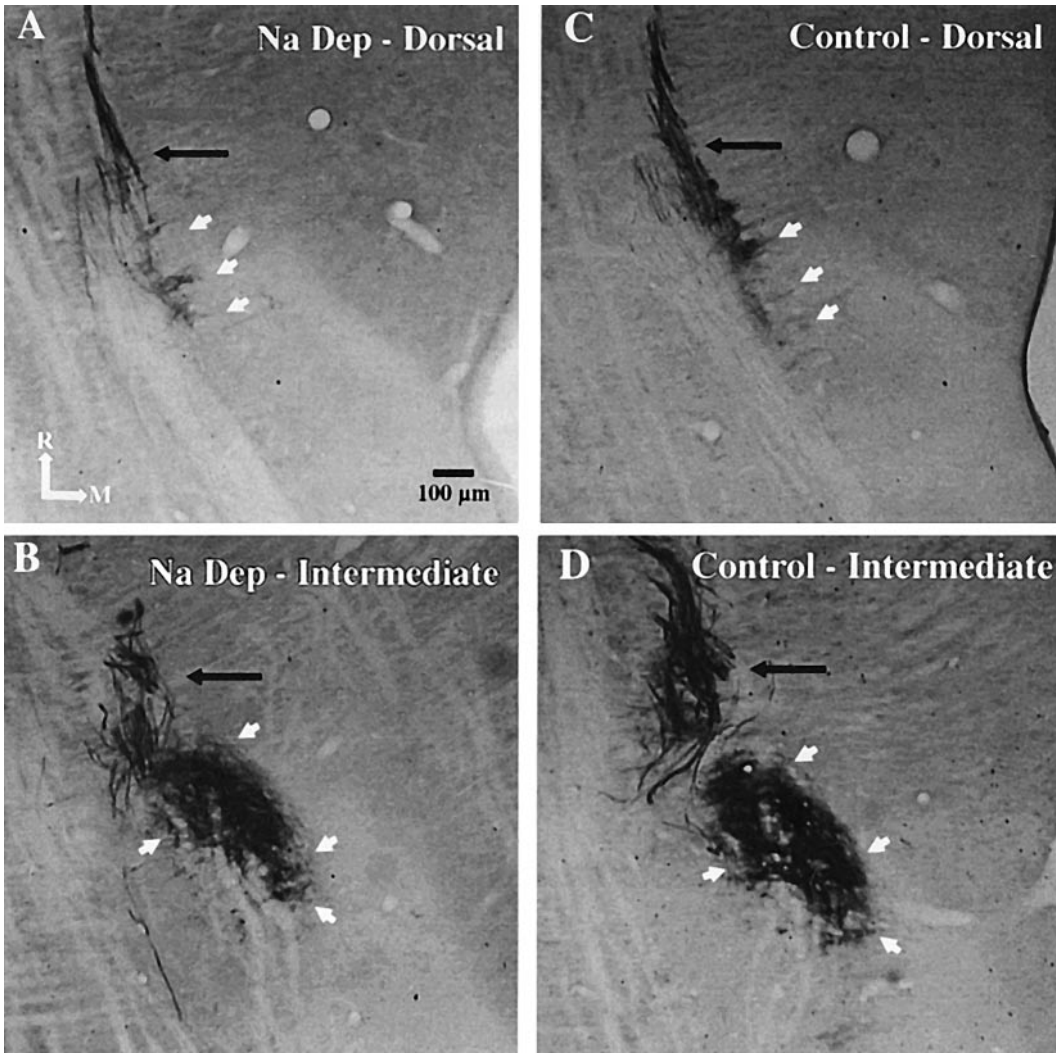

Figure 5 Examples of GSP terminal field label in the rostral portion of the NTS. The GSP was labeled with 3 kDa biotinylated dextran amine. Sections from the dorsal and intermediate levels of the NTS are shown for one NaDep **(A, B)** and one control **(C, D)** rat. Black arrows indicate GSP fibers rostral and lateral to the NTS. White arrows indicate the region of GSP terminations within the rostral NTS. Rostral (R) is toward the top. Medial (M) is toward the right side of each photomicrograph. The scale bar in (A) applies to all panels.

The lack of diet-induced alteration in the function of amiloride-sensitive sodium channels in the palate suggests fundamental differences in the regulation of the channel in fungiform as opposed to palatal taste receptor cells. The difference could relate to early developmental processes and/or the maintenance of the local environmental milieu. Restriction of sodium in the maternal diet during gestation has widespread effects. For example, developmental sodium restriction results in decreases in circulating levels of insulin-like growth factor 1 (personal observation), which regulates amiloride channel function in non-gustatory tissues (Blazer-Yost *et al.*, 1989). In addition, there are dietary-induced alterations in other circulating factors, such as renin and aldosterone (Roy-Clavel *et al.*, 1999). We have argued in the past that the lack of amiloride sensitivity in the chorda tympani nerve of NaDep rats is not due to lack of sodium *per se* (Stewart *et al.*, 1997); rather, alterations in some of these physiological systems subsequently impact on amiloride-sensitive sodium channels in later developing taste receptor cells. Indeed, Lin *et al.* (Lin *et al.*, 1999) recently demonstrated that aldosterone enhanced the immunoreactivity of beta and gamma subunits of the amiloride-sensitive sodium channel in fungiform taste buds in adult rats and increased the number of taste cells with amiloride-sensitive currents along with increasing the amplitude of such currents. These findings demonstrate that sodium-regulation systems are also operable in regulation of the amiloride-sensitive sodium channel in taste receptor cells. It is possible that these and/or other regulatory factors have a differential effect on lingual and palatal taste receptor development via the local environment of taste buds or in the geniculate ganglion. Although analyses of amiloride-sensitive subunit

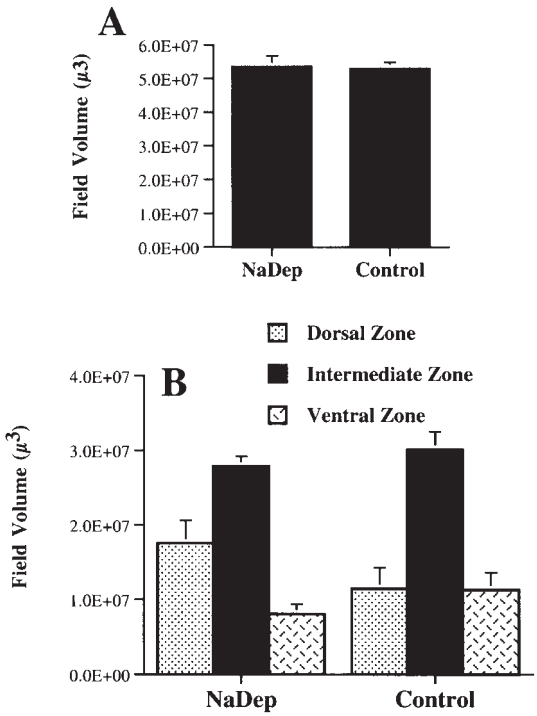

Figure 6 GSP terminal field volume (mean ± SE) in NaDep (n = 5) and control rats (n = 5). **(A)** Average volume of total terminal field label. **(B)** Volumes of GSP terminal field in the dorsal, intermediate and ventral regions of the NTS.

complementation have not been done comparing palatal taste buds with those on the anterior tongue, there are clear differences between taste receptor cells located on the anterior compared with posterior receptor cells (Kretz *et al.*, 1999). Similar receptor-field-specific differences in subunit composition may exist between the palate and anterior tongue that could account for the developmental differences in the dietary susceptibility noted here. That is, the factors and/or processes involved in regulation of the channel in fungiform taste receptor cells may not be the same for palatal taste receptors.

Another possibility may be related to the sodium-restricted diet itself. Although low in sodium, the sodium-restricted diet used in this study is high in sucrose (67%). Since the GSP in adults is highly sucrose responsive, while the chorda tympani nerve is minimally responsive, palatal taste receptor cells may receive relatively normal, overall amounts of activity as opposed to the less stimulated fungiform taste receptor cells. This would suggest an activity-dependent mechanism that serves to maintain overall function in palatal taste receptors, even though not all transduction pathways are stimulated directly (i.e. sodium-elicited responses). The extension of this hypothesis is that the GSP may have the same altered functional development as the chorda tympani during early postnatal periods in sodium-restricted rats but becomes normal upon weaning, at which time sucrose-elicited activity has its stabilizing effect. Such an effect is observed for chorda tympani function if NaDep rats are given sodium to drink or are placed on regular chow at weaning; sodium responsivity and amiloride sensitivity of the chorda tympani 'recovers' to normal values (Przekop *et al.*, 1990; Stewart and Hill, 1996). While there are no comparable data concerning regulation of the amiloride-sensitive sodium channel in other systems, it is clear that apical membrane permeability to sodium through the amiloride-sensitive sodium channel in other epithelial tissues is regulated by factors such as cell metabolism, pH and feedback mechanisms that include intracellular calcium and sodium itself [see Turnheim for a review (Turnheim, 1991)]. Similar regulatory mechanisms may exist in taste receptor cells. It is possible that transduction of non-sodium stimuli could involve intracellular changes in pH, intracellular calcium and even sodium. For example, sugars which strongly stimulate palatal but minimally stimulate fungiform taste buds may maintain amiloride-sensitive sodium channels in palatal taste receptor cells of NaDep rats. Studies of transduction pathways using sugars and artificial sweeteners have identified multiple transduction pathways in a variety of species, involving second-messenger systems and ionic channels, including the amiloride-sensitive sodium channel (Margolskee, 1995). The relationship between these transduction pathways and the amiloride-sensitive sodium channel have not been explored in taste bud cells.

While differences in taste receptor cell activity may have a role in the functional differences between the GSP and chorda tympani, it is more difficult to argue that neural activity explains differences in terminal field alterations. That is, it follows from other sensory systems that decreased afferent activity, as seen in the chorda tympani nerve, results in altered central morphologies (Moore, 1985; Garraghty *et al.*, 1986; Renehan *et al.*, 1989). Accordingly, the lack of altered activity in the GSP would be consistent with normal terminal fields in the NTS. However, the argument for activity dependence shaping terminal field organization for the chorda tympani is difficult to make. First, the altered field organization of the chorda tympani occurs even if rats are fed the sodium-restricted diet only from E3 through E12 and are then maintained on regular chow throughout life (Krimm and Hill, 1997). This 'sensitive' period is long before presumptive taste cells could be stimulated directly by factors in the diet, since presumptive taste cells do not appear until approximately E20 (Mistretta, 1972). Second, the birth of geniculate ganglion neurons that give rise to the chorda tympani and GSP occurs around E11 (Altman and Bayer, 1982). Therefore, this age is near the end of the 'sensitive' period of the chorda tympani terminal field susceptibility to the sodium-restricted diet. Finally, plasma sodium levels are protected in pregnant rats and their offspring when fed low-sodium diets (Hill, 1987), and milk electrolyte levels are protected in lactating rats (Stewart *et al.*, 1993). Therefore, at least to weaning, sodium levels

appear normal. This does not rule out postweaning, activity-dependent mechanisms and/or selective changes in local sodium levels provided by different circulatory and/or salivary influences. Indeed, the terminal field of the chorda tympani appears normal until the age when the nerve normally responds best to sodium salts (i.e. between postnatal days 15 and 25) (Walker and Hill, 1995). Furthermore, when dietary sodium content is reduced only at weaning, alterations in the dendritic organization of NTS neurons is produced, suggesting that taste-elicited activity at weaning shapes gustatory neuron morphologies (Liu *et al.*, 1999).

In summary, these remarkably different functional and morphological outcomes between the chorda tympani nerve and GSP in NaDep rats should allow a better understanding of the role of activity- and non-activity-dependent mechanisms in taste receptor cell development and central gustatory development. Based on the results from the current study, 'recovery' of sodium responses and amiloride sensitivity of the chorda tympani in NaDep rats would be predicted to occur if fungiform taste buds could be stimulated with non-sodium taste stimuli. Taste responses from the GSP and from the chorda tympani have not been recorded during early postnatal development in sodium-restricted rats; therefore, it is not clear what the developmental patterns are for the two nerves. However, it is clear that the same influences on sodium salt taste development do not occur for lingual and palatal taste buds. Further experiments that exploit these differences may lead to identification of the mechanisms that regulate amiloride-sensitive sodium channels in taste receptor cells.

Acknowledgements

This research was supported in part by National Institutes of Health Grants DC03500 (S.I.S.), DC00407 and DC03576 (D.L.H.). The authors wish to thank Susan J. Hendricks for comments on an earlier version of this manuscript.

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Accepted April 25, 2000