

Sensitive periods for the effect of dietary sodium restriction on intact and denervated taste receptor cells

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McCluskey, Lynnette Phillips, and David L. Hill. Sensitive periods for the effect of dietary sodium restriction on intact and denervated taste receptor cells. *Am J Physiol Regul Integr Comp Physiol* 283: R1275–R1284, 2002. First published July 18, 2002; 10.1152/ajpregu.00282.2002.—Unilateral chorda tympani nerve (CT) section combined with dietary sodium restriction leads to striking alterations in sodium taste function. The regenerated rat CT exhibits deficits in sodium sensitivity, and surprisingly, there are also functional alterations in the intact, contralateral nerve. The studies presented here describe the functional “sensitive periods” for these aberrations and the number of taste buds present during corresponding stages. The regenerated CT is sensitive to dietary sodium restriction during the first 2 wk after denervation, whereas the intact CT is sensitive to dietary manipulation during the first week postsection. Therefore, distinct mechanisms are responsible for the effects of sodium restriction combined with denervation, because separate sensitive periods exist for the regenerated and intact CT nerves. Identification of mature taste buds with an antibody directed at anti-keratin 19 revealed that there is a loss of ~85% of taste buds on the denervated side of the tongue under control and low-sodium diets within the first week postsection. Thus, sodium restriction does not differentially affect the loss of taste buds following denervation.

amiloride; chorda tympani nerve; keratin 19; electrophysiology; neural degeneration

TASTE RECEPTOR CELLS LOCATED within fungiform papillae on the anterior tongue are innervated by the chorda tympani nerve (CT). One function of the CT, in addition to conduction of neural impulses to the central nervous system, is to maintain the structural and functional fitness of associated taste receptor cells. When the CT is unilaterally sectioned, taste buds degenerate and gustatory function is ipsilaterally abolished. However, taste receptor cells eventually reappear following reinnervation and normal taste function is restored (4, 5, 12).

Previously, we demonstrated that the function of taste receptor cells that regenerate under dietary sodium restriction is dramatically different from the normal postregeneration state (18). Specifically, dietary sodium restriction in combination with CT section se-

lectively affects gustatory afferent responses to sodium. Neurophysiological responses to sodium stimuli recorded from the regenerated CT of sodium-restricted rats were greatly depressed compared with sectioned and dietary control rats, and the attenuation in sodium responses appeared to be permanent. Surprisingly, the intact, contralateral CT displayed hypersensitive responses to sodium, despite the lack of a peripheral neural connection between the two sides of the tongue (23).

A detailed examination of sodium taste function in the intact CT revealed that responses to sodium were extremely low during the first week after the contralateral nerve was sectioned and the sodium-restricted diet was initiated. However, there was a gradual, linear increase in sodium sensitivity, so that hypersensitive responses were observed by ~50 days after sectioning the contralateral CT. Because primary afferent taste responses reflect taste receptor cell function (3, 17), it is likely that alterations occur in taste receptor cells as well as in the CT. Thus, taste receptor cells that newly form under dietary sodium restriction after regeneration appear to display a long-term and stable reduction in sodium taste function. Conversely, the intact, contralateral CT shows dramatic functional changes with time. Importantly, in our hands, neither initiation of sodium restriction alone (diet controls) in adults nor CT section alone (cut controls) had any influence on peripheral taste responses (18).

These experiments also demonstrated that the cellular mechanism responsible for attenuated or supersensitive sodium taste responses in taste receptor cells involves changes in the epithelial sodium channel (ENaC). This channel is sensitive to the pharmacological antagonist amiloride and is thought to be primarily responsible for sodium taste transduction in the adult rodent (14, 39). In the study by Hill and Phillips (18), lingual application of amiloride eliminated all experimentally induced differences in CT responses to sodium salts and had no effect on responses to other stimuli. Finally, multiple sectioning of the CT did not change the predictable increase in sodium sensitivity by the intact nerve, indicating that reinnervation by

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the cut CT does not mediate the increased sodium taste responses in the intact CT (18).

From the results discussed above, it is apparent that important events subsequent to unilateral CT section occur if adult rats are placed on a sodium-deficient diet. However, the timing of such events is unknown because the sodium-restricted diet was always instituted immediately after sectioning. To begin determination of the relevant physiological processes responsible for the dramatic response alterations, it is necessary to know when the diet has its influence. The first study presented here was designed to determine the period of vulnerability of CT function to dietary sodium restriction following nerve sectioning. Therefore, the goal was to define the onset of the "sensitive period" for the regenerated and intact CT nerves by systematically varying when rats were placed on the low-sodium diet. Responses were then recorded from both CT nerves starting at 50 days after unilateral CT section. The period of 50 days was chosen because the sectioned CT nerve regenerates during that period (i.e., demonstrates robust neural responses to multiple stimuli) (18). Moreover, at 50 days postsectioning, the hypersensitivity to sodium in the uncut nerve is evident when dietary restriction is instituted immediately after nerve section (18). Such a strategy has been extremely useful in developmental studies of taste function (19), and knowing when the diet is effective will further subsequent determination of the underlying mechanisms.

Our second goal in these experiments was to investigate taste bud degeneration in sodium-restricted and control rats during the corresponding functional sensitive period(s). To accomplish this, a monoclonal antibody to keratin 19 was used to immunohistochemically label taste buds at various times after unilateral CT section. Keratins are intermediate filament proteins expressed in a range of epithelial tissue, with specific sequences present during various states of differentiation and tumorigenesis (1, 8, 34). Keratin 19-like immunoreactivity has been demonstrated in rat fungiform taste buds, which contain the taste receptor cells innervated by the CT nerve (24, 44). Importantly, keratin 19 is restricted to fusiform cells located within the limits of the taste bud, and the immunopositive cells are thought to represent mature, functional taste receptor cells (44).

Various histological methods have been used to examine mammalian taste buds at several time periods following nerve section, although there is a lack of consensus as to whether taste buds completely disappear, merely become atrophic, or contain remnants of taste receptor cells (2, 5, 31). The identification of keratin 19-like immunoreactivity as a marker for fully differentiated taste cells is advantageous for identifying taste buds to complement the neurophysiological experiments described here. These studies will focus future work on mechanisms underlying alterations in intact and regenerated taste receptor cells and provide information concerning plasticity in the adult taste system.

METHODS

Experiment 1: Determination of the "Sensitive Period"

Nerve sectioning procedure. Female Sprague-Dawley rats (Harlan Sprague-Dawley, Dublin, VA) were 30–40 days old at the time of CT section. Rats received an injection of atropine sulfate (0.5 mg/ml ip) and were anesthetized with sodium Brevital (60 mg/kg ip). The left or right CT nerve was exposed in the neck and sectioned between the anterior belly of the digastric and the masseter muscles where the CT nerve bifurcates from the lingual branch of the trigeminal nerve. Thus, the lingual branch of the trigeminal nerve remained intact. In all groups receiving CT nerve sectioning, the cut ends of the nerve were left in place, and regeneration was allowed to proceed.

Groups. Groups included rats receiving: 1) CT nerve section on *day 1* followed by dietary sodium restriction from *day 7* to *day 50*; 2) CT nerve section on *day 1* followed by dietary sodium restriction from *day 14* to *day 50*; 3) CT nerve section followed by control diet; and 4) dietary sodium restriction without nerve section. Thus, experimental *groups 1* and *2* received the same surgical treatment as in earlier work (18), but dietary manipulations were delayed for 1 or 2 wk postsectioning before being continued until neurophysiological recording. Figure 2 also includes data previously obtained from rats that received both CT nerve section and dietary sodium restriction on *day 1* for comparison (18).

Dietary manipulations. Rats that received nerve section were placed on standard laboratory chow (1.0% NaCl; pellet form, Purina) and tap water. On *day 7* postsection, rats were given sodium-deficient chow (0.03% NaCl; pellet form, ICN Biochemicals) and distilled water as in previous work (18) and maintained on this dietary regimen until the time of neurophysiological recording at *day 50* postsection. In addition, on *days 7* and *8* postsection, rats were injected with furosemide (Aldrich; 2 injections of 10 mg each within 24 h). The 0.03% NaCl diet has been used in a number of previous studies (16, 19, 35, 45) and is known to induce bilateral changes in CT responses to sodium following denervation and regeneration (18). The period between surgical and dietary manipulations was then extended, if necessary, until taste responses from both the regenerated and uncut CT nerves were like those of controls at 50 days postsection. That is, the onset of the period of vulnerability was found when reduced or hypersensitive responses to sodium from the regenerated and uncut nerves, respectively, were prevented by the delay in initiation of the sodium-restricted diet.

Neurophysiology. Beginning 50 days after unilateral CT section or institution of the sodium-restricted diet in diet controls, rats were anesthetized with pentobarbital sodium (10 mg/kg body wt ip) and maintained at a surgical anesthetic level with additional injections as needed. When possible ($n = 7$ of 24 rats), sequential bilateral recordings of both the regenerated and intact CT nerves were performed within the same rat, with the order of recording varied between rats. We did not observe differences in CT nerve responses from rats within the same surgical and dietary groups that received bilateral recordings instead of unilateral recordings. Body temperature was maintained between 36 and 38°C with a pad heated by circulating water. After a tracheotomy was made, rats were secured in a nontraumatic headholder. The CT nerve was exposed from its bifurcation with the lingual nerve at its entry into the tympanic bulla, cut, and the exposed length was freed of surrounding connective tis-

sues. The connective sheath was then removed from the CT, and the nerve was placed on a platinum electrode.

Standard multifiber recordings from the CT nerve were performed (16). Neural activity was amplified, integrated (time constant = 1.0–2.0 s), and displayed on an oscilloscope and chart recorder. The steady-state portion of the integrated taste responses was measured 20 s after stimulation and expressed relative to the response to 0.50 M NH_4Cl . This measure of the neural response reflects the sum of single-fiber responses and is an appropriate measure for studying responses from a large population of taste receptor cells (3, 17).

Stimulation procedures. Responses of the CT nerve were recorded while the anterior tongue was stimulated with concentration series (0.05, 0.10, 0.25, and 0.50 M) of NaCl, sodium acetate (NaAc), KCl, and NH_4Cl . In addition, 0.01 M quinine hydrochloride (QHCl), 1.0 M sucrose, and 0.01 N HCl were applied to further examine nonsodium stimuli. All chemicals were dissolved in distilled water and kept at room temperature. Approximately 3 ml of each stimulus were applied to the tongue with a syringe, followed by at least a 1-min rinse with distilled water before application of the next

stimulus (18). Responses to a series of NaCl were also recorded after the tongue was preadapted for 10 min with 100 μM amiloride hydrochloride (Sigma). All stimulation procedures were performed as above, except that the rinse for this series also consisted of 100 μM amiloride. To monitor the stability of each series, 0.50 M NH_4Cl was applied at the beginning and end of each concentration series. NH_4Cl was chosen as the standard stimulus because CT responses to this salt are consistent during development, after developmental dietary sodium restriction, and after nerve section combined with sodium restriction (16–18). Only data obtained from stable series, in which the responses to NH_4Cl did not differ by more than 10%, were used for data analysis.

Data analysis. Mean relative response ratios were calculated for each group and compared with values for pooled controls using planned, a priori, unpaired *t*-tests. That is, mean responses from both the regenerated and uncut CT nerves from rats placed on the sodium-restricted diet at 7-day intervals were compared with controls. The α -level was adjusted to account for the number of comparisons at each concentration (0.05/4 comparisons) (18).

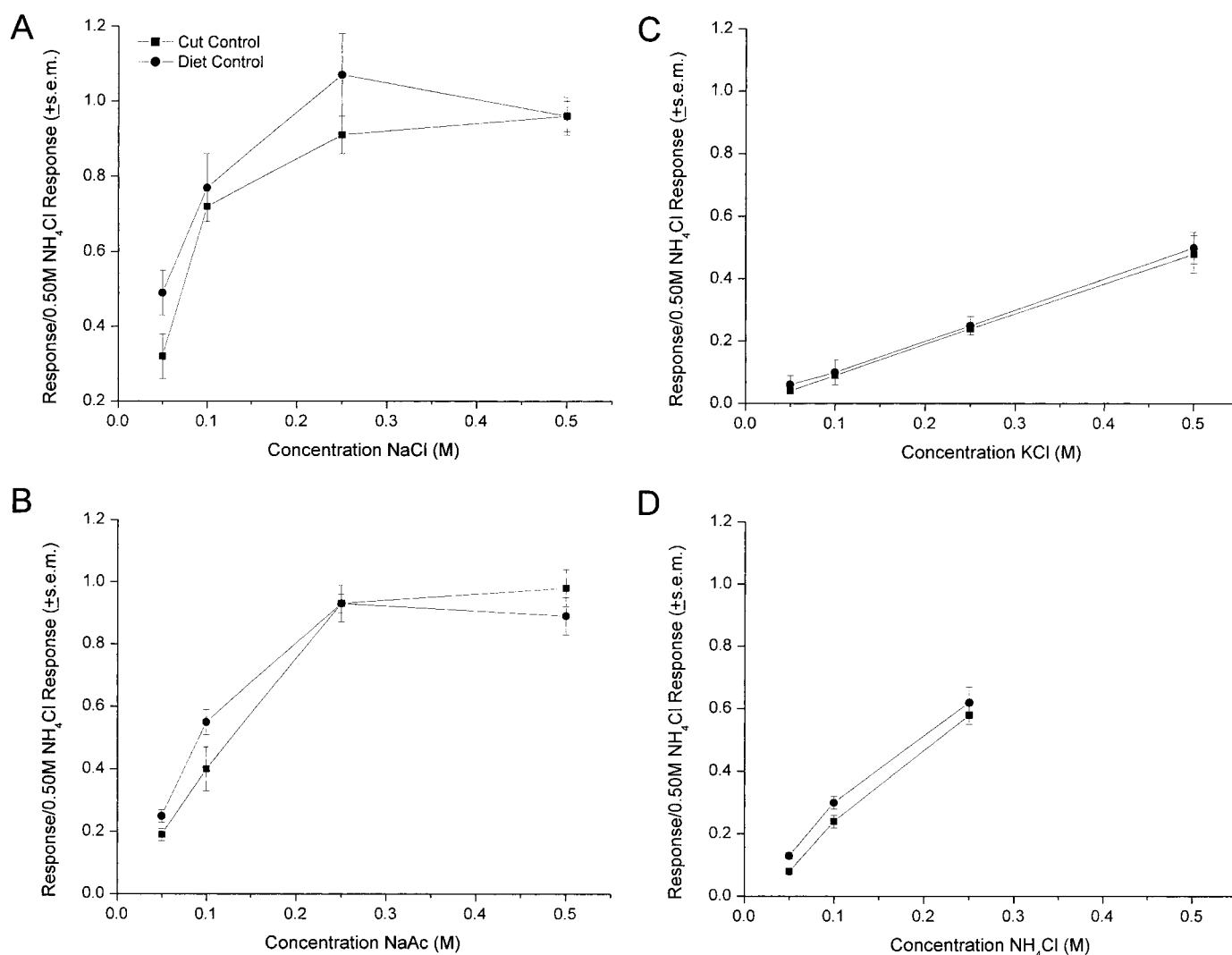


Fig. 1. Mean chorda tympani (CT) relative responses (\pm SE) from control rats receiving either unilateral nerve sectioning and control diet or dietary sodium restriction alone. Neurophysiological responses to concentration series of NaCl (A), sodium acetate (NaAc; B), KCl (C), and NH_4Cl (D) were not significantly different between groups and were pooled for further analyses.

Experiment 2: Keratin 19 Immunohistochemistry

Rats received unilateral CT section as above and immediately after nerve section were 1) injected with furosemide (2 injections of 10 mg each within 24 h) and placed on the sodium-restricted diet as above 2) or maintained on control diet.

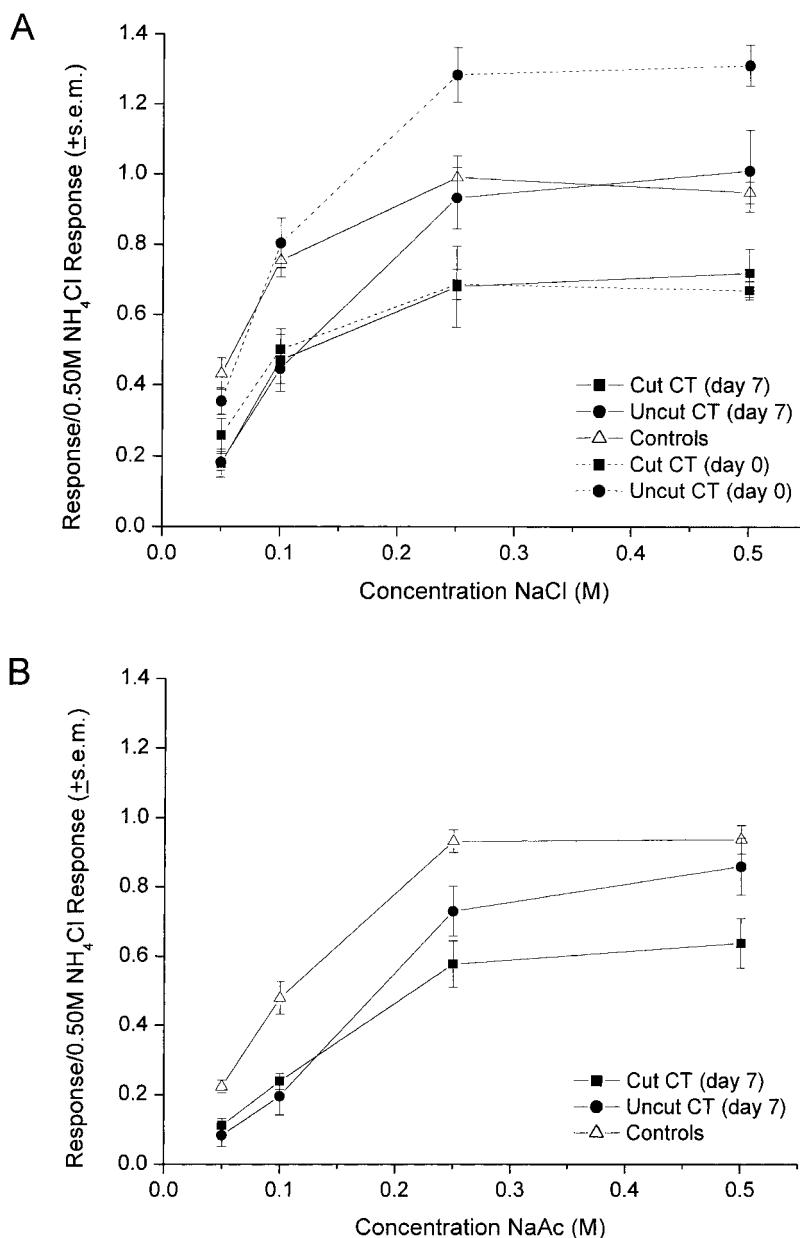
Tissue collection and immunolabeling. Tongues were collected following death with urethane anesthetic (160 mg/kg ip; Sigma) at 0 (i.e., no nerve section), 2, 5, 7, and 14 days after CT section. Blocked tissue was snap-frozen in 2-methylbutane chilled to -30°C . Tongues were sectioned coronally at 8 μm on a cryostat and thaw-mounted on gelatin-subbed slides. Approximately 120 serial sections were taken from the tip of the tongue, while ~ 60 sections each were collected from the mid and caudal regions. Tissue sections were then desiccated under a vacuum at 4°C for 18–48 h.

To begin immunohistochemical labeling, tissue was hydrated with three rinses for 3 min each in 10 mM PBS (3×3 PBS; pH 7.5) and then fixed for 1 min in 0.2% glutaraldehyde in phos-

phate buffer (pH 6.4) and for 45 s in 4% formaldehyde in borate buffer (pH 11.0). Nonspecific binding was minimized with a 45-min incubation in 1% normal goat serum (Jackson ImmunoResearch). Keratin 19 (mAb 4.62; Sigma) was diluted to a concentration of 1:400 with a solution of 1% Triton X-100 (Sigma) in PBS (pH 7.5) and applied to slides for a 2-h incubation at room temperature. Sections were then rinsed and incubated in biotin-conjugated goat IgG (Jackson ImmunoResearch) diluted to 1:250 in 1% Triton X-100 solution for 1 h. Antibodies were visualized with an avidin-biotin-peroxidase reaction (Vector Research) that used 3',3'-diaminobenzidine (ICN Biochemicals) as the chromogen. Tissue was cleared in xylenes, dehydrated with alcohols, lightly counterstained with hematoxylin, and placed under coverslips with Histomount. Control sections were incubated in primary-omitted diluents to examine nonspecific binding.

Mapping immunolabeled taste buds. Each serial section was traced using NeuroLucida software (Microbrightfield),

Fig. 2. CT relative responses (means \pm SE) to NaCl (A) or NaAc (B). Responses from the regenerated ("Cut CT day 7") and contralateral, intact ("Uncut CT day 7") nerves were recorded 50 days after unilateral nerve section in rats that had been placed on the sodium-restricted diet 7 days postsectioning. Control responses represent rats that had either CT sectioning alone or dietary sodium restriction alone. Responses from the regenerated, "Cut CT day 0" and contralateral, "Uncut CT day 0" were from rats placed on the restricted diet immediately after nerve sectioning in previous work and are shown solely for comparison (18). Sodium sensitivity exhibited by the Uncut CT was similar to that of controls, whereas a 1-wk delay of sodium restriction failed to induce recovery of normal responses to sodium by the regenerated, Cut CT.



and the position of fungiform papillae with immunopositive fungiform taste buds was marked (not shown). Subsequently, traced sections were compiled and the resulting maps were used to count keratin 19⁺ taste buds. Taste buds were defined as “onion-shaped” accumulations of fusiform epithelial cells in the apical portion of fungiform papillae (22) and were considered immunopositive when reaction product was observed in at least four serial sections, which contain at least 50% of the total taste bud diameter (30). The presence of the entire papillae was also used as a criterion for data analysis, which eliminated false categorization of incomplete papillae and/or taste buds at the beginning or end of collected tissue sections. Maps were used to determine the number of taste buds, and the number of keratin 19⁺ buds was expressed as a percentage of the total number of taste buds and/or fungiform papillae. In addition, the number of keratin 19⁺ buds on the midline of the tongue (defined as the area on the map within 1 cm on each side of the midline) was compiled across regions. These measures were determined for both the cut and uncut sides of the tongue where appropriate, in sodium-restricted and control rats at each time point detailed above.

Data analysis. Differences in the percentage of keratin 19⁺ taste buds between sodium-restricted and control rats were analyzed with *t*-tests at 2, 5, 7, and 14 days post-CT section. The α -level was set at $P < 0.05$.

RESULTS

Experiment 1: Neurophysiology

Control groups. CT responses from rats receiving unilateral CT section then maintained on a control diet (“Cut Controls”) were similar to responses from rats that were placed on the sodium-restricted diet as adults (“Diet Controls”) (Fig. 1). There were no significant differences in neural responses to any of the taste stimuli tested ($P = 0.03$ – 0.97), and responses were similar to those of normal, unmanipulated adult rats. Therefore, control data from the two groups were pooled and are subsequently referred to as “controls.”

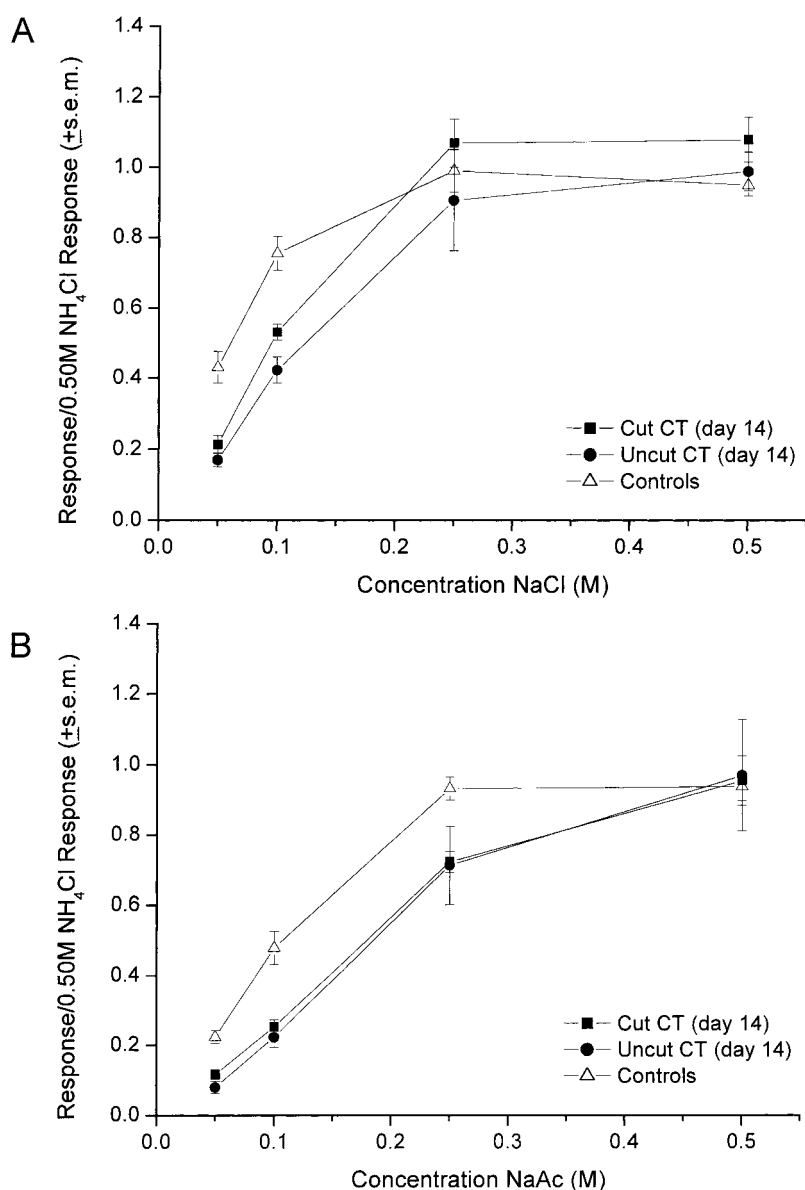


Fig. 3. Relative CT responses (means \pm SE) to concentration series of NaCl (A) and NaAc (B) from the regenerated nerve of rats that were placed on the sodium-restricted diet 14 days after unilateral CT sectioning (“Cut CT day 14”). Sodium responses from the uncut CT after a 2-wk delay in instituting sodium restriction were also like those of control rats, as expected.

Effects of dietary manipulations. The regenerated and intact CT nerves were vulnerable to the institution of the sodium-deficient diet at different periods after nerve section. When the dietary manipulation was begun 7 days after surgery, responses from the intact, "uncut" nerve to 0.25 and 0.50 M NaCl ($n = 6$) were like those of controls ($n = 10$) at 50 days postsection, as shown in Fig. 2A ($P = 0.32$ and 0.90, respectively). That is, a delay of 1 wk postsectioning before placing rats on the sodium-restricted diet prevented sodium hypersensitivity in the uncut nerve. In contrast, the regenerated, "cut" CT ($n = 6$) exhibited reduced sensitivity to 0.05, 0.10, and 0.50 M NaCl compared with controls ($P = 0.0015$ – 0.0048), like the responses observed when the dietary manipulation occurred immediately after nerve section. Mean CT responses from rats placed on the low-sodium diet immediately after sectioning (Fig. 2A) are from previous experiments and are included solely to clarify the functional consequences of delaying sodium restriction (18).

NaAc elicited a similar pattern of responses, in that the intact, "uncut" nerve of rats placed on the diet 7 days postsectioning demonstrated sodium sensitivity like controls (Fig. 2B). Responses to all concentrations of NaAc from the regenerated, "cut" CT were significantly lower than control responses ($P = 0.00003$ – 0.001 ; Fig. 2B). In addition, the sodium channel blocker amiloride eliminated group-related differences in responses to NaCl between groups ($P = 0.137$ – 0.994 ; data not shown). This finding indicates that disparities in the function of the amiloride-sensitive sodium channel are largely responsible for these alterations in taste function.

Although the intact CT displayed normal sodium sensitivity when the sodium-deficient diet was started at 7 days after section, the regenerated CT continued to exhibit reduced sodium taste function at that time. Consequently, the period between nerve section and initiation of the dietary manipulation was lengthened to 14 days. As shown in Fig. 3A, relative responses to 0.25 and 0.50 M NaCl from the regenerated CT were restored to control levels ($n = 8$, $P = 0.411$ and 0.072, respectively) when rats were placed on the sodium-restricted diet 2 wk after nerve cut. A similar recovery of normal sodium sensitivity by the regenerated CT was demonstrated in response to 0.50 M NaAc ($P = 0.838$; Fig. 3B). As expected, mean taste responses of the uncut CT ($n = 3$) to higher concentrations of NaCl and NaAc were like those of controls ($P = 0.230$ – 0.775 ; Fig. 3) when the sodium-deficient diet was initiated 14 days after nerve section, just as when sodium restriction was begun 7 days postsectioning (Fig. 2). Furthermore, application of amiloride reduced whole nerve taste responses by ~ 70 – 90% in each group.

Taste sensitivity to nonsodium stimuli, including NH_4Cl , KCl, QHCl, and sucrose (Fig. 4), was similar among groups regardless of when rats were placed on the sodium-restricted diet ($P = 0.022$ – 0.951). As in related experiments (18), the alterations in gustatory function that result from CT section and manipulation of dietary sodium are specific to sodium stimuli. How-

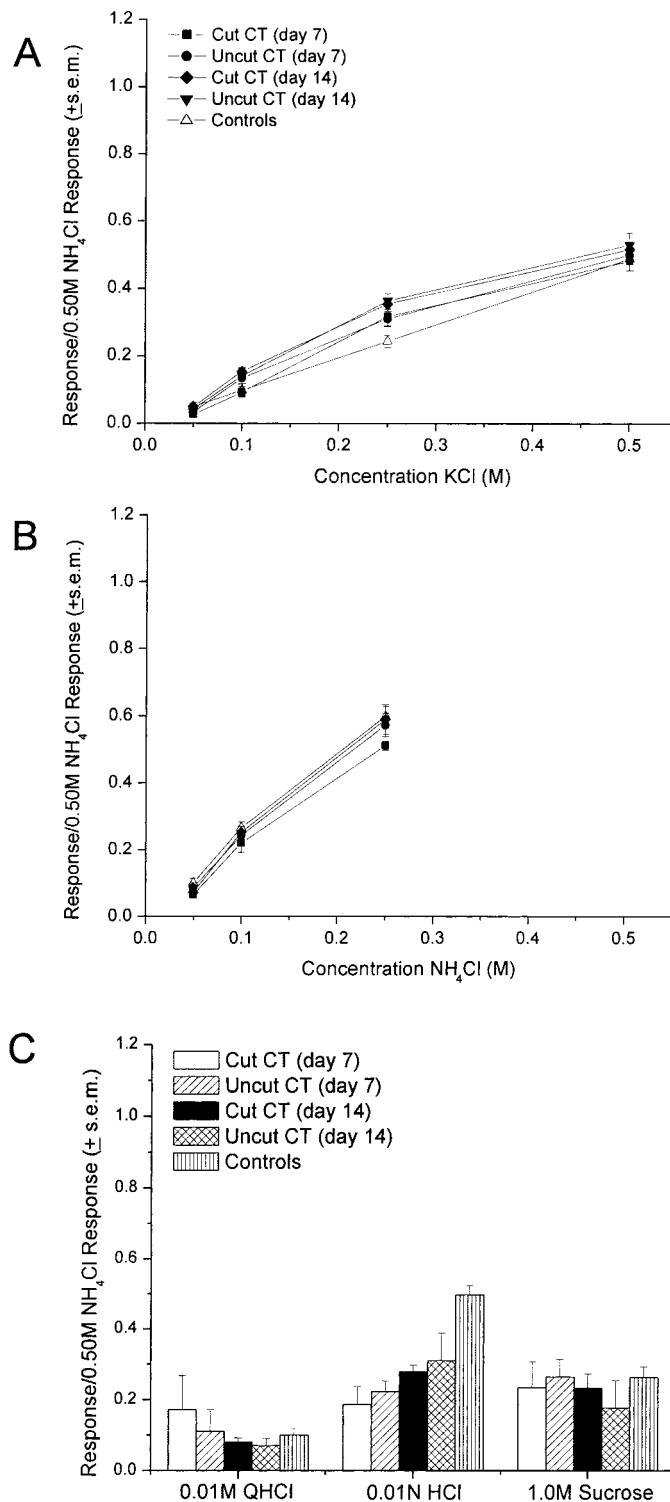


Fig. 4. CT relative responses (means \pm SE) to concentration series of KCl (A), NH_4Cl (B), and 0.01 M quinine hydrochloride (QHCl), 0.01 N HCl, and 1.0 M sucrose (C). Neural responses from rats receiving nerve section followed by a 1-wk (day 7) or 2-wk (day 14) delay in initiating sodium restriction are shown. Response magnitudes to these nonsodium stimuli did not differ between groups, with the exception of responses to HCl, which were significantly higher in control rats.

ever, an exception to the sodium specificity of functional changes was that responses to HCl from control rats were significantly higher compared with those from the regenerated (i.e., cut) or uncut CT ($P = 0.0000098-0.0124$; Fig. 4C).

Experiment 2: Keratin 19 Immunolabeling

Examples of representative tissue sections containing keratin 19⁺ taste buds are presented in Fig. 5. Staining was robust, and immunopositive taste buds were easily distinguished from negligible background staining. There was no significant loss of keratin 19⁺ taste buds from the intact side of the tongue in either control or sodium-restricted rats at any time postsection ($n = 4$ sodium-restricted and 4 control rats at each time point; Fig. 6). However, there was a rapid decrease in the percentage of keratin 19⁺ buds on the denervated side of tongues from both groups. By the first week after sectioning, the maximum loss of immu-

noreactivity was evident, as only ~20% of fungiform papillae contained keratin 19⁺ taste buds. Importantly, dietary sodium restriction did not alter the rate of disappearance of immunopositive taste buds. There were no significant diet-related differences between groups when examining the intact or denervated sides of the tongue at any time following CT section ($P = 0.129-0.903$).

Similar decreases in keratin 19⁺ taste buds occurred in each region of tongue examined. However, there were ~10 times the number of fungiform papillae located within the tip region of the tongue compared with other areas, so mid and caudal regions of the tongue did not contribute to the total pattern of loss as much as tip regions did. In addition, the percentage of keratin 19⁺ taste buds on the midline of the tongue was scored to determine if a greater proportion of surviving immunopositive taste buds was located closer to the innervated region of the tongue than to the denervated

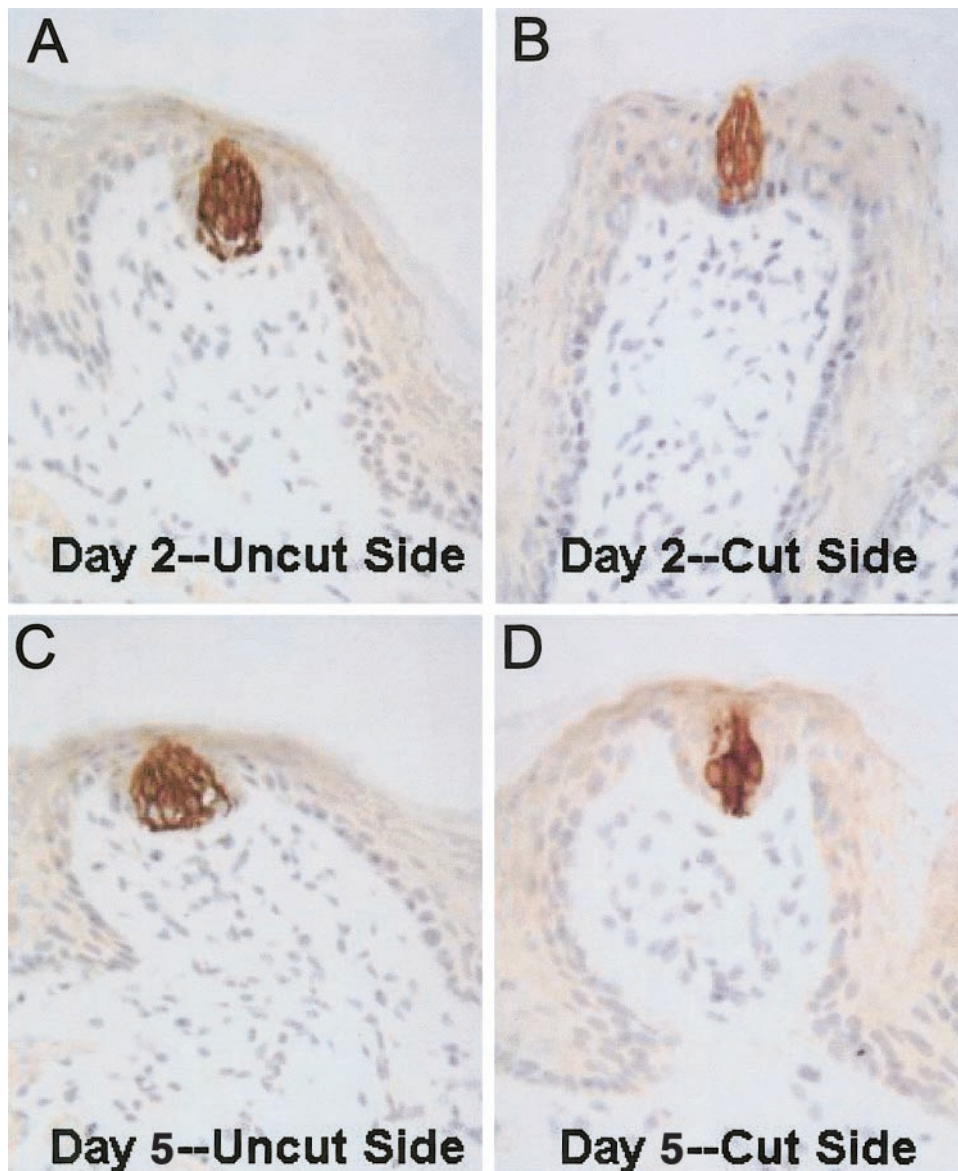


Fig. 5. Keratin 19⁺ taste buds following denervation. Representative sections of fungiform papillae from the intact (A and C) and denervated (B and D) sides of the tongue. Keratin 19⁺ taste buds appear normal on the uncut side of the tongue. In contrast, by day 5 post-CT sectioning, taste buds contain fewer keratin 19⁺ cells on the denervated side of the tongue (D). There is no difference in keratin 19⁺ staining in tongues of sodium-restricted rats after CT section (not shown).

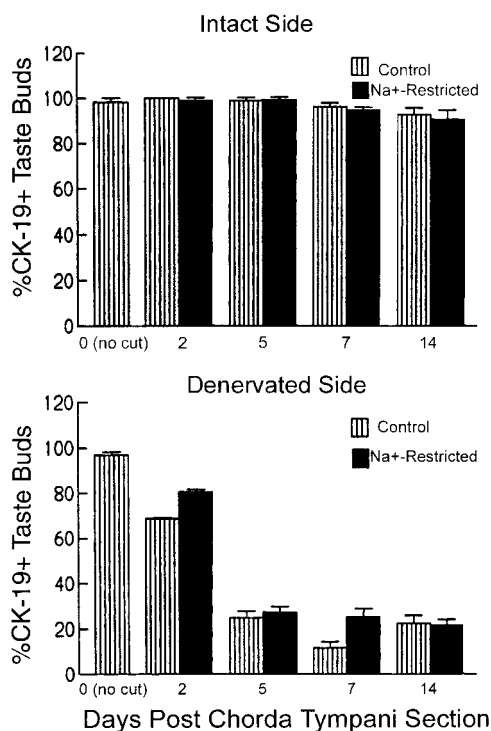


Fig. 6. Percentage of the total number of taste buds/fungiform papillae that are keratin 19⁺ as a function of time post-CT sectioning on the intact (*top*) or denervated (*bottom*) side of the tongue. There was a negligible loss of immunopositive taste buds on the intact side, but both sodium-restricted and control rats demonstrated taste bud loss on the denervated side.

side. This did not occur. There was only a slight decrease in the percentage of keratin 19⁺ buds present near the midline of the cut side (mean = 3.61%) compared with either the number of positive buds on the midline of the uncut side (mean = 9.01%) or to tongues from rats with no nerve section (mean = 8.34%).

DISCUSSION

These results demonstrate that two distinct functional “sensitive periods” exist for the regenerated compared with the uncut, contralateral CT nerves in the same animal. Dietary sodium must be restricted in rats within the first week after nerve section to show altered sodium sensitivity (i.e., hypersensitivity) in the intact CT. Conversely, the regenerated CT is vulnerable to the effects of sodium restriction within 2 wk after it is originally sectioned. Examination of taste buds during the 2 wk after cut, which corresponds to the functional sensitive period, reveals that there is a similar loss of keratin 19⁺ taste buds in both sodium-restricted and control rats. That is, dietary sodium restriction does not protect taste buds from degenerating after denervation nor does it speed their degeneration.

The temporal dissociation in the sensitive periods of the regenerated and intact CT nerves suggests that different mechanisms are responsible for the variations in sodium taste function. During the second week following nerve section, corresponding to the sensitive

period of the regenerated CT, processes involved in late degeneration and/or early regeneration may be involved. For example, the structural and physiological state of ENaCs in newly emerging taste receptor cells may be sodium dependent. At 2 wk postsection, the diet may have been instituted after newly reformed taste receptor cells are susceptible to environmental influence. The first week following denervation, corresponding to the sensitive period of the intact, contralateral CT, is likely to involve processes of degeneration. Inflammatory events, including clearance of tissue debris by phagocytes (11, 32, 41), infiltration by leukocytes (13, 21, 38), and the secretion of cytokines known to affect neural and epithelial cells (25, 37, 42), occur soon after neural injury or transection and are also likely to transpire following CT section. Whatever the mechanisms underlying these events, we showed here that the number of immunopositive taste buds remaining after denervation does not depend on dietary sodium content.

As Oakley and colleagues (24, 44) previously demonstrated, keratin 19 immunoreactivity is a useful and reliable means of examining taste buds. Approximately 80–85% of the total number of taste buds degenerated within the first 2 wk. This is in agreement with other studies that also demonstrated a number of remaining taste buds using hematoxylin staining (43) or electron microscopy (7) in rat following unilateral CT damage. Although ~15% of fungiform papillae on the denervated side of the tongue remained keratin 19⁺, it was qualitatively noted that these immunopositive taste buds were not equivalent to taste buds on the intact side of the tongue. Far fewer immunopositive sections were present in denervated papillae (i.e., usually 4 or 5), even when the complete taste bud was scored as positive. Oakley and colleagues (31) also found that keratin 19⁺ “remnant” or “atrophic” taste buds remained after chorda-lingual nerve section. Nevertheless, there was a similar and substantial loss of keratin 19⁺ taste buds that was independent of the dietary regimen.

Functional alterations are largely specific to sodium. A wide range of nonsodium stimuli, including NH₄Cl, KCl, QHCl, and sucrose, did not elicit differences in CT responses between the sides of the tongue or between dietary manipulations. However, there was a significant decrease in HCl responses from the cut and uncut CT nerves of all experimental sodium-restricted groups compared with HCl responses from control rats. This is the first time that we observed changes in HCl responses following any dietary or surgical manipulation, and the explanation is currently unclear. Although species diversity exists (10), the consensus of studies in rat fungiform taste receptor cells is that responses to HCl are voltage and amiloride insensitive (27, 28). Thus, it is unlikely that deficits in apical ENaC underlie variations in acid responses. Moreover, in the current experiments, decreased responses to HCl were observed even in groups that did not exhibit changes in responses to sodium. Perhaps sodium restriction and/or unilateral CT section influence acid

transduction through proton channels, the Na⁺-H⁺ exchanger, or the intracellular pH of taste receptor cells (26–28, 40).

The studies presented here show that previously described (18) effects of CT cut and sodium restriction on sodium taste function in the two sides of the tongue are likely to be due to different mechanisms. The intact CT is susceptible to sodium restriction in the first week after sectioning, while the regenerated nerve is sensitive to the diet in the first 2 wk after sectioning. This work presents a narrowed time period (i.e., 2 wk post-sectioning) to focus further attention on possible mechanisms involved in changes in sodium sensitivity and eliminates an effect of sodium restriction on the number of taste buds as a candidate mechanism. Although alterations in sodium taste function were assessed by recording from the CT nerve, it is likely that the initial site of these changes is in taste receptor cells. The effects of dietary sodium restriction and CT section were largely specific to sodium, suggesting that sodium transduction through ENaC on taste receptor cells is responsible. However, we cannot rule out the possibility that additional functional alterations in the CT nerve contribute to changes in taste responses. In future work, we hope to determine mechanisms responsible for altered CT responses to sodium, including the identification of sites where the effects of CT section and sodium restriction initially take place.

Although the mechanism for dietary related differences in sodium taste function following nerve section has yet to be discovered, there is an indication that the immune system may play a role in maintaining normal sodium responses in intact taste receptor cells contralateral to denervated taste receptor cells. We showed that upregulation of immune function with systemic LPS reverses the dramatic decrease in sodium sensitivity exhibited by the intact CT shortly after contralateral denervation (33). We suggest that sodium restriction leads to abnormalities in immune-derived factors liberated by neural damage. In fact, there is evidence to suggest that macrophages are present in both the denervated and intact taste epithelium following CT sectioning, but their numbers are substantially lower in sodium-restricted compared with control rats (29). The soluble products of immune cells may then modulate changes in the number or function of ENaCs in the intact population of taste receptor cells.

Macrophages and other leukocytes secrete an array of cytokines and growth factors that are known to influence injured neural and epithelial cells (15, 32, 36). More specifically, the proinflammatory cytokine tumor necrosis factor- α increases amiloride-sensitive sodium transport in the alveolar epithelium of murine lung (9). In contrast, the anti-inflammatory cytokine transforming growth factor- β prevents aldosterone-stimulated sodium transport through ENaC in the collecting duct of rat kidney (20). LPS-stimulated macrophages (or macrophage-conditioned media) also inhibit ENaC activity and mRNA levels in rat distal lung epithelial cultures (6). Thus, leukocytes and cytokines

regulate ENaC function in nonlingual epithelial cells and may do so in taste receptor cells as well.

The basic mechanism of changes in the function of denervated taste receptor cells is also likely to involve channel function. However, the difference in the periods of sensitivity to sodium restriction indicates that processes diverge in the regenerated and intact nerves before the effects on channel function. For example, alterations in channel function induced by sodium restriction and denervation in regenerated taste receptor cells may be caused by factors similar to those that occur during developmental sodium restriction (16, 17, 19, 35, 45). A current challenge is to determine the cellular events that are responsible for this remarkable functional plasticity exhibited by both the regenerated and intact nerves as well as to investigate behavioral implications.

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