Amiloride Blocks Acid Responses in NaCl-Best Gustatory Neurons of the Hamster Solitary Nucleus

JOHN D. BOUGHTER, JR. AND DAVID V. SMITH
Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, Maryland 21201-1509

Boughter, John D., Jr. and David V. Smith. Amiloride blocks acid responses in NaCl-best gustatory neurons of the hamster solitary nucleus. J. Neurophysiol. 80: 1362–1372, 1998. Biophysical studies of isolated taste receptor cells show that one mechanism of Na⁺ salt transduction involves the inward movement of Na⁺ through amiloride-blockable ion channels on the apical receptor cell membrane, which leads to a direct depolarization. Hamster taste receptor cells with amiloride-blockable Na⁺ responses also show an amiloride-sensitive H⁺ current. Thus one mechanism for the transduction of acid taste involves the amiloride-sensitive channel. We investigated the effects of amiloride on responses to acids in neurons of the nucleus of the solitary tract (NST) of the hamster. The responses of 47 NST neurons were recorded extracellularly while the anterior tongue was stimulated with solutions representing the four taste qualities (NaCl, sucrose, HCl, quinine), which were used to characterize each cell on the basis of its best stimulus. The effects of amiloride on responses to 10 mM HCl, 10 mM citric acid, 100 mM NaCl, and 100 mM sucrose were then investigated. Stimuli were presented alone for 30 s (control trials) and also presented for 10 s, followed by a mixture of the stimulus with 10 µM amiloride for 10 s, followed by the stimulus alone again for 10 s (amiloride trials). The effects of amiloride were assessed by comparing the responses of cells with the stimulus + amiloride with that of the stimulus alone. In neurons classified as NaCl-best, amiloride reversibly blocked responses to NaCl, HCl, and citric acid. In HCl-best neurons, amiloride had no effect on responses to any of these stimuli. In sucrose-best neurons, amiloride blocked the response to NaCl but not to sucrose or to either acid. These results support the hypothesis that acids are transduced by at least two different receptor mechanisms in the hamster, amiloride sensitive and amiloride insensitive. At the NST, these inputs are tightly maintained in two separate populations of neurons. Sucrose-best neurons, which show amiloride effects on NaCl but not acids, appear to receive converging inputs from both amiloride-sensitive (N-best) and amiloride-insensitive (H-best) chorda tympani nerve fibers.

INTRODUCTION

Biophysical studies of isolated taste receptor cells and of taste buds in situ show that one mechanism of Na⁺ salt transduction involves the inward movement of Na⁺ through apical amiloride-blockable ion channels, which leads to a direct receptor cell depolarization (Avenet and Lindemann 1988, 1991). Application of amiloride to the anterior tongue in micromolar concentrations produces as much as a 60% reduction in the integrated whole chorda tympani (CT) nerve response to NaCl in rats (Brand et al. 1985; Heck et al. 1984; Ye et al. 1993) and hamsters (Herness 1987; Hettinger and Frank 1990). Furthermore, amiloride reduces the response to NaCl only in those single CT fibers classified as NaCl-best (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988), suggesting an organization of taste receptor cell inputs that may be important for the coding of Na⁺ salt taste. In the hamster nucleus of the solitary tract (NST), sucrose-best neurons are also responsive to NaCl and amiloride attenuates responses to Na⁺ salts in both NaCl-best and sucrose-best neurons but not in neurons classified as HCl- or quinine-best (Smith et al. 1996). Although both peripheral and central gustatory neurons of the hamster are broadly tuned across taste qualities (Frank et al. 1988; Smith et al. 1983a; Travers and Smith 1979), there is clearly an organization to their sensitivities.

The transduction of acids by vertebrate taste receptors was shown to involve either the blockage of apical K⁺ channels or the influx of protons through amiloride-sensitive Na⁺ channels (Gilbertson 1993; Kinnamon 1993). In the hamster, the response to citric acid in fungiform taste cells was blocked by amiloride in both in situ (Gilbertson et al. 1992) and isolated cell (Gilbertson et al. 1993) preparations. This amiloride-sensitive H⁺ current was present only in cells that also showed an amiloride-sensitive Na⁺ current, suggesting that the same channel is partially involved in the transduction of both NaCl and citric acid. Recordings from CT fibers (Frank 1973; Frank et al. 1988) or brain stem neurons (Smith et al. 1983b; Travers and Smith 1979) in the hamster demonstrate that NaCl and HCl stimulate many of the same single cells. Additionally, Hettinger and Frank (1990) demonstrated that 10 µM amiloride reduced the response to HCl in a NaCl-best hamster CT fiber. However, in the nucleus of the solitary tract (NST) of the hamster, adaptation to amiloride generally had no effect on responses to a subsequent presentation of HCl (Smith et al. 1996). Hamsters showed slightly less aversion to citric acid when it was mixed with amiloride (Gilbertson and Gilbertson 1994), suggesting that the effects of amiloride have consequences for behavior. Although H⁺ currents can be blocked by amiloride at the taste receptor cell, it is not clear how this transduction mechanism contributes to acid responses in the hamster CNS.

The purpose of this study was to examine the effects of
amiloride treatment on the responses of central gustatory neurons to acids and to determine how input from amiloride-sensitive transduction mechanisms is organized across the various gustatory neuron types in the medulla. To this end, we recorded responses from hamster NST cells to NaCl, HCl, and citric acid and assessed the effects of lingual application of amiloride on these responses. A portion of these results was presented at the joint meeting of the International Symposium on Olfaction and Taste and the Association for Chemoreception Sciences in San Diego, CA, July 1997.

**Fig. 1.** Across-neuron patterns of the responses to each of the 4 basic stimuli. Responses are adjusted for spontaneous rate, which is also shown in the figure. Neurons are grouped from left to right into best-stimulus classes and ranked within each group according to the magnitude of the response to the best stimulus. The response of any one neuron can be read vertically. Neurons 1–16 are S-best (●), 17–33 are N-best (□), 34–44 are H-best (■), and 45–47 are Q-best (○).
FIG. 2. Trains of action potentials for an individual NaCl-best neuron (A and B) and an HCl-best neuron (C and D). Distilled water was flowing across the tongue before the initial stimulus. --- ---, the time when the stimuli were switched (every 10 s) from stimulus to stimulus + amiloride, back to stimulus, and finally to water. In all records, 10 μM amiloride was applied for 10 s mixed with the stimulus during the steady-state phase of the response (i.e., after 10 s of stimulation). Amiloride reversibly suppressed the response to 0.1 M NaCl (A) and 0.01 M HCl (B) in the NaCl-best neuron. On the other hand, amiloride had no effect on the response to either NaCl (C) or HCl (D) in the HCl-best cell.

METHODS

Animals and surgery

Thirty-three young adult male hamsters, Mesocricetus auratus, weighing a mean of 164.5 ± 4.4 (SE) g, were deeply anesthetized with urethan (1.7 g/kg ip). Additional anesthetic was given as needed during the course of each experiment. The animal was tracheotomized, and the hypoglossal nerve was transected bilaterally to eliminate tongue movements. The animal was mounted in a nontraumatic head holder (Erickson 1966), and the snout was angled downward 27° from horizontal to straighten the brain stem and minimize brain movement associated with breathing (Van Buskirk and Smith 1981). Body temperature was monitored and maintained at 37°C with a heating pad. A midline incision was made through the tissue overlying the posterior skull, and part of the occipital bone just dorsal to the foramen magnum was removed to reveal the cerebellum. After the dura was removed, the posterior portion of the cerebellum was aspirated to expose the floor of the fourth ventricle for 3- to 4-mm anterior to obex.

Single-neuron recording and stimulation

Tungsten microelectrodes (0.2–2.0 MΩ) were used to record action potentials extracellularly. The mean (±SD) coordinates (in mm) for taste cell recordings were 2.13 ± 0.16 anterior and 1.36 ± 0.14 lateral to obex and between 0.5 and 1.2 ventral to the surface of the brainstem. Action potentials were differentially amplified (Grass P511), discriminated with a dual time-amplitude window discriminator (Bak DDIS-1), displayed on a storage oscilloscope, and monitored with an audio monitor. The amplified action potentials were recorded along with voice cues on VCR tape. An IBM-compatible computer, configured with a Lab Master direct memory access board (Scientific Solutions, Solon, OH) and custom software, controlled chemical stimulus delivery and on-line data acquisition and analysis.

Taste-evoked NST activity was initially identified by a change in neural activity associated with the application of anodal current pulses (40 μA, 0.5 s) to the anterior tongue. Single units were identified and isolated during stimulation of the anterior tongue with a mixture containing 100 mM sucrose, 1 mM quinine hydrochloride, 30 mM sodium chloride (NaCl), and 10 mM citric acid; this search stimulus was applied from a syringe. Stimuli were made from (Sigma Chemical, St. Louis, MO) NaCl, hydrochloric acid (HCl), citric acid, sucrose, quinine hydrochloride (QHCl), and amiloride-HCl (amiloride). The stimuli were dissolved in distilled water; for amiloride trials, the stimuli were dissolved in 10 μM amiloride. Stimuli were delivered to the anterior tongue at room temperature (21°C) by a gravity-flow system composed of two-way solenoid valves connected via Tygon tubing to a distilled water rinse reservoir and stimulus reservoirs; the flow rate was 2.6 ml/s. The animal’s tongue hung vertically over the side of the mandible and was held in place with a small serrefine clamp attached to the ventral lingual epithelium. The stimulus delivery tube was positioned directly over the dorsal anterior tongue within 2 mm of the surface. The posterior tongue region containing the foliate and vallate papillae remained in the oral cavity and was not exposed to the stimuli.
**Best-stimulus classification**

Once a unit was isolated, its taste response profile was determined by stimulation through the gravity-flow system with 100 mM sucrose, 30 mM NaCl, 3 mM HCl (pH ~2.52), and 1 mM QHCl; the order of these stimuli was random. These concentrations elicit half-maximal responses in the hamster CT nerve for these compounds (Frank 1973). The stimulation sequence for these four trials, during which the computer acquired data, was a continuous flow initiated by the delivery of 15 s of distilled water, followed by 10 s of stimulus and then by 20 s of distilled water. After each chemical stimulation, the tongue was rinsed with an additional 50 ml of distilled water, and individual stimulations were separated by ~2 min to avoid adaptation effects. Each neuron was classified as sucrose-, NaCl-, HCl-, or QHCl-best by determining which stimulus evoked the greatest net response (calculated as the number of impulses during the 1st 5 s of stimulus application minus the number of impulses during the immediately preceding 5 s of distilled water).

**Amiloride treatment**

The effects of amiloride on responses to 10 mM HCl (pH ~2), 10 mM citric acid (pH ~2.6), 100 mM NaCl, and 100 mM sucrose were subsequently investigated. Pairs of trials for each stimulus were conducted, one with amiloride (amiloride treatment) and one without (control). Both the order of stimulus presentation and the order of treatment (control or amiloride) for each stimulus were

![Peristimulus histograms showing the responses of a single N-best neuron to an ascending series of citric acid concentrations (■) with the addition of amiloride (■) 10 s after the start of citric acid stimulation. Distilled water (□) flowed immediately before and after citric acid. Magnitude of the response to citric acid increased slightly with concentration and amiloride completely and reversibly blocked each response.](http://jn.physiology.org/)

FIG. 3
randomly determined. The control trial was a continuous flow initiated by the delivery of 10 s of distilled water, followed by 30 s of stimulus, followed by 10 s of distilled water. The amiloride trial was a continuous flow initiated by the delivery of 10 s of distilled water, followed by 10 s of stimulus, followed by 10 s of stimulus + 10 μM amiloride, followed by 10 s of stimulus, followed by 10 s of distilled water. After a trial was finished, the tongue was washed with an additional 50 ml of distilled water, and trials were separated by ≥3 min to avoid adaptation effects and to ensure recovery from amiloride treatment.

Whenever possible, we attempted to assess the effect of amiloride on the responses to NaCl, HCl, and citric acid for each neuron. However, we also analyzed data from neurons for which we conducted trials on NaCl and only one of the acids. In every instance, comparisons between amiloride and control trials were made only on cells that were tested under both conditions. Additionally, the effect of amiloride on the response to sucrose was only assessed in neurons classified as sucrose-best because 100 mM sucrose did not typically evoke a response in N- or H-best neurons lasting more than 10–15 s. In a subset of neurons, we also assessed the effect of 10 μM amiloride on a two-logstep range of acid concentrations (0.3–30 mM).

**Data analysis**

The window-discriminated action potentials were converted into frequency counts (imp/s). For cell classification, the response measure was net impulses/5 s. For the amiloride trials, the effect of amiloride on the responses to NaCl, HCl, citric acid, or sucrose in a particular cell was assessed as follows. Because it typically took a few seconds for the addition of amiloride to reduce the NaCl response in NaCl-best cells, we counted the net impulses/5 s during the last 5 s of amiloride treatment. This response was compared with the net response during an equivalent time period in the control trial. An average of the number of impulses during the last 5 s of the distilled water rinse immediately preceding the control and amiloride trials was determined; this local mean spontaneous rate was subtracted from the responses for both trials to determine each net response. Mean responses under the two conditions were compared with the Student’s t-test for unpaired data.

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Peristimulus histograms showing the responses of 2 different S-best neurons to sucrose, NaCl, and acid with the addition of 10 μM amiloride 10 s after the start of each stimulus. Conventions and shading are the same as those in Fig. 3. One neuron responded strongly to 100 mM sucrose (A) and 100 mM NaCl (B) and weakly 10 mM citric acid (C). Amiloride reversibly blocked the NaCl response in this cell but did not appear to affect the response to either sucrose or citric acid. A 2nd S-best neuron responded strongly to both 100 mM sucrose (D) and 10 mM HCl (F); response to NaCl (E) was smaller. Amiloride did not affect any of the gustatory responses in this cell.
conditions were compared with the use of two-tailed paired $t$-tests (with Bonferroni correction).

**RESULTS**

**Cell classification**

Action potentials were recorded from 47 individual NST neurons. These cells were classified as responding best to sucrose (S-best; $n = 16$), NaCl (N-best; $n = 17$), HCl (H-best; $n = 11$), or QHCl (Q-best; $n = 3$) on the basis of their net responses to these stimuli. The responses of all neurons to these four stimuli are shown in Fig. 1. In this figure, the neurons are arranged left to right along the abscissa in four groups: S-best (neurons 1–16), N-best (17–33), H-best (34–44), and Q-best (45–47). Within each best-stimulus group, the units are arranged in order of the response magnitude to their best stimulus. Most units responded to more than one of these compounds. In particular, the N-best neurons had a substantial response to 3 mM HCl, and H-best units responded well to 30 mM NaCl. S-best neurons had a strong response to NaCl but responded less strongly to 3 mM HCl. Of interest are the three units classified as Q-best. In each case, the response to 1 mM QHCl was only slightly stronger than the response to 3 mM HCl. Many of the H-units responded well to QHCl. Because we recorded from so few Q-best cells, and based on the similarity in the responsiveness of these cells to that of H-best units (including their lack of amiloride sensitivity), we combined the H-best and Q-best units when analyzing the effects of amiloride treatment. Also plotted at the bottom graph of Fig. 1 is the spontaneous rate (during the last 5 s of the prestimulus water rinse) for each neuron. The N-best neurons had a significantly higher mean ($\pm$SE) spontaneous discharge ($34.0 \pm 5.9$ imp/5 s) than S-best cells ($12.8 \pm 2.6$; 2-tailed independent $t$-test, $t = 3.269$, df = 31, $P < 0.01$).

**Effects of amiloride on NaCl and acid responses in individual neurons**

Traces of action potentials from a representative N-best (Fig. 2, A and B) and a typical H-best (Fig. 2, C and D) neuron are shown in Fig. 2. This N-best neuron was spontaneously active and responded vigorously when 100 mM NaCl was applied (Fig. 2A). When 10 $\mu$M amiloride was added after 10 s of NaCl stimulation (1st $\square$), the evoked response was completely blocked. The inhibitory effect of amiloride was reversed within a few seconds after reapplica-

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**FIG. 5.** Mean peristimulus histograms for N-best neurons. Seventeen neurons were tested with 100 mM NaCl alone (A) and with amiloride added during the response (B). Conventions and shading are the same as those in Fig. 3, except that error bars show $+1$ SE and the histograms on the left (A, C, and E) represent responses during an uninterrupted stimulus flow (without amiloride). Amiloride completely and reversibly blocked the response to NaCl in these cells (B). A subset of cells was also tested with 10 mM HCl (C and D; $n = 13$) and 10 mM citric acid (E and F; $n = 14$). Amiloride addition strongly attenuated the response to acids in these N-best cells. See Fig. 9 for a statistical summary of these effects.
tion of NaCl (2nd − − −). This cell responded less strongly to 10 mM HCl (Fig. 2B), but the HCl-evoked response was also reversibly reduced by amiloride. This cell and others showed a sustained response after HCl when water was flowing over the tongue (after 3rd − − −). Such afterdischarges are common after acid stimulation in this species (Van Buskirk and Smith 1981). The H-best neuron had less spontaneous activity than the N-best neuron; both 100 mM NaCl (Fig. 2C) and 10 mM HCl (Fig. 2D) provoked a strong response. However, amiloride had no effect on the response to either stimulus in this H-best cell.

Figure 3 shows peristimulus histograms of the responses of a single N-best neuron to an ascending series (0.3–30 mM; pH 3.3–2.3) of citric acid concentrations with the addition of 10 μM amiloride. Citric acid flowed during the 6- to 35-s period (■), with amiloride added during the 16- to 25-s interval (■■). This cell responded well to citric acid; slightly larger responses were evoked with increasing concentration. At each concentration, addition of 10 μM amiloride (■■) effectively blocked the citric acid response in a reversible manner. The response at the end of the 10-s amiloride treatment was at or below the spontaneous activity of the cell before citric acid stimulation (□). In contrast, the addition of amiloride did not reduce the response to citric acid in H-best neurons at any concentration.

Responses to NaCl and the acids were more varied in S-best neurons than those in N- and H-best cells. Some units had a stronger NaCl response, whereas others responded more vigorously to acid than to NaCl. This was not especially apparent when stimulating with the set of basic compounds (including 30 mM NaCl and 3 mM HCl; see Fig. 1), but 30 mM HCl or citric acid activated some of the S-best cells strongly compared with 100 mM NaCl. Peristimulus histograms from two S-best neurons are shown in Fig. 4. Both neurons responded strongly to 100 mM sucrose, and the addition of amiloride had no effect on this response (Fig. 4A and D). NaCl (100 mM) elicited a strong response in one cell, and this response was reversibly blocked by amiloride (Fig. 4B). The response of this cell to 10 mM citric acid was much smaller, but amiloride had no effect on this response (Fig. 4C). The other cell classified as S-best had a relatively small response to 100 mM NaCl (Fig. 4E), but responded strongly to 10 mM HCl (Fig. 4F). Amiloride did not reduce the response to either compound, although the
Mean responses of each neuron type

Mean peristimulus histograms (+SE) for NaCl-best neurons responding to 100 mM NaCl, 10 mM HCl, and 10 mM citric acid are shown in Fig. 5. Seventeen NaCl-best neurons were tested with NaCl alone for 30 s (Fig. 5A, ■) and with the addition of 10 μM amiloride after 10 s of NaCl stimulation (Fig. 5B, □). In this and all other figures, the responses with and without amiloride were always tested on the same cells. Amiloride reversibly blocked NaCl responses in all N-best neurons relative to control trials. Subsets of these NaCl-best cells were also tested with 10 mM HCl (n = 13) and 10 mM citric acid (n = 14). Acids elicited a smaller response in these neurons relative to NaCl (Fig. 5, C and E). Amiloride reversibly reduced the responses to both HCl (Fig. 5D) and citric acid (Fig. 5F) in all N-best neurons.

Responses of 11 H-best and 3 Q-best neurons were combined to create the peristimulus histograms in Fig. 6. Thirteen of these cells were tested with 100 mM NaCl, which produced a small response in these neurons (Fig. 6A). Twelve cells were tested with 10 mM HCl (Fig. 6C), and 13 were tested with 10 mM citric acid (Fig. 6E), which produced larger responses than NaCl. Amiloride did not reduce the response to NaCl (Fig. 6B) or to either acid (Fig. 6, D and F) in any H- or Q-best cell.

Figure 7 contains mean peristimulus histograms for S-best neurons responding to 100 mM sucrose, 100 mM NaCl, 10 mM HCl, and 10 mM citric acid. In 15 S-best cells, amiloride reduced the response to NaCl relative to the NaCl response alone (Fig. 7, C and D). Amiloride did not affect the response to sucrose (n = 15; Fig. 7, A and B). Subsets of S-best cells were tested with HCl (n = 9) and citric acid (n = 10). Acids elicited sustained activity in these cells (Fig. 7, E and G), but amiloride had no effect on these responses (Fig. 7, F and H).

**Fig. 7.** Mean peristimulus histograms for S-best neurons. Fifteen S-best neurons were tested with sucrose alone (A) or with the addition of amiloride (B). Conventions and shading are the same as in Fig. 6. Amiloride did not affect the response to sucrose in these cells. Fifteen S-best neurons were also tested with NaCl alone (C) or with the addition of amiloride (D). Amiloride treatment significantly reduced the response to NaCl in these cells. Subsets of S-best cells were tested with HCl (E, n = 9) and citric acid (G, n = 10). In these cells the acid responses were unaffected by amiloride treatment (F and H). See Fig. 9 for a statistical summary of these effects.
These effects of amiloride are summarized for each cell type in Fig. 8. For each stimulus, the mean response during the last 5 s of amiloride treatment was compared with the equivalent 5-s period in the control trial. In neurons classified as N-best (Fig. 8A), amiloride produced a complete block of the NaCl response (2-tailed paired t-test, t = 7.08, df = 16, P < 0.0001). For HCl, the amiloride effect was not a complete block, but the reduction was large and significant (t = 3.77; df = 12, P < 0.01). Amiloride also produced a significant decrease in the response to citric acid (t = 3.04; df = 13, P < 0.01). Unlike N-units, NaCl and acid responses in H- and Q-best neurons were completely unaffected by amiloride (t < 1.2; P > 0.2; Fig. 8B). In S-best neurons (Fig. 8C) the NaCl response was significantly reduced by amiloride (t = 4.29; df = 14, P < 0.001). However, responses to sucrose (t = 0.35; df = 14, P > 0.5), HCl (t = 1.66; df = 8, P > 0.1), and citric acid (t = 0.24; df = 9, P > 0.5) were unaffected.

**DISCUSSION**

In this study, we investigated the effects of lingual application of the passive Na\(^+\) channel blocker amiloride on responses to acids and NaCl in gustatory neurons in the hamster NST. When added during an ongoing response to NaCl, HCl, or citric acid, 10 \(\mu\)M amiloride reversibly blocked the response to these stimuli in neurons that responded best to NaCl (N-best). Amiloride treatment did not affect the responses to any of these stimuli in H- or Q-best neurons. Amiloride reduced the NaCl response in S-best neurons; however, the responses of these cells to sucrose, HCl, and citric acid were unaffected. Thus in a subset of NST gustatory neurons, amiloride clearly reduces the responses to both NaCl and the two acids. These data reflect the contribution of at least two different mechanisms for the transduction of acids in this species.

The effects of amiloride on the responses of hamster NST neurons is expected from what is known about the effects of this Na\(^+\) channel blocker on hamster taste receptor cells. In recordings from isolated receptor cells (Gilbertson et al. 1993) or from taste receptors in situ (Gilbertson et al. 1992), cells with amiloride-blockable Na\(^+\) currents were also shown to have amiloride-blockable H\(^+\) currents. Because these H\(^+\) currents are only seen in conjunction with amiloride-sensitive Na\(^+\) currents and because previous investigators showed that amiloride-sensitive NaCl responses are restricted to N-best CT fibers in both rats (Ninomiya and Funakoshi 1988) and hamsters (Hettinger and Frank 1990), it is not surprising that N-best cells of the hamster NST show amiloride-sensitive responses to HCl and citric acid. The responses to both NaCl and the two acids in H- and Q-best cells are not amiloride sensitive, suggesting that the receptor mechanisms providing input to these neuron types are segregated from the amiloride-sensitive apical ion channel. Little is known about the transduction mechanisms for acids in mammalian taste cells, although there is some evidence that an apical K\(^+\) conductance is involved in the transduction of acids by mudpuppy taste cells (Kinnamon 1993; Kinnamon et al. 1988). On the other hand, Na\(^+\) salt transduction in mammals appears to involve both the apical amiloride-sensitive channel and a paracellular transduction pathway that produces an anion-dependent (but amiloride-insensitive) response to Na\(^+\) (Elliott and Simon 1990; Harper 1987; Ye et al. 1991, 1993) and other cations (Kloub et al. 1997; Ye et al. 1994). These data suggest strongly that taste receptor cells with amiloride-sensitive transduction mechanisms (for both Na\(^+\) and H\(^+\)) provide input to N-best cells of the gustatory NST, whereas the H- and Q-best neurons receive input from taste receptors lacking amiloride-sensitive mechanisms. These latter cells could transduce Na\(^+\) and H\(^+\) responses by paracellular mechanisms and/or by yet-unidentified apical cation channels. The majority of the information about acids arises from other than the amiloride-sensitive pathway (see Figs. 1 and 6). The minor contribution of this transduction mecha-

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**Fig. 8.** Mean responses (net imp/5 s) to stimuli and stimuli + amiloride for each gustatory neuron type. N = 100 mM NaCl, H = 10 mM HCl, C = 10 mM citric acid, and S = 100 mM sucrose. For N-best units (A), n = 17 for NaCl, n = 13 for HCl, and n = 14 for citric acid. For H- and Q-best units (B), n = 13 for NaCl, n = 12 for HCl, and n = 13 for citric acid. For S-best units (C), n = 15 for NaCl, n = 9 for HCl, n = 10 for citric acid, and n = 15 for sucrose. Error bars show +1 SE; asterisks indicate a significant difference in the means of the stimulus alone vs. the stimulus mixed with amiloride (Bonferroni t, P < 0.01; see text for details).
nism to the coding of acid taste is compatible with the small effect of amiloride on the hamster’s behavioral response to citric acid (Gilbertson and Gilbertson 1994).

One interesting result of this study was the discrepancy between the effects of amiloride on the responses to acids and NaCl in S-best neurons. As previously shown for this species (Smith et al. 1996), amiloride significantly reduced the response to NaCl in S-best cells (Figs. 7 and 8). However, the striking suppression of the responses to acids by amiloride that was evident in N-best neurons (Figs. 5 and 8) did not occur in S-best neurons (Figs. 7 and 8). This distribution of amiloride sensitivity suggests a pattern of convergence of CT fibers onto NST neurons, as depicted schematically in Fig. 9. Fibers of the hamster CT nerve are more narrowly tuned to the four basic stimuli than are NST cells of this species (Travers and Smith 1979). The increased breadth of tuning of NST neurons probably occurs through convergence of peripheral fibers onto these cells, which was shown directly in a number of mammalian species (Sweazey and Smith 1987; Travers et al. 1986; Vogt and Mistretta 1990). The patterns of amiloride sensitivity in hamster NST cells suggest that the amiloride-sensitive transduction mechanism for Na\(^+\) and H\(^+\) projects, via N-best CT fibers, to both N- and S-best NST neurons, although the projection to N-best neurons is clearly the greater (Fig. 9).

The amiloride-insensitive transduction mechanism for H\(^+\) and Na\(^+\) projects via H-best CT fibers to H- and S-best NST neurons. Input from sucrose-sensitive receptor mechanisms travels to the NST predominantly in S-best CT fibers, although some of this input is reflected in the slight sucrose sensitivity of N- and H-best fibers as well (Frank et al. 1988). It is currently unknown whether the responses of S-best CT fibers to either NaCl or acids are amiloride sensitive; these responses are typically quite small (Frank 1973; Frank et al. 1988). Because the responses of S-best NST neurons to acid are not reduced by amiloride, the great majority of the information provided to these cells about acids must arise from input from H-best CT fibers. Conversely, the amiloride-sensitive NaCl responses of the S-best NST cells must arrive primarily via the N-best CT fibers. Interestingly, it is the S-best cells that show the greatest increase in breadth of tuning between the CT nerve and the NST (Travers and Smith 1979; Van Buskirk and Smith 1981).

These data show that the organization of taste sensitivity in NST neurons reflects a remarkable amount of specificity; that is, input from a particular transduction mechanism—the amiloride-sensitive channel—is specifically funneled into two NST cell types and completely restricted from two others. Previous studies in the rat also showed that the responses to Na\(^+\) and Li\(^+\) salts are reduced by amiloride only in cells that are more responsive to NaCl than to HCl (Giza and Scott 1991; Scott and Giza 1990). Such exquisite specificity is not likely to be accidental. The amiloride-sensitive input appears to be critical for the neural and behavioral discrimination of NaCl and other Na\(^+\) and Li\(^+\) salts from non-Na\(^+\) salts and acids. After amiloride treatment, rats cannot make a behavioral discrimination between NaCl and KCl (Spector et al. 1996), and the information contained in the across-neuron patterns within the rat NST is insufficient to discriminate Na\(^+\) from non-Na\(^+\) salts (Giza and Scott 1991; Scott and Giza 1990). In addition, when rats are conditioned to avoid NaCl in the presence of amiloride, they generalize that aversion to KCl (Hill et al. 1990). Similar data are not available for the hamster. In spite of the striking specificity of the distribution of receptor inputs to NST neurons, however, the same level of specificity does not extend to stimulus coding. Both NaCl and acid responses are blocked by amiloride and the effects of amiloride on the response to NaCl are seen in two different neuron types (N- and S-best). This organization suggests that any one neuron type alone or any particular transduction mechanism alone is not sufficient to provide for the discrimination among different tasting stimuli. For an animal to discriminate NaCl from KCl, differential activity must be generated across the afferent neurons. When the response to NaCl is blocked in N-best (and S-best) neurons, the remaining neurons (H- and Q-best) cannot provide for the NaCl/KCl discrimination because these salts are not differentiated by these neurons. Such discrimination must ultimately depend on the relative activity in different neuron types (Erickson 1968, 1982; Pfaffmann 1959; Smith 1985; Smith and Frank 1993; Smith et al. 1983a).

This work was supported in part by National Institute of Deafness and Other Communication Disorders Grant DC-00353-13 to D. V. Smith.

Address for reprint requests: J. D. Boughter, Dept. of Anatomy and Neurobiology, University of Maryland School of Medicine, 685 West Baltimore St., Baltimore, MD 21201-1509.

Received 4 March 1998; accepted in final form 3 June 1998.

FIG. 9. Schematic diagram showing the pattern of convergence onto cells of the nucleus of the solitary tract (NST) from chorda tympani (CT) nerve fibers receiving input from three distinct receptor mechanisms. N-best NST neurons receive information about Na\(^+\) and, to a much lesser extent, H\(^+\) stimuli from amiloride-sensitive transduction pathways via N-best CT fibers, which also impart amiloride-sensitive responses (predominantly to NaCl) to S-best NST neurons. H-best NST neurons receive information about H\(^+\) and, to a much lesser extent, Na\(^+\) stimuli from amiloride-insensitive transduction pathways via H-best CT fibers, which also impart amiloride-insensitive responses (predominantly to acids) to S-best NST neurons. S-best CT fibers project primarily to S-best NST neurons, with some contribution to N- and H-best cells. The S-best NST cells are significantly more broadly tuned than S-best CT fibers (Travers and Smith 1979; Van Buskirk and Smith 1981). There is likely to be some convergence between taste receptor cells and CT fibers (see), although the organization of sensitivities among receptor cells is not well understood.
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