Neural Representation of Salts in the Rat Solitary Nucleus: Brain Stem Correlates of Taste Discrimination

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Received 4 November 1999; accepted in final form 19 April 2000

St. John, Steven J. and David V. Smith. Neural representation of salts in the rat solitary nucleus: brain stem correlates of taste discrimination. *J Neurophysiol* 84: 628–638, 2000. One mechanism of salt taste transduction by gustatory receptor cells involves the influx of cations through epithelial sodium channels that can be blocked by oral application of amiloride. A second mechanism is less clearly defined but seems to depend on electroneutral diffusion of the salt through the tight junctions between receptor cells; this paracellular pathway is insensitive to amiloride. Because the first mechanism is more sensitive to sodium salts and the second to nonsodium salts, these peripheral events could underlie the ability of rats to discriminate sodium from nonsodium salts on the basis of taste. Behavioral experiments indicate that amiloride, at concentrations that are tasteless to rats, impairs a rat's ability to discriminate NaCl from KCl and may do so by making both salts taste like KCl. In the present study, we examined the neural representation of NaCl and KCl (0.05–0.2 M), and mixtures of these salts with amiloride (0, 3, and 30 μM), to explore the neural correlates of this behavioral result. NaCl and KCl were represented by distinct patterns of activity in the nucleus of the solitary tract. Amiloride, in a concentration-dependent manner, changed the pattern for NaCl to one more characteristic of KCl, primarily by reducing activity in neurons responding best to NaCl and sucrose. The effect of amiloride concentration on the response to 0.1 M NaCl in NaCl-best neurons was virtually identical to its effect on behavioral discrimination performance. Modeling the effects of blocking the amiloride-insensitive pathway also resulted in highly similar patterns of activity for NaCl and KCl. These results suggest that activity in both the amiloride-sensitive and -insensitive pathways is required for the behavioral discrimination between NaCl and KCl. In the context of published behavioral data, the present results suggest that amiloride-sensitive activity alone is not sufficient to impart a unique signal for the taste of sodium salts.

INTRODUCTION

Two transduction mechanisms have been described for sodium salts: an amiloride-sensitive and -insensitive pathway (see Herness and Gilbertson 1999; Lindemann 1996; Stewart et al. 1997). Amiloride-sensitive transduction occurs when sodium enters taste receptor cells directly via apical epithelial sodium channels similar to those found in kidney and colon (Benos et al. 1996). The influx of sodium can be competitively inhibited by the lingual application of the diuretic drug amiloride (DeSimone and Ferrell 1985). Amiloride-insensitive transduction, in contrast, is believed to begin with electroneutral diffusion of the salt across the tight junctions between taste receptor cells and sodium entry into the cells via unspecified basolateral ion channels (DeSimone and Ferrell 1985; Elliott and Simon 1990; Ye et al. 1993). This paracellular pathway is not blocked by mucosal application of amiloride. Sodium salts with large anions, such as sodium gluconate, have less access to the paracellular pathway, making their evoked responses smaller and more amiloride sensitive than the response to NaCl (Elliott and Simon 1990; Formaker and Hill 1988; Ye et al. 1991).

Taste responses evoked by nonsodium salts, such as KCl or NH_4Cl, are predominantly insensitive to amiloride treatment, although there is evidence in both rats (Minear et al. 1996) and hamsters (Boughter et al. 1999) that amiloride reduces the response to KCl. In rodent taste tissue, however, the amiloride-sensitive channel is far more permeable to Na^+ than K^+ (Brand et al. 1985; Heck et al. 1984; Herness 1987; Ninomiya and Funakoshi 1988). Nonsodium salts are thought to stimulate taste receptors predominantly through the paracellular pathway (Kloub et al. 1997; Stewart et al. 1997), although the cellular mechanisms of this transduction pathway are not completely known. In behavioral generalization studies, nonsodium salts and acids are categorized similarly by both rats and hamsters (Nowlis et al. 1980; Smith et al. 1979) and tend to generate similar patterns of activity across gustatory afferent neurons (Perrotto and Scott 1976; Smith et al. 1983); both are perceptually and neurally distinct from sodium salts (Erickson 1963; Krieckhaus and Wolf 1968; Morrison 1967; Nowlis et al. 1980).

Input from the amiloride-sensitive and -insensitive transduction mechanisms remains largely segregated in fibers of the chorda tympani (CT) nerve (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988) and in cells of the CNS of rodents (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996). If amiloride is added to an ongoing response to NaCl (N), the response of N-best neurons in the hamster CT nerve (Hettinger and Frank 1990) and in the nucleus of the solitary tract (NST) (Boughter and Smith 1998; Boughter et al. 1999) is suppressed to prestimulus levels. These data suggest that the sodium responses of N-best cells are completely amiloride sensitive. Conversely, there is absolutely no effect of amiloride on the

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responses of HCl (H)-best CT fibers or NST neurons (Boughter and Smith 1998; Boughter et al. 1999; Hettinger and Frank 1990). The response to NaCl is partially reduced in sucrose (S)-best NST neurons of the hamster (Boughter and Smith 1998; Boughter et al. 1999). Because these two receptor mechanisms are differentially sensitive to sodium and nonsodium salts and because their inputs remain segregated in the CNS, the relative activation of these two pathways could underlie the ability of rodents to discriminate among salts.

Direct behavioral support for the involvement of the amiloride-sensitive pathway in salt discrimination was recently presented (Spector et al. 1996; see also Hill et al. 1990). Water-restricted rats were trained to lick a drinking spout to obtain NaCl or KCl (0.05, 0.1, or 0.2 M) and to press one of two levers associated with each stimulus (Spector et al. 1996). Discrimination performance (the percentage of correct lever responses) was then measured over sessions when amiloride (1, 3, 10, 30, or 100 μM) was mixed with the salt solutions. Amiloride at the two highest doses completely prevented rats from discriminating NaCl from KCl. Because amiloride is tasteless to rats at 100 μM (Markison and Spector 1995), Spector et al. (1996) concluded that amiloride reduced discrimination performance by virtue of its selective effect on the amiloride-sensitive transduction pathway.

The present study was conducted to examine the relative contribution of these two transduction pathways to salt discrimination. We recorded responses to NaCl and KCl from cells in the NST, the first central synapse for gustatory information, to determine the central neural representation of the taste of these salts after blocking the amiloride-sensitive or -insensitive pathway. We used the same concentrations of salts and amiloride as in the aforementioned behavioral work (Spector et al. 1996). In addition, given our knowledge of the relative distribution of these two transduction inputs to different cell types, we modeled the neural response patterns that would occur if we had the appropriate agent to block the amiloride-insensitive transduction pathway. The present experiment suggests that activity in both the amiloride-sensitive and -insensitive pathways subserves the behavioral discrimination between NaCl and KCl.

A portion of these results was presented at the 1999 meeting of the Association for Chemoreception Sciences, Sarasota, FL.

METHODS

Subjects and recording

Twenty-nine male Sprague Dawley rats, weighing 210–574 g, were deeply anesthetized with urethan (1.7 g/kg ip) and prepared for electrophysiological recording. Supplemental injections of anesthesia were occasionally given as necessary. Following bilateral hypoglossal neurectomy, the rat was tracheotomized and secured in a nontraumatic headholder that deflected the head downward at a 27° angle. These preparations served to minimize brain stem movements associated with breathing. The brain stem was exposed for recording by removal of a portion of the occipital bone and aspiration of the posterior part of the cerebellum. Throughout the procedure, body temperature was monitored and maintained at 37 ± 1°C with a heating pad.

Extracellular recordings from single neurons in the gustatory zone of the NST were made using tungsten microelectrodes (0.4–0.8 MΩ). The search for taste-responsive neurons typically began 2.8 mm anterior to obex, 1.8 mm lateral to the midline, and approximately 1 mm ventral to the surface of the brain stem. Initially taste-responsive neurons were identified by a change in neural activity evoked by anodal current pulses (40 μA, 0.5 s) applied to the anterior tongue and then confirmed with chemical stimulation of the tongue. Action potentials were 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 digital PCM-VCR tape. An IBM-compatible computer configured with a Lab Master DMA board (Scientific Solutions; Solon OH) and custom software controlled chemical stimulus delivery and on-line data acquisition and analysis.

Stimulus protocol

Once a single neuron was isolated, it was tested with three groups of stimuli: *group A*, basic stimuli; *group B*, a standard array of 18 salt-amiloride mixtures, and *group C*, an array of six 1.0 M salt-amiloride mixtures (Table 1). The three groups of stimuli were always tested sequentially, but presentation within these groups was random for any given neuron. If a neuron did not remain viable throughout the testing of *group A* and *B* stimuli, it was excluded from analysis. Thus every cell was tested with *group A* and *B* stimuli, whereas a subset was tested also with *group C*.

Tastants were delivered at room temperature to the anterior tongue via gravity flow at a rate of 2.6 ml/s. During a trial, the tongue was first rinsed with distilled water for at least 15 s (the last 5 s of which were used to determine the cell's spontaneous rate), followed immediately by the taste stimulus for 10 s. The tongue was then rinsed with at least 50 ml of distilled water and greater than 2 min were allowed to elapse between trials to preclude adaptation effects from confounding interpretation of neuronal responses (Smith et al. 1975, 1978). To more fully characterize the concentration-dependent effects of amiloride on single NST cells, five N-best neurons were tested for their responses to 0.1 M NaCl mixed with all five amiloride concentrations used in the Spector et al. (1996) experiment: 1, 3, 10, 30, and 100 μM.

Whereas amiloride added to the stimuli would be effective in blocking the amiloride-sensitive pathway, there is currently no comparable drug known that will effect a reversible block of the paracellular pathway. Although lanthanum chloride will presumably block the tight junctions (Holland et al. 1989), it has nonspecific effects on both amiloride-sensitive channels and calcium channels, and its effects on taste responses are varied and not readily reversible (unpublished observations). However, since we know the relative contribution of the amiloride-sensitive and -insensitive pathways to the various neuron types from our previous work on the hamster NST (Boughter and Smith 1998; Boughter et al. 1999), which are paralleled in the effects of amiloride on rat NST cells in the current data, we are

TABLE 1. *Taste stimuli used*

Group A (basic stimuli)
0.5 M sucrose
0.1 M sodium chloride (NaCl)*
0.01 M hydrochloric acid (HCl)
0.02 M quinine hydrochloride (QHCl)
Group B (salt-amiloride mixtures)†
0.05 M NaCl
0.1 M NaCl*
0.2 M NaCl
0.05 M potassium chloride (KCl)
0.1 M KCl
0.2 M KCl
Group C (1 M salt-amiloride mixtures)†
1.0 M NaCl
1.0 M KCl

* Every neuron was tested twice with 0.1 M NaCl; once with *group A* stimuli and once with *group B* stimuli. † Each salt was mixed with 0, 3, and 30 μM amiloride hydrochloride.

able to model the effects of such a blocker. For this modeling, we assume that the hypothetical blocker of the amiloride-insensitive pathway produces the same effect on H-best cells that amiloride does on N-best cells (i.e., a 75.5% reduction of the response to NaCl with the mixture protocol used in the current study), whereas it has half that effect (37.75%) on S-best cells. This assumption is based on data showing that responses to NaCl and KCl are completely unaffected by amiloride in H-best NST cells and that about half or less of the response to these stimuli is blocked by amiloride in S-best neurons. Although the effect of amiloride is less when it is applied before or mixed with NaCl (Scott and Giza 1990; Smith et al. 1996), as in the present data, the complete inhibition that occurs when amiloride is added to an ongoing NaCl response (Boughter and Smith 1998; Boughter et al. 1999; Hettinger and Frank 1990) suggests that all of the input to N-best cells arises from the amiloride-sensitive transduction pathway. In addition, NaCl and KCl produce comparable responses in the non-N-best neurons (Boughter et al. 1999) (see also RESULTS), suggesting a common mechanism. We assume that the amiloride-insensitive mechanism is the paracellular pathway described by Ye et al. (1993), although there could be other, yet unknown, transduction mechanisms for these salts. In summary, for this hypothetical treatment we reduced the responses to both NaCl and KCl in H-best neurons by 75.5% (as in the mixture protocol with amiloride, see RESULTS) and in S-best neurons by 37.75%, mimicking the effect of a specific blocker of the amiloride-insensitive pathway.

Data analysis

The window-discriminated action potentials were converted into frequency counts. Net responses were derived by subtracting the number of impulses to water alone during the last 5 s prior to stimulus onset (multiplied by 2) from the frequency count during 10 s of taste stimulation.

For some analyses, neurons were classified into best-stimulus categories based on which of the *group A* (basic) stimuli evoked the greatest net response (Frank 1973). A two-way ANOVA (salt concentration × amiloride concentration) was conducted separately for NaCl and KCl to determine whether the responses of neurons responding best to NaCl (N-best), sucrose (S-best), or HCl (H-best) were significantly modified by salt or amiloride concentration. The breadth of tuning of cells in each best-stimulus category was measured using the formula for entropy introduced by Smith and Travers (1979). Entropy, which ranges from 0 (i.e., a neuron responding exclusively to 1 of the 4 stimuli) to 1 (a neuron responding equally to all 4) was determined using the four *group A* stimuli.

To examine the neural representation of the *group A* and *B* stimuli, across-neuron patterns were derived. Two measures were used to quantify the similarity of across-neuron patterns for pairs of stimuli: across-neuron correlations (Erickson 1963) and neural mass differences (NMDs) (Erickson 1986; Gill and Erickson 1985). Across-neuron correlations were Pearson product-moment correlations between the 10-s net firing rates to stimulus pairs across all neurons. Across-neuron correlations have been shown to be high between stimuli that animals treat as qualitatively similar and low between stimuli that can be behaviorally discriminated on the basis of taste quality (Erickson 1963; Smith et al. 1979).

The NMD is the sum of the differences in firing rates (over 10 s) to two stimuli across all neurons. NMDs are high between easily discriminated stimuli and low between stimuli that are behaviorally similar to one another (Dahl et al. 1997; Gill and Erickson 1985). The NMD may be a more reliable metric than across-neuron correlations when low levels of activity are generated (e.g., as with 30 μM amiloride), because low correlations resulting from low response levels can sometimes give a misleading implication of high discriminability (Erickson 1986; Gill and Erickson 1985). However, the NMD is not merely a reflection of qualitative differences between stimuli. Large NMDs can be generated between stimuli of different qualities or between stimuli of the same quality at different intensities.

Each measure has its strengths and weaknesses. The across-neuron correlation is thought to be an index of qualitative differences between taste stimuli, whereas the NMD reflects differences in either quality or intensity. Because both quality and intensity may be relevant in the behavioral discrimination task being modeled, both measures are presented. Both measures were also used in multidimensional scaling routines (SPSS for Windows, v. 9.0; SPSS, Chicago IL), which produced stimulus spaces representing the relative differences in the across-neuron patterns among the *group A* and *B* stimuli.

For five cells tested with the wider array of amiloride concentrations, the concentration-response data for amiloride's effects on 0.1 M NaCl were compared with the corresponding behavioral data from Spector et al. (1996). For the electrophysiological measure, a sigmoidal curve was fit to the average percent response of the cells (net spikes in 10 s) when NaCl was mixed with amiloride relative to the no amiloride condition. The same function was applied to the behavioral data, where the "response" was taken as overall performance (percent of correct responses) during behavioral sessions with amiloride relative to the no amiloride condition. The sigmoidal function was

$$f(x) = ((100 - d)/(1 + (x/c)^b)) + d$$

where d is the asymptotic minimum response, c is the concentration of amiloride that produced a half-maximal decrease in response, and b is the slope.

RESULTS

Group A stimuli

Action potentials were recorded from 37 NST neurons in response to both *group A* and *B* stimuli (see Table 1). Based on the response to *group A* stimuli, the neurons were classified as 21 N-best, 7 S-best, and 9 H-best; none of the cells responded best to 0.02 M QHCl. Figure 1 shows representative responses from an H-best neuron (Fig. 1*A*) and an N-best neuron (Fig. 1*B*) to the *group A* stimuli and two *group B* stimuli (0.1 M NaCl +30 μM amiloride and 0.1 M KCl). As previously shown (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996), N-best NST neurons are characterized by amiloride sensitivity, whereas H-best neurons are predominantly amiloride insensitive. That is, adding 30 μM amiloride reduced the response to NaCl in the N-best neuron (*B*), but not in the H-best cell (*A*). In general, all neurons were quite broadly tuned to the *group A* stimuli (Fig. 1*C*), with mean entropy of 0.80, 0.83, and 0.86 for S-, N-, and H-best neurons, respectively.

Group B stimuli

Concentration-response functions for NaCl and KCl demonstrate that N-best neurons are far more responsive to NaCl (Fig. 2*A*) than KCl (Fig. 2*B*). Two separate ANOVAs (salt concentration × amiloride concentration) confirmed that, in N-best neurons, NaCl responses were significantly modified by both NaCl concentration ($F[2,40] = 57.2$, $P < 0.001$) and amiloride concentration ($F[2,40] = 68.9$, $P < 0.001$) and that KCl responses were affected by KCl concentration ($F[2,40] = 43.8$, $P < 0.001$) and amiloride concentration ($F[2,40] = 48.8$, $P < 0.001$). Preplanned contrasts indicated that all levels of amiloride and salt concentration differed significantly from one another, except that 3 and 30 μM amiloride did not differentially suppress responses to KCl.

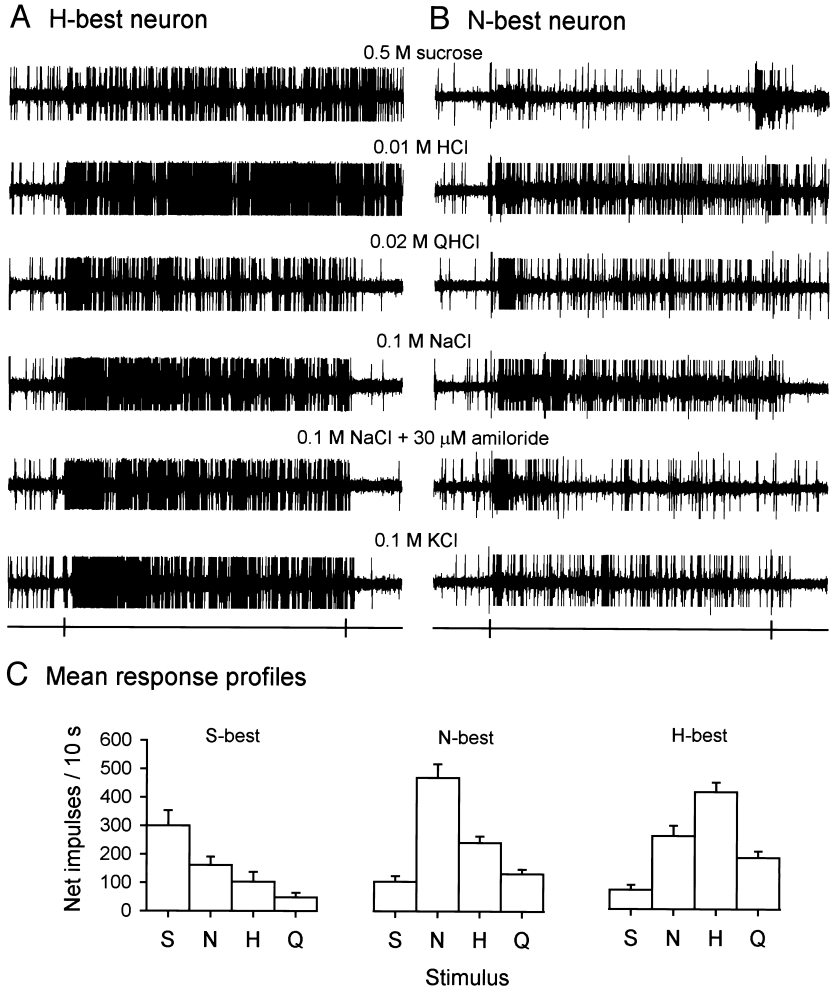

FIG. 1. Trains of action potentials for individual neurons in the rat nucleus of the solitary tract (NST) that responded best to HCl (*A*) or NaCl (*B*). The beginning and end of the 10-s stimulus presentation is indicated by the line below the records. Both neurons were broadly tuned; the responses of these 2 cells are characteristic of HCl (H)-best and NaCl (N)-best cells. H-best cells were about equally responsive to isomolar NaCl and KCl, and their responses were not substantially suppressed by amiloride (*bottom 3 traces, A*). N-best cells, like the one shown, responded much better to NaCl than KCl and were dramatically suppressed by amiloride treatment (*bottom 3 traces, B*). Also shown (*C*) are mean + SE response profiles of the 3 neuron types: sucrose-best (S-best; $n = 7$), N-best ($n = 21$), and H-best ($n = 9$), defined by their responses to the *group A* stimuli (0.5 M sucrose, S; 0.1 M NaCl, N; 0.01 M HCl, H; 0.02 M QHCl, Q). Action potentials were summed over 10 s of stimulation and counts were adjusted for spontaneous rate.

In contrast, H-best neurons were approximately equally responsive to isomolar concentrations of NaCl (Fig. 2*C*) and KCl (Fig. 2*D*). The responses of H-best neurons were affected by NaCl concentration ($F[2,16] = 25.8$, $P < 0.001$) and slightly by amiloride concentration ($F[2,16] = 5.0$, $P = 0.021$). Preplanned contrasts revealed that 3 μM amiloride differed significantly from 0 μM ($F[1,8] = 5.5$, $P = 0.042$), but there was no difference in the effects of 3 and 30 μM amiloride. It is clear, however, that the effects of amiloride were far less dramatic on NaCl responses in H-best cells than in N-best cells (Fig. 2, *A* and *C*; Table 2). H-best neurons were also modified by KCl concentration ($F[2,16] = 16.8$, $P < 0.001$), but KCl responses were unaffected by amiloride ($F[2,16] = 0.4$, $P = 0.69$) in these cells.

Salts were not strong stimuli for S-best neurons (Fig. 2, *E* and *F*); nevertheless, this neuron type responded to both NaCl ($F[2,12] = 21.0$, $P < 0.001$) and KCl ($F[2,12] = 14.6$, $P = 0.001$) in a concentration-dependent manner. Amiloride significantly reduced both NaCl responses ($F[2,12] = 12.6$, $P = 0.001$) and KCl responses ($F[2,12] = 7.8$, $P = 0.007$) in S-best cells. All levels of salt concentration and amiloride concentration differed significantly from one another.

In summary, N-best neurons, unlike H- and S-best neurons, are driven by NaCl to a much greater degree than by KCl. Amiloride reduced NaCl responses in all three neuron types, but the effect of amiloride was far more pronounced in N-best cells than in S-best cells and in S-best cells than in H-best cells (Fig. 2, *A*, *C*, and *E;* Table 2). Likewise, amiloride inhibited KCl-evoked responses in N-best neurons to a greater degree than in S-best neurons, whereas the KCl response of H-best neurons was unaffected by amiloride (Fig. 2, *B*, *D*, and *F;* Table 2).

Neural representation of salt stimuli

Across-neuron patterns for two pairs of stimuli are presented in Fig. 3, which depicts (■) the differences between the patterns for NaCl and KCl. Neurons are arranged along the abscissa by best-stimulus classification; the S-best cells on the left, the N-best cells in the middle, and the H-best cells on the right. Within these groups, the neurons are arranged in order of their response to 0.1 M NaCl (Fig. 3*A*); the arrangement is the same in each graph. In Fig. 3*A*, isomolar NaCl and KCl responses are shown, and Fig. 3*B* shows responses to 0.2 M NaCl and 0.05 M KCl, concentrations which evoke the greatest difference in across-neuron patterns among the *group B* stimuli. NaCl and KCl evoke very different patterns of activity across NST neurons (Fig. 3, *A* and *B*, *top*). However, amiloride produces a pronounced, concentration-dependent reduction in the distinction between these neural patterns. At the highest dose of amiloride (30 μM; *bottom*), the across-neuron patterns are virtually indistinguishable.

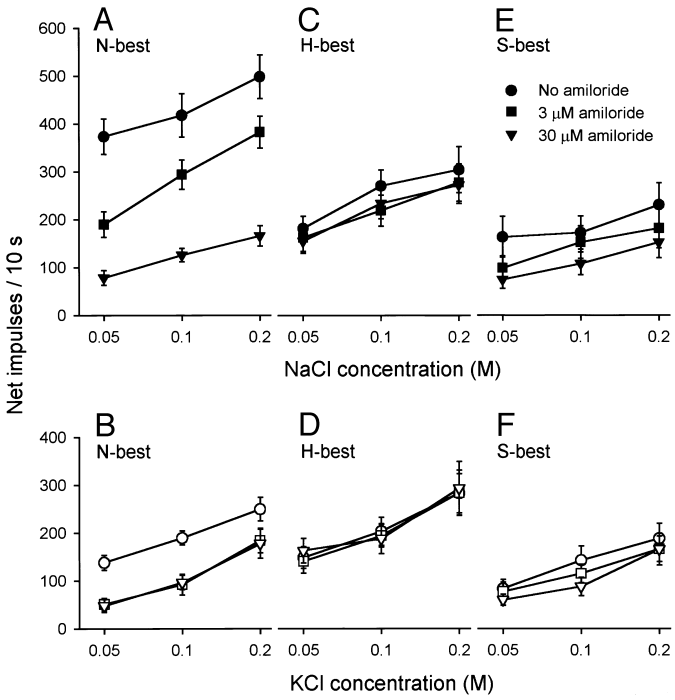

FIG. 2. Mean net responses (± SE) evoked by concentration series of NaCl (●, ■, ▼; *A*, *C*, and *E*) and KCl (○, □, ▽; *B*, *D*, and *F*) in physiologically defined neuron types: N best (*A* and *B*), H best (*C* and *D*), and S best (*E* and *F*). Salts were applied for 10 s and were mixed with 3 doses of amiloride: 0 (● and ○), 3 (■ and □), and 30 (▼ and ▽) μM.

The NMDs (Fig. 3, ■) for some selected stimulus pairs are shown in Fig. 4. The absolute value of the NMD is not meaningful, but as a relative measure, the higher the NMD the more easily discriminated are two stimuli. Thus the NMDs between 0.1 M NaCl and sucrose, HCl, and QHCl (Fig. 4*A*; open circles) indicate the level of NMD characteristic of stimuli that rats can easily discriminate on the basis of taste. This range of NMDs is highlighted in the figure by the upper shaded zone. The NMD for a replication of NaCl (Fig. 4*A*; solid circle) gives an indication of the biological and experimental variability in the experiment and reflects the value of NMD characteristic of stimuli that cannot be behaviorally discriminated. The lower shaded zone highlights this indiscriminable range in the NMD. As seen in Fig. 4*B*, the NMD between isomolar pairs of NaCl and KCl are all within the discriminable range (open circles). However, amiloride has a concentration-dependent effect on the NMD, so that at 30 μM (triangles), it is within the indiscriminable range. This level of NMD suggests that NaCl and KCl would not be discriminable on the basis of either qualitative or intensive differences (compare 0.1 M NaCl with itself, filled circle, Fig. 4*A*). At the intermediate concentration of amiloride (squares), there was also an effect of salt concentration, such that the NMD for 0.05 M salts was closer to the indiscriminable zone and for 0.1 and 0.2 M salts closer to the discriminable zone.

Although it is clear from the NMD measure that amiloride reduces the neural discrimination between NaCl and KCl, an analysis of the across-neuron correlations between stimuli provides additional information (Fig. 5). Across-neuron correlations are relatively low between stimuli that taste different and high between stimuli with similar taste. Figure 5 shows the effect of amiloride on the correlation of 0.1 M NaCl with itself (●) and with 0.1 M KCl (○). With no amiloride (0 μM), NaCl correlates only moderately with KCl ($r = +0.54$) but highly with an independent presentation of NaCl ($r = +0.85$). At the highest concentration of amiloride (30 μM), the situation is reversed: NaCl in amiloride correlates poorly with an unadulterated presentation of NaCl ($r = +0.30$) and actually correlates *better* with unadulterated KCl ($r = +0.76$). In other words, when mixed with amiloride, NaCl is represented by an across-neuron pattern progressively more similar to that evoked by KCl and less like the pattern for NaCl (cf. Fig. 3).

The NMDs were used to create a multidimensional space for all 22 *group A* and *B* stimuli (Fig. 6). Although presented here in three separate panels, these relationships were derived from a single multidimensional scaling analysis of all 22 stimuli. Such spaces represent the similarities and differences in the across-neuron patterns evoked by these stimuli (Bieber and Smith 1986). KCl and NaCl were initially separated from one another (Fig. 6*A*), but amiloride moved the NaCl stimuli (filled triangles) toward the KCl stimuli (open triangles) in a concentration-dependent manner (Fig. 6, *B* and *C*). At 30 μM, the KCl and NaCl stimuli were grouped together in this two-dimensional space, reflecting the similarities in the patterns of neural activity depicted in Fig. 3. A taste space based on the across-neuron correlations showed a similar dramatic effect of amiloride on the neural representation of NaCl and KCl (see Fig. 7).

Whereas treatment with amiloride produced across-neuron patterns for NaCl and KCl that were different from those produced by unadulterated NaCl stimuli (Figs. 3 and 6), modeling the effects of a paracellular pathway blocker resulted in across-neuron patterns for both NaCl and KCl that were highly similar to those produced by unadulterated NaCl. A multidimensional space representing both the effects of amiloride and the results of modeling the effects of blocking the amiloride-insensitive pathway is shown in Fig. 7. This space was based on the across-neuron correlations among the stimuli. As in the NMD space, treatment of the stimuli with 30 μM amiloride produced similar across neuron correlations among NaCl and KCl stimuli (Fig. 7, triangles), which were grouped away from unadulterated NaCl. Mimicking the effects of a paracellular blocker, however, resulted in across-neuron patterns for both NaCl and KCl stimuli (Fig. 7, squares) that were virtually indistinguishable from unadulterated NaCl. Thus whether the amiloride-sensitive or -insensitive pathway is blocked, cells in the rat NST cannot distinguish between NaCl and KCl.

One subtle difference in the taste spaces based on NMD and correlation is worth noting. As shown in Fig. 6*A*, KCl grouped near QHCl (Q) in the NMD-generated space. In the taste space based on correlations, KCl (open circles) did not appear as near

TABLE 2. *Average percent reduction in firing rate to NaCl and KCl produced by 30 μM amiloride*

	NaCl Concentration, M			KCl Concentration, M		
Neuron Type	0.05	0.1	0.2	0.05	0.1	0.2
N best	75.5	65.5	63.5	68.7	50.8	34.2
H best	14.5	13.2	7.8	−8.5	7.3	−1.5
S best	60.0	37.1	31.6	9.1	32.6	13.5

H-best responses to KCl were not significantly affected (2-way ANOVA, see text). Negative numbers indicate percent increases in evoked response rate. N, NaCl; H, HCl; S, sucrose.

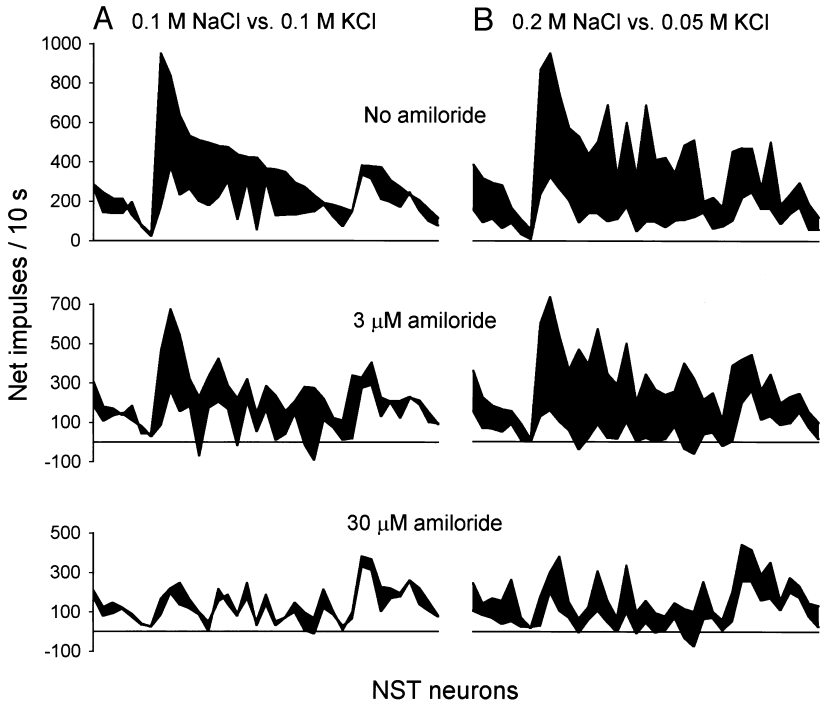

FIG. 3. Across-neuron patterns for 0.1 M NaCl and 0.1 M KCl (*A*) and 0.2 M NaCl and 0.05 M KCl (*B*) in 3 conditions: without amiloride (*top*), mixed with 3 μM amiloride (*middle*), and mixed with 30 μM amiloride (*bottom*). Neural response is taken as the net impulses produced in 10 s. Neurons are arranged along the abscissa by neuron type (S best, then N best, then H best) and within each type by decreasing response to 0.1 M NaCl (as in *A*, *top*); the arrangement of neurons is the same in all graphs. The area between the 2 patterns is shaded to highlight the difference between them; this area is mathematically proportional to the neural mass difference (NMD). Amiloride eliminates the difference in the across-neuron patterns between these salts in a concentration-dependent manner.

to QHCl, whereas QHCl was located closer to HCl. This difference reflects the way NMDs and correlations quantify differences in the across-neuron patterns: NMDs reflect both differences in intensity and in the patterns of responses across neurons, whereas correlations are insensitive to intensity differences. Indeed, across this sample of NST neurons, HCl and QHCl correlated +0.83 but maintained a relatively large NMD because, in general, the neurons were much more responsive to HCl than QHCl. However, the NMDs between QHCl and KCl were quite low (Fig. 6*A*), reflecting the lower response rates to these two stimuli relative to the others. In that regard, it is noteworthy that rats can discriminate between KCl and concentrations of QHCl somewhat lower than those used in the current study (0.01–0.1 mM) (St. John and Spector 1998).

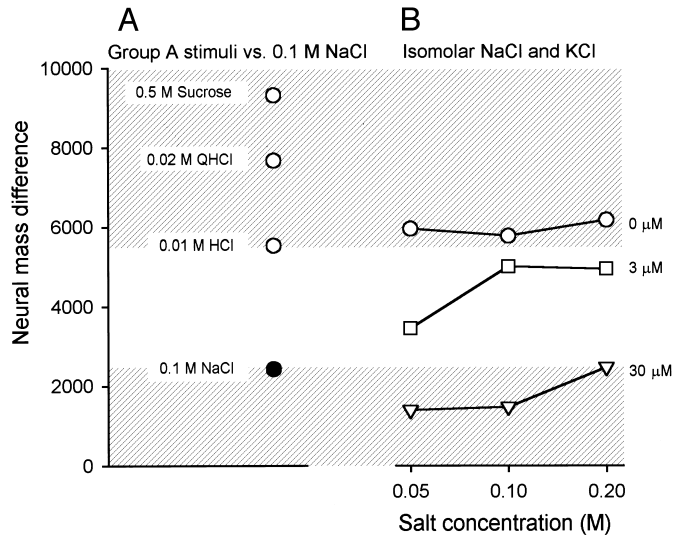

FIG. 4. NMDs between 0.1 M NaCl and the 4 *group A* stimuli (*A*) and between pairs of isomolar salts (*B*) mixed with no amiloride (open circles), 3 μM amiloride (squares), and 30 μM amiloride (triangles). The filled circle in *A* represents the NMD for 2 independent presentations of 0.1 M NaCl and thus represents biological and experimental variability and the level of NMD characteristic of stimuli that are not behaviorally discriminable (bottom shaded zone). The open circles in *A* represent the NMD for behaviorally discriminable taste stimuli (sucrose, QHCl, and HCl vs. NaCl) and thus define the NMD expected of behaviorally discriminable taste stimuli (top shaded zone). In *B*, isomolar pairs of NaCl and KCl are in the discriminable zone, but when mixed with 30 μM amiloride, these same stimulus pairs become indiscriminable. This effect is dependent on both amiloride and salt concentration, and reflects the effect of amiloride in the behavioral experiment (Spector et al. 1996).

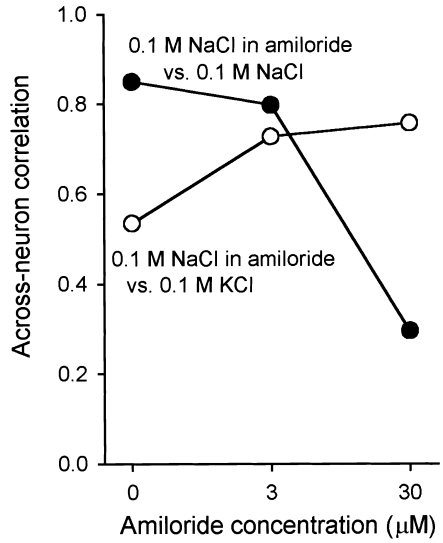

FIG. 5. The effect of amiloride on the across-neuron correlation of 0.1 M NaCl with 0.1 M KCl (○) and 0.1 M NaCl (●). When no amiloride (0 μM) is present, NaCl does not correlate well with KCl ($r = +0.54$) but correlates highly with an independent presentation of NaCl ($r = +0.85$). As amiloride concentration increases, this situation reverses: NaCl in 30 μM amiloride correlates highly with unadulterated KCl ($r = +0.76$) but poorly with unadulterated NaCl ($r = +0.30$). The across-neuron pattern of NaCl mixed with amiloride is more similar to that of KCl than that of NaCl.

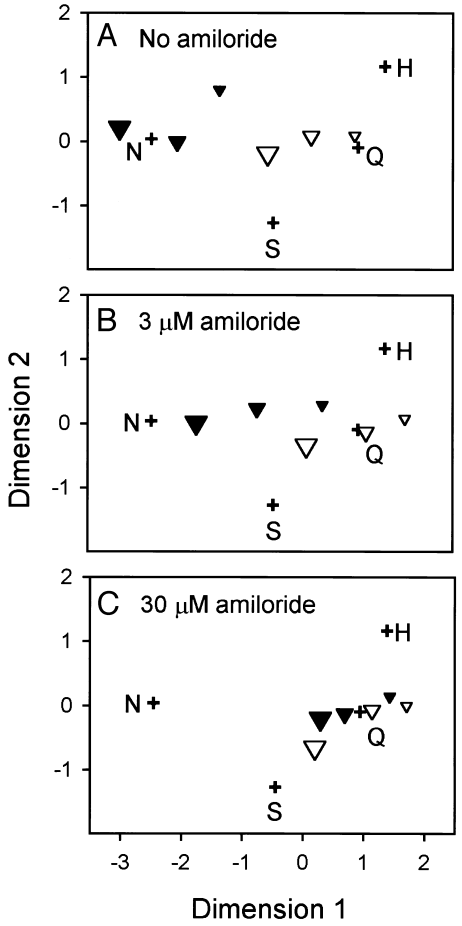

FIG. 6. The matrix of neural mass differences among all 22 *group A* and *B* stimuli were used to construct a 2-dimensional taste space using multidimensional scaling (SPSS for Windows, v. 9.0). Although stimulus relationships are plotted in 3 panels to highlight the effect of increasing amiloride concentration, the relationships among all 22 stimuli were derived from a single multidimensional scaling analysis. *Group A* stimuli are represented by the plus symbol and identified by letter (sucrose, S; NaCl, N; HCl, H; QHCl, Q). These stimuli are repeated on all 3 panels to facilitate comparison; they were never mixed in amiloride. The concentrations of *group B* stimuli, NaCl (filled triangles) and KCl (open triangles), are represented by symbol size (0.05 M, smallest; 0.1 M, intermediate; 0.2 M, largest). Amiloride treatment caused these 2 sets of salt stimuli, originally far apart in the taste space, to group together, reflecting an increasing similarity in their across-neuron patterns.

Amiloride: extended concentration range

Amiloride inhibited the responses of N-best cells to 0.1 M NaCl across the extended amiloride concentration range. Figure 8*A* shows the mean response of five N-best neurons to 0.1 M NaCl mixed with different concentrations of amiloride relative to the response to 0.1 M NaCl alone. There was a striking similarity between this effect and the efficacy of amiloride in disrupting behavioral responses in the Spector et al. (1996) study (Fig. 8*B*). Where comparable, the values of the parameters defining the curves fit to the concentration-response data are remarkably similar. That is, both have virtually the same slope (*b*) and half-maximum (*c*). The only difference was in the minimum response (*d*), but this difference is due to the scale of the ordinate. For the neurophysiological measure, the minimum that could be expected is zero spikes in 10 s (without considering the possibility of inhibitory responses). On the other hand, the minimal behavioral performance that could be expected is 50%, indicating rats performing at chance levels in the discrimination task (see Spector et al. 1996 for further details).

Group C stimuli

Although N-best neurons respond differentially to NaCl and KCl at midrange concentrations (Fig. 2, *A* and *B*), higher concentrations of KCl drive N-best neurons quite well. For the subset of neurons tested with *group C* stimuli, 1.0 M KCl correlated strongly with 0.1 M NaCl in N-best neurons (Fig. 9; $r = +0.85$), showing that these neurons alone cannot provide a unique signal for sodium taste. Moreover, the overall across-neuron correlation between NaCl and KCl remains moderate ($r = +0.60$) at these concentrations due to the differential input provided by the other neuron types (H- and S-best neurons). Whether rats can behaviorally discriminate these particular concentrations of NaCl and KCl has not been tested, but if they can, it seems unlikely that the activity of N-best neurons alone could provide the discriminative signal.

Salt responses across neuron types

The contribution of each neuron type to the overall patterns of response to each stimulus under both the amiloride and the amiloride-insensitive treatment is depicted in Fig. 10. Here, the mean response of each neuron type is shown for 0.1 M NaCl and 0.1 M KCl in the untreated condition (□), when mixed with amiloride (▨), and after the hypothetical block of the amiloride-insensitive pathway (▩). As reflected in the MDS

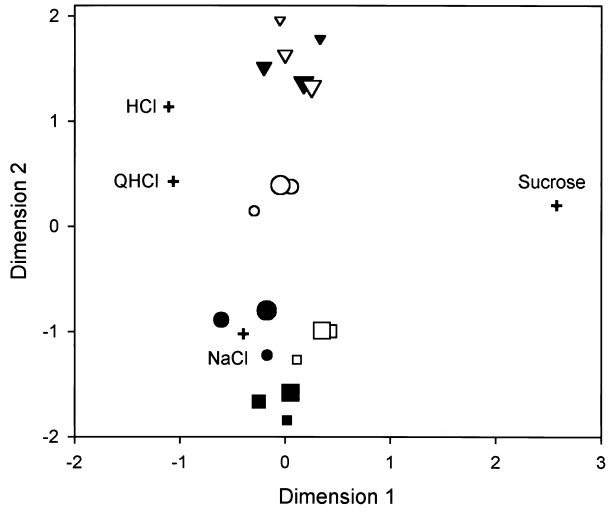

FIG. 7. The matrix of across-neuron correlations among *group A* and *B* stimuli (except those mixed with 3 μM amiloride) were used to construct a 2-dimensional taste space using multidimensional scaling (SPSS for Windows, v. 9.0). *Group A* stimuli are represented by the plus symbol and identified by letter (sucrose, S; NaCl, N; HCl, H; QHCl, Q). The concentrations of *group B stimuli*, NaCl (filled circles, triangles, or squares) and KCl (open circles, triangles, or squares), are represented by symbol size (0.05 M, smallest; 0.1 M, intermediate; 0.2 M, largest). Amiloride treatment (triangles) caused these 2 sets of salt stimuli, originally far apart in the taste space (circles), to group together, reflecting an increasing similarity in their across-neuron patterns and a distinct difference from the pattern to unadulterated NaCl (+). The theoretical paracellular block (squares) also resulted in the patterns for NaCl and KCl being highly similar and located together in multidimensional space but close to unadulterated NaCl (+).

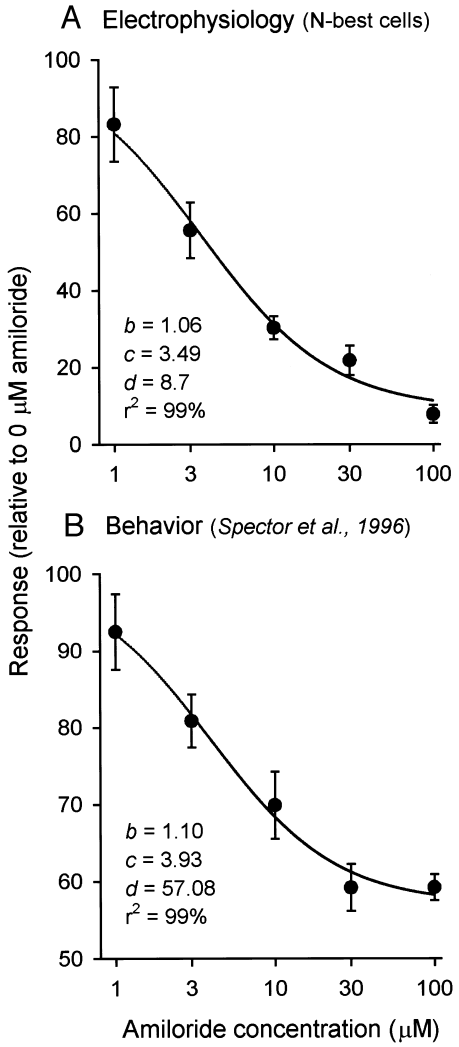

FIG. 8. A direct comparison of the effect of amiloride on responses evoked by 0.1 M NaCl in 5 N-best neurons (*A*) and behavioral performance in a 2-lever NaCl vs. KCl (0.05, 0.1, and 0.2 M salts) discrimination paradigm (*B*) (from Spector et al. 1996). In *A*, net responses in 10-s trials were standardized to responses in the no amiloride condition. In *B*, percentage of correct responses in amiloride sessions were standardized to performance in no amiloride control sessions. Three-parameter sigmoidal curves were fit to both concentration-response data sets; the values for slope (*b*) and half-maximum amiloride concentration (*c*) were virtually identical.

analyses (Figs. 6 and 7), amiloride makes the across-neuron type patterns for both NaCl and KCl more similar to untreated KCl than to NaCl. On the other hand, the amiloride-insensitive treatment makes these patterns more similar to untreated NaCl than to KCl. These shifts in pattern are due to the reduction primarily of the responses of N- and S-best cells after amiloride and of H- and S-best cells after the model treatment. Nevertheless, the responses to both stimuli are characterized by substantial activity in all three neuron types. The similarity between NaCl and KCl, for example, after the model treatment (as reflected in the MDS space of Fig. 7) is not due to the large response in N-best cells but in the relative response among the three cell types. The response of the N-best cells to KCl after this model treatment is actually no greater than the response of these cells to untreated KCl, yet KCl after this treatment produces an across-neuron pattern highly similar to untreated

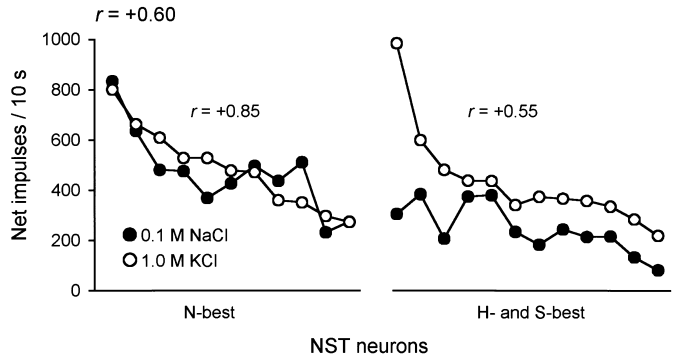

FIG. 9. Across-neuron patterns evoked by 0.1 M NaCl (●) and 1.0 M KCl (○) in a subset of NST neurons ($n = 23$). Neurons are arranged along the abscissa by decreasing response to 1.0 M KCl in 2 groups: N-best neurons on the *left* and H- and S-best neurons on the *right*. At these concentrations, N-best neurons respond similarly to these 2 salts, whereas the other neuron types respond differentially. The correlation across only N-best cells is high ($r = +0.85$) as occurs across all cells between 2 replications of 0.1 M NaCl (Fig. 5). However, the correlation across all neurons between 0.1 M NaCl and 1.0 M KCl is modest ($r = +0.60$) due to the differential input of the other neurons. This modest correlation is characteristic of easily discriminated stimuli (Erickson 1963; Smith et al. 1979).

NaCl (Fig. 7). Similarly the response of H-best neurons to NaCl after amiloride is no different from the response of these neurons to untreated NaCl, yet the across-neuron pattern for NaCl after amiloride is like that evoked by KCl either untreated or after amiloride (Figs. 3 and 7).

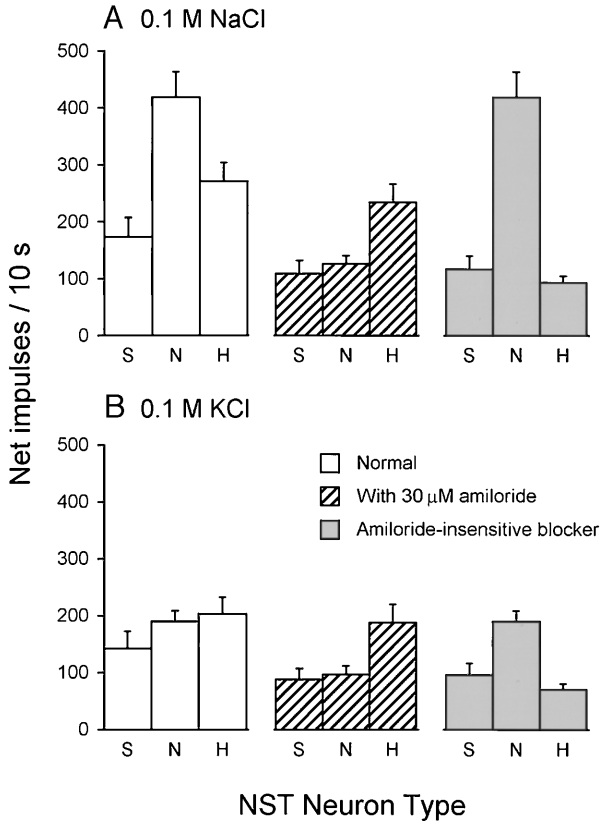

FIG. 10. Profiles of activity across gustatory neuron types. *A*: response to 0.1 M NaCl in S-, N-, and H-best neurons under 3 treatment conditions: normal (□), amiloride (▨), and the hypothetical amiloride-insensitive treatment (▤). *B*: responses to 0.1 M KCl in S-, N-, and H-best neurons under the same 3 treatment conditions. Error bars = +1 SE.

DISCUSSION

Segregation of amiloride-sensitive and -insensitive responses in the rat NST

Consistent with studies of peripheral taste fibers (Brand et al. 1985; Formaker and Hill 1988; Heck et al. 1984; Herness 1987; Hettinger and Frank 1990; Ninomiya and Funakoshi 1988), NaCl responses in the NST were partially inhibited by amiloride. Amiloride predominantly affected responses in N-best neurons (Fig. 2 and Table 2). These results are consistent with other work demonstrating the segregation of amiloride-sensitive and -insensitive activity in the NST (Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996) and extend earlier findings in the rat by demonstrating amiloride effects across a dosage range (3–30 μM) substantially lower than used previously (500 μM) (Giza and Scott 1991; Scott and Giza 1990).

In the present study, the segregation of amiloride-sensitive and -insensitive input was not absolute. Average responses to NaCl were significantly reduced by amiloride in all neuron types, suggesting some convergence of amiloride-sensitive and -insensitive peripheral fibers onto H- and S-best NST neurons (Fig. 2). Nonetheless it is also apparent that the bulk of amiloride-sensitive input is to N- and S-best neurons (Fig. 2 and Table 2); the input to H-best cells is minimal at best. Using a different cell classification scheme, Giza and Scott (1991) found that amiloride inhibited NaCl responses in two groups of neurons that responded best to NaCl but not in two other groups that were relatively more responsive to acids. Thus there is general agreement that amiloride predominantly affects N-best neurons. A striking difference to the results of Giza and Scott (1991) was that amiloride significantly reduced responses to KCl in the present experiment (Fig. 2). An effect of amiloride on KCl responses has been reported in studies on the rat chorda tympani nerve (Lundy and Contreras 1997; Minear et al. 1996; Ninomiya and Funakoshi 1988) and the hamster NST (Boughter et al. 1999).

Relationship to discrimination behavior

A primary goal of the present study was to relate the neural representation of NaCl and KCl to the behavioral results of Spector et al. (1996). In that experiment, rats were trained to press one lever after tasting NaCl and another one after tasting KCl. There were several significant results in the behavioral study: discrimination performance deteriorated when increasing concentrations of amiloride were mixed with NaCl and KCl; rats performed better on trials with higher salt concentrations; rats responded to NaCl mixed with high concentrations of amiloride as if it was KCl (i.e., they pressed the KCl lever); and the concentration of amiloride with a half-maximal effect on discrimination performance was in the range of the inhibition constant for amiloride's effects on NaCl-evoked activity in the CT.

The current neurophysiological results relate directly to the behavioral findings. First, amiloride had a concentration-dependent effect on the neural representation of NaCl and KCl (Figs. 3–6). Across-neuron patterns for NaCl and KCl are very different in the NST, which presumably allows the qualitative discrimination of these salts behaviorally (Erickson 1963). The difference in these across-neuron patterns declines when the salts are mixed with 3 μM amiloride and virtually disappears at 30 μM (Figs. 3–6).

Second, the neurophysiological data suggest why behavioral performance improved at higher salt concentrations. The NMD measure, which takes into account both quality and intensity information to provide an overall index of discriminability, decreases as a function of amiloride concentration but increases slightly with salt concentration (Fig. 4*B*). More dramatically, the taste spaces based on the NMD (Fig. 6) clearly separate NaCl (filled triangles) from KCl (open triangles) in the no amiloride condition (*A*) but not at 30 μM amiloride (*C*). However, at 3 μM amiloride (*B*), 0.2 M NaCl is located closer to unadulterated NaCl, whereas 0.05 M NaCl is located nearer to KCl. This suggests that higher concentrations of NaCl, even mixed with amiloride, should be easier for rats to recognize than lower concentrations.

The third behavioral finding was that rats tended to press the KCl-appropriate lever when the stimulus was NaCl mixed with high concentrations of amiloride. In the derived taste spaces (Figs. 6 and 7), amiloride shifted NaCl stimuli closer to unadulterated KCl rather than vice versa. This shift reflects the fact that amiloride had a relatively larger effect on responses to NaCl than to KCl (Figs. 2 and 3), which changed the across-neuron pattern evoked by NaCl to one more characteristic of KCl (Figs. 3 and 5). This result is consistent with the interpretation that NaCl, when mixed with amiloride, tastes like KCl.

Finally, Spector et al. (1996) found that the effect of amiloride on discrimination could be described by a sigmoidal function (Fig. 8*B*), with amiloride producing a half-maximal effect on behavior at 3.93 μM. This value was in the range of the inhibition constant for amiloride's effect on NaCl responses in the rat chorda tympani nerve (Brand et al. 1985; DeSimone and Ferrell 1985) and in rat and hamster taste receptor cells (Avenet and Lindemann 1991; Gilbertson et al. 1992) when stimulated with midrange concentrations of NaCl. When a subset of N-best neurons was tested with the full range of amiloride concentrations, we found the concentration-response function for amiloride's effect on 0.1 M NaCl responses to be well described by a similar function (Fig. 8*A*), with an inhibition constant of 3.49 μM. Thus there is a striking correspondence in amiloride's action on taste receptor cells, peripheral nerve fibers, N-best cells in the NST and the rat's ability to make a behavioral discrimination between sodium and nonsodium salts.

Other investigators have shown that amiloride reduces the neural differentiation of sodium and nonsodium salts (Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990). However, comparison of earlier rat electrophysiological data to the Spector et al. (1996) experiment is limited by the salt concentrations used and by the very high amiloride concentration employed (500 μM). Amiloride has a half-maximal effect on taste receptor cells near 1 μM (Avenet and Lindemann 1991; Gilbertson et al. 1992); such strong concentrations could produce nonspecific effects (Lindemann 1996; Lundy and Contreras 1997; Lundy et al. 1997; Smith and Benos 1991).

Implications for taste quality coding

Our results are consistent with the interpretation that amiloride makes NaCl taste like KCl to rats. In addition, when we

modeled the effect of blocking the amiloride-insensitive transduction pathway (see METHODS and Fig. 7), the neural distinction between NaCl and KCl was reduced. Given that this manipulation caused KCl and NaCl to cluster near unmodified NaCl in the multidimensional space (Fig. 7), it is indeed possible that the perceptual effect of such a blocker would be to make KCl taste like NaCl.

Does this mean that N-best cells, which are substantially inhibited by amiloride, are responsible for coding the "sodium taste" (cf. McCaughey and Scott 1998), whereas H-best neurons are responsible for coding the "nonsodium taste" of salts? In other words, are N- and H-best cells labeled lines for these two qualities of salt taste?

We believe such an interpretation ignores several salient features of the rodent taste system, as well as available information on behavioral responses to NaCl and KCl. For one, a labeled-line interpretation ignores the fact that both salts stimulate all neuron types. As shown in Fig. 10, information about NaCl is indeed carried predominately through N-best cells, but it is difficult to support the opposite claim for KCl. That is, KCl evokes roughly equivalent levels of activity in N- and H-best neurons and certainly more activity in N- and S-best cells combined than in H-best cells. Taken to its logical conclusion, a labeled-line interpretation (that N-best neuron activity signals a sodium taste and H-best neuron activity a "potassium taste") would imply that both NaCl and KCl are complex tastes, perceptually representing mixtures of sodium and potassium taste to rats. Since it is known that rats will avoid a conditioned stimulus in proportion to its concentration in a mixture (Smith and Theodore 1984), the labeled-line notion would predict (by virtue of the broad tuning displayed in Fig. 10) that taste aversions conditioned to NaCl should generalize to KCl and vice versa. However, there is strong behavioral evidence that NaCl and KCl do not cross-generalize (Hill et al. 1990; St. John et al. 1997).

Second, interpreting the effects of amiloride as due to the specific inhibition of N-best neuronal activity is an oversimplification at best. As summarized in Fig. 10, amiloride (▨) does not simply reduce the N-best neural activity to zero while having no effect on H- or S-best units. Instead, N-best *and* S-best responses are considerably reduced, but activity in neither neuron type is completely eliminated. It is important to note that when an identical stimulus protocol is used in behavioral experiments, rats cannot distinguish NaCl from KCl (Spector et al. 1996).

Why then does amiloride cause NaCl to taste like KCl? We believe the most straightforward explanation, and one that accounts for the greatest amount of neural and behavioral data, is that the relative activity across all neurons is responsible for encoding the taste of these salts. Note the similarity in the across neuron patterns after amiloride in Fig. 3. In Fig. 10, where across-neuron patterns have been simplified as "across neuron type patterns," the same case can be made. It is not the amount of activity in any one neuron type that conforms to the predicted results, otherwise unadulterated NaCl (□), which has the greatest activity in H-best units of the six stimuli presented, should taste the most like KCl. Instead it is the relative activity that appears critical: amiloride treatment renders the patterns of both NaCl and KCl (▨) similar to that of unadulterated KCl and dissimilar to that of NaCl. Behaviorally (Spector et al. 1996) and neurally, the results of amiloride treatment are consistent with the perceptual effect of making both salts taste like KCl. In addition, after the paracellular treatment (▤) NaCl and KCl are similar in their patterns across cell types, but the amount of activity in the N-best cells is dramatically different. These data, along with the lack of behavioral generalization between NaCl and KCl, suggest strongly that it is not the absolute activity in any one neuron type but the relative activity across neurons that represents the taste of these salts.

We thank Dr. John D. Boughter, Jr., for valuable comments on the manuscript.

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

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