with ketamine (30 mg/kg) and maintained areflexic with pentobarbital administered intravenously (~20.0 mg/kg the first hour, then 2 to 4 mg/kg per hour thereafter). Digitally synthesized sounds were presented dichotically by means of sealed, calibrated delivery systems in a sound-attenuating chamber. Each action potential and various temporal features of the stimulus were detected with 1-μs resolution. Recording sites were verified histologically.
10. No units studied entrained to monaural phase modulation in absence of the reference signal. Identical responses were obtained when the signals at the two ears were switched, if the direction of modulation was reversed. Responses similar to those obtained with triangular modulation were obtained with sinusoidal modulation.
11. Cat neurons tuned to frequencies above 2.0 kHz typically show pronounced adaptation to static IPD stimuli with long durations. Nonadapting units commonly respond most sensitively to frequencies below 2 kHz. Similar adaptation was rarely seen at any frequency in the gerbil.
12. J. E. Rose, N. B. Gross, C. D. Geisler, J. E. Hind, *J. Neurophysiol.* **29**, 288 (1966).
13. L. A. Jeffress, *J. Comp. Neurol.* **41**, 35 (1948).
14. W. E. Sullivan, *Brain Behav. Evol.* **28**, 109 (1986).
15. J. M. Goldberg and P. B. Brown, *J. Neurophysiol.* **32**, 613 (1969).
16. G. L. Roth, R. K. Kochhar, J. E. Hind, *J. Acoust. Soc. Am.* **68**, 1643 (1980).
17. J. A. Altman, *Exp. Neurol.* **22**, 13 (1968).
18. D. W. Grantham, *J. Acoust. Soc. Am.* **79**, 1939 (1986).
19. We thank S. Kaiser for technical support and L. Kitzes and R. Robertson for critical review of the manuscript. Supported by NIH grant DC00364.

24 April 1991; accepted 1 August 1991

The Anion Paradox in Sodium Taste Reception: Resolution by Voltage-Clamp Studies

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Sodium salts are potent taste stimuli, but their effectiveness is markedly dependent on the anion, with chloride yielding the greatest response. The cellular mechanisms that mediate this phenomenon are not known. This "anion paradox" has been resolved by considering the field potential that is generated by restricted electrodiffusion of the anion through paracellular shunts between taste-bud cells. Neural responses to sodium chloride, sodium acetate, and sodium gluconate were studied while the field potential was voltage-clamped. Clamping at electronegative values eliminated the anion effect, whereas clamping at electropositive potentials exaggerated it. Thus, field potentials across the lingual epithelium modulate taste reception, indicating that the functional unit of taste reception includes the taste cell and its paracellular microenvironment.

THE PERCEIVED SALTINESS OF SODIUM salts varies significantly with the anion present. For anions other than chloride, saltiness is attenuated or gustatory qualities other than saltiness appear (*1*). Because the anion effect can be seen in recordings from the chorda tympani (CT) nerve of animals, its origin seems to be peripheral (*2*). Neurophysiological investigations (*3*), lingual epithelial ion transport studies (*4*), and patch-clamp recordings from taste-bud cells (*5*) have established that a major Na^+-transducing element is an amiloride-blockable ion channel in the plasma membrane of taste receptor cells. An amiloride-insensitive Na^+ transducer may also exist (*6*). The effects of the anions of salt stimuli are less well understood. Various blockers of Cl^- cotransport (electroneutral anion exchange involving Cl^-) and antibodies against Cl^- channels have no effect on the CT response of the rat to NaCl (*7*). These results suggest that specific cellular Cl^- transport processes are unlikely to be the major cause of the anion effect. The large effect of the anion on the neural response makes this result seem paradoxical. The alternative that anions might affect taste reception through diffusion potentials across shunts between receptor cells has been proposed (*7, 8*), but the hypothesis has not been tested.

We used the technique of Heck *et al.* (*9*) to test whether relative changes in paracellular anion permeability could affect the transepithelial potential across the taste buds and, in turn, the neural response. This method permits either voltage-clamping or current-clamping a macroscopic patch of rat dorsal lingual epithelium while simultaneously monitoring the CT response from the taste fibers innervating the patch (*10*). We used three stimuli: NaCl, sodium acetate (NaAc), and sodium gluconate (NaGlu). Figure 1A shows the change in transepithelial potential across the patch (*11*) evoked by 0.5 M NaCl, NaAc, and NaGlu. NaCl caused an electropositive change in the potential and a decreased resistance relative to the rinse solution (0.03 M NaCl in this case). The CT response, recorded simultaneously, is shown below in Fig. 1A. Changing the salt to NaAc produced a more electropositive potential, a smaller decrease in resistance, and the typically lower neural response. With NaGlu as the stimulus the electropositive potential increased further, the resistance decrease was smallest, and the neural response was the lowest of the three test salts.

This pattern of CT response for the test salts occurred for concentrations from 0.05 to 0.5 M so that NaCl > NaAc > NaGlu (Fig. 1B). The pattern for the transepithelial potential change over the same concentration range (Fig. 1C) was exactly opposite: NaCl < NaAc < NaGlu. Changes with concentration in transepithelial conductance paralleled those of the neural response with the same rank order (not shown). The lingual epithelium transports both Na^+ and Cl^- ions (*4, 12*). Pathways for ion flow are both active and passive, and some evidence indicates that a major passive route is paracellular (*4, 12, 13*). The data in Fig. 1 are consistent with the anion effect on the neural response being mediated through a paracellular shunt. Chloride, the most conductive of the three anions, provides a shunting current that minimizes the electropositive potential created by the transcellular and paracellular transport of Na^+. Once established, the electropositive potential could act as a hyperpolarizing field potential depressing the receptor potential of the taste cell (*7, 8*).

If this hypothesis is correct, then it should be possible to voltage-clamp the field potential so that anion diffusion potentials do not influence the receptor potentials or, therefore, the neural responses. This setup can be achieved by clamping the inside of the lingual epithelium at electronegative potentials. At −90 mV, the neural responses to NaCl, NaAc, and NaGlu did not differ significantly from one another (Fig. 2). The evolution and the direction of the stimulus-evoked currents at negative clamping voltages were similar for all three test salts. These data are consistent with the convergence of the neural response magnitudes and indicate that the major electrical consequences of anion shunting were eliminated.

In contrast, with a clamping voltage of 90 mV the neural responses to NaCl, NaAc, and NaGlu were significantly reduced (Fig. 2). Responses to NaAc and NaGlu were especially inhibited, rising just above baseline. To sustain a clamping voltage of 90 mV with NaCl, an inward current was required because of the high shunt Cl^- conductance. The smaller inward current required to sustain 90 mV in the case of the lower-conductance acetate ion was consistent with this result. Gluconate, the ion with the lowest conductance, provided the poorest shunting so that a small outward clamp-

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ing current was required to keep the potential from becoming more electropositive. Thus, a hyperpolarizing field potential imposed on the taste cells attenuated responses to all test salts and was increasingly more effective as the conductance of the shunting anion decreased. When clamped at various voltages from −90 to 90 mV, the CT responses to NaCl, NaAc, and NaGlu tended to converge at electronegative potentials and to diverge at electropositive potentials.

The lingual epithelium actively transports Na^+ through amiloride-sensitive transcellular pathways (*4, 12*), which have been shown to be channels in taste cells (*5*). The chemosensory cells are connected by tight junctions near the apical pole of the taste bud (*13, 14*). As with other epithelia, tight junctions serve as paracellular shunt pathways for ion transport. Ouabain eliminates the entire short-circuit current without significantly affecting the resistance (*4, 12*). This fact and the linearity of the current-voltage curves indicate that the transepithelial resistance is dominated by the paracellular shunt resistance. Reducing the shunt conductance to anions must reduce sodium current through the taste cells while producing a more electropositive transepithelial potential (*4, 7*). Our data show that the shunt-regulated transepithelial potential acts as a field potential on the taste sensory cells and that modulation of this field potential causes the anion effect in salt taste.

With salts, the stimulus ions are the major determinants of the field potential. However, active ion transport in situ can produce a metabolically regulated field potential (*4*). Metabolic or hormonal regulation of the sensitivity of taste receptor cells may be exerted through the field potential across the receptor cells. The regulation could be exerted through variations in the ion permeability of the paracellular shunts, in electrogenic pump activity, or in both. The paracellular ion pathway acts as a shunt in parallel with transcellular active ion movement in various other epithelia (*15, 16*). Our

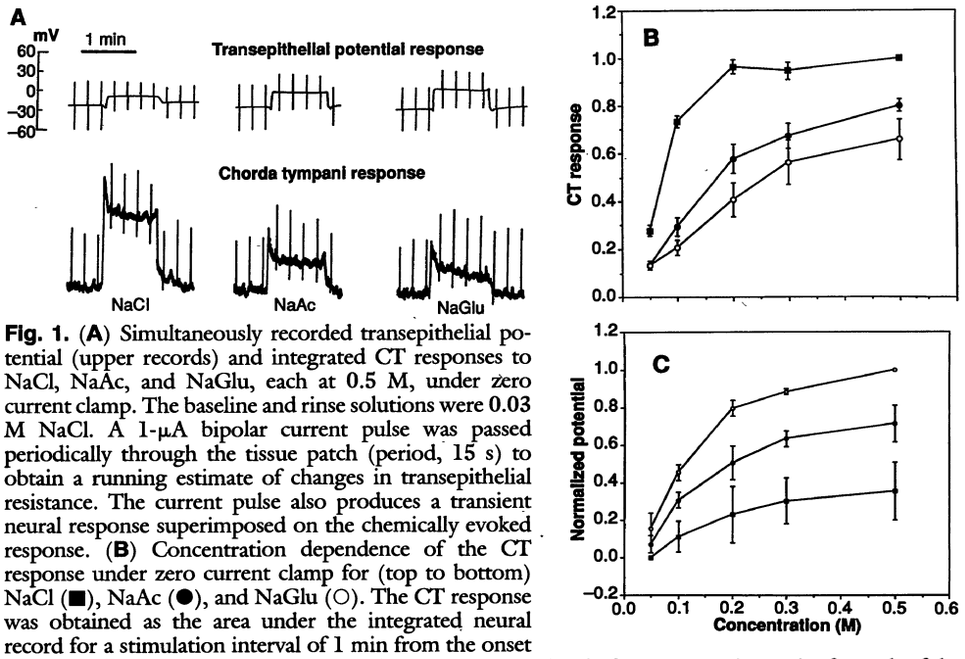

Fig. 1. (**A**) Simultaneously recorded transepithelial potential (upper records) and integrated CT responses to NaCl, NaAc, and NaGlu, each at 0.5 M, under zero current clamp. The baseline and rinse solutions were 0.03 M NaCl. A 1-μA bipolar current pulse was passed periodically through the tissue patch (period, 15 s) to obtain a running estimate of changes in transepithelial resistance. The current pulse also produces a transient neural response superimposed on the chemically evoked response. (**B**) Concentration dependence of the CT response under zero current clamp for (top to bottom) NaCl (■), NaAc (●), and NaGlu (○). The CT response was obtained as the area under the integrated neural record for a stimulation interval of 1 min from the onset of chemically evoked neural activity. Each experiment consisted of a concentration series for each of the test salts. The order of presentation of a series was varied. The response to 0.5 M NaCl was assigned a value of 1, and all other responses were normalized to it. Each point represents the mean ± SEM (n = 4). The baseline and rinse solutions were 0.01 M NaCl. (**C**) Concentration dependence of the normalized change in transepithelial potential under zero current clamp for (bottom to top) NaCl (■), NaAc (●), and NaGlu (○). The potential achieved by 0.5 M NaGlu (always the greatest in a test salt series) was assigned the value unity, and all other potentials were normalized to it. Each point represents the mean ± SEM (n = 4). The baseline and rinse solutions were 0.01 M NaCl.

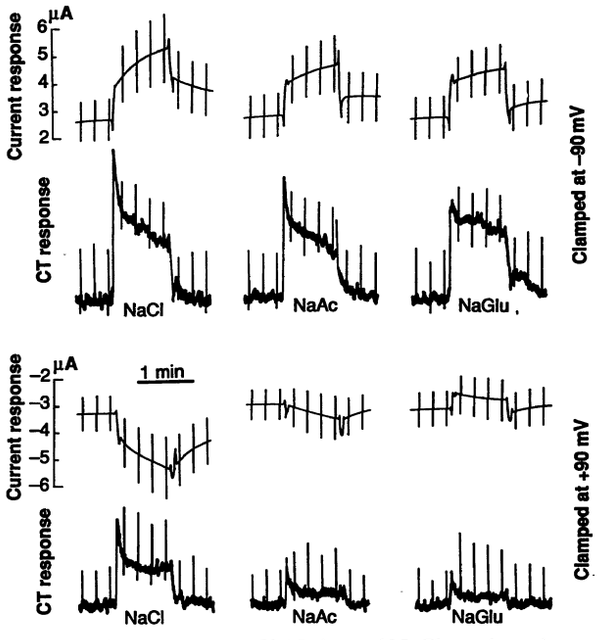

Fig. 2. The neural response to NaCl, NaAc, and NaGlu under voltage-clamp. The top two rows show the current and CT response for a clamping voltage of −90 mV (inside electronegative). The CT responses are not significantly different. In each case inward current is positive. The bottom two rows show the current response and the CT response at a clamped transepithelial voltage of 90 mV. The baseline and rinse solutions were 0.03 M NaCl. A constant bipolar voltage pulse was passed at 15-s intervals to obtain a running measure of the transepithelial conductance.

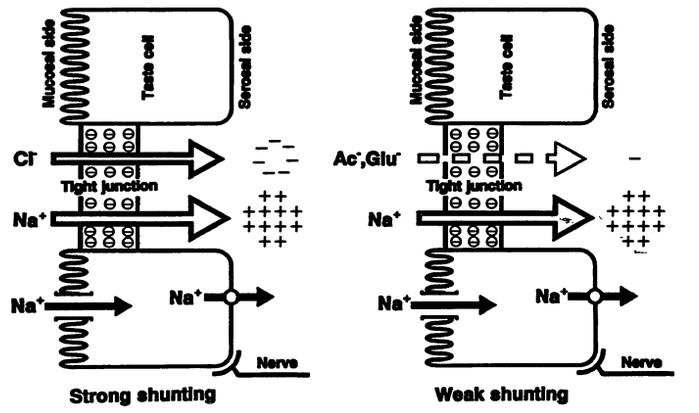

Fig. 3. The functional unit for salt taste reception. The unit includes receptor cells that contain apical Na^+ channels and basolateral sodium pumps connected functionally in series. A shunt current across tight junctions flows parallel to the transcellular Na^+ current. The strong shunting Cl^- ion largely compensates the electropositive field potential due to active and passive Na^+ transport. The weak shunting acetate and gluconate ions poorly compensate the field potential, resulting in hyperpolarization of the receptor cells.

results indicate that paracellular electrodiffusional processes can exert a direct influence on the sensory receptor potential of taste-bud cells. This is further evidence that the fundamental functional unit in taste reception is not the single taste-bud cell (*17*), but at least includes the receptor cell and its paracellular microenvironment (Fig. 3).

REFERENCES AND NOTES

1. D. L. Hill, B. K. Formaker, K. S. White, *Behav. Neurosci.* **104**, 734 (1990); S. S. Schiffman, A. E. McElroy, R. P. Erickson, *Physiol. Behav.* **24**, 217 (1980); C. Murphy, A. V. Cardello, J. G. Brand, *ibid.* **26**, 1083 (1981); L. M. Bartoshuk, in *Handbook of Behavioral Neurobiology*, vol. 1, *Sensory Integration*, R. B. Masterson, Ed. (Plenum, New York, 1978), pp. 503–567.
2. L. M. Beidler, in *Olfaction and Taste*, T. Hayashi, Ed. (Pergamon, New York, 1967), vol. 2, pp. 509–534; B. K. Formaker and D. L. Hill, *Am. J. Physiol.* **255**, R1002 (1988).
3. S. S. Schiffman, E. Lockhead, F. W. Maes, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6136 (1983); G. L. Heck, S. Mierson, J. A. DeSimone, *Science* **223**, 403 (1984); J. A. DeSimone, G. L. Heck, S. Mierson, S. K. DeSimone, *J. Gen. Physiol.* **83**, 633 (1984); J. G. Brand, J. H. Teeter, W. L. Silver, *Brain Res.* **334**, 207 (1985); D. L. Hill and T. C. Bour, *Dev. Brain Res.* **20**, 310 (1985); T. P. Hettinger and M. E. Frank, *Brain Res.* **513**, 24 (1990); T. R. Scott and B. K. Giza, *Science* **249**, 1585 (1990).
4. J. A. DeSimone, G. L. Heck, S. K. DeSimone, *Science* **214**, 1039 (1981); S. A. Simon and J. L. Garvin, *Am. J. Physiol.* **249**, C398 (1985); S. Mierson, G. L. Heck, S. K. DeSimone, T. U. L. Biber, J. A. DeSimone, *Biochim. Biophys. Acta* **816**, 283 (1985); S. Mierson, S. K. DeSimone, G. L. Heck, J. A. DeSimone, *J. Gen. Physiol.* **92**, 87 (1988).
5. P. Avenet and B. Lindemann, *J. Membr. Biol.* **97**, 223 (1987); *ibid.* **105**, 245 (1988); J. H. Teeter, K. Sugimoto, J. G. Brand, in *Chemical Senses*, J. G. Brand, J. H. Teeter, R. H. Cagan, M. R. Kare, Eds. (Dekker, New York, 1989), vol. 1, pp. 151–170.
6. Y. Ninomiya, N. Sako, M. Funakoshi, *J. Comp. Physiol. A* **166**, 1 (1989).
7. E. J. Elliott and S. A. Simon, *Brain Res.* **535**, 9 (1990).
8. H. W. Harper, *Ann. N.Y. Acad. Sci.* **510**, 349 (1987).
9. G. L. Heck, K. C. Persaud, J. A. DeSimone, *Biophys. J.* **55**, 843 (1989).
10. The procedure has been described in detail (*9*). Female Sprague-Dawley rats were anesthetized by intraperitoneal (IP) injection of sodium pentabarbital [60 mg per kg of body weight (mg/kg)]. IP booster injections (12 mg/kg) were given as needed during the course of the experiment. A tracheal cannula facilitated free breathing, and normal body temperature was maintained. Standard procedures were used to expose the CT and prepare it for recording (*9*). Taste stimuli were applied through a Lucite chamber that circumscribed a patch of the anterior dorsal tongue 6 mm in diameter. The chamber was affixed to the tongue by vacuum and was fitted with separate Ag/AgCl electrodes for potential and current measurement. Reference electrodes were placed noninvasively beneath the tongue rather than in the muscle as reported (*9*). The current-passing electrode in the chamber served as a virtual ground, ensuring that only current passing across the epithelial patch was collected. For stimulation or rinsing, 3-ml aliquots were injected at a rate of 1 ml/s.
11. The potential differences are displayed as submucosa relative to mucosa with increases corresponding to an upward pen deflection. In the normal rinse solution, the potentials were stable. In some experiments the rinse was 0.01 M NaCl, in others it was 0.03 M NaCl. Increases in stimulus concentration always produced an electropositive shift in potential. Because of the sublingual placement of the reference electrodes, the potential included stimulus invariant contributions. These were not a problem because we were primarily concerned with the relative changes evoked by stimuli differing only in anion identity.
12. S. A. Simon, R. Robb, S. S. Schiffman, *Pharm. Biochem. Behav.* **29**, 257 (1988).
13. V. F. Holland, G. A. Zampighi, S. A. Simon, *J. Comp. Neurol.* **279**, 13 (1989).
14. J. C. Kinnamon, B. T. Taylor, R. J. Delay, S. J. Roper, *ibid.* **235**, 48 (1985).
15. V. L. Lew, H. G. Ferreira, T. Moura, *Proc. R. Soc. London Ser. B* **206**, 53 (1979); M. L. Fidelman and D. C. Mikulecky, *J. Theoret. Biol.* **130**, 73 (1988).
16. M. L. Fidelman and S. Mierson, *Am. J. Physiol.* **257**, G475 (1989).
17. C. H. K. West and R. A. Bernard, *J. Gen. Physiol.* **72**, 305 (1978); J. H. Teeter, in *Proceedings of the 19th Japanese Symposium on Taste and Smell*, S. Kimura, A. Miyoshi, I. Shimada, Eds. (Assai University, Gifu, Japan, 1985), pp. 29–33; J. Yang and S. D. Roper, *J. Neurosci.* **7**, 3561 (1987).

18. We thank S. Price for helpful discussions and criticism. Supported by NIH grant DC00122 and the Campbell Institute for Research and Technology.

26 June 1991; accepted 13 September 1991

Pre-Bötzinger Complex: A Brainstem Region That May Generate Respiratory Rhythm in Mammals

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The location of neurons generating the rhythm of breathing in mammals is unknown. By microsection of the neonatal rat brainstem in vitro, a limited region of the ventral medulla (the pre-Bötzinger Complex) that contains neurons essential for rhythmogenesis was identified. Rhythm generation was eliminated by removal of only this region. Medullary slices containing the pre-Bötzinger Complex generated respiratory-related oscillations similar to those generated by the whole brainstem in vitro, and neurons with voltage-dependent pacemaker-like properties were identified in this region. Thus, the respiratory rhythm in the mammalian neonatal nervous system may result from a population of conditional bursting pacemaker neurons in the pre-Bötzinger Complex.

THE RHYTHM OF BREATHING ANIMATES mammalian life, and the source of this rhythm, the *noeud vital* (*1*), is unknown. The basic oscillator lies within the brainstem, but technical limitations of experiments in the mammalian nervous system in vivo have hindered localization of the neurons generating the rhythm (*2*). An in vitro preparation of neonatal mammalian brainstem and spinal cord that spontaneously generates respiratory rhythm (*3, 4*) allows application of a broader range of techniques and has provided insights into neural mechanisms controlling breathing (*5*). Analysis of synaptic mechanisms in this preparation has led to the hypothesis that conditional pacemaker neurons in the medulla are the kernel for rhythm generation (*6*). Further tests of this hypothesis and network-based models (*2, 7*) of rhythmogenesis require identification of the sites and specific cells producing the rhythm. In our experiments we have systematically microsectioned the in vitro neonatal rat brainstem and precisely localized regions with neurons critical for rhythmogenesis (*8*).

Serial transverse microsections (50 to 75 μm thick) were made with a Vibratome, either caudally along the brainstem, starting from the pontomedullary junction, or rostrally from the spinomedullary junction, and perturbations of rhythmogenesis were analyzed (*9*). Rostral to caudal sectioning (n = 20 experiments) did not perturb the frequency of inspiratory phase motor discharge (of phrenic and other respiratory motoneurons) until the level of caudal retrofacial nucleus; further sectioning induced instabilities in the rhythm and then eliminated rhythmic motor output (*10*) (Fig. 1). Perturbations of rhythmogenesis occurred only with the removal of sections at this level of the medulla. More caudal medullary regions were not essential for rhythm generation, because sectioning rostrally from the spinomedullary junction (n = 11) did not disrupt respiratory motor output on cranial nerves (*11*) until a level rostral to the obex, within 200 μm of the level causing cessation of respiratory output in the rostral to caudal sectioning experiments. Transections in horizontal planes (n = 11), which removed regions dorsal to nucleus ambiguus (Fig. 1) but left intact motor circuits of the ventral medulla (*2*), did not alter the rhythmic discharge of medullary motoneurons (*12*).

Neurons essential for respiratory rhythmogenesis thus appear localized in the ventral medulla just caudal to the level of retrofacial nucleus. Rhythmically active neurons are localized within this critical area (*2, 4,*

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