strains were provided by P. Nguyen-Dinh and C. C. Campbell of the Centers for Disease Control. The FCR-3 strain was provided by W. Trager of Rockefeller University.
11. W. Trager, M. Tershakovec, L. Lyandvert, H. Stanley, N. Lanners, E. Gubert, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6527 (1981).
12. I. J. Udeinya, J. A. Schmidt, M. Aikawa, L. H. Miller, I. Green, *Science* **213**, 555 (1981).
13. J. B. Jensen, *Am. J. Trop. Med. Hyg.* **27**, 1274 (1978).
14. G. R. Cokelet, in *Biomechanics: Its Foundations and Objectives*, Y. C. Fung, N. Perrone, M. Anliker, Eds. (Prentice-Hall, Englewood Cliffs, N.J., 1972), p. 63.
15. M. A. Pfaller, A. R. Parquette, D. J. Krogstad, P. Nguyen-Dinh, *Exp. Parasitol.* **54**, 391 (1982); A. Kilejian, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3695 (1980); C. Lambros and J. P. Vanderberg, *J. Parasitol.* **65**, 418 (1979).
16. J. R. Williamson, C. Kilo, S. P. Sutera, R. A. Gardner, C. W. Boylan, K. C. Chang, *Horm. Metab. Res. (Suppl. Ser.)* **11**, 103 (1981).
17. R. A. Gardner, G. L. Carroll, S. P. Sutera, in *Developments in Mechanics* (vol. 12, *Proceedings of the 18th Midwestern Mechanics Conference, Iowa City, Iowa, 16 to 18 May 1983*), pp. 115–117.
18. R. M. Hochmuth, P. R. Worthy, E. A. Evans, *Biophys. J.* **26**, 101 (1979).
19. O. Linderkamp and H. J. Meiselman, *Blood* **59**, 1121 (1982); O. C. Pfafferot, R. Wenby, H. J. Meiselman, *Blood Cells* **8**, 65 (1982); T. M. Fischer, C. W. M. Haest, M. Stoehr, D. Kamp, B. Deuticke, *Biochim. Biophys. Acta* **510**, 270 (1978).
20. H. Ginsburg, M. Krugliak, O. Eidelman, Z. I. Cabantchik, *Mol. Biochem. Parasitol.* **8**, 177 (1983); S. Kutner, H. Ginsburg, Z. I. Cabantchik, *J. Cell Physiol.* **114**, 245 (1983).
21. S. Chien, K. L. P. Sung, R. Skalak, S. Usami, A. Toezeren, *Biophys. J.* **24**, 463 (1978).
22. N. Mohandas, M. R. Clark, M. S. Jacobs, S. B. Shohet, *J. Clin. Invest.* **66**, 563 (1980).
23. J. G. Sandza, Jr., R. E. Clark, C. S. Weldon, S. P. Sutera, *Trans. Am. Soc. Artif. Int. Organs* **20**, 457 (1974).
24. R. T. Card, N. Mohandas, P. L. Mollison, *Br. J. Haematol.* **53**, 237 (1983); G. K. Driessen *et al.*, *Pfluegers Arch.* **392**, 261 (1982).
25. B. Schnitzer, T. Sodeman, M. L. Mead, P. G. Contacos, *Science* **177**, 175 (1972).
26. T. C. Quinn and D. J. Wyler, *J. Clin. Invest.* **63**, 1187 (1979); D. J. Wyler, T. C. Quinn, L. T. Chen, *ibid.* **67**, 1400 (1981).
27. P. H. David, M. Hommel, L. H. Miller, I. J. Udeinya, L. D. Oligino, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5075 (1983).
28. Presented in part at the 58th Annual Meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, Texas, December 1983. We thank P. R. Rao for help with the rheoscope, C. D. Fitch for review of the manuscript, W. Trager, P. Nguyen-Dinh, and C. C. Campbell for providing the strains used to perform these studies, and J. P. Miller for help with the statistics. The research was supported in part by grant HL 12839 from the National Heart, Lung, and Blood Institute, by grant AI 18911 from the National Institute of Allergy and Infectious Disease, and by the Kilo Diabetes and Vascular Research Foundation.
* To whom requests for reprints should be addressed.

9 November 1983; accepted 16 November 1983

Salt Taste Transduction Occurs Through an Amiloride-Sensitive Sodium Transport Pathway

Abstract. *An important early event in mammalian gustatory transduction with respect to sodium chloride has been found to be the passage of sodium ions through specific transport pathways in the apical region of the taste bud. The inward current caused by sodium chloride placed on the mucosal surface of an in vitro preparation of rat dorsal lingual epithelium can be substantially reduced by the blocker of sodium ion transport, amiloride. The data show (i) that amiloride is a specific blocker of the chorda tympani response to sodium chloride, but not to potassium chloride, (ii) that the sodium and potassium gustatory systems are largely independent at the peripheral level, and (iii) that the classical ion taste "receptor" is actually a specific transport pathway permitting the cation to enter the taste-bud cell and thereby to spread depolarizing current.*

The classical interpretation of the events surrounding the excitation of mammalian taste-bud cells by salt is based on two fundamental assumptions:

1) The dorsal lingual epithelium, including the taste-bud cells, acts as an impermeable barrier against ions and other tastants.

2) The essential interaction between stimulus ions and taste buds is adsorption to an apical receptor.

From these assumptions follow models of salt taste transduction that have regarded penetration of the stimulus ions into the taste-bud cells as nonessential and in some cases impossible (*1*). However, the canine lingual epithelium actively transports ions, and the active transport system is particularly stimulated by NaCl concentrations spanning the gustatory range of 1 mM to 1M (*2, 3*). Furthermore, stimulation of the transport system by hyperosmotic NaCl can be blocked by amiloride (*3*). These results call into question the general validity of assumption 1, particularly regarding a barrier against ions. There remained the possibility, however, that assumption 1 might be valid for taste buds only. We now present evidence that the amiloride-sensitive sodium transport system includes the apical regions of the taste buds. The finding has important consequences for gustatory transduction. The conclusions are drawn from studies on the rat in which amiloride (i) inhibited the short-circuit current (I_{sc}) that results from placing hyperosmotic NaCl on the rat lingual epithelium in vitro and (ii) likewise inhibited the neural response to NaCl in vivo without effects on the KCl response.

Sprague-Dawley rats were used in both the in vitro and in vivo experiments. In the first case, rats were decapitated and the tongues removed. A section of the anterior dorsal epithelium was freed from the underlying skeletal muscle and placed between plexiglass chambers (*4*). Under symmetrical conditions in Krebs-Henseleit buffer (*5*), a steady open-circuit potential (V_{oc}) was achieved within 1 hour. The mean (± standard error) V_{oc} for 14 preparations was 13.4 ± 0.7 mV (inside positive). The I_{sc} was 8.2 ± 0.9 μA/cm^2, and the resistance was 1686 ± 147 ohm-cm^2. When the mucosal solution was replaced by a series of NaCl solutions ranging from 0.01M to 2M, I_{sc} increased in a graded manner (Fig. 1A). Like canine epithelium (*2, 3*), the rat epithelium responded to hyperosmotic NaCl with a two-component increase in V_{oc} when the adapting mucosal NaCl concentration of 0.001M was replaced by 1.5M NaCl (upper trace in Fig. 1B). This hyperosmotic NaCl response could be sharply attenuated by placing $10^{-4}M$ amiloride in the mucosal adapting solution. The lower trace in Fig. 1B is the response to 1.5M NaCl after a 5-minute exposure to $10^{-4}M$ amiloride in 0.001M NaCl. Both the rapid rise and the quasi-steady component were reduced. The corresponding reduction in I_{sc} extended over the entire hyperosmotic range (Fig. 1A). The effect was selective for NaCl over KCl (*3*).

If the amiloride-sensitive inward current extends to the taste-bud cells, and if it is coupled to early events in gustatory transduction and subsequent neural events, it should be possible to reversibly and specifically block the neural response to NaCl by rinsing the rat's tongue in amiloride. The entire NaCl

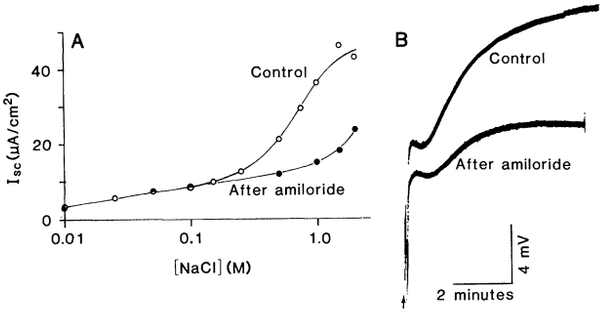

Fig. 1. (A) Short-circuit current (I_{sc}) resulting from NaCl solutions placed in the mucosal chamber. (B) Time course of the open-circuit potential after 0.001M NaCl in mucosal chamber was replaced with 1.5M NaCl.

concentration–neural response curve should be sharply attenuated over the hyperosmotic range, similar to the amiloride effect on I_{sc} in vitro. This should occur without corresponding effects on the KCl response.

We have demonstrated this phenomenon by observing the effects of amiloride on the integrated chorda tympani responses of rats stimulated with NaCl and KCl. The rats were anesthetized with sodium pentobarbital, and the chorda tympani was exposed according to conventional microsurgical methods (*6*). The nerve was suspended over a platinum electrode and recorded impulses were amplified, half-wave rectified, and integrated (time constant, 5 seconds). The results were displayed on a strip-chart recorder. Responses to NaCl (Fig. 2) and KCl were obtained by flowing salt solutions over the tongue in ascending order of concentration. Each trial was terminated by a water rinse followed by a second rinse, which was allowed to drain freely from the tongue for at least 1 minute. In Fig. 3A, the record on the left shows a typical response to 1*M* NaCl and on the right, the response after a 5-minute exposure to $10^{-4}M$ amiloride in water (*7*). Both the early phasic and later quasi-steady portions of the response were markedly attenuated, much as I_{sc} was when measured in vitro. The reduced amplitude of the chorda tympani response was observed at each tested NaCl concentration in the hyperosmotic range (Fig. 2). The neural response recovers from amiloride treatment within minutes (*7*).

The specificity of the suppression of the NaCl response is illustrated by the lack of an effect on the KCl response. In Fig. 3B, the record on the left shows a control response to 1*M* KCl. After treatment with amiloride, the response was nearly identical in both phasic and quasi-steady components. Drawing on the known effects of amiloride as a specific blocker of apical Na entry in a variety of transporting epithelia (*8*) and its specific effects on lingual Na current (*2*, *3*), it is reasonable to conclude (i) the taste-bud cells also contain an amiloride-sensitive Na transport pathway; (ii) this pathway plays a key role in NaCl taste transduction; (iii) the pathway serves simultaneously as a specific Na recognition site and the means by which depolarizing current is transmitted to the taste-bud cells; and (iv) this Na-selective pathway is distinct from that for K, indicating that K current is spread by alternative routes.

The general validity of these findings seems to extend to human taste. Schiffman *et al.* (*9*) have shown that amiloride can selectively attenuate NaCl perception in humans at comparable NaCl concentrations. The fact that NaCl taste is mediated in part by a Na current, largely of stimulus origin, removes one of the former major concerns surrounding the transduction mechanism—the mode of cell depolarization after first contact between stimulus and cell. Our findings that the peripheral Na taste apparatus is an ion transport system resembling other epithelial transport systems promises a fresh approach to the study of gustatory transduction.

Note added in proof: Teeter *et al.* (*10*) have recently shown that the chorda tympani response of the gerbil to both NaCl and LiCl is reduced by amiloride without significant effect on the KCl response. These results in the gerbil confirm ours in the rat and provide evidence for a common Na-Li transduction-transport pathway.

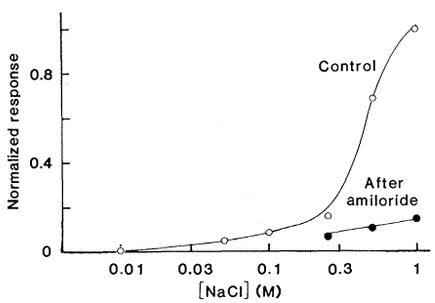

Fig. 2. Integrated chorda tympani response normalized to that of 1*M* NaCl (maximum) as a function of the NaCl concentration on the tongue. The response was taken as the mean displacement above baseline 1 minute after stimulus presentation.

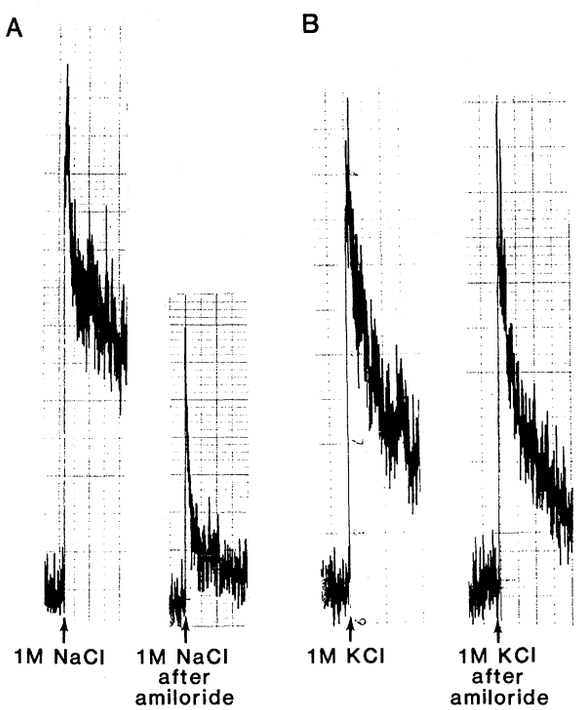

Fig. 3. Integrated chorda tympani responses to NaCl (A) and KCl (B). In both (A) and (B), the record on the left is the control and the record on the right shows the effect of a 5-minute exposure to $10^{-4}M$ amiloride. Excess amiloride was washed from the tongue before testing. The first 30 seconds ater stimulus presentation are shown.

GERARD L. HECK
SHEELLA MIERSON
JOHN A. DESIMONE
Department of Physiology and Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298

References and Notes

1. C. M. Mistretta, *Am. J. Physiol.* **220**, 1162 (1971); L. M. Beidler in *Olfaction and Taste* (Pergamon, New York, 1967), vol. 2, pp. 509–534; N. Kamo, M. Miyake, K. Kurihara, Y. Kobatake, *Biochim. Biophys. Acta* **367**, 1 (1974); L. M. Beidler and G. W. Gross, in *Contributions to Sensory Physiology* (Academic Press, New York, 1971), vol. 5, pp. 97–127; K. Kurihara, in *Transduction Mechanisms in Chemoreception* (Information Retrieval, London, 1974), pp. 163–176; J. A. DeSimone and G. L. Heck, *Chem. Senses* **5**, 295 (1980); T. Sato, *Jpn. J. Physiol.* **32**, 459 (1982).
2. J. A. DeSimone, G. L. Heck, S. K. DeSimone, *Science* **214**, 1039 (1981).
3. J. A. DeSimone, G. L. Heck, S. Mierson, S. K DeSimone, *J. Gen. Physiol.*, in press.
4. The tongue was removed caudal to the circumvallate region and placed dorsal side down on a hard rubber dissecting board. It was pinned along its periphery, and the muscle fibers were removed with microdissecting scissors and forceps under a dissecting microscope, beginning at the posterior end of the tongue and moving to the tip. A section of the dorsal epithelium, anterior to glandular portions of the epithelium, was placed between Lucite chambers, each with a volume of 5 ml. The tissue was held between silicone rubber gaskets. The open cross-sectional area of the chamber was 0.4 cm^2. Potentials were measured between 0.15*M* NaCl-agar bridges in series with saturated calomel electrodes and recorded with a Keithley 610C electrometer with a strip-chart recorder. Current was passed through Ag-AgCl electrodes.

5. The buffer consisted of 118 m*M* NaCl, 6 m*M* KCl, 2 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 1.3 m*M* NaH_2PO_4, 25 m*M* $NaHCO_3$, and 5.6 m*M* glucose. When exposed to 95 percent O_2–5 percent CO_2 at 34°C, the *p*H was 7.4.
6. L. M. Beidler, *J. Neurophysiol.* **16**, 595 (1953).
7. Contact between the tongue and the amiloride solution was maintained by blocking the drain tube of the plexiglass flow chamber. The effect of amiloride was assessed by testing the three highest concentrations of NaCl and KCl. Recovery was monitored by sampling the response to 1*M* NaCl at various intervals. The phasic component recovered 72 percent and the steady state 50 percent in 15 minutes.
8. D. J. Benos, *Am. J. Physiol.* **242**, C131 (1982).
9. S. S. Schiffman, E. Lockhead, F. W. Maes, *Proc. Natl. Acad Sci. U.S.A.* **80**, 6136 (1983).
10. J. H. Teeter, W. L. Silver, J. G. Brand, *Soc. Neurosci. Abstr.* **9**, 1022 (1983).
11. We thank B. K. Coplon and S. K. DeSimone for technical assistance. Supported by NIH research grant NS 13767 and by the Brown and Williamson Tobacco Corporation.

20 October 1983; accepted 25 October 1983

Algal Chemical Defense Against Herbivores: Allocation of Phenolic Compounds in the Kelp *Alaria marginata*

Abstract. *Higher concentrations of phenolic compounds were found in the reproductive fronds (sporophylls) of the intertidal kelp* Alaria marginata *than were found in the vegetative blades. The sporophylls were consumed by herbivorous snails at a lower rate than were the vegetative blades, in both field and laboratory studies. These results indicate that differential internal production of defensive compounds in a marine alga can significantly affect the pattern of herbivory on the plant.*

Ecological and evolutionary theories often predict that the internal resources of an organism (such as energy, nutrients, and metabolites) should be allocated to different structures or functions in ways that maximize the organism's fitness (*1, 2*). Since fitness depends ultimately on the survival and growth of reproductive products, organisms should be under strong selection to allocate resources for protection of these reproductive parts against natural enemies.

Terrestrial plants have a wide variety of chemical and mechanical defenses in their fruits, seeds, or spores, that deter their natural enemies (*3*). The chemical defenses in the reproductive parts of terrestrial plants often differ quantitatively or qualitatively from those produced in the leaves or other vegetative parts of the same plant (*4*). Although many secondary compounds are common in marine plants (*5*), the interactions between these potentially defensive compounds and the herbivores that feed on them are just beginning to be investigated (*6–8*). Phenolic compounds, especially polyphenolics or tannins, are present in most or all species of brown algae (*9*) and deter feeding by invertebrate herbivores (*6, 7*). Differential feeding by invertebrate herbivores on vegetative and reproductive parts of marine algae has also been shown (*10*). I now report that the marine brown alga *Alaria marginata* allocates more phenolic compounds to reproductive parts than to vegetative blades, giving increased protection to its reproductive fronds against invertebrate herbivores.

Alaria marginata is a large (up to 3 m long), intertidal kelp (brown algae in the order Laminariales) from the Northeast Pacific Ocean (*11, 12*). Near the holdfast in the thallus of mature *A. marginata* are several to many pairs of reproductive fronds (sporophylls), which are morphologically distinct from the alga's single, large, vegetative blade (*11*).

In laboratory feeding experiments conducted over 2 years, sporophylls and vegetative fronds of *A. marginata* were offered to the intertidal herbivorous gastropod *Tegula funebralis* (*13*). The snails ate significantly more of the vegetative blade than of the sporophylls [*t*-test (*14*), $P < 0.001$ for both years] (Fig. 1A). The vegetative and reproductive tissues also differed chemically and physically. The sporophylls were significantly higher in total phenolics and tanning ability (*t*-test, $P < 0.001$ for both measures in both years) (Fig. 1B) (*15, 16*), not significantly different in nitrogen content (Fig. 1C), but somewhat tougher (*t*-test, $P < 0.05$) (Fig. 1D) than the vegetative blades.

I also assessed experimentally the amounts of herbivore damage on sporophylls and vegetative blades in the field by transplanting mature *A. marginata* plants into the intertidal in areas of "high" and "low" densities of *T. funebralis* (high, 35 to 105 snails per square meter; low, 8 to 15 snails per square meter) (Tables 1 and 2) (*17*). Algal tissue losses were analyzed by a two-way analysis of variance (herbivore density by tissue type; unequal but proportional cell sizes).

The results for measures of absolute (in square centimeters) and relative (as percentage of area) tissue loss are similar. The effect of tissue type was highly significant. More vegetative than sporophyllic tissue was lost in both high- and low-density herbivore treatments. The density of *T. funebralis* present was not a significant effect. The interaction between herbivore density and tissue type was significant. Loss of sporophyllic tissue was similar in the two levels of herbivores, but much more vegetative tissue was lost when herbivores were present in the higher density. These results show that when only a few herbivores or none are present, there is little tissue loss from both sporophylls and vegetative blades. Much of this is likely

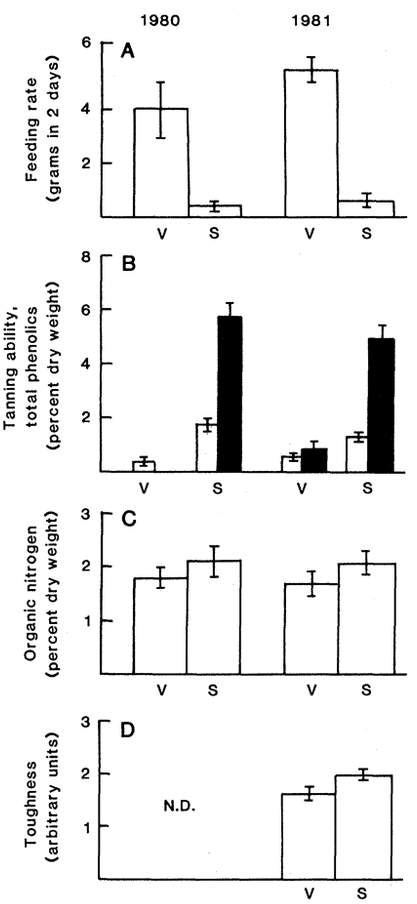

Fig. 1. Laboratory feeding experiments (A) and chemical (B and C) and physical (D) analyses of *A. marginata*; *V*, vegetative blades; *S*, sporophylls. Data are means ± standard errors (S.E.) for six plants. (A) Ten large (wet weight, about 10 g) *T. funebralis* were offered equal weights (about 10 g each) of *A. marginata* sporophylls and vegetative blades in 36-liter aquaria. After 48 hours, the algae were weighed again. Control aquaria contained algae but no snails. (B) For total phenolic content and tanning ability measurements, fresh algal tissue was ground in 50 percent methanol in a tissue macerator and extracted in the dark for 24 hours. Total phenolics were assayed by the Folin-Denis technique with phloroglucinol as the reference compound (*23, 24*). Tanning ability was measured by hemanalysis, with high molecular weight tannins from *Fucus vesiculosus* as reference compounds (*24–26*). (C) Organic nitrogen content was measured by the Kjehdahl technique (*27*). (D) Thallus toughness was measured with a "penetrometer" (*28*).