Influences of Dietary Sodium on Functional Taste Receptor Development: A Sensitive Period

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Restriction of maternal dietary sodium on or before embryonic day 8 reduced taste responses of the chorda tympani nerve to sodium chloride in the offspring. The response attenuation was substantial; responses to sodium chloride in the offspring of deprived rats were approximately 40 percent of those in control animals. Instituting the low sodium diet at embryonic day 10 or later did not produce functional changes. Thus, a sensitive period for the gustatory system exists, and the abrupt transition from maximal environmental susceptibility to no susceptibility occurs during a 2-day prenatal period. Moreover, events important in determining the developmental fate of taste membrane components occur before the initial formation of taste buds.

MATURATION OF NORMAL SENSORY function depends on proper environmental stimulation during well-defined periods of development. Such "sensitive periods" often occur when the developing system is especially susceptible to environmental modification (*1*). Sensory deprivation strategies have been used to characterize sensitive periods and to derive insights into both the developmental end points of these periods and the epigenetic factors important in normal development (*2*). Until recently, however, such strategies have not been used to characterize these periods for the sense of taste (*3*).

Given the characteristics of sensitive periods and the knowledge of normal functional development, restricted exposure to NaCl during early postnatal development should have a profound influence on the developing gustatory system (*4*). Indeed, there are dramatic alterations in normal behavioral and functional taste responses when NaCl exposure is restricted during early postnatal development. Specifically, sodium taste responses of the chorda tympani nerve in rats recorded after they are 28 days old are attenuated when they are fed a low sodium diet beginning at embryonic day 3, whereas responses to other taste stimuli are unaffected. Alterations in functional responses do not occur in rats deprived as adults (*3*).

A possible prenatal sensitive period was studied by instituting maternal sodium deprivation at various ages after conception and then comparing multifiber chorda tympani responses among deprivation groups. Pregnant Sprague-Dawley rats were fed a NaCl-replete diet (1.0% NaCl) from conception to the embryonic day on which deprivation began. At embryonic day 3, 8, 10, 12, or 15, a NaCl-replete diet was exchanged for a diet in which the dietary NaCl content was 0.03% (*5*). After birth the offspring were continually maintained on the 0.03% diet for at least 28 more days (*6*).

Multifiber neural activity from the whole chorda tympani nerve was recorded (*7*) while the anterior tongue was stimulated with a concentration series (0.05*M* to 0.5*M*) of NaCl, NH_4Cl, and KCl. All chemicals were dissolved in distilled water and kept at room temperature during experiments. Fifteen milliliters of each stimulus were applied to the anterior tongue with a gravity flow system (8 ml/s), and approximately 20 s later, a distilled water rinse was applied. After responses to the three concentration series were completed, chromatography paper soaked in 500 μ*M* amiloride hydrochloride was placed on the anterior two-thirds of the tongue for 5 min. The paper was then removed, and the tongue was stimulated again with the concentration series of NaCl. However, the solvent and rinse for these concentration series contained 500 μ*M* amiloride. Amiloride can affect a host of cellular processes involving sodium in epithelia but has no apparent effect on excitable tissue such as neural tissue (*8*). It was not our intent to identify which process is involved in sodium taste transduction. Rather, we were interested in learning whether any of the cellular processes are affected by the dietary manipulation.

For data analysis, the amplified neural signal was passed through an integrator with a time constant of 0.5 s, the output of which was displayed on a rectilinear pen recorder (*9*). The steady-state portion of the response was measured from the output of the pen recorder. This measure of neural response reflects the sum of single-fiber responses (*10*) and is an appropriate measure for studying responses from a large population of taste receptors (*9*).

The embryonic age at which the NaCl-replete maternal diet was exchanged for the 0.03% NaCl diet had a profound influence on the outcome of sodium taste sensitivities in adulthood. Our results indicate that the developing peripheral gustatory system changes in its susceptibility to sodium deprivation within a period of 2 days. When neural recordings were made at least 50 days after diets were switched from 1.0% to 0.03% NaCl, relative responses to NaCl were attenuated only in rats initially deprived at embryonic days 3 and 8. In contrast, NaCl responses in rats deprived at 10, 12, and 15 days were similar to responses of rats that had only been fed the 1.0% NaCl (control) diet (Figs. 1 and 2). For example, responses to 0.5*M* NaCl in rats deprived at embryonic day 8 were 50% of those in rats always fed the 1.0% NaCl diet, whereas responses in rats deprived only 2 days later were at least 90% of the control response. Responses to KCl and NH_4Cl were unaffected for all deprivation groups; this finding confirmed earlier results (*3*). Mean KCl and NH_4Cl responses in the 8-day group were within 15% of the respective control mean ($P > 0.10$). Thus, the effects described here occur only for sodium, the dietary constituent that was manipulated.

The differential sensitivities to NaCl among deprivation groups were eliminated after lingual application of amiloride (Fig. 3). Amiloride suppressed responses to NaCl by at least 50% in rats in which the maternal diet was switched to the low sodium diet at 10 days or later. In contrast, amiloride suppressed NaCl responses in the 3- and 8-day deprivation groups by less than 12%. This difference in NaCl response suppression by amiloride resulted in similar responses among groups (Fig. 3A). Since amiloride affects transport properties on the apical

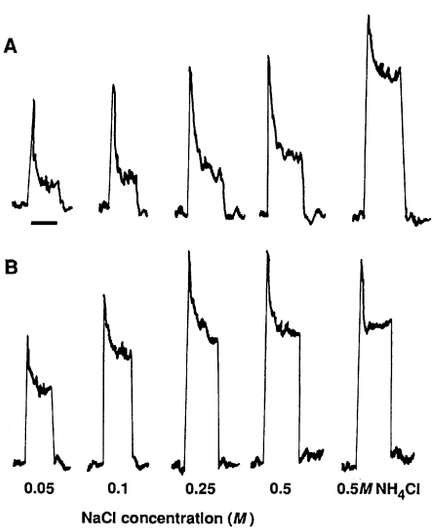

Fig. 1. Integrated responses from the chorda tympani nerve to a concentration series (0.05*M* to 0.5*M*) of NaCl and 0.5*M* NH_4Cl in a rat that the 0.03% NaCl diet was imposed at (**A**) embryonic day 8 or (**B**) embryonic day 10. Scale bar is 15 s.

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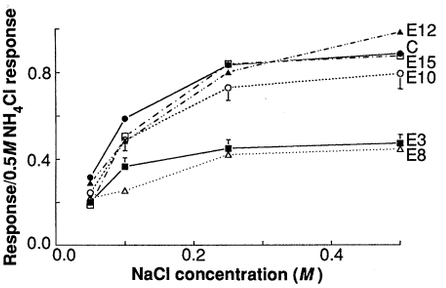

Fig. 2. Mean response ratios from the chorda tympani nerve to a concentration series of NaCl in rats deprived at embryonic day (E) 3 (n = 16), day 8 (n = 7), day 10 (n = 6), day 12 (n = 14), and day 15 (n = 12), and from rats fed the control (C) diet (n = 28). Standard error bars are shown for the embryonic day 3 and 10 groups. Each group was composed of rats from at least two different litters.

taste receptor membrane and it eliminates differences among our deprivation groups, we suggest that the dietary manipulations affect the functional development of specific membrane components sensitive to sodium. Thus, even though our measurements are from the primary afferent nerve and not directly from the receptors, we infer that the receptors must be affected (*9*).

The sensitive period in which maternal dietary manipulations have such profound influences on the development of sodium taste sensitivities is especially intriguing when placed within the time course of normal gustatory system development. Taste buds first appear in fungiform papillae at approximately 21 days after conception (at birth) (*11*). Amiloride-sensitive membrane components important in sodium taste transduction are first expressed at approximately 32 days after conception, as inferred from chorda tympani recordings (11 days after birth) (*12*). Thus, the end of the sensitive period is 13 days before the appearance of the first taste buds and 24 days before expression of amiloride-sensitive membrane components.

Given the rapidity with which rats experience the physiological effects of sodium deprivation (*13*), it seems likely that such effects would occur before the period of taste bud formation. Even if the effects of deprivation instituted during the sensitive period occurred after taste bud formation, it is unlikely that direct stimulation of taste receptors by differing amounts of sodium would determine the length of the period. Sodium levels in a variety of fetal tissues and fluids are protected from sodium deprivation by the mother (*14*). That is, even though maternal sodium levels are depleted under dietary NaCl restriction, sodium levels in the offspring are similar to controls. Thus, the sensitive period probably does not depend on direct contact of taste receptors by sodium (*15*). Alternative mechanisms may relate to factors in the mother or embryo during the sensitive period that would preclude normal maturation of sodium taste sensitivity. Low dietary NaCl levels before embryonic day 8 may have significant effects on maternally produced substances that cross the placenta and exert an influence on the offspring. For example, levels of maternal hormones or growth factors important in inducing amiloride-sensitive membrane components could be altered in the mother and expressed in the offspring by a lack of sodium taste sensitivity. Additionally, NaCl deprivation instituted before day 8 may alter the synthesis or action of factors in the embryo that are not directly related to sodium levels (*16*). For example, metabolic substrates of hormones and growth factors synthesized in the embryo could be altered as a result of a less than optimal maternal diet. Presumably, after embryonic day 8, the system would be protected from the effects of maternal dietary NaCl depletion. Regardless of the specific mechanism, the process would be much different from that proposed for other developing sensory systems where the susceptibility to environmental influences focuses on sensory stimulus-receptor interactions (*2*). The existence of such a process, however, does not rule out the coexistence of direct stimulus-receptor interactions.

We have demonstrated environmentally induced functional changes in the peripheral gustatory system. Furthermore, the magnitude of environmentally induced alterations in developing taste responses is similar to that seen only at higher neuronal levels in other sensory systems. Receptor systems are virtually unaffected by enviromental manipulations (*17*). Thus, the peripheral gustatory system seems unique in that the formation of a functional transducing unit can be influenced by environmental conditions, and the formation of this unit may be directly dependent on systemic factors that require sodium for their synthesis and expression.

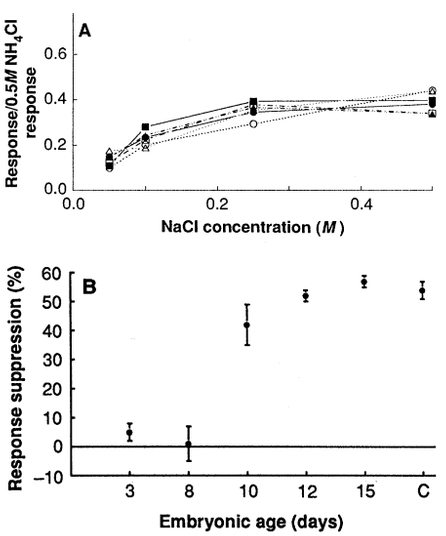

Fig. 3. (**A**) Mean response ratios from the chorda tympani nerve to a concentration series of NaCl in rats deprived at embryonic day 3 (■) (n = 9), day 8 (△) (n = 5), day 10 (○) (n = 6), day 12 (▲) (n = 13), and day 15 (□) (n = 11), and from rats fed the control (●) diet (n = 23) after lingual application of amiloride. Each group was composed of rats from at least two different litters. (**B**) Percentage inhibition of responses to 0.5M NaCl by amiloride. Amiloride was ineffective in suppressing 0.5M NaCl responses in rats deprived at embryonic days 3 and 8 (less than 5% suppression), whereas most of the responses in the other four groups were suppressed by at least 40%. Standard error bars are shown for each group. C, control.

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5. Diet was from ICN Nutritional Biochemicals. All rats had free access to distilled water throughout the study.
6. Recordings were begun at 28 days after birth because mature responses do not occur before this age.
7. Rats were anesthetized with an intraperitoneal injection of urethane (0.9 g per kilogram of body weight), and supplementary injections were given as needed to maintain a surgical level of anesthesia. The left chorda tympani nerve was then exposed, cut near its entrance into the tympanic bulla, and dissected from underlying tissues. The nerve was desheathed and placed on a platinum electrode with an indifferent electrode in nearby tissues. Standard ac electrophysiological techniques were used. Each rat was killed with a lethal dose of urethane after completion of the experiment. Detailed descriptions of surgical and recording procedures are in (*3*).
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the age of the rat, absolute measures are not used in such recordings. Rather, within-experiment procedures are used to make comparisons among animals and groups. The procedure used almost exclusively in chorda tympani nerve recordings is to express each response (for example, NaCl response) relative to a standard response (for example, 0.5*M* NH_4Cl response) recorded from the same preparation in the same stimulation series. Responses were used only when the response to 0.5*M* NH_4Cl deviated less than 10% within a preparation.
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Middle Archaic Period Domestic Architecture from Southern Peru

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Domestic or residential structures ranging in age from 6040 to 6850 years old have discovered at the open air site of Asana in the high sierra of southern Peru. These are the earliest domestic structures known from the high elevation zones of the south central Andes, and they are contemporaneous with sites with structures on the north Chilean littoral. Analysis of site structure and content suggests that during the Middle Archaic the site was a logistical camp within a dry *puna*–high sierra settlement system.

NO DOMESTIC ARCHITECTURE AT open air sites dating to the Archaic Period (10,000 to 4,000 years ago) has been discovered at elevations above 3000 to 4800 m in the south central Andes, nor from models of settlement patterns in the region would the existence of such be expected (*1*). Although some activities were performed at open air sites on the dry *puna* and sierra, caves and rockshelters were thought to be the residential bases. Sites in the open air would, therefore, be expected to be ephemeral, and materials found there would reflect daily food-gathering activities. Artifact density and diversity should be very low, and structures would not be expected (*2*). Furthermore, Santoro and Nuñez (*1*) argued that the high elevation zones of the region were abandoned during the Middle Archaic as a result of climatic deterioration and vulcanism. The discovery of domestic structures at an open air site of Middle Archaic date has not only forced a major revision of current hypotheses on sierra and *puna* land use, but has also necessitated a reexamination of models of the sedentarization process—the causes of the changes in the frequency and scale of residential mobility seen throughout the Archaic in this region (*3*).

The site of Asana is located at 17°06′S, 70°38′W at an elevation of 3450 m. It is situated on the northern side of the Rio Asana, one of the major tributaries of the Rio Osmore. The site is covered with 3 m of blocky colluvium deposited by a landslide that occurred within the past 200 years. Although the total size of the site is not known, it is estimated to be at least 800 to 1000 m^2.

Excavations in 1986 and 1987, exposing a total of 40 m^2, have revealed a stratified archeological deposit that ranges in thickness from 2 to 3 m. More than 75 natural soil strata have been identified and, within these, at least 36 distinct occupations can be distinguished (Fig. 1). Occupation at the site ranges from about 3600 to 9600 years ago.

Domestic structures have been discovered in three levels (XIVB, XVIIA-C, and XVIIA-B), and charcoal obtained from middens, hearths, or other features has been dated to the Middle Archaic. The soil matrix in which the structures are found is a fine sand with a small proportion of silt. When floors are stratigraphically contiguous, as they are for the eastern structure in levels XVIIC to XVIIIB, they are separated by 1 to 3 cm of culturally sterile sands and very fine colluvial gravels. When they are not, they can be separated by as much as 15 cm of homogeneous sands and fine gravels.

The structures, circular or ovoid in form (Fig. 2), have been constructed through the puddling, pooling, and shaping of a locally available white clay on a cleaned, prepared surface. This results in a floor elevated slightly above the substrate upon which it has been placed. In some levels, the clay is mixed with fine alluvial sands. The thickness of the floors varies from 2 to 4 cm. The floors have been placed between large colluvial boulders that served as windbreaks. The margins of the floors are surrounded at irregular intervals by post molds that range in size from 4 to 8 cm in diameter and from 2 to 5 cm in depth. No burned clay or other preserved material that could have served as a daub or wall filler is found in association with either the floors or post molds. This suggests that the walls of these structures were constructed of brush. Small colluvial rocks were placed along the edges of the puddled clay floors and were probably used to support post molds or to anchor the brush walls. None of these rocks show any signs of modification or use. In some of the structures, there is a large post mold (about 10 to 15 cm in diameter and 5 to 8 cm in depth), located near or at the center of the structure, which probably supported a brush roof.

In levels with multiple structures, they are archeologically contemporaneous. Each floor is well delineated, and there is no overlap in features or post-mold alignments indicative of a palimpsest reoccupation of the site. This inference is further supported by noting that those floors presumed to be contemporaneous follow the natural southward slope of this portion of the site. There is no evidence of rebuilding or changing the size or shape of the prepared clay floors. The sizes of the floors vary but remain relatively constant through time (Table 1), and the floor dimensions are similar to those of structures found at short-term camps of ethnographically known arid lands foragers (*4*).

Associated nonarchitectural features in-

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