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Ion Transport in Rat Tongue Epithelium In Vitro: A Developmental Study

A. MARK SETTLES AND SHEELLA MIERSON[1]

School of Life and Health Sciences, University of Delaware, Newark, DE 19716

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SETTLES, A. M. AND S. MIERSON. *Ion transport in rat tongue epithelium in vitro: A developmental study.* PHARMACOL BIOCHEM BEHAV **46**(1) 83–88, 1993.—The responsiveness of the rat gustatory system to monochloride salts changes during development. Neurophysiological recordings in the chorda tympani indicate that a) the taste responses to NaCl and KCl in early postnatal rats are small relative to NH_4Cl, b) both salts become more potent stimuli as the animal matures, and c) the developmental increase is accompanied by an increase in sensitivity of the NaCl response to the sodium transport blocker amiloride. We measured ion transport properties of in vitro tongue epithelia from Wistar rats. When the tissue is mounted in an Ussing chamber, the short-circuit current responses to NaCl and KCl are small in the neonatal rat and increase during development in postweaning and adult animals. Amiloride sensitivity of the NaCl response also increases with age. This study confirms that increased sensitivity of the rat gustatory system to NaCl with age reflects changes in the peripheral membranes. The results support the hypothesis that the increased sensitivity is due to amiloride-sensitive membrane components being added or becoming functional.

Salt taste Amiloride Transduction Lingual epithelium Ion transport Development

TASTE nerve recordings indicate that the responsiveness of the rat gustatory system to monochloride salts changes during development. Whole chorda tympani nerve responses to NaCl, LiCl, and KCl in early postnatal rats are small relative to NH_4Cl; they become more potent stimuli as the animal matures (7,22). In recordings from single chorda tympani fibers, response frequencies to NaCl and LiCl increase during development (11). As shown in Fig. 1, the developmental increase is accompanied by an increase in sensitivity of the NaCl or LiCl response to the Na^+ ion transport blocker amiloride, as measured in whole chorda tympani recordings (10). Hill and Bour (10) hypothesized that amiloride-sensitive peripheral membrane components underlying transduction for NaCl and LiCl are added or become functional during development. This study was conducted to test that hypothesis.

We used an in vitro preparation of the dorsal rat tongue epithelium. This preparation has been used extensively to study amiloride sensitivity in both rat and dog tongue epithelia (4–6,9,13,14,18,19). Patch clamp recordings confirmed that an amiloride-sensitive ion channel in taste cell membranes is a major Na^+ transducing element (1–3). We studied the responses to NaCl, KCl, and NH_4Cl, as well as the accompanying amiloride sensitivity, in the epithelium from rats of different ages. The results show age-related changes in ion transport and support Hill and Bour's hypothesis that the increase in amiloride sensitivity resides in the peripheral membranes.

METHOD

Tissue preparation and recording apparatus were similar to those described previously (15). Wistar rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were killed either with sodium pentobarbital or by placing in a CO_2 atmosphere; no difference was observed in the epithelial response between the two methods. The tongue was pinned dorsal side down on a rubber dissecting board and the muscle fibers removed under a dissecting microscope. The tissue was sandwiched between two flat silastic gaskets to which it was attached with cyanocrylate adhesive and clamped in a modified Ussing chamber. Tissue cross-sectional area was 0.24 cm^2; the chamber was approximately 5 ml on each side. Voltage was measured between 0.9% NaCl-agar bridges in series with calomel electrodes using an automatic voltage clamp (Physiologic Instruments, San Diego, CA); current was passed through 0.9% NaCl-agar bridges in series with Ag-AgCl electrodes. Short-circuit current (I_{sc}) was monitored on a strip-chart recorder; transepithelial resistance (R) was determined by pulsing current for 1 s (± 5 mV); potential difference (PD) was calculated from I_{sc} and R. The tissue was bathed in Krebs-Henseleit buffer (K-H), consisting of 118 mM NaCl, 5.6 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.3 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 5.6 mM glucose; pH was 7.4 when the solution was bubbled with 95% O_2/5% CO_2. The chamber was kept at 34°C. All chemicals were reagent grade.

[1] To whom requests for reprints should be addressed.

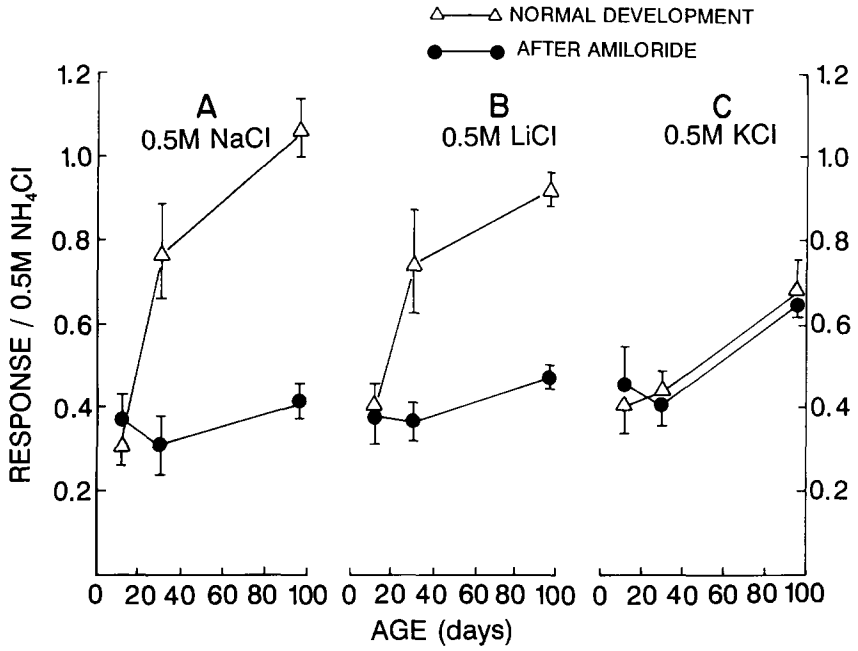

FIG. 1. Average response ratios from whole chorda tympani recordings to 0.5 M NaCl (A), 0.5 M LiCl (B), and 0.5 M KCl (C) compared to 0.5 M NH_4Cl in rats aged 12–13 days, 29–31 days, and 90–110 days before and after lingual application of amiloride (0.5 mM). SE bars are denoted for each mean; $n = 5$. Reprinted from (10) with permission.

Amiloride HCl was obtained from Sigma Chemical Co. (St. Louis, MO).

The tissue was mounted with K-H on both sides; after voltage and temperature reached steady state (30–60 min), the tissue was clamped to zero voltage. To obtain concentration-response functions, the mucosal solution was replaced by increasing concentrations of the first salt beginning with 0.01 M and waiting for I_{sc} to come to a steady state (at least 5 min) before going to the next higher concentration. At the end of one series, the tissue was allowed to equilibrate with K-H on the mucosal side, and then the series was begun for a second salt. All three salt series were presented in one tissue: The order of presentation among the three salts (NaCl, KCl, and NH_4Cl) was systematically varied from one experiment to an-

TABLE 1

STEADY-STATE ELECTRICAL PARAMETERS OF RAT TONGUE EPITHELIUM IN VITRO

	Age (days)		
	11–13	29–31	90–110
I_{sc} ($\mu A/cm^2$)	4.2 ± 0.3	7.5 ± 0.7	9.9 ± 0.9
PD (mV)	6.0 ± 0.4	10.1 ± 0.4	10.6 ± 0.6
R (Ω-cm^2)	1,455 ± 103	1,517 ± 144	1,162 ± 99
(*n*)	(22)	(19)	(18)

Values are means and SEMs, measured in an Ussing chamber with Krebs-Henseleit buffer on both sides. I_{sc}, short-circuit current; PD, transepithelial potential difference; *R*, transepithelial resistance; (*n*), number of experiments.

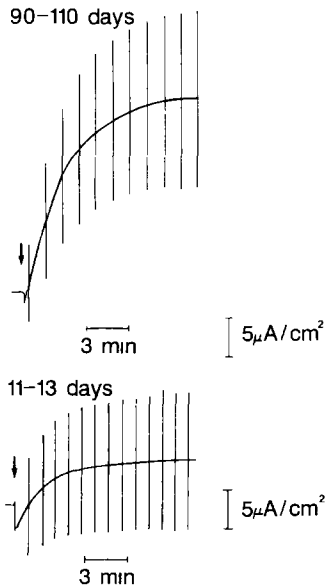

FIG. 2. Typical I_{sc} responses to 0.5 M NaCl in in vitro tongue epithelia from rats aged 11–13 days (A) and 90–110 days (B). The rinse solution is 0.01 M NaCl. The bottom of the vertical bar indicating the scale for I_{sc} is at 0 $\mu A/cm^2$ for each graph. Current excursions are in response to a bipolar 5-mV voltage pulse; the length of the excursion is proportional to tissue conductance.

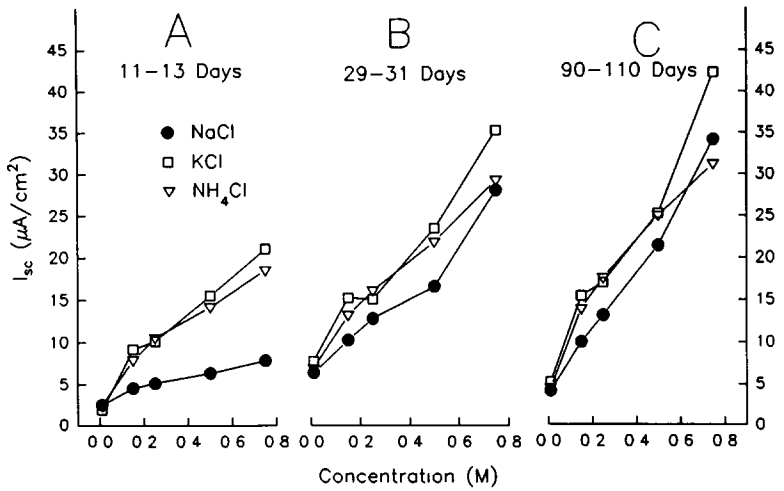

FIG. 3. Steady-state I_{sc} vs. concentration for three salts in in vitro lingual epithelia from rats aged 11–13 days (A), 29–31 days (B), and 90–110 days (C); $n = 3$.

other. Serosal K-H was changed at least once an hour to assure adequate supply of nutrients to the tissue.

For the measurements of amiloride sensitivity, the response of each tissue to 0.5 M concentration of two salts was measured before and after exposure to amiloride. The protocol was as follows: a) Change the mucosal solution from 0.01 M NaCl to 0.5 M of the first salt (NaCl, KCl, or NH_4Cl); b) return to symmetrical K-H until I_{sc} reaches steady state; c) repeat (a) and (b) for a second salt; d) replace the mucosal solution with 0.01 M NaCl; e) after I_{sc} reaches steady state, add 0.5 mM amiloride (in 0.01 M NaCl) to the mucosal solution for 5 min; f) repeat steps (a)–(c) to determine the response to the same two salts after amiloride. The epithelium from the adult animal is hardy and responds to 0.5 M NaCl for many hours with no diminution in response; however, the tissue from the neonatal animal shows a diminution in response after only a few stimuli. Hence, only two salts were presented to one tissue rather than all three; to be consistent, the same protocol was followed for all age groups. In addition, it was necessary to carry out control experiments to correct for length of time in the chamber. These were identical to the above protocol except no amiloride was added. Using the control data, a correction factor was derived for each age group, salt, and electrical parameter (I_{sc} or *PD*). This factor consisted of the average value of the ratio of the first response to a salt divided by its second response. Five amiloride experiments and five control experiments were done for each salt in each age group. The response after exposure to amiloride was

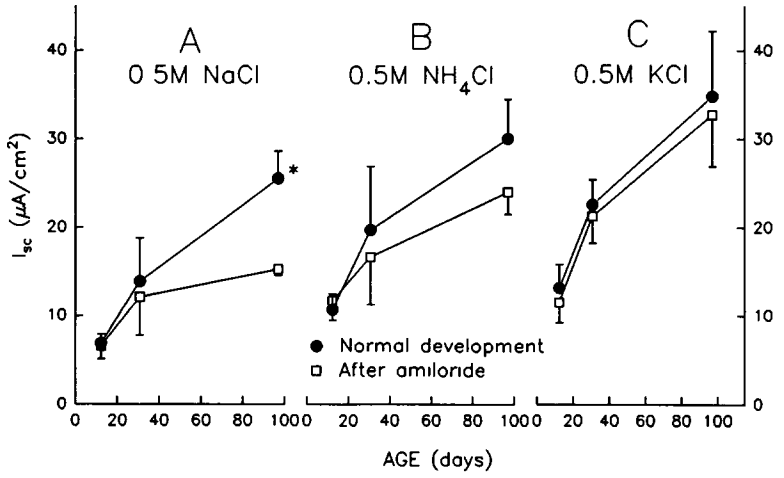

FIG. 4. Steady-state I_{sc} responses to three salts in in vitro tongue epithelia from rats aged 11–13 days, 29–31 days, and 90–110 days before and after exposure to amiloride (0.5 mM) in the mucosal solution. SE bars are shown for each mean ($n = 5$). Data after amiloride are corrected for length of time in the Ussing chamber (see text). *Statistically significant decrease due to amiloride; the percentage decrease was compared between amiloride and control experiments ($p < 0.05$, Student's *t*-test).

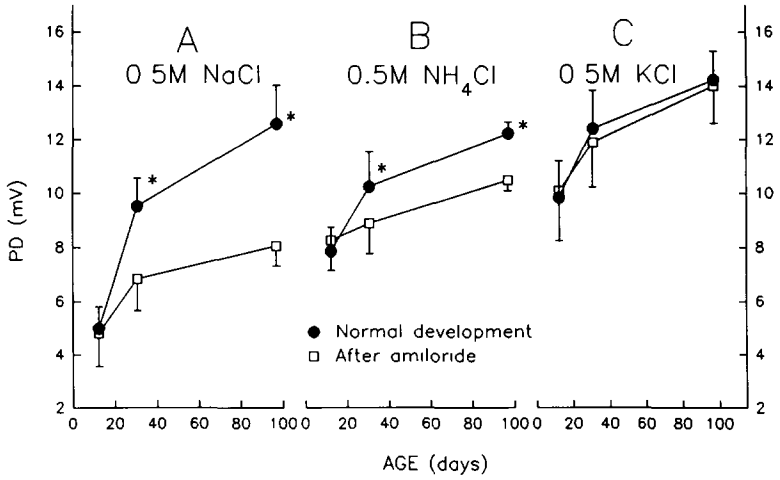

FIG. 5. PD responses to three salts in rat tongue epithelia. Conditions are the same as in Fig. 4.

multiplied by the correction factor from the control experiments. To determine the statistical significance of the decrease in response after amiloride, the percentage decrease from the first application to the second application of a salt was compared between amiloride experiments and control experiments.

RESULTS

The electrical parameters in symmetrical K-H show developmental changes. As can be seen in Table 1, both I_{sc} and *PD* increase with age. The I_{sc} response to a hyperosmotic salt solution is small for the neonatal rat relative to that for the adult animal, as shown in Fig. 2. I_{sc} concentration-response functions for three salts are given in Fig. 3 for the three age groups. The slope of the function is less for each salt in the neonatal animal than that in the adult, with the biggest change occurring for NaCl. In the young animal, at all concentrations above 0.01 M the response to NaCl is smaller than the response to KCl or NH_4Cl; in the adult animal, the responses to all three salts are more comparable.

The steady-state I_{sc} response of the tissue to 0.5 M NaCl, NH_4Cl, and KCl before and after amiloride is shown in Fig. 4 for each age group. Figure 5 shows the potential difference and Fig. 6 the transepithelial resistance for the same experiments. Amiloride has no effect on the response to any salt in the neonatal rat. The effect of amiloride on the I_{sc} response to NaCl is pronounced in the 3-month-old animal. In the 1-month-old rat, the PD response shows amiloride sensitivity that is statistically significant; the amiloride sensitivity of the I_{sc} response at that age is not significant. Both I_{sc} and *PD* responses to KCl are not amiloride sensitive at any age. The response to NH_4Cl shows a small amiloride sensitivity in the 1- and 3-month-old.

To determine if the developmental change is hormone mediated, we exposed the neonatal tissue to serum from an adult animal. Serum was prepared from blood collected by cannulating the femoral artery of adult, male Wistars. The serum

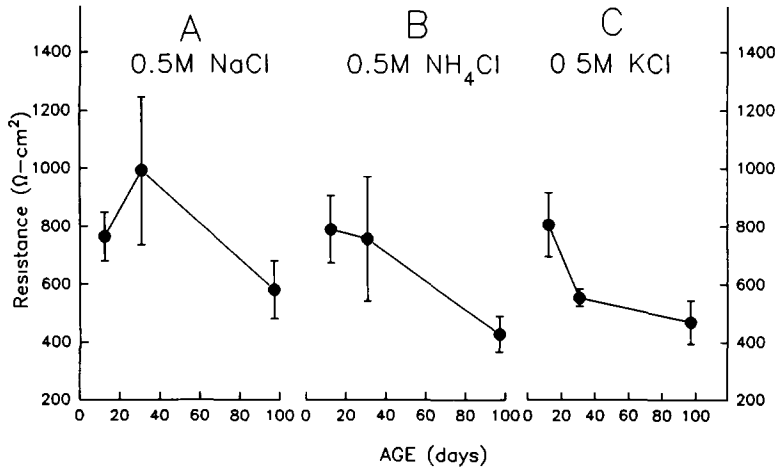

FIG. 6. Transepithelial resistance in response to three salts in rat tongue epithelia before amiloride presentation (n = 5). There was little or no change after amiloride.

was frozen overnight and used the next day. Epithelia from 13- to 22-day-old rats were mounted in the Ussing chamber and exposed to mucosal 0.01 M NaCl followed by 0.5 M NaCl to get a baseline response. After returning the tissue to symmetrical K-H, the serosal solution was replaced by either 10% serum diluted in K-H or 100% serum. The I_{sc} response to mucosal 0.5 M NaCl was measured once an hour; fresh serum was placed on the serosal side every hour. No effect was seen due to either concentration of serum over a 3-h exposure, which was as long as the tissue from the young animal was viable in the Ussing chamber.

DISCUSSION

This study shows that there is an increased sensitivity of the in vitro lingual epithelium to NaCl, KCl, and NH_4Cl as measured by I_{sc} in the Ussing chamber. Amiloride sensitivity of the NaCl response is nonexistent in the neonatal rat and develops with age, approximately paralleling the development of amiloride sensitivity as measured in whole chorda tympani recordings (10). These results confirm that increased sensitivity of the rat gustatory system to NaCl with age reflects changes in the peripheral membranes and provide support for the hypothesis that the increased sensitivity is due to amiloride-sensitive membrane components either being added or becoming functional.

Although the experiments reported here cannot distinguish between amiloride-sensitive apical Na^+ channels being added or becoming functional, the latter is the more likely possibility. Immunohistochemical evidence shows that amiloride-sensitive Na^+ channels are present in the taste buds of even 1-day-old rats, suggesting that the onset of amiloride sensitivity is due to activation of quiescent channels already present in taste receptor cell membranes (21).

It is not known what factors cause the activation of the apical channels. The experiment reported here with whole blood serum from adult animals was a beginning attempt to identify those factors. However, the negative result cannot be interpreted to mean that hormones are not involved. Three hours after adding serum may be insufficient time for the hormone(s) to have an observable effect. The relevant hormones may be present or at elevated concentrations only at particular stages in development. The effect could be due to a combination of hormone(s) plus some other factor. Alternatively, access to the basolateral membrane may be inadequate through the muscle and connective tissue on this preparation (14).

It is possible that membrane components besides the apical Na^+ channel also change with age. The fact that the I_{sc} responses to all three salts increase with age (Fig. 4) suggests that other components may vary. It is not known if there are changes in the activity of the Na,K-ATPase, in the structure of the tight junction, etc. In the literature, there are examples of other epithelia that show a variety of transport-related changes with development (8,12,16,17,20).

Changing sensitivities to monochloride salts with age have been observed not only in taste nerve recordings but also in behavioral studies of taste in rodents and humans [see (10) for a list of references]. This study shows that the in vitro Ussing chamber preparation can be useful for studying development of salt taste. It is hoped that the preparation can be used further to shed light on the factors that activate the Na^+ channels.

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