Lingual Epithelium of Spontaneously Hypertensive Rats Has Decreased Short-Circuit Current in Response to NaCl

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SUMMARY Alterations in ion transport associated with hypertension have been found in a variety of organs. We used a modified Ussing chamber to compare the NaCl dependence of the short-circuit current across the dorsal lingual epithelium in vitro from spontaneously hypertensive rats (SHR) with that from Wistar-Kyoto rats (WKY). The short-circuit current in response to mucosal NaCl was less in SHR than in WKY at hyperosmotic concentrations (above 0.15 M and up to 2.0 M). Since ion transport in the lingual epithelium has been found to play a role in early events of salt taste transduction, the attenuation in the short-circuit current in hypertensive animals may be a factor in the enhanced salt preference of SHR compared with WKY. (Hypertension 11: 519–522, 1988)

KEY WORDS • lingual epithelium • ion transport • hypertension • taste • spontaneously hypertensive rats

THE epithelial cells of the mammalian dorsal tongue transport sodium and other ions, and a notable body of evidence indicates that ion flow through taste bud cells mediates salt taste.1–7 In vitro measurements of ion transport in rat and canine lingual epithelia and recordings from rat chorda tympani suggest that salt taste transduction is mediated in part by apical membrane ion transport pathways and that sodium and potassium traverse the membrane by different routes.1,2 Hyperosmotic NaCl on the mucosal side causes an increase in inward current due largely to an increase in sodium influx.3 The increase in transepithelial inward current is also seen in vivo in the rat and is temporally correlated to the chorda tympani response to NaCl.3 Although the lingual ion transport system has now been characterized in a number of respects, virtually nothing is known about its regulation or possible alteration in diseases. In this article we report modifications of lingual ion transport associated with hypertension.

Alterations of ion transport associated with hypertension have been found in a variety of organs. We hypothesized that ion transport might be attenuated in the lingual epithelium,9 given its role in NaCl taste transduction, and that such a modification might be a factor in the enhanced salt preference in hypertensive versus normotensive rats.10,11 To test this hypothesis, we used a modified Ussing chamber to investigate the response of the voltage-clamped epithelium in vitro from spontaneously hypertensive rats (SHR) and control Wistar-Kyoto rats (WKY). We measured the electrical parameters of each tissue in response to test salt solutions. Our hypothesis was verified in the case of NaCl: The short-circuit current in response to NaCl in SHR was less than that in WKY at hyperosmotic concentrations. The implications for taste phenomena will be discussed.

Materials and Methods

The preparation of the tissue and the experimental apparatus have been described previously.1 Briefly, the animals were killed with sodium pentobarbital; the tongue was pinned dorsal side down on a hard rubber dissecting board, and the muscle fibers were removed under a dissecting microscope. The modification for this study was that the tissue was attached to a flat Teflon washer on the mucosal side with cyanoacrylate adhesive; this rested on a second identical washer on the serosal side, and the resulting sandwich was clamped by silicone rubber gaskets between two Lucite chambers. Each chamber had a volume of 5 ml; the
open cross-sectional area of the washer was 0.2 cm². Potentials were measured between 0.15 M NaCl-agar bridges in series with saturated calomel electrodes, using an automatic voltage clamp (Physiologic Instruments, Houston, TX, USA); current was passed through Ag-AgCl electrodes. The short-circuit current (Isc) was monitored on a strip-chart recorder; resistance was determined by pulsing current for 1 second (± 10 mV); and the potential difference was calculated from Isc and resistance. The tissue was bathed in Krebs-Henseleit (K-H) buffer, consisting of 118 mM NaCl, 5.6 mM KCl, 1.9 mM CaCl₂, 1.2 mM MgSO₄, 1.3 mM NaH₂PO₄, 25 mM NaHCO₃, and 5.6 mM glucose; this solution was bubbled with 95% O₂, 5% CO₂ and maintained at 34°C. All chemicals were reagent grade. Ouabain octahydrate was obtained from Sigma Chemical (St. Louis, MO, USA).

Rats were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA). SHR and WKY were paired by age and sex. Animals were received at age 8 weeks and were acclimated to blood pressure measurements two to three times per week for a minimum of 2 weeks. Experiments were performed between 11 and 17 weeks of age, with most occurring between 11 and 13 weeks of age. The last blood pressure measurement before the experiment was used for each animal. Blood pressure was measured using the tail-cuff method recorded on a Natsume rat tail manometer system (Model KN-0090, from Peninsula Laboratories, Belmont, CA, USA). Animals were preheated for 1 minute at 70°C to maximize tail flow. Blood pressure was then recorded in a quiet room by the same person with minimal handling. The average systolic blood pressure (± SEM) for WKY was 132 ± 8 mm Hg and for SHR was 185 ± 11 mm Hg. Thirteen pairs of rats were used in this study. The animals were fed standard laboratory chow (Type RMH 3000, Agway, Syracuse, NY, USA), which is 0.44% sodium by weight, and water ad libitum.

The protocol was to mount the tissue with K-H buffer on both sides and wait for Isc to come to a steady state (± 20 minutes). NaCl solutions were presented to the mucosal side in order of increasing concentration (0.01, 0.15, 0.5, 1.0, and 2.0 M), after waiting at each concentration for Isc to come to steady state (at least 5 minutes). After an equilibration period in K-H buffer, the same series of KCl solutions was used. The tissue was then exposed to K-H buffer on the mucosal side and ouabain (10⁻² M) in K-H buffer on the serosal side for 1 hour. The NaCl and the KCl series were repeated. In half the pairs of rats, the KCl series was presented first before and after treatment with ouabain; order of presentation of salts made no difference to the final results. The entire experiment took 5 to 7 hours; control experiments with no ouabain present showed that the response to a hyperosmotic salt solution was reproducible over that period.

Results

The electrical parameters for the dorsal lingual epithelia from WKY, with K-H buffer on both sides of the tissue, were Isc, 6.8 ± 0.7 µA/cm²; resistance, 1428 ± 203 Ω-cm²; and potential difference, 9.5 ± 1.2 mV. Values for SHR were not significantly different.

We measured the electrical parameters of each tissue before and after treatment with ouabain to examine the contribution of the Na⁺-K⁺ pump to Isc. Figure 1 shows the current before and after ouabain treatment as a function of NaCl concentration in WKY. Following DeSimone et al.,² we define the ouabain-sensitive part of the current, Iₐ, as

\[ Iₐ = Iₛₚ - (Rₛ/R)Iₒₛₚ \]

where R and Rₛ are the values of resistance at a given concentration before and after ouabain treatment, respectively, and Iₒₛₚ is the ouabain-resistant short-circuit current. This definition eliminates errors introduced by liquid junction potentials at the bridge-solution interface. The same functions for KCl in WKY are shown in Figure 2.

For NaCl test solutions, Iₛ in the SHR was decreased compared with WKY, as shown in Figure 3. This decrease was due primarily to a decrease in Iₛₚ and was consistent for both sexes and across the age range. For hyperosmotic concentrations, the decrease in Iₛ was approximately 20%. The decrease in Iₛ was statistically significant (p < 0.05) for all hyperosmotic concentrations tested, using the paired Student's t test. However, the use of multiple t tests can result in a false significance. Hence, a generalized multivariate analysis of variance (GMANOVA),¹² based on the 13 paired differences, was used to test for a positive trend between the SHR and WKY groups. Based on the likelihood ratio test for a patterned covariance structure,¹³ an unweighted analysis was determined appropriate with a test statistic of 0.294 (p = 0.091). The unweighted analysis transforms the GMANOVA
model to a classic multivariate analysis of variance (MANOVA) model. The one-sided MANOVA test for trend on the transformed data indicated a significant trend away from zero \( p = 0.029 \) based on the construction of simultaneous confidence intervals. Therefore, we can consider that there is a significant difference between the SHR and WKY groups.

In the case of KCl, \( I_{\text{Na}} \) showed no significant change between strains; however, \( I_{\text{Na}} \) was decreased in SHR compared with WKY above 0.15 M (Figure 4). For both salts, the SHR tissues tended to have higher values of resistance at all concentrations and both before and after ouabain treatment when compared with WKY; however, this increase was not significant at all concentrations. In all cases the magnitude of the increase was greater the lower the salt concentration. For KCl in the untreated tissue, the increase was significant at the two lowest concentrations (0.15 M and below). For KCl in the ouabain-treated tissue, the increase was significant at all but the lowest concentration; at 0.15 M KCl, for example, the resistance increased from 952 ± 76 Ω·cm² for WKY to 1144 ± 74 Ω·cm² for SHR \( p = 0.015 \).

**Discussion**

This study shows that there are alterations in rat lingual epithelial ion transport of NaCl associated with hypertension. The response measure, \( I_{\text{Na}} \), in the in vitro tissue from SHR was significantly less than that from WKY at hyperosmotic NaCl concentrations. For KCl the modifications were more ambiguous: \( I_{\text{Na}} \) in the untreated tissue showed no change; however, the ouabain-insensitive short-circuit current, \( I_{\text{Na}} \), was decreased in SHR compared with WKY above 0.15 M, and resistance was increased in the untreated tissue for 0.15 M and below.

Hypertension has been found by some investigators to be associated with alterations in ion transport in erythrocytes, vascular smooth muscle, and renal tubules. The alteration in transport has been attributed either to a change in a transporting enzyme system, to an increase in ion permeability, or to a circulating factor inhibiting the \( \text{Na}^+,\text{K}^- \)-adenosine triphosphatase, depending on the particular organ, animal, or type of hypertension. There is not yet agreement on whether an abnormality of sodium transport is involved in the pathogenesis of essential hypertension. Meyer and Garay hypothesized that the primary defect in hypertension could consist of a genetically transmitted membrane defect impairing the intracellular sodium homeostatic mechanisms that normally compensate sodium load. Whether alterations occur in ion transport in alimentary tract tissues other than the
lingual epithelium is unknown. Interestingly, Rodriguez-Sargent et al. 22 recently reported a possible ion transport defect in the ciliary processes or lens epithelium (or both) that may contribute to cataract formation in Dahl salt-sensitive hypertensive rats.

SHR show increased taste preferences and decreased chorda tympani responses to NaCl and KCl when compared with WKY. 10 11 The decrease in I Na in response to NaCl seen in this study is in the right direction to correlate with decreased chorda tympani responses to NaCl seen by Priehs et al., 10 suggesting that the decreased neural response may be due in part to a modification of lingual epithelial ion transport. A similar correlation for KCl was not seen. However, sodium and potassium follow different transport pathways across this epithelium. 2

For humans, high NaCl consumption may be associated with the development of some types of hypertension in genetically susceptible individuals. 15 It has been hypothesized that untreated hypertensive persons may have elevated recognition thresholds for NaCl compared with normotensive persons. (See Reference 24 for a review of the literature.) In attempting to draw parallels with human essential hypertension, there is always the question of which animal to use. Humans with mild to moderate essential hypertension and rats with spontaneous hypertension have several similar abnormalities, including abnormalities of sodium transport by red blood cell membranes. 24 Genetic analysis of blood pressure in SHR indicates a multifactorial disease attributed to at least three to five major genes. 19 SHR become hypertensive spontaneously without any additional inducements such as salt loading. High sodium loads exacerbate the spontaneous hypertension already present; potassium attenuates the deleterious effects of high NaCl; however, neither is essential to the development of hypertension. 11, 19

Contreras et al. 26 showed that enhanced NaCl preference of sodium-deprived and adrenalectomized rats is accompanied by a specific decrease in gustatory neural activity. They hypothesized that this decrease is due to a receptor change at the peripheral level, causing a shift in suprathreshold NaCl intensity, which could influence the animal to increase salt consumption. A similar phenomenon may be responsible for the increased salt intake and decreased chorda tympani response to salts of the SHR. Previous studies indicate that one sodium taste receptor is an ion transport pathway. The results of this report suggest that this transport system is attenuated in the SHR.

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