Effects of osmolarity on taste receptor cell size and function

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Lyall, Vijay, Gerard L. Heck, John A. DeSimone, and George M. Feldman. Effects of osmolarity on taste receptor cell size and function. Am. J. Physiol. 277 (Cell Physiol. 46): C800–C813, 1999.—Osmotic effects on salt taste were studied by recording from the rat chorda tympani (CT) nerve and by measuring changes in cell volume of isolated rat fungiform taste receptor cells (TRCs). Mannitol, cellobiose, urea, or DMSO did not induce CT responses. However, the steady-state CT responses to 150 mM NaCl were significantly increased when the stimulus solutions also contained 300 mM mannitol or cellobiose, but not 600 mM urea or DMSO. The enhanced CT responses to NaCl were reversed when the saccharides were removed and were completely blocked by addition of 100 µM amiloride to the stimulus solution. Exposure of TRCs to hyperosmotic solutions of mannitol or cellobiose induced a rapid and sustained decrease in cell volume that was completely reversible, whereas exposure to hypertonic urea or DMSO did not induce sustained reductions in cell volume. These data suggest that the osmolyte-induced increase in the CT response to NaCl involves a sustained decrease in TRC volume and the activation of amiloride-sensitive apical Na+ channels.

TASTE RECEPTOR CELLS (TRCs) provide for sensory transduction of chemical stimuli normally found in foods and initiate quality coding in the gustatory neuraxis (32). Taste quality, a key factor in the decision to accept or reject a substance as a meal, is a complex entity that includes significant influences from environmental and cultural, as well as physiological, factors (30). Taste quality perception may also be influenced by factors of long-term duration, such as metabolic or hormonal status, that often reflect individual nutritional and health factors (28). In the taste periphery, variations in the physicochemical properties of taste stimuli, including mixtures, are probable sources of acute and widely experienced variations in taste intensity and quality. Stimulus properties such as viscosity (7) or tonicity (10) do not activate the peripheral taste organs directly, but they may exert a modulatory effect on the response of TRCs.

In the case of tonicity, TRCs are exposed to osmolarities ranging from nearly zero to >2,500 mosmol/kg, all under physiological conditions (10). TRCs must be robust, as evidenced by their ability to transduce stimulus quality and intensity under extremes that would incapacitate many cells. On exposure to anisotonic conditions, most cells initially behave as osmometers and alter their volumes according to the tonicity of the extracellular compartment. However, some cells do not demonstrate short-term recovery from volume perturbations (26, 36), whereas many other cell types are capable of actively restoring their volumes, despite continuous hypotonic and hypertonic challenge (17). Littke is known regarding the reaction of individual TRCs to rapid changes in osmotic pressure and possible mechanisms of cell volume recovery. In most cells, recovery from volume perturbations involves the activation of a variety of solute transport mechanisms. Given that many of these solutes are normal stimuli for TRCs, one might reasonably expect osmotic changes in the oral cavity fluid to have consequences for the encoding of taste sensation. This would suggest mechanisms in polarized epithelial cells permitting stimulus-induced changes in one cell membrane to be communicated to the contralateral membrane via changes in cell volume, intracellular ion activities, and membrane voltages (36).

Although not polarized epithelial cells, supraoptic neurons are well-studied examples of osmoreceptors that serve as cell volume transducers (6). Although TRCs are not osmoreceptors per se, studies have shown that active solute transport and arterial-venous exchange of solutes along the length of cat fungiform papillae exposed to isotonic fluids result in the papilla tips becoming hypertonic (15). Thus TRCs may experience changing osmotic pressure gradients depending on the permeability properties of various solutes and their effects on cell metabolism and papillary blood flow. We have investigated the effects of osmotic pressure on taste at the level of the sensory afferents by recording from the rat chorda tympani (CT) and on the cellular level by measuring changes in cell volume of isolated rat fungiform TRCs with use of imaging techniques. Because taste stimuli have their own intrinsic osmotic pressures, we have employed conditions that separate the osmotic pressure variables from those relating to taste stimulus intensity. Correlation of the electrophysiological results with the cell volume measurements indicates that a sustained decrease in TRC volume is the necessary precursor to the enhancement of the taste neural response to isotonic NaCl by certain osmolytes.

MATERIALS AND METHODS

Recording CT Responses

Nerve preparation and recording. Female Sprague-Dawley rats (150–200 g) were anesthetized by brief exposure to ether
followed by injection of pentobarbital sodium (60 mg/kg ip). Supplemental pentobarbital sodium (60 mg/kg) was administered as necessary to maintain surgical anesthesia. Body temperatures were maintained at 36–37°C with a circulating water heating pad. The left CT was exposed laterally as it exited the tympanic bulla, as previously described (38). After the CT was dissected free from surrounding tissue, it was cut proximally, desheathed, and placed onto a 32-gauge platinum-iridium wire electrode. An indifferent electrode was placed in nearby tissue. Neural responses were differentially amplified with a custom-built, optically coupled isolation amplifier and recorded on a modified Toshiba DX-900 videocassette recorder. For display, responses were filtered using a band-pass filter with cutoff frequencies of 40 Hz–3 kHz and fed to an oscilloscope. Responses were then full-wave rectified and integrated with a time constant of 1 s. The voltage output of the integrator is a measure of the neural response (the number of individual nerve fibers firing at a given time) and is proportional to the number of spikes per second (4). Integrated neural responses and current and voltage records were recorded on a chart recorder (model TY 7045, Linseis, Princeton Junction, NJ). For display, the integrated neural records were plotted in scaled arbitrary chart units relative to baseline in rinse solutions. An upward pen excursion corresponds to an increase in magnitude of the integrated neural response (i.e., increased spike frequency) at a given point in time.

Stimulation chamber and in situ transepithelial potential recording. Solutions were injected (3 ml, 1 ml/s) into a Lucite chamber affixed by vacuum to a 28-mm2 patch of anterior tongue epithelium. The chamber was fitted with a Ag-AgCl electrode for current passing and a salt-bridge electrode for measuring the in situ lingual transepithelial potential (Vts). Corresponding reference electrodes were placed noninvasively on the ventral lingual epithelium. The Vts and applied currents were measured and programmed, respectively, using a voltage-current-clamp amplifier (model VCC600, Physiologic Instruments, San Diego, CA). All experiments were performed while the lingual epithelium was maintained under zero current-clamp mode, and all voltages were referenced to the mucosal side. The current-passing electrode within the chamber served as a virtual ground, ensuring that only current passing through the stimulated patch was collected. A periodic (15-s) bidirectional constant-current pulse (4 µA) was generated across the lingual receptive field contained in the stimulation chamber. The current also perturbed the steady-state Vts, and this yielded a measure of the relative changes in situ tissue resistance (Rtis). For comparison purposes the data are presented as relative changes in Riis and Vts with respect to their values in the rinse solution rather than their absolute values. This is because it is necessary to place the voltage and current-passing reference electrodes noninvasively along the ventral lingual surface rather than embed them in the muscle close to the dorsal surface (16). This avoids injury and inflammation, which is essential in preserving normal peripheral taste sensory function. Sublingual electrode placement adds another series resistance, due to muscle and connective tissue, that varies among animals because of variations in muscle thickness and the position of the reference electrodes. However, on stimulating the tongue with taste stimuli, the added series resistance does not change with solution composition, so measured changes in Vts and Riis in vivo correlate well with changes in transepithelial potential and resistance, respectively, as determined from previous studies (34, 38, 39).

The time course of lingual potentials, programmed currents, and integrated CT responses were also captured on disk during an experiment by use of Labview software and then analyzed off-line in a manner similar to that previously described (38). The numerical value of an integrated CT response was obtained as the area under the integrated CT response curve for a time interval of 75 s from the onset of chemically evoked neural activity. The area under the integrated CT response curve under control conditions was normalized to 100%, and the increase or decrease in the area under experimental conditions was expressed relative to control.

The protocol for stimulation of the lingual epithelium was as follows: Before the experiment was started, the responses to three reference stimuli were tested. The reference solution was injected into the chamber and allowed to remain on the tongue for 40–60 s. The reference solution was then rinsed with several applications of rinse solution. Next, the stimulus series with solutions of different osmolarities containing mannitol, cellobiose, urea, or DMSO was applied to the tongue. At the end of this series the three reference stimuli were reapplied and rinsed. The data from the stimulus series with various osmolarities were accepted if the nerve responses to three reference stimuli did not differ by >10% before and after the stimulus series.

For comparison purposes the data are presented as relative changes in Vts with respect to their values in the rinse solution rather than their absolute values. This is because it is necessary to place the voltage and current-passing reference electrodes noninvasively along the ventral lingual surface rather than embed them in the muscle close to the dorsal surface (16). This avoids injury and inflammation, which is essential in preserving normal peripheral taste sensory function. Sublingual electrode placement adds another series resistance, due to muscle and connective tissue, that varies among animals because of variations in muscle thickness and the position of the reference electrodes. However, on stimulating the tongue with taste stimuli, the added series resistance does not change with solution composition, so measured changes in Vts and Riis in vivo correlate well with changes in transepithelial potential and resistance, respectively, as determined from previous studies (34, 38, 39).

Preparation of TRCs. Female Sprague-Dawley rats weighing 150–200 g were anesthetized with methoxyflurane and then killed by cervical dislocation. The tongues were rapidly removed and stored in ice-cold HEPES-buffered solution (pH 7.4) preequilibrated with 100% O2. The lingual epithelium was isolated by injection of collagenase (Boehringer-Mannheim, Indianapolis, IN) and incubation in a Ca2+-free solution (3). Then taste bud fragments (TBFs) and TRCs were prepared from the fungiform papillae, as described previously (19, 34).

Perfusion chamber. The open perfusion chamber consisted of a standard glass slide onto which a piece of silicone rubber sheet with a 4-cm2 cutout window in the center was glued (19). Cells were affixed to the slide with Cell-Tak (1 µg/cm2; Collaborative Research, Bedford, MA), and a fresh chamber was prepared for each experiment. The chamber was perfused at 4 ml/min.

Measurement of cell dimensions. After an initial wash perfusion for 15 min with HEPES-buffered solution, TRCs were visualized through a ×40 objective (Zeiss; 0.9 numerical aperture) with a Zeiss Axioskop. Transmitted images were acquired with a video camera (model ITC 510, Ika-gami) and digitized at 10-s intervals with a software-controlled frame grabber board (Digidata 2000 Image Lightning Board and Imaging Workbench, Axon Instruments, Foster City, CA). Changes in length of the cell major and minor axes were measured using Transform (Fortner Research, Sterling, VA). With the assumption that the TRC body has the shape of an...
ellipsoid, the TRC volume (V) was calculated using the following formula: $V = 4\pi a^2/3c$. This formula is based on the following relations: $S = 4\pi a^2/3c$, where $S$ is surface area, $a = \sqrt{1 - e^2}$ and $e$ is the ratio $a/c$, where $a$ is one-half the minor axis length and $c$ is one-half the major axis length.

Measurement of calcein fluorescence. In some experiments, relative changes in cell volume were monitored using the fluorophore calcein, because the calcein fluorescence varies inversely with its concentration (36, 37). TRCs in the perfusion chamber were loaded with calcein in its AM form (25 µM) at 4°C overnight. Before the experiment was started, the cells were superfused with room temperature control solution for 30 min. The imaging setup, described above, was used with the addition of an image intensifier (Videoscope, Washington, DC), an epifluorescent light source (TILL Photonics Plochrome II, Applied Scientific Instrumentation, Eugene, OR), a 515-nm dichroic beam splitter (Omega Optical), and a 535-nm emission filter (20-nm band pass, Omega Optical). The cells, illuminated with 490-nm light, were imaged at 10-s intervals, and 16 frames were averaged. Small regions of interest (~5 µm²) in cells were chosen in which fluorescence was monitored. Photobleaching of calcein was <5% (see Figs. 11 and 12).

In separate experiments, TBFs and TRCs were imaged with a confocal laser scanning imaging system (LSM 410 or LSM 510, Carl Zeiss, Heidelberg, Germany). The excitation light was 488 nm, and the light emitted above 515 nm was measured. To evaluate the relationship between calcein fluorescence and cell size, TRCs were exposed to hypertonic NaCl. Images were obtained at 20-s intervals, and in each image cell size and mean calcein fluorescence intensity were measured. In four TRCs the mean changes in calcein fluorescence were linearly related to changes in cell size with a slope of 0.58. A similar linear relationship between calcein fluorescence intensity and cell size has been reported in gallbladder epithelial cells (36) and in rat hepatocytes (37).

Solutions. HEPES-buffered control solutions (pH 7.4) contained (in mM) 140 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 sodium pyruvate, 10 glucose, and 10 HEPES. The hypotonic solution was NaCl free. The solution osmolality was increased by the addition of mannitol, cellobiose, urea, DMSO, or NaCl.

Statistical Analyses

Values are means ± SE; n represents the number of animals from which CT recordings were made in the group. In in vitro experiments n represents the number of TRCs in an experiment. Statistical significance was assessed with the paired Student’s t-test, and significance was achieved when two-tailed $P < 0.05$.

RESULTS

In Vivo Studies

Effects of osmolarity on CT activity. To verify that d-mannitol does not evoke a neural response in the rat CT, the tongue was superfused first with a solution that approximated normal saliva, i.e., 20 mM KCl, and then with a solution containing 300 mM mannitol + 20 mM KCl. As shown in Fig. 1A, apart from a mechanical rinse artifact (rapid transient upward deflection in the baseline neural record), no significant chemically evoked neural response occurred. In the bottom trace, exposure of the tongue to mannitol solution also caused a small positive increase in $V_\text{muc}$ (referred to the mucosal side) and an increase in $R_\text{in}$ (i.e., increase in the amplitude of the transient voltage excursions in response to the periodic bipolar current pulses). The observed changes in $R_\text{in}$ cannot, of course, be attributed to intrinsic properties of the added nonelectrolyte osmolyte. Similar changes in $R_\text{in}$ and $V_\text{muc}$ were observed when cellobiose (which also did not evoke a CT response) was used rather than mannitol (data not shown). The data from both saccharides were, therefore, pooled and analyzed. In 10 animals the tongues were initially superfused with a solution containing 300 mM mannitol, i.e., 20 mM KCl. As shown in Fig. 1A, apart from a mechanical rinse artifact (rapid transient upward deflection in the baseline neural record), no significant chemically evoked neural response occurred. In the bottom trace, exposure of the tongue to mannitol solution also caused a small positive increase in $V_\text{muc}$ (referred to the mucosal side) and an increase in $R_\text{in}$ (i.e., increase in the amplitude of the transient voltage excursions in response to the periodic bipolar current pulses). The observed changes in $R_\text{in}$ cannot, of course, be attributed to intrinsic properties of the added nonelectrolyte osmolyte. Similar changes in $R_\text{in}$ and $V_\text{muc}$ were observed when cellobiose (which also did not evoke a CT response) was used rather than mannitol (data not shown). The data from both saccharides were, therefore, pooled and analyzed.
with a rinse solution containing 20 mM KCl. On increase in the osmolarity of the rinse solutions with 300 mM mannitol (n = 7) or cellobiose (n = 3), R_{IS} increased by 53.3 ± 17.1% (P < 0.025). These changes were accompanied by an increase in V_{IS} by 4.7 ± 1.4 mV (P < 0.01). In seven additional animals, increasing the saccharide concentration from 300 to 600 mM increased R_{IS} further by 23.0 ± 3.4% (Fig. 1B).

In parallel experiments the tongue was first superfused with 20 mM KCl, which was then replaced by another solution containing 20 mM KCl + 600 mM urea (n = 3) or DMSO (n = 3). As was the case with mannitol (cf. Fig. 1A), no significant chemically evoked neural responses occurred with urea or DMSO. Application of urea or DMSO did not significantly alter V_{IS} and R_{IS} (data not shown).

In the next series of experiments we investigated the effect of mannitol on the neural response to stimulation with 150 mM NaCl. The first stimulation series was performed under near-isosmotic conditions. The tongue was superfused with a solution containing 20 mM KCl + 300 mM mannitol for several minutes and then stimulated with a solution in which 300 mM mannitol was replaced with 150 mM NaCl (an isosmotic change). As shown in Fig. 2A (also see Figs. 3–5), the 150 mM NaCl stimulus solution induced an increase in the CT response (top trace). These changes were accompanied by a small decrease in V_{IS} (bottom trace) and R_{IS}. After several minutes the salt stimulus was added once again, and after an infusion artifact the CT response assumed its original time course. In the third step the salt stimulus was replaced by a hypertonic salt stimulus containing 150 mM NaCl + 20 mM KCl + 300 mM mannitol. The CT record shows a further increase in activity (upward deflection) that follows a new time course of elevated neural activity compared with that seen in the absence of mannitol. On rinsing the hypertonic salt stimulus with the solution containing 20 mM KCl + 300 mM mannitol, the original baseline was reestablished and V_{IS} and R_{IS} returned to values close to their baseline. Similar results were obtained when the solutions contained cellobiose in place of mannitol (data not shown).

The CT responses from several animals are summarized in Fig. 2B. The integrated CT responses were calculated as the area under the response curve in the presence of 150 mM NaCl and in the presence of 150 mM NaCl + 300 mM mannitol or cellobiose. The percent change in area in the presence of 150 mM NaCl + 300 mM mannitol or cellobiose is expressed relative to that in the presence of 150 mM NaCl alone. The data show that superfusing the tongue with a second salt stimulus (150 mM NaCl + 20 mM KCl) does not change the steady-state CT response (bars 1 and 2). However, on superfusion, a similar salt stimulus containing 300 mM saccharide (bar 3) induced an increase in CT response by 49.0 ± 17.9% (solid bar; P < 0.025, n = 8). Although during a second stimulation with hypertonic stimulus solution the CT response decreased (bar 4), it was still 26.7 ± 5.2% greater (P < 0.005) than in the absence of saccharides.

Changing from a solution with 300 mM mannitol to a stimulus solution containing 150 mM NaCl caused an electronegative change in $V_{IS}$ by 8.8 ± 1.8 mV (P < 0.001). The changes in $V_{IS}$ were in the opposite direction from those observed when saccharides were adminis-
However, on superfusion, the salt stimulus containing, in addition, 300 mM mannitol did not change $R_{\text{is}}$ ($AR_{\text{is}} = 2.1 \pm 2.1\%$, $P > 0.05$, $n = 8$) or $V_{\text{is}}$.

To investigate whether the lowering of tonicity of the salt stimulus would attenuate the nerve response, the same experiment was done under hypertonic salt stimulus. The tongue was rinsed with a rinse solution containing 20 mM KCl $+$ 600 mM mannitol for several minutes and then stimulated with hypertonic salt solution containing 20 mM KCl $+$ 150 mM NaCl $+$ 300 mM mannitol (no change in tonicity, because the rinse and the salt stimulus have an osmotic pressure of ~640 mosM). As shown in Fig. 3A, there was an increase in the CT response (top trace) due to NaCl that maintained its time course during a second exposure to the hypertonic salt stimulus. These changes were accompanied by a small decrease in $V_{\text{is}}$ (bottom trace) and a small decrease in $R_{\text{is}}$. This was replaced by an isosmotic salt stimulus without mannitol. It caused a significant decrease in neural activity, but in this case the decrease in neural activity was more protracted in time. Similar responses were obtained with cellobiose (data not shown). The data from several animals are presented in Fig. 3B, in which the relative CT response is expressed as the area under the CT response curve in the presence and absence of saccharides. The data show that superfusing the tongue with a second salt stimulus (150 mM NaCl $+$ 20 mM KCl $+$ 300 mM saccharide) does not change the steady-state CT response (bars 1 and 2). However, superfusing a similar salt stimulus without the saccharide (bar 3) induced a decrease in CT response by $17.9 \pm 3.2\%$ (solid bar; $P < 0.005$, $n = 8$). Subsequently, a second stimulation with the isotonic salt stimulus (bar 4) caused the CT response to decrease further and was $43.6 \pm 9.7\%$ lower (solid bar; $P < 0.005$) than stimulation with the hypertonic stimulus.

As observed in the previous experiment, changing from a rinse solution with 600 mM mannitol to a stimulus solution containing 150 mM NaCl $+$ 300 mM mannitol caused an electronegative change in the transepithelial potential and a decrease in $R_{\text{is}}$ by $49.6 \pm 5.5\%$ ($n = 9$) relative to the rinse solution (data not shown). The changes in $V_{\text{is}}$ were in the opposite direction from those observed when saccharides were administered in the absence of NaCl (Fig. 1). However, superfusing the salt stimulus containing 150 mM NaCl alone induced a further decrease in $R_{\text{is}}$ by $3.9 \pm 1.5\%$ ($P < 0.05$, $n = 9$) without a change in $V_{\text{is}}$.

When the lingual epithelium was rinsed with solutions containing mannitol, a subsequent salt stimulus in which 150 mM NaCl replaced an equivalent amount of mannitol decreased $R_{\text{is}}$ but caused an electronegative shift in $V_{\text{is}}$. However, we observed that when the lingual epithelium was rinsed with rinse solutions without the saccharides, a subsequent NaCl stimulus always induced a positive shift in $V_{\text{is}}$ (39). In tongues rinsed with 15 mM KCl $+$ 15 mM KHCO$_3$, a subsequent exposure to 300 mM NaCl decreased $R_{\text{is}}$ by $58.3 \pm 4.5\%$ ($P < 0.001$) and increased $V_{\text{is}}$ by $9.2 \pm 1.6$ mV ($P < 0.001$, $n = 10$). Similarly, in tongues rinsed with 10 mM KHCO$_3$, a

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**Fig. 3.** A: effect of hyposmolality on CT response to 150 mM NaCl (top trace) and $V_{\text{is}}$ and $R_{\text{is}}$ (bottom trace) in vivo. CT responses are measured as voltage output of a spike integrator and displayed graphically in arbitrary chart units. Tongue was initially treated with a hypertonic rinse solution containing 20 mM KCl $+$ 600 mM mannitol, and CT response was taken as baseline. Perfusion of tongue with a salt stimulus with same osmolality as rinse solution (300 mM mannitol replaced with 150 mM NaCl) represents neural response to 150 mM NaCl under hypertonic conditions. In next step, perfusion with an isotonic salt stimulus containing 20 mM KCl $+$ 150 mM NaCl decreased CT response. In final step, on exposure of tongue to rinse solution, CT response declined to baseline value. B: summary of data from several such experiments represented as percent change in CT response calculated from changes in relative area under response curve. Percent decrease in area in presence of 150 mM NaCl alone is expressed relative to that of 150 mM NaCl $+$ 300 mM saccharide. Values are means $\pm$ SE of number of animals in parentheses. Solid bars, paired differences in CT responses between sets of data (see text for details).
subsequent exposure to 100 mM NaCl induced a decrease in $R_{is}$ by $25.9 \pm 5.6\%$ ($P < 0.025$), with a positive shift in $V_{is}$ by $6.2 \pm 2.1$ mV ($P > 0.05$, $n = 5$). However, in additional experiments, when tongues exposed to 10 mM NaCl were subsequently treated with 100 mM NaCl, $R_{is}$ decreased by $33.5 \pm 2.9\%$ ($P < 0.005$) and $V_{is}$ increased by $8.6 \pm 1.4$ mV ($P < 0.025$, $n = 5$). These data suggest that NaCl-induced changes in $V_{is}$ depend on the rinse solution composition. This effect is certainly not restricted to NaCl, since we observed that, in

![Diagram of CT Response (0.5 unit) and $V_{is}$ (20 mV)](image)

![Diagram of CT Response (1 unit) and $V_{is}$ (20 mV)](image)
the same five rats, when 10 mM NaCl was replaced with 300 mM cellobiose, $R_{is}$ increased by 9.5 ± 2.1% (P < 0.025) and $V_{is}$ decreased by 2.6 ± 0.5 mV (P < 0.025). This change in $V_{is}$ is opposite from that observed with mannitol (Fig. 1A) or cellobiose when the rinse solution was 20 mM KCl. In contrast to this, in the presence of 150 mM NaCl (Figs. 2A and 3A), addition or removal of the saccharides had only minimal effects on $R_{is}$ and $V_{is}$.

In the above experiments, altering the osmolarity of the NaCl stimulus solution with cellobiose or mannitol modulated the CT response to 150 mM NaCl. Because most cells respond to changes in external osmolarity with changes in cell volume (17), we hypothesized that mannitol and cellobiose modulate the CT response to NaCl via changes in TRC volume. To investigate this possibility, in the next set of experiments we altered the osmolarity of the NaCl stimulus solution with urea and DMSO, both of which have been shown to have high permeability across cell membranes (14, 24) and are expected to induce no significant changes in TRC volume. The tongue was rinsed with 20 mM KCl + 300 mM urea for several minutes and then stimulated with a solution in which 300 mM urea was replaced with 150 mM NaCl (an isosmotic change, since both solutions have an osmotic pressure of ~340 mosM). As shown in Fig. 4, there was an increase in CT response (top trace) and a relative decrease in $R_{is}$ and $V_{is}$ (bottom trace). After several minutes the salt stimulus was added once again. After an infusion artifact, the response assumed its original time course. In the third step the salt stimulus was replaced by a hypertonic salt stimulus containing 150 mM NaCl + 20 mM KCl + 600 mM urea. There was no change in the neural activity or its time course compared with that seen in the absence of urea. On rinsing the hypertonic salt stimulus with the rinse solution, the original baseline was reestablished. When the infusion solutions contained 600 mM DMSO in place of urea, once again there was no change in the neural activity or its time course compared with that in the absence of DMSO (cf. Fig. 4). In additional experiments the steady-state CT response in the presence of a hypertonic salt stimulus containing 150 mM NaCl + 20 mM KCl + 300 mM DMSO (or urea) did not show any change in neural activity or its time course compared with the response in the presence of 150 mM NaCl + 20 mM KCl. In eight such experiments when the salt stimulus contained 300 mM urea ($n = 4$) or DMSO ($n = 4$), the integrated CT responses (cf. Figs. 2B and 3B) were increased by only 3.4 ± 2.9% (P > 0.05) compared with the steady-state CT activity in the presence of the salt stimulus alone.

The CT nerve responses to NaCl are inhibited by the application of amiloride (32), suggesting the presence of apical amiloride-sensitive Na$^+$ channels on TRCs innervated by the CT. To investigate whether apical amiloride-sensitive Na$^+$ channels in TRCs may modulate osmotically induced changes in NaCl responses, CT responses were monitored in stimulus solutions without and with amiloride. As shown in the experiment in Fig. 5A, when amiloride was absent the CT responses to 150 mM NaCl and 150 mM NaCl + 300 mM mannitol were similar to those shown previously in Fig. 2A. In the second part of the experiment the same sequential protocol was used, but 100 µM amiloride was added to the stimulus solutions. As shown in Fig. 5B, amiloride completely blocked the CT responses to 150 mM NaCl and to 150 mM NaCl + 300 mM mannitol. Similar amiloride inhibition of the CT responses was observed in two additional animals (data not shown). These data suggest that the amiloride-sensitive Na$^+$ channels present in the apical membranes of TRCs are involved in osmotically induced modulation of CT responses to NaCl.

In summary, during stimulation with 150 mM NaCl, changing directly to a solution containing 150 mM NaCl, but with 300 mM saccharide, caused an increase in the CT response. In contrast, during stimulation with 150 mM NaCl + 300 mM saccharide, changing directly to a solution containing 150 mM NaCl, but without the saccharide, caused a decrease in the CT response. On the other hand, urea and DMSO had no significant effect on the steady-state CT responses to 150 mM NaCl. On the basis of the differences in the permeability of saccharides vs. DMSO and urea across cell membranes (14, 17, 24), these results suggest that taste responses to NaCl may be modulated in part by osmotically induced changes in TRC volume. This hypothesis was tested directly by measuring changes in TRC volume with mannitol, cellobiose, urea, and DMSO.

**In Vitro Studies**

Effect of osmolarity on TRC volume. Figure 6 shows three-dimensional reconstructed images of a TBF and isolated TRCs perfused in control solution without mannitol (A and C) and after a 1-min perfusion with a similar solution containing 600 mM mannitol (B and D). The images demonstrate that TRCs shrink in hypertonic solutions and that the cell shrinkage occurs in major and minor axes. Also, the images demonstrate that some TRCs are rounded and that they too respond to changes in osmolarity.

To evaluate the time course of the nontastant saccharide-induced changes in cell volume, TRCs were initially perfused with hypotonic NaCl-free solution, then they were superfused with a similar solution containing mannitol or cellobiose. Figure 7 shows a representative experiment in which solution tonicity was varied with cellobiose. Although 300 mM cellobiose decreased cell volume by 17.8 ± 5.6% (P < 0.05, n = 5), further raising the concentration to 600 mM induced an additional decrease in cell volume by 19.9 ± 3.4% (P < 0.01). The total decrement in cell volume in 600 mM cellobiose was 37.7 ± 6.6%, and as shown in Fig. 8, the addition of 600 mM mannitol decreased cell volume by 34.3 ± 6.1% (P < 0.001, n = 11). To assess whether mannitol had the same effect on cell volume when Na$^+$ was in the bathing solution, 300 mM mannitol was added to a solution containing 150 mM Na$^+$. In 9 TRCs (Fig. 9), addition of mannitol induced a 22.6 ± 6.3% (P < 0.01) decrease in cell volume. In these experiments the decrements in volume were complete within
30 s of solution change. Similar results were obtained with cellobiose (data not shown).

These data suggest that TRCs behave as osmometers. The changes in cell volume are rapid and completely reversible (cf. Fig. 8). Although no spontaneous volume compensation was observed when solution tonicity was increased, on reducing tonicity from 600 mM mannitol to zero mannitol, TRC volume increased rapidly (Fig. 8), transiently exceeding the starting volume (i.e., volume overshoot) and then relaxing to the

Fig. 7. Effect of solution osmolarity on TRC volume. TRCs were initially perfused with NaCl-free solution. As indicated by horizontal bar, cells were perfused with NaCl-free solution containing, in addition, 300 or 600 mM cellobiose. Percent changes in TRC volume are shown relative to TRC volume in presence of 300 mM cellobiose. In 5 TRCs, 300 and 600 mM cellobiose decreased cell volume by 17.8 ± 5.6% (P < 0.05) and 37.7 ± 6.6% (P < 0.01), respectively.

Fig. 8. Effect of mannitol on TRC volume. TRCs were initially perfused with NaCl-free solution. As indicated by horizontal bar, perfusion solution was switched to NaCl-free solution containing, in addition, 600 mM mannitol. Values represent paired differences in individual TRC volume (means ± SE; n = 11) between 2 sets of data. Presence of 600 mM mannitol decreased TRC volume by 34.3 ± 6.1% (P < 0.01). On reperfusion of TRCs with solution without mannitol, TRC volume increased rapidly, transiently exceeding starting volume (P < 0.05) and then relaxing to initial value within 30 s.
initial volume within 30 s. These data suggest that TRCs are capable of regulatory volume decrease (17). However, it is important to note that in these experiments the determination of cell volume occurred at 10-s intervals, thus limiting the detection of more rapid events.

Because volume changes were similar with mannitol or cellobiose (Figs. 7 and 8) and were not affected by the presence and absence of NaCl (Figs. 7 and 9), the data were pooled to analyze the distribution of volume responses to increasing osmolarity. As shown in Fig. 10A, changing from a 340 to a 640 mosM solution reversibly decreased TRC volume by 26.8 ± 3.5% (P < 0.001, n = 42). The decrements in cell volume were grouped into the following volume decrease categories: 0–9, 10–19, 20–29, 30–39, 40–49, and >50%. As shown in Fig. 10B, the cell number (n), corresponding to an observed relative decrease in volume, follows a Poisson distribution \( f(x) = \frac{42e^{-\mu}x^x}{x!} \), where \( x = 0, 1, 2, 3, 4, 5,... \), are the volume decrease categories chosen above. The mean, \( \mu = 2.35 \), was found from \( \mu = 42/\ln(4) \), where 42 and 4 are the total number of cells and the number of cells observed in the zeroth category, respectively. This corresponded to a mean decrease in TRC volume of 23.5%, a value close to the experimental value of 26.8%. The distribution of volume decreases suggests that TRCs may form a heterogeneous population with respect to water and solute permeability.

We also monitored relative changes in TRC volume using calcein, a fluorescent compound. Because the calcein fluorescence self-quenches as its concentration increases, intracellular calcein fluorescence varies with cell volume and serves as a convenient marker for changes in cell volume (36, 37). Figure 11A shows a representative experiment in which seven TRCs were initially bathed in isotonic solution containing 150 mM NaCl; after an increase in mannitol concentration from 0 to 600 mM, the mean calcein fluorescence decreased in all seven TRCs. The maximum decrease in fluorescence was complete within 30 s after the change in perfusion solution and was sustained in the presence of mannitol. On return to the initial control 150 mM NaCl solution, the calcein fluorescence recovered to near its resting

![Fig. 9. Effect of mannitol on TRC volume in presence of NaCl. TRCs were initially perfused with control solution (containing 140 mM NaCl). As indicated by horizontal bar, solution was switched to a similar solution containing, in addition, 300 mM mannitol. Values represent paired differences in individual TRC volume (means ± SE; n = 9) between 2 sets of data. Addition of mannitol decreased TRC volume by 22.6 ± 6.3% (P < 0.01) in 30 s.](http://ajpcell.physiology.org/)

![Fig. 10. A: changes in cell volume in 42 TRCs that were initially bathed in an isotonic solution (solution with or without NaCl + 300 mM saccharide) and then perfused with a hypertonic solution (solution with 140 NaCl + 300 mM saccharide or NaCl-free solution + 600 mM saccharide). Values are means ± SE. Solid bar, paired differences between 2 data sets. B: number of TRCs that responded to a decrease in volume of 0–9, 10–19, 20–29, 30–39, 40–49, and >50%. Cell number corresponding to an observed value of shrinkage follows a Poisson distribution \( f(x) = \frac{42e^{-\mu}x^x}{x!} \), where \( x = 0, 1, 2, 3, 4, 5,... \), discrete states representing above 6 categories in which TRCs were arbitrarily divided with a mean value of 2.35 (corresponding to a mean decrease in TRC volume of 23.5%). Values in parentheses above bars represent theoretically predicted values and are close to those observed experimentally.](http://ajpcell.physiology.org/)
value, demonstrating the reversibility of the saccharide-induced volume changes. In 19 TRCs investigated (including the 7 TRCs shown in Fig. 11A), the mean decrease in calcein fluorescence intensity was 19.5 ± 2.9%. These data indicate that relative changes in calcein fluorescence reflect changes in TRC volume and follow the same time course observed in Figs. 7–9.

To determine whether changing osmolarity with NaCl affects TRC volume differently from the changes induced by saccharides, the NaCl concentration was increased from 140 to 500 mM. As shown in Fig. 11B, increasing NaCl concentration induced a rapid decrease in calcein fluorescence in four TRCs, indicating a rapid reduction in cell volume. Unlike the effect of the saccharides, the fluorescence spontaneously but slowly increased with time, indicating a spontaneous recovery of cell volume. On superfusion of TRCs with control solution (140 mM NaCl), the calcein fluorescence recovered to near its resting value. In 12 TRCs (including the 4 TRCs shown in Fig. 11B), immediately after the increase in NaCl concentration the calcein fluorescence intensity decreased to 54.6 ± 7.0% of its baseline value. However, in all cells, calcein fluorescence intensity increased spontaneously with time to 69.8 ± 5.6% of its baseline value (Δcalcein fluorescence = 15.2 ± 1.7%, paired difference, P < 0.001). Thus it appears that TRCs demonstrate spontaneous regulatory volume increase in the presence of the salt but not in the presence of saccharides. Although such differences in solute-induced changes in cell volume have been observed in some cell types (17), the mechanisms involved in regulatory volume decrease and increase in TRCs are not known.

To evaluate whether urea or DMSO affects cell volume differently from NaCl or saccharides, calcein fluorescence was monitored as osmolarity was increased by urea or DMSO. As shown in Fig. 12A, on exposure to 600 mM urea, there was a rapid decrease in calcein fluorescence in all seven TRCs that was followed by a spontaneous recovery of fluorescence to near its resting value. In 19 TRCs investigated (including the 7 TRCs shown in Fig. 12A), the mean maximum transient decrease in calcein fluorescence intensity was 7.9 ± 0.4% (P < 0.001). In all 19 TRCs the calcein fluorescence intensity increased spontaneously and stabilized to a new steady-state value, which was 4.0 ± 0.5% (P < 0.001) above its control value. Although urea had transient effects on TRC volume, it had no sustained effects on TRC volume, which is similar to the lack of sustained effects of urea on cells derived from rabbit thick ascending limb of Henle's loop (14). As shown in Fig. 12B, exposure to 600 mM DMSO did not alter calcein fluorescence in three TRCs. In nine TRCs (including the 3 TRCs shown in Fig. 12B), the mean increase in calcein fluorescence intensity in the presence of DMSO was 2.1 ± 0.7%. These data are consistent with the notion that DMSO and urea (14, 24) exhibit a significant permeability across cell membranes and thus induce only transient or minimal changes in TRC volume.

DISCUSSION

Taste responses arising from a meal normally result from mixtures of chemicals released from foods during chewing and swallowing. Such mixtures of taste stimuli often evoke taste responses that are not easily interpret-
able in terms of the responses of their isolated components. These mixture interactions may arise in quality and intensity and may have their origin in the taste periphery and/or in higher gustatory centers. Mixture suppression is said to occur when the intensity of the response of a mixture is less than that of the sum of its components presented individually. A well-studied example of mixture suppression, arising at the taste cell level, is the suppression of rat CT responses to Na\(^+\) salts when presented together with potassium benzoate (23, 29) or potassium gluconate (33). On the other hand, mixture enhancement occurs when the intensity of the response of a mixture is greater than that of the sum of its components presented individually. The mixtures of mannitol or cellobiose with NaCl, used in the present study, fall into the latter category.

Although numerous accounts of mixture interaction are to be found in the literature, the mechanisms underlying it remain, in most cases, poorly understood. In this study we present evidence that substances, at hyperosmotic concentrations, that produce sustained TRC shrinkage (mannitol or cellobiose) enhanced the rat CT response to NaCl when presented in mixture with NaCl. The reductions in TRC volume were not only sustained, but they also varied with the concentration of saccharide, suggesting that the mechanism of TRC shrinkage is osmotic and that mannitol and cellobiose have high reflection coefficients (perhaps approaching unity, i.e., nearly TRC membrane-impermeable solutes). This is in contrast to the effects of hyperosmotic concentrations of urea and DMSO, which did not sustain a decrease in TRC volume and had no effect on the CT response to isosmotic NaCl. In their ability to produce no more than transient TRC shrinkage, it may be concluded that urea and DMSO have low reflection coefficients; i.e., they readily enter TRCs. These results suggest that the capacity of hyperosmotic agents to cause enhancement in the CT response to NaCl is, therefore, more a function of their ability to sustain TRC shrinkage and, therefore, not the result of cellular processes critically dependent on the permeation of the agents across TRC membranes. On this basis, it seems reasonable to propose that changes in cell volume can directly affect the sensory function of TRCs.

An important consideration in such a proposal is, of course, the relative time courses of CT response enhancement and TRC shrinkage after solution composition changes involving the saccharides. From our measurements the time course of TRC shrinkage seems sufficiently fast to be considered a possible precursor of an enhanced sensory nerve response. What may appear to be differences between the time courses in the data between the two procedures are accounted for by the slower image acquisition rate (1 image every 10 s) in the cell volume studies compared with CT nerve records (integrator time constant of 1 s). Also, limits on the fluid exchange characteristics of the perfusion chamber impose delays in the attainment of steady-state cell volume values that do not exist under in vivo conditions (19, 34). The comparable time courses of cell shrinkage and changes in neural activity, therefore, further support the conclusion that the mixture enhancement in the NaCl response due to mannitol or cellobiose is a consequence of osmotically induced changes in TRC volume. Although it is also possible that transient changes in TRC volume also induce changes in CT responses, our data suggest that sustained changes in TRC volume are more effective than

Fig. 12. Effect of solution osmolarity on relative changes in TRC volume measured with calcein. A: 7 TRCs were initially superfused with control solution (containing 140 mM NaCl). As indicated by horizontal bar, cells were superfused with a similar solution containing, in addition, 600 mM urea. Urea induced a rapid transient decrease in calcein fluorescence that was followed by a rapid spontaneous increase in fluorescence that stabilized to a value slightly above its baseline value in control solution. B: 3 TRCs were initially perfused with control solution (containing 140 mM NaCl). At time period indicated by horizontal bar, cells were superfused with a similar solution containing, in addition, 600 mM DMSO. At each time period, percent change in calcein fluorescence intensity of individual TRC was calculated relative to baseline fluorescence in control solution. Error bars, SE. DMSO induced only minor changes in overall fluorescence intensity.
transient changes. For example, the transient changes in TRC volume in response to urea (Fig. 12A) were significant; however, as shown in Fig. 4, a similar concentration of urea did not produce transient changes in the steady-state CT responses to 150 mM NaCl. Hence, it is tempting to speculate that transient changes in volume are significantly attenuated in vivo (see below) and occur on a faster time scale than shown in Fig. 12A. If the volume decrease transients in vivo were small and of very short duration, the CT nerve response transients might not be observed because of the small volume change and the fact that an integrator time constant of 1 s precludes the resolution of faster neural transients. It is important to note that the mechanisms that link TRC volume changes to TRC sensory activity, including their sensitivities and time resolutions, are unknown.

Further evidence in support of osmotically induced cell volume change is indicated by the increased resistance of the anterior lingual receptive field when hypotonic KCl rinse solution was replaced by 300 mM mannitol (cf. Fig. 1). It is known that increasing the osmotic pressure of the mucosal bathing solution of leaky ion-transporting epithelia in vitro results in increased transepithelial resistance. Studies on frog gallbladder showed that the resistance increased by ~40% when mucosal tonicity was increased by addition of 200 mM sucrose (5). This is comparable to the 53% increase in resistance we observed in rat tongue in vivo with 300 mM cellulobiose or mannitol. In frog gallbladder, ultrastructural studies revealed that the increase in resistance was related to the collapse of the lateral intercellular spaces and the shrinkage of the epithelial cells (5). Similar studies with Necturus gallbladder epithelium mounted in an Ussing-type chamber showed that increasing the apical solution osmolarity induced a small positive increase in transepithelial voltage as well as an increase in tissue resistance (36). The increase in transepithelial voltage is comparable to our observation of an increase in $V_{16}$ of ~5 mV in the lingual epithelium. From studies of CT responses to NaCl under lingual voltage clamp (38), it is unlikely that changes in $V_{16}$ of such small magnitude will significantly influence TRC membrane voltages and, therefore, neural responses. However, the changes observed in the in situ lingual electrical parameters are nonetheless similar to changes observed in vitro in other epithelia under osmotic stress. On this basis, one might anticipate TRC volume to be affected by exposure to anisotonic conditions in situ. As seen in Figs. 6–12, TRC volume decreased with increasing osmotic pressure in a reversible manner. In recent preliminary observations, using immunocytochemical techniques and RT-PCR, Gilbertson et al. (12) identified aquaporins (AQP1, AQP2, and AQP5), which are molecules involved in water flow across cell membranes, in rat and hamster TRCs. In addition, they observed that changes in external osmolarity induced voltage-activated currents in TRCs: hypertonic solutions increased inward currents, whereas hypotonic solutions increased outward currents. In preliminary studies from our laboratory (20), changes in TRC volume were associated with changes in intracellular pH and activation of a large anion pathway across TRC membranes. It is quite likely that the above mechanisms are involved in TRC volume regulation.

Cell volume changes are central to the osmoreceptor function of supraoptic neurons, resulting in the release of vasopressin (6). There are, in addition, afferent neural pathways originating in the oropharyngeal/laryngeal mucosa and terminating on neurons in the hypothalamus that release vasopressin (1). This release depends on the molarity of NaCl in the stimulus and is amiloride sensitive. The receptors in the mouth for hyperosmotically evoked thirst have not been identified, but the observations suggest that NaCl-induced changes in receptor cell volume could play a role.

In the present study, all the osmotic agents were nonelectrolytes, and the critical cell transport parameter distinguishing those that produced mixture enhancement from those that did not was their respective reflection coefficients. In cases involving nonisomotic electrolyte solutions, the relationship between TRC volume and excitation of the taste nerves is, however, far from a simple one, as can be seen from the variety of dilution or water responses reported in single taste fibers of various species (9, 31, 40) and psychophysically in humans (2). It would appear that TRCs in these cases are not functioning as simple osmometers, because dilution response often depends specifically on the solute present. In the cat CT (9) and rabbit superior laryngeal nerve (31), observations of the dilution or water response depend on the anion present in the solution applied to the receptive field. Increasing Cl$^-$ concentration inhibits the response from single water units, whereas increasing sulfate activates it. If modulation of a Cl$^-$ channel is an important part of the water response, as suggested (31), the key osmotic effect may be in recovery from osmotic swelling, not the initial swelling itself. The Cl$^-$ channel involved could be related to the swelling-activated anion channel, which is affected by the transmembrane Cl$^-$ gradient, the presence of foreign anions in the extracellular medium, and anion channel blockers (35). Evidence for this was obtained by Okada et al. (25), who found that increasing Cl$^-$ concentration and applying the Cl$^-$ channel blockers 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid and 4,4'-disothiocyanostilbene-2,2'-disulfonic acid inhibited the water response in frog taste nerves and the depolarization potential in TRCs resulting from the application of water to frog tongue. Recent studies in rat TRCs suggest that an anion channel (12) and a pathway for the exit of large anions (20) may be activated during cell swelling.

Osmotically induced changes in cell volume can exert mechanical stress on membranes and channel proteins (8). In this regard, it is interesting to note that rabbit laryngeal water-sensitive single units are also mechanosensitive (31). In amphibians it has been observed that cell swelling activates epithelial Na$^+$ channels and cell shrinkage suppresses them (11). The cloned rENaC (epithelial Na$^+$ channel), isolated from the rat colon,
was responsive to changes in external osmotic pressure when expressed in Xenopus oocytes. However, in this case, oocyte swelling decreased the channel activity, whereas use of mannitol to shrink oocytes increased the activity of eNaC (18). In our studies, amiloride effectively blocked CT responses to 150 mM NaCl and the subsequent increase in the CT response to 150 mM NaCl + 300 mM mannitol (Fig. 5). These data suggest that an osmotically induced increase in amiloride-sensitive Na\(^+\) channel activity in the apical membrane of TRCs is the basis of the taste mixture enhancement reported here. An increase in apical Na\(^+\) channel activity and the accompanying decrease in cell volume in hypertonic solutions should result in an increase in intracellular Na\(^+\) activity in TRCs, where the salt concentration of the applied stimulus remains constant. These changes in turn could influence cell potentials or other factors affecting receptor excitability. However, this hypothesis remains to be tested explicitly.

If a high reflection coefficient is sufficient to account for the mixture enhancement potential of mannitol and cellobiose, then it is likely that other nonelectrolytes with this property will have similar effects on taste responses. Support for this view emerges in the extensive literature on the taste effects of polyose, a mixture of short-chain polysaccharides with a mean molecular weight of 1,000 (27). Polyose, which is highly preferred by rats, gives a strong neural response in single units of the nucleus tractus solitarius at mean concentrations of 100–200 mM (13). Surprisingly, the units most stimulated are those that respond best to salts and acids and not those most sensitive to sucrose. In a subsequent study, Rehnberg et al. (27) tested undialyzed and dialyzed polyose on the CT response of hamsters. They found that removing the ionic contaminants from the polyose eliminated the CT response. However, the concentration of ionic contaminants could not alone account for the response; i.e., the presence of saccharide seems necessary to amplify the ionic response. Our results suggest that this may also be accomplished through osmotic shrinkage of the salt-sensitive receptor cells. Accordingly, the effects of polyose and other nonelectrolytes of high reflection coefficient merit further investigation along the lines presented here.

In the in vitro experiments, isolated TRCs are exposed to solutions on apical and basolateral membranes. However, it should be emphasized that TRCs are structurally polarized columnar epithelial cells with an apical projection of microvilli above the tight junctions and a smooth basolateral membrane below. The lingual epithelium actively transports Na\(^+\) from the mucosa to the submucosa, an indication that structural polarity has functional consequence (22). In a polarized preparation of lingual epithelium, we have observed in preliminary studies that a unilateral increase in NaCl concentration on the apical side induced a decrease in TRC volume, although a similar change in NaCl concentration on the basolateral side alone caused a significantly greater decrease in TRC volume. These unpublished observations suggest that the changes in TRC volume do occur in the intact epithelium and are significantly attenuated when the tissue is exposed unilaterally to hypertonic solutions from the apical side.

In summary, we observed that osmotically effective substances that produce sustained TRC shrinkage (mannitol or cellobiose) enhanced the rat CT response to NaCl when presented in mixture with NaCl. In contrast, osmotically ineffective substances that did not produce sustained TRC shrinkage (urea and DMSO) had no effect on the CT response to NaCl. These results indicate that changes in TRC volume directly affect the sensory function of TRCs.

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