Size Matters:

Anion Influences on Hamster Chorda Tympani Responses to Na^+ Salts

Riley E. Greene

Size Matters:

Anion Influences on Hamster Chorda Tympani Responses to Na^+ Salts

Riley E. Greene

May 15, 2000

On my honor, I have neither given nor received any unacknowledged aid on this paper.

Table of Contents

Abstract 3

Introduction 4

Gustatory Anatomy and Physiology: An Overview 6

Sweet Taste 16

Bitter Taste 19

Amino Acid Taste Including Umami 22

Sour Taste 24

Salt Taste
- **K^+ Salts** 26
- **Na^+ Salts** 27

Methods 32

Results 36

Discussion 38

Figure Legends 47

Figures 49

References 54

Abstract

Over the past two decades, researchers have shown repeatedly that salt taste reception depends, in numerous mammals, on the passive diffusion of Na^+ ions through apical, Na^+-selective ion channels that can be blocked by the diuretic drug amiloride. Oral application of amiloride suppresses the sodium responsiveness of the chorda tympani nerve, which innervates taste buds in fungiform papillae on the anterior portion and in rostral foliate papillae on the sides of the mammalian tongue. Using a powerful *in vivo* technique that permits control of the electrical potential across the epithelium of the tongue, Ye et al. (1991) identified two topologically separate Na^+ sensing mechanisms in rat fungiform papilla taste buds. Despite impressive gains in understanding Na^+ sensing in the rat taste system, less is known about sodium stimulus transduction in the hamster, which is widely used in studies of gustatory neural coding. The present study used *in vivo* voltage clamping with simultaneous chorda tympani neurophysiology to examine sodium sensing in the intact hamster. As expected, we observed an anion influence on relative chorda tympani response magnitudes mediated through indirect anion influences on apical Na^+ channel properties. The anion effect on Na^+ channel properties was evident in the lower K_m obtained for NaCl relative to NaGlu. Thus, our results suggest that differences in the transepithelial potentials generated by NaCl and NaGlu, which originate from differences in paracellular anion permeability, meditate stimulus-dependent modulation of apical Na^+ channel affinity.

Introduction

The chemical sense of taste is activated during the ingestion of food or liquid and may be thought of as fulfilling two disparate roles. On one hand, the sense of taste functions in the identification of essential nutrients including minerals, carbohydrates, proteins, and fats. Arguably, from an evolutionary perspective, the more important role of peripheral gustatory reception is to identify harmful and potentially toxic compounds prior to ingestion (Gilbertson, 1998). In response to the two demands placed on the peripheral gustatory system, those of nutrient detection and toxin avoidance, taste receptor cells must detect a wide variety of chemicals. These chemicals range from simple ions like sodium (Na^+) and hydrogen (H^+) to complex bitter and sweet tastant compounds. Thus, taste receptor cells function primarily to take the information contained in these chemical signals and translate it into the "language" of the cell. Accordingly, the mechanisms by which taste receptor cells transduce these diverse chemicals are numerous and varied (Gilbertson, 1998).

Taste receptor cells perform the formidable task of transforming stimulus energy into neural information about stimulus quality and concentration. The elucidation of the mechanisms by which taste receptors transduce and detect taste stimuli has occupied gustatory research efforts throughout the twentieth century. However, the pace of this delineation of the specific molecular interactions which occur in taste transduction can be described as sluggish at best due to the difficulties associated with studying taste receptor cells *in situ* (Stewart et al., 1997). Recently, elucidation of the transduction pathways for salty, sweet, bitter, and sour stimuli has progressed more rapidly due to the increased implementation of biochemical and molecular biological

techniques in studies of the taste system. For the aforementioned taste categories, potential transduction mechanisms have been proposed that range from ion channels for stimulus translocation to the transduction of sweet and bitter taste stimuli through various second messenger-linked cell surface receptors (Stewart et al., 1997).

While early, reductionist models championed the notion of transduction of the diverse classes of taste stimuli through individual taste receptor cells specific to only one of the basic tastes, the data are equivocal. These early models asserted that unique classes of taste receptor cells accomplished this specificity by possession of only a specific subset of the cellular machinery required for the transduction of a specific tastant. However, in recent studies, impalement of individual taste receptor cells with microelectrodes has produced data that suggest that individual taste cells respond to more than a single taste quality (Roper, 1992). Therefore, based on these and other data, investigators currently assert that individual classes of tastants may be transduced by several different, but possibly overlapping, mechanisms. The multiple and specific, newly elucidated mechanisms of transduction undoubtedly contribute to the perception of many subtle tastes in foods and may enable the discrimination of different compounds within a single taste category. The following sections will focus on the mechanisms by which individual classes of tastants (salty, sweet, sour, bitter) produce receptor potentials in taste receptor cells. I begin with a brief overview of gustatory anatomy and taste receptor cell physiology.

Gustatory Anatomy and Physiology: An Overview

The cells responsible for the transduction of taste stimuli in the oral cavity are the taste receptor cells. These cells are polarized neuroepithelial cells which, in clusters of 50-100 cells each, form taste buds. Taste receptor cells are generally embedded in specializations of surrounding epithelium, termed papillae (Stewart et al., 1997). Individual taste receptor cells are joined at the apical end by tight junctions which, hypothetically, form an impenetrable barrier to most taste stimuli. The apical membranes of taste receptor cells lie above the tight junctions and protrude microvillar processes though a small opening, the taste pore, into the oral milieu. While tight junctions have been viewed as impermeable to taste stimuli historically, recent data suggest that the tight junctions function as a selectively permeable barrier between taste cells (Stewart et al., 1997). Moreover, this selective permeability repudiates the notion that taste stimulus-receptor interactions occur only on the apical membrane domain. In fact, recent data show that tight junctional selective permeabliity allows a number of stimuli to interact meaningfully with the basolateral regions of receptor cells (Gilbertson, 1998). This is discussed in more detail in later sections.

Taste buds reside both in the tongue and throughout the oral cavity. The tongue bears three distinct regions of unique papillae, all of which house taste buds. The fungiform papillae are found on the anterior two-thirds of the tongue, with each papilla containing typically one (rodents), but as many as four (ungulates and primates), taste buds. In mammals, 20% of the taste buds in the oral cavity are contained within fungiform papillae (Gilbertson, 1998). Afferent innervation of the fungiform papilla taste buds is supplied by the chorda tympani branch of the

facial (VIIth cranial) nerve. In many mammals, the two foliate papillae are located further caudal to the fungiform papillae and on the lateral surface of the tongue (Stewart et al., 1997). These trench-like specializations are studded with 33% of the taste buds in the oral cavity (Gilbertson, 1998). Foliate receptor cells receive mixed innervation: the more rostral foliate receptor cells are innervated by the chorda tympani nerve, while the more caudal cells receive innervation from the lingual tonsilar branch of the glossopharyngeal (IXth cranial) nerve (Gilbertson, 1998). Finally, located most caudally on the tongue are the circumvallate papilla(e). Circumvallate papillae are semicircular trench-like epithelial structures, the number of which ranges from one in rat to a dozen or more in higher primates, including humans. The walls of the circumvallate papillae are densely packed with taste buds, often with several hundred per papilla, all of which are innervated by the glossopharyngeal nerve (Gilbertson, 1998; Stewart et al., 1997).

Receptors in the tongue, however, are not the only mediators of taste. Taste receptor cells are also found in other areas of the oral cavity, including the soft palate, epiglottis, esophagus, and the nasopharynx. Those cells found in the soft palate are, among the cells located in the aforementioned areas, the most important contributors to taste perception, based upon sheer number of taste cells. The taste buds distributed in the epiglottis, esophagus, and nasopharynx are innervated by a branch of cranial nerve X (vagus), while the palatal taste buds are innervated by the superficial petrosal branch of the VIIth cranial nerve (facial) (Gilbertson, 1998).

Among the taste receptor cells within the taste buds are several morphologically distinct cell types. The currently posited cell types have spurred recent debate due to their inablility to be categorized solely on the basis of unambiguous morphological characteristics. However, although there is disagreement over categorization of all taste cells, consensus generally holds

that there are at least two distinct types of receptor cells in mammals (Type I or dark and Type II or light). Type I cells present a characteristically long and narrow morphology and extend from the base of the taste bud to the taste pore. Most investigators concur that Type I cells are the most numerous cells in vertebrate taste buds, comprising roughly 55-75% of the total cell population (Roper, 1989). Moreover, Type I cells may be distinguished from other cell types by the presence of membrane-bound granules in the apical cytoplasm. Light cells, or Type II cells, have similar ultrastructural morphology to Type I cells save for the absence of cytoplasmic granules. Light cells represent a smaller fraction of cells in the taste bud than Type I cells (Roper, 1989). Based on the finding that both Type I cells and Type II cells synapse onto afferent nerve fibers, Kinnamon (1986) asserts that both light and dark cells are chemosensory.

Additionally, many researchers name two other distinct types of taste cells within the taste buds. Type III cells are morphologically similar to Type II cells, but contain numerous dense- cored vesicles in the basal cytoplasm, and are believed to perform a sustentacular rather than chemosensory function (Kinnamon, 1986). However, the sporadic observation of this cell across species has limited the number of investigators who agree upon its separate identity. Finally, basal, or Type IV cells, have a characteristically flattened or oblate morphology, lie at the base of the taste bud, and do not send processes to the taste pore (Roper, 1989). Many investigators assert that, while the basal cell may seem to be a morphologically distinct cell type, it may only represent an immature state of one of the other cellular elements of the taste bud (Kinnamon, 1986).

Taste cells are part of a dynamic system and are subject to an ongoing turnover at a rate comparable to that in the surrounding stratified epithelium. A current debate pits Kolmer's

(1910) unifying hypothesis that the vertebrate taste bud is comprised of only a single cell line which adopts different morphological features during its lifespan, against the hypothesis that multiple cell lines exist and perform chemosensory, sustentacular, or secretory functions, respectively (Roper, 1989). However, Delay et al. (1986) provided strong evidence for the hypothesis that basal cells function as stem cells. Over succeeding days, Delay et al. (1986) found a thymidine label first in basal cells, then in the nuclei of Type I cells, then in the nuclei of an intermediate (transition) cell type, and finally, within Type II cells' nuclei. Thus, Delay et al.'s (1986) results suggest that as the basal cell differentiates and progresses through its lifespan, it transforms into a Type I cell, then to an intermediate cell, and lastly to a Type II cell. As an interesting aside, chemical synapses between serotonergic basal cells and taste receptor cells have been described suggesting that basal cells may serve an interneuronal function (Roper, 1992). Thus, basal cells may provide the physiological mechanism for "cross talk" or lateral synaptic interactions between taste receptor cells. Lateral synaptic interactions may process initial signals or modulate the output of the taste buds, thus functioning much like the horizontal and amacrine cells in the visual system (Roper, 1992).

Taste receptor cells provide the mechanism for the transduction of gustatory stimuli--that is, the interaction of taste stimuli with the receptor cell induces the release of neurotransmitter onto the gustatory afferent nerve fibers. While the initial event in taste receptor cell activation involves a taste stimulus-receptor cell interaction, there is no one mechanism underlying taste transduction. Instead, there are myriad membrane events that occur during taste stimulation, including stimulus permeation through ion channels, G-protein activation, probable intracellular Ca^{2+} release, and combinations of these and other events (Roper, 1992). However, the end result

of stimulus-receptor cell interactions is often a depolarization, or receptor potential, within the cell (Gilbertson, 1998). Then, much like a prototypical neuron, the receptor potential generated within the taste cell activates a number of voltage-dependent Na^+ and potassium (K^+) channels, followed by action potential propagation. Next, voltage-activated Ca^{2+} channels may open, or Ca^{2+} may be released from intracellular stores, leading to an increase in intracellular free Ca^{2+} concentration and, finally, modulation of neurotransmitter release onto afferent nerve fibers (Gilbertson, 1998). *Nota bene* that while taste receptor cell depolarization followed by action potential propagation has been repeatedly observed across species in response to many stimuli, action potential generation is not invariant, as some stimuli seem to elicit neurotransmitter release without concomitant receptor cell depolarization (Gilbertson, 1998).

While recent evidence suggests that tastants elicit action potentials from taste receptor cells in many species, the role of action potentials in taste coding is uncertain. Some investigators suggest that action potential frequency imparts some information about stimulus intensity: the more intense the stimulus, the greater the action potential frequency generated both at the level of the taste bud and in the isolated cells (Gilbertson, 1998). Action potentials may also provide the depolarization necessary to open voltage-activated Ca^{2+} channels facilitating Ca^{2+} influx and subsequent neurotransmitter release (Gilbertson, 1998; Stewart et al., 1997). Thus, as a general paradigm for the transduction of taste stimuli, Roper (1989) suggests the following steps: (1) chemical stimulus presentation; (2) ligand-receptor interactions on the apical [and in some cases, basolateral] membrane of taste cells; (3) an intracellular membrane potential change (receptor potential); (4) alteration in neurotransmitter release at taste cell synapses; (5) diffusion of transmitter across the synaptic cleft; (6) transmitter binding to the postsynaptic

(gustatory afferent) membrane; (7) postsynaptic membrane potential change; and finally (8), alteration in impulse activity in a sensory afferent axon.

As Roper (1989) suggests, one of the final steps in the transduction of taste stimuli involves the release of neurotransmitter from the basolateral region of the receptor cell onto associated gustatory afferent nerves. Typically, up to six taste receptor cells of a single morphological class (i.e. Type I or Type II) are innervated by a single nerve fiber. This pattern of innervation suggests that a single taste bud may be parsed into smaller organizational units consisting of cells with similar morphology, which are synaptically connected to a single afferent output (Gilbertson, 1998). Currently, there is no definitive identification of the neurotransmitter(s?) which mediates transmission between taste receptor cells and afferent fibers in any species. However, there have been a number of neurotransmitters identified which meet several of the criteria required for chemical synaptic transmission. These include acetylcholine, amino acids, neuropeptides, and biogenic amines.

Histochemical studies revealed to early investigators the presence of acetylcholinesterase at the base of taste buds where the nerve supply enters. However, application of cholinergic agonists and antagonists produced alterations in reception that investigators deemed resultant from pharmacological actions unrelated to cholinergic neurotransmission. Furthermore, choline acetyltransferase (ChAt), a critical enzyme for acetylcholine (ACh) synthesis, has yet to be observed in vertebrate taste buds. Thus, the bulk of the current data argues against the utilization of acetylcholine for neurotransmission by taste receptor cells (Roper, 1992).

The observation that amino acids are taste stimuli as well as potent central nervous system neurotransmitters (glutamate, etc.) has led many investigators to suggest a possible role

for amino acids in taste receptor cell neurotransmission. However, while GABA and glutamate containing axons have been identified in the taste bud, data from systematic studies on their proposed action at synaptic sites in taste buds is lacking (Roper, 1992).

The neuropeptides bombesin, cholecystokinin, calcitonin gene-related peptide (CGRP), galanin, gastrin-releasing peptide, peptide HI, substance P, and vasoactive intestinal peptide have been localized to taste cells and to their nerve supply. Historically, much attention has been focused on substance P which is present in nerve fibers that penetrate the taste buds and pass near, but do not directly innervate, taste cells. The absence of direct synaptic contacts between substance P-containing fibers and taste receptor cells seems to repudiate the notion that substance P is uniquely responsible for neurotransmission. However, current research has focused on various axillary functions substance P may play in taste, including: (1) regulation of the 5-HT content of basal cells, and (2) modulation of gustatory afferent nerve responses to salt and acid stimuli. These data suggest that, while not solely responsible for neurotransmission, substance P may play a modulatory role at the level of the taste receptor cell (Roper, 1992). Additionally, some authors have suggested that substance P plays a trophic role in the maintenance and regeneration of taste cells (Roper, 1992).

The presence of noradrenaline and 5-HT in vertebrate taste buds has also been shown repeatedly. The current consensus is that serotonin is present in basal cells in lower vertebrates and in Type III cells in mammalian taste buds. Moreover, as serotonergic mechanisms are known to be involved in reducing food intake, many investigators suggest (part and parcel to its effects in the gut and CNS) that the aforementioned anoretic effect may localize at the peripheral taste receptor level by 5-HT-mediated peripheral sensory sensitivity enhancement (Roper, 1992).

Additionally, many investigators have concluded that 5-HT is the transmitter between taste cells and sensory afferent fibers; yet, others assert the contradictory conclusion that noradrenaline serves this function (Roper, 1992). Thus, the role of biogenic amines in peripheral taste processing remains clouded.

Although the debate persists as to which neurotransmitter(s?) is released from taste receptor cells causing the subsequent generation of afferent neuronal action potentials, a general consensus exists for the pathway that taste information follows from the peripheral taste receptors to the CNS. In vertebrates, the gustatory afferents terminate within the nucleus tractus solitarius (NTS) of the medulla. From the NTS a tripartite pattern of connections emerges including a local reflex system, an ascending lemniscal system, and a diffuse visceral limbic system.

In its role of detection and subsequent rejection of particularly toxic or otherwise inedible compounds, the local reflex system predominates in taste information processing. Stimulation of the taste buds and processing through the local reflex system can, as Finger (1986, p. 334) suggests, "invoke ingestive reflexes such as swallowing, salivation, and gastric changes, or oropharyngeal rejection such as spitting, coughing, or gagging." In order to illicit these responses, the NTS nuclei project axons to the salivatory nuclei, nucleus ambiguus, and dorsal motor nucleus of the vagus, all of whose visceral motor or premotor neurons mediate the aforementioned responses. Moreover, the vagal part of the NTS in its local reflex function, makes connections which permit the evaluation of the ingestability of potential foodstuffs. On the other hand, the facial-glossopharyngeal rostral part of the NTS controls food selection, taste hedonics to some extent, and oral foodstuff manipulation (Finger, 1986).

Much like the dorsal column-medial lemniscus somatosensory system, the geniculo-striate visual pathway, and the cochlear nucleus-lateral lemniscus auditory system, the gustatory system comprises a sensory lemniscal channel, defined as "a multisynaptic labeled-line sensory pathway which terminates in a specific telencephalic target zone" (Finger, 1986, p. 334). Within the lateral NTS, the chorda tympani nerve, the lingual tonsilar branch of the glossopharyngeal nerve, and the vagus nerve project mainly to unique, representative areas. The chorda tympani input predominates within the anterior third of the NTS, glossopharyngeal inputs are heavier just caudally, while the vagally innervated gustatory fields have their heaviest representation most caudally (Finger, 1986).

Gustatory information arrives at the thalamus from the NTS via one of two routes. In the direct perceptual pathway, taste information passes from the NTS directly to the ventral thalamic gustatory relay nucleus. In the second route, taste information follows projections from the NTS to the dorsal pons (pontine gustatory nucleus) whose axons, in turn, project to the same gustatory nucleus in the ventral thalamus that receives the direct perceptual pathway information. Subsequent to its arrival in the thalamic gustatory nuclei, taste information travels to a telencephalic gustatory zone (Area 43 or insular cortex). As a further specialization, Scott & Giza (1990) concluded that as NTS neurons can be categorized into four groups on the basis of their response profiles to a broad stimulus array (i.e. Group 1-salt-sweet, Group 2-salt, Group 3-salt-acid, and Group 4-acid-salt-bitter), the segregation of function that is maintained from receptor to the CNS implies the existence of discrete information channels in the taste system. Finally, the affective (hedonic) aspects of taste are mediated physiologically by extensive connections with many limbic and supratentorial visceral regulatory areas. Finger (1986) points

out that the "ascending gustatory fiber systems terminate extensively within the lateral hypothalamus and basal forebrain areas, including the amygdala"(p. 336).

While the physiological mechanism by which taste information ascends the neuraxis is lucid, the nature of the neural representation, or code, of taste quality has been debated for many years. The coding issue refers to whether the activity in a given sensory neuron is an unambiguous representation of the quality of the stimulus applied to its receptors or whether this activity is meaningful only in the context of activity in other afferent fibers. Two contrasting coding theories currently vie for dominance. The labeled-line hypothesis of taste-quality coding suggests that activity in a particular fiber type represents a specific taste quality, whereas the across-fiber pattern theory holds that a particular pattern of activity across the entire ensemble of afferent fibers represents a taste quality (Smith & Frank, 1993). Ample evidence has been given in support of each hypothesis, providing the impetus for many investigators to suggest a possible synthesis of the two wherein some tastes (i.e. salt) may be coded on a labeled-line while others may utilize an across-fiber approach. Given recent advances in the neuroanatomical and neurochemical delineation of the gustatory nuclei and more comprehensive gustatory stimulating techniques, a resolution to this issue may be imminent.

While numerous chemicals function as taste stimuli by eliciting activity from taste receptor cells, there are considered to be only four or five basic tastes. Humans group their perceptions of various tastant molecules into categories of salty, sour, sweet and bitter. Additionally, umami, the unique taste which corresponds to monosodium glutamate (MSG), has been proposed as a fifth taste category (Gilbertson, 1998). In the sections which follow, we review the currently hypothesized transduction mechanisms for taste stimuli in each of the five

aforementioned categories.

Sweet Taste

Sweet substances include mono-, di-, and polysaccharides and polyalcohols, in addition to some amino acids, peptides, and proteins. Behavioral and electrophysiological experiments have shown that disaccharides are comparatively more potent sweet stimuli than mono- and polysaccharides (Stewart et al., 1997). In parallel with most general classes of taste stimuli, numerous cellular mechanisms appear to mediate sweet taste transduction. Considerable evidence suggests that sweet tastants bind to G-protein-linked receptors wherein the G-protein α-subunit subsequently stimulates the activity of adenylate cyclase leading to cAMP production. Cyclic adenosine monophosphate, in turn, activates protein kinase A (PKA) which phosphorylates, and thus, closes a basolateral K^+ channel, causing taste receptor cell depolarization (Cummings et al., 1993).

Early evidence for this mechanism was provided by Tonosaki & Funakoshi (1988) who showed that sucrose depolarized mouse taste cells with a concomitant increase in membrane resistance, and that intracellularly injected cAMP and cyclic GMP (cGMP) also induced depolarization accompanied by an increase in membrane resistance. Moreover, Tonosaki & Funakoshi (1988) showed that these effects could be mimicked by intracellular applications of the K^+ channel blocker tetraethyl ammonium (TEA). Further support for this model came from biochemical studies by Striem and colleagues (1989), who showed that some sweet tasting compounds stimulate the formation of cAMP in a rat tongue membrane preparation. Moreover, Striem et al. (1989) showed that the activation of adenylate cyclase by sugar sweeteners requires

guanine nucleotides. Thus, Striem et al.'s (1989) results suggest that the sweet receptor is a heptahelical transmembrane protein wherein G-protein activation leads to increased adenylate cyclase activity (Lindemann, 1995).

In patch-clamp experiments on frog taste cells, injection of cAMP-dependent protein kinase into taste receptor cells caused depolarization through an inhibition of outward K^+ currents (deceased K^+ conductance). Also, Cummings et al. (1993) reported results based on whole cell recordings from isolated taste cells of hamster which were consistent with sweet compound-induced closure of K^+ channels. Therefore, these combined results support a paradigm for saccharide taste transduction in several species which consists of sweet stimulus G-protein activation followed by cAMP production leading a to blockade of the resting K^+ conductance and concomitant taste receptor cell depolarization.

Notably, a taste receptor cell-specific G-protein, gustducin, was recently cloned. Gustducin is 80% homologous to transducin, the G-protein that activates phosphodiesterase in photoreceptor cells. In order to determine whether gustducin plays a role in sweet-taste transduction, Wong et al. (1996) generated and characterized mice deficient in the gustducin α-subunit (α-gustducin). Wong et al. (1996) noticed, unexpectedly, that null mice showed decreased preference responses to two sweet substances, sucrose and the sweetener SC45647, compared to their wild-type siblings. Next, Wong et al. (1996) performed recordings from the chorda tympani nerve to confirm a peripheral taste deficit in the null mice. The chorda tympani responses to the sweet compounds sucrose and SC45647 were significantly lower in the null mice than in the wild-type mice. Thus, on the basis of their behavioral and electrophysiological results taken together, Wong et al. (1996) conclude that α-gustducin, in part, mediates the

transduction of sweet taste stimuli.

Although the *sin qua non* for natural saccharide transduction may be the activation of a G-protein followed by second messenger production, artificial sweeteners, unlike saccharides, do not appear to stimulate cAMP formation. These findings, coupled with the data from Cummings et al.'s (1993) cross adaptation studies, suggest one of the following possibilities: (1) different sweet receptor cell types exist which correspond to only one type of sweetener; (2) multiple types of sweet receptor molecules inhabit sweet-receptive cells; or (3) multiple binding sites on a given sweet receptor molecule respond to differently structured sweeteners. Thus, while a single class of receptors appear to bind glucose, fructose, and sucrose and transduce this information through the cAMP second messenger, another family of sweet receptors may exist which utilizes a different mechanism for artificial sweetener transduction.

The non-sugar sweeteners saccharin and SC-45647 appear to bind to taste receptor cell receptors which stimulate the production of the second messenger inositol 1,4,5-trisphosphate (IP_3) and not cAMP, in rat. Thus, artificial sweeteners may bind to a sweet receptor linked via G-proteins to the activation of phospholipase C (Gilbertson, 1998). Moreover, IP_3 has been shown to increase intracellular Ca^{2+} release from intracellular stores, probably even without receptor cell depolarization. However, Kolesnikov & Margolskee (1995) proposed an alternate model based on the observation that many of these same cells respond to sucrose with increases in intracellular Ca^{2+} concentration:

> ...a tastant-activated receptor activates transducin; activated transducin, in turn, activates cAMP-specific PDE; the ensuing drop in taste receptor cell cAMP concentration activates a cyclic nucleotide monophosphate-suppressible channel, leading to depolarization, increased Ca^{2+} influx and therefore increased intracellular Ca^{2+} concentration. The cascade may be turned off, as with the

cGMP cascade in photoreceptors, via receptor inactivation, hydrolysis of GTP by α-transducin and Ca^{2+} enhanced cNMP cyclase activity.

Gilbertson (1998) argues the antithesis of Cummings et al. (1993), as he stresses that the activation of heterotrimeric G-proteins has been shown to stimulate both adenylate cyclase to produce cAMP and phospholipase C to produce IP_3, simultaneously: the α-subunit may activate adenylate cyclase while the βγ-subunits enable PLC activation at higher agonist concentrations. However, Gilbertson (1998) does cede that sugar and non-sugar sweetener cross-adaptation (shown by Cummings et al., 1993) suggests that these dual pathways must converge at some common target. Thus, while artificial and natural sweeteners may be linked to disparate second messenger systems, their coexpression within individual taste cells and their possible convergence on a common target ensures reliability in sweet taste (Stewart et al., 1997).

As a final caveat, some investigators suggest that sweet transduction does not involve second messenger-mediated systems at all. Experiments in canine lingual epithelium have shown that sugars stimulate an amiloride-sensitive cation influx. Thus, in dog and possibly in carnivores generally, a ligand-gated, nonselective, apical membrane cation channel may function as one type of saccharide receptor. Further studies are required to investigate this novel result (Stewart et al., 1997).

Bitter Taste

The tremendous diversity of bitter stimuli, from simple salts like K^+ and Ca^{2+} to complex structures such as quaternary amines (denatonium) and acetylated sugars (sucrose octaacetate), makes the existence of an omnibus bitter receptor unlikely (Gilbertson, 1998). Furthermore, the identification of a genetic dimorphism in the ability of humans to taste phenylthiocarbamide

(PTC) as bitter suggests that there are at least two, most likely protein (coded for by DNA), receptors involved in bitter taste (Akabas, 1993).

The detection of bitter substances is extremely important for the survival of an organism, as sensitivity to bitter stimuli is a protective mechanism for poison avoidance. Thus, as many plant and environmental toxins taste bitter, there is strong evolutionary pressure for high bitter sensitivity (McLaughlin & Margolskee, 1994). The most intensely bitter substances include the plant alkaloids atropine, caffeine, coniine, morphine, nicotine, quinine, and strychnine (Stewart et al., 1997). Due to their high toxicity, it is not surprising that many species have evolved taste systems to detect bitter substances at lower concentrations than salts and saccharides. Recent investigations of bitter transduction mechanisms provide evidence for the constellation of transduction mechanisms expected for the diversity of bitter-tastant structures.

Current evidence points to a common transduction mechanism for bitter salts and the alkaloid quinine in mudpuppy. These bitter stimuli have been shown to block K^+ channels in taste receptor cells, inhibiting K^+ efflux, and thus, depolarizing the cell. The direct interaction of bitter tastants with sites on the K^+ channel seems to mediate this depolarization. Furthermore, as compounds which have the ability to block K^+ channels appear to have taste qualities similar to those evoked by classical bitter stimuli, these behavioral data further support the aforementioned transduction mechanism (Gilbertson, 1998).

In contrast, recent data support the notion that most bitter taste stimuli do not directly inhibit K^+ channels, but rather exert effects through receptor-mediated transduction mechanisms. Using the intensely bitter stimulus denatonium benzoate, Akabas et al. (1988) showed that in the presence of denatonium, a subset of rat circumvallate taste cells responded with an increase in

intracellular Ca^{2+}. This increase was independent of extracellular Ca^{2+} levels, indicating the release of Ca^{2+} from intracellular stores. Akabas et al. (1988) speculate that due to denatonium's membrane impermeance, the first step in its transduction mechanism must involve binding to an apical cell surface receptor. This interaction between denatonium and its receptor (assumed to be G-protein-linked) results in the activation of phospholipase C (PLC) and the subsequent generation of the second messengers diacylglycerol and IP_3. Inositol trisphosphate, in turn, stimulates the release of Ca^{2+} from intracellular stores, which may subsequently trigger neurotransmitter release from the taste cell without a change in membrane potential (Gilbertson, 1998).

The recent cloning of gustducin, and the subsequent discovery that α-gustducin's base sequence is 80% identical to rod cell α-transducin, has led some investigators to suggest that α-gustducin has an analogous function to α-transducin. Rulz-Avila and colleagues (1995) directly demonstrated that rod transducin is present in taste cells where it is activated by a denatonium-responsive taste receptor which, in turn, stimulates a taste-tissue specific phosphodiesterase (PDE). However, Rulz-Avila et al. (1995) were not able to show analogous stimulation of taste-tissue PDE by gustducin. To date, the only data supporting the role of gustducin in bitter transduction was provided by Wong et al. (1996) in the investigation of gustducin knockout mice. Mice lacking the gustducin gene are much less responsive to denatonium and quinine both behaviorally and electrophysiologically.

The effects of the decrease in cAMP by PDE activation are presently unclear. Recent hypotheses, such as that offered by Kolesnikov & Margolskee (1995), name possible targets including cyclic nucleotide-gated ion channels in taste cells. By this mechanism for example, the

fall in cAMP could inhibit cAMP-activated cation channels in rat taste receptor cells (Kolesnikov & Margolskee, 1995). The diversity of mechanisms for detecting bitterness may reflect evolution's attempt to accommodate the myriad compounds in nature that could pose a threat of toxicity (Stewart et al., 1997).

Amino Acid Taste Including Umami

The comparatively little which is known about how amino acids, including glutamate, are transduced comes largely from investigations on non-mammalian aquatic species. To humans, D-amino acids and their L-enantiomers have a sweet and bitter taste, respectively (Stewart et al., 1997). The unique taste of L-glutamate and also of L-aspartate prompted Ikeda to propose a fifth basic taste, which he termed "umami." Presently, amino acid sensing in the channel catfish *Ictalurus punctatus,* is the only well understood amino acid sensing system.

In the catfish facial taste system, three independent amino acid receptor sites, each linked to unique transduction mechanisms, have been described (Stewart et al., 1997). Brand et al. (1991) described a G-protein linked receptor site for L-alanine and short-chain neutral amino acids. As is the case with bitter stimuli, cAMP and IP_3 appear to be involved in amino acid transduction but the steps following second messenger production remain to be elucidated in the future. Two other high-affinity receptors exist for L-arginine and L-proline which do not appear to be linked to a second messenger system. Instead, each comprises a ligand-gated cation-nonselective channel (Brand et al., 1991; Stewart et al., 1997). Binding of L-arginine or L-proline to its respective receptor may facilitate the entry of cations. Thus, amino acid sensing in

I. punctatus appears to occur through several, stimulus-variant pathways.

Few detailed studies on the transduction mechanisms of amino acids in mammalian systems have been performed save a few focusing upon L-glutamate transduction. L-glutamate has been shown to activate cation channels in reconstituted mouse taste membranes with synergistic effects produced by the addition of guanosine 5'-monophosphate (5'-GMP) (Gilbertson, 1998). In order to determine whether taste receptor sites for L-glutamate possess features similar to brain glutamate receptor sites, Faurion (1991) observed chorda tympani responses in hamster to lingually applied glutamate as well as to agonsits and antagonists of N-methyl-D-aspartate (NMDA) and non-NMDA receptors. L-glutamate and L-aspartate, part and parcel to their potential value as taste stimuli, produce CNS excitation through a variety of receptors in the glutamate receptor family. Faurion's (1991) results suggest that NMDA brain receptor agonists most effectively stimulate the glutamate receptor on the hamster anterior tongue. Thus, Faurion (1991) concludes that the glutamate taste receptor and NMDA CNS receptors share homologous ultrastructural characteristics.

Recently, after the cloning of mGluR4, a glutamate receptor from rat, many investigators have asserted that this glutamate receptor may represent the umami receptor. Behavioral experiments have shown that the mGluR4 agonist, 2-amino-4-phosphonobutyrate (L-AP4), has a similar taste to MSG. L-glutamate acting through the mGluR4 receptor would, most likely, decrease intracellular cAMP since the inhibition of adenylate cyclase by the mGluR4 receptor has been demonstrated in other cell types (Gilbertson, 1998). As a relative paucity of data exist, except for a few divergent results regarding glutamate transduction, much future research will need to focus on the elucidation of the many likely mechanisms of amino acid taste transduction.

Sour Taste

In a dose-dependent manner with decreasing pH, acidic stimuli (specifically, protons) elicit action potentials from taste cells, which confer the perception humans refer to as sour. Significant differences in the mode of sour stimulus transduction have been observed across species. It is not surprising that a number of taste transduction mechanisms have been proposed for acids, as protons enact multipartite effects on ion channels while dynamicisity in intracellular pH bequeaths modified signal transduction (Gilbertson, 1998).

In the mudpuppy, *Necturus*, detailed analyses of sour transduction mechanisms have been performed. Kinnamon & Roper (1988) extensively pursued the role of K^+ conductance changes in the transduction of sour stimuli in *Necturus*. Kinnamon & Roper (1988) performed whole cell and loose-patch clamp studies *in situ* and *in vitro* in order to demonstrate that by decreasing a resting outward K^+ conductance, acidic stimuli elicit action potentials. Kinnamon & Roper (1988) suggest that depolarization arises from H^+ block of a voltage-sensitive K^+ channel. To support further their conclusion, Kinnamon & Roper (1988) illustrated the effect of the potassium channel blocker TEA on responses to weak acids, and showed that TEA completely and reversibly blocks the response to acidic stimuli.

The results of the majority of studies concerning sour transduction in *Necturus* suggest two essential features of voltage-gated K^+ channels. First, a hyperpolarizing potassium efflux at rest facilitates H^+ block-induced depolarization. Second, an enrichment of channels in the apical domain must facilitate a significant depolarization if H^+ can in fact access only apical channels (Stewart et al., 1997). Various studies have repeatedly demonstrated that these requirements are

met by the mudpuppy taste system.

Since H^+ ions are able to permeate the apical amiloride-sensitive Na^+ channel in other tissues, Gilbertson et al. (1992) reasoned that H^+ conductance through a similar channel in hamster taste cells could account for acid stimulus-induced depolarization. Support for this hypothesis came from the finding that amiloride mostly eradicates action currents in response to citric acid application. Also, a single amiloride concentration proved to block equally both Na^+ and H^+ action currents in single cells. These results suggest that a single amiloride-sensitive channel provides H^+ and Na^+ access to taste receptor cells, reflecting a potential unitary mechanism for sour and salt stimulus transduction in hamster (Stewart et al., 1997). However, in a recent study, Stewart et al. (1998) showed, using simultaneous *in vivo* voltage clamping and chorda tympani recording, that chorda tympani responses to various concentrations of HCl in the intact hamster taste system were not significantly influenced by voltage perturbation. Furthermore, Stewart et al. (1998) demonstrated that neither amiloride nor benzamil significantly inhibited HCl chorda tympani responses. Thus, the combined results of this study led Stewart et al. (1998) to conclude that, in hamster, acid stimuli may interact with transduction sites in both the apical and basolateral domains of taste receptor cells. While it is still possible that Na^+ and H^+ ions share a single transduction pathway, wherein a complex coding mechanism affects discrimination between the two, further research will be necessary to determine the physiology behind salt and acid stimulus transduction in hamster.

In striking contrast to hamsters, early evidence suggested that, in rat, H^+ flux through taste cell apical Na^+ channels does not occur at all. These findings prompted DeSimone et al. (1995) to investigate the nature of apical H^+ conductance in rat fungiform papillae, using voltage

clamping and chorda tympani recording simultaneously. DeSimone et al. (1995) showed that while NaCl responses show sensitivity to epithelial voltage perturbations, HCl responses, unlike NaCl responses, were not suppressed by serosal positive voltages. Furthermore, negative voltage clamp caused an aberrant reduction in HCl response magnitude. These data discredit the hypothesis of passive diffusion of H^+ through an apical ion channel in rat taste cells. Furthermore, as amiloride does not inhibit chorda tympani responses to HCl, apical H^+ conductance via amiloride-sensitive ion channels is unlikely (Stewart et al., 1997). These and other observations prompted DeSimone et al. (1995) to conclude that H^+ must utilize tight junctional selective permeability to effect basolateral transduction sites of taste cells. Further studies will be necessary to support DeSimone et al.'s (1995) conclusions, but a concrete hypothesis regarding the specific cellular processes that transduce acid stimuli in rat taste cells seems to be on the horizon.

Salt Taste

K^+ Salts

Ye et al. (1994), using an *in vivo* voltage clamp technique, showed that in the case of KCl, the voltage-dependent modulation of the CT response is absent, and they reported a nearly zero voltage sensitivity index (VSI), the difference in chorda tympani responses at two distinct voltage clamp conditions. Moreover, Ye et al. (1994) reported that the response magnitude and time course of the K^+ salt response is strongly anion dependent. Finally, they showed that K^+ channel blockers fail to alter K^+ responses when applied to the tongue. *In toto*, this evidence

supports the conclusion that apical K^+ channels in rat taste cells are not a major factor in K^+ salt transduction; instead, Ye et al. (1994) assert that the paracellular shunt is a principal transduction pathway for salts and the response, therefore, is diffusion limited. Further support for the diffusion-limited paracellular model for K^+ transduction came from the observation that potassium gluconate stimulated small neural responses virtually identical to those of potassium benzoate (Stewart et al., 1997). Also, as would be expected for a diffusion-controlled process, under zero current clamp, potassium gluconate responses reached half-maximum approximately 30 times more slowly than KCl responses. Ye et al. (1994) also noted that under large negative voltage clamp, potassium gluconate chorda tymapani responses were comparatively fast, as stimulus K^+ most likely electrophoreses through the tight junctions in response to the voltage differential, independent of the slowly diffusing gluconate anion (Ye et al., 1994). Thus, to account for their data, Ye et al. (1994) suggest that electroneutral diffusion of K^+ and Cl^- through the tight junctions promotes transduction through basolateral K^+ channels.

Na^+ Salts

Over the past two decades, researchers have shown repeatedly that salt taste reception depends on the passive diffusion of Na^+ ions through apical, Na^+-selective ion channels that can be blocked by the diuretic drug amiloride. Oral application of amiloride suppresses the sodium responsiveness of the chorda tympani nerve, which innervates taste buds in fungiform papilla on the anterior portion and, in rostral foliate papillae, on the sides of the mammalian tongue. In addition to this apical ion conductive pathway, recent evidence has suggested that an additional

Na^+ sensing mechanism exists below taste cell tight junctions within the taste cell basolateral membrane.

Classically, two assumptions dictated the interpretation of the manner by which salt affects excitation of mammalian taste receptor cells: (1) the dorsal lingual epithelium is an impermeable barrier against ions and other tastants ; (2) adsorption to an apical receptor comprises the stimulus-receptor cell interaction. From these assumptions, early models of salt taste transduction dispensed with the notion of transduction mediated by stimulus ion penetration into the taste receptor cells (Heck et al., 1984). However, DeSimone et al. (1981) demonstrated that the canine lingual epithelium actively transports ions. Furthermore, Heck et al. (1984) largely eliminated the integrated chorda tympani response of rats stimulated with NaCl with the diuretic drug amiloride. Thus, Heck et al. (1984) concluded that (1) taste receptor cells contain an amiloride-sensitive sodium transport pathway which functions in NaCl taste transduction; (2) the pathway provides the means by which taste receptor cells are depolarized; and (3) the Na^+-selectivity of this pathway indicates that K^+ provides depolarization through an alternate mechanism.

Further support for this newly evolving paradigm of salt taste transduction was provided by Palmer (1987) who showed that, in frog epithelia, ion channels confer the selectivity of the apical membrane for Na^+ over K^+. Furthermore, Palmer (1987) reasoned that the selectivity properties of these epithelial Na^+-channels are determined by three factors:

> ...[1] a high field-strength anionic site, most likely a carboxyl residue of glutamic or aspartic acid residues on the channel protein, which accounts for the high conductance of Na^+ and Li^+ and for the low conductance of K^+; [2] a restriction in the size of the pore at its narrowest point bequeaths the low conductance of organic cations; and finally, [3] the outer mouth of the channel carries negative

charge and may serve as a preliminary selectivity filter, attracting cations over anions.

Moreover, studies have shown that the sodium channel protein consists of at least three subunits, α, β, and γ, wherein the α-subunit displays amiloride-sensitive channel activity. Thus, *in toto*, these data support the assertion that salt taste reception depends on the passive diffusion of Na^+ ions through apical, Na^+-selective and amiloride-blockable ion channels.

However, the observation that whole nerve and single fiber chorda tympani responses to high concentrations of some Na^+ salts are not wholly inhibited even at high amiloride concentrations led some investigators to propose the existence of a second, amiloride-insensitive, Na^+ sensing mechanism (Brand et al., 1985). Further research showed that the amiloride-insensitive component of the chorda tympani response to Na^+ salts was highly anion dependent (Formaker & Hill, 1988; Elliot & Simon, 1990). Moreover, as the hydrated diameter of the anion decreases, the residual chorda tympani response (i.e. the amiloride-insensitive component) to various sodium salts increases. Consequently, the insensitive component of the chorda tympani response to high concentrations of NaCl is large, while the responses to sodium salts of organic acids (e.g., NaGlu, and sodium acetate) can be largely eliminated by amiloride (Formaker & Hill, 1988).

Using the powerful technique of *in vivo* voltage clamping, Ye et al. (1991) determined that the Na^+ sensing mechanism sensitive to applied voltage perturbations corresponds to the apical, amiloride-sensitive Na^+ channels. Ye et al. (1991) hypothesized that, as a consequence of the polarized epithelial topology of rat taste buds, changes in transepithelial potential could modulate receptor potential, which, in turn, could modulate the chorda tympani response. This

hypothesis arose from the observation that when Na^+ salts containing anions of limited junctional mobility are applied to the tongue, large electropositive transepithelial field potentials are observed. These large serosal positive field potentials result essentially from the failure of the stimulus anion to co-diffuse with sodium through the paracellular shunt (i.e., tight junctional) pathway. This notion was verified when Ye et al. (1991) were able to voltage-clamp the field potential so that anion diffusion potentials did not influence the receptor potentials or, therefore, the neural response. As field potential modulates Na^+ movement through apical amiloride-sensitive Na^+ channels, the apical Na^+ sensing pathway is, consequently, subject to stimulus (i.e. anion-dependent) self-modulation.

Beyond this anion effect, Ye et al. (1993) noticed that under positive voltage clamp conditions, chorda tympani responses to high concentrations of NaCl remained significantly higher than those to NaGlu. This indicated that voltage clamp of the transepithelial potential was not sufficient to eliminate the anion effect and suggests that an additional, second anion effect, insensitive to voltage clamp, emerges at high Na^+ salt concentration. This second anion effect relates to a Na^+ sensing mechanism that is thought to rely upon concentration-dependent, electroneutral diffusion of Na^+ and the stimulus co-anion through the taste cell tight junctions, and the consequent activation of stimulation sites within the taste cell basolateral domain. The amiloride-insensitive component, therefore, presumably occurs as Na^+ passes into these intercellular regions below the tight junctions, where basolateral stimulation occurs and where amiloride fails to penetrate. In support of this idea, Ye et al. (1993) showed that, in the case of NaCl, where the chloride anion is relatively small, electroneutral diffusion could occur, and basolateral stimulation was independent of the clamp voltage. However, for those anions with

low shunt permeability, where co-diffusion of Na^+ and the stimulus anion is severely limited (such as for NaGlu), the basolateral sites are not activated, and an amiloride-insensitive component is not produced.

Despite impressive gains in the understanding of mammalian Na^+ sensing, less is known about Na^+ stimulus transduction in the hamster, which is widely used in studies of gustatory neural coding. Thus far, in unpublished work, Stewart has shown that the voltage sensitivity index (VSI) of hamster chorda tympani responses at high concentrations of NaCl (i.e. [NaCl]>200 mM) is depressed with respect to the observed VSI at lower stimulus concentrations. To account for this finding, Stewart reasons that, in a fashion analogous to that already elucidated in rat, at high stimulus concentrations, chemical potential drives the electroneutral diffusion of Na^+ and the stimulus co-anion through the tight junctions. As this electroneutral diffusion allows sodium ions to interact with the basolateral domain of the taste cell, the observed decrease in voltage sensitivity of the chorda tympani response may result from stimulus-receptor cell interaction through basolateral transduction sites. Furthermore, Rehnberg et al. (1993) demonstrated that the hamster chorda tympani response to various sodium salts is anion dependent. The current study, much like that of Ye et al. (1993), utilizes lingual epithelial voltage clamping with simultaneous chorda tympani recording in order to acquire data pertaining to the relative response magnitudes evoked by equimolar concentrations of sodium salts that bear anions of different size. These data will be used to test the hypotheses that (1) the Na^+ salt stimulus co-anion modulates the magnitude of chorda tympani response to Na^+ salts by influencing transepithelial field potential, and (2) that anion size modulates Na^+ salt response voltage sensitivity at high stimulus concentrations. Furthermore, these concentration-response

data will be subjected to kinetic analyses where prediction holds that fitting the data with the modified Beidler equation will produce a K_m value which varies as a function of the field potential relating to the various anion species employed.

Methods

Animals. Syrian Golden hamsters at 70-100 days of age and 215-230 days of age were used in these studies. All hamsters were raised in our colony. These adult animals, and all breeding stock, were obtained from Harlan-Sprague-Dawley. Hamsters were provided standard Purina laboratory chow and water ad libitum and maintained on a 12h:12h light:dark photoperiod (lights on at 0700).

Surgery. Hamsters were deeply anesthetized by intraperitoneal injection of pentobarbital (65 mg/kg). Surgical anesthesia was maintained by supplemental injections of pentobarbital (25 mg/kg) as needed. Body temperature was maintained at 37 ± 1.5 °C by a circulating water, clinical heating pad. Animals were then tracheotomized to facilitate free-breathing, and the hypoglossal nerves were transected to eliminate tongue movements. Animals were secured by placement in a non-traumatic head holder, then, using an epimandibular approach, the left chorda tympani was exposed from its bifurcation with the lingual nerve to its entrance into the tympanic bulla. The exposed nerve was cut at the tympanic bulla, freed of surrounding connective fascia, and desheathed in preparation for neural recordings. The desheathed nerve was placed onto a 28G platinum wire electrode, and a reference electrode was placed in nearby tissue. The wound

was filled with a mixture of petroleum jelly and mineral oil to prevent drying.

Chorda tympani recording. Neural activity was fed to a fixed gain isolation amplifier (BioAmp 100, Axon Instruments) and then to a Grass P511 preamplifier (AstroMed). The amplified signal was monitored with an oscilloscope and an audio loud speaker. Amplified neural activity was RMS-rectified and integrated with a time constant of 1-1.33 s. The integrated output was recorded on one channel of a Linseis L6514 four channel rectilinear chart recorder (Linseis).

Lingual receptive field voltage clamp. Simultaneous lingual epithelial voltage clamping and chorda tympani neural recordings have been described in detail (Ye et al., 1993; Stewart et al., 1996). Briefly, a portion of the anterior dorsal lingual epithelium was enclosed in a vacuum-applied, cast acrylic stimulation chamber. The vacuum is applied to an annular groove along the outer diameter of the chamber, drawing a ring of tissue into the groove. Compression of the tissue within the groove creates a mechanically stable, hydraulically and electrically tight seal between the chamber and lingual epithelium. Within this affixed ring is an effective inner stimulating chamber of approximately 6 mm in diameter that typically encloses between 25 and 40 taste buds of the hamster anterior tongue. Stimulus and rinse solutions (see below) were injected (4 ml; 1 ml/s) into the chamber via tubing fitted to a dedicated port (dead space: approx. 0.5 ml). The chamber was fitted with separate Ag-AgCl electrodes for measurement of current and potential, while reference electrodes were placed non-invasively on the ventral lingual epithelium. The current passing electrode in the chamber served as virtual ground, ensuring that only current passing through the stimulated patch was collected. Command currents and

potentials were delivered by a voltage current amplifier (Physiologic Instruments VCC600). Periodic current and voltage pulses delivered by the VCC600 permitted the monitoring of transepithelial resistance and conductance during zero-current clamp and voltage clamp, respectively. Finally, transepithelial potential and current responses were recorded on two channels of a Linseis L6514 4-channel rectilinear chart recorder.

Stimulus solutions and stimulus application. All solutions were prepared from reagent grade chemicals dissolved in glass-distilled H_20. The rinse solution contained 15 mM $KHCO_3$ (pH 8.3). A Na^+-depleted Krebs-Henseleit solution (DKH) that contained (in mM) 6 KCL, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.3 NaH_2PO_4, 25 $NaHCO_3$, and 5.6 glucose (pH 7.5) was applied after each stimulus series to help maintain a stable transepithelial potential. Transepithelial potential in the presence of 150 mM NaCl rarely fluctuated more than ±5 mV during the course of an experiment. In addition, those preparations that exhibited absolute transepithelial potentials greater than ±20 mV in the presence of 150 mM NaCl were excluded from analysis. Stimuli were concentration series (10, 50, 100, 250, and 500 mM) of NaCl and NaGlu.

Stimuli were applied under zero-current clamp (equivalent to open circuit conditions), and steady-state potentials were recorded from the front panel display of the voltage-current amplifier. Next, chorda tympani responses to the stimuli were obtained under voltage clamp at +60 and -60 mV, relative to the zero-current clamp potential recorded for each stimulus. In this way, neural responses to concentration series were obtained under three membrane potential conditions: effective open circuit and +60 and -60 mV voltage clamp. Each stimulus series was bracketed by the application of a 150 mM NaCl reference stimulus under zero-current clamp, and

intervening data was retained for analysis only when the magnitudes of the 150 NaCl reference responses before and after the stimulus series varied by less than 10%. Steady-state response magnitudes, measured as the height of each integrated chorda tympani response 30 s after application of the stimulus, were expressed relative to the mean NaCl response magnitude bracketing a stimulus series.

The protocol for stimulation of the lingual epithelium was generally as follows. The reference NaCl stimulus was applied and allowed to remain in the chamber for 40-50 s. The solution was then rinsed from the chamber by repeated application of the $KHCO_3$ rinse solution for at least 60 s. Then, the stimuli of interest (NaCl or NaGlu) were applied as a concentration series, with each concentration being allowed to remain in the chamber for 40-50 s before being rinsed from the chamber repeatedly with $KHCO_3$, as noted above. About 60 s after the final stimulus in a concentration series was rinsed from the chamber, the reference 150 mM NaCl solution was reapplied and then rinsed. At this point, DKH was injected into the chamber, allowed to remain on the tongue for 60 s, and then rinsed from the chamber with repeated applications of the $KHCO_3$ rinse solution. Lastly, another reference stimulus was applied and rinsed, and the next concentration series was begun.

Data analyses. Data are expressed as mean ±SEM, unless otherwise noted. Voltage-dependent differences in chorda tympani relative response magnitudes at each stimulus concentration were determined using t-tests. Likewise, anion-dependent differences in zero-current clamp relative response magnitudes at each stimulus concentration were assessed using t-tests. Finally, chorda tympani relative response data was fit with the modified Beidler taste equation in order to obtain

kinetic parameters that describe the behavior of apical Na^+ transduction elements.

Results

Figure 1 shows chorda tympani responses to 100 mM and 500 mM NaCl and NaGlu under zero current clamp and ±60 mV voltage clamp. Responses to both salts at 100 and 500 mM show considerable voltage sensitivity, with responses suppressed under serosal positive clamp and elevated under serosal negative clamp.

Figures 2 and 3 show that Na^+ salt chorda tympani responses are subject to modulation by imposed transepithelial voltage perturbations. For NaCl concentrations of 10, 50, and100 mM, +60 mV voltage clamp produced chorda tympani responses that were significantly suppressed with respect to the zero current clamp condition ($t \geq 3.07$, $ps \leq .03$). Moreover, under -60 mV voltage clamp, the chorda tympani responses to 10, 50, and 100 mM NaCl were significantly elevated ($t \geq 2.30$, $ps \leq .05$). However, while the mean chorda tympani responses to 250 mM and 500 mM NaCl were significantly suppressed under serosal positive clamp ($t \geq 4.07$, $ps \leq .01$), the chorda tympani responses to 250 and 500 mM NaCl under serosal negative clamp did not differ significantly from the zero current clamp condition ($t = .76$, $ps > .05$).

Under serosal positive clamp, chorda tympani NaGlu responses at concentrations of 100, 250 and 500 mM were significantly suppressed with respect to the zero current clamp condition responses and significantly elevated under serosal negative clamp ($t \geq 4.28$, $ps < .02$). At concentrations of 10 and 50 mM, no significant suppression or elevation of the chorda tympani NaGlu response was observed under either voltage clamp condition ($t \leq 1.81$, $ps > .05$).

Upon comparison of the neural responses of NaCl to NaGlu under zero current clamp, a

threshold concentration between 50 and 100 mM emerged. That is, above this threshold, NaCl chorda tympani responses were significantly greater than NaGlu responses to equivalent concentrations (i.e., 100, 250, and 500 mM) ($t \geq 2.81$, $ps < .03$), while, below the threshold, NaCl and NaGlu chorda tympani responses to stimulus concentrations of 10 and 50 mM did not differ ($t \leq .99$, $ps > .05$). The greater chorda tympani response to NaCl under the zero current clamp condition probably relates to the first anion effect of Ye et al. (1991, 1993), whereby the transepithelial potential (TEP) modulates the receptor potential and thus the chorda tympani response. No significant differences were found between NaCl chorda tympani response magnitudes and NaGlu response magnitudes under serosal positive clamp at stimulus concentrations of 250 and 500 mM ($t \leq 1.81$, $ps > .05$).

I expected the observed response suppression under serosal positive clamp, and response enhancement under serosal negative clamp, for NaCl and NaGlu following the assumption that the initial event in taste cell excitation is the influx of Na^+ through apical membrane ion channels down its electrochemical potential gradient. The driving force for Na^+ influx depends on electrical potential as well as ion concentration (Ye et al., 1993). That is, *electrochemical concentration* modulates Na^+ flux through apical channels. Electrochemical concentration may be written:

$$C_e = Ce^{-\delta\varphi} \qquad \text{(Eq. 1)}$$

where, C is the stimulus Na^+ concentration, φ is $F\Delta V/RT$, where ΔV is the applied clamp voltage ($J \cdot C^{-1}$), at 35 °C (i.e., 308 °K), the approximate tongue surface temperature, $F = 9.65 \times 10^4$ $C \cdot mol^{-1}$, and $R = 8.314$ $J \cdot mol^{-1} \cdot K^{-1}$. Finally, δ is the fraction of φ that is dropped across the apical cell membrane (Ye et al., 1993). If I regard the effective stimulus intensity as the

electrochemical concentration (i.e., the force for Na^+ influx into the taste cell) and assume that the resultant Na^+ influx into the taste cells is the rate-limiting event in the evoked neural response to Na^+, then the Beidler taste equation (Beidler, 1953) becomes the apical channel model equation:

$$R = CT_{max}C_e/(K_m + C_e) \quad \text{(Eq. 2)}$$

where, K_m is the stimulus concentration that yields a half-maximal chorda tympani response, and CT_{max} is the maximum value of R, which is the neural response obtained at a given Na^+ electrochemical concentration, C_e.

Figures 4 and 5 depict the chorda tympani response versus electrochemical concentration functions for NaCl and NaGlu, respectively. Clearly, the three chorda tympani response-stimulus concentration curves that correspond to the three voltage conditions in both Figures 2 and 3 collapse to single response-electrochemical concentration curves. The best fit to the NaCl data arose when $CT_{max} = 1.44$, $\delta = 1.00$, and $K_{NaCl} = 63.1$ mM ($R^2 = .91$), while the best fit to the NaGlu data resulted from $CT_{max} = 1.54$, $\delta = .94$, and $K_{NaGlu} = 252.04$ mM ($R^2 = .94$). This transformation of the data reveals that the driving force for Na^+ ions through the apical amiloride-sensitive Na^+ channel manifests in a single intensity dimension for Na^+, the electrochemical concentration, which encompasses both the stimulus concentration and the apical membrane potential difference.

Discussion

In the present study, simultaneous in vivo lingual voltage clamping and chorda tympani recording were used to examine whether the Na^+ salt stimulus co-anion modulates the magnitude

of the chorda tympani response to Na^+ salts by influencing transepithelial field potential. The primary finding from these experiments is that, for hamster, the chorda tympani Na^+ response results from a transduction mechanism driven by stimulus electrochemical concentration. In general, consistent with previously published results for rat Na^+ sensing (Ye et al., 1993), chorda tympani responses to Na^+ salts at all concentrations were elevated under serosal negative voltage clamp, which increases the electrochemical driving force for Na^+ into the cell. The enhancement is, however, limited to the system's natural response maximum. Under serosal positive clamp, which decreases the driving force for Na^+ into the cell, chorda tympani responses were significantly suppressed.

Analogous to the data Ye et al. (1993) developed in rat, the data from the current study suggest a threshold concentration in the hamster salt taste system somewhere between 50 and 100 mM, above which the NaCl response significantly exceeds the NaGlu response under zero current clamp. This difference is presumably due to the first anion effect. The first anion effect, or modulation of the receptor potential by the transepithelial potential (TEP), arises from electroneutral diffusion of Na^+ ions and their stimulus co-anion across the paracellular shunt between taste cells. The paracellular shunt (i.e., tight junctions) has the properties of a weak cation exchanger at physiological pH, and serves as the primary mediator of the paracellular resistance and diffusion barrier (Stewart et al., 1997). While the first anion effect accounts for the greater NaCl response at concentrations above the threshold, the existence of the observed threshold concentration for this response component suggests that the intercellular Na^+ concentration must be exceeded for an influx of stimulus to occur (Ye et al., 1993). This would require the mean intercellular NaCl concentration to be below plasma levels (assuming a plasma

Na^+ concentration of approximately 150 mM), possibly achieved by equilibration across the tight junctions between a plasma-like submucosal region and the oral cavity, where the NaCl concentration is set low by secretion of saliva of 6.6 mM sodium concentration (Rehnberg et al., 1992). Then, at stimulus concentrations above threshold where the aforementioned condition is met, the TEP is established, in part, by electroneutral diffusion of Na^+ and the stimulus anion through the tight junctions. Receptor potential modulation arises, in turn, from stimulus co-anion diffusion limitations imposed by the shunt cationic exchanger, which lies in parallel with the apical Na^+-selective channel. Therefore, a change in TEP, ΔV_t (referenced to the mucosa), produces a change in receptor potential, ΔV_r (referenced to the submucosa), in the amount

$$\Delta V_r = -(1-\delta)\Delta V_t \qquad \text{(Eq. 3)}$$

where δ retains its aforementioned parameters. Since large organic anions, such as gluconate in the current study, have low shunt permeability, the concomitant evoked changes in ΔV_t are more electropositive than those evoked by small anions such as Cl^-. Thus, in the presence of large organic anions such as gluconate, hyperpolarizing changes in ΔV_t contribute to producing the observed smaller chorda tympani responses of NaGlu versus NaCl under zero current clamp (Stewart et al., 1997).

Kinetic analyses were performed on the data to examine my prediction that fitting the data with the modified Beidler equation will produce a K_m value that varies as a function of the field potential relating to the various anion species employed. In support of this assertion, my kinetic analyses of the chorda tympani response data predict divergent K_m values for NaGlu and NaCl (K_{NaGlu} = 252.04 mM, K_{NaCl} = 63.1mM). Interestingly, if K_m describes the affinity of Na^+ for a Na^+ specific channel, the K_m values predicted by the model for the two salts should be

identical. That is, the data beg the question: why is the apparent affinity of Na^+ for Na^+ channels so much greater for NaCl than for NaGlu? In general, the stimulus-dependent shifts in K_m support the net effect of stimulus- and thus, TEP-dependent differences in the driving force for apical Na^+ flux. In rat, Stewart et al. (1996) demonstrated potent stimulus-dependent TEP influence on the Michaelis constant, K_m, in the intact system upon application of the apical channel model. Moreover, Stewart et al. (1996) assert that maintenance of the polar topology of taste buds, achieved by studying the intact taste system in vivo, allows for the conclusion, based on the stimulus-dependent K_m, that the polar topology of the taste bud constrains the *in situ* function of ion channels. In the current study, the data support identical conclusions for hamster. Thus, it appears that for rat and hamster, the kinetic data support the following evolving paradigm for taste receptor self modulation: As large organic anions, such as gluconate in the current study, have low shunt permeability, the concomitant evoked changes in ΔV_t are more electropositive than those evoked by Cl^-, which is apparently sufficiently mobile to result in NaCl electroneutral diffusion into the intercellular spaces. The lower TEP evoked by NaCl results in a greater depolarization of the basolateral taste receptor cell membrane and a larger hyperpolarization across the apical taste receptor cell membrane relative to NaGlu. Thus, in the presence of Cl^-, a relative increase in the driving force for Na^+ ions into the taste cell through amiloride-sensitive Na^+ channels results, as Na^+ ions 'see' hyperpolarization of this membrane as an increased intracellular electronegative potential. However, when gluconate is the stimulus anion, the more electropositive evoked change in V_t causes a relative depolarization of the apical membrane and a concomitant decrease in the driving force for Na^+ ions into the taste cell (Hendricks et al., in press).

Application of the apical channel model taste equation to the experimental data provided effective binding dissociation constants for the two sodium salts, viz K_{NaCl} = 63.1 mM and K_{NaGlu} = 252.04 mM. Next, regression analysis of the stimulus generated field potentials across the apical membrane at all concentrations for each salt produced a hyperbolic fit for both the NaCl and NaGlu data. Next, using the specific K_{salt} values in their respective hyperbolic ΔTEP versus concentration formulae the corresponding ΔTEP was predicted. This analysis resulted in an observed ΔTEP (TEP_{NaGlu} - TEP_{NaCl}) of 19.7 mV. Then, in order to analyze the predictive utility of the anion channel model, I used the K_{NaGlu} and K_{NaCl} values to predict a theoretical voltage difference corresponding to the change in NaGlu- and NaCl-stimulated field potentials across the apical membrane. As these values reflect a K_m that varies as a function of the field potential that prevails in the presence of the two respective anion species employed, K_{salt} reflects the *effective* binding dissociation constant for Na^+. Therefore, the observed effective binding dissociation constants for Na^+ actually represent a potential-independent constant (i.e. the actual channel K_m) multiplied by the factor $e^{\varphi\ apical}$, where φ_{apical} is the stimulus-generated transepithelial potential. Thus, the stimulus-specific effective K_m is a function of the field potential generated by that stimulus such that:

$$K_{NaCl} = Ke^{\varphi apical,\ NaCl} \qquad \text{(Eq. 4)}$$

and

$$K_{NaGlu} = Ke^{\varphi apical,\ NaGlu} \qquad \text{(Eq. 5)}$$

As application of the modified Beidler taste equation to my experimental data provided values of K_{NaCl} and K_{NaGlu}, the theoretical difference between $\varphi^{apical,\ NaCl}$ and $\varphi^{apical,\ NaGlu}$ was calculated using the equation:

$$\varphi^{apical,\ NaGlu} - \varphi^{apical,\ NaCl} = \ln[K_{NaGlu}/K_{NaCl}] \quad \text{(Eq. 6)}$$

The voltage difference that corresponds to the apical φ difference here obtained was found by:

$$\Delta V = \varphi RT/F \quad \text{(Eq. 7)}$$

The apical channel model-generated theoretical ΔV was found to be equal to 36.6 mV.

At the outset, I expected the theoretical values predicted by the modified Beidler equation to closely correspond to differences in NaCl- and NaGlu- stimulated field potentials measured experimentally. Not only would this result strongly support the first anion effect notion of anion-generated field potential modulation of the apical amiloride-sensitive Na^+ channel, but comparable observed and theoretical values would validate the predictive utility of the modified Beidler taste equation. While there seems to be a disparity in the observed (ΔTEP = 19.7 mV) and theoretical values (ΔTEP = 36.6 mV), the large magnitude of the ΔTEP values observed experimentally and gleaned from the model reflect the significant effect of receptor cell self modulation through stimulus generated field potentials. The disparity in the theoretical and observed values likely reflects that while the theoretical value predicts a change in TEP manifested at the site of the apical amiloride-sensitive sodium channel exclusively, experimentally, measurement of the TEP is across the entire lingual epithelium, wherein taste cells comprise less than 1% of the total area. Thus, in light of the excellent fit of the model to the data reflected in R^2 values of .91 and .94 for NaCl and NaGlu, respectively, the predictive accuracy of the anion channel model remains uncompromised as the disparity between the observed and theoretical values likely stems from inaccurate assessment of stimulus-induced changes in transepithelial potential observed experimentally.

As a secondary aim for the current study, I sought to test the hypothesis that anion size

modulates Na^+ salt response voltage sensitivity at high stimulus concentrations, the so-called second anion effect. In rats, Stewart et al. (1996) and Ye et al. (1993) have shown that the second anion effect accounts for the observed amiloride-insensitive component of the neural response at high concentrations to Na^+ salts with anions of high junctional mobility. In support of this assumption, the current literature cites a significantly increased response to NaCl over NaGlu under serosal positive clamp at concentrations in excess of 250 mM (Ye et al., 1993). Moreover, the current data in rat also support a significant decrease in the VSI at high concentrations of NaCl. Hendricks et al. (in press) speculate that in the presence of high NaCl stimulus concentrations, the paracellular diffusion potential becomes so high that Na^+ and Cl^- ions diffuse with low resistance electroneutrally through the shunt. Presumably, this increase in the intercellular Na^+ concentration is great enough to permit stimulation at the basolateral taste cell membrane. Also, as the diffusion of Na^+ and Cl^- is driven by chemical potential (i.e. diffusion is electroneutral), the second anion effect is relatively voltage insensitive. In response to NaGlu, a voltage-insensitive component has not been demonstrated in rat or hamster, presumably due to the co-anion size-dependent permeability limitations imposed by the paracellular shunt.

Quite unexpectedly, my data do not support analogous conclusions to those made by Ye et al. (1993). That is, neither the chorda tympani response magnitude data nor the kinetic analysis data support a second anion-derived voltage insensitive component of the chorda tympani response to NaCl at high concentrations. At serosal positive voltage clamp, I expected the chorda tympani response magnitudes to stimulus concentrations of 250 and 500 mM NaCl to exceed those to NaGlu at equivalent concentrations. However, as no significant differences in

response magnitudes were found under the aforementioned conditions, the data do not support the conclusion of a significant second anion effect on chorda tympani response in hamster. Furthermore, kinetic analyses produced the theoretical value maximum δ for each salt. Based on prior studies in rat (Hendricks et al., in press), I expected the δ values for NaCl to be smaller than those for NaGlu as a consequence of the higher shunt permeability of Cl^- relative to that of gluconate. However, the predicted δ values for NaCl and NaGlu proved to be similar (δ_{NaCl} = 1.00 and δ_{NaGlu} = .94). Thus, as the NaCl and NaGlu δ values are, for all intents and purposes, equivalent, the data do not support the higher shunt permeability of Cl^- over gluconate, a prerequisite for second anion effect-modulation of chorda tympani response magnitude. Moreover, the high δ values for both NaCl and NaGlu suggest that the taste bud tight junctions in hamster are extremely tight. As a consequence, even as NaCl concentration increases, the fraction of the clamp voltage that is dropped across the paracellular shunt does not increase, but rather, the majority of the clamp voltage continues to be dropped across the apical Na^+ channel. Thus, the data suggest that extremely tight tight junctions, which may have been naturally selected for in the xerophytic hamster to prevent water loss to its environment via the lingual epithelium, prevent the voltage insensitive component of the chorda tympani response to high NaCl concentrations that has been previously reported in rat (Ye et al, 1993; Stewart et al., 1996).

The results from this study that contradict any rat-hamster homology with respect to the second anion effect may be anomalous. Hendricks et al. (in press) report that, throughout rat development, the voltage sensitivity of the chorda tympani response to high stimulus concentrations of NaCl decreases. Moreover, Hendricks et al. (unpublished work) present a

marked decline in δ for NaCl over time, which implies that taste bud shunt properties change as a function of postnatal age. Specifically, taste bud tight junctions become leakier with advancing postnatal age. As a comprehensive study on postnatal development of hamster chorda tympani responses has yet to be performed, the data in the current study may suffer from the confounding effect of using groups of hamsters of significantly different postnatal age. That is, if the hamster taste system does not reach maturity until postnatal day 100 or later, and similar trends are found with respect to the relationship between tight junction permeability and postnatal age in hamster as Hendricks et al. (in press) reported in rat, the δ values predicted from my data will likely prove erroneously elevated. However, in the future, studies utilizing amiloride may directly investigate my unexpected results concerning the absence of a voltage-insensitive, and thus, amiloride-insensitive chorda tympani response component to high stimulus concentrations of NaCl in hamster.

Figure Legends

Figure 1. Integrated chorda tympani responses to 100 mM (A) and 500 mM (B) NaCl and NaGlu under zero current clamp (CC_0) and ±60 mV voltage clamp. Responses to both salts at 100 mM show considerable voltage sensitivity, with responses suppressed under serosal positive clamp and elevated under serosal negative clamp. In contrast, at 500 mM, NaGlu responses appear to remain very voltage sensitive, while those to NaCl have blunted voltage sensitivity. Time markers correspond to 30s; superimposed spikes are caused by voltage (CC_0) or current (VC) injected to monitor transepithelial resistance.

Figure 2. Response-concentration functions of mean NaCl relative response data under three transepithelial potential conditions (symbols). It can be seen clearly that perturbation of transepithelial voltage has a sizeable impact on chorda tympani response magnitude to NaCl. It is especially notable that the apparent greatest effect of voltage clamp is observed at *low* concentrations of NaCl. The compression of voltage sensitivity at high concentrations of NaCl may relate to clamp current dissipation caused by electroneutral diffusion of Na^+ and Cl^- through the paracellular shunt. Data presented are mean ±SEM (N=3-6).

Figure 3. Response-concentration functions of mean NaGlu relative response data under three transepithelial potential conditions (symbols). It can be seen clearly that perturbation of transepithelial voltage has a sizeable impact on chorda tympani response magnitude to NaGlu. It is especially notable that the apparent greatest effect of voltage clamp is observed at *high* concentrations of NaGlu. In the case of NaGlu, enhanced voltage sensitivity at high concentration may relate to the gluconate anion's inability to traverse

the tight junctions, thus preventing any clamp current dissipation which would result from electroneutral diffusion of Na^+ and Glu^- through the paracellular shunt. Data presented are mean ±SEM (N=3-5).

Figure 4. Chorda tympani response-electrochemical concentration plot of mean NaCl relative response data. NaCl chemical concentrations were transformed to electrochemical concentration with Eq. 1 using the best fit parameters shown, and the data were replotted. The resulting theoretical line indicates that the 3 curves in Fig. 2, which correspond to the 3 voltage conditions, collapse to a single curve. The fit of the model to the data for NaCl reflects that the appropriate stimulus dimension for a Na^+ stimulus is electrochemical concentration (R^2 = .91). Data presented are mean ±SEM.

Figure 5. Chorda tympani response-electrochemical concentration plot of mean NaGlu relative response data. NaGlu chemical concentrations were transformed to electrochemical concentration with Eq. 1 using the best fit parameters shown, and the data were replotted. The resulting theoretical line indicates that the 3 curves in Fig. 3, which correspond to the 3 voltage conditions, collapse to a single curve. The fit of the model to the data for NaGlu reflects that the appropriate stimulus dimension for a Na^+ stimulus is electrochemical concentration (R^2 = .94). Data presented are mean ±SEM.

Figure 1

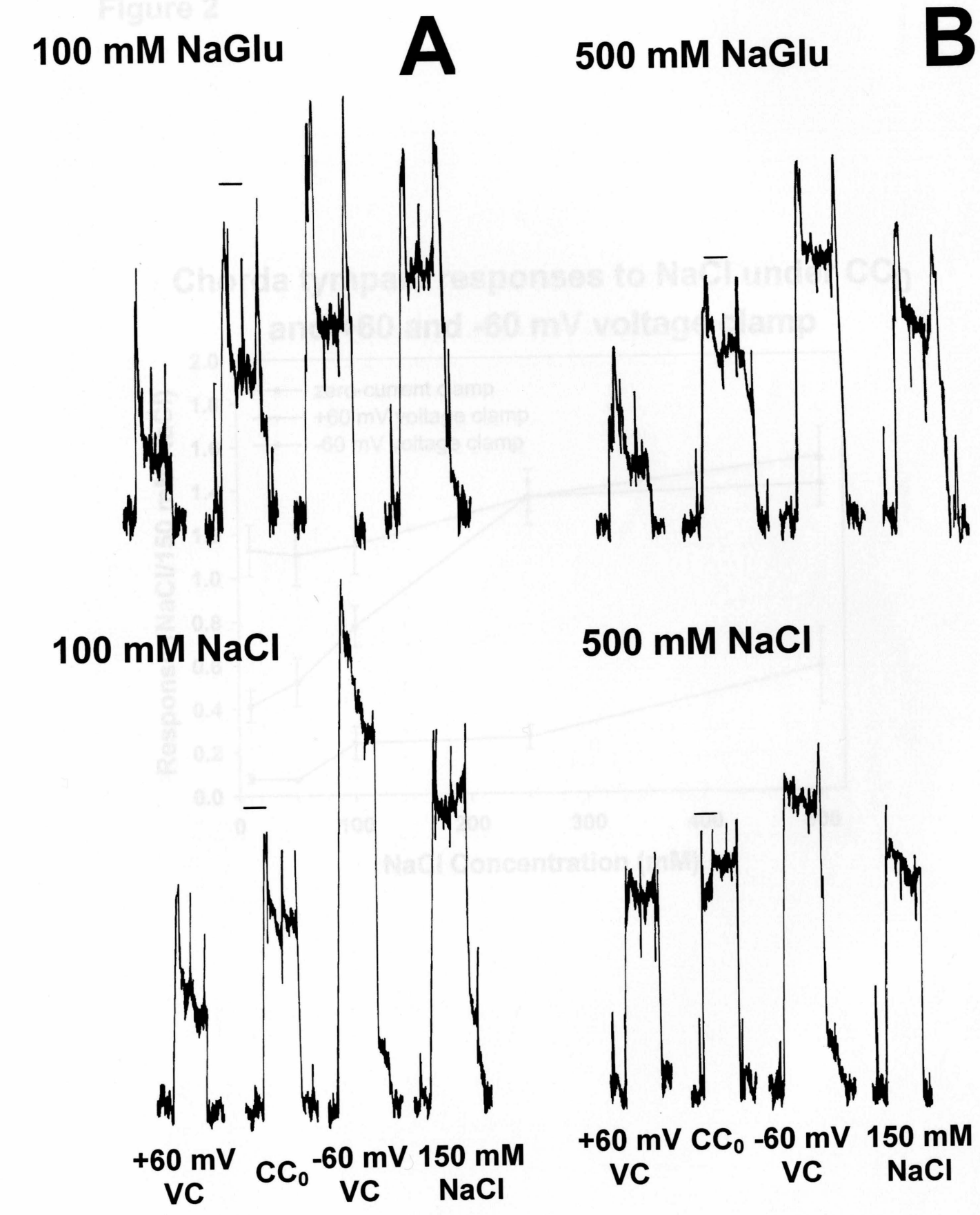

100 mM NaGlu
A
500 mM NaGlu
B
100 mM NaCl
500 mM NaCl
+60 mV VC
CC0
-60 mV VC
150 mM NaCl
+60 mV VC
CC0
-60 mV VC
150 mM NaCl

Figure 2

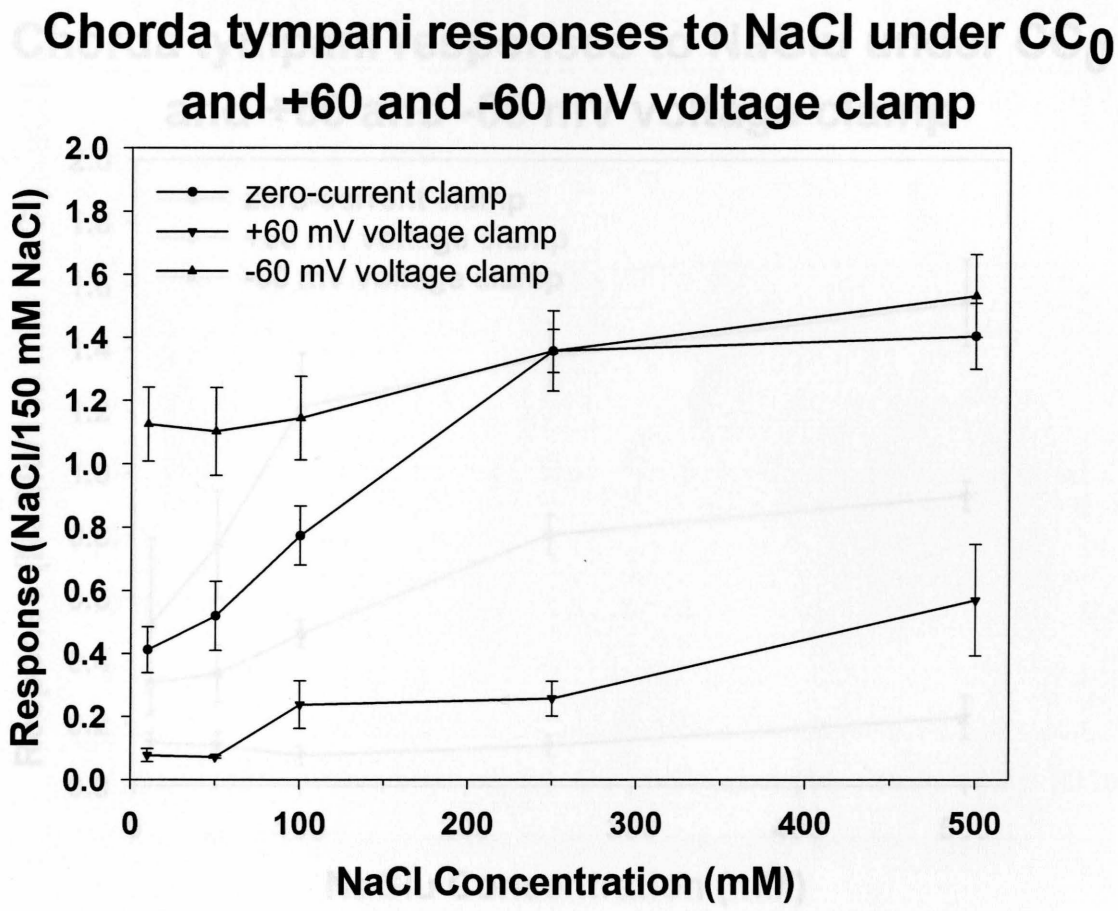

Chorda tympani responses to NaCl under CC0 and +60 and -60 mV voltage clamp
zero-current clamp
+60 mV voltage clamp
-60 mV voltage clamp
Response (NaCl/150 mM NaCl)
NaCl Concentration (mM)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
100
200
300
400
500

Figure 3

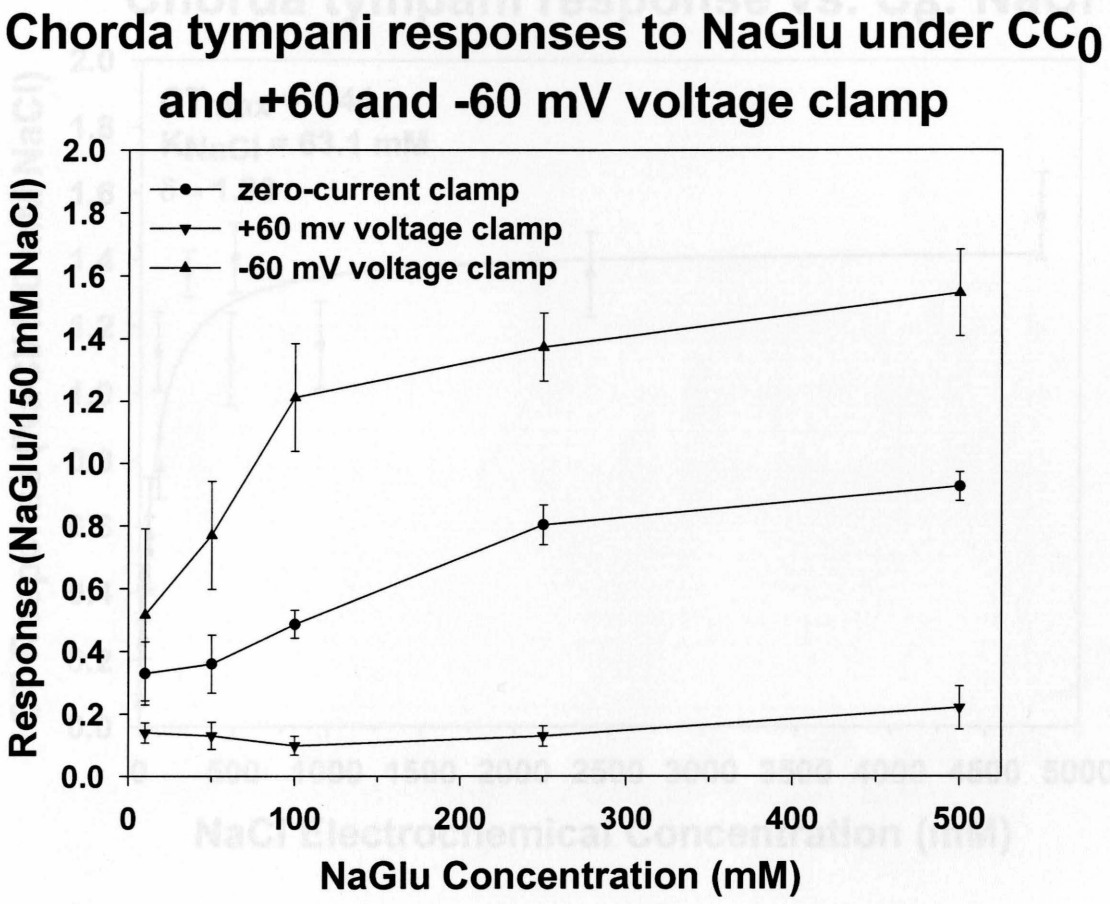

Chorda tympani responses to NaGlu under CC0 and +60 and -60 mV voltage clamp
zero-current clamp
+60 mv voltage clamp
-60 mV voltage clamp
Response (NaGlu/150 mM NaCl)
NaGlu Concentration (mM)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
100
200
300
400
500

Figure 4

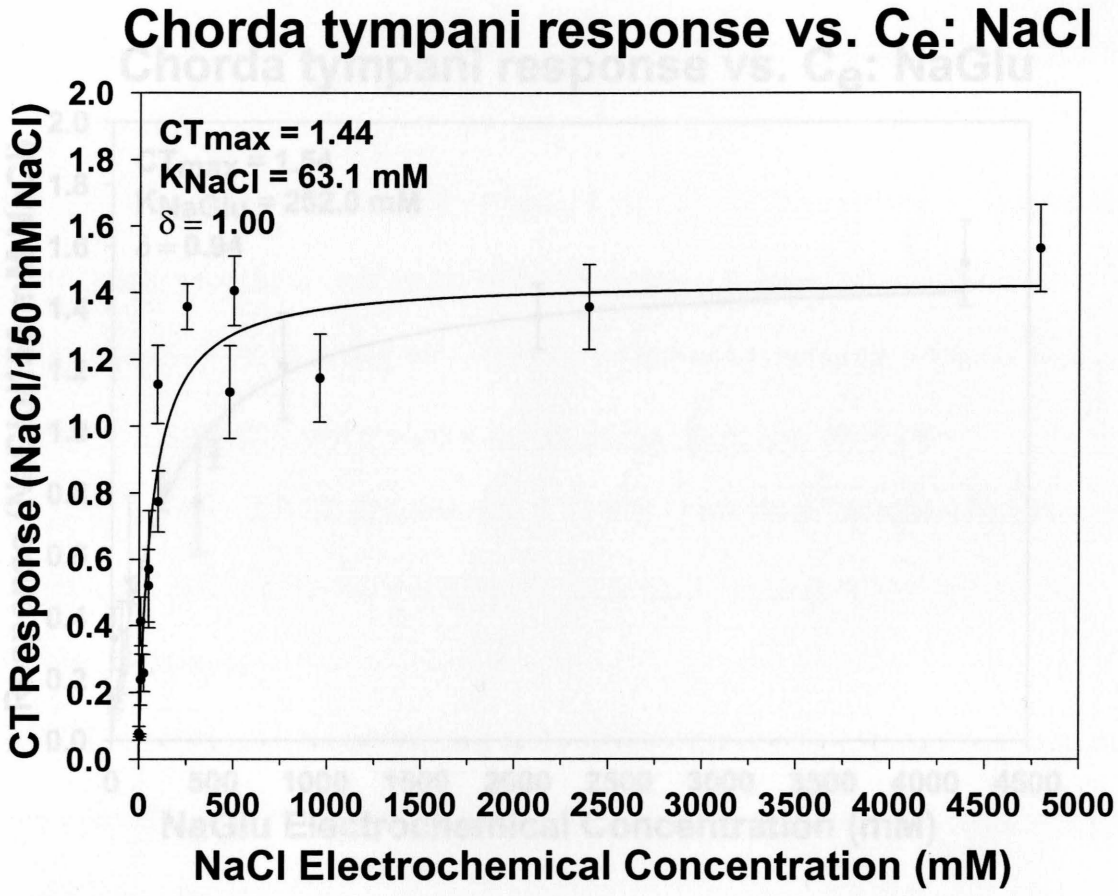
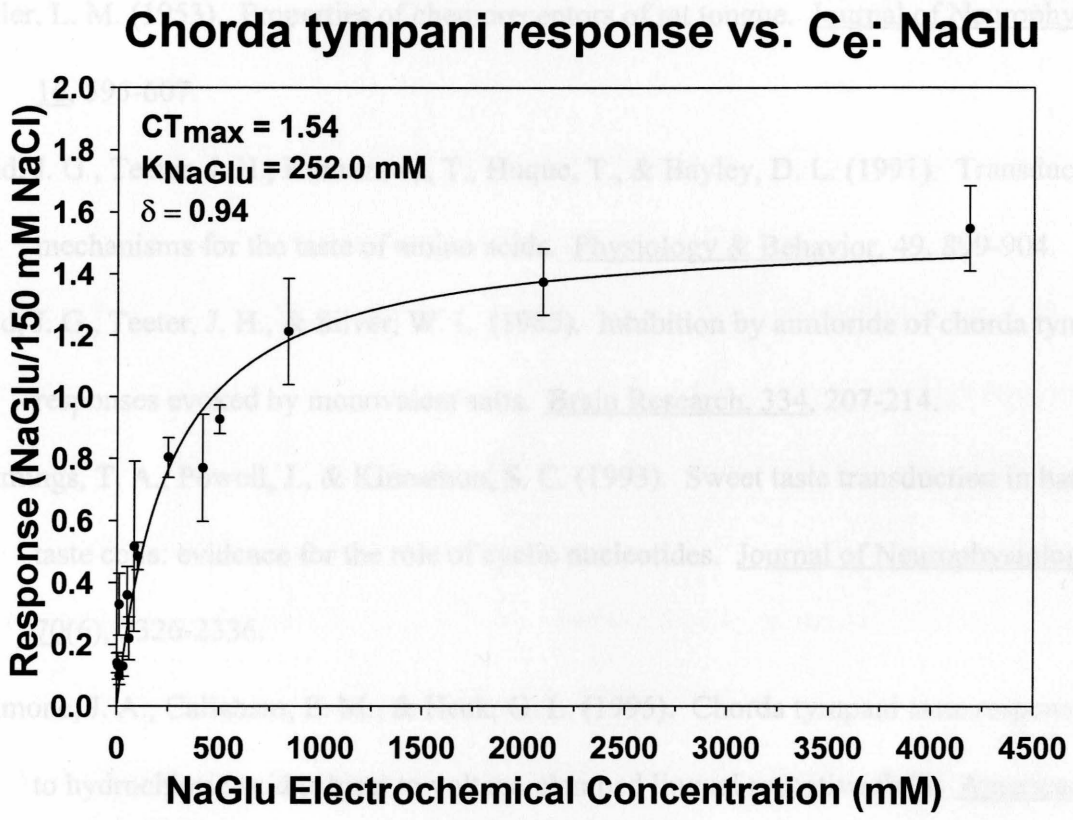

Chorda tympani response vs. C_e: NaCl
CT_{max} = 1.44
K_{NaCl} = 63.1 mM
δ = 1.00
CT Response (NaCl/150 mM NaCl)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
NaCl Electrochemical Concentration (mM)

Figure 5


Chorda tympani response vs. C_e: NaGlu
CT_{max} = 1.54
K_{NaGlu} = 252.0 mM
δ = 0.94
Response (NaGlu/150 mM NaCl)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
NaGlu Electrochemical Concentration (mM)

References

Akabas, M. H. (1993). The molecular biology of chemotransduction. See Simon & Roper (1993), pp. 175-200.

Akabas, M. H., Dodd, J., & Al-Awqati, Q. (1988). A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. Science, 242, 1047-1050.

Beidler, L. M. (1953). Properties of chemoreceptors of rat tongue. Journal of Neurophysiology, 16, 595-607.

Brand, J. G., Teeter, J. H., Kumazawa, T., Huque, T., & Bayley, D. L. (1991). Transduction mechanisms for the taste of amino acids. Physiology & Behavior, 49, 899-904.

Brand, J. G., Teeter, J. H., & Silver, W. L. (1985). Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. Brain Research, 334, 207-214.

Cummings, T. A., Powell, J., & Kinnamon, S. C. (1993). Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. Journal of Neurophysiology, 70(6), 2326-2336.

DeSimone, J. A., Callaham, E. M., & Heck, G. L. (1995). Chorda tympani taste response of rat to hydrochloric acid subject to voltage-clamped lingual receptive field. American Journal of Physiology, 268, 1295-1300.

DeSimone, J. A., & Ferrell, F. (1985). Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. American Journal of Physiology, 249, 52-61.

DeSimone, J. A., Heck, G. L., & DeSimone, S. K. (1981). Active ion transport in dog tongue: a possible role in taste. Science, 214, 1039-1041.

Elliot, E. J., & Simon, S. A. (1990). The anion in salt taste: a possible role for paracellular

pathways. Brain Research, 535, 9-17.

Faurion, A. (1991). Are umami taste receptor sites structurally related to glutamate CNS receptor sites. Physiology & Behavior, 49, 905-912.

Finger, T. E. (1986). Gustatory nuclei and pathways in the central nervous system. See Finger & Silver (1986), pp. 331-351.

Finger, T. E., & Silver, W. (1986). Neurobiology of taste and smell. New York: Academic Press.

Formaker, B. K., & Hill, D. L. (1988). An analysis of residual NaCl taste response after amiloride. American Journal of Physiology, 255, 1002-1007.

Gilbertson, T. A. (1998). Peripheral mechanisms of taste. See Linden (1998), pp. 1-28.

Gilbertson, T. A., Avenet, S. C., & Roper, S. D. (1992). Proton currents through amiloride-sensitive Na^+ channels in hamster taste cells. Role in acid transduction. Journal of General Physiology, 100, 941-960.

Gilbertson, D. M., & Gilbertson, T. A. (1994). Amiloride reduces the aversiveness of acids in preference tests. Physiology & Behavior, 56(4), 649-654.

Heck, G. L., Mierson, S., & DeSimone, J. A. (1984). Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science, 223, 403-404.

Hendricks, S. J., Stewart, R. E., Heck, G. L., DeSimone, J. A., & Hill, D. L. (In press). Postnatal development of rat chorda tympani sodium responses: Evidence from in vivo voltage clamp for age-dependent changes in global amiloride-sensitive Na^+ channel kinetics. Journal of Neurophysiology.

Kinnamon, J. C. (1986). Organization and innervation of taste buds. See Finger & Silver

(1986), pp. 277-297.

Kinnamon, S. C., & Roper, S. D. (1988). Evidence for a role of voltage-sensitive apical K^+ channels in sour and salt taste transduction. Chemical Senses, 13(1), 114-121.

Kolesnikov, S. S., & Margolskee, R. F. (1995). A cyclic-nucleotide-suppressible conductance activated by transducin in taste cells. Nature, 376, 85-88.

Kolmer, W. (1910). Über strukturen im epithel des sinnesorgane. Anat. Anz., 36, 281-299.

Lindemann, B. (1995). Sweet and salty: transduction in taste. News In Physiology Science, 10, 166-170.

Linden, R. W. A.(Ed.). (1998). The scientific basis of eating. Basel: Karger.

McLaughlin, S., & Margolskee, R. F. (1994). The sense of taste. American Scientist, 82, 538-545.

Ossebaard, C. A., & Smith, D. V. (1995). Effect of amiloride on the taste of NaCl, Na-gluconate and KCl in humans: implications for Na^+ receptor mechanisms. Chemical Senses, 20, 37-46.

Palmer, L. G. (1987). Ion selectivity of epithelial Na channels. The Journal of Membrane Biology, 96, 97-106.

Rehnberg, B. G., Hettinger, T. P., & Frank, M. E. (1992). Salivary ions and neural taste responses in the hamster. Chemical Senses, 17(2), 179-190.

Rehnberg, B. G., MacKinnon, B. I., Hettinger, T. P., & Frank, M. E. (1993). Anion modulation of taste responses in sodium-sensitive neurons of the hamster chorda tympani nerve. Journal of General Physiology, 101, 453-465.

Roper, S. D. (1992). The microphysiology of peripheral taste organs. The Journal of

Neuroscience, 12(4), 1127-1134.

Roper, S. D. (1989). The cell biology of vertebrate taste receptors. Annual Review Neuroscience, 12, 329-353.

Ruiz-Avila, L., McLaughlin, S. K., Wildman, D., McKinnon, P. J., Robichon, A., Spickofsky, N., & Margolskee, R. F. (1995). Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells. Nature, 376, 80-85.

Scott, T. R., & Giza, B. K. (1990). Coding channels in the taste system of the rat. Science, 249, 1585-1587.

Simon, S. A., Holland, V. F., Benos, D. J., & Zampighi, G. A. (1993). Transcellular and paracellular pathways in lingual epithelia and their influence in taste transduction. Microscopy Research and Technique, 26, 196-208.

Simon, S. A., & Roper, S. D. (1993). Mechanisms of taste transduction. Boca Raton: CRC Press.

Smith, D. V., & Frank, M. E. (1993). Sensory coding by peripheral taste fibers. See Simon & Roper (1993), pp. 295-338.

Stewart, R. E., DeSimone, J. A., & Hill, D. L. (1997). New perspectives in gustatory physiology: transduction, development, and plasticity. American Journal of Physiology, 272, C1-C26.

Stewart, R. E., Heck, G. L., & DeSimone, J. A. (1996). Taste-mixture suppression: functional dissection of cellular and paracellular origins. Journal of Neurophysiology, 75(5), 2124-2128.

Striem, B.J., Pace, U., Zehavi, U., Naim, M., & Lancet, D. (1989). Sweet tastants stimulate

adenylate cyclase coupled to GTP-binding protein in rat tongue membranes. Biochemistry Journal, 260, 121-126.

Tonosaki, K., & Funakoshi, M. (1988). Cyclic nucleotides may mediate taste transduction. Nature, 331, 354-355.

Wong, G. T., Gannon, K. S., & Margolskee, R. F. (1996). Transducin of bitter and sweet taste by gustducin. Nature, 381, 796-800.

Ye, Q., Heck, G. L., & DeSimone, J. A. (1994). Effects of voltage perturbation of the lingual receptive field on chorda tympani responses to Na^+ and K^+ salts in the rat: implications for gustatory transduction. Journal of General Physiology, 104, 885-907.

Ye, Q., Heck, G. L., & DeSimone, J. A. (1993). Voltage dependence of the rat chorda tympani response to Na^+ salts: implications for the functional organization of taste receptor cells. Journal of Neurophysiology, 70(1), 167-178.

Ye, Q., Heck, G. L., & DeSimone, J. A. (1991). The anion paradox in sodium taste reception: resolution by voltage-clamp studies. Science, 254, 724-726.