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Biochemistry of sweet taste transduction

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Recent biochemical and biophysical studies point to mechanistic hypotheses for sweet taste transduction involving either second messengers or stimulus-gated ion channels. Biochemical studies have shown that sweet tasting stimuli enhance the production of the second messenger, cyclic AMP, in a GTP-dependent manner in taste tissue homogenates. The cyclic AMP thus produced apparently stimulates a protein kinase A which may phosphorylate ion channels, leading ultimately to depolarization, an increase in intracellular calcium ion activity, and release of neurotransmitter. Recent studies show that some sweeteners also induce the production of inositol 1,4,5-trisphosphate (IP_3). Other sweet transduction processes not associated with second messenger production may exist. For example, evidence for a stimulus-gated type ion channel for sweet taste can be inferred from ion transport studies on lingual epithelia and from psychophysics. A recent study demonstrated that certain amphiphilic sweeteners are capable of directly stimulating purified G proteins in an *in vitro* assay. Perhaps these and other amphiphilic intense sweeteners cross the plasma membrane and directly stimulate the G protein, inducing production of second messenger and bypassing the receptor. A number of sweeteners are capable of forming ion channels or of simply perturbing the membrane, actions which could operate during stimulation of the sweet receptor cell. This type of action could explain the relatively longer response times and lingering taste intensity associated with many amphiphilic sweeteners. Copyright ©1996 Elsevier Science Ltd.

INTRODUCTION

Research over the past decade suggests that unique receptor mechanisms exist for each of the presumed taste modalities. (For recent reviews, see Akabas, 1990; Avenet & Kinnamon, 1991; Brand & Bryant, 1994; Corey & Roper, 1992; Gilbertson, 1993; Kinnamon & Cummings, 1992; Kurihara, 1990; Margolskee, 1993; Naim, 1993; Sato *et al.*, 1995*a*, 1995*b*; Tonosaki, 1990.) These receptor processes can be roughly divided into three types: (i) those that contain a presumed receptor protein which transduces its interaction with a stimulus to changes in the levels of various intracellular signalling compounds, i.e. a second messenger system; (ii) those making use of existing plasma membrane-associated ion channels in taste cells, whereby the stimulus traverses the membrane through these channels or modulates the activity of these channels; and (iii) those making use of a stimulus-gated ion channel, whereby a taste stimulus binds to a receptor site on a receptor–ion channel complex and alters the flux of ions through the ion channel. Each of these mechanisms alters the ion balance within the receptor cell by changing the ion flux across the plasma membrane of the taste receptor cell.

There is a reasonable consensus that the taste system recognizes some four or five general qualities: salty, sour, sweet, bitter and umami. Research into the transduction mechanisms of each of these qualities suggests that each has a particular transduction mechanism(s) that can be experimentally isolated from the others. While each modality is served by a particular mechanism(s), there may be some similarities among mechanisms used by different qualities. Thus, while salty taste utilizes an epithelial sodium channel, sour taste may also use these channels in at least some species (Gilbertson, 1993). Likewise, the stimulus-gated ion channel type mechanism has been implicated in umami taste (Brand *et al.*, 1991; Teeks *et al* 1991) and amino acid taste in aquatic species (Caprio *et al.*, 1993; Teeks *et al* 1991). One difficulty with generalizing the results of studies of transduction mechanisms from experimental animals to humans is the species' specificity now being recognized with taste transduction mechanisms. This specificity goes beyond the well-known stimulus specificity to actual differences among species in the transduction schemes used for reception of an identical stimulus.

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Both of these challenges need to be addressed in the study of sweet taste transduction mechanisms.

Sweetness is a powerful signal for ingestion. There is a great deal of interest in defining the molecular parameters important to a sweet tasting stimulus and in discerning the mechanisms that define the signal transduction sequences for sweet taste. A number of hypotheses have been generated to explain sweet taste transduction, and several of these are reviewed here.

THE CELLULAR RESPONSE TO TASTE STIMULI

The taste receptor cell is a modified epithelial cell, innervated by sensory nerve fibres from cranial nerves VII, IX or X. Taste cells are grouped in a multicellular array called the taste bud (Fig. 1). Not every cell within the bud participates in the receptive process at any given time. Of the 100 or so cells within a typical taste bud, in mammals only a small number of them reach the area of the taste pore at any given time (Kinnamon *et al.*, 1985; Spielman *et al.*, 1992*b*). The actual plasma membrane area exposed to the oral environment from these active receptor cells is quite small, probably of the order of 2–5%. Yet it is likely that this small area is theonly available surface for contact of larger taste stimuli with the receptor cells, since the presence of tight or occluding junctions between the receptor cells essentially seals the oral space from the lower baso-lateral space, preventing the diffusion of all but only the smallest ions and compounds from the oral or apical space into the baso-lateral space. The taste bud is, therefore, effectively a polarized structure, with the apical region bathed in the hypotonic medium of saliva and the baso-lateral space bathed in interstitial fluid (Roper, 1992) (Fig. 1).

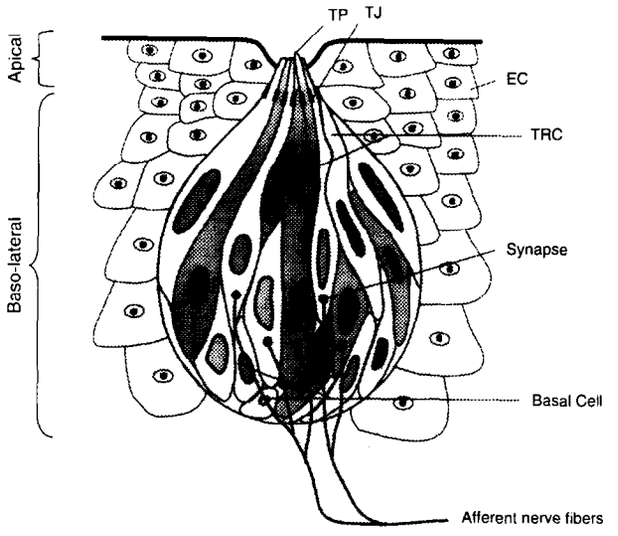

Fig. 1. Drawing of a typical mammalian taste bud. The taste bud is composed of approximately 100 specialized epithelial cells, some of which make synaptic contact with innervating sensory nerve fibers. Only a few of the cells of the taste bud are exposed to the oral environment at the taste pore (TP) at any given time. Because of the presence of tight junctions (TJ) just below the TP, the taste bud, like other epithelial cells (EC), functions as a partial barrier to the diffusion of large molecules and large ions. These tight junctions effectively divide the taste bud into a small apical region and a larger baso-lateral region, polarizing the cells with respect to their extracellular environment. The taste receptor cells presumably insert receptors for taste stimuli into the plasma membrane, with transductive elements located in the membrane and in the cytosol. A sufficiently large transductive wave can initiate secretion of neurotransmitter from the taste receptor cell to the innervating sensory nerve at the synapse. There is evidence also for synaptic contact between apparent taste receptor cells and basal cells within the taste bud (Reutter, 1978; Roper, 1992), with the basal cells then showing synaptic contact with sensory nerve fibers. The function of this intervening cell is not understood, but it is possible that it may act as a bipolar cell does in the visual system, allowing a hyperpolarizing response of the receptor cell to be transformed into an excitatory response at the neural level.

In order to report the presence of a taste stimulus to the brain, the active receptor cell must recognize the stimulus, transduce this chemical recognition into an electrical signal, and spill neurotransmitter across a synaptic cleft. Sufficient changes in the activity of calcium ion must occur within the taste cell to trigger this neurotransmitter release. It is probably this change in intracellular calcium ion which is the ultimate signal that arises from each unique transduction sequence.

SWEET TASTE TRANSDUCTION

A number of chemicals with a seemingly wide array of structures can impart a sweet taste. There appear to be some basic structural determinants that can be used to predict sweetness of compounds, and by following these rules within a given chemical class, it is possible to rationalize the taste of these molecules and even to design 'super-sweeteners' (see Walters *et al.*, 1991). While such rules for predicting sweetness from chemical structure would imply the existence of sweet taste receptors, there are, nevertheless, compounds which impart a sweet taste but which do not fit conveniently into the accepted chemical structural framework. One such compound is lead acetate, another is chloroform. In addition it is appreciated that each enantiomer of almost every monosaccharide tastes sweet. These discrepancies lead to the consideration that sweetness may be at least partly due to an interaction of some stimuli with proteins that may not be unique to the taste receptor cell, but may, nevertheless, be able to participate in a transduction sequence that ultimately leads to second messenger production or to sufficient change in the intracellular ionic environment to allow for neurotransmitter release (for discussions, see DuBois *et al.*, 1993 and Simon, 1991).

The cyclic AMP hypothesis of sweet taste transduction

The prevailing hypothesis for sweet taste transduction states that cellular response is brought about by a

receptor-mediated, G protein-coupled, second messenger cascade (Fig. 2). This hypothesis is perhaps more widely accepted than others because evidence in favour of it has been obtained from a number of animal models using a variety of techniques. The sweet stimulus is hypothesized to interact with a unique, taste cell-specific protein receptor, probably of the seven transmembrane domain type, which couples to a heterotrimeric GTP-binding regulatory protein (G protein) (Hepler & Gilman, 1992) of the G_s type. The α-subunit of this G protein then presumably activates an adenylyl cyclase which brings about an increase in the concentration of the intracellular second messenger, adenosine 3′:5′-cyclic monophosphate (cyclic AMP). The messenger molecule, cyclic AMP, may then stimulate a protein kinase A which phosphorylates ion channels causing a depolarization of the cell. The depolarization would activate voltage-gated channels, causing further depolarization and an increase in intracellular calcium ion (Ca^{2+}) activity, leading ultimately to neurotransmitter release (Fig. 2).

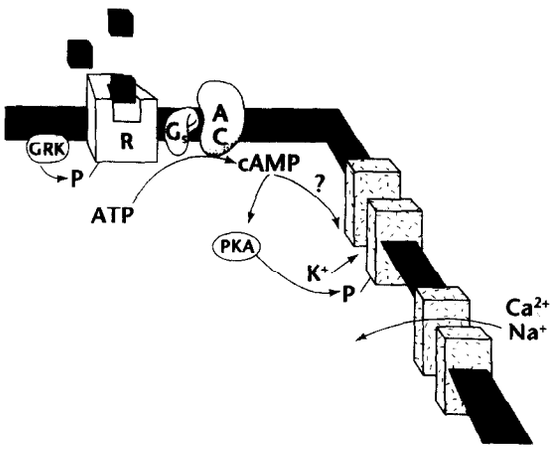

Fig. 2. Schematic representation of the cyclic AMP-mediated transduction mechanism for sweet taste. Taste receptor elements are located at the apical processes of taste receptor cells. It is likely that the entire plasma membrane of the taste cell contains receptor elements, but for non-penetrating stimuli, only those receptor elements above the tight junction level will be activated. In this scheme, the solid band represents the plasma membrane of the receptor cell. Elements of the transduction processes, such as receptor proteins, ion channels, and proteins of the second messenger system, are located either within this membrane or within the cytosol. In this mechanism the stimulus (box) binds to a receptor (R) which then activates a G protein (G_s) which, in turn, activates the enzyme adenylyl cyclase (AC). This enzyme produces the second messenger, cyclic AMP. This second messenger can activate protein kinase A (PKA), which phosphorylates proteins, including ion channels and possibly the receptor, activating some, and inhibiting others. The agonist-occupied receptor may also be phosphorylated by a G protein receptor kinase (GRK). Phosphorylation of the receptor prevents its efficient coupling to the G protein thus contributing to the process of adaptation. As a result of the activation of inhibition of ion channels by phosphorylations, a depolarization is realized, which, if sufficient, triggers release of neurotransmitter. The question of whether cyclic AMP directly gates an ion channel in taste receptor cells that signal sweetness is still being evaluated.

Several biochemical studies have shown that sweet tasting stimuli enhance the production of the second messenger, cyclic AMP, in a GTP-dependent manner in tissue homogenates derived primarily from those areas of the tongue containing taste buds (Naim *et al.*, 1991; Streim *et al.*, 1989, 1991). These stimuli were found to be generally less effective at inducing cyclic AMP production in lingual epithelia devoid of taste receptors. These studies are, however, not without some interesting inconsistencies. For example, while sucrose and other sugars induced an increase in cyclic AMP levels in tissue preparations from anterior tip of rat tongue (containing fungiform taste papillae and other non-taste epithelium) (Streim *et al.*, 1989) and in preparations from circumvallate tissue of rat, cow and pig (Naim *et al.*, 1991; Streim *et al.*, 1991), no increase in cyclic AMP was reported in sugar-stimulated tissue using fungiform taste papillae of pig (Streim *et al.*, 1991). This observation suggests several possibilities including the one that the anterior field in porcine tissue uses a non-cyclic AMP mechanism for sweet response, or that the concentration of sweet taste receptors in this material was too low for the preparation to show a response. The sweet taste inhibitor, methyl 4,6-dichloro-4,6-dideoxy--D-galactopyranoside, was effective in preventing the accumulation of cyclic AMP due to stimulation by sugars in taste tissue of rats, cows and pigs (Streim *et al.*, 1990, 1991). On the other hand, the non-carbohydrate sweetener, saccharin, for which the rat shows a preference, did not enhance the specific accumulation of cyclic AMP in rat taste tissue homogenates (Naim, 1993). This observation suggests the presence of additional messenger systems for sweet taste transduction, and a recent study by Bernhardt *et al.* (1996) supports this hypothesis (see below). Taken together, these biochemical studies support the hypothesis that cyclic AMP is one intermediate in the transduction of sweet tasting sugar stimuli. However, they also point to other mechanisms for sweet taste transduction.

The cyclic AMP thus produced must initiate events that depolarize the receptor cell to a degree sufficient to eventually allow for transmitter release. Several experiments support such a role for cyclic AMP in this process. Injection of cyclic AMP or cyclic GMP, along with ATP, into taste cells of frog and mouse, induced depolarization of these cells with decreased membrane conductance (Avenet & Lindemann, 1987; Tonosaki & Funakoshi, 1988). This action of cyclic AMP may be achieved through its stimulation of a cyclic AMP-dependent protein kinase which presumably phosphorylates and inactivates potassium channels (Avenet *et al.*, 1988). If this depolarization develops rapidly it could initiate Ca^{2+} uptake into the cell, triggering release of neurotransmitter. Uptake of Ca^{2+} from the extracellular space in taste cells from the rat vallate has been observed when these cells were stimulated by sucrose (Bernhardt *et al.*, 1996).

Electrophysiological studies also support a role for cyclic nucleotides in sweet taste transduction. Using a loose-patch technique that allowed recordings from

hamster fungiform taste buds *in situ*, Cummings *et al.* (1993) reported action currents to sucrose and three sweeteners. These action currents were mimicked by addition of membrane permeable analogues of cyclic AMP and cyclic GMP. Interestingly, while the sweeteners self-adapted to repeated stimulation, they did not cross-adapt, implying separate transduction mechanisms for each. Also every taste bud cell that responded to sweet stimuli also responded to the cyclic nucleotide analogs, whereas those buds that did not respond to sweet compounds also did not respond to the analogues. In addition, the phosphodiesterase inhibitor, isobutyl-1-methylxanthine (IBMX), and the adenylyl cyclase stimulator, forskolin, were able to elicit responses in those cells sensitive to sweeteners. Using a whole nerve recording technique in gerbils, Schiffman *et al.* (1994*a*,*b*) reported that the neural responses to some sweet stimuli (as well as stimuli of other modalities) were moderately affected by bathing the tongue in modulators of the cyclic nucleotide cascade sequence. Interestingly, NaF, an activator of the G proteins, enhanced the neural response to many of the sweeteners (Schiffman *et al.*, 1994*b*). These observations strongly suggest that the two processes — response to sweeteners and response to components of the cyclic nucleotide cascade — are linked in the cell.

The taste response is distinguished by a rapid appearance and a rapid desensitization. The onset is assumed to be due to a rapid build-up of the second messenger molecule, cyclic AMP. A preliminary study of cyclic AMP production in the millisecond time frame has been reported only for sucrose in mouse, where no change was observed in cyclic AMP levels at 200 ms (Nagai *et al.*, 1995). As noted above, earlier studies had reported cyclic AMP accumulation in response to sugars, the most rapid being measured after 6 min incubation (Streim *et al.*, 1991). Clearly additional experiments at other time points and with other stimuli are warranted.

Given the speed of sweet taste perception, it is likely that, if cyclic AMP is the second messenger in sweet taste transduction, then it should be produced within 50–200 ms after interaction of the sweet stimulus with the receptor. A logical question to ask is the nature of the signals that then desensitize the cell to further stimulation. As with other receptor-mediated second messenger signalling systems, two obvious possibilities are a decrease in the concentration of cyclic AMP and a decrease in the affinity of the receptor for its ligand. The enzyme phosphodiesterase breaks down cyclic AMP into AMP, and would be active in the cytosol, particularly at the higher Ca^{2+} concentrations that would be achieved with depolarization. In addition, during the signal transduction cascade, agonist-occupied receptors are often phosphorylated, decreasing their ability to couple with G proteins. This process is often mediated by specialized G protein-coupled receptor kinases (GRKs). One member of this family of kinases, the GRK5, was recently cloned from taste tissue of cows (Premont *et al.*, 1994). Unlike many other members of the GRK family, GRK5 apparently is not regulated by the β,γ-subunit of G proteins and is associated directly with the membrane. Together, these two events, metabolism of cyclic AMP and phosphorylation of the receptor, could bring about the termination of the signalling event and an eventual return of the cell to a resting state.

Other second messengers in sweet taste transduction

The likely involvement of cyclic AMP in sweet taste transduction raises the question as to the participation of the other major cyclic nucleotide second messenger, cyclic GMP. Many of the studies that used cyclic AMP also revealed equivalent responses to cyclic GMP. It is known that, for many signalling systems investigated *in vitro*, these two cyclic nucleotides are often closely related. Whether or not both are stimulated *in vivo* remains to be determined. While their roles are analogous, they are not equivalent. For example, in the olfactory system, both cyclic AMP and cyclic GMP are produced in response to certain stimuli, but it appears that they are generated under different time courses and by different mechanisms, cyclic GMP being produced as a consequence of nitric oxide (or carbon monoxide) production (Breer & Shepherd, 1993). The nitric oxide pathway has not been investigated in detail in the taste system, although one biochemical study reports its presence in the taste system of the catfish (Huque & Brand, 1994).

Some recent studies have suggested that the second messenger, inositol 1,4,5-trisphosphate (IP_3), may also be formed during stimulation by sweet tasting compounds. Artificial sweeteners induced the production of IP_3 when epithelium from the vallate papilla of the rat was used as the tissue source (Bernhardt *et al.*, 1996). It has further been reported (Bernhardt *et al.*, 1996) that this IP_3 production occurs rapidly, probably peaking before 500 msec. Only the non-sugar sweeteners, saccharin and the guanidine sweetener SC-45647 (to both of which the rat displays a preference), enhanced the production of IP_3. Sucrose was without effect on IP_3. These are intriguing preliminary results, and need to be strengthened by simultaneous measurements of the production of both IP_3 and cyclic AMP in the millisecond time frame. Consistent with these biochemical studies, recent calcium imaging experiments on isolated taste buds of rat vallate papillae have shown that sucrose enhances uptake of Ca^{2+} from the extracellular space while the non-sugar sweeteners, saccharin and SC-45647, cause release of Ca^{2+} from intracellular stores (Bernhardt *et al.*, 1996).

Direct activation of G proteins by sweet stimuli

While the traditional receptor-second messenger hypothesis assumes the presence of a G protein-linked receptor, this is not an obligatory pairing. A number of compounds are known that can enhance production of second messengers by directly activating the G protein, in essence, bypassing the receptor step (e.g. Mousli *et al.*, 1990). Indeed, several of the bitter tasting peptides

are known to be direct stimulators of G proteins, and the hypothesis was made a number of years ago that such a direct G protein stimulation could be one mechanism for bitter taste transduction (Spielman *et al.*, 1992*a*). Recent work by Naim *et al.* (1994) lends support for this hypothesis for sweet tasting stimuli. They found that the sweet compounds saccharin, neohesperidin dihydrochalcone, aspartame, cyclamate and monellin stimulated the GTPase activity of a mixture of G_i/G_o proteins as well as that of the G protein, transducin. Interestingly a higher sensitivity to these stimuli was seen with transducin compared with that seen to the other G proteins. In this regard it is worth noting that transducin, along with its analogous taste cell-specific G protein, gustducin, is present in the cells of the taste bud (Ruiz-Avila *et al.*, 1995).

The stimulus-gated ion channel hypothesis of sweet taste transduction

Other transduction processes for sweetness may exist that are not necessarily associated with second messenger production. For example, the existence of a stimulus-gated type ion channel for sweet tasting carbohydrates can be inferred from ion transport studies on lingual epithelia of dog, chorda tympani recordings from dog, and psychophysical studies on human (Mierson *et al.*, 1988; Schiffman *et al.*, 1983; Simon *et al.*, 1989) (Fig. 3). In each of the studies, the ability of the diuretic, amiloride, to suppress, ion transport, neural response and sweet taste, suggested the existence of a receptor/(sodium) ion channel complex for sweet taste.

The psychophysical study of Schiffman *et al.* (1983) showed a marked reduction in total taste intensity for several sweeteners, both carbohydrates and other types, after the tongue was adapted to the diuretic, amiloride. Amiloride is a well-studied inhibitor of passive sodium transport through epithelial-type sodium ion channels. Therefore, this psychophysical study predicted that the sweet taste receptor in human may be of the type which when bound by a sweet ligand, allows the passage of sodium ion from the oral space into the cellular space. This movement of sodium is then at least partly responsible for a cellular depolarization. Since the perception of sweet taste could be blocked by amiloride, the suggestion is made that this initial depolarization is due to sodium flux through these passive channels.

Support for this mechanism came from studies on canine lingual transport and from recordings of the chorda tympani nerve in dog (Mierson *et al.*, 1988; Simon *et al.*, 1989). In these studies, both mono- and disaccharides presented at the mucosal side of the isolated lingual epithelium stimulated an increase in short circuit current across the epithelium, suggesting that these sugars caused an increase in the flux of positive ions across the epithelial sheet. This current was carried primarily by sodium ion and could be partially blocked by amiloride on the mucosal side and almost completely blocked by ouabain (an ATPase inhibitor) on the serosal side. These

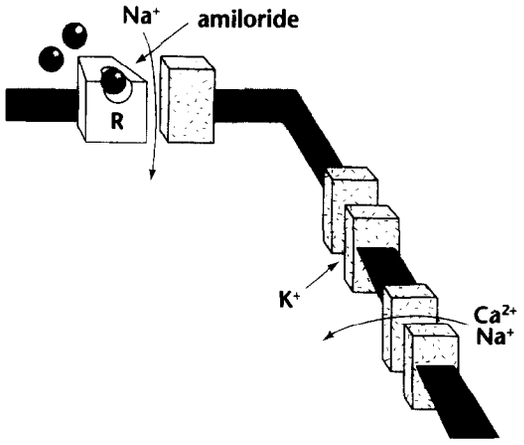

Fig. 3. A stimulus-gated ion channel transduction scheme for sweet taste. In this mechanism, the stimuli (balls) bind to a recognition site on a receptor (R) which is contiguous to or closely associated with an ion channel. When the stimulus binds to the receptor site, the ion channel opens and (in the example shown here) positive charge flows into the cell. This influx of positive charge brings about a depolarization, which, if sufficient, could trigger the opening or closing of voltage-dependent ion channels in the baso-lateral portion of the cell. In this scheme, channels are opened and Na^+ and Ca^{2+} flow into the cell, leading to further depolarization and release of neurotransmitter. In studies of this type of channel mechanism for sweet taste, the diuretic amiloride was shown to be an effective blocker of the inward sodium current.

blockers did not affect sugar transport, indicating that these effects on current were not due to coupled transport of sugar. An analogous block of the sugar-stimulated neural discharge by amiloride was also observed, providing further evidence that what was being measured in the transport studies was a taste-related event.

This type of amiloride-sensitive response to sweeteners has only been documented in humans and canines. However, a recent study has shown a sugar and sodium coupled transport across frog tongue epithelium measured by changes in potential across the epithelium (Soeda *et al.*, 1995). On the other hand, rodents do not display an amiloride-sensitive sugar taste response nor do some other primates. While no other direct evidence for this type of receptor in sweet taste transduction has been published, other stimulus-gated ion channel-type receptors in taste have been demonstrated or inferred for the recognition of L-arginine and L-proline in catfish (Brand *et al.*, 1991; Caprio *et al.*, 1993; Teeter *et al.*, 1990, 1992), and L-glutamate in mouse (Brand *et al.*, 1991; Teeter *et al.*, 1992).

Receptors for sweet taste transduction

Evidence for cyclic nucleotide mediation of sweet taste is strong. However, several problems remain to achieving a better understanding of this hypothesized transduction pathway. Primary among these is the elucidation of the receptor step. To date, no taste receptors of mammals have been isolated and identified for sweet taste. Their eventual identification will help in clarifying such questions as the number of taste

receptors and the interactions which these receptors have with G proteins and second messenger systems.

Protein receptors unique to the taste systems of mammals have yet to be identified. While it is likely that receptors exist for certain modalities, no candidates have been directly and functionally linked to taste transduction. For certain modalities, including sweet, bitter and umami taste in mammals and amino acid taste in fish, there is circumstantial evidence for unique receptors. There is also reasonable evidence for the existence of receptor proteins in lower forms of life, including insects and unicellular animals (Ozaki, 1988; Van Houten, 1994) and fishes (Caprio *et al.*, 1993; Kalinoski *et al.*, 1994). The fact that sweet taste inhibitors are known and that some can act to suppress the sweet-induced generation of cyclic AMP is good evidence for the existence of specific sweet taste receptors. Yet the marked species specificity shown by the known sweet taste inhibitors and the known non-carbohydrate sweeteners argues for major differences in the receptor and, possibly, even major differences in the entire transduction scheme among members of the animal kingdom.

Direct purification of taste receptors from taste tissue of mammals has not, as yet, met with success. Based on the success at identifying the clones of olfactory receptors (Buck & Axel, 1991; Lancet & Ben-Arie, 1993), several groups have undertaken similar searches in taste and lingual cDNA libraries, confining their investigation to clones that might display the classic seven transmembrane domain-type sequences. Several groups have reported results from these approaches, and the sequences show homology with the known olfactory receptor sequences (Abe *et al.*, 1993; Matsuoka *et al.*, 1993). *In situ* hybridization studies show that their expression is not unique to taste receptor cells. It is likely that taste cell specific receptors exist. Given the low abundance of taste cells and the probably low level of expression of receptors in these cells, their identification may prove difficult. Using an alternative approach, Tal *et al.* (1995) have reported the existence of unique G protein coupled receptor-like sequences expressed preferentially in neural and taste sensory tissues.

Given the apparent structural requirements for a compound to act as a sweetener, it is only reasonable to postulate the existence of sweet taste receptors. The fact that carbohydrates do not show strict enantiomeric specificity in their sweet structure/activity relationships does not immediately rule out the existence of receptors. As discussed by DuBois *et al.* (1993), any biochemical or biophysical event that induces the requisite changes anywhere in the sweet transduction cascade will signal sweet, provided the change in the cell response is sufficient to initiate neurotransmitter release. This statement then implies that an agent can be perceived as sweet if it can alter the sweet receptor cell's activity at any level, from the receptor level to the secretion of neurotransmitter. (Likewise, sweetness can be blocked at any level along this cascade.) One example of this type of action of taste stimuli from the information above is the ability of some compounds to directly stimulate the G protein. Conceivably, these and other stimuli could bypass the receptor step, provided they were sufficiently permeable to interact with the G protein or other elements of the cascade further downstream. In order to effect non-receptor stimulation, these compounds need to initiate a physico-chemical process, such as one induced by osmotic effects, or they need to be amphiphilic, and membrane-permeable or membrane-perturbable. We now show that several sweeteners possess these properties and are capable of forming ion channels and perturbing the integrity of model membranes.

Ion channel formation by sweeteners

Many of the known intense sweeteners are amphiphilic. While amphiphilicity cannot be a requisite for sweet taste, nor are all sweeteners amphiphilic, it is possible that those that are may derive some of their sweet taste properties from their ability to either induce ion channels or simply perturb general membrane integrity. In the preliminary studies reported here, we show that some of these high intensity sweeteners are capable of inducing ion channel-like activity in planar lipid bilayers.

Planar lipid bilayers were formed from pure phospholipids. Synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphoserine (DOPS) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were obtained from Avanti Polar Lipids, Inc. (Pelham, AL). All electrolytes were reagent grade and water was doubly distilled and deionized. Salt solutions for bilayer experiments were 100 mM NaCl buffered by 5 mM MOPS to pH 6.5. Sweeteners used in this study were a gift of the Nutrasweet Co. (now NSC Technologies) (Dr. Grant DuBois).

Virtually solvent-free membranes were prepared as described by Montal & Mueller (1972). Two symmetrical halves of a Teflon chamber with solution volumes of 1 cm^3 were divided by a 15 μm-thick Teflon partition containing a round aperture of about 30 μm diameter. Hexadecane in *n*-hexane (1:10, v/v) was used for aperture pretreatment. 'Virtual ground' was maintained at the *trans* side of the bilayer. Hence positive voltages mean that the *cis* side compartment is positive with respect to the *trans* side. Positive currents are therefore those of cations flowing from *cis* to *trans*. All experiments were performed at room temperature. Detailed descriptions of the method used for membrane preparation have been published (Bezrukov & Vodyanoy, 1993; Teeter *et al.*, 1990).

Sweeteners were added to the aqueous phase at one (*cis*) side of the bilayer from stock solutions in water or in 40% ethanol (neohesperidin dihydrochalcone only).

Figure 4 shows ion channel activity induced by two sweeteners, monoammonium glycyrrhizinate (MAG) and the aryl urea sweetener, NC-00274-01. The current fluctuations shown by MAG alternated between discrete levels, consistent with the opening and closing of MAG channels. A large variability in the amplitude of current fluctuations was observed, corresponding to single channel conductances of from 40 pS to several hundreds of pS. The channels formed by NC-00274 are more

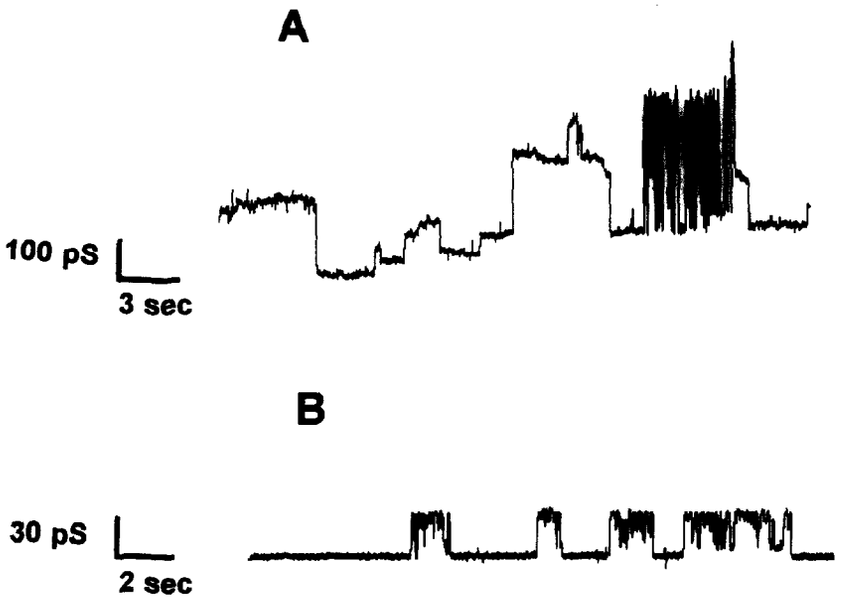

Fig. 4. Ion channels induced by intense sweeteners. Single channel activity induced by the addition of 500 μg ml^{-1} of monoammonium glycyrrhizinate (A) and NC-00274-01 (B) to the *cis* side of the lipid bilayers formed from the mixture of DOPE: DOPS = 7:3. Bath solutions: 0.1 M NaCl, 5 mM MOPS, pH 6.5. The voltage across the membrane was + 150 mV (A) and + 100 mV (B). A positive voltage sign corresponds to the *cis* side being positive. Signals were filtered at 200 Hz by a low-pass Bessel filter.

regular showing a conductance level of 30 pS, although in other experiments we observed a variety of channel conductances with this compound also. Ion channel activity was also induced by rebaudioside A. While no distinct ion channel activity was observed with neohesperidin dihydrochalcone, this compound did increase the general conductance of the planar lipid bilayers in an irregular manner.

While it is known that MAG can affect membrane integrity (Reardon & Audus, 1993) its ability to form ion channels in lipid bilayers has not been previously documented. Likewise the ion channel properties of the other sweeteners studied here have not been reported. Whether or not these properties contribute to the sweet taste profile of these stimuli remains to be evaluated. It is known that many of the sweeteners evaluated here show an intense sweetness with a slow onset and a lingering sweet quality. While the ability to form ion channels in planar lipid bilayers is certainly not a requisite for transducing the actual quality of sweetness, it may be possible that some of the taste characteristics of these sweeteners are related to their ability to alter the integrity of the plasma membrane surrounding the sweet taste receptor, and that this change in membrane integrity, coupled with the activity of the receptor process, may be responsible for some of the taste profile properties of these sweeteners.

CONCLUSIONS

The initial event in the perception of sweetness involves the interaction of the sweet taste stimulus with the apical process of an appropriate taste receptor cell in one of the taste buds of the oral cavity. Since the taste cell is a specialized epithelial cell, this interaction of the cell with a sweet tasting stimulus needs to be transduced through the cell for eventual release of neurotransmitter. The release of neurotransmitter then alters the firing rate of the innervating nerve fibre, signalling to the brain that a taste stimulus is present in the oral cavity. The sequence of metabolic and ionic events that constitute this transduction process is under intense investigation. While the bulk of the evidence favours the cyclic AMP hypothesis as the major transduction mechanism for sweet taste, other mechanisms are also likely. In particular, it now appears that both cyclic AMP and IP_3 may act as second messengers in sweet taste transduction, each perhaps stimulated by different sweeteners. Other data suggest the presence of a stimulus-gated ion channel type mechanism for sweet taste transduction in some species. While sweet substances may interact with unique receptors of the taste receptor cell, these have not, to date, been identified. Some taste stimuli could directly activate the intervening G protein, thereby bypassing the receptor step. In addition, some amphiphilic sweeteners can directly perturb a lipid bilayer, suggesting the possibility that they may interact directly with the plasma membrane of the taste cell in addition to their binding to a presumed taste receptor. While the many studies carried out at the biochemical and electrophysiological levels indicate the presence of a receptor (or receptors) for sweet taste, interaction of all sweet stimuli with that receptor may not be an obligatory step for initiating the transduction process.

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