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CELLULAR SIGNAL TRANSDUCTION OF SWEETENER-INDUCED TASTE

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I. INTRODUCTION

In humans, the response to sweet taste is believed to be innate. Studies examining facial expressions of neonates following taste stimulation indi-

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cated appealing responses to sweet taste and aversion to bitter taste (Steiner, 1973). Newborns prefer sugar solutions to water and their preference increases with sugar concentration (Desor *et al.,* 1973). Furthermore, their preference for different sugars corresponds to adult perception (Maller and Desor, 1974). The high threshold for the appealing taste of sugar (above 10 m*M*) and actual preference level (0.3–0.5 *M*) (Cagan and Maller, 1974) lead mammals to select high-caloric foods and consequently have had a significant effect on survival during phylogenetic development. Moreover, the preference for sugars may change with physiological state. Human subjects prefer a 5% sucrose solution to a 30% sucrose solution, but can be induced to prefer the more concentrated solution once blood glucose level is decreased to 50 mg% following insulin injection (Mayer-Gross and Walker, 1946). Similar results were found in rats (Jacobs, 1958). A strong preference for sweet high-fat foods and fat is associated with opioids (Drewnowski, 1991; Drewnowski *et al.,* 1992; Marks-Kaufman and Kanarek, 1981; Marks-Kaufman and Lipeles, 1982). For example, administration of naloxone, an opioid antagonist, reduced the hedonic preference for sugar in high-fat food (Drewnowski *et al.,* 1995). In fact, oral stimulation by sweet-taste substances may, via a cephalic phase, release insulin, which in turn may participate in stimulating eating (Brand *et al.,* 1982). Individual human subjects whose eating was most responsive to cues associated with food showed the largest insulin release in response to the sight and smell of steaks being grilled (Rodin, 1978), and insulin release was higher after food presentation in obese than in nonobese subjects (Sjostrom *et al.,* 1980). The selection of sweet tasting and other foods is therefore complex: it may be governed by innate and learned responses, and it is often controlled by the physiological state of the organism.

High sugar intake, which has increased significantly in the present century, has been linked to metabolic disorders such as hypertension, diabetes, and obesity as well as to dental caries, suggesting that sugar intake should be limited (e.g., Grand, 1974). As a result, chemical studies were initiated to explore alternative sweeteners and research, especially in the past 10 years, has led to the availability of very potent ones (e.g., Tinti and Nofre, 1996). In fact, in the West low-calorie soft-drink consumption has increased significantly in recent years, creating a multibillion dollar economic target. However, the sweet taste of sugars, especially that of sucrose, is regarded as pure, whereas many nonsugar sweeteners possess inferior sweet quality. Almost all of the latter have undesirable sensory properties such as slow taste onset and lingering (sweet persistence) aftertaste. (Birch *et al.,* 1980; DuBois *et al.,* 1981; Larson-Powers and Pangborn, 1978; Naim *et al.,* 1986; Schiffman *et al.,* 1979). To date, the molecular basis for these phenomena is unknown. One may hypothesize that modifications in sweet intensity–time

relationships involve events at the taste-cell level, either at the receptor or along the signal transduction chain. Therefore, studies initiated in recent years on taste transduction (see below) may lead to the identification of factors responsible for temporal sweet taste.

Many mammals prefer the sweet taste of sugars. With other sweeteners, however, variability among species is evident (Fisher *et al.,* 1965). Aspartame, monellin, thaumatin, and neohesperidin dihydrochalcone (NHD) are potent taste stimuli for humans and old-world monkeys (Table I), whereas they elicit little or no taste responses in new-world monkeys, guinea pigs, or rats (Brouwer *et al.,* 1973; Glaser *et al.,* 1978, 1992, 1996; Hellekant *et al.,* 1976; Naim *et al.,* 1982). This implies a phylogenetic relationship between the responses to sweeteners of old-world monkeys and humans and suggests multiple mechanisms for sweet-taste transduction in different mammalian species.

Taste-papillae-containing taste buds located on the dorsal surface of mammalian tongues and additional taste buds located in other parts of the oral cavity (e.g., the palate) are the organelles responsible for taste chemoreception (Kinnamon, 1987; Miller and Spangler, 1982). Taste buds contain about 50–150 cells, some of which are sensory cells (Fig. 1). A taste pore is located at the apical end of each taste bud and it has long been suggested that the initial stage in taste chemoreception is the interaction between a taste stimulus and microvilli located at the apical end of the taste-receptor cells. Axons of specific sensory neurons enter the buds through special openings at the bottom, where they form a synapse with the taste-receptor cells. Following the chemical interaction between a taste stimulus and the taste-receptor cell, the cell responds with membrane depolarization, which leads to the release of a putative neurotransmitter at the synapse. The taste nerves carry the signals to neurons of the solitary tract nucleus. Subsequently, these signals are carried via nerve projections to

TABLE I

RESPONSES OF SOME MAMMALIAN SPECIES TO CERTAIN SWEETENERS

	Monellin	Aspartame	NHD	Saccharin	SC45647	Sucrose
Humans	++	++	++	++	++	++
Old-world monkeys	++	++	++	++	++	++
New-world monkeys	−−	−−	+−	+−	??	++
Rats	−−	−−	−−	++	++	++

Note. +, Positive; −, negative; +−, partial behavioral and/or electrophysiological response. Sources: Brouwer *et al.* (1973); Glaser *et al.* (1978, 1992); Hellekant and Danilova (1996); Hellekant *et al.* (1976); Naim *et al.* (1982).

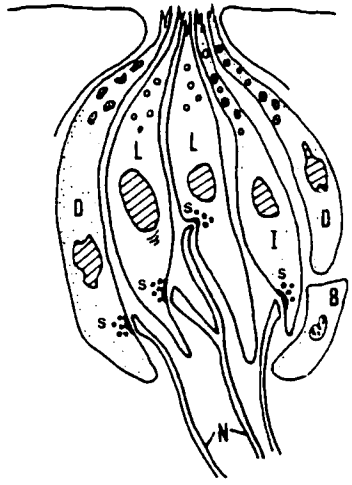


FIG. 1. Diagrammatic representation of mouse taste bud. B, basal cells; D, dark cells; I, intermediate sensory cells; L, light sensory cells. Synaptic contacts (s) are present between dark, intermediate, and light cells and nerve fibers (N). Reprinted from Kinnamon (1987). *In* T. E. Finger, ed., "Neurobiology of Taste and Smell," pp. 227–297, by kind permission of John Wiley & Sons, Inc.

specific nuclei and cortical areas of the central nervous system where they are interpreted as a specific taste sensation. The objective of this review is to discuss the complex cellular events related to mechanisms of sweet-taste transduction, focusing on the biochemical processes leading to the release of chemical signals known as second messengers. Additional taste qualities and taste cellular electrophysiology have been reviewed (Brand and Feigin, 1996; Kinnamon and Margolskee, 1996; Lindemann, 1996a,b).

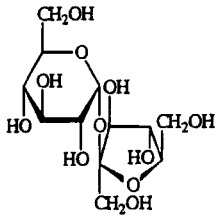
II. RECOGNITION STAGE AT THE TASTE-RECEPTOR CELL

An understanding of the mechanisms via which sweeteners are recognized by taste-receptor cells requires a close examination of both the functional groups in the sweetener molecule that form the pharmacophore (chemical aspects) needed to elicit the sweet taste and the receptor molecule(s) in the sweet-taste-receptor cell (biochemical and physiological aspects) that may interact with a given pharmacophore. Surprisingly, very little interaction has occurred, to date, between scientists in these two research areas.

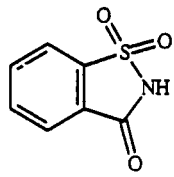
A. CHEMICAL ASPECTS OF THE SWEET MOLECULE

Many chemical studies have been conducted on the chemical structure–sweet taste relationships of various sweeteners (see DuBois, 1997, for a recent review). The main objective of these studies was to explore a pharmacophore(s) that stimulates some common sweet-taste receptor(s). Sweet taste substances include a large collection of diverse synthetic and naturally occurring organic compounds such as sugars, sulfamates, oximes, amino acids, peptides, proteins, guanidines, and terpenoids (Fig. 2) as well as inorganic salts, especially when used at low concentrations. Interestingly, the sweet potency of these compounds is also extremely diverse.

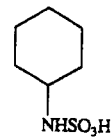
Whether a single or multiple receptor mechanism is responsible for eliciting sweet-taste sensation by these structurally diverse compounds is a peculiar and unsolved question. Most of the structure–function relationship studies have proposed a single sweet-taste receptor for all sweeteners. The Shallenberger/Acree AH–B model (Shallenberger and Acree, 1967) suggested that a sweet molecule must contain a hydrogen-donor group (AH) and a hydrogen-acceptor group (B) and that these two groups interact via hydrogen bonding with the corresponding groups on the sweet-taste receptor. The fact that some D-amino acids are sweet and L-amino acids are bitter led Kier (1972) to assume that a third binding site, characterized by a dispersion binding group (X), is needed, hence the AH–B–X triangle model of sweetness with 2.5 Å between AH and B binding groups, 5.5 Å between B and X, and 3.5 Å between AH and X groups. Belitz and colleagues (Rohse and Belitz, 1991) proposed that e–n (electrophile–nucleophile) groups should replace the AH–B designation since some sweet-tasting compounds do not exhibit H-bond-donating or H-bond-accepting groups. Based on the AH–B–X model, van der Heijden and colleagues (1985a,b) proposed several different receptor sites for sweetness due to some variation in the distances between AH, B, and X groups of different sweeteners. Tinti and Nofre, often regarded as the "sweet people" due to the extremely potent sweeteners they have synthesized, proposed the multipoint attachment (MPA) model of sweetness (Nofre and Tinti, 1996; Tinti and Nofre, 1996) (Fig. 3). This theory postulates the existence of eight optional cooperative recognition sites in the sweet receptor that are able to interact with sweeteners via ionic and hydrogen bonds, as well as via hydrophobic interactions. These occur as a set of three receptor recognition sites (B, AH, and XH) in which ionic and hydrogen bonds are involved and a second set of four sites (G1, G2, G3, and G4), involved in the steric fit with the sweet molecules. The eighth D receptor recognition site is a hydrogen-donor group. A sweetener need not bear all eight binding sites to elicit the sweet taste. High sweet-taste potency may result, however,



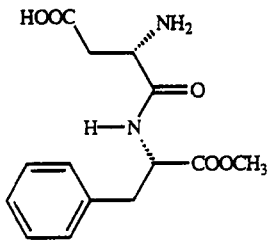
SUCROSE



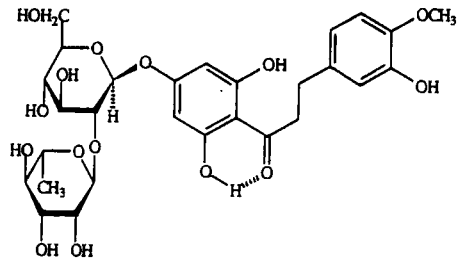
SACCHARIN



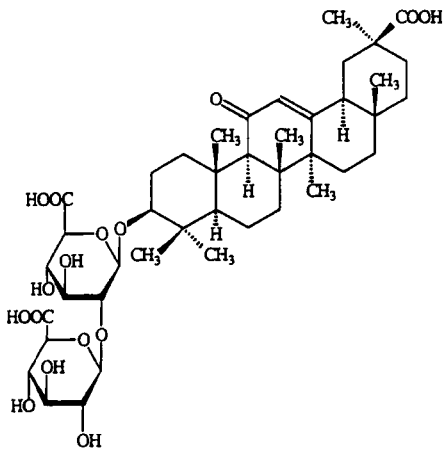
CYCLAMATE



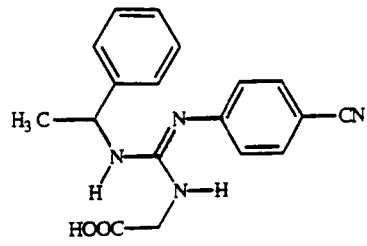
ASPARTAME



NEOHESPERIDIN DIHYDROCHALCONE



GLYCYRRHIZIN



GUANIDINEACETIC ACID (SC45647)

FIG. 2. The diverse chemical structures of some sweeteners.

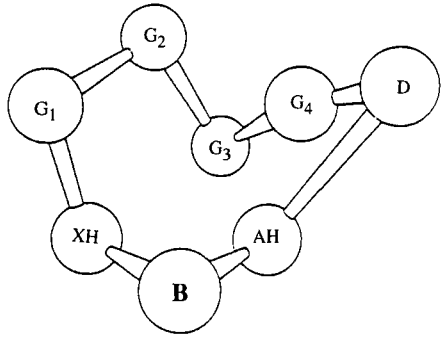


FIG. 3. Spatial arrangement of the eight sites of interaction according to the multipoint attachment theory. Reprinted from Nofre and Tinti (1996) *Food Chem.* **56,** 263–274, by kind permission of The Lancet Ltd.

from the cumulative presence of these binding sites. Recently, Birch and colleagues (1993, 1996) introduced the importance of water and molar volumes in sweet-taste perception. Molecular volumes have been shown to be important in the efficacy of drug actions (McGowan and Mellors, 1986) and in sulfamate sweeteners (Spillane and McGlinchey, 1981). Using experimental biophysical parameters and computer modeling, Birch and colleagues (1993) have proposed that the interaction of a sweet stimulus with the sweet receptor requires specific volumes of the stimulus in water and biophase. Apparent specific volume defines taste quality and specific volumes define hydrostatic packing of the sweet molecules among the water molecules, whereas the related intrinsic viscosities define the hydrodynamic behavior of the sweet molecules.

Among the aformentioned models, the Nofre–Tinti model appears to have made the most significant contribution, leading to the discovery of highly potent sweeteners such as superaspartame (10,000 × sucrose) (Nofre and Tinti, 1987), guanidineacetic acid sweeteners (200,000 × sucrose), *N*-acylglutamylanilide sweeteners (9000 × sucrose), and *N*-alkyldipeptide sweeteners (10,000 × sucrose) (Nofre and Tinti, 1994).

B. POSSIBLE RECEPTORS AT THE PLASMA MEMBRANE

An initial study by Dastoli and Price (1966) was the first to claim the isolation of a sweet-sensitive protein from bovine tongue epithelium. Additional studies (e.g., Hiji and Sato, 1973; Shimazaki *et al.,* 1986) proposed the existence of such proteins in rats and monkeys. The binding of sugars and nonsugar sweeteners to plasma membranes prepared from bovine and human taste papillae was also demonstrated (Cagan, 1971; Cagan and

Morris, 1979). Moreover, the application of proteolytic enzymes such as pronase E to receptor membranes blocked the sweet response (Hiji, 1975). Hence, in analogy to hormones and neurotransmitters, it has been suggested that at least some sweeteners interact with specific receptors located on the plasma membranes, and that intake of stimulus into the cell is not necessary for the recognition stage. However, the low affinity ($K_D \sim 10^{-1}$–10^{-3} M) of sugars to taste receptors (Cagan, 1971) made binding experiments very difficult to conduct. Although the affinity of some nonsugar sweeteners is much higher ($K_D \sim 10^{-5}$ M) (Cagan and Morris, 1979), complications arose in the preparation of sufficient amounts of membrane for these experiments and in the "noise" often produced by the nonspecific binding of stimuli to sites other than taste receptors. Studies in recent years (see discussion below) have suggested the involvement of heterotrimeric guanine nucleotide-binding proteins (GTP-binding proteins, G-proteins) in a putative signal transduction chain in sweet-responsive cells corresponding to: receptor → G-protein → effector enzyme → intracellular signal molecule → ionic channels → cell depolarization → neurotransmitter release. As a result, molecular biology approaches have been used in an attempt to clone the putative seven transmembrane taste receptors. Some polymerase chain reaction (PCR) experiments on rat lingual epithelia and bovine taste tissue (Abe *et al.,* 1993a,b; Matsuoka *et al.,* 1993) identified such receptors that either exhibited significant homology with putative olfactory G-protein-coupled receptors (Buck and Axel, 1991) or were also expressed in nonsensory epithelium. An additional seven transmembrane receptor related to neuropeptides was found to be expressed in gustatory tissue (Tal *et al.,* 1995). A collection of circumvallate (CV) papillae-specific PCR-derived cDNAs, encoding a putative G-protein-coupled receptor, were identified by distinguishing PCR products amplified from the cDNA of nonsensory epithelial sheets lacking taste buds. A novel G-protein-coupled receptor gene, expressed in the CV taste buds but not in the nonsensory sheets, which may or may not encode a taste receptor, was cloned. Ligands to the above receptors have not yet been identified and, to date, no sweet-taste receptor has been identified or cloned. Thus, the putative sweet-taste receptors may differ in sequence from known G-protein-coupled receptors, or may not be coupled to G-proteins at all; if so, a new approach needs to be taken.

As already indicated, chemical studies in general have concluded that a single sweet-taste receptor mediates sweet sensation for all sweeteners. However, cross-adaptation studies in humans (Lawless and Stevens, 1983; Schiffman and Cahn, 1981), electrophysiological recordings in monkeys and rodents (Faurion *et al.,* 1980; Hellekant, 1975; Jakinovich, 1982), and biochemical sweet-taste transduction studies (see below) leave little doubt

that either multiple receptors or multiple transduction mechanisms exist for sweet taste.

III. COMPONENTS OF THE DOWNSTREAM TRANSDUCTION PATHWAY

The hypothesis that sweet-taste receptors occur on the surface of the plasma membrane of taste cells is generally accepted. However, due to the aforementioned difficulties in identifying and isolating such taste receptors, available information is limited. Neurotransmitters and hormones, as well as some sensory signals of vision, olfaction, and taste, bind to specific membrane receptors, then initiate biochemical events through membrane components leading to the formation of intracellular signals (Berridge, 1984; Gilman, 1984; Pace *et al.*, 1985; Striem *et al.*, 1989; Stryer, 1986). In many cases, G-protein is a coupling component between receptors and effector enzymes (Simon *et al.*, 1991). G-proteins transmit either stimulatory (e.g., via G_s) or inhibitory (e.g., via G_i) signals from receptors to adenylyl cyclase, cGMP phosphodiesterase, phospholipases, and several types of ion channels (Schultz *et al.*, 1990; Yatani *et al.*, 1987). A significant feature of such mechanisms is that the G-proteins temporally uncouple the detection of external signals at the receptor from the activation of the effector. This allows the system to amplify the signal manyfold. In vision, a single photoexcited rhodopsin molecule can activate hundreds of phosphodiesterase (PDE) molecules, due to facilitated binding of GTP to a large number of transducin molecules (Stryer, 1986). The cellular amplification of transduction signals following binding at the receptor makes the measurement of the transduction response more sensitive, and perhaps more relevant than binding experiments. Furthermore, components along the signal transduction chain may, in fact, be the site at which taste sensation is primarily modulated. Taste adaptation may be a type of receptor desensitization, whereas sweet intensity and persistence may be dependent on the modulation of transduction systems.

A. INVOLVEMENT OF THE ADENYLYL CYCLASE CASCADE IN SUGAR-TASTE TRANSDUCTION

The idea that cyclic adenosine monophosphate (cAMP) is involved in chemosensory transduction as a second messenger was advanced following results showing its involvement in the transduction of hormones (Robison *et al.*, 1971). Available knowledge at that time indicated that adenylyl cyclase catalyzes the conversion of ATP to cAMP, that a specific PDE

breaks cAMP down to 5′-AMP, and that both membrane enzymes alter the intracellular level of cAMP. High adenylyl cyclase (Kurihara and Koyama, 1972) and PDE (Kurihara, 1972) activity was found in the gustatory epithelium. The presence of these enzymes in the microvilli of cells in rabbit taste buds was also shown (Asanuma and Nomura, 1982). Furthermore, cAMP content could increase in intact bovine taste papillae in response to sucrose stimulation (Cagan, 1974). However, due to the very limited information available at the time on the significant role of G-proteins in the adenylyl transduction pathway, these studies provided no direct evidence for the involvement of cAMP as a second messenger in sweet-taste transduction. The tools needed to test the involvement of the adenylyl cyclase cascade directly became available when the signal-chain components were identified, leading to controlled biochemical experiments (e.g., adding ATP, guanine nucleotides, and other effectors) using plasma membrane preparations (Striem *et al.*, 1989). In addition, the patch-clamp technique became available to record electrophysiological responses in single taste cells following the intracellular administration of cAMP (e.g., Avenet *et al.*, 1988).

A few basic criteria needed to be met prior to drawing a conclusion regarding the involvement of the adenylyl cyclase cascade (e.g., Sutherland, 1972) in sweet-taste transduction. The involvement of G-protein in the mediation of cellular cAMP formation had to be shown. The response also had to be tissue and stimulus specific. In fact, due to the species specificity of the sweet responses in mammals, biochemical studies were needed to follow behavioral and electrophysiological responses where taste responses by a given animal to specific sweeteners had been proven. Time-course experiments needed to be conducted to show that the increase in cAMP or other messengers occurs in real time. Indeed, various sugars stimulated the activity of adenylyl cyclase in crude taste-membrane preparations from the apical anterior of rat tongues (Figs. 4C and 4E) and the formation of cAMP in response to sugar stimulation was dependent on the presence of GTP (Fig. 4E), suggesting that the formation of cAMP is mediated by G-proteins (Striem *et al.*, 1989). Because the reaction mixture contained the PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX), the possible involvement of the G_s type of G-protein, which is present in taste cells (McLaughlin *et al.*, 1993), was proposed. Similar results were found with membranes prepared from pig CV papillae (Naim *et al.*, 1991), and in neither rats nor pigs did sucrose stimulate adenylyl cyclase activity in membranes prepared from nonsensory lingual epithelium. In a similar taste-membrane preparation from rats, sucrose stimulation of adenylyl cyclase activity was inhibited by Cu^{2+} and Zn^{2+} but not by Cd^{2+}, Co^{2+}, or Ni^{2+} (Striem *et al.*, 1989), in agreement with the effect of these ions on the neural taste responses

to sugar stimulation in rats and mice (Kasahara *et al.,* 1987; Yamamoto and Kawamura, 1971). Furthermore, methyl-4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (MAD), an inhibitor (probably competitive) of the sucrose stimulation of the chorda tympani nerve in gerbils and rats (Blochaviak and Jakinovich, 1985; Jakinovich, 1983), and a suppressor of the perception of several sweeteners' sweet intensity in humans (Schiffman *et al.,* 1987), also inhibited sucrose stimulation of adenylyl cyclase activity in taste membranes (Fig. 4C), in complete agreement with the chorda tympani electrophysiological responses (Striem *et al.,* 1990b). As the concentration of MAD increased, stimulation of adenylyl cyclase by sucrose decreased, with the effect of sucrose being completely abolished in the presence of 60 m*M* MAD. The inhibitor itself had no effect on adenylyl cyclase activity.

The above results were in line with the required criteria that sugar stimulation of adenylyl cyclase activity in lingual membranes be GTP dependent, tissue specific, and inhibited by sweet-taste inhibitors. However, the taste membranes prepared from the tip of the tongue of rats, although containing >90% of the tongue's fungiform taste papillae (Miller, 1976; Miller and Spangler, 1982), or membranes prepared from the whole CV papillae of pigs, (Naim *et al.,* 1991) containing about 6000 taste buds (Tuckerman, 1888), were contaminated with membranes derived from epithelial nonsensory, connective, and perhaps even muscle membranes.

An additional study in rats was aimed at obtaining much cleaner sensory tissue and intact taste cells, in which changes in the intracellular level of cAMP in response to sweetener stimulation could be evaluated (Striem *et al.,* 1991). A CV papilla containing a large number of taste buds, located within an epithelial layer covering connective and muscle tissue, was selected. Following subepithelial collagenase treatment, the taste-bud sheet containing condensed taste buds was separated from the connective and muscle tissues. A similar size sheet, separated from the surrounding epithelium of the same papilla, but lacking taste buds, was used as the control nonsensory epithelial tissue. Exposure of CV taste-bud sheets for 6 min to sucrose resulted in a two to threefold increase in cAMP accumulation (Fig. 4F). There was no such response in the nonsensory epithelial tissue derived from the same taste papilla of the same animals. The accumulation of cAMP in these taste buds in response to sucrose was dose dependent and inhibition (65%) of sucrose-dependent cAMP formation was observed in the intact tissue after application of 50 m*M* MAD. The use of IBMX in these biochemical experiments enables the measurement of cAMP formation on a scale of minutes, evidently beyond the expected below-1-sec real time for taste responses (Bernhardt *et al.,* 1996; Spielman *et al.,* 1996). Thus, the temporal dynamics of cAMP signals, as shown in the neural circuit (Hempel

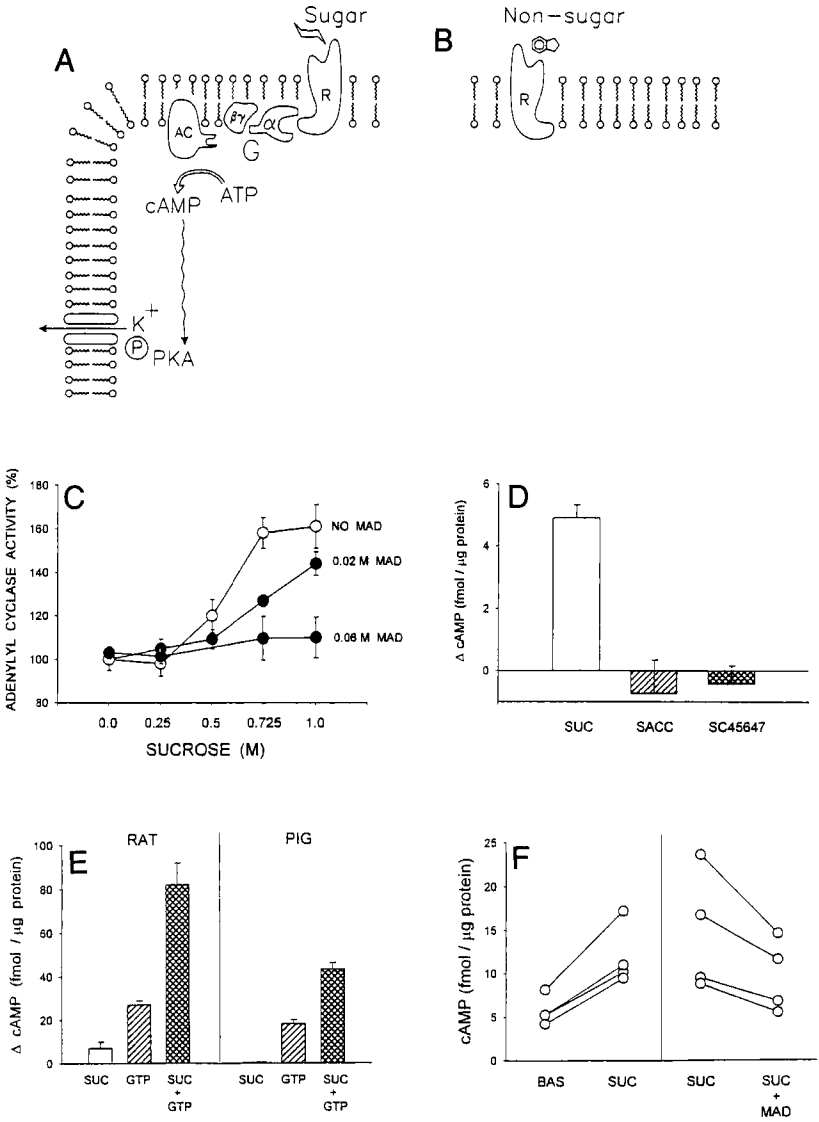


FIG. 4. Biochemical data supporting the model (A) for adenylyl cyclase-induced cAMP formation in sugar-taste transduction. Sucrose (SUC) stimulates adenylyl cyclase activity in taste-membrane preparations of rats and pigs (C,E). The sucrose-stimulated adenylyl cyclase activity is GTP-dependent (E) and is inhibited by the sweet-taste inhibitor MAD (C). In taste-bud sheets, cellular cAMP is increased above basal (BAS) levels (D,F); it is inhibited by MAD (F). cAMP through the activation of PKA induces phosphorylation of the K^+ channel, leading to decreased conductance. This transduction pathway appears not to be

et al., 1996), has to be determined in taste cells. Indeed, preliminary observations indicated a sucrose-stimulated cAMP formation in CV taste-bud sheets within the 500 msec time course (Naim *et al.*, unpublished). Interestingly, stimulation by SC45647, a nonsugar sweetener (Nofre and Tinti, 1987), in humans and rats (Hellekant and Walters, 1993), like saccharin, did not stimulate intracellular formation of cAMP in rat CV taste-bud sheets (Naim *et al.*, 1996) (Fig. 4D).

Important support for the suggestion that cAMP may be a second messenger in sweet-taste transduction came from cellular electrophysiological studies. Lindemann and co-workers (Avenet *et al.*, 1988), using whole-cell recordings from isolated taste cells of frogs (inside-out membrane patches) indicated that cAMP causes depolarization via the action of cAMP-dependent protein kinase A (PKA), which inactivates potassium channels. At the same time, Tonosaki and Funakoshi (1988) found that cGMP, and to a lesser extent cAMP, injected into mouse taste cells decreases potassium conductance, leading to depolarization, and sucrose stimulation depolarized the same cells via potassium conductance. More recently, Kinnamon and co-workers (Cummings *et al.*, 1993) showed in hamsters that fungiform taste buds, which responded to stimulation by sucrose and some nonsugar sweeteners by inducing action potentials, were those which responded in the same way to stimulation by membrane-permeant analogs of cAMP and cGMP. Similar results were also observed with hamster-isolated taste cells (Cummings *et al.*, 1996). Based on these results, a model for sugar-taste transduction has been hypothesized (Fig. 4A). The transduction pathway involves sugar stimulation of specific receptors located on the taste cell's apical membrane. The adenylyl cyclase (AC) cascade is then stimulated via the mediation of G_s-type G-proteins, leading to the production of cAMP, which, via PKA, induces phosphorylation of potassium channels located in the basolateral membranes. The reduced potassium conductance causes membrane depolarization, eventually leading to neural transmission.

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affected by saccharin (SACC) or SC45647 sweeteners (B, D). Abbreviations: R, putative receptors; G, G-protein with the $\alpha,\beta\gamma$-subunits; AC, adenylyl cyclase; PKA, protein kinase. (C) Adapted from Striem *et al.* (1990b) *Chem. Senses* **15,** 529–536, by kind permission of Oxford University Press; (D) reproduced from Naim *et al.* (1996). *In* W. Pickenhagen, C.-T. Ho, and A. M. Spanier, eds. "The Contribution of Low- and Nonvolatile Materials to the Flavor of Foods," pp. 65–75, by kind permission of Allured Publishing Corporation; (E) adapted from Striem *et al.* (1989) *Biochem. J.* **260,** 121–126, by kind permission of The Biochemical Society and Portland Press; (E) adapted from Naim *et al.* (1991) *Comp. Biochem. Physiol. B* **100,** 455–458, by kind permission of Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington OX5 1GB, UK; (F) adapted from Striem *et al.* (1991) *Cell. Physiol. Biochem.* **1,** 46–54, by kind permission of S. Karger AG, Basel.

B. ROLE OF THE PHOSPHOINOSITIDE TRANSDUCTION PATHWAY IN TASTE INDUCED BY SACCHARIN AND SC45647 SWEETENERS

The above-described results suggested a role for cAMP in sugar-taste transduction in rats and pigs, and electrophysiological experiments (Cummings *et al.,* 1996) suggested that some artificial sweeteners elicit action potentials in hamster taste cells and that these responses are mimicked by membrane-permanent analogs of cAMP and cGMP. However, as already indicated, neither SC45647 nor saccharin sweeteners stimulated cAMP formation in intact taste-bud sheets prepared from the CV papilla of rats (Fig. 4D). Such observations have led to the conclusion that an additional signal transduction pathway may be operative for sweet taste. Since psychophysical and chemical studies have suggested that sweet and bitter tastes are interrelated (Bartoshuk, 1975; Birch and Mylvaganam, 1976), the possible stimulation of the phosphoinositide pathway was tested next (Bernhardt *et al.,* 1996).

1. Phospholipase C Activity

The phosphoinositide pathway is known to involve receptor stimulation of phospholipase C (PLC) via G_q- or G_i-type G-proteins to stimulate the formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG) as second messengers (Berridge, 1984; Simon *et al.,* 1991). Its involvement in the transduction of bitter taste in rats and mice has also been proposed (Hwang *et al.,* 1990; Spielman *et al.,* 1994, 1996). IP_3 may, in turn, release Ca^{2+} from intracellular Ca^{2+} stores via IP_3-sensitive receptors (Hwang *et al.,* 1990), as found following stimulation by the bitter tastant, denatonium (Akabas *et al.,* 1988). In contrast to the case of cAMP, an efficient inhibitor of intracellular IP_3 degradation is not available. In view of the fact that the release of second messengers in response to olfactory and taste stimulations observed in membrane experiments is very rapid (Breer *et al.,* 1990; Spielman *et al.,* 1996), a fast-reaction timer instrument was used, thus enabling the monitoring of IP_3 level in intact taste cells in close to real time, less than 1 sec (Bernhardt *et al.,* 1996).

Incubation of intact CV taste-bud sheets with SC45647 and saccharin for 0.5 sec significantly stimulated the formation of intracellular IP_3 (Fig. 5C) whereas no significant IP_3 response was observed in the nonsensory sheets. Moreover, the response was, as expected, time dependent, with maximal IP_3 formation within 0.5 sec under the experimental conditions (Bernhardt *et al.,* 1996). The effects of both SC45647 and saccharin on IP_3 formation were concentration dependent, validating the assumption of a physiological

phenomenon. In contrast to SC45647 and saccharin, sucrose, which is known to stimulate cAMP, produced only very small amounts of IP_3 (Fig. 5C). The phenomenon whereby sugars use cAMP as an intracellular messenger whereas nonsugar sweeteners such as saccharin and SC45647 use IP_3, the latter having been proposed to mediate bitter-taste signaling, has raised a significant question related to taste specificity. The following Ca-imaging experiments conducted by Lindemann and co-workers (Bernhardt *et al.*, 1996) were helpful in assessing this question.

2. *Imaging of Intracellular Ca^{2+} Following Stimulation by Sweeteners*

Since exposure of CV taste buds to the nonsugar sweeteners SC45647 and saccharin stimulated intracellular IP_3 formation, one would suspect that IP_3, in turn, will release Ca^{2+} from intracellular stores by acting on IP_3-sensitive receptors (Irvine *et al.*, 1986). The permeant fluorescent indicator fura 2 acetoxymethyl ester (Tsien, 1983) was used to monitor changes in cellular Ca^{2+} content in CV taste buds isolated from the CV taste-bud sheets of rats by a brief low-calcium treatment (Bernhardt *et al.*, 1996). Under a fluorescence microscope, single taste buds were subjected to digital fluorescence ratio imaging following superfusion with sweeteners. The imaging technique provided significant information because the Ca^{2+} level could be monitored in living taste cells in response to stimulation by a few taste stimuli applied in sequence, each stimulus followed by a Tyrode wash.

Stimulation of isolated taste buds by SC45647 increased the cellular content of Ca^{2+} (Fig. 5D). This release of Ca^{2+} must be from intracellular sources because the same response was observed when Ca^{2+} ions were removed from the extracellular medium by ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetraacetic acid (EGTA; Fig. 5D, hatched bars). The same taste cells that responded to SC45647 also responded to stimulation by saccharin with Ca^{2+} release, again independent of the presence of Ca^{2+} in the extracellular medium. Thus these nonsugar sweeteners, which stimulated the formation of IP_3 in CV taste-bud cells, also stimulated the release of Ca^{2+} from intracellular stores of these cells. Unexpectedly, sucrose, which did not (or only slightly) stimulate IP_3 formation in the biochemical experiments, also stimulated an increase in Ca^{2+} content in the same taste cells that responded to saccharin and SC45647 (Fig. 5D, open bars). However, when Ca^{2+} ions were removed from the extracellular medium by EGTA, the increase in cellular Ca^{2+} content in response to sucrose was no longer seen (Fig. 5D, hatched bars). Hence, the same cells, which could now be classified as sweet-responsive cells, responded to all sweeteners by elevating cellular Ca^{2+} levels. However, sucrose increased Ca^{2+} from an external source, whereas the nonsugar sweeteners increased Ca^{2+} from

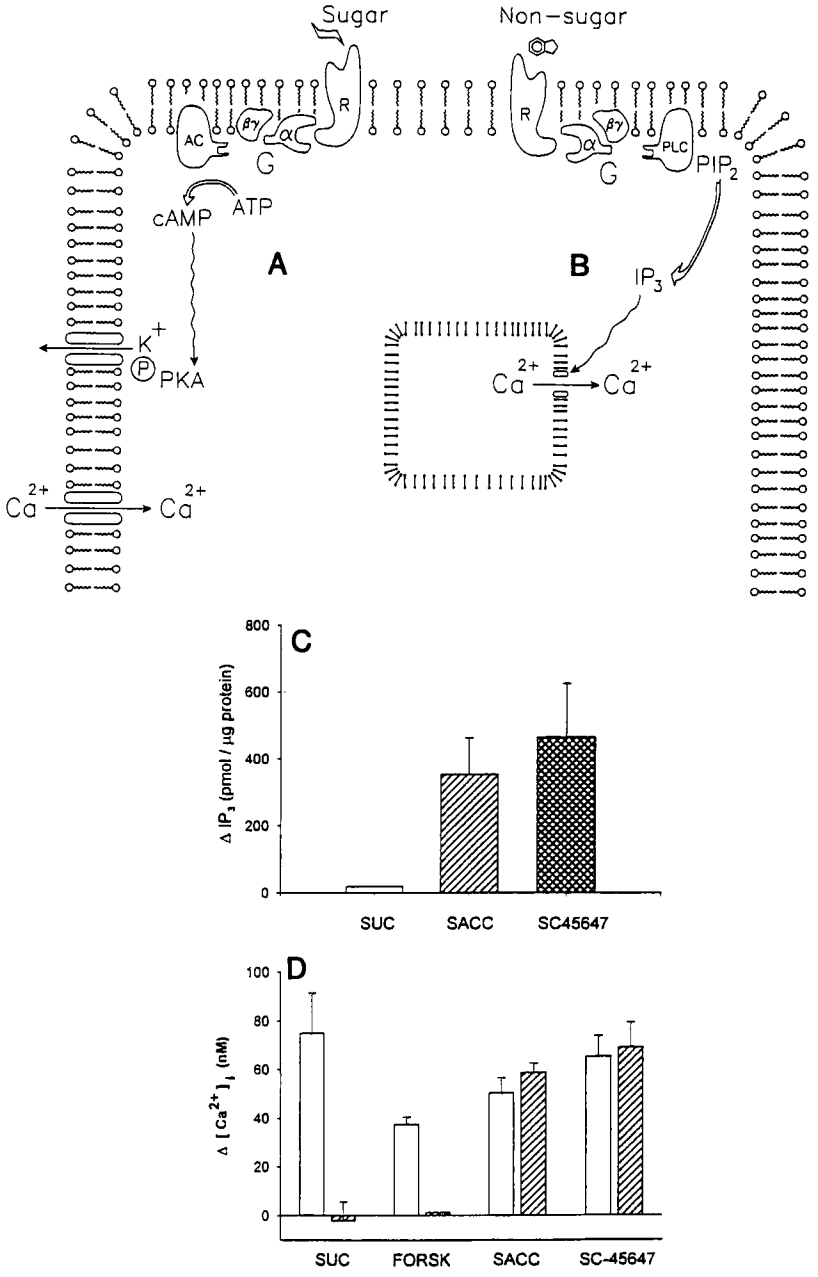

FIG. 5. Data supporting the model for sugar-induced cAMP-dependent Ca^{2+} entry from extracellular medium (A) and for nonsugar-sweetener-induced IP_3-dependent Ca^{2+} elevation from intracellular stores (B). Stimulation of isolated CV taste buds by saccharin (SACC) and SC45647 sweeteners, but not by sucrose, increased cellular IP_3 content (C). Stimulation by

intracellular stores. When the membrane-permeant diterpene forskolin, a direct activator of adenylyl cyclase (Bouhelal *et al.,* 1985; Seamon *et al.,* 1981), was applied to the sweet-responsive cells (i.e., those cells that responded to SC45647, saccharin, and sucrose), the cellular Ca^{2+} level was elevated, and as with sucrose, this elevation depended on the presence of Ca^{2+} in the extracellular medium (Fig. 5D). This result supports the notion that the sucrose-induced entry of Ca^{2+} is mediated by cAMP. Interestingly, taste cells that responded with elevated Ca^{2+} following stimulation by the sweeteners sucrose, saccharin, and SC45647 did not respond to stimulation by the bitter tastant denatonium. The opposite was also true: cells that responded with elevated Ca^{2+} to stimulation by denatonium never responded to these sweeteners. Thus, although saccharin, SC45647, and denatonium stimulate the same transduction pathway in rats (i.e., IP_3), Ca^{2+}-imaging of individual cells suggests that these events occur in two different subpopulations of taste cells. These results could explain sweet- and bitter-taste specificity and are in line with recent results in chimpanzees indicating that sweet-responding fibers of the chorda tympani nerve did not respond to bitter compounds (Hellekant and Ninomiya, 1994). The slight bitterness that accompanies the sweetness of saccharin applied at high concentrations might therefore be due to the generation of IP_3 in bitter-responsive cells, in addition to the IP_3 generated in sweet-responsive cells.

Thus in a sweet-responsive cell, in addition to the adenylyl cyclase cascade, which is apparently stimulated by sugars and which induces cAMP-dependent Ca^{2+} entry from the extracellular medium (Fig. 5A), a second pathway for sweet taste may be occurring (Fig. 5B). The proposed second pathway involves nonsugar sweeteners that stimulate the phosphoinositide transduction pathway to form IP_3 via G_q-like G proteins. IP_3 then acts on intracellular Ca^{2+} stores to release Ca^{2+}.

IV. INVOLVEMENT OF GUSTDUCIN/TRANSDUCIN IN SWEET-TASTE TRANSDUCTION

G-proteins such as G_s, G_i, G_q, G_{14}, and transducin have been identified in taste buds, and a taste-specific G-protein, gustducin, was cloned by

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sucrose (SUC), forskolin (FORSK), saccharin, or SC45647 increased the cellular content of Ca^{2+} (D, open bars). However, only saccharin and SC45647 increased cellular Ca^{2+} in the absence of Ca^{2+} in the extracellular medium (D, hatched bars). Abbreviations: R, putative receptors, G, G-protein with the $\alpha,\beta\gamma$-subunits; AC, adenylyl cyclase; PKA, protein kinase A; PLC, phospholipase C; PIP_2, phosphatidylinositol bisphosphate. (C, D) Adapted from Bernhardt *et al.* (1996) *J. Physiol.* **490,** 325–336, by kind permission of Cambridge University Press.

Margolskee and co-workers (McLaughlin *et al.*, 1992, 1993). Gustducin has recently been found to be expressed in intestinal cells (Hofer *et al.*, 1996). It is closely related to the transducins, and both activate a PDE purified from taste cells (Kolesnikov and Margolskee, 1995; Ruiz-Avila *et al.*, 1995) in a manner similar to the activation of rod transducin in vision. A PDE activation is expected to reduce cellular cyclic nucleotides, as proposed for bitter stimulation (Kurihara, 1972; Price, 1973), rather than increase in cAMP content via the sugar-stimulated adenylyl cyclase activity described earlier. Alternatively, PDE activation may suppress the sugar-stimulated cellular cAMP formation, thereby playing a role in the extinction of the sweet-taste response. There are some data supporting the role of gustducin and/or transducin in both bitter- and sweet-taste transduction. The bitter tastant denatonium, in the presence of bovine CV papillae membranes activated α-transducin but not G_i-protein, suggesting that the putative reduction in cAMP is related to the PDE pathway rather than to the inhibitory pathway of adenylyl cyclase (Ruiz-Avila *et al.*, 1995). In frogs, the nonsugar sweeteners saccharin and NC-01 appear to stimulate the transducin–PDE pathway, suggesting that such a sweet-transduction pathway occurs in a subpopulation of cells different from that containing the adenylyl cyclase pathway; alternatively in frogs these sweeteners stimulate bitter-taste transduction (Kolesnikov and Margolskee, 1995). Most important, Margolskee and co-workers (Wong *et al.*, 1996) have recently found that α-gustducin knockout mice (mutant mice in which the gene encoding the α-subunit of gustducin has been eliminated) exhibit reduced (though not completely eliminated) behavioral and electrophysiological responses to both bitter- and sweet- (see the effect on sweet taste; Fig. 6), but not salty- or sour-taste stimulations. These results suggest that gustducin is involved in both bitter- and sweet-taste transduction. This conclusion appears to contradict the aforementioned data suggesting that the adenylyl cyclase and PLC pathways mediate sweet-taste transduction. However, as was hypothesized by Kinnamon (1996), the α-subunit of gustducin may activate the proposed pathways of bitter-taste transduction (e.g., PDE-reduced cAMP), while the $\beta\gamma$-subunits may activate PLC following nonsugar stimulation. Such a hypothesis is in line with data indicating dual signaling via G-proteins, whereby the α-subunit may involve one pathway, for example, adenylyl cyclase (Zhu *et al.*, 1994), while the $\beta\gamma$-complex stimulates PLC (Gierschik and Camps, 1993; Katz *et al.*, 1992). One problem with the α-gustducin knockout mice is the possibility that $\beta\gamma$-subunits may be in excess. In the α-gustducin knockout mice, excess $\beta\gamma$-subunits may interfere indirectly with α-subunits of other G-proteins (Kinnamon and Margolskee, 1996; Neer, 1995), leading to impaired bitter- and sweet-taste responses. Gustducin, as already mentioned, may also play a role in sweet-taste extinction,

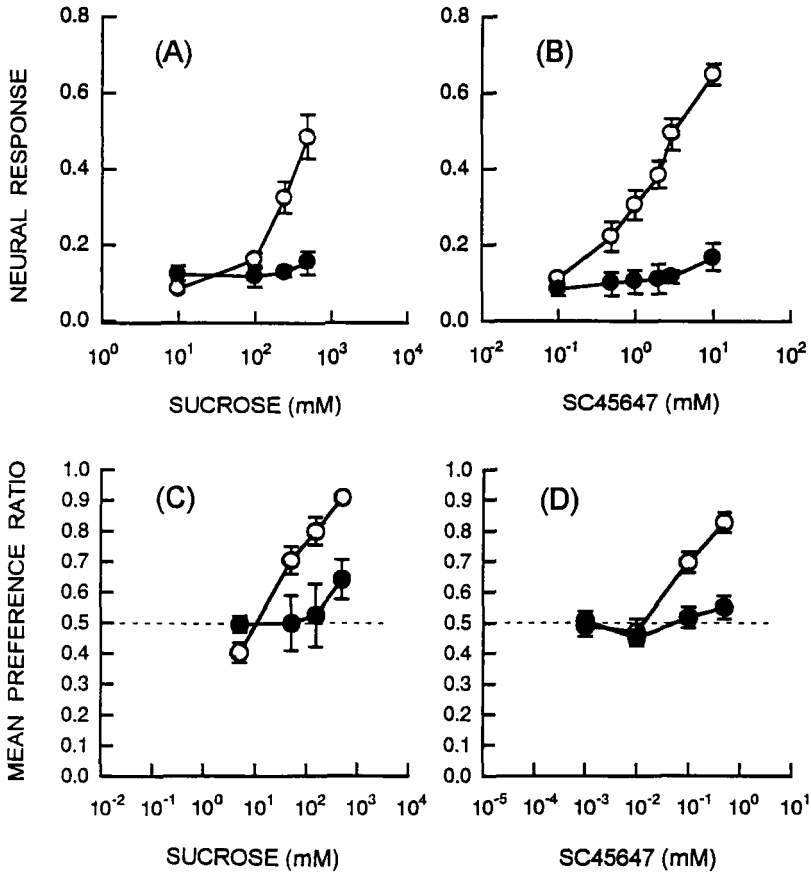

FIG. 6. Relative chorda tympani nerve responses to lingual sweet-taste stimulation (A,B) and 48-hr two-bottle (Sweetener vs distilled water) preference tests (C,D) of control (open circles) and α-gustducin knockout (filled circles) mice. Reproduced from Wong *et al.* (1996) *Nature* **381,** 796–800, by kind permission of Macmillan Magazines Ltd.

for example, by reducing the cAMP level after it has been elevated due to sugar stimulation. Such questions will probably be resolved by detailed time-course measurements of second messengers released by the proposed pathways.

V. AMILORIDE-SENSITIVE SWEET-TASTE TRANSDUCTION

DeSimone and co-workers (1981) proposed a role for active transepithelial ion transport in taste transduction. Such a mechanism was proposed

for sugar sweet-taste transduction in dogs (Mierson *et al.,* 1988; Simon *et al.,* 1986). In response to sugar stimulation, the mucosa of dog tongue has been found to generate a macroscopic inward current through the apical membrane of taste receptor cells. Amiloride (a heterocyclic carboxy guanidinium compound that is a reversible blocker of epithelial channel-mediated Na^+ transport) caused partial blockage of the sugar-evoked current and also suppressed the neural response to sugar-taste stimulation (Mierson *et al.,* 1988). Furthermore, the presence of NaCl in the dog-tongue mucosa enhanced the taste response to sucrose (Kumazawa and Kurihara, 1990), although its presence is not mandatory for the sweet response. The application of amiloride reduces sweet-taste intensity in humans as well (Schiffman *et al.,* 1983). Amiloride inhibited the sensory nerve responses to sugars in dogs (Mierson *et al.,* 1988) but not in rats, gerbils, or hamsters (Herness, 1987; Jakinovich, 1985). This sugar-taste transduction pathway is proposed to be occurring in dogs, rabbits (Simon *et al.,* 1986), and perhaps humans, but not in rats, and vice versa: the cAMP pathway proposed for sugar taste transduction in rats (Striem *et al.,* 1989) may not be involved in the canine response to sucrose (Simon *et al.,* 1989). It should be noted, however, that amiloride may interact with G-proteins and affect the inhibitory pathway of adenylyl cyclase (Anand-Srivastava, 1989) and, therefore, may modify cellular cAMP content.

VI. THE HYPOTHESIS OF RECEPTOR-INDEPENDENT ACTIVATION OF SWEET TASTE BY AMPHIPATHIC NONSUGAR SWEETENERS

The understanding of signal-transduction mechanisms of taste is further complicated by the hypothesis that some bitter and sweet stimuli may initiate taste via receptor-independent mechanisms (Koyama and Kurihara, 1972; Naim *et al.,* 1994; Spielman *et al.,* 1992). It is proposed that some amphipathic (i.e., having both hydrophobic and hydrophilic domains) tastants may activate G-proteins directly, act directly on effector enzymes along the transduction pathway, or even act directly on ionic channels. The idea of a direct activation of G-proteins may be of particular relevance. The heterotrimeric G-proteins act as switches that regulate information-processing circuits connecting cell surface receptors to a variety of enzyme effectors (Simon *et al.,* 1991). These proteins contain three subunits, G_α and $G_{\beta\gamma}$, which link together. Following a receptoral signal, G-proteins bind GTP via the G_α subunit which dissociates from the $\beta\gamma$-subunits. The intrinsic GTPase activity of the G_α-subunit terminates the activation and converts the G-protein to an

inactive $G_{\alpha\beta\gamma}$-GDP-bound form. As discussed earlier, it is now evident that both the G_α- and $G_{\beta\gamma}$-subunits are capable of activating effector enzymes such as adenylyl cyclase and PLC (Clapham and Neer, 1993; Katz *et al.*, 1992). A variety of amphipathic neuropeptides, venom peptides (e.g., substances P, bradykinin, mastoparan), and nonpeptide substances can activate G proteins directly, leading to cellular responses such as histamine and insulin release (Avidor *et al.*, 1993; Higashijima *et al.*, 1990; Mousli *et al.*, 1990). The amphipathic properties of such compounds allow them to penetrate deep into the plasma membrane and activate G-proteins directly, thereby mimicking receptoral stimulation of cellular responses (Mousli *et al.*, 1990). Bitter stimuli can depolarize N-18 mouse neuroblastoma cells, which are unrelated to taste (Kumazawa *et al.*, 1985), and some bitter drugs (e.g., propranolol) and neuropeptides (bradykinin is bitter; Spielman *et al.*, 1992), are direct activators of G-proteins (Hagelueken *et al.*, 1994). In contrast to sugars, nonsugar sweeteners are, like bitter stimuli, chemically diverse, that is, they are aromatic compounds, sulfamates, dipeptides, and guanidines, and they are also amphipathic (Fig. 2). Indeed, some sweet amphipathic tastants such as saccharin, NHD (e.g., Fig. 7), cyclamate, and the bitter tastant quinine have been found to activate the GTPase of either purified transducin or a mixture of purified G_i/G_o-proteins (and G_i/G_o-proteins reconstituted into phospholipid vesicles) *in vitro* in a concentration-dependent manner (Naim *et al.*, 1994). The concentrations of the taste substances that activated G_i/G_o-proteins and transducin correlated closely with those needed to elicit taste. If these tastants could bypass the taste receptors and permeate the plasma membrane, then taste specificity might be achieved at intracellular targets (e.g., G-proteins) and/or be dependent on the permeation of the amphipathic tastant through the plasma membrane, the composition of which may vary among mammals (Maddy, 1966). Changes in lipid composition affected the interaction of some bitter tastants with liposomes (Kumazawa *et al.*, 1988).

The above *in vitro* experiments are consistent with the slow taste onset and lingering aftertaste that are common among nonsugar sweeteners (Birch *et al.*, 1980; Larson-Powers and Pangborn, 1978) and that may result from a process of stimulus penetration through the plasma membrane. Furthermore, some of these taste compounds elicit taste and taste nerve responses (Bradley, 1973; Fishberg *et al.*, 1933; Hellekant *et al.*, 1987) following intravenous or intralingual administration, independent of stimulation of putative receptors at the apical surface of the tongue. For example, Fishberg and colleagues (1933) used intravenous injection of sodium saccharin to measure the blood circulation time in humans from the time of saccharin injection into the peripheral vein until subjects indicate that they

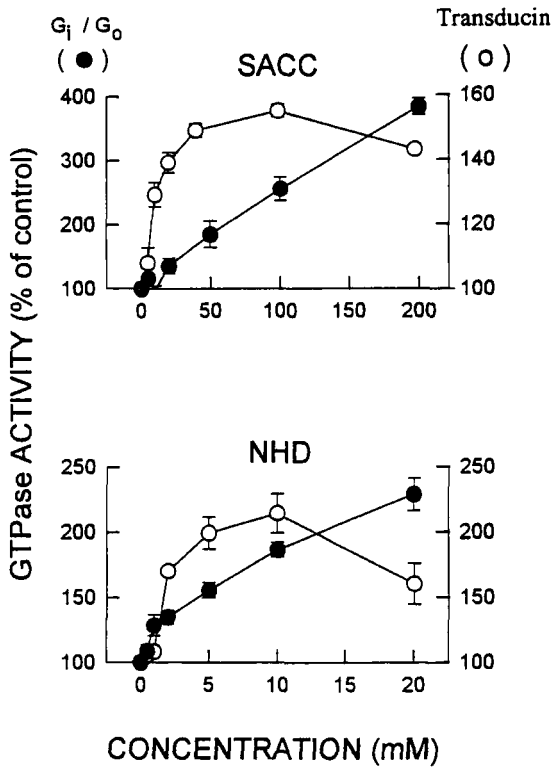

FIG. 7. *In vitro* stimulation of GTPase of G_i/G_o-proteins (filled circles) and transducin (open circles) by saccharin (SACC) and neohesperidin dihydrochalcone (NHD). Reproduced from Naim *et al.* (1994) *Biochem J.* **297,** 451–454, by kind permission of The Biochemical Society and Portland Press.

taste the compound. Saccharin modifies adenylyl cyclase activity in membranes derived from the muscle and liver, which are unlikely to contain taste receptors (Striem *et al.,* 1990a), and it has been proposed to affect the adenylyl cyclase catalytic unit in fat cells (Dib *et al.,* 1996). Moreover, the nonsugar sweetener acesulfame K acts directly on pancreatic islets, potentiating insulin release (Liang *et al.,* 1987). These observations have generally been interpreted as stimulation of putative receptors in various tissues. However, the lack of tissue specificity for such tastants may also be indicative of receptor-independent mechanisms. Thus, if some of these amphipathic sweeteners are proven to be taste-cell permeants, they may affect taste-transduction pathways independently of receptors located on the plasma membrane.

VII. SUMMARY AND RESEARCH NEEDS

In addition to the innate strong preference of most mammals for sweet stimuli, oral sweet-taste signals of a particular food are coupled with postingestive signals of that food to participate in the control of the release of some hormones, such as endorphins and insulin, and in the regulation of diet selection. As such they have become nutritionally important. From a nutritional and food research point of view, an understanding of the molecular mechanisms of sweet taste is even more essential as sweetness has always been associated by humans with an appealing sensation. Because a restriction in the intake of sugar (sucrose), bearing the most appealing sweet taste, has been recommended, the need to discover new, alternative sweeteners with a desirable sensation, close to that of sucrose, is thus imperative. The ability to synthesize very high-potency sweeteners appears to be available in terms of advanced chemical considerations, but very little is known about molecular factors affecting slow taste onset and lingering aftertaste, which are common in nonsugar sweeteners and which reduce sweet-taste quality. Recent progress in the understanding of sweet-taste transduction at the taste-cell level has opened the way to monitoring the signal-transduction output following stimulation by new sweeteners. A collaboration between chemists and taste physiologists has therefore become relevant to identify sites affecting sweet-taste quality.

Despite research efforts in recent years, the initial step in sweet-taste sensation, that is, identification or cloning of the receptor molecule(s) in the taste cell responsible for sweet specificity, has not yet been performed. Studies employing molecular biology techniques are expected to be the main tool to overcome this important obstacle. Do certain amphipathic nonsugar sweeteners bypass the taste receptors *in vivo* to act on intracellular targets along the downstream transduction pathway? This is a hypothesis that has been raised for bitter taste as well, but requires much more investigation, though *in vitro* experiments have suggested that some sweeteners are direct activators of G-proteins. The permeation of amphipathic tastants through and/or their interacting with components of the plasma membrane of taste-receptor cells still needs to be shown.

Accumulated data suggest that multiple transduction pathways at the taste-cell level may be activated by sweet-taste stimulation. The understanding of such mechanisms is particularly complicated by the diversity of species specificity in the taste response to nonsugar sweeteners and the different mechanisms that may be operative for sugar and nonsugar sweeteners in different mammals. For example, in rats and apparently in hamsters, measurements of cellular chemical signals (i.e., second messengers) and cellular electrophysiological studies suggest the involvement of cyclic nucle-

otides such as cAMP (via the adenylyl cyclase cascade) and perhaps cGMP, as second messengers of sugar-taste transduction. The phosphoinositide pathway (e.g., formation of IP_3) is activated by saccharin and SC45647 and perhaps by other nonsugar sweeteners. However, the amiloride-sensitive sweet-transduction pathway may be operative in dogs and rabbits, and perhaps in humans, but not in rats. Ca-imaging experiments suggest that the cAMP and IP_3 pathways occur in the same sweet-responsive cell; both pathways elevate cellular Ca level. Could cross-talk between these pathways, as shown in other systems, be needed for optimal sweet-taste response? Additional studies are required to simultaneously quantify the time course of the release of these two second messengers, to determine whether they are sequentially co-released.

A major challenge in the near future is to explore the pathway by which gustducin and/or transducin, two G-proteins known to activate PDE, are involved in sweet-taste transduction. The recent results showing that sweet and bitter sensation are impaired in α-gustducin knockout mice suggest such an involvement and appear to contradict the proposed cAMP and IP_3 pathway hypothesis. One may suggest that gustducin activates an additional, different pathway(s). A more likely possibility is that the gustducin–PDE components participate in the aforedescribed mechanisms. Perhaps gustducin is important in steps occurring later in the transduction sequence rather than at the initial stage, for example, in metabolic and other functions (neurotransmitter release) of the stimulated taste cell. The α-gustducin–PDE system may be important in reducing cAMP level after it has been elevated via sugar-induced adenylyl cyclase activity. Furthermore, α-gustducin may be responsible for maintaining a low level of cAMP under resting conditions, thus allowing the cAMP level to be elevated following sugar-induced adenylyl cyclase activity. If so, the resting cAMP level in taste cells of the α-gustducin knockout mice may be too high, resulting in an impaired sugar-stimulated cAMP signal. As suggested (Lindemann, 1996a), this phenomenon may also be related to the impaired sweet response to stimulation by SC45647 (stimulating the PLC–IP_3 pathway) in the α-gustducin knockout mice. If cross-inhibition occurs between the cAMP and IP_3 pathways, as in other systems (Liu and Simon, 1996), then the IP_3 level may increase in response to SC45647 stimulation only if the cAMP level is kept low.

In summary, based on studies utilizing rodent taste tissue, possible transduction pathways may be proposed (Fig. 8). In a sweet-responsive cell, there are at least two pathways for sweet-taste transduction. The first involves sugar stimulation of putative receptors to stimulate the adenylyl cyclase cascade, which forms cAMP via the mediation of a G_s-type of G-protein (Fig. 8, left). cAMP, in turn, depolarizes the taste cell by reducing

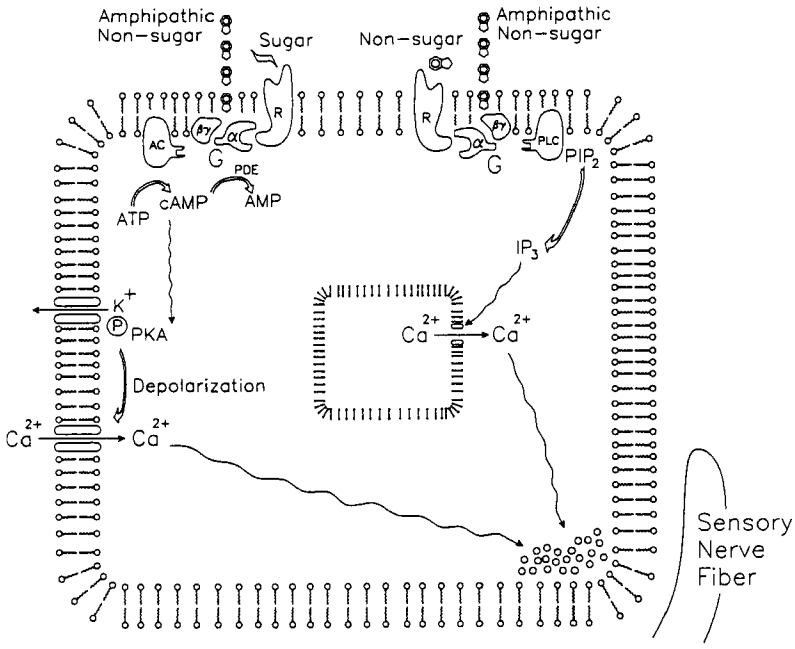

FIG. 8. Proposed pathways of sweet-taste transduction in a sweet-responsive cell in the rat circumvallate taste papilla. Abbreviations: R, putative receptors; G, G-protein with $\alpha,\beta\gamma$-subunits; AC, adenylyl cyclase; PKA, protein kinase A; PDE, phosphodiesterase; PLC, phospholipase C; PIP_2, phosphatidylinositol bisphosphate.

potassium conductance. The resulting depolarization may lead to the entry of Ca^{2+} from the extracellular medium via voltage-dependent Ca^{2+} channels. The α-subunit of gustducin, acting on PDE, may keep the cAMP resting level low before and after sugar stimulation. The second proposed pathway involves nonsugar sweeteners, which stimulate the phosphoinositide transduction pathway to form IP_3 via the mediation of the α-subunits of G_q-like G-proteins or by the $\beta\gamma$-subunits of gustducin. IP_3 then acts on intracellular Ca^{2+} stores to release Ca^{2+}. It is still possible that both transduction pathways are initiated by a single taste receptor. Recent studies list a number of cloned, G-protein-coupled receptors (for thyrotropin, luteinizing hormone, calcitonin, parathyroid hormone, vasopressin, catecholamines) that are able to activate both adenylyl cyclase and PLC (Gierschik and Camps, 1993; Zhu *et al.*, 1994). In at least some of these cases, the α-subunit of the heterotrimeric G-proteins activates adenylyl cyclase while, at a higher agonist concentration, the $\beta\gamma$-complex activates PLC. Amphipathic nonsugar sweeteners, in addition to their action on putative receptors, may permeate

the membrane and act directly on G-proteins or other targets along the transduction pathways.

The increase in the cellular content of Ca^{2+}, either from the extracellular medium due to sucrose or from intracellular stores due to the nonsugar sweeteners SC45647 and saccharin, probably leads to the release of a neurotransmitter in the synapse with sensory nerve fibers that carry the signal to the brain.

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