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Effect of Modulators of the Adenylate Cyclase System on Sweet Electrophysiological Taste Responses in Gerbil

SUSAN S. SCHIFFMAN*[1] MARK S. SUGGS* AND MICHAEL L. LOSEE†

Departments of Psychiatry and Psychology, Duke University, Durham, NC 27706
†The Nutrasweet Co., Mt. Prospect, IL 60056

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SCHIFFMAN, S. S., M. S. SUGGS AND M. L. LOSEE. *Effect of modulators of the adenylate cyclase system on sweet electrophysiological taste responses in gerbil.* PHARMACOL BIOCHEM BEHAV **48**(4) 991-998, 1994.—The adenylate cyclase system has been implicated in sweet taste transduction. The purpose of this study was to determine whether application of modulators of the adenylate cyclase system to the tongue alters sweet taste responses. Integrated chorda tympani (CT) recordings were made in gerbils to sweet tastants before and after a 4-min application of four types of modulators of the adenylate cyclase system. The four types of modulators tested were: a) NaF, a compound that promotes dissociation of GTP-binding protein; b) forskolin, a powerful stimulant of adenylate cyclase; c) 8-bromoadenosine 3′ : 5′-cyclic monophosphate sodium salt (8BrcAMP) and N^6,2′-O-dibutyryladenosine 3′ : 5′-cyclic monophosphate sodium salt (DBcAMP), two membrane permeable forms of cAMP; and d) 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride (H-7) and *N*-(2-[methylamino]ethyl)-5-isoquinolinesulfonamide dihydrochloride) (H-8), which are protein kinase inhibitors. The sweet compounds tested were: sucrose (30 mM and 100 mM), glucose (300 mM), fructose (300 mM), maltitol (150 mM and 300 mM), mannitol (300 mM and 500 mM), sodium saccharin (10 mM), D-tryptophan (6.5 mM), dulcin (0.88 mM, 1.75 mM, and 3.5 mM), and stevioside (0.55 mM and 1.1 mM). NaCl (30 mM and 100 mM) and KCl (300 mM and 500 mM) were used as control stimuli. The main findings were as follows. Application of NaF (20 mM) for 4 min as a rinse significantly enhanced all of the sweet compounds by at least 23%, except for 10 mM sodium saccharin and 6.5 mM D-tryptophan, while all control compounds were suppressed. NaCl (20 mM), which was used as a control for NaF, did not significantly enhance any of the responses when applied as a rinse. 8BrcAMP (1.16 mM) enhanced 30 mM sucrose by 16%, 300 mM glucose by 36%, 300 mM maltitol by 18%, and 6.5 mM D-tryptophan by 24%. DBcAMP had a minimal effect on most of the compounds tested with a 26% enhancement of 300 mM mannitol and a 17% blockage of 300 mM KCl. H-7 (300 μM) enhanced 30 mM sucrose and 1.75 mM dulcin, but this may not be due to an effect on the adenylate cyclase system. H-8 (147 μM) was used in a single trial with no consistent changes. These data indicate that modulation of the adenylate cyclase system can increase the intensity of some sweet taste responses.

Adenylate cyclase Taste Electrophysiology Sweeteners Gerbils

THE sweet taste sensation is produced by a wide range of compounds including saccharides, diterpene glycosides, polyols, amino acids, dipeptides, and other nonsugars (24). However, our understanding of the chemical properties of these sweeteners and the biochemical mechanisms responsible for signal transduction of sweet taste is limited. Recent biochemical studies have investigated the role the adenylate cyclase system, a second messenger system that utilizes cAMP as an intracellular messenger, in sweet taste tranduction. These studies suggest that binding of sweeteners to cell surface receptors elevates intracellular cAMP (15,26) and inactivates potassium channels (1,27,31).

In the adenylate cyclase system, the ligand (here the sweetener molecule) binds to a membrane receptor which interacts with a G protein to stimulate adenylate cyclase and, thus, enhance the rate of cyclic AMP (cAMP) synthesis within the cell (11). G proteins are heterotrimers and consist of three distinct subunits: α (molecular mass = 39-46 kDa), β (37 kDa), and γ (8 kDa). The β and γ subunits are tightly associated and function as a complex (see Fig. 1). The α-subunit is

[1] To whom requests for reprints should be addressed.

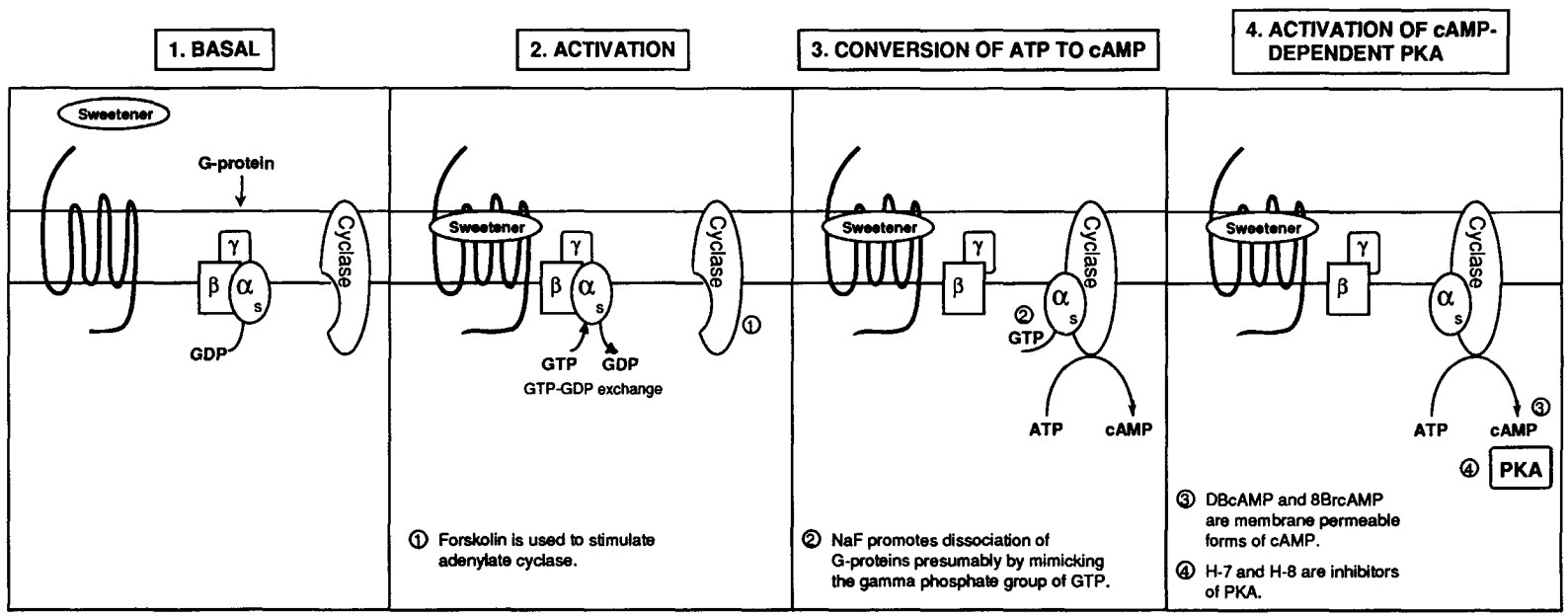

FIG. 1. Activation of adenylate cyclase system.

used to identify the heterotrimer because the same β-γ complex can be shared with different α subunits. α Subunits contain a single, high affinity binding site for guanine nucleotides (guanosine diphosphate or guanosine triphosphate).

In the inactive or basal state (when the receptor is unoccupied), the binding site on α is occupied by guanosine diphosphate (GDP), and the α subunit binds tightly to the β-γ complex. When the receptor is occupied or activated, the receptor interacts with the heterotrimer causing a conformational change. GDP is displaced from the binding site on α and is replaced by guanosine triphosphate (GTP). Binding of GTP to α causes the GTP-bound form of a to dissociate from the β-γ complex, thus freeing α-GTP to modulate adenylate cyclase. Activation of adenylate cyclase leads to the conversion of ATP to the second messenger cyclic AMP which exerts nearly all its effects by activating cAMP-dependent protein kinase (PKA). PKA is ubiquitous in cells and phosphorylates many intracellular proteins (5).

In addition, binding of GTP induces a conformational change that leads to the dissociation of the G protein from the stimulus-receptor complex, thus reducing the affinity of the stimulus for the receptor and freeing the receptor for another binding event. The α-subunit can also be activated by aluminum tetrafluoride (AlF_4^-), together with Mg^+, which interact with the α-bound GDP to mimic GTP.

To date, 21 distinct G protein α subunits have been identified; in addition, four different β units and six γ units have been described. A novel G protein named gustducin (G_g) that is expressed only in taste buds has recently been identified (18). The structure of gustducin is similar to the G protein called transducin which is located in photoreceptor rod outer segments.

The purpose of this study this study was to determine if modulation of the adenylate cyclase system could modify sweet taste. Pharmacologic probes known to alter the adenylate cyclase cascade were applied to the tongue of gerbil to determine the effect on electrophysiologic responses to sweet taste stimuli recorded from the chorda nerve of the gerbil.

METHOD

Animals

Female Mongolian gerbils, *Meriones unguiculatus*, were obtained from Tumblebrook Farm, West Brookfield, MA. The gerbils were 10–12 weeks old and weighed from 45–60 g (avg. 50 g).

Stimuli

Six modulators of the adenylate cyclase system were tested for their effect on sweet taste. The compounds tested were: NaF at 20 mM; forskolin at 0.12 mM; 8-bromoadenosine 3′ : 5′-cyclic monophosphate sodium salt (8BrcAMP) at 1.16 mM; N^6,2′-O-dibutyryladenosine 3′ : 5′-cyclic monophosphate sodium salt (DBcAMP) at 5 mM; 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride (H-7) at 300 μM; and *N*-(2-[methylamino]ethyl)-5-isoquinolinesulfonamide dihydrochloride) (H-8) at 147 μM. NaCl was tested at 20 mM as a control for the NaF experiment. Each of these compounds can gain intracellular access in nonpermeabilized cells (17).

These six modulators affect the adenylate cylase system in the following manner (see Fig. 1). NaF promotes dissociation of GTP-binding protein (7,9,13) and has been used at concentrations from 1–20 mM . Forskolin is an alkaloid extract from the Indian herb Coleus forskohlii (3,6), which is a powerful stimulant of adenylate cyclase at concentrations from 1 μM to 1 mM. 8-Bromoadenosine 3′ : 5′-cyclic monophosphate (3,6) is a membrane-permeable form of cAMP which has been used at concentrations from 0.1 mM to 5 mM. N^6,2′-O-dibutyryladenosine 3′ : 5′-cyclic monophosphate (8,21) is another membrane-permeable form of cAMP which is applied at concentrations from 2 mM to 10 mM. H-7 (23,29) and H-8 (30) are protein kinase inhibitors which have been used at concentrations of 50 μM to 300 μM. The concentrations used for the six inhibitors in the present experiment have been employed previously in other biological systems as well as the taste system (25).

All of the modulators were dissolved in deionized water, with exception of forskolin, which was dissolved in 100% DMF (*N,N* dimethyl formamide) and diluted to 1% DMF, and H-7 which was dissolved in 100% ethanol and diluted to 1% ethanol. The pH of the solutions ranged from 6.0 to 7.0.

The effect of these modulators on 14 sweet solutions was examined. The stimuli tested were: 30 mM and 100 mM sucrose, 300 mM glucose, 300 mM fructose, 150 mM and 300 mM maltitol, 300 mM and 500 mM mannitol, 10 mM sodium saccharin, 6.5 mM D-tryptophan, 0.88 mM and 3.5 mM dulcin, and 0.55 mM and 1.1 mM stevioside. NaCl (30 mM and 100 mM) and KCl (300 mM and 500 mM) were used as controls for the experiments. For some modulators, only a subset of the sweeteners were tested due to the prohibitive cost for running each of the concentrations for some of the modulators. All solutions were tested at room temperature.

Experimental Procedure

Gerbils were anesthetized with an intraperitoneal injection of ketamine HCl (Ketalar 50 mg/ml) at a dose of 330 mg/kg body weight. This dosage was administered in two doses with 15 min in between each dose. Supplementary injections of sodium pentobarbital (Nembutal at 5 mg/ml) were delivered to maintain a surgical level of anesthesia. Integrated electrophysiological recordings from the chorda tympani nerve were made using the techniques described by Jakinovich and Oakley (12).

Recordings from 4–10 animals (with the exception of H-8 with one trial) were obtained to evaluate the effect of a single concentration of each modulator of the adenylate cyclase system on the sweet stimuli. At each of the four trials NaCl, sucrose, and KCl were applied to the gerbil tongue followed by the series of sweet compounds with 1 min interstimulus rinses of deionized water (except forskolin for which 1% DMF was used and H-7 for which 1% ethanol was used). The stimuli were delivered in 2.0 ml samples by a gravity flow system at a rate of 0.20 ml per second. Next, the tongue was adapted for 4 min with the adenylate cyclase system modulator followed by a reapplication of the taste solutions with interstimulus rinses of the modulator.

RESULTS

Adaptation of the tongue to NaF (20 mM) for 4 min resulted in significant increases in most of the sweet responses and decreases in the controls (NaCl and KCl). There were significant increases in the responses to: 30 mM and 100 mM sucrose (30% and 23%, respectively), 300 mM glucose (65%), 300 mM fructose (62%), 150 and 300 mM maltitol (75% and 40%, respectively), 300 mM mannitol (71%), 0.88 mM and 3.5 mM dulcin (94% and 64%, respectively), and 0.55 mM and 1.1 mM stevioside (58% and 67%, respectively). NaCl (30

mM and 100 mM) were inhibited by 35% and 12%, respectively. KCl (300 mM) was also inhibited by 24% (see Fig. 2).

Adaptation of the tongue for 4 min to NaCl (20 mM) was also tested as a control on the same stimuli as NaF. Several responses were significantly suppressed by the application of NaCl for 4 min to the gerbil tongue. The suppressions were: 30 mM and 100 mM NaCl (54% and 23%, respectively), 300 mM and 500 mM KCl (48% and 35%, respectively), 300 mM glucose (40%), 300 mM maltitol (33%), 500 mM mannitol 18%), and 0.88 mM dulcin (12%). No other responses were significantly affected (see Fig. 3). Sample electrophysiological traces of taste solutions used in the experiments with NaF and NaCl are shown in Figs. 4 and 5. Each of the responses are shown before and after a 4-min application of 20 mM NaF and 20 mM NaCl to the gerbil tongue.

NaF (20 mM) and NaCl (20 mM) were also tested as dilutants for each of the stimuli. The response to the mixture when NaF and a sweetener was dissolved in the same solution did not show enhancement like that found for the adaptation experiment. The response to the mixture was less than the sum of individual responses for NaCl and the sweetener.

Application of 0.12 mM forskolin for 4 min to the tongue of the gerbil had no significant effects on any stimulus other than 150 mM maltitol with a 14% blockage (see Fig. 6).

Application of 1.16 mM 8BrcAMP for 4 min to the tongue significantly blocked the response to 300 mM KCl by 15% and enhanced responses to: 30 mM sucrose (16%), 300 mM glucose (36%), 300 mM maltitol (18%), and 6.5 mM D-tryptophan (24%). It had no significant effect on any of the other stimuli tested (see Fig. 7).

When 5 mM DBcAMP was applied to the tongue for 4 min, the mean responses to three compounds were significantly changed. 30 mM NaCl was decreased by 12% and 300 mM KCl was decreased by 17%. Response to 300 mM mannitol was enhanced by 26%. None of the remaining stimuli were significantly affected (see Fig. 8).

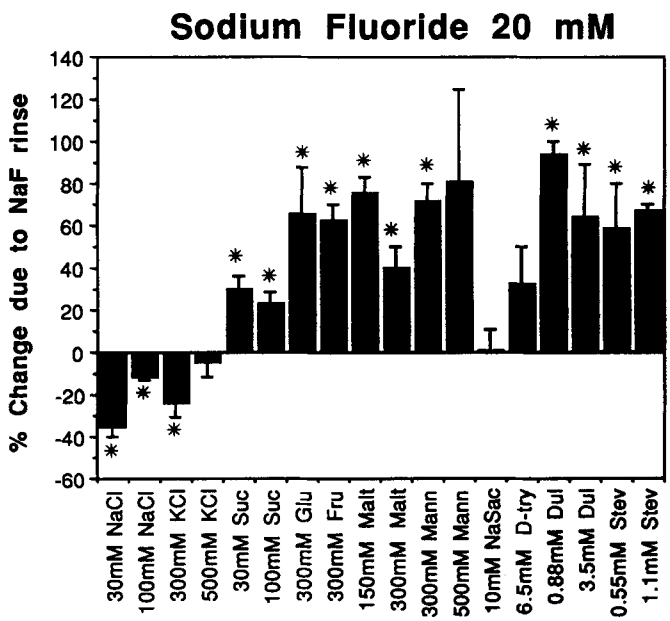

FIG. 2. Percent change in integrated chorda tympani responses after a 4-min application of 20 mM NaF. Abbreviations: NaCl, sodium chloride; KCl, potassium chloride; Suc, sucrose; Glu, glucose; Fru, fructose; Malt, maltitol; Mann, mannitol; NaSac, sodium saccharin; D-Try, D-tryptophan; Dul, dulcin; Stev, stevioside.

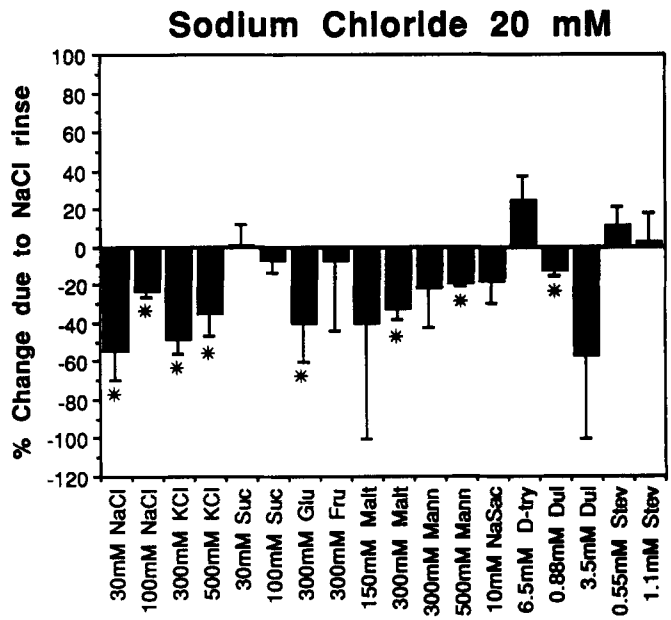

FIG. 3. Percent change in integrated chorda tympani responses after a 4-min application of 20 mM NaCl.

H-7 was applied to the tongue at 300 μM for 4 min yielding significant increases in responses to 30 mM sucrose (21%) and 1.75 mM dulcin (28%). No other stimuli were significantly affected (see Fig. 9). These results are similar to those found for H-7 in pretesting when the tongue was adapted for 30 min with H-7 rather than 4 min.

H-8 was applied at 147 μM during one trial with no consistent results.

DISCUSSION

The main finding of this study is that the intensity of some sweet taste responses can be enhanced by pretreatment of the tongue with NaF and 8 BrcAMP. Simultaneous application of sweeteners with NaF, i.e., mixtures of NaF and sweeteners, did not lead to enhancement probably because the sweet receptor was activated before NaF permeated the cell. The data obtained when the tongue was pretreated with NaF and 8 BrcAMP, however, suggest that the intensity of sweet taste is dependent on dissociation of a G-protein as well as cAMP levels inside of cells. The finding that forskolin did not enhance sweet taste responses may be due to limited permeability in the taste system of the gerbil. The suppression of responses to NaCl and KCl by both NaF and NaCl is due to adaptation that results from repeated exposure to the sodium ion.

The finding that H-7 increased the intensity of the responses to two sweet tastants was unexpected, given the actions of NaF and membrane permeable forms of cAMP. However, H-7 inhibits protein kinase C as well as protein kinase A; protein kinase C is active in another second messenger system, the phosphoinositide system (5,23). Thus, H-7 may produce increased amplitude for sucrose and dulcin through the phosphoinositide system system rather that the adenylate cyclase system, an effect that requires further study.

It is unlikely that enhancement of the sweet responses

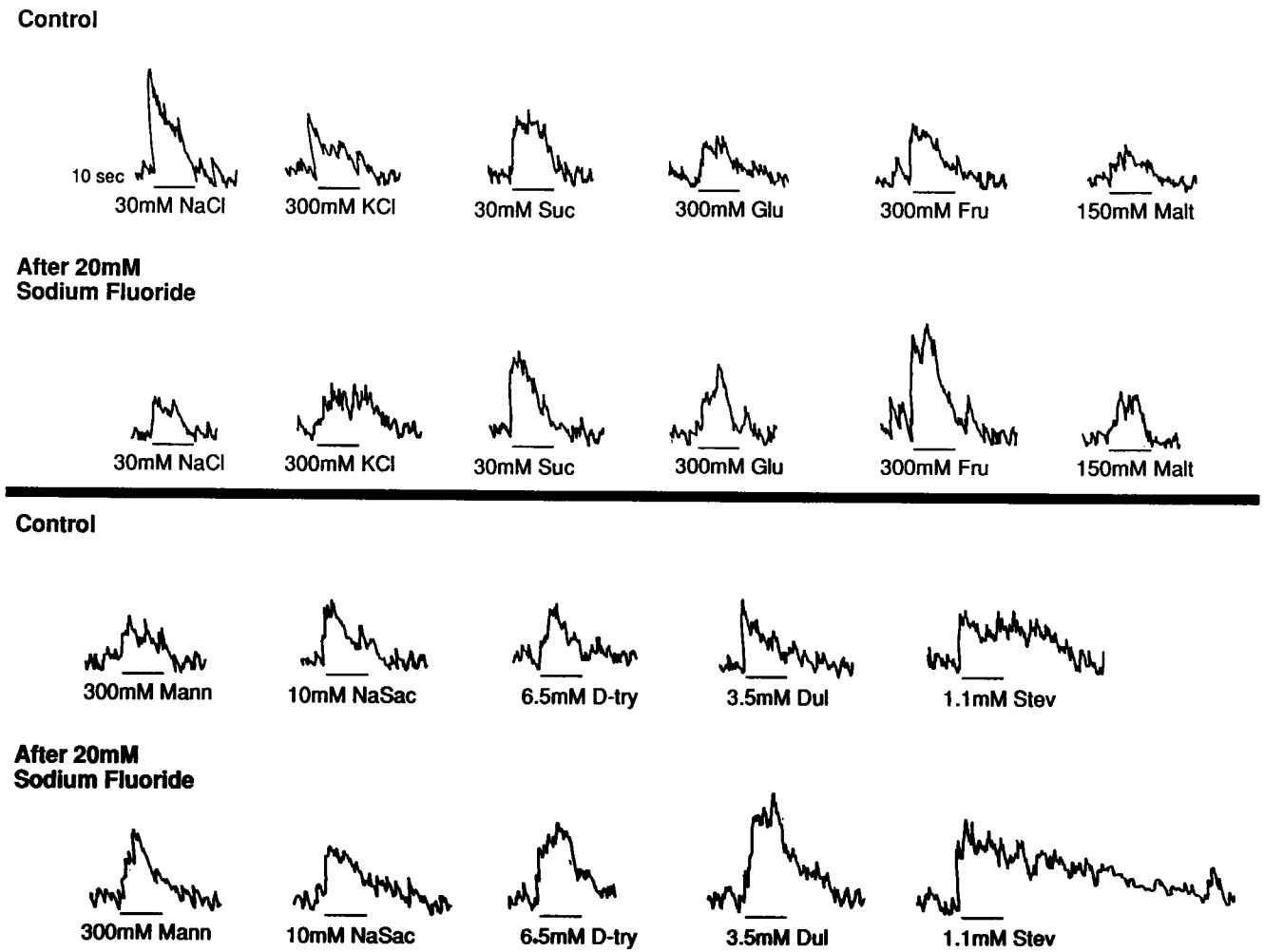

FIG. 4. Sample integrated chorda tympani responses before and after a 4-min rinse of 20 mM NaF. Abbreviations: NaCl, sodium chloride; KCl, potassium chloride; Suc, sucrose; Glu, glucose; Fru, fructose; Malt, maltitol; Mann, mannitol; NaSac, sodium saccharin; D-Try, D-tryptophan; Dul, dulcin; Stev, stevioside.

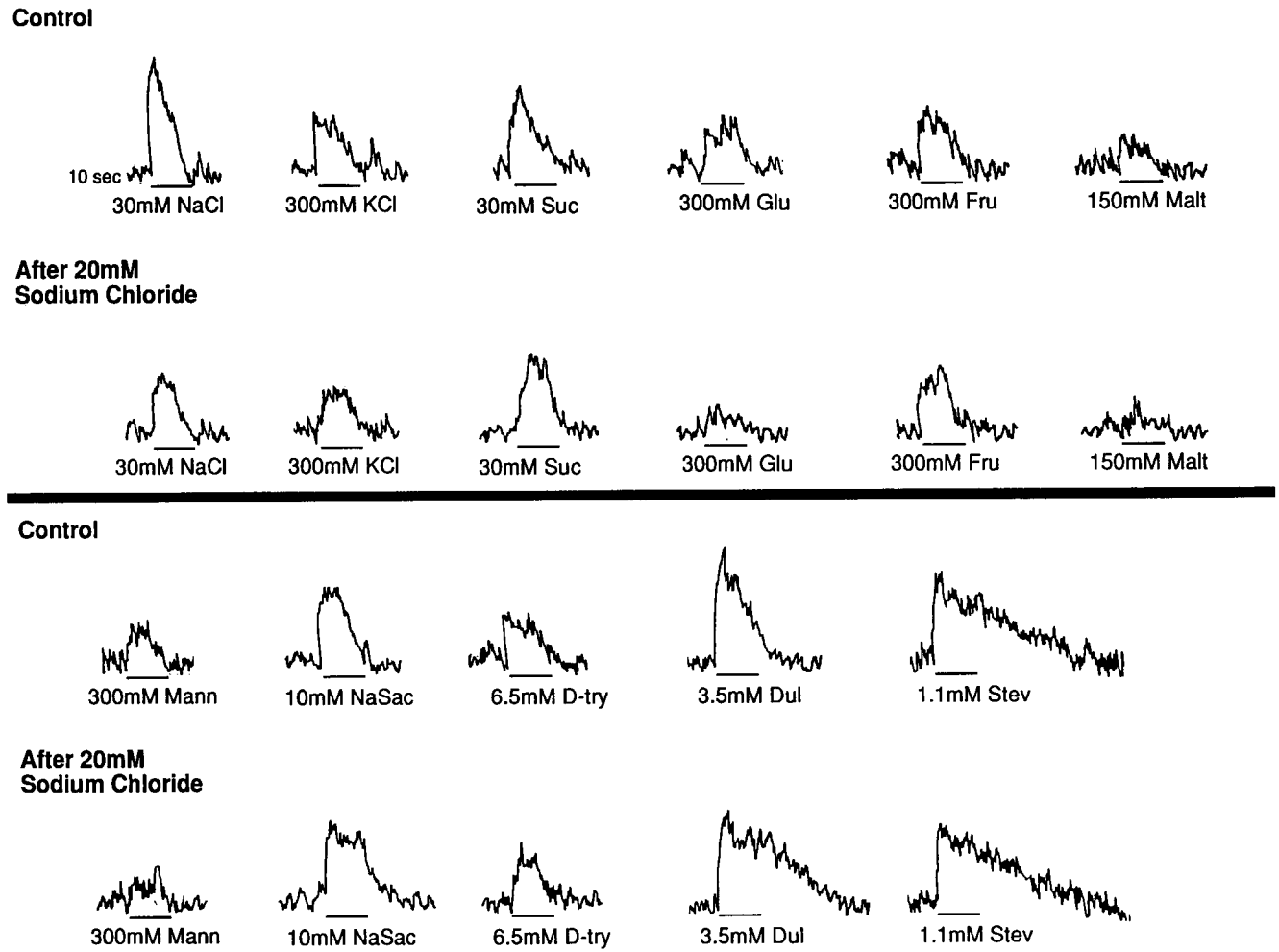

FIG. 5. Sample integrated chorda tympani responses before and after a 4-min rinse of 20 mM NaCl.

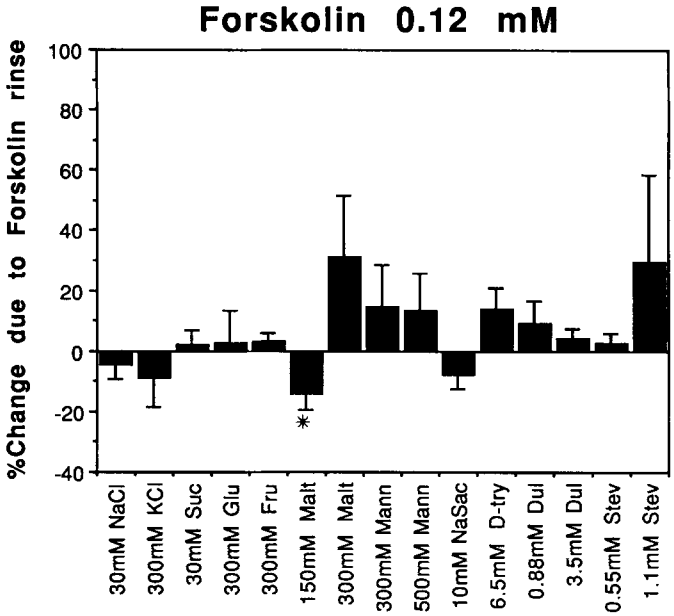

FIG. 6. Percent change in integrated chorda tympani responses after a 4-min application of 0.12 mM forskolin.

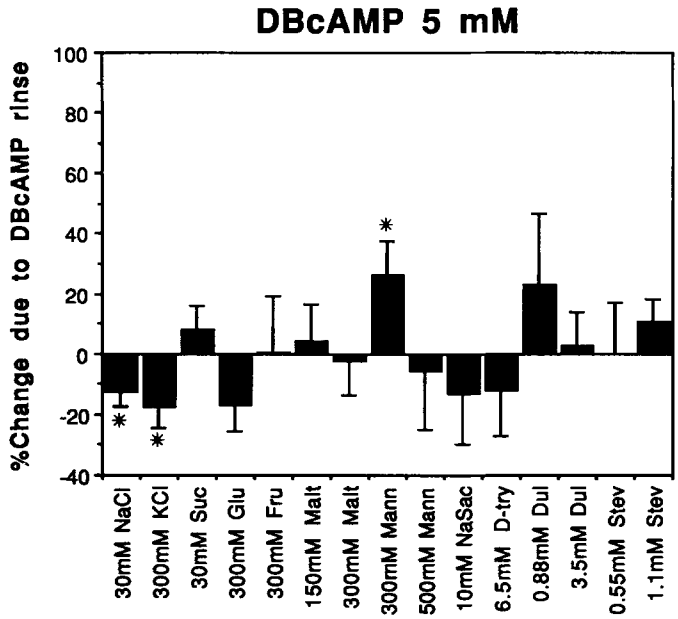

FIG. 8. Percent change in integrated chorda tympani responses after a 4-min application of 5 mM DBcAMP.

found by NaF is due to activation of the novel G protein named gustducin. This G protein, which has a novel α subunit unique to the taste system (18), resembles the G proteins in rod and cone photoreceptor cells called transducins. In the retina, transducin promotes activation of cGMP phosphodiesterase (cGMP-PDE) (19,20) which leads to breakdown of cGMP and closure of cyclic nucleotide gated channels (10,32). McLaughlin et al. (18) suggested that gustducin acts in a manner similar to transducin by activating taste cell PDE. Taste tissue has previously been found to contain high levels of cAMP PDE (14,16,22). If gustducin binds to an inhibitory subunit of taste cell cAMP-PDE, the enzyme would be activated and decrease intracellular cAMP levels. Thus, activation of gustducin would be expected to inhibit sweet responses, which is opposite of that found with NaF. In addition, it is unlikely that gustducin activates adenylate cyclase since trans-

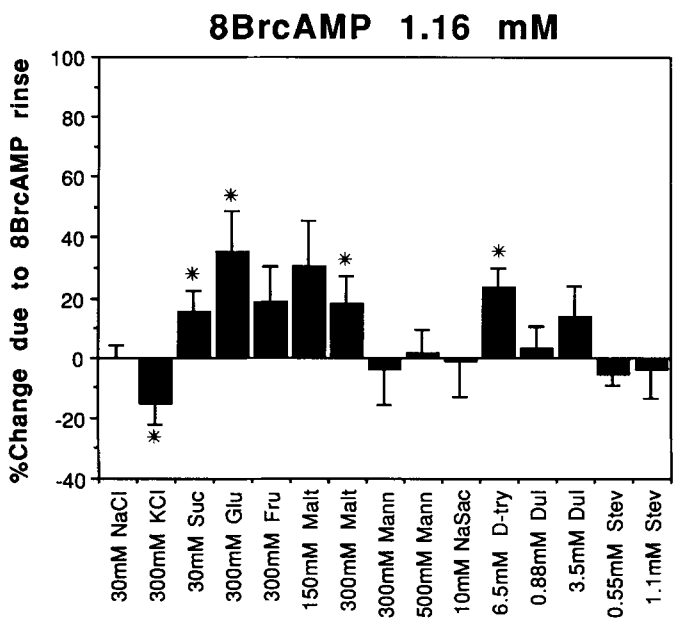

FIG. 7. Percent change in integrated chorda tympani responses after a 4-min application of 1.16 mM 8BrcAMP.

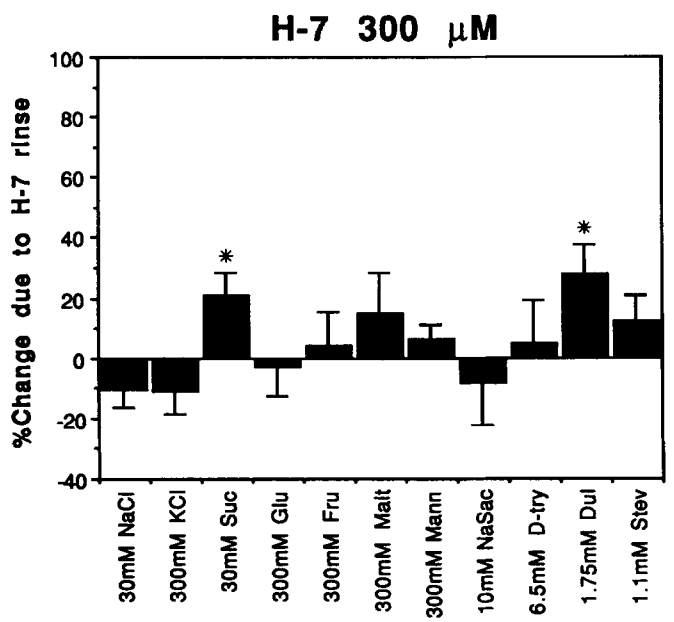

FIG. 9. Percent change in integrated chorda tympani responses after a 4-min application of 300 μM H-7.

ducin does not. The bulk of biochemical (27,28) and electrophysiological (1,2,31) evidence suggests that the G protein involved in sweet taste is the G_s type that activates adenylate cyclase.

The data found in the present study lend support for the adenylate cyclase system as a transduction system for sweet taste because modulators of this system modify sweet taste responses. A further implication may be that the receptors for sweet taste are seven-hydrophobic-domain polypeptides, and each subunit of the receptor carries a G protein recognition sequence on its intracellular face. Three structural types of membrane-spanning receptors have been identified for signal transduction: channel-enclosing oligomers, seven-hydrophobic-domain polypeptides, and single-hydrophobic-domain polypeptides (4). Only seven-hydrophobic-domain polypeptides utilize G proteins in the transduction process.

Use of modulators may ultimately be useful in amplifying taste for persons with hypogeusia. Although NaF and 8BrcAMP are not appropriate for this purpose, food grade modulators may ultimately be developed that will be helpful in compensating for taste losses that occur with age, diseases, and pharmaceutical interventions.

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