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# Effect of Lipid-Derived Second Messengers on Electrophysiological Taste Responses in the Gerbil

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SCHIFFMAN, S. S., M. S. SUGGS, M. L. LOSEE, L. A. GATLIN, W. C. STAGNER AND R. M. BELL. *Effect of lipid-derived second messengers on electrophysiological taste responses in the gerbil.* PHARMACOL BIOCHEM BEHAV 52(1) 49–58, 1995. – Integrated chorda tympani (CT) recordings were made to salty, sour, sweet, bitter, and glutamate tastants before and after a 4-min application of modulators of lipid-derived second messenger systems. The modulators included two membrane-permeable analogues of DAG, 1-oleoyl-2-acetyl glycerol (OAG) and dioctanoyl glycerol (DiC8); thapsigargin, which releases $Ca^{++}$ from intracellular stores; ionomycin, a calcium ionophore; lanthanum chloride, an inorganic calcium channel blocker; nifedipine, a dihydropyridine calcium channel blocker; quinacrine diHCl, a phospholipase $A_2$ antagonist; melittin, a phospholipase $A_2$ agonist; and indomethacin, which decreases the release of prostaglandins by inhibiting the enzyme cyclo-oxygenase. The main findings were: OAG (125 μM) and DiC8 (100 μM) blocked the responses of several bitter compounds while enhancing the taste response to several sweeteners. Lanthanum chloride blocked all responses, which may be due to the fact that it blocks tight junctions. Quinacrine (1 mM) suppressed several bitter responses while enhancing the response to several sweeteners. The enhancement of sweet taste responses by DAG analogues suggests that there is cross-talk between the adenylate cyclase system and one (or more) pathways involving lipid-derived second messengers in taste cells.

Taste Second messengers Phosphotidylinositol system Arachidonic acid system Cross-talk Diacylglycerol

A VARIETY OF mechanisms have been shown to play a role in taste transduction including sodium channels, potassium channels, and two second messenger systems, the adenylate cyclase system and the phosphatidylinositol system [see (1, 11,23,38,39,49,62,72) for reviews]. The influx of $Na^+$ ions through amiloride-sensitive sodium channels in taste cell membranes is involved in transduction of sodium salts (5,6,18, 19,28,29,31,64,67,69). Diffusion of anions across tight junctions through paracellular pathways modulates taste responses to cations (63,73,87). Sour taste can result from blockage of amiloride-sensitive $Na^+$ channels in some (24,25) but not all (40,67,82) species. Alteration of potassium conductance is implicated in the tastes of KCl (36,37,68) as well the taste qualities of sweet (4,8,30,84), sour (40,82), and bitter (41,77,78).

The adenylate cyclase system has been implicated in both sweet (4,43,52,80,81,84) and bitter (10,58) taste transduction. Modulation of the adenylate cyclase system using membrane-permeable forms of the intracellular second messenger cAMP suppressed certain bitter tastes and enhanced some sweet tastes in gerbil (65,66,70). Amiloride-sensitive sodium channels (19,67,74) and receptor-independent activation of G-proteins (53) also appear to play a role in sweet taste transduction. Numerous mechanisms in addition to the adenylate cyclase system play a role in bitter taste transduction including

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activation of the phosphatidylinositol system, causing production of $IP_3$ (32,76,77); activation of phophodiesterase by a G-protein called gustducin, resulting in activation of phosphodiesterase, decreased cAMP concentration, and hyperpolarization of taste cells (49,50); direct closure of $K^+$ channels by bitter-tasting compounds (39,77,78); modification of sodium channels (14,27); and receptor-independent activation of G-proteins (53).

Biochemical investigations have firmly established the existence and activation of components of the adenylate system in taste buds including adenylate cyclase (43,52,55,81), cAMP phosphodiesterase (46,55), cAMP (80), and cAMP-dependent kinase (4). Certain sweeteners including sucrose and saccharin cause a stimulation in adenylate cyclase activity leading to elevated levels of cAMP (80). Certain bitter compounds may decrease levels of intracellular cAMP by activation of cAMP phosphodiesterase through a novel G-protein called gustducin (49,50).

Transduction of extracellular signals across plasma membranes in numerous cell types can also be induced by pathways involving a variety of lipid-derived second messengers produced from membrane phospholipids (16,17,21,34,47,54). The best understood of the signal-activated phospholipase pathways is the phosphatidylinositol system. In the phosphatidylinositol system, a ligand binds to a receptor, activates a G-protein or a tyrosine kinase, which subsequently activate specific isoforms of phospholipase C [see (7,59) for reviews], leading to the hydrolysis of the phospholipid phosphatidylinositol 4,5-biphosphate ($PIP_2$). The hydrolysis of $PIP_2$ generates two products, the sugar phosphate inositol-triphosphate $IP_3$ and diacylglycerol (DAG). $IP_3$ and DAG influence intracellular functions that can lead to the subsequent depolarization of a cell. $IP_3$ binds to a receptor on the endoplasmic reticulum that triggers the mobilization of calcium from intracellular stores. Calcium together with DAG activates protein kinase C, and protein kinase C phosphorylates key proteins that regulate the response of the target cell to the ligand. In addition, calcium may activate other cellular enzymes as well as ion channels. DAG can also be derived from membrane phospholipids other than $PIP_2$. DAG can be produced directly from phosphatidylcholine (PC) by phospholipase C or produced indirectly from PC via another phospholipase called phospholipase D. Calcium mobilization occurs in the phosphatidylinositol system but not in PC pathways.

The finding that the bitter compound denatonium leads to the release of $Ca^{2+}$ from intracellular stores (2) suggests a role for pathways involving $PIP_2$-derived messengers in bitter taste transduction. Biochemical studies indicate that the phosphatidylinositol system is involved taste transduction in rat circumvallate papillae because the bitter compounds denatonium, sucrose octaacetate, and strychnine stimulate increases in $IP_3$ levels in taste cells (32,76,77).

Arachidonic acid is another messenger derived from phospholipids by activation of phospholipases involving G-proteins and other mechanisms (17). Little is known about its potential role in taste transduction. In this system, a ligand-receptor complex is coupled to phospholipase $A_2$ activation, thereby generating arachidonic acid and its metabolites that act as messengers [see (17)]. Phospholipase $A_2$ is a protein that directly releases arachidonic acid from a variety of phospholipids including phophatidylcholine, phosphatidylethanolamine, phosphatidylinositides, phosphatidylserine, and phosphatidic acid. Arachidonic acid can also be generated by phospholipase C, which yields arachidonyldiglyceride from phosphoinoside or phosphatidylcholine. Diglyceride lipase liberates arachidonate from the diglyceride.

Arachidonate liberated by phospholipase $A_2$ elicits effects commonly associated with phopholipase C activation including activation of protein kinase C and elevation of cytosolic calcium levels. Arachidonic acid metabolites including prostaglandins (PGs), leukotrienes (LTs), and hydroxyeicosatethraenoic acids (HETEs) can serve as intercellular messengers.

The purpose of the present study was to determine if modulators of signal-activated phospholipase pathways, including DAG analogues, alter electrophysiological responses to taste stimuli in the gerbil. The results suggest that DAG analogues clearly alter some taste responses. However, more research is necessary to specifically identify which of the signal-activated phopholipases and membrane lipids is involved in taste perception.

## 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 45–65 g (avg. 50 g).

### *Stimuli*

Six modulators of the phosphoinositide system were tested for their effect on bitter taste. The compounds tested were: 1-oleoyl-2-acetyl glycerol (OAG, $C_{23}H_{42}O_5$) at 125 $\mu$ (15,35); 1,2-dioctanoyl glycerol (C8:0) (DiC8, $C_{19}H_{36}O_5$) at 100 $\mu$M (45); thapsigargin ($C_{34}H_{50}O_{12}$) at 6 $\mu$M (83); ionomycin ($C_{41}H_{70}O_9Ca$) at 7 $\mu$M (57); lanthanum chloride ($LaCl_3 \cdot 7H_20$) at 0.01, 0.1, 1, and 10 mM (61); and nifedipine ($C_{17}H_{18}N_2O_6$) at 10 and 100 $\mu$M (61). OAG and DiC8 were dissolved in 0.8% ethanol; thapsigargin in 0.10% dimethyl sulfoxide; ionomycin in 0.20% dimethyl sulfoxide; lanthanum chloride in $diH_20$; and nifedipine (10 and 100 $\mu$M) in 0.20% and 0.40% DMF (*N,N*-dimethyl formamide), respectively. The pH of the solutions ranged from 6.0 to 7.0.

Four modulators of the arachidonic acid pathway were tested for their effect on bitter taste. The compounds tested were: quinacrine diHCl ($C_{23}H_{30}ClN_3O \cdot 2HCl$) at 1 mM (79); melittin ($C_{131}H_{229}N_{39}O_{31}$) at 70 $\mu$M (13); indomethacin ($C_{19}H_{16}ClNO_4$) at 10 $\mu$M (56); and arachidonic acid ($C_{20}H_{32}O_2$) at 500 $\mu$M. Quinacrine diHCl and melittin were dissolved in $diH_2O$, whereas indomethacin and arachidonic acid were both dissolved in 1% ethanol.

The effect of these modulators on 31 taste solutions with salty, sweet, sour, and bitter tastes were examined. The compounds with salty or sour qualities were: NaCl (30 and 100 mM), KCl (300 and 500 mM), $NH_4Cl$ (100 mM), monosodium glutamate (MSG) (50 and 100 mM), and HCl (5 and 10 mM). The sweet compounds tested were: sucrose (30 and 100 mM), glucose (300 mM), fructose (300 mM), maltitol (150 and 300 mM), mannitol (300 and 500 mM), sodium saccharin (10 mM), D-tryptophan (6.5 mM), dulcin (0.88 and 3.5 mM), and stevioside (0.55 and 1.1 mM). The compounds with a bitter component were: quinine HCl (30 mM), $MgCl_2$ (30 and 100 mM), $CaCl_2$ (30, 100, and 300 mM), erythromycin (0.7 mM), and urea (2 M). All solutions were tested at room temperature.

### *Experimental Procedure*

Gerbils were anesthetized with an IP injection of ketamine HCl (Ketalar at 50 mg/ml) at a dose of 330 mg/kg body weight. This dosage was administered in two doses with 15 min inbetween each dose. Supplementary injections of sodium pentobarbital (nembutal at 5 mg/ml) were delivered to main-

tain a surgical level of anesthesia. Integrated electrophysiological recordings from the chorda tympani nerve were made using the techniques described by Jakinovich and Oakley (33).

Due to the large number of taste stimuli to be evaluated, the experiment was divided into two parts. At each trial in both parts, NaCl, KCl, and sucrose were applied to the gerbil tongue followed by a series of taste solutions with a 1-min interstimulus rinse of the same concentration of solvent used to dissolve each compound. In part 1 the taste series consisted of $NH_4Cl$, MSG, HCl, quinine HCl, $MgCl_2$, $CaCl_2$, erythromycin, and urea. In part 2, the series of taste solutions consisted of all of the sweet compounds including sucrose, glucose, fructose, maltitol, mannitol, sodium saccharin, D-tryptophan, dulcin, and stevioside. In some instance, the number of solutions in a taste series did not include all of the stimuli used in that part (1 or 2) due to the prohibitive cost for running each of the concentrations for some of the modulators. 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 with a modulator of the phosphatidylinositol system or the arachidonic acid system modulator followed by a reapplication of the taste solutions with interstimulus rinses of the compounds.

Recordings were obtained from a minimum of four and a maximum of 10 animals to evaluate the effect of a single concentration of each modulator of the phosphatidylinositol system and the arachidonic acid system.

## RESULTS

### *Phosphotidyl Inositol System*

*Analogues of DAG.* OAG, a membrane-permeable analogue of DAG, permeates whole cells within 1 min. Application of 125 μM OAG to the gerbil tongue for 4 min during one trial produced several significant changes in the responses to taste stimuli. There were significant increases in the responses to 300 mM fructose (21.16%), 300 mM maltitol (37.70%), and 0.55 mM stevioside (18.15%). OAG also significantly blocked the responses to 30 $MgCl_2$ (28.75%) and 2 M urea (40.35%) (Fig. 1). DiC8 (C8:0), another analogue of DAG, was applied at 100 μM, resulting in enhancement of most sweet responses and blockage of most bitter responses. Increases in response were seen for 300 mM fructose (15.98%), 150 and 300 mM maltitol (7.63% and 8.61%, respectively), 10 mM sodium saccharin (3.19%), 0.88 and 3.5 mM dulcin (6.55% and 9.78%, respectively), and 0.55 mM stevioside (8.89%). NaCl (30 mM) was slightly increased by 7.35%. DiC8 also decreased the responses to 500 mM KCl (17.04%), 100 mM $NH_4Cl$ (18.02%), 5 mM HCl (17.50%), 30 mM QHCl (20.22%), 100 mM $MgCl_2$ (15.97%), 300 mM $CaCl_2$ (8.86%), and 0.7 mM erythromycin (50.67%) (Fig. 2).

*Alteration of intracellular calcium levels.* Thapsigargin releases $Ca^{++}$ from intracellular stores. Application of 6 μM thapsigargin to the gerbil tongue for 4 min resulted in a reduction in response to 300 mM glucose (22.97%), 300 mM maltitol (28.47%), 30 mM QHCl (33.65%), 30 mM $MgCl_2$ (23.56%), and 0.7 mM erythromycin (30.06%) (Fig. 3).

Ionomycin, a calcium ionophore, was tested at 7 μM with only a slight effect on the taste responses. $MgCl_2$ (30 mM) was slighty enhanced by 13.73%. There was a reduction in the response to 30 mM NaCl (10.28%), 30 mM maltitol (22.22%), 10 mM sodium saccharin (14.28%), and 2 M urea (26.25%) (Fig. 4).

The inorganic calcium channel blocker lanthanum chloride was applied at four different concentrations for 4 min to the gerbil tongue. Application of 0.01 mM lanthanum chloride resulted in significant changes to several of the taste responses. There was an increase in response to 300 mM KCl (21.71%). A significant blockage in response was obtained with 300 mM fructose (23.33%), 10 mM sodium saccharin (29.32%), 50 and 100 mM MSG (11.03% and 12.05%, respectively), 30 mM $MgCl_2$ (10.63%), 30 and 300 mM CaCl2 (8.33% and 10.37%,

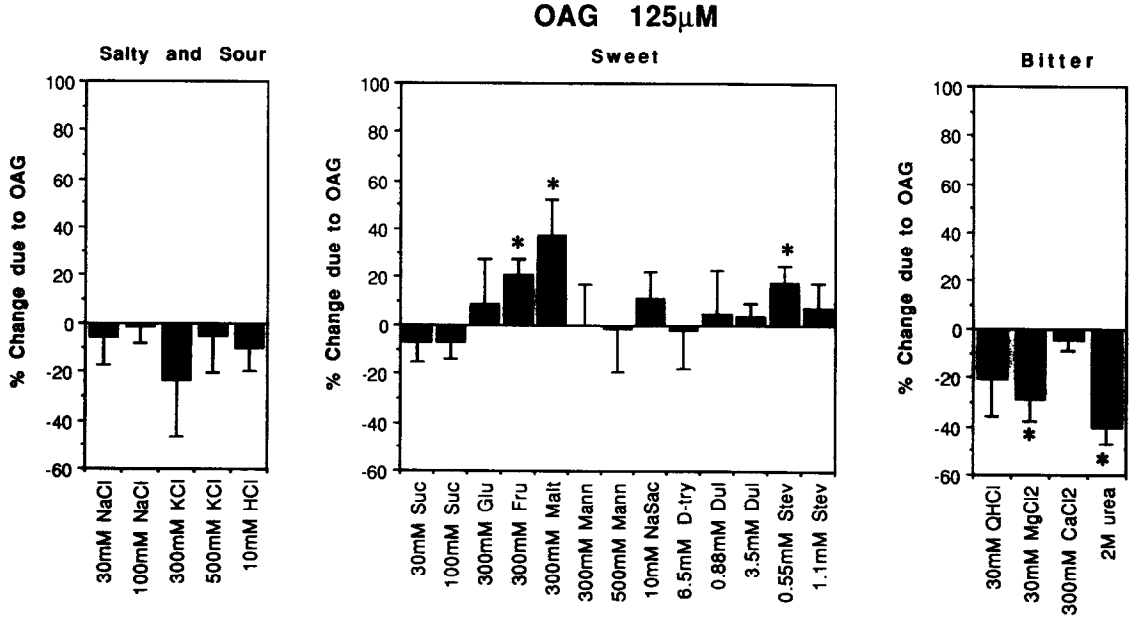


FIG. 1. Percent change in integrated chorda tympani responses after a 4-min application of 125 μM OAG. Abbreviations: NaCl, sodium chloride; KCl, potassium chloride; Suc, sucrose; Glu, glucose; Fru, fructose; Malt, maltitol; Mann, mannitol; NaSac, sodium saccharin; D-try, D-tryptophan; Dul, dulcin; Stev, stevioside; HCl, hydrochloric acid; QHCl, quinine hydrochloride; $MgCl_2$, magnesium chloride; $CaCl_2$, calcium chloride; Urea, urea.

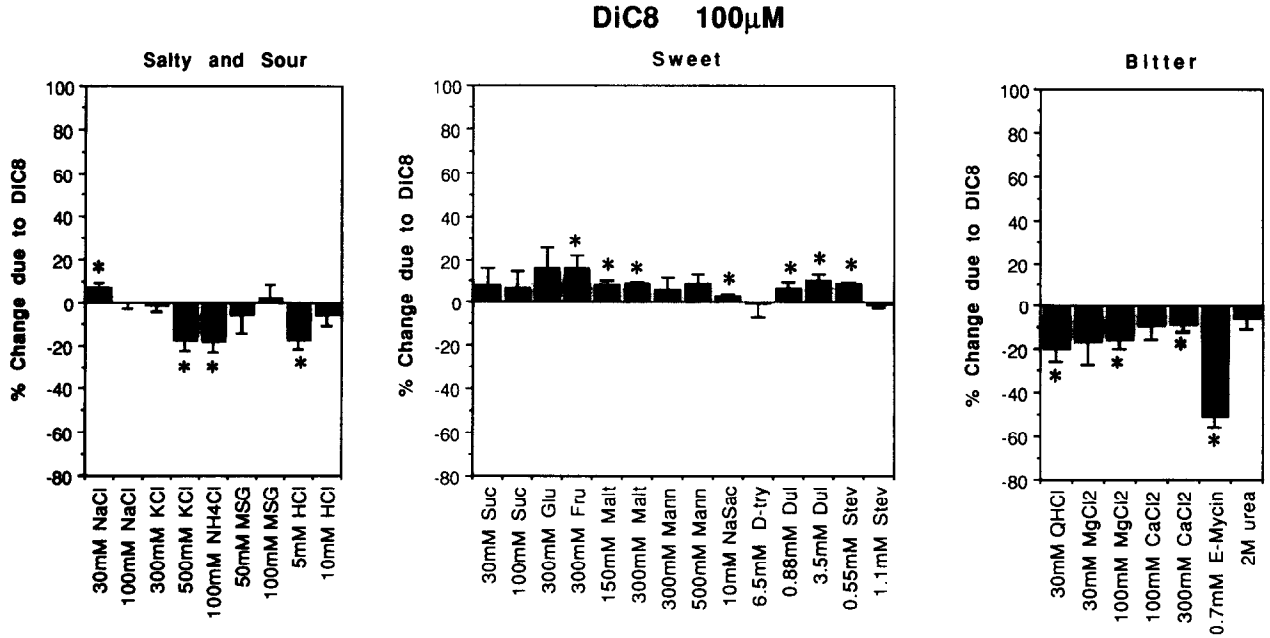


FIG. 2. Percent change in integrated chorda tympani responses after a 4-min application of 100 μM DiC8. Abbreviations: NaCl, sodium chloride; KCl, potassium chloride; Suc, sucrose; Glu, glucose; Fru, fructose; Malt, maltitol; Mann, mannitol; NaSac, sodium saccharin; D-try, D-tryptophan; Dul, dulcin; Stev, stevioside; $NH_4Cl$, ammonium chloride; MSG, monosodium glutamate; HCl, hydrochloric acid; QHCl, quinine hydrochloride; $MgCl_2$, magnesium chloride; $CaCl_2$, calcium chloride; E-Mycin, erythromycin; Urea, urea.

respectively). All other responses were not significantly affected (Fig 5). Lanthanum chloride was also applied to the gerbil tongue for 4 min at 0.10, 1.0, and 10 mM, resulting in a significantly higher rate of blockage to most of the taste responses. The overall average reduction in taste responses for 0.01, 0.1, 1.0, and 10 mM lanthanum chloride were 13.97%, 35.17%, 42.03%, and 70%, respectively (Fig. 5).

Nifedipine, a dihydropyridine calcium channel blocker, was tested at 10 and 100 μM. Application of 10 μM nifedipine for 4 min to the gerbil tongue resulted in an increase in response to 100 mM $NaCl_2$ (13.93%), 50 mM MSG (38.67%), and 100 mM $MgCl_2$ (6.02%). There were reductions in responses to 500 mM mannitol (9.77%), 3.5 mM dulcin (4.53%), and 100 mM $CaCl_2$ (23.33%). No other taste re-

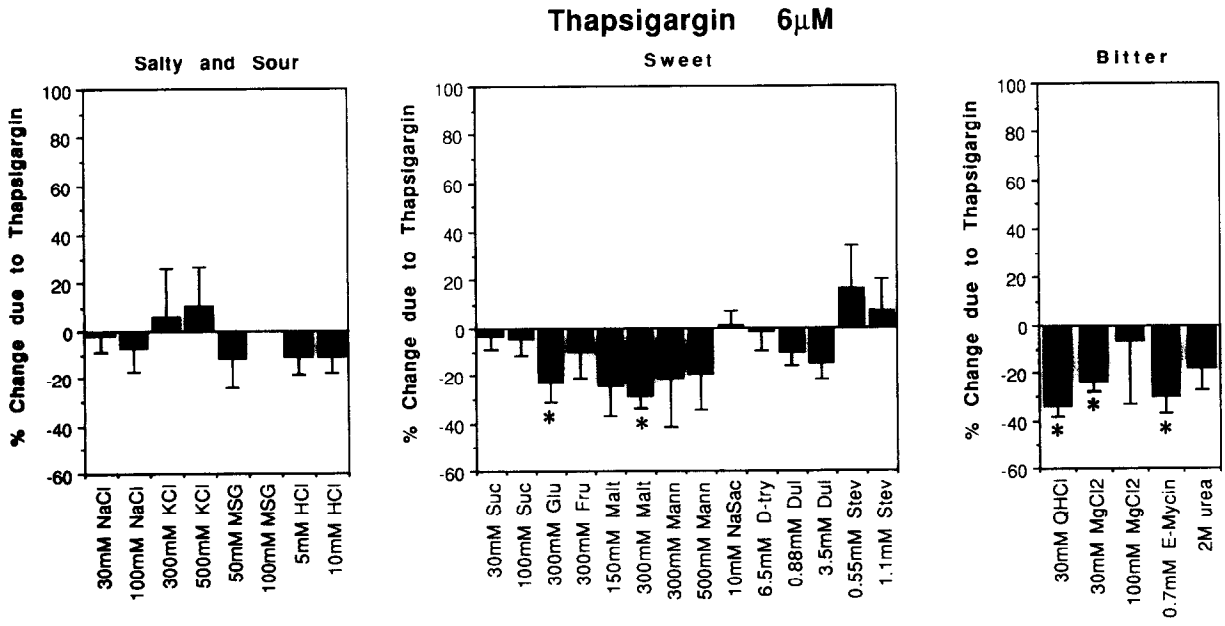


FIG. 3. Percent change in integrated chorda tympani responses after a 4-min application of 6 μM thapsigargin.

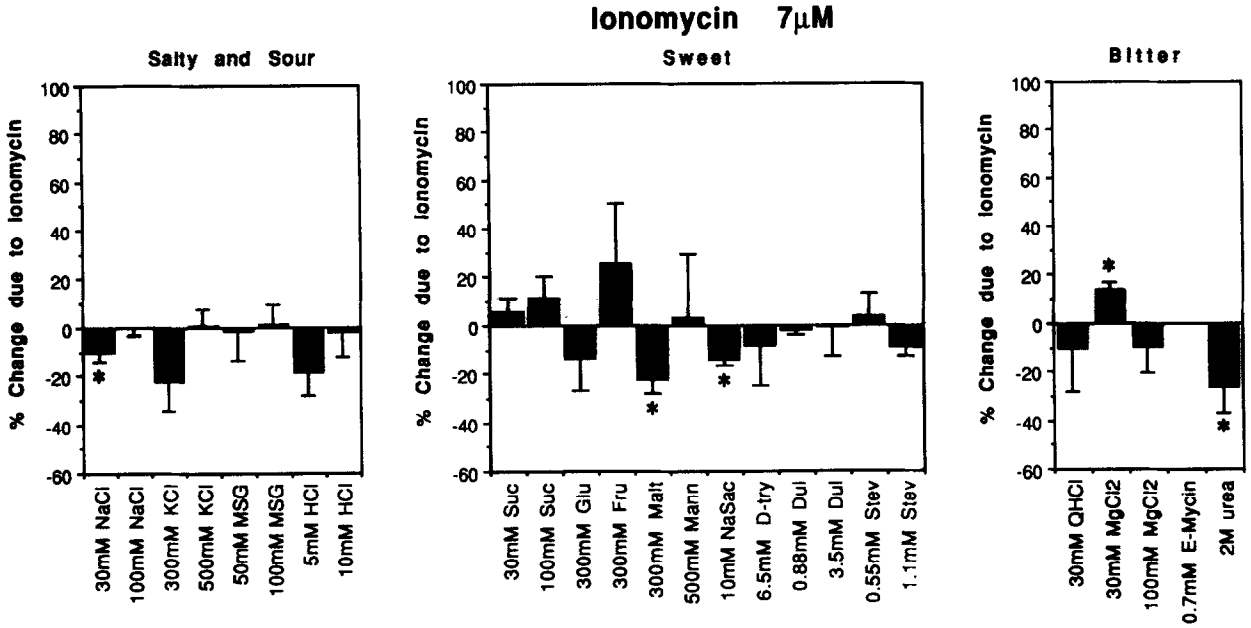


FIG. 4. Percent change in integrated chorda tympani responses after a 4-min application of 7 μM ionomycin.

sponses were significantly affected (Fig. 6). Nifedipine was also applied at 100 μM with only a slight effect on four of the responses. There were reductions in responses to: 300 mM fructose (12.96%), 100 mM MSG (12.78%), 30 mM QHCl (22.9%), and 300 mM $CaCl_2$ (14.14%). No other taste solutions were significantly affected.

*Arachidonic Acid System*

*Antagonist of phospholipase $A_2$.* Quinacrine diHCl, also known as mepacrine, is a drug used to treat malaria. It has also been shown to be a phospholipase $A_2$ antagonist. Application of 1 mM quinacrine diHCl to the gerbil tongue for 4 min had a significant effect on several of the taste stimuli. Several responses were blocked by the application of 1 mM quinacrine diHCl including 30 mM NaCl (17.18%), 500 mM KCl (25.82%), 0.88 mM dulcin (18.95%), 5 mM HCl (42.64%), 30 mM QHCl (50.38%), 100 mM $MgCl_2$ (38.4%), and 300 mM $CaCl_2$ (33.47%). Quinacrine (1 mM) also enhanced the responses to 10 mM sodium saccharin (22.9%) and 1.1 mM stevioside (17.34%) (Fig. 7).

Melittin is a toxin from bee venom that can form ion chan-

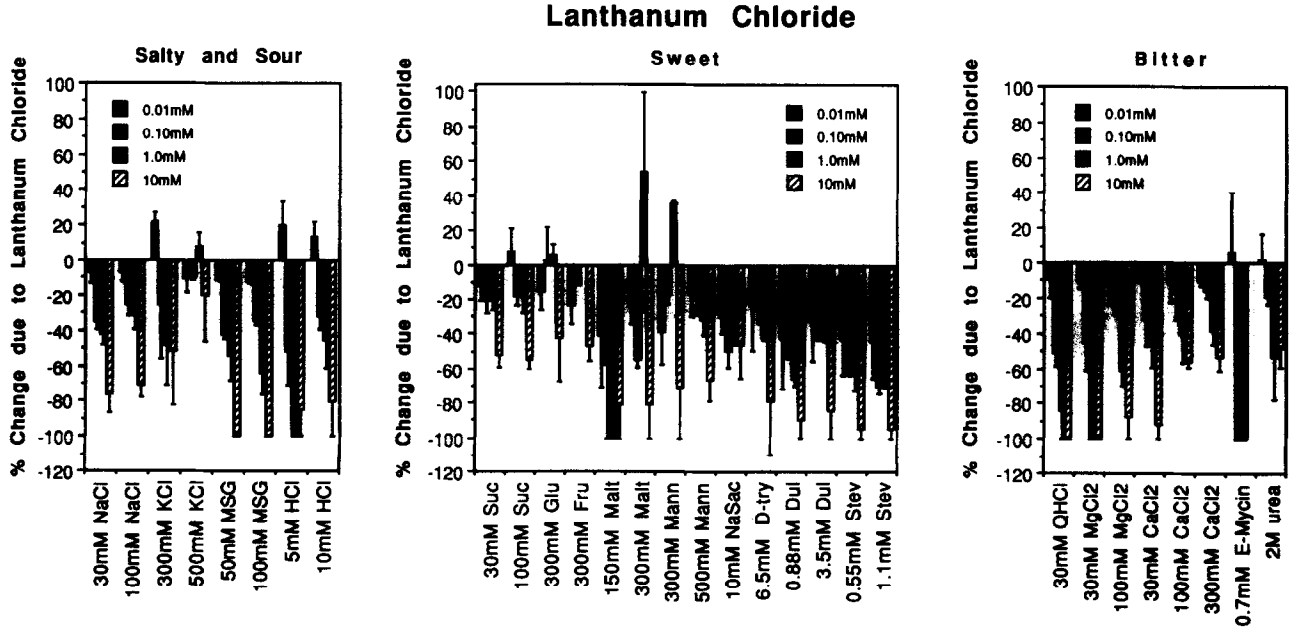


FIG. 5. Percent change in integrated chorda tympani responses after a 4-min application of 0.01, 0.10, 1, and 10 mM lanthanum chloride.

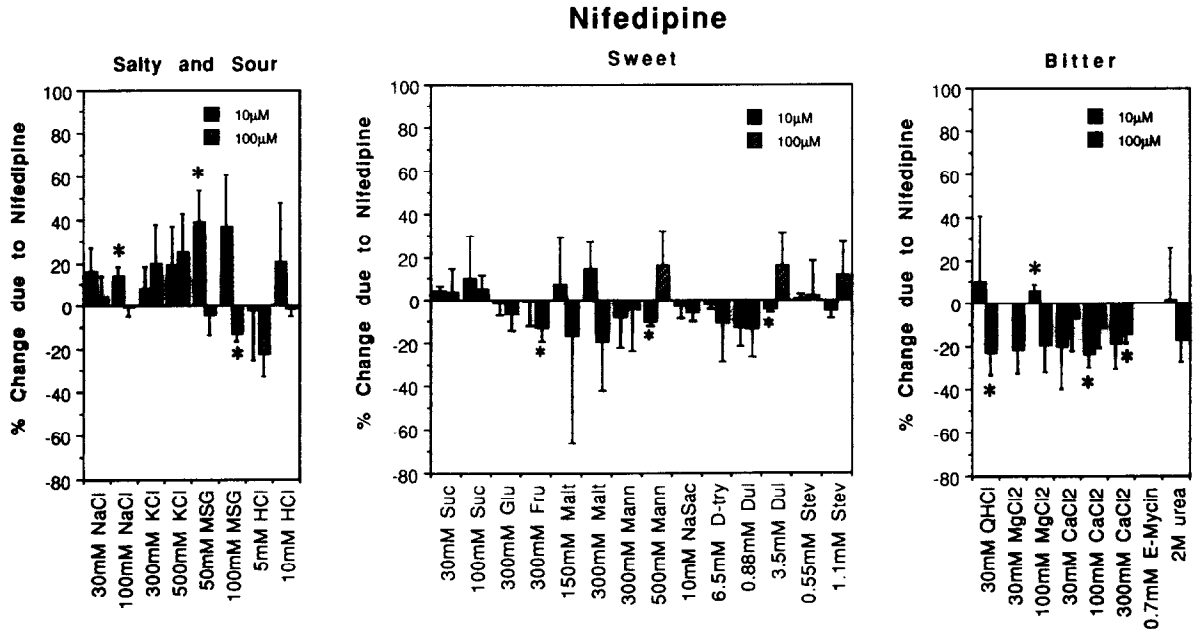


FIG. 6. Percent change in integrated chorda tympani responses after a 4-min application of 10 and 100 μM nifedipine.

nels in biological membranes. Application of 70 μM melittin to the gerbil tongue resulted in significant reductions in all of the taste responses tested except for 300 and 500 mM KCl, 300 mM fructose, and 300 mM mannitol. Reduction is responses seen were 30 and 100 mM NaCl (65.45% and 59.58%, respectively), 30 and 100 mM sucrose (53.95% and 57.21%, respectively), 150 and 300 mM maltitol (39.23% and 100%, respectively), 500 mM mannitol (36.54%), 10 mM sodium saccharin (65.62%), 6.5 mM D-tryptophan (71.43%), 0.88 and 3.5 mM dulcin (65.58% and 56.85%, respectively), 0.55 and 1.1 mM stevioside (75% and 72.92%, respectively), 100 mM $NH_4Cl$ (43.7%), 50 and 100 mM MSG (67.11% and 63.16%, respectively), 5 and 10 mM HCl (53.03% and 43.75%, respectively), 30 mM QHCl (80%), 100 mM $MgCl_2$ (61.32%), 300 mM $CaCl_2$ (30.67%), and 2 M urea (78.57%) (Fig. 8). The average reduction for all taste responses was 50.26%.

Application of 10 μM indomethacin to the tongue for 4 min resulted in the reduction in the response to 300 mM glucose (11.67%) and 2 M urea (37.65%). Indomethacin also increased the responses to 3.5 mM dulcin (7.64%), 0.55 mM stevioside (11.82%), and 300 mM $CaCl_2$ (7.34%) (Fig. 9).

Arachidonic acid (500 μM) was also applied to the gerbil

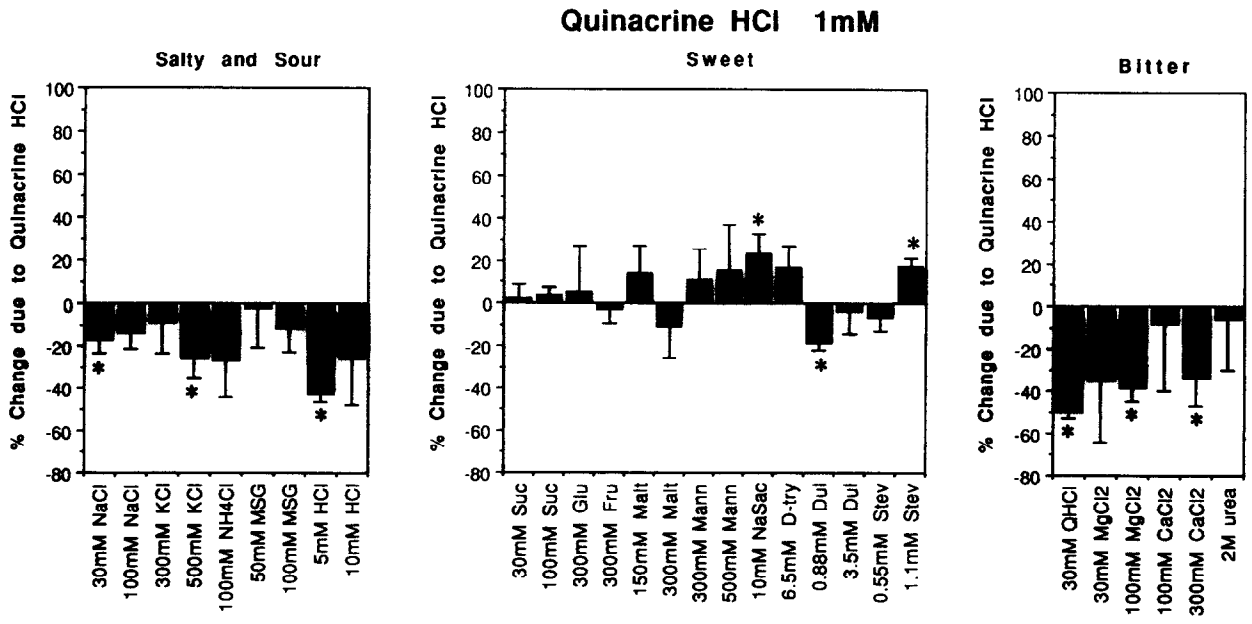


FIG. 7. Percent change in integrated chorda tympani responses after a 4-min application of 1 mM quinacrine diHCl.

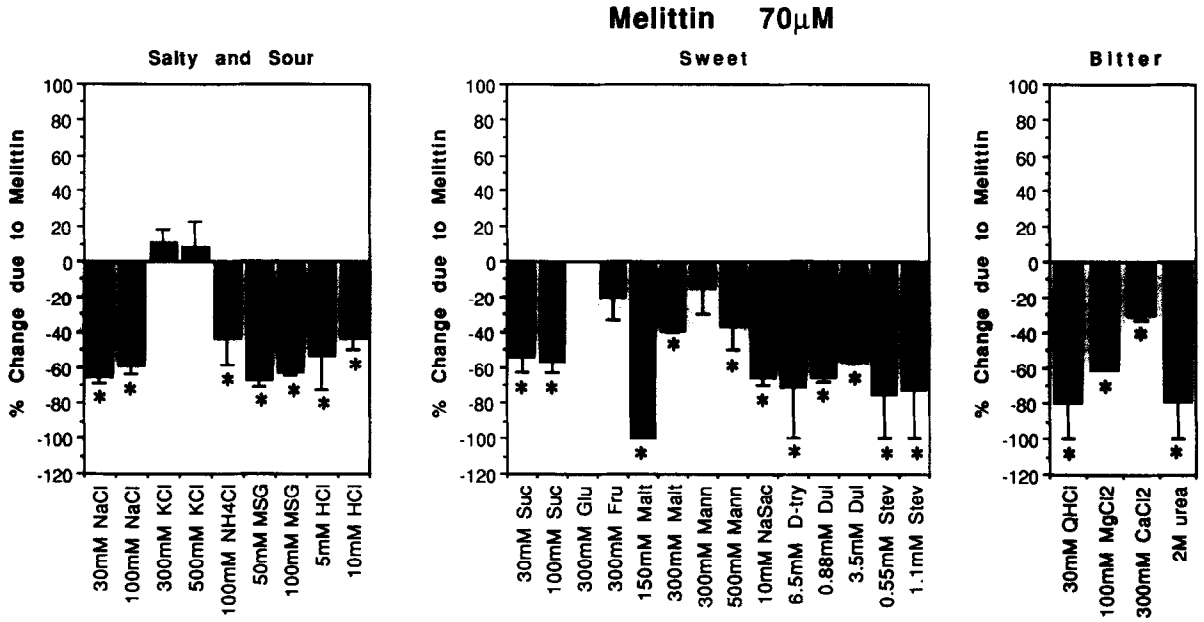


FIG. 8. Percent change in integrated chorda tympani responses after a 4-min application of 70 μM melittin.

tongue for 4 min to assess its effect on the taste responses. The only responses affected were 30 mM sucrose, which showed a 11.94% increase in response, and 100 mM MSG with a 7.88% blockage in response (Fig. 10).

## DISCUSSION

The main finding of this study is that many bitter and sweet taste responses can be modulated by analogues of the second messenger diacylglycerol (DAG). Cell-permeable forms of DAG tended to increase responses to sweet stimuli and decrease responses to bitter stimuli. OAG (125 μM) significantly enhanced responses to the sweeteners fructose, maltose, and stevioside whereas DiC8 (100 μM) significantly enhanced responses to the sweeteners fructose, maltose, sodium saccharin, dulcin, and stevioside. OAG (125 μM) significantly suppressed responses to the bitter compounds $MgCl_2$ and urea whereas DiC8 (100 μM) significantly suppressed responses to the bitter compounds quinine HCl, $MgCl_2$, and erythromycin. DiC8 also suppressed responses to taste compounds with bitter com-

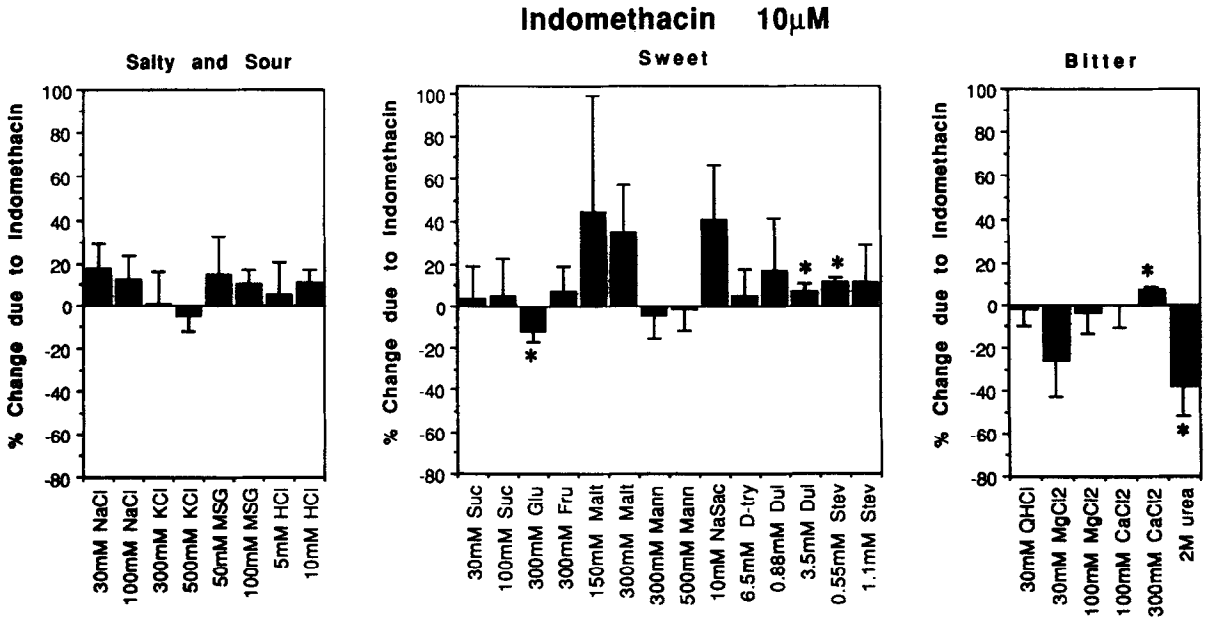


FIG. 9. Percent change in integrated chorda tympani responses after a 4-min application of 10 μM indomethacin.

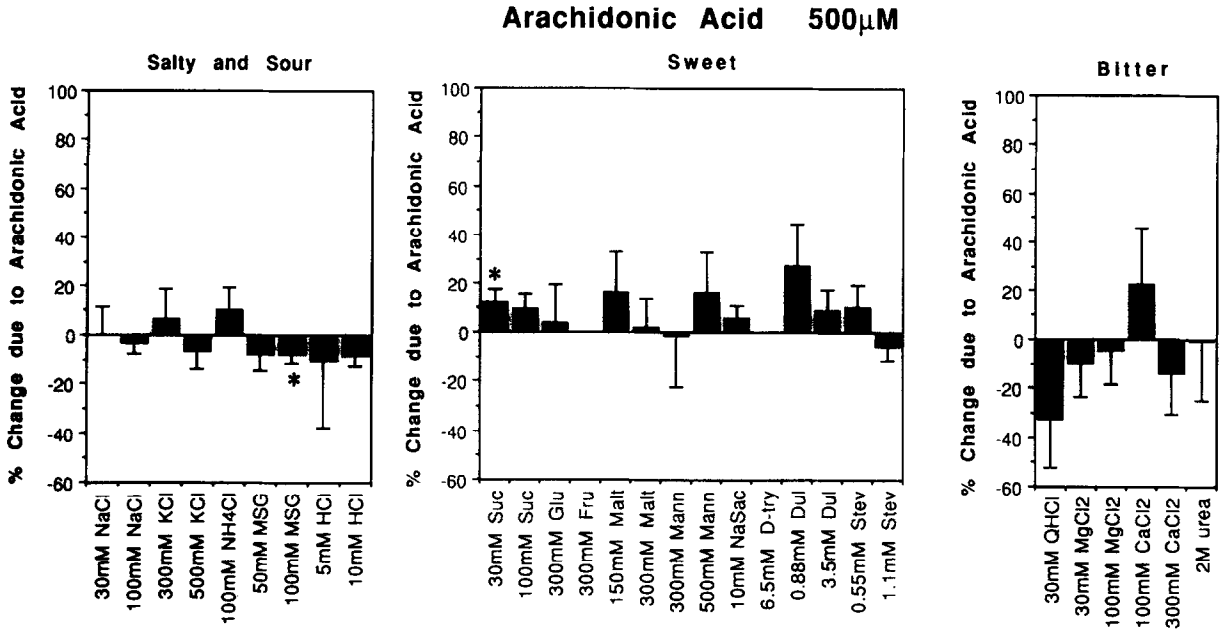


FIG. 10. Percent change in integrated chorda tympani responses after a 4-min application of 500 μM arachidonic acid.

ponents such as KCl, $NH_4Cl$, $CaCl_2$, and the highest concentration of stevioside.

The finding that cell membrane forms can suppress bitter taste was not unexpected because the phosphatidylinositol system has been implicated in bitter taste transduction (2,32). Thus, treatment of the tongue for 4 min with a cell-permeable form of DAG may be equivalent to adapting the tongue to a bitter compound that releases DAG.

The finding that DiC8 significantly enhanced sweet responses suggests that there is cross-talk between lipid-derived second messengers and the adenylate cyclase system [see (9) for discussion of cross-talk]. Previous studies have shown that stimulation of receptors coupled to the phosphatidylinositol system can enhance cAMP production mediated by stimulation of the β-adrenergic receptor (51,85). Production of cAMP is important in the transduction of sweet taste responses (52,80,81). Cross-talk has previously been shown to occur in the olfactory system (3) via a calmodulin, found in olfactory neurons, that potently activates olfactory adenylate cyclase. In addition, protein kinase C has been reported to sensitize olfactory adenylate cyclase (22). It appears that mechanisms analogous to those in olfaction also exist in the taste system because DiC8 enhances sweet taste. Cross-talk in the taste system may also explain the efficacy of the empirical use of sugar or sweeteners to suppress bitterness in foods, beverages, and drugs.

$LaCl_3$ tended to block all taste responses. The probable reason for this finding is that in addition to its role as an inhibitor calcium channels, it also blocks tight junctions. A previous study (73) has shown that electrophysiological responses recorded from the trigeminal nerve to salts were inhibited by 3.5 mM $LaCl_3$ while responses to nonpolar stimuli such as menthol were not affected.

Application of the $PLA_2$ stimulator mellitin, a 26 amino acid amphipathic peptide from bee venom, also suppressed most taste responses. The probable reason for this suppression is not its well-known $PLA_2$ activity (60,75) but rather some of its other effects. Mellitin, in addition to stimulating $PLA_2$, also serves as a pore-forming agent (20), stimulates calcium influx (12,71), activates certain G-proteins (42), inhibits protein kinase C (26), binds calmodulin (48), and induces mast cell degranulation (42). Thapsigargin also tended to suppress both sweet and bitter taste responses. Thapsigargin can also produce an elevation in $[Ca^{2+}]$ by inhibiting endoplasmic reticular $Ca^{2+}$-ATPase (86). No consistent trends were found for treatment of the gerbil tongue with inomycin, nifedipine, indomethacin, quinacrine, or arachidonic acid.

Overall, these data suggest that one or more lipid-derived second messengers are involved in taste transduction in the gerbil. However, further studies must be performed to determine the specific phospholipases and phopholipids involved. In addition, the relative differences in modulation for specific sweet and bitter compounds must be further investigated.

*Main Conclusions*

1. Both sweet and bitter taste responses can be modified by membrane-permeable forms of the second messenger diacylglycerol (DAG). DAG analogues tend to increase responses to sweet stimuli and decrease responses to bitter stimuli. Thus, a DAG generating second messenger system plays a role in taste perception.
2. $LaCl_3$, an inhibitor of tight junctions, blocks all taste responses, emphasizing the importance of tight junctions in maintenance of taste cell integrity.
3. Mellitin blocks all taste responses, probably due to the fact that it induces calcium influx in taste cells.

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