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7. Three presumptive motor neurons were used: LP5, LP6, and LP7. These cells were identified by their motor effects on the tail, and their size, location, and electrophysiological characteristics (*3*). During each test the motor neuron was hyperpolarized to the same membrane potential (about 25 mV from the initial pretest level) with a second intracellular electrode to prevent spike initiation.
8. EPSP's elicited by single action potentials at regular 5-minute intervals (except in trial 5 where the interval was 6 minutes) were used to assay associative and nonassociative effects of training rather than using the CS itself. This procedure minimized the effects of testing on the acquisition and retention of associative effects, and allowed the examination of the effects of the US alone (Sens responses) during training.
9. For the first five animals, a coin flip was used to decide which of the two cells with EPSP's closest to each other in amplitude was to be CS+, and which CS−, with the remaining cell used as Sens. For the remaining five animals, CS+ and CS− assignments were made so as to minimize the difference between the CS+ and CS− groups in their mean pretest scores (Figure 2A, top). No obvious differences in the effects of training were observed between the first and second five animals.
10. Constant-current pulses lasting 20 msec were applied in 400-msec trains (20 Hz) through the recording electrode (2 to 6 megohms) (WPI capillaries TW150F). Current intensity (about 4 nA) was adjusted to about twice the spike threshold before the pretest. Since sensitizing stimulation often changes the excitability of the sensory neurons (*3*, *12*), current intensity was sometimes readjusted during training to maintain a relatively constant number of spikes in response to each CS. Differences in CS intensity cannot account for differences between CS+ and CS− effects since in the overall study the average number of spikes evoked by the CS on each trial was the same in each group (9.1 ± 1.9 and 9.1 ± 2.3, respectively, mean ± standard deviation).
11. EPSP's were measured by an observer blind to both the cell's training history and the purpose of the experiment. Similar results were obtained in pilot studies ($N = 6$); see also (*12*).
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Methyl 4,6-Dichloro-4,6-Dideoxy-α-D-Galactopyranoside: An Inhibitor of Sweet Taste Responses in Gerbils

Abstract. *The sugar methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (DiCl-gal) is a new type of inhibitor of the gerbil's electrophysiological taste response to sucrose or saccharin. Saturated solutions of this compound alone barely stimulate the gerbil's taste nerve. But, when mixed with sucrose or saccharin, DiCl-gal suppresses the gerbil's taste response to these two sweeteners. In contrast, when mixed with sodium chloride or hydrochloric acid, DiCl-gal does not affect the taste responses to these compounds. However, unlike other inhibitors of sweet taste, the DiCl-gal taste suppression is short-lived and occurs only when the inhibitor is combined with the sweetener.*

My colleagues and I have been investigating the electrophysiological taste responses of gerbils to sugars and artificial sweeteners (*1–3*), seeking inhibitors that would provide some insight into the mechanism of the sweet taste response. We discovered a new type of inhibitor when we were comparing the taste responses to sucrose with those to tetrachloro-*galacto*-sucrose (4,6-dichloro-4,6-dideoxy-α-D-galactopyranosyl 1,6-dichloro-1,6-dideoxy-β-D-fructofuranoside). This chlorine-containing sucrose derivative is about 100 times as effective as sucrose in producing an electrophysiological response (*2*, *4*). Therefore, in an attempt to produce a "super sweetener," we synthesized methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (DiCl-gal), a monosaccharide that contains chlorine atoms and also closely resembles the chlorinated sucrose derivative. This sugar barely stimulated the gerbil's taste receptors even at a concentration of 0.1*M*, a saturated solution. In our earlier research, whenever we found

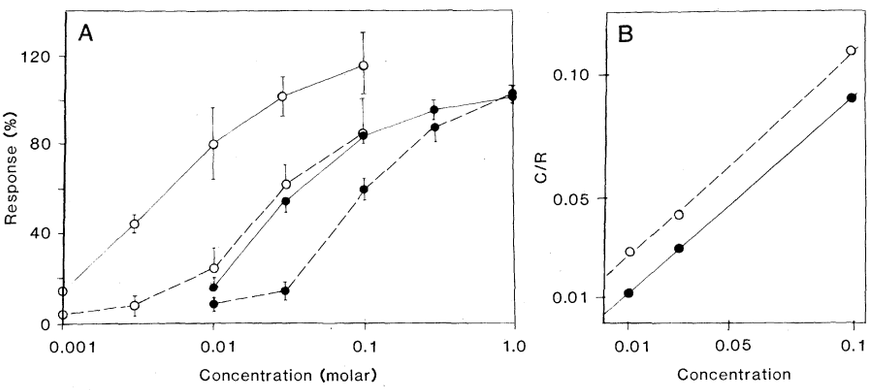

Fig. 1. (A) A comparison of the mean integrated nerve responses to sweetener solutions with the responses to mixtures of the sweetener and DiCl-gal. The sucrose solutions were prepared with deionized water: ●——●, sucrose ($N = 5$); ●– – –●, mixture of sucrose and 0.1*M* DiCl-gal ($N = 5$). The H-saccharin solutions were prepared with 0.1*M* potassium phosphate buffer, *p*H 7 : ○——○, H-saccharin ($N = 4$); ○– – –○, mixture of H-saccharin and 0.1*M* DiCl-gal ($N = 4$). The bars indicate ± 2 standard errors. (B) A Beidler plot of integrated nerve responses to H-saccharin (●) and mixtures of H-saccharin and 0.1*M* DiCl-gal (○); *C* represents concentration and *R* is response.

a nonstimulating sweetener derivative, our normal practice had been to mix it with sucrose or saccharin and test for inhibition. Until now, this practice had not been fruitful. In this case, however, the gerbil's electrophysiological taste responses to sucrose or saccharin were inhibited by DiCl-gal. In contrast, DiCl-gal did not inhibit the gerbil's taste responses to sodium chloride or hydrochloric acid.

The method for recording discharges from the intact chorda tympani nerve (*5*) consists of touching the nerve with a Nichrome electrode (100 μm in diameter) connected to a differential amplifier (Grass P-511). The integrated discharge of the nerve is used to characterize the gustatory system since it represents a summation of activity from many receptor cells (*6*). A response in this study was defined as the difference between spontaneous activity and the greatest integrated potential elicited by a given solution applied to the tongue. The time constant of the integrator (Grass model 73PA) was set at 0.5 second, full wave rectification. The indifferent electrode was placed on a nearby piece of moist tissue within the auditory bulla.

Chemical stimulation of the tongue was effected by a gravity-flow funnel tubing system in which deionized water or a buffered solution was flowing (0.13 to 0.17 ml/sec) over it. Test solutions (2 to 4 ml) were alternated with water without interruption of the flow. The water and taste solution temperatures were the same, 25° ± 1°C.

I prepared the taste solutions by dissolving the compounds in deionized water or 0.1*M* potassium phosphate buffer (*p*H 7). These solutions were either used immediately or were stored at 2°C for up to 7 days for later use, at which time they were brought to room temperature.

The synthesized crystals of DiCl-gal had a melting point of 158°C and an $[\alpha]_{25}^{D}$ (specific rotation relative to the sodium D line at 25°C) of +178.0°. These values are in agreement with those in the literature (*7*).

The taste responses of the gerbils to the various stimuli were affected differently by DiCl-gal. For example, taste responses to a mixture of sucrose and DiCl-gal were suppressed at low concentrations, but the suppression diminished at higher concentrations (Fig. 1A), until, at the highest concentration tested, 1.0*M*, DiCl-gal did not inhibit at all. This result suggests that DiCl-gal was acting as a competitive inhibitor of the sucrose taste response. The inhibition constant (K_i) was 0.051*M*.

Taste responses to H-saccharin, dissolved in phosphate buffer, were also suppressed by DiCl-gal. [The free acid, hydrogen saccharin (Aldrich Chemical), was used instead of the salt, sodium saccharin, to eliminate the possibility of Na^+ involvement in the taste response.] In this instance, unlike the case for sucrose, the DiCl-gal suppression of the saccharin responses appeared to be the same at all concentrations tested. Unfortunately, the limited solubility of saccharin prevented testing at concentrations higher than 0.1*M*. Because of this, I did a Beidler (*8*) plot (Fig. 1B) of the H-saccharin responses, which revealed the parallel shift of responses to the mixture of saccharin and DiCl-gal typical of competitive inhibition. The K_i for this inhibition was 0.021*M*.

The suppression of the sucrose taste response was short-lived and occurred only when DiCl-gal was mixed with sucrose. The application of DiCl-gal to the gerbil's tongue for 2 minutes followed by a 1-minute water rinse did not diminish the animal's subsequent sucrose taste response (Fig. 2C). The taste responses to sodium chloride and hydrochloric acid were not suppressed by DiCl-gal (Fig. 2, A and B).

To my knowledge, this is the first report of the suppression of the mammalian sweet taste response by a rapidly reversible inhibitor; earlier inhibitors of sweet taste, whether in electrophysiological or behavioral experiments, continued to suppress the taste response for many minutes after application (*9–11*). For example, alloxan applied to the tongue of the rat caused long-lasting suppression of the electrophysiological response to sucrose (*11*). Gymnemic acid had the same effect on the human's behavioral response to sucrose (*9*). Even rinsing the tongue with water did not immediately reverse the inhibition in those cases.

According to Beidler's taste theory, a single-stimulus molecule binds to a single receptor site to form a stimulus-receptor complex (*8*). He assumed that the integrated response of the chorda tympani nerve was proportional to the number of complexes formed between the stimulus and receptor. Beidler later expanded his theory to describe what might happen if two stimulus molecules with different intrinsic effectiveness were competing for the same receptor site (*12*). The molecule with the low intrinsic effectiveness would probably produce a very small taste response, but, when mixed with a molecule of high intrinsic effectiveness, it would be a rea-

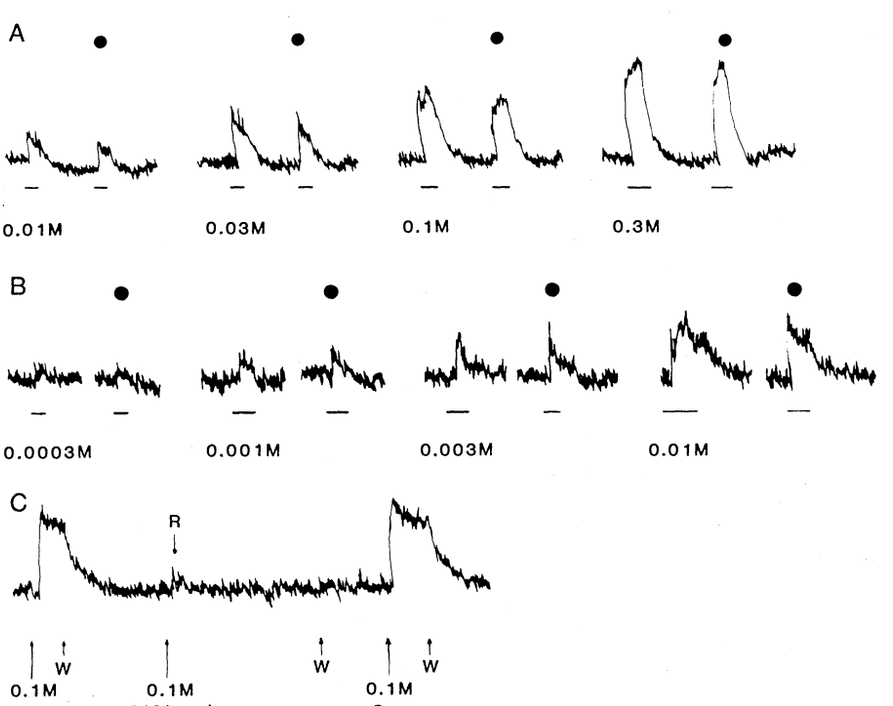

Fig. 2. (A) A comparison of the integrated nerve responses to sodium chloride with the responses to mixtures of sodium chloride and 0.1*M* DiCl-gal (●). The bars indicate the addition of the stimuli to the flow system. (B) A comparison of the integrated nerve responses to hydrochloric acid with the reponses to mixtures of hydrochloric acid and 0.1*M* DiCl-gal (●). (C) The effect of preexposure to DiCl-gal on the response to sucrose. The long arrows indicate the addition of 0.1*M* sucrose or 0.1*M* DiCl-gal to the flow system. The short arrows labeled "*W*" indicate the addition of the water rinse to the flow system. The small response produced by the DiCl-gal is labeled "*R*". The DiCl-gal addition to the flow system is for 2 minutes followed by a 1-minute water rinse.

sonably good inhibitor of the taste response. The suppression by DiCl-gal of the response to sweeteners of the gerbil's chorda tympani is consistent with the inhibitory mechanism proposed by Beidler for the following reasons: (i) DiCl-gal alone barely stimulates the gerbil's taste nerve and (ii) the kinetic plots of the taste to DiCl-gal mixed with sucrose or saccharin suggest that each sweetener and DiCl-gal are competing for the same receptor site.

The mechanism for the DiCl-gal suppression of sweet taste is puzzling despite the fact that kinetic plots indicate competitive inhibition. The reason is that, unless saccharin, sucrose, and DiCl-gal possess some common molecular feature, it is difficult to understand how DiCl-gal can be acting simultaneously as a competitive inhibitor with both sucrose and saccharin, especially since the two sweeteners are believed to bind to separate receptor sites (*3*). Because the molecular similarities are not evident, a possible explanation is that DiCl-gal is acting as an allosteric inhibitor which is diminishing only the rate constant (K_d) but not the maximum taste response (*13*). Its allosteric site could be independent, or it could be the sucrose site itself. The latter possibility, which takes into account the molecular similarities between DiCl-gal and sucrose, suggests that, when DiCl-gal binds to the sucrose site, not only is DiCl-gal a competitive inhibitor at that site, but also, at the same time it allosterically affects the saccharin binding site.

These results indicate that there are at least two reactions causing the sweet taste response. The first reaction is the binding of the sweetener to the receptor. The second reaction is activation of the receptor by the bound sweetener. The experimental results indicate that DiCl-gal was binding to the receptor but not activating it. On the other hand, sucrose was binding to the receptor as well as activating it. In these respects the mechanism of the sweetener taste response resembles the drug-receptor interaction mechanisms (*14*).

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Net Primary Productivity in Coral Reef Sponges

Abstract. *Nine of the ten most common sponge species on the fore-reef slope of Davies Reef (Great Barrier Reef) contain symbiotic cyanobacteria. Six of the ten are net primary producers, with three times more oxygen produced by photosynthesis than is consumed during respiration. Light interception is enhanced by morphological flattening, thereby increasing the potential for phototrophic nutrition, a factor crucial in the ecology of most sessile coral reef invertebrates.*

Extensive tracts of coral reefs in nutrient-deficient tropical waters owe their existence to the symbiosis between corals and eukaryotic algae, the zooxanthellae (*1*). This symbiosis results in the translocation to the host of considerable amounts of fixed carbon (*2*) as well as nitrogen (*3*) and phosphorus (*4*).

Sponges are the second largest biomass component of the coral reef benthic fauna (*5, 6*), but the mechanisms by which these large sponge populations obtain sufficient nutrition from low-nutrient tropical waters has not been resolved (*7*). A symbiosis comparable to that in corals occurs between sponges and cyanobacteria (blue-green algae); these prokaryotic symbionts may be either uni- or multicellular (*8, 9*). This symbiosis is widespread in marine sponges in shallow tropical and warm temperate environments (*5, 6*). Speculation on the role of these cyanobacteria in sponge ecology has included light shielding, "farming," with subsequent intracellular digestion by the host, and translocation of glycogen (*8, 10*) or glycerol (*11*). I have found that these cyanobacterial symbioses are crucial in the nutrition and ecology of coral reef sponges.

Sponges with symbiotic cyanobacteria constitute approximately 80 percent of individuals and of the biomass to 20 m on the fore-reef slope of Davies Reef (Great Barrier Reef; latitude 18°15′S, longitude 147°38.5′E) (*6*). Nine of the ten most common sponge species contain symbiotic cyanobacteria morphologically similar to those in sponges from the southern Great Barrier Reef, Red Sea, and Mediterranean (*8, 9*). The proportion of cyanobacteria varies considerably from one species to the next, as is evidenced by the chlorophyll a content (Table 1). In *Pericharax heteroraphis* and *Jaspis stellifera*, the cyanobacteria are restricted to a thin layer of tissue exposed to light, whereas in *Neofibularia irata* and *Carteriospongia (Phyllospongia)* species, cyanobacteria are distributed throughout the tissue. Only one of these ten sponges, *Spongia* sp., had no photosynthetic symbionts.

The importance of the cyanobacteria was tested by examining the photosynthetic potential of the ten most common sponges. Seven of these showed ratios of instantaneous gross productivity to respiration (P/R) that were greater than 1 in the light (photon flux density of 200 microeinsteins (μE) per square meter per second, indicating net primary production under the experimental conditions (Table 1) (*12*). Indeed, six of these sponges showed P/R ratios in excess of 3 at the maximum light level at 20 m where the largest sponge population occurs (*6*).

Quantitatively similar results were obtained for parallel measurements of net $^{14}CO_2$ fixation and production of oxygen (Table 2) (*13*). In the seven sponges with large cyanobacterial populations, dark fixation rates were between 2.4 and 6.6 percent of light fixation, indicating that most of the incorporation occurs through photosynthetic pathways (Table 2). These sponges released between 1 and 5 percent of fixed ^{14}C as dissolved organic carbon during the first hour, indicating a small contribution to reef nutrition (Table 2).

The data summarized in Table 1 demonstrate that the majority of sponges on Davies Reef can obtain much of their nutrition from their symbiotic cyanobacteria. Extrapolation of instantaneous