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Cyclic AMP Tastes Aversive, Not Sweet, to Rats

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HOUPT, T. A., S. P. FRANKMANN AND R. BERLIN. *Cyclic AMP tastes aversive, not sweet, to rats.* PHYSIOL BEHAV **59**(3) 495–498, 1996.—Electrophysiological and biochemical evidence suggests that cAMP mediates sweet taste transduction. Neural recordings from anesthetized rats and in vitro preparations demonstrate that membrane-permeable cAMP analogues mimic the effects of sucrose and artificial sweetners. We presented solutions of sodium 8-(4-chlorophenylthio)-adenosine 3′-5′-cyclic monophosphate (8cpt-cAMP), a water-soluble, membrane-permeable cAMP analogue to freely behaving rats in short-term lickometer tests. Rats licked significantly less to 8cpt-cAMP than to sucrose or palatable saccharin solutions. Rats could taste 8cpt-cAMP solutions, however, because they licked less to 8cpt-cAMP in mixture with sucrose than to sucrose alone. Because 8cpt-cAMP decreased licking when mixed with sucrose, we conclude that the taste of 8cpt-cAMP is aversive, not sweet, to freely behaving rats.

8cpt-cAMP Saccharin Sucrose Second messenger Lickometer Licking

ACTIVATION of taste cells by sucrose, saccharin, and other sweeteners causes activation of G-proteins and adenylate cyclase, leading to the formation of the second messenger cAMP. Sucrose and saccharin activate adenylate cyclase in vitro in rat (10,12) and pig (4) tongue tissue. Saccharin and other artificial sweeteners activate G-proteins in bovine brain (5) and rat liver and muscle membranes (11), possibly by direct interactions with G-proteins across lipid membranes, even in the absence of sucrose receptors. Ionophoretic injection of cAMP analogues or the addition of membrane-permeable cAMP analogues to the medium causes isolated sweet-responsive hamster taste cells to depolarize, thus electrophysiologically mimicking the presence of sucrose or saccharin in the medium (1). Integrated chorda tympani responses to sucrose in rats are attenuated with an adenylate cyclase inhibitor (13), whereas some (but not all) G-protein-, adenylate cyclase-, and cAMP-dependent protein kinase stimulators enhance the gerbil chorda tympani response to sucrose (7). The psychophysical rating of sweeteners correlates well with the sweeteners' potencies in activating G-proteins in vitro (5).

These in vitro results lead to a straight-forward behavioral prediction: membrane-permeable, water-soluble cAMP analogues should taste sweet to the rat when placed on the tongue. Our study was an attempt to test this prediction by measuring the licking of rats to a concentration range of 8cpt-cAMP, a membrane-permeable, water-soluble cAMP analogue, which mimics the depolarizing action of saccharin on isolated hamster taste cells. Licking was measured during repeated brief presentations (15 s) of seven concentrations of 8cpt-cAMP from 0.003 to 30 m*M*. The resulting concentration–lick curve for 8cpt-cAMP was compared to the lick rate elicited by a range of saccharin and sucrose concentrations. The lick rate produced during presentation of combined sucrose and 8cpt-cAMP solutions was also measured, to determine if the cAMP analogue enhanced or reduced the palatability of sucrose.

METHOD AND RESULTS

Lickometer Training

Eight adult male Sprague–Dawley rats (250–300 g) without prior experience with sucrose or saccharin were group housed (two per cage, each tail marked for identification) with ad lib access to Purina rodent chow and tap water under a 12-h light, 12-h dark cycle. All tests were conducted 1–4 h after lights on. The rats were trained to lick sodium saccharin concentrations during brief presentations in a MS80 multistation lick analysis system (Dilog Instruments, Tallahassee, FL), described in detail elsewhere (9). Briefly, the drinking spouts of eight bottles were individually presented in arbitrary order (computer controlled) at one end of a Plexiglas test chamber. A shutter opened and closed at an access portal, as the motorized rack moved to change the bottle to be presented from trial to trial. The time of the onset of

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each individual lick was recorded to the nearest millisecond when the rat's tongue contacted the drinking spout, thus completing a computer-monitored electronic circuit. A white noise generator in the test room masked outside sounds. The rats' behavior was monitored remotely by video camera to minimize disturbance by the experimenter.

Saccharin solutions were employed during training so that rats would have experience of presentations of a solution with both palatable and aversive qualities, and to minimize postingestive (caloric) effects. Rats were not food or water deprived during training or testing. Training sessions consisted of a total of nine 60-s presentions of one to three concentrations of saccharin (5, 10, and 50 m*M*). All rats received two training sessions a day for 14 days prior to experimental testing.

Experiment 1: Saccharin, 8cpt-cAMP, and Sucrose Concentration–Lick Rate Curves

The trained, nondeprived rats were individually tested with the MS80 system on 3 consecutive days with either sodium saccharin (Sigma), sodium-8-(4-chlorophenylthio)-adenosine 3′-5′-cyclic monophosphate (8cpt-cAMP; Sigma), or sucrose (Fisher) solutions. Stock saccharin and sucrose solutions were stored at 4°C; crystalline 8cpt-cAMP was stored at −20°C, and fresh solutions were mixed the day of testing and used within 90 min. All tastants were dissolved in charcoal-filtered deionized water and presented at room temperature. Eight concentrations of each taste solution were presented three times in a single session: saccharin at 0, 0.1, 1, 5, 10, 50, 100, 1000 m*M*; sucrose at 0, 1, 10, 50, 100, 1000, 2000 m*M*; 8cpt-cAMP at 0, 0.003, 0.15, 0.3, 1.5, 3, 30 m*M*. The concentration ranges were centered around the reported peaks of the palatability curves for saccharin (10 m*M*) and sucrose (100 m*M*) (2), and around the 8cpt-cAMP concentration that was maximally effective at depolarizing isolated hamster taste cells (0.5 m*M*) (1). Each concentration was presented for 15 s after the first lick, with a 10-s interval between consecutive presentations; if the rat failed to lick within 15 s, the next solution was presented after a 10-s interval. The order of presentation was randomized for each individual rat.

On the first day of testing, half the rats were tested with a range of saccharin concentrations and half with 8cpt-cAMP. On the second day, those rats first tested with saccharin were tested with 8cpt-cAMP and vice versa. On the third day, all rats were tested with a range of sucrose concentrations.

The mean number of licks recorded for each rat at each tastant concentration was averaged across all rats. Presentations that were not sampled (i.e., with zero licks) were not included in the means.

Results of Experiment 1

The mean numbers of licks during 15-s access to the range of saccharin, sucrose, and 8cpt-cAMP concentrations are shown in Fig. 1. The licking response to saccharin was an inverted U-shape with a sharp peak of maximal licking (> 100 licks/15 s for some rats) at 10 m*M*, although some rats licked 5, 50, or 100 m*M* solutions at a maximal rate as well. Saccharin concentrations below and above 10 m*M* on average generated less licking. In some instances rats licked only once when given 1 *M* saccharin solutions and then terminated their licking to the presentation.

Sucrose also elicited maximal licking rates but at concentrations over 1 log unit higher than the maximally palatable saccharin concentration. Above 100 m*M*, the licking response to sucrose plateaued at the maximal rates of licking observed (65–80 licks per 15 s), with no evidence of a decline in licking at higher concentrations.

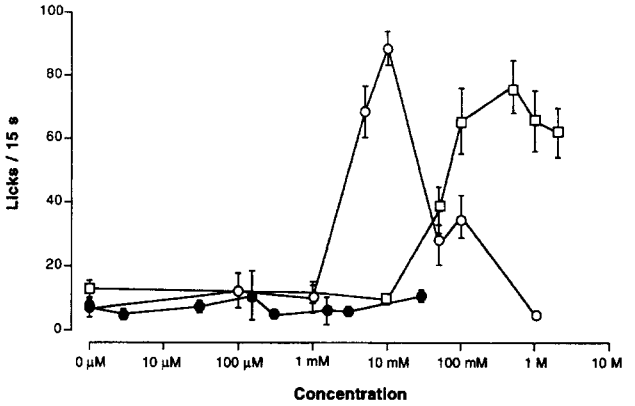

FIG. 1. Mean ± SEM total licks during 15-s access to varying concentrations of saccharin (empty circles), sucrose (empty squares), and 8cpt-cAMP, a membrane-permeable, water-soluble cAMP analogue (filled circles). Because not all rats ($n = 8$) sampled all concentrations, the number of rats providing lick data varied for each concentration of each tastant: $n = 6$–7 rats per point for saccharin, 7–8 for sucrose, and 3–8 for 8cpt-cAMP.

In contrast to both saccharin and sucrose, no concentration (3 μM–30 m*M*) of 8cpt-cAMP was able to elicit more licking than water, even though 50 μM 8cpt-cAMP is sufficient to depolarize isolated hamster taste cells (1). Only one rat licked more than 16 times at any concentration of 8cpt-cAMP (23 licks at 0.15 m*M*).

There were no differences in the number of times rats sampled (i.e., licked at least once to the presentation of a bottle) the first four presentations of any tastant during the different test sessions (saccharin = 3.0 ± 0.4, sucrose = 2.3 ± 0.5, 8cpt-cAMP = 2.6 ± 0.4). Sampling of all 24 presentations of each tastant, however, was variable and dependent on the tastant (saccharin = 11.5 ± 1.8, sucrose = 16.3 ± 1.4, 8cpt-cAMP = 5.8 ± 0.5). Thus, the difference in the number of presentations sampled across the test session occurred after the rats had already sampled early presentations of the tastant being offered, and on average rats sampled less during the 8cpt-cAMP session than they sampled during the saccharin or sucrose sessions.

In contrast to sucrose and saccharin, 8cpt-cAMP failed to elicit any more licking than water did (see 0 μM points in Fig. 1). Not only did 8cpt-cAMP fail to sustain licking when rats initiated drinking, but rats consistently sampled tubes less frequently when only 8cpt-cAMP was presented. Therefore, 8cpt-cAMP in the concentration range 0.003–30 m*M* either 1) does not generate any sweet taste, or 2) any sweet taste that might be present is masked by an aversive quality that suppresses licking.

A second experiment was designed to distinguish these two possibilities by presenting mixtures of 8cpt-cAMP/sucrose and saccharin/sucrose. If rats cannot detect 8cpt-cAMP at the concentrations presented in Experiment 1, then they should not respond to a 8cpt-cAMP/sucrose solution differently than to a pure sucrose solution. Conversely, if 8cpt-cAMP has an aversive taste quality, it should reduce licking to a sucrose solution. Furthermore, subthreshold concentrations of saccharin increase licking in mixture with sucrose (6) or glucose (8); 8cpt-cAMP might also potentiate sweet taste without tasting sweet itself. We therefore compared responses to 8cpt-cAMP/sucrose mixtures with responses to saccharin/sucrose mixtures.

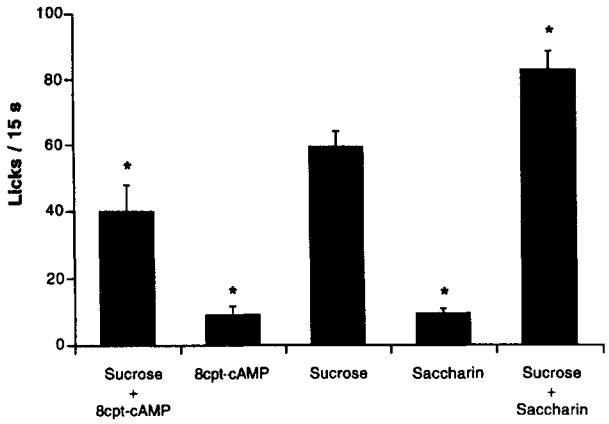

FIG. 2. Mean ± SEM total licks to 50 m*M* sucrose mixed with 1.5 m*M* 8cpt-cAMP or 1 m*M* saccharin, and to each tastant alone. $n = 7$–8 rats per mean per solution. * $p < 0.05$ vs. sucrose.

Experiment 2: Sucrose-8cpt/cAMP and Sucrose/Saccharin Mixtures

Twenty-four hours after the last test of Experiment 1, rats were tested with presentations of five solutions: 50 m*M* sucrose; a mixture of 50 m*M* sucrose and 1.5 m*M* 8cpt-cAMP; a mixture of 50 m*M* sucrose and 1 m*M* saccharin; 1.5 m*M* 8cpt-cAMP; and 1 m*M* saccharin, presented in that order. The order of presentation was selected to maximize the contrast between the pure sucrose solution and the mixed solutions. The sucrose and mixed solutions were each presented six times; the pure 8cpt-cAMP and saccharin solutions were each presented three times. The number of licks at each sampled presentation for each solution was averaged within rats, and the individual means averaged across all eight rats. Comparisons between the sucrose alone and the four other solutions were analyzed by *t*-test.

Results of Experiment 2

Because in Experiment 1 rats did not lick to 8cpt-cAMP more than to water, our second experiment was an attempt to determine if rats either could not detect 8cpt-cAMP or found the taste of 8cpt-cAMP aversive by presenting mixtures of 8cpt-cAMP/sucrose and saccharin/sucrose. The mean numbers of licks to 15-s presentations of each of the three sucrose-containing solutions are shown in Fig. 2. When sucrose was presented in a mixture with 1.5 m*M* 8cpt-cAMP, the number of licks was significantly smaller than the number of licks during presentation of the pure sucrose solution, $t(7) = 3.63$, $p < 0.05$; seven out of eight rats licked less to the combination than to the sucrose alone. The addition of 1 m*M* saccharin potentiated licking to 50 m*M* sucrose: the number of licks to the mixture was significant greater than the number of licks to unmixed 50 m*M* sucrose, $t(7) = -5.33$, $p < 0.05$. As in Experiment 1, 8cpt-cAMP and the low saccharin concentration both elicited very few licks, both significantly less than the sucrose alone [$t(7) = 7.96$ (8cpt-cAMP) and 7.32 (saccharin), both $p < 0.05$].

Mixing the 8cpt-cAMP with a submaximal concentration of sucrose (50 m*M*) could have: 1) increased licking if 8cpt-cAMP increases palatability, 2) had no effect on licking if 8cpt-cAMP has a neutral taste, or 3) decreased licking if 8cpt-cAMP decreased palatability. 8cpt-cAMP decreased licking when in solution with 50 m*M* sucrose, suggesting that its taste had adulterated the sweet taste of sucrose to make it less palatable. We conclude that not only is the taste of 8cpt-cAMP in a short-access test not sweet or palatable to rats, but it is aversive.

In contrast, a subthreshold concentration of saccharin raised the licking rate to the submaximal sucrose solution to the maximum rate of licking. The potentiation of the palatibility of a low concentration of sucrose by the addition of a low concentration of saccharin (6) parallels the synergistic effects of mixing low concentration glucose and saccharin solutions (8). The biochemical or neural mechanism by which a mixture of natural and artifical sweeteners is perceived as more sweet than the combined intensity of the two alone is currently unknown.

DISCUSSION

There are three possible explanations as to why 8cpt-cAMP failed to mimic the effects of sweeteners when tasted by freely behaving rats.

1. Detection of cAMP-induced sweet taste may be confounded by employing normal licking as the route of administration. For example, orally ingested 8cpt-cAMP may not have access to the taste cells, or may not be able to penetrate the taste cell membranes fast enough to cause depolarization [up to 5–15 min in vitro (1)] during the 15-s presentation. In contrast, sucrose might taste sweet because it needs access only to the external ligand binding site of its receptor. This explanation is not consistent with the rapid effects of the artificial sweetener saccharin, however, which has been suggested to act by partially penetrating the membrane and activating G-proteins independent of the external ligand binding site of the sucrose receptor (5); the rapidly detected sweet taste of saccharin might be mediated by an external receptor, however. Furthermore, even when the duration of exposure to 8cpt-cAMP was extended by "sugar-coating" the compound with a palatable sucrose solution, there was a decrease rather than an increase in palatability. Rats thus responded differentially to 8cpt-cAMP/sucrose mixtures during a 15-s presentation, suggesting that 8cpt-cAMP can be detected within 15 s.
2. cAMP might be a necessary component of the intracellular transduction cascade of sweet taste, but not sufficient to replicate the full effects of a sweetener itself. Activation of G-proteins and adenylate cyclase may be an obligatory correlate of sweet transduction, but the depolarization of the taste cell caused by cAMP may not be sufficient to stimulate the gustatory nerves transynaptically. Furthermore, cGMP is equipotent at stimulating isolated hamster taste cells and may be a required second messenger, with or without cAMP (1).
3. 8cpt-cAMP might induce a sweet taste, but this is overwhelmed by other aversive effects of the general and nonspecific activation of the numerous cells of the taste buds, tongue, nerves, etc. In isolated cells, cAMP analogues modulate the responses of bitter and sodium responsive cells (3), and some bitter substances stimulate adenylate cyclase activity (5). A general and nonspecific activation of numerous cells in the tongue and mouth might generate a noxious effect.

Although the results presented here do not parallel those obtained electrophysiologically or biochemically in vitro, they do not exclude a role for cyclic nucleotides in the transduction of sweet taste in vivo. Elevated cAMP levels may be just one component of a complex second messenger cascade underlying sweet taste. It should ultimately be possible to manipulate the behavioral perception of taste by manipulating the biochemical processes of transduction modeled in the isolated taste cell.

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