Theory for the mechanism of action of "miracle fruit"

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The action of miracle fruit in changing the quality of a sour stimulus to that of sweet is hypothesized as being caused by blocking of the sour receptor sites. It is assumed, according to a new theory that holds that proton acceptors are sweet-evoking, that many sour stimuli are also simultaneously stimuli for the sweet receptors. However, the resulting sweet information is strongly inhibited by the sour system, so that sweet is not reported.

The purpose of this paper is to propose a mechanism for the gustatory quality change that occurs, in response to certain stimuli, as a result of the action on the tongue of the active principle in "miracle fruit" (*Synsepalum dulcificum*). In brief, this quality change is that a substance that is normally designated as sour, e.g., citric acid, is reported as sweet after an application of miracle fruit to the tongue.

The active principle has recently been purified and characterized by Henning, Brouwer, van der Wel, and Francke (in press), Kurihara and Beidler (1968), and Kurihara, Kurihara, and Beidler (in press). The former group has termed the principle, "miraculin," and the latter groups, "taste modifying protein." The term miraculin will be used in this paper, for purposes of convenience. The results of the above investigations were in agreement that miraculin is a glycoprotein, with a molecular weight of about 44,000. In addition, its psychophysical action has been studied by these groups, and also by another, Bartoshuk, Cateo, Vandenberg, Buttrick, and Long (in press). Before these results are considered, and because the proposed mechanism will be in terms of a new theory concerning the mechanism of initiating the sweet response (Dzendolet, 1968), a brief review of the new theory will be given first, along with recent evidence to support it.

In essence, the theory states that a sweet-evoking substance has the property of being a proton-acceptor, and that the specific mechanism at the receptor site is the removal of a proton at that point. The development of the theory showed that the same mechanism should be active in the case of the sweet taste of weak concentrations of inorganic salts, e.g., LiCl or NaCl, and of such artificial sweeteners as saccharine, as well as that of the usual organic compounds like sucrose.

There are data that can be interpreted as supporting the assumption that the same mechanism is active in these diverse situations. For example, it has been shown many times that an aqueous decoction of *Gymnema sylvestre* leaves, when applied to the tongue, abolishes the sweet taste of sucrose, and also that of saccharine. Bartoshuk and McBurney (Bartoshuk et al, in press) have shown that a similar decoction abolished the sweet taste of a weak solution of NaCl. Unless the *Gymnema sylvestre* can act in a number of different ways to produce the same result, which does not seem likely, these findings are consistent with the idea of a single operative mechanism.

Another observation by Bartoshuk and McBurney is also consistent with this theory of sweet initiation. The observation is that a water rinse to the tongue, after adaptation to an acid, results in the report of a sweet taste. This effect can be abolished by an earlier application of a *Gymnema sylvestre* decoction. The

result can be explained in the following way: A prolonged rinse with an acid solution, i.e., adaptation to an acid, can be considered as loading sweet receptor sites with protons to a degree greater than is normally the case. No sweet quality is reported in the course of this process because the mechanism for producing sweet, it will be remembered, is the removal of protons. (There are separate sites which react during the adsorption of a proton, i.e., the sites for sour.) When the mouth is rinsed with water, the sweet taste appears, because protons are then removed from the receptor sites, due to the much lower concentration of protons in the water.

At this point, a hypothesis can be advanced as to the mode of action of *Gymnema sylvestre*. It has already been concluded by Diamant, Oakley, Ström, Wells, and Zotterman (1965), on the basis of electrophysiological recordings from human subjects, that the active substance works peripherally. From the point of view of the new theory of sweet, and of the data discussed above, it seems likely that the active principle of *Gymnema sylvestre* straddles the sweet sites, and prevents the protons from being removed. This possibility seems more reasonable than a hypothesis that the active principle displaces the proton. The latter hypothesis implies that a sweet taste would be reported for the taste of the decoction, whereas a bitter one is actually given.

Although the above data are easily consistent with the new theory, the results of an investigation by Dastoli and Price (1966) initially appeared to argue against this mechanism. These investigators found a protein fraction in bovine tongue papillae that appeared to form a complex with various sugars. Evidence for the complex was based on a difference in the ultraviolet spectrograms of the protein fraction, with and without the addition of a sugar. They concluded that this protein fraction was the component of the taste bud that is responsible for the initial reaction, i.e., the formation of a sugar-protein complex, in producing a sweet taste. However, such a difference in spectrograms could also have resulted from the removal of protons from the protein fraction (S. Price, personal communication). Thus, the observations of Dastoli and Price on the presumed existence of a sweet-complexing protein, presumably from the taste bud, are not inconsistent with the new theory of sweet.

The main hypothesis of this paper, the mode of action of miraculin, can now be stated. It is that miraculin blocks the receptor sites for sour, so that protons cannot attach themselves to these sites and, thus, initiate the quality of sour. Therefore, if a weak acid, e.g., citric acid, is tasted after an application of miraculin, it will not taste very sour. Apparently not all the sites will be blocked, because Bartoshuk et al (in press) have shown that some sourness is still present, but much reduced in magnitude. The gustatory quality that will be present, in addition to the remaining sour, will depend on the quality that the anion is capable of evoking. With citric acid, it would be sweet, because citrate is a strong proton acceptor, and would remove protons from sweet sites in accordance with the new theory.

An alternate explanation of the effect of miraculin would be that it converts the sour quality into sweet in some simple way, using the hydrogen ion of the citric acid. If this were so, then the

effect would occur at any or all the concentrations of the citric acid at which sour had been reported. Kurihara et al (in press) reported, however, that citric acid does not taste sweet after the mouth is treated with miraculin unless the acid concentration exceeds 1.5×10^{-3} M. Visual inspection of the data presented by Bartoshuk et al (in press) also suggests this approximate value as the concentration above which there is a distinct sweet aspect to the taste of citric acid.

Further support for the role of citrate in evoking the sweet quality was also reported by Bartoshuk et al. Three of their five subjects did not report a sweet response to HCl, whereas they did to citric acid. Chloride ion is not a proton acceptor, so that it should not have evoked sweet. It is not clear why the remaining two subjects did report sweet.

The sweet quality produced by miraculin and citric acid was investigated further by Bartoshuk et al (in press) and Kurihara et al (in press) by applying *Gymnema sylvestre* extracts to the tongue after the application of miraculin. Both groups reported that the *Gymnema sylvestre* abolished the sweet quality, and that the sour quality then appeared at its full earlier intensity. The major part of the mechanism here seems clear. The *Gymnema sylvestre* blocked most or all of the sweet sites so that only the unblocked sour sites remained to be stimulated by the hydrogen of the citric acid.

The interesting aspect of the above phenomenon is why the magnitude estimate of the sour quality returned to the value it had before any treatment. It should be remembered that the mechanism proposed here is that fewer sour sites are available after treatment with miraculin than before, so that the sour intensity, at first thought, should remain the same after treatment with *Gymnema sylvestre* as before.

An explanation for this apparently anomalous behavior is suggested in investigations reported by Békésy. He had shown first (Békésy, 1964a) that gustatory qualities appeared to fall into two groups: one, a sweet-bitter group, and the other, a salty-sour one. The basis for this grouping was that simultaneous stimulation of two places on the tongue, one by a salty, and the other by a sour stimulus, produced a quality fusion of the two, and the report of an actual spatial localization midway between the two points of stimulation. Such an interaction, however, did not occur with sour and sweet stimuli, for example. Later, Békésy (1964b) showed that electrical stimulation of single papillae, which are sensitive to different qualities, produced similar results. Particularly noteworthy was the result when two papillae belonging to the separate groups were stimulated. With subjectively equal intensities, simultaneous stimulation produced a complete inhibition of both qualities. Relatively small

differences in intensity of stimulation have also been shown to inhibit one or the other site of stimulation (Békésy, 1967, pp. 100-103), and, therefore, the quality which is present there, leaving the other to be reported.

Returning to the effect produced by miraculin and *Gymnema sylvestre*, it can be explained as follows: Before treatment with anything, both sweet and sour sites are stimulated by the citric acid, but the sour system inhibits the sweet quality because of the greater intensity of the sour. After treatment with miraculin, the number of sour sites capable of being stimulated is reduced considerably, so that the sweet quality is not completely inhibited and is reported as being present. There is still mutual inhibition between the two systems, however. Finally, with application of the *Gymnema sylvestre*, essentially all the sweet sites are blocked, leaving only the sour ones to be stimulated. There is an increase now in the subjective intensity of the sour, because there is no longer any simultaneous inhibition of it by the sweet system.

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