Do Unique Proteins Exist in Taste Buds?

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ABSTRACT Proteins in papillae on the bovine tongue were analyzed by semimicro, polyacrylamide gel electrophoresis. All the proteins in the papillae with taste buds were observed to be common to proteins in the surrounding epithelium without taste buds. The protein band which was reported to form a weak complex with compounds called sweet by man was also found in all parts of the tongue epithelium. The receptor molecules for chemical stimuli may be distributed in all the cells of the tongue epithelium or the content of receptor molecules in taste bud papillae may be extremely low.

INTRODUCTION

It has been postulated that the initial event in taste stimulation is the formation of a weak complex of the receptor molecule with the stimulus compound (1). Recently, proteins which form complexes in vitro with compounds called sweet and bitter by man were extracted from the epithelium of bovine (2, 3), porcine (4), and rat tongues (5, 6), and it was claimed that these extracted proteins were the receptors for sweet and bitter compounds. On the other hand, Hansen insisted that the primary process of sugar reception in the blowfly, *Phormia regina*, is identical with the formation of a sugar-glucosidase complex similar to the enzyme-substrate complex of Michaelis and Menten (7).

In mammals, taste stimulation is induced in taste buds which occur in fungiform, circumvallate, and foliate papillae on the surface of the tongue. An early question that arose was whether or not receptor molecules for the stimulus compounds are localized only in the papillae with taste buds.

Since the content of taste buds in a single papilla of the bovine tongue is fairly high (for example, a single circumvallate papilla of bovine tongue contains about 1,500 taste buds [8]), we suggest that an appreciable portion of proteins from the papillae seems to be accounted for by proteins from taste buds. In the present study, we aimed to compare the number of protein bands of the papillae containing taste buds with those of the surrounding epithelium without taste buds. The technique of polyacrylamide gel electrophoresis was

chosen to compare protein patterns because of its high resolution of complex protein mixtures. Since the papillae are fairly small, a semimicrotechnique was devised for the protein preparation and electrophoresis.

EXPERIMENTAL

26 circumvallate papillae and about 200 fungiform papillae were seen on the epithelium of a bovine tongue used in this study. Circumvallate papillae, fungiform papillae, or the surrounding epithelium including filiform papillae which contain no taste buds were cut off with a small scalpel from the bovine tongue, and homogenized with a small motor-driven glass homogenizer in 0.3 ml of a 0.06 M buffer solution (Tris-HCl, pH 8.7, or acetate-KOH, pH 4.5). The homogenate was centrifuged in a microcentrifuge for 30 min at 10,000 × *g*. The protein concentration of the supernatant was determined on an aliquot of the supernatant by the method of Gornall et al. (9). Half volume of the supernatant (0.13 ml) was subjected to polyacrylamide gel electrophoresis with a 3 (I.D.) × 70 mm glass tubing. Gels for electrophoresis at pH 8.7 and those at pH 4.5 were prepared according to the method of Davis (10) and that of Reisfeld et al. (11), respectively. Electrophoresis was carried out at 1 ma/tube for 80 min at pH 8.7 and at 1 ma/tube for 150 min at pH 4.5. Gels were stained with Coomassie blue (12) which affords high sensitivity detection of protein bands. The minimum amount of protein detected by the electrophoresis technique used in the present study was determined to be 0.1 ± 0.05 μg per gel by using serum albumin (Nutritional Biochemical Corp., Cleveland, Ohio) as a standard protein sample. For precise comparison of protein bands in gels, electrophoresis was also carried out by running two different samples on a single gel column (referred to as a "split gel" [13]). All the experimental operations were performed at 4°C.

After extraction with the buffer the sediment was subjected to further extraction by a 8 M urea solution containing 1 % sodium dodecyl sulfate (SDS). After 1 hr of extraction at room temperature, the extract was centrifuged and the supernatant was subjected to electrophoresis on 8 M urea gels. In this case, gels were stained with Amido Schwarz (10).

The protein reported by Dastoli et al. was prepared according to their method (3) from the whole epithelium of bovine tongues by ammonium sulfate fractionation, followed by gel filtration with a column of Bio-Gel P-150 (Bio-Rad Laboratories, Richmond, Calif.).

RESULTS AND DISCUSSION

Electrophoretic patterns at pH 8.7 of proteins extracted with the buffer are shown in Fig. 1. The photographs of A, B, and C represent the patterns of proteins from fungiform papillae, circumvallate papillae, and the surrounding epithelium, respectively. These patterns are also shown schematically in Fig. 2. The widths of the bands in the figure are indicative of relative widths of the staining bands and dark-, cross-, and parallel-hatching indicate the relative staining intensity of the bands. As seen from the figures, all the protein bands from the fungiform and circumvallate papillae are common to those from the

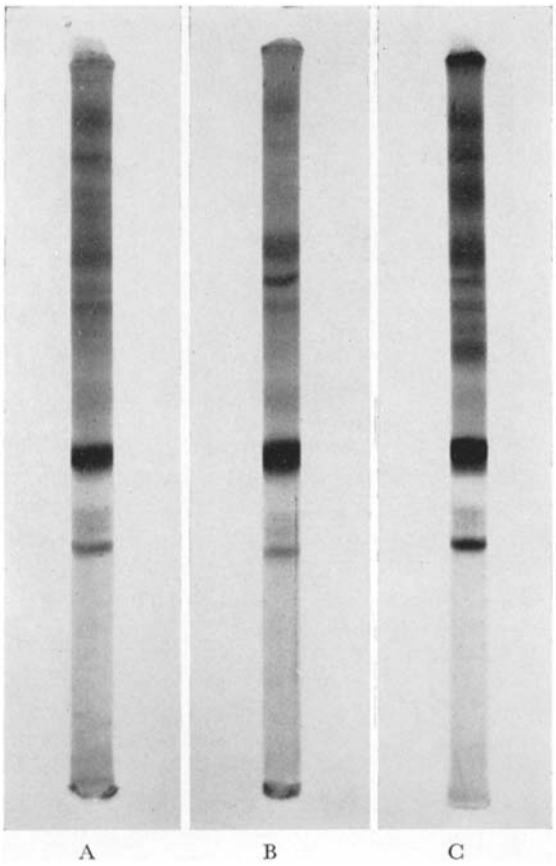

FIGURE 1. Electrophoretic patterns of the proteins extracted with the buffer from fungiform papillae (A), circumvallate papillae (B), and the surrounding epithelium without taste buds (C). The amounts of protein applied to a gel were: A, 170 μg from seven fungiform papillae (wet weight, 14 mg); B, 160 μg from two circumvallate papillae (12 mg); C, 160 μg from the surrounding epithelium (13 mg). Electrophoresis was carried out at pH 8.7. All gels were stained with Coomassie blue.

surrounding epithelium. Although the protein of AB-16 (Fig. 2) is more abundant in the papillae with taste buds, the protein seems to have no direct relation to taste reception, because the protein was confirmed to be serum albumin by electrophoresis with purified serum albumin in split gel.

It is known that in man the sweet taste is most easily sensed at the tip of the tongue, the bitter at the back, the sour at the edge, and the salt both on the tip and at the edge. However, a distinct difference was not found among the protein patterns from the fungiform papillae on different sides of the tongue.

Since most basic proteins do not migrate anodically on electrophoresis at pH 8.7, a protein solution extracted with the buffer was also subjected to electrophoresis at pH 4.5. Some of the proteins which migrated at pH 8.7 also

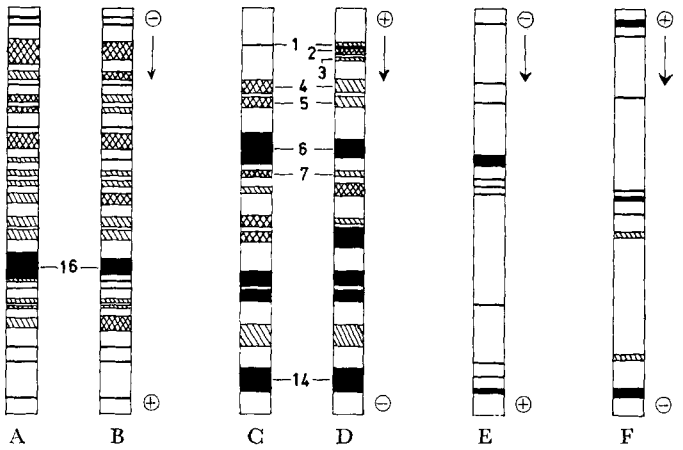

FIGURE 2. Schematic representation of polyacrylamide gels of proteins from fungiform papillae and the surrounding epithelium without taste buds. A, fungiform papillae, electrophoresis at pH 8.7 (same as A in Fig. 1); B, epithelium, electrophoresis at pH 8.7 (same as C in Fig. 1); C, fungiform papillae, electrophoresis at pH 4.5; D, epithelium, electrophoresis at pH 4.5; E, proteins extracted with a 8 M urea solution containing 1% SDS from fungiform papillae, electrophoresis at pH 8.7; F, the same proteins as E, electrophoresis at pH 4.5. Amounts of protein applied to a gel were: C, 170 μg from 7 fungiform papillae (13 mg); D, 180 μg from the epithelium (14 mg); E, 50 μg from 20 fungiform papillae (42 mg); F, 45 μg from 20 fungiform papillae (38 mg). A, B, C, and D were stained with Coomassie blue; E and F stained with Amido Schwarz.

migrated at pH 4.5. For example, the band of CD-6 which is more abundant in papillae with taste buds was confirmed again to be that of serum albumin by split gel. The diagrams C and D represent the protein patterns of fungiform papillae and the surrounding epithelium, respectively. As in the electrophoretic patterns at the alkaline pH, all the protein bands from the fungiform papillae were found in the surrounding epithelium, while two bands (D-2 and D-3) which were lacking in the fungiform papillae were found in the surrounding epithelium.

Since the protein reported by Dastoli et al. (3) was reported to be a basic protein, the band of this protein must be contained in the electrophoretic pattern at the acidic pH. In order to find the band of the protein in the gel, the protein was prepared from the whole epithelium of bovine tongues according to the method of Dastoli et al. (3). The protein preparation obtained gave one major band which was found to be CD-14 in split gel, accompanied by five minor bands (CD-1, 2, 4, 5, 7). The results indicated that the content of the proteins reported by Dastoli et al. in the epithelium is sufficiently high to be detected by electrophoresis and also that the proteins are homogeneously distributed in the whole epithelium of the bovine tongue surface. Since all the protein bands from fungiform papillae are common to those from the surrounding epithelium, the protein which was reported to form a weak complex

with bitter compounds (4) must also be homogeneously distributed in the whole epithelium of the tongue surface.

Some proteins may be firmly bound to the cell membrane and thus not solubilized with buffer solution. Therefore, after extraction with buffer the sediment was subjected to extraction by a 8 M urea solution containing detergents such as Triton X-100, deoxycholate, and SDS. Electrophoresis of the extract was carried out at pH 8.7 and at pH 4.5. Since gels containing detergents often became opaque in the process of staining with Coomassie blue, gels were stained with Amido Schwarz, although the sensitivity of this stain was less than that of Coomassie blue. The electrophoretic patterns of the extraction by a 8 M urea solution containing 1% SDS, which was found to solubilize most strongly the proteins in the sediment, are shown in diagrams E (pH 8.7) and F (pH 4.5). Again, no difference was found between the protein pattern of the papillae with taste buds and that of the surrounding epithelium.

It was concluded from the present study that all the proteins in the papillae with taste buds are common to those in the surrounding epithelium without taste buds. It is unlikely that the proteins from the epithelium without taste buds would happen to show the same mobilities as those from the papillae with taste buds, since polyacrylamide gel electrophoresis affords a very high resolution of protein bands. Various interpretations may be placed on the present conclusion. One of these interpretations is as follows. The receptor molecules for chemical stimuli are distributed in all parts of the tongue epithelium. Although the stimuli may bind to the receptor molecules at all parts of the epithelium, only the binding of the stimuli at taste buds produces effective taste stimulation.

An alternative interpretation is that the content of receptor molecules in the papillae is too low to be detected by polyacrylamide gel electrophoresis. Any new protein band in addition to the bands of diagrams A and C in Fig. 2 was not detected even when 260 μg of protein extracted from 10 fungiform papillae (wet weight, 21 mg) was applied to a single gel without urea for the electrophoresis at pH 8.7 and pH 4.5, respectively. The minimum amount of protein detectable by the electrophoretic technique used in the present study was determined to be around 0.1 μg per gel by using serum albumin as a standard protein sample; therefore, the content of receptor molecules in a single fungiform papilla may be lower than 0.01 μg, if a receptor molecule is a protein unique to taste buds and is extractable in aqueous buffer. Since the content of the proteins reported by Dastoli et al. (2, 3) in the papillae and the surrounding epithelium was sufficiently high to be detected by electrophoresis, we are forced to deny that the proteins are true receptor molecules, if we admit the above interpretation. Further study will be needed to clarify the actual nature of taste receptor molecules.

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