BBA 76054

MECHANISM OF BITTER TASTE RECEPTION: INTERACTION OF BITTER COMPOUNDS WITH MONOLAYERS OF LIPIDS FROM BOVINE CIRCUMVALLATE PAPILLAE

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(Received April 4th, 1972)

SUMMARY

Monolayers of lipids from bovine circumvallate papillae were prepared as a model system for the gustatory receptor membrane. Interaction of the monolayers with bitter compounds was examined by measuring a surface pressure increase in the monolayers. It was found that there is a good correlation between the concentration of the bitter compounds to give an identical increase in surface pressure in the monolayers and their taste thresholds. From the results obtained in the present study it is proposed that bitter taste is induced by penetration of bitter compounds into the non-polar region of the lipid layer of the gustatory receptor membrane.

It has been postulated that the initial event in taste stimulation is the formation of a weak complex of the receptor molecules with the stimulus compound[1]. Dastoli *et al.*[2] reported that the protein extracted from epithelium at the back of the porcine tongue forms complex *in vitro* with compounds called bitter by man. Koyama and Kurihara[3], however, failed to find any protein unique to taste buds or to the back of the tongue epithelium by polyacrylamide gel electrophoresis of proteins in the bovine tongue papillae.

On the other hand, Hoshishima[4] reported that phospholipid in taste cells may be involved in the reception of bitter stimuli. When the phospholipid fractions obtained from the tongue surface tissue of male and female mice were shaken with water containing propylthiouracil, the fraction from female mice, which are more sensitive than male mice to the bitter taste of propylthiouracil, adsorbed significantly more, compared with the fraction from male mice. Faull and Halpern[5] stated that the lipid fraction from bovine circumvallate papillae, which contain many taste buds, adsorbed more quinine or quinidine, compared with the fraction from tongue epithelium without taste buds.

It is generally accepted that taste reception occurs on gustatory receptor membrane[1,6]. The above findings, therefore, suggest that lipids in the gustatory receptor membrane may be involved in the reception of bitter stimuli. In the present study, monolayers of lipids from bovine circumvallate papillae were prepared as a model system for the gustatory receptor membrane, and interaction of the monolayers with bitter compounds was examined by measuring a surface pressure increase in the monolayers. The results obtained in the present study indicated that there is good correlation between the concentration of the bitter compounds to give an identical surface pressure increase in the monolayers and their taste thresholds.

MATERIALS AND METHODS

Lipid extracts

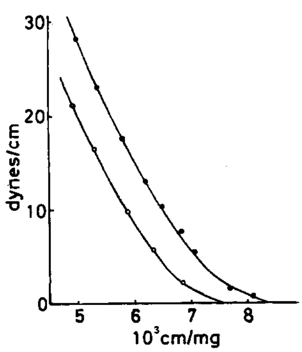
Circumvallate papillae (approx. 0.3 g) were cut off with a small scalpel from two fresh bovine tongues. The papillae obtained were homogenized with a glass homogenizer in distilled water and the homogenates were lyophilized. Lipids were extracted three times with 30 ml of chloroform–methanol (2:1, v/v) from the lyophilized homogenates and the extracts were washed according to the method of Folch *et al.*[7].

Preparation and surface pressure measurements of lipid momolayers

The lipid monolayers were spread from a dilute solution (0.3–0.5 mg/ml) in the extraction medium on the surface of a Ringer solution containing 154 mM NaCl, 5.6 mM KCl and 2.2 mM $CaCl_2$ and buffered to pH 7.3 with 20 mM $NaHCO_3$–HCl buffer. The trough was made of 'Teflon'. To stir subsolution adequately, a trough with a deep depression 170 mm × 150 mm × 10 mm) and a shallow depression (230 mm × 150 mm × 0.5 mm) was devised.

The surface pressure–area curves were determined on the lipid monolayers on the Ringer solution and the solution containing $3.0 \cdot 10^{-6}$ M brucine.

The relation between the concentration of bitter compounds and the surface pressure increase was examined as follows. Both sections of the trough were filled with Ringer solution and the monolayers of the lipid were spread on the whole surface of the trough. After leaving the monolayers for 10 min, they were compressed so as to occupy only the surface area on the deep section by moving a 'Teflon' barrier from the rigth side of the shallow section to the left side. The 'Teflon' barrier was set to make a narrow clean area with about 5 mm width between the barrier and the right side of the deep section. The amount of lipid applied to Ringer solution was determined so that a



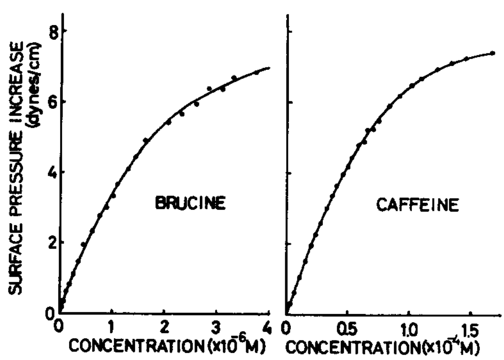


Fig. 1. Surface pressure–area curves of lipids from bovine circumvallate papillae on Ringer solution (○—○) and on Ringer solution containing $3.0 \cdot 10^{-6}$ M brucine (●—●).

Fig. 2. Relation between the concentration of brucine and caffeine and surface pressure increase in the lipid monolayers.

surface pressure of the monolayers gives 10 dynes/cm at the above compressed state. An aqueous solution of the bitter compound under investigation was injected in a Ringer solution with a microsyringe through the clean narrow area and an equal volume of Ringer solution was removed from the subsolution. The bitter compound was evenly distributed by gentle stirring with a magnetic stirrer. 2 min after stirring, the surface pressure of the monolayers was measured using the vertical plate method[8]. The water used in this study was twice distilled in an all-glass apparatus and all reagents were analytical reagent grade. All measurements were made at 23 ± 0.2 °C.

Determination of taste thresholds

Taste thresholds of bitter compounds were determined on five subjects, according to the method of Harris and Kalmus[9] as modified by Fischer *et al.*[10].

RESULTS AND DISCUSSION

Total lipids extracted from bovine circumvallate papillae were used to prepare the lipid monolayers as model membrane for the gustatory receptor membrane. Fig. 2 shows a typical example of surface pressure–area diagrams of the lipid monolayers spread on Ringer solution and the solution containing the bitter compound (brucine). They reveal that the bitter compound produces pressure increase in the monolayers. The relation between the concentration of bitter compounds and the surface pressure increase which they produce in the monolayers was examined by measuring the pressure increease in the monolayers when the bitter compounds were added to the subsolution under the monolayers giving the initial surface pressure of 10 dynes/cm. Fig. 3 shows the relations obtained by addition of brucine and caffeine. The effect of the bitter compounds on surface pressure of the Ringer solution without the lipid monolayers was practically negligible in the concentration range studied.

For comparison of relative strength of affinity of bitter compounds for the lipid monolayers, the concentrations of the compounds which give a pressure increase of 1.0 dyne/cm are listed in Table I. The right-hand column of the table shows the taste thresholds of the compounds for human, which include those taken from the literature[11], and those determined in the present study. It appears from the data in the table that there is a good correlation between the concentration giving an identical pressure increase and taste thresholds.

Dastoli *et al.*[2] reported that the protein extracted from the porcine tongue epithelium forms complexes *in vitro* with bitter compounds. Price[11], however, criticized the paper in the following points of view. (1) Dissociation constants of the bitter compounds (brucine, quinine and caffeine) with the protein differ from each other by factors of less than 2-fold, while the taste thresholds of the compounds differ from each other by factor of 40- to 1000-fold. (2) The dissociation constants are much larger than what one might expect on the basis of their taste thresholds.

On the other hand, much better correlation was found between the concentration of the bitter compound giving an identical surface pressure increase in the lipid monolayers and taste thresholds. It was also found that bitter compounds bind to the lipid monolayers in much lower concentration range of the compounds than the range in which the compounds bind with the protein extracted by Dastoli *et al.*[2]. While the formation of complexes of the protein with bitter compounds followed the Beidler's

TABLE I

CONCENTRATIONS OF BITTER COMPOUNDS GIVING A SURFACE PRESSURE INCREASE OF 1.0 dyne/cm AND TASTE THRESHOLDS FOR THE COMPOUNDS

Bitter compound	*Concentrations giving a pressure increase of 1.0 dyne/cm (M)*	*Taste thresholds (M)*
Brucine	$2.4 \cdot 10^{-7}$	$7 \cdot 10^{-7}$ *
Quinine·HCl	$1.4 \cdot 10^{-6}$	$3 \cdot 10^{-6}$ **
Strychnine·HCl	$1.7 \cdot 10^{-6}$	$1.6 \cdot 10^{-6}$ **
Picric acid	$4.5 \cdot 10^{-6}$	$3.7 \cdot 10^{-6}$ *
Nicotine	$5.2 \cdot 10^{-6}$	$1.9 \cdot 10^{-5}$ **
Naringin	$7.5 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$ *
Theobromine	$9.3 \cdot 10^{-6}$	$7.5 \cdot 10^{-4}$ *
Caffeine	$1.3 \cdot 10^{-5}$	$7 \cdot 10^{-4}$ **
Formanilide	$1.9 \cdot 10^{-5}$	$4.0 \cdot 10^{-3}$ *
Phenylurea	$2.0 \cdot 10^{-4}$	$2.5 \cdot 10^{-2}$ *
Urea	$4.1 \cdot 10^{-3}$	$1.2 \cdot 10^{-1}$ **
Formamide	$1.2 \cdot 10^{-1}$	$7.5 \cdot 10^{-2}$ *

* The thresholds were determined in the present study.
** The thresholds were taken from the literature[11].

taste equation[1], the relation shown in Fig. 2 did not satisfy the taste equation. It should be remembered that no electrophysiological data indicating that the taste equation applies in the case of bitter stimuli have been reported.

It is known that cations bind to the polar groups of phospholipids and produce surface pressure increase in the lipid monolayers[13,14]. On the other hand, bitter compounds seem to expand the lipid membrane by penetration into the non-polar region of the membrane, because most bitter compounds are uncharged and more soluble in organic solvents than in water. From the results obtained in the present study, the authors propose that bitter taste is induced by penetration of bitter compound into the non-polar region of the lipid layer of the gustatory receptor membrane. Since biological membrane is composed mainly of protein and lipid, protein molecules of the gustatory receptor membrane may also play a role in discrimination of taste compounds, as a barrier to control the penetration of chemical compounds into the lipid layer of the receptor membrane. In the above proposal on the mechanism of bitter taste reception, it is not postulated that there exists a specific receptor molecule for bitter compounds. In this context, it is interesting that bitter compounds stimulate the olfactory organ[15], provided that the compounds can reach the organ. The results on analysis of lipids in bovine tongue papillae will be described elsewhere.

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Biochim. Biophys. Acta, 288 (1972) 22–26