INHIBITION OF CYCLIC 3′, 5′-NUCLEOTIDE PHOSPHODIESTERASE IN BOVINE TASTE PAPILLAE BY BITTER TASTE STIMULI

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1. Introduction

In a previous paper [1], it was reported that bovine taste papillae and rabbit olfactory epithelium contain high adenyl cyclase activity comparable to the activity found in rat brain. This finding, together with the fact that photoreceptors exhibit high activities of adenyl cyclase [2, 3] and cyclic 3′,5′-nucleotide phosphodiesterase [4], suggested that adenosine cyclic 3′,5′-monophosphate (cyclic AMP) may play an important role in the transducer process common to the sensory organs.

It is known that phosphodiesterase is inhibited by caffeine, theophylline, theobromine [5] and papaverine [4]. Considering the fact that these compounds are typical bitter taste stimuli, it may be conceivable that bitter taste stimulation is associated with inhibition of phosphodiesterase in the gustatory cells. In the present study, the effect of bitter compounds on the activity of phosphodiesterase in bovine taste papillae was examined and it was found that all the bitter compounds examined in the present study inhibit the phosphodiesterase.

2. Experimental

Gymnemic acid A_1 was prepared according to Kurihara [6]. Other bitter compounds were purchased from Tokyo Kasei Co.

Bovine fungiform papillae, circumvallate papillae or the surrounding epithelium including filiform papillae were homogenized with a motor driven glass homogenizer in 40 mM Tris-HCl (pH 7.4) containing 30 mM KCl and 5 mM $MgCl_2$ and the homogenates were used for assay of phosphodiesterase activity. For examination of inhibitory effect of bitter compounds, the homogenate from the whole taste buds bearing papillae was used.

3. Results

Fig. 1 shows Lineweaver–Burk graph [8] of the kinetics of phosphodiesterase in bovine fungiform papillae, circumvallate papillae and the surrounding epithelium. As seen in the figure, fungiform and circumvallate papillae which bear taste buds contain much higher activity of phosphodiesterase than that found in tongue epithelium without taste buds. This result correlates with the fact that taste buds bearing papillae contain much higher activity of adenyl cyclase than the tongue epithelium [1]. The apparent K_m for phosphodiesterase in fungiform and circumvallate papillae was 1.2×10^{-6} M. Phosphodiesterase in these papillae was about 30% particulate.

It was found that the bitter compounds inhibit phosphodiesterase activity in the taste buds bearing papillae. The nature of inhibition by the bitter compounds, which was examined by varying cyclic AMP concentration in the presence of bitter compounds [8], was competitive for picric acid and naringin, noncompetitive for gymnemic acid A_1 and bacitracin and mixed type for quinine hydrochloride.

In table 1, inhibitory effect of the bitter compounds on phosphodiesterase in the taste buds bearing papillae is represented. No matter how there is great diversity in structure of the bitter compounds, all the bitter com-

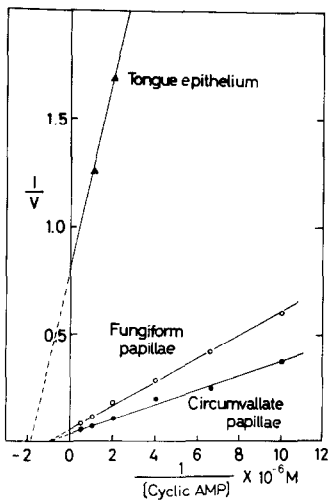

Fig. 1. Lineweaver–Burk [8] graph of the kinetics of phosphodiesterase activity in bovine circumvallate papillae, fungiform papillae and the surrounding epithelium. V is expressed as nanomoles of cyclic AMP hydrolyzed per mg protein per 10 min. The protein concentration: circumvallate papillae (130 μg/50 μl), fungiform papillae (130 μg/50 μl), epithelium (180 μg/50 μl). Phosphodiesterase activity was measured essentially according to Ward and Fain [9]. For determination of the activity, 10 μl aliquots of the homogenate were added to 20 μl of incubation medium containing 40 mM Tris-HCl (pH 7.4), 30 mM KCl and 5 mM $MgCl_2$. For examination of inhibitory effect of bitter compounds, the compounds (0.075–15 mM) were added in the incubation medium. 10 μl 0.02 μCi ^{3}H-labeled cyclic AMP (Sigma) and 10 μl unlabeled cyclic AMP in quantities sufficient to make a final concentration from 10 to 200 μM were then added. After a 10 min incubation at 37° the tubes were chilled and 125 μl of carrier cyclic AMP (0.2 mg/ml) was added. A barium–zinc precipitation (44 μl 0.2 M $ZnSO_4$, 22 μl saturated $Ba(OH)_2$) was done [10] and 100 μl of the supernatant was added to 15 ml of a liquid scintillation fluid (0.4% PPO, 0.01% POPOP in toluene–methanol (7:3, v/v)).

pounds examined in the present study showed inhibitory effect on the phosphodiesterase. For reference, the taste thresholds of the bitter compounds in man are listed in the table.

4. Discussion

In the previous paper [11] it was reported that bitter compounds strongly interact with monolayers of the lipids from bovine taste papillae and that there is a good correlation between the penetrating potency of the compounds into the lipid membrane and their taste thresholds. From these results, it was concluded that the initial event of bitter taste reception is penetration of bitter compounds into the lipid layer of the gustatory cell membrane. This suggests the possibility that bitter taste stimuli applied on the tongue surface can contact with phosphodiesterase inside the gustatory cells. The fact that all the bitter compounds examined inhibited phosphodiesterase may indicate that bitter taste stimulation is associated with inhibition of phosphodiesterase in the gustatory cells by the bitter compounds.

Since the taste thresholds of the bitter compounds are primarily defined by their penetrating potency into the lipid membrane [11], the inhibitory potency itself does not directly correlate with the taste thresholds. For example, brucine has relatively weak inhibitory potency while its taste threshold is lowest among the bitter compounds and the taste thresholds of papaverine hydrochloride and naringin are relatively high while they have a strong inhibitory potency. This discrepancy seems to come from the fact that brucine has the strongest penetrating potency into the lipid membrane among the bitter compounds and the potencies of papaverine hydrochloride and naringin are 10-fold and 30-fold lower, respectively, than that of brucine [11].

On the other hand, it is known that nucleotide triphosphates, which do not elicit bitter taste, inhibit phosphodiesterase [12]. The reason why nucleotide triphosphates do not elicit bitter taste seems to be because these compounds do not penetrate the gustatory cell membrane.

Ozeki [13] reported that quinine produced a decrease in the conductance of the gustatory cell membrane together with an increase in the receptor potential magnitude, whereas sodium chloride, sucrose and hydrochloric acid produced an increase in the membrane conductance. The present results, together with the above fact, suggest that cyclic AMP may have a mediatory function to regulate permeability of the gustatory cells to ions.

Table 1
Inhibitory effect of bitter compounds on the activity of phosphodiesterase.

	Concentration of bitter compounds (M)						
	3×10^{-5} (%)	6×10^{-5} (%)	3×10^{-4} (%)	6×10^{-4} (%)	3×10^{-3} (%)	6×10^{-3} (%)	Taste threshold (M)
Bacitracin	90.2	74.4	14.7				2.5×10^{-6} *
Papaverine-HCl	60.9	48.6	34.8				7.5×10^{-5} *
Naringin	87.9	86.7	61.6	51.0			2.5×10^{-5}
Gymnemic acid A_1		83.8	64.6	58.1	20.7		1.2×10^{-6} *
Picric acid		89.3	75.0	54.2	38.2		3.7×10^{-6}
Brucine			90.7	60.8	47.3	27.1	7×10^{-7}
Quinine-HCl			101.1	93.1	41.7	27.1	3×10^{-6}
Strychnine-HNO_3			92.1	60.0	52.8	38.7	1.6×10^{-6}
Theophylline		88.9	80.1	67.5	39.7	38.4	2.0×10^{-4} *
Caffeine			90.9	78.6	52.1	40.3	7×10^{-4}
Theobromine			90.1	84.3	58.8		7.5×10^{-4}

The activities in the presence of bitter compounds are expressed as percentage of control. The effects of some bitter compounds at higher concentration could not be examined because of their limited solubilities.

* The thresholds were determined in the present study as described in the previous paper [11]. Other values were taken from the previous paper.

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