Biochimica et Biophysica Acta 904 (1987) 187–190
Elsevier

BBA 73738

Depolarization induced by injection of cyclic nucleotides into frog taste cell

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(Received 28 April 1987)

Key words Membrane potential, Depolarization, cyclic AMP, cyclic GMP, (Frog taste cell)

In order to identify the intracellular transmitter involved in the taste transduction process, cyclic nucleotides were iontophoretically injected into the frog taste cells while membrane potentials were recorded intracellularly. Injection of either cyclic GMP or cyclic AMP induced a depolarizing response of about 5 mV in the taste cells, but injection of Cl^- had no effect. The rate of a repolarization after the depolarization elicited by cyclic GMP was larger than that after cyclic AMP. The possible role of cyclic nucleotide in the taste transduction was discussed.

Introduction

The first event in the transduction process from the taste stimulation to the electric signal of a taste cell is the adsorption of a taste substance to the receptor sites on the taste receptor membrane [1], and the final event is the depolarization of the receptor or basolateral membrane in the taste cell [2]. Since the receptor site existing in the tongue surface is apart from the depolarizing site on the basolateral membrane, the existence of an intracellular transmitter is supposed for connecting the two events. It has been reported that bovine taste buds exhibit high adenyl cyclase activity which is related to the synthesis of cyclic AMP [3]. One of the effective approaches for identifying the intracellular transmitter is to inject a possible substance into a taste cell while recording intracellularly the membrane potential [4–6].

In the experiments, we found that cyclic nucleotides injected into a frog taste cell induced a depolarization. The preliminary report has been previously published [7].

Materials and Methods

Bullfrogs (*Rana catesbeiana*) weighing 200–500 g were used for the experiment in winter (November–February). The animals were anesthetized with an intraperitoneal injection of 50% (w/v) urethane-Ringer solution (3 g/kg body wt.). The hypoglossal nerves and hyoglossal muscles were cut bilaterally to prevent spontaneous contraction of the tongue. The glossopharyngeal nerves on both sides were cut. The tongue was fully pulled from the mouth, the base of which was fixed with steel pins on a cork plate in an experimental chamber. All the experiments were carried out with in vivo preparations.

The intracellular potential in a taste cell was recorded by inserting a single glass capillary microelectrode into the taste disk of the fungiform papilla. Whether the cell impaled by a microelectrode was a taste cell or not was identified according to the electrophysiological criteria by Akaike

Abbreviation Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid

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et al. [8]. The glass microelectrodes were made with glass capillaries (outer diameter, 1 mm) into which a microfilament was inserted.

To inject Cl^- into the taste cell, negative current was passed into the cell through the microelectrode filled with 0.5 M KCl and 5 mM Hepes (50–100 MΩ). Similarly, cyclic GMP or cyclic AMP was injected by negative current using the microelectrodes filled with 0.5 M KCl, 5 mM Hepes plus 25 mM cyclic GMP or 25 mM cyclic AMP (50–100 MΩ), respectively. In these experiments, the membrane potential was simultaneously measured during the injection by the same microelectrode. As an indifferent electrode, a glass capillary (outer tip diameter, 100–150 μm) filled with 3 M KCl–3% agar was inserted into the lingual muscle. Details of the methods for recording the electrical potential have previously been described by Sato and Beidler [9].

The tongue surface was always adapted to a frog normal saline solution whose composition was 115 mM NaCl, 2.5 mM KCl, and 1.8 mM $CaCl_2$, adjusted to a pH of 7.2 by 5 mM Hepes-NaOH. Quinine hydrochloride (10 mM) (Wako, Osaka) dissolved in deionized water was used for taste stimulation. The stimulating solution and normal saline were flowed on the tongue surface at a rate of 0.1 ml/s. Cyclic GMP (potassium salt) was purchased from P-L Biochemicals (Milwaukee), and cyclic AMP (sodium salt) from Sigma (St. Louis).

All the experiments were carried out at a room temperature of 20–25 °C.

Results

When cyclic GMP was iontophoretically injected into a frog taste cell, the cell was depolarized transiently. The rising phase of the depolarization was very rapid. The magnitude and duration of the depolarization were increased with an increase of injected current intensity (Fig. 1). In the taste cell in Fig. 1, injection of cyclic GMP by 2 nA initiated a depolarization whose magnitude was about 5 mV and whose duration was about 25 s. Injection of cyclic AMP depolarized the cell as well. The longer the duration of the single injection, the larger the depolarization arose (Fig. 2A). The repetitive injections sustained the

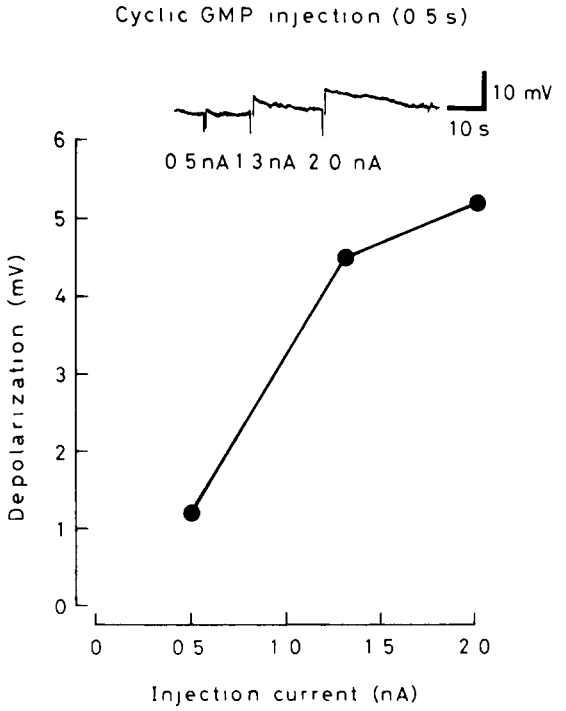

Fig 1 Relation between the current intensity for intracellular cyclic GMP injection and the magnitude of depolarization in a taste cell The actual depolarizing responses are shown over the plot The numeral under each dot means injected current intensity The duration for injection was 0 5 s

duration of the depolarization by the summation (Fig. 2B).

Injection of Cl^- did not induce the slow depolarization. Sometimes, an anodal break spike potential [10] was observed after the taste cell had

TABLE I

COMPARISON BETWEEN DEPOLARIZATIONS BY CYCLIC NUCLEOTIDES AND RECEPTOR POTENTIALS BY 10 mM QUININE HYDROCHLORIDE

Cyclic nucleotides were injected by a 5 s pulse of 1 5 nA Values indicate means ± S E of seven cells Q-HCl, quinine hydrochloride

Injected nucleotide or taste stimulus	Depolarization (mv)
Cyclic GMP	5 2±1 5
10 mM Q-HCl	5 0±1 7
Cyclic AMP	6 0±0 8
10 mM Q-HCl	6 3±0 6

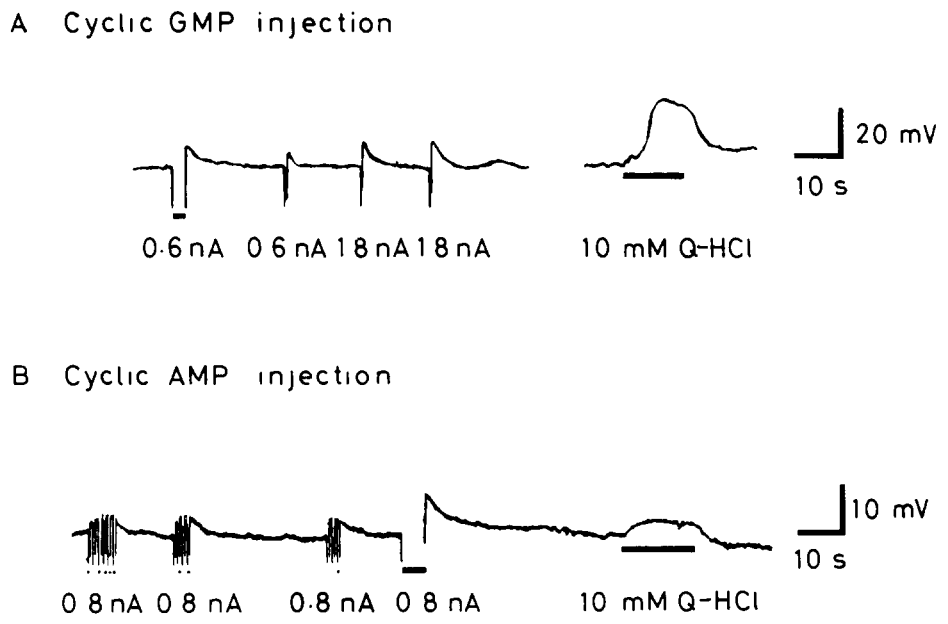

Fig 2 Depolarizations induced by cyclic GMP and cyclic AMP injected into taste cells In A, cyclic GMP was injected by single pulses (2 5 s and 0 5 s) In B, cyclic AMP was injected by repetitive (1 Hz, 0 5 s) and single (5 s) pulses 10 mM quinine hydrochloride (Q-HCl) was given on the tongue surface Dots and horizontal bars under records indicate the duration of cyclic nucleotide injections and of quinine hydrochloride applications

been hyperpolarized during an injection of Cl^-. Since the duration of the spike potential was shorter than 20 ms, the slow depolarization induced by cyclic nucleotide injection was quite different from the spike potential.

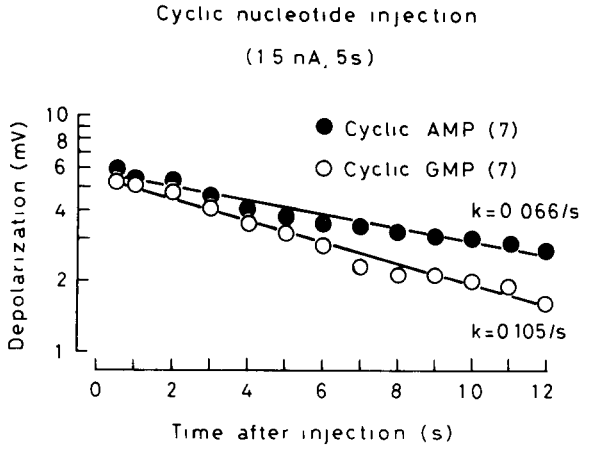

Fig 3 Time course of repolarization after the peak of the depolarization induced by single injeciton of cyclic nucleotide The averaged values are plotted on a semi-logarithmic scale Assuming that first-order kinetics was present, the rate constant of repolarization (k) was calculated from the best-fitted line

Of 13 taste cells depolarized by 10 mM quinine hydrochloride, the depolarization elicited by injection of cyclic GMP (1.5 nA, 5 s) was found in only seven cells. Cyclic AMP was injected into 14 taste cells depolarized by 10 mM quinine hydrochloride and the depolarization was found in seven cells. A depolarization of 5–6 mV in response to 10 mM quinine hydrochloride was the same value as that induced by a single injection (1.5 nA, 5 s) of cyclic nucleotide (Table I).

The time courses of repolarizations after the peaks of cyclic nucleotide-induced depolarizations were shown in Fig. 3. The rate of the repolarization was calculated on the assumption of first-order kinetics. The rate for cyclic GMP was 60% larger than that for cyclic AMP.

Discussion

Intracellular injection of cyclic nucleotides into a frog taste cell induced a depolarizing response whose magnitude and duration depended on the current intensity. Like the retinal rod [4,5], the rising time of the depolarization was very fast (Figs. 1 and 2). Since the dendrite-like processes

of a frog taste cell have a very small diameter, a microelectrode used for both the cyclic nucleotide injeciton and the membrane potential measurement was assumed to be impaled into the cell body. It is unlikely that injected cyclic nucleotides could diffuse rapidly from the cell body to the receptor membrane situated at the top of the dendrite-like processes, so that the fast rising phase of the depolarization suggests that the acting site of cyclic nucleotides might be the basolateral membrane in the taste cell.

Cyclic GMP directly activates a cationic channel which exists in the outer segment of the retinal rod [11]. This mechanism supports the fast sensory response in vision. The fast rising of cyclic nucleotide-induced depolarizations in the frog taste cell may be due to the direct action of the drug to an ionic channel of the basolateral membrane which is not coupled with the phosphorylation of a protein by protein kinase.

Cyclic AMP as well as cyclic GMP depolarized the frog taste cell to the same degree (Table I). Recently, the excised patch method exhibited that a cationic conductance in the ciliary membrane of toad olfactory receptor is activated by both of cyclic AMP and cyclic GMP [12].

The present experiments suggest that when a taste substance binds to a receptor site on the taste receptor membrane, a cyclic nucleotide will be produced in the cell, and then the nucleotide will depolarize the cell by activating ionic channels in the receptor and basolateral membranes.

Application of various analogs of cyclic nucleotide into the taste cell using a patch pipet will prove the presence of cyclic nucleotide-activated ion channel in the cell [13].

Acknowledgements

This work was supported in part by Grants-in-Aid (Nos. 5923011, 60480402 and 61771461) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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