ELECTRICAL RESPONSE OF THE FROG'S GUSTATORY CELLS TO CENTRIFUGAL STIMULATION

A. I. Esakov and A. L. Byzov

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In response to stimulation of the lingual nerve, an intracellular hyperpolarization potential of the taste receptor cell and intracellular spikes from synaptic thickenings of efferent nature were recorded.

Some special features which distinguish the region of synaptic contact of the taste receptor cell [5, 16, 14] and the existence of efferent synapses on the receptor cell [4, 11] suggest the possibility of centrifugal regulation of the activity of the receptor structures themselves.

In investigations of gustatory reception, efferent regulation of the activity of the taste receptors in the frog's tongue was shown to be possible [1-3]. Spike activity in the taste fibers was studied in those investigations, but on this basis alone it was impossible to state whether the change in the character of the afferent flow of impulses was the result of effects on the receptor structures (sensory epithelial cells) themselves, in which the primary process of reception takes place, or of effects on the region of spike generation in the afferent fiber in contact with the gustatory cell.

An intracellular response of the gustatory cells to stimulation of the tongue by substance possessing taste qualities was described by Beidler et al. [8, 9, 13] in experiments on rats and hamsters, and also by Sato [12] in frogs. The criterion of the intracellular position of the microelectrode in these investigations was the presence of a resting potential (RP) and of a depolarization potential wave in response to application of various solutions to the tongue. However, all cells possess an RP, so that by itself it cannot be used to prove that the recording comes from inside the receptors. Furthermore, the electrical response recorded from the tongue to the application of solutions to its surface, as control experiments show, may be physicochemical in nature and unconnected with the process of reception. For this reason, in the present investigation attention was concentrated on the problem of identification of the receptors.

EXPERIMENTAL METHOD

Experiments were carried out on the tongue of frogs (Rana temporaria) with intact lingual nerves and blood supplies. A glass microelectrode, filled with 2.5 M Na_2SO_4 solution and with an impedance of 100-400 MΩ, was inserted into the fungiform papillae of the tongue under a binocular microscope. The reference electrode (Ag–AgCl) was located in the opposite side of the tongue. The microelectrode was connected to a cathode follower and S1-19 oscilloscope, from the screen of which the responses were photographed. Electrical stimulation of the lingual nerve at a frequency of 1-100 Hz (from an ÉSL-1 stimulator) was applied through a pair of platinum electrodes.

The cells were tagged by an intracellular labeling method using the dye Niagara sky blue [7]. The dye, with which the microelectrode was filled, was expelled from its tip under visual control by a short (2 msec) pulse of current (−120 V on the microelectrode). The area of the tongue containing the tested fungiform papilla was fixed with glutaraldehyde, dehydrated in alcohol, embedded in Epon, and cut into sections

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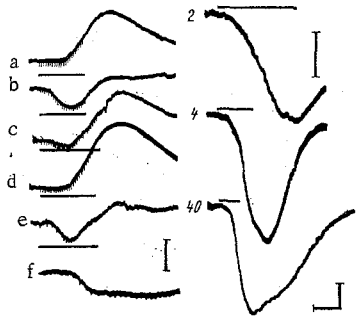

Fig. 1

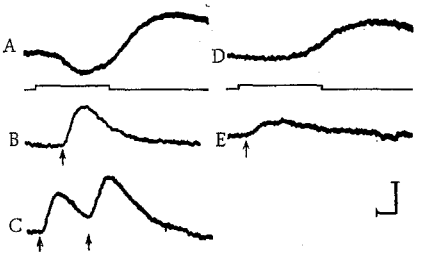

Fig. 2

Fig. 1. Electrical response of cells of a fungiform papilla to stimulation of the lingual nerve. On left: a) microelectrode on surface of papilla; b) intracellular hyperpolarization of gustatory cell, resting potential 30 mV; c) microelectrode leaves cell (resting potential and intracellular hyperpolarization are reduced); d) microelectrode outside cell (extracellular response is visible); e) microelectrode again in the same cell. Frequency of stimulation for records a-e 8 Hz; f) hyperpolarization response to prolonged stimulation at 2 Hz. Lines below curves show duration of stimulation. Right: hyperpolarization response of gustatory cell (in another experiment) to stimulation of nerve at different frequencies (numbers on left show frequency in Hz). Calibration 10 mV, time marker 2 sec in all cases; in bottom right curve (40) amplification is reduced.

Fig. 2. Response of cells of taste papilla to CO_2: A) hyperpolarization response to nerve stimulation; B) depolarization response to brief stimulation with CO_2; C) the same to repeated stimulation; D) response to nerve stimulation after microelectrode had left cell; E) response to same dose of CO_2 after microelectrode had left cell. Top marker shows duration of electrical stimulation of nerve, arrows indicate times of administration of CO_2. Below on right, calibration: 5 mV, 2 sec.

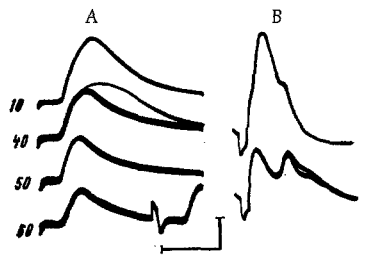

Fig. 3. Intracellular recording of potentials from papilla in response to stimulation of lingual nerve: A) shape of impulses for different frequencies of stimulation (numbers on left show frequency in Hz); B) changes in shape of impulse during experiment, frequency of stimulation 2 Hz; superposition of 10-30 sweeps of the beam. Calibration: 10 mV; 5 msec.

8 μ in thickness. In sections stained with eosin, the blue label inside the cell was clearly visible against the background of the pink color of the tissue.*

Other details of the method will be mentioned during the description of the results.

EXPERIMENTAL RESULTS AND DISCUSSION

An electrical response to stimulation of the lingual nerve can be recorded as soon as the microelectrode touches the surface of the tongue. The response consisted of a positive wave, which could be recorded not only from the papillae but also from all parts between them (Fig. 1a). It appeared after a long latent period (1.5-2 sec) and sometimes reached an amplitude of tens of millivolts. An analysis of its origin, which is published separately, showed that this response is generated in the surface epithelium of the whole tongue and is unconnected with the activity of the taste cells themselves.

* All the histological work was done by I. A. Utina, to whom the writers are sincerely grateful.

Insertion of the microelectrode into the papillae to a depth of 20-40 μ often led to the appearance of an RP of 20-40 mV. In some cases stimulation of the nerve was accompanied by the same electrical response as that from the surface of the papilla, so that the recorded potential was extracellular in origin. In other cases, however, a hyperpolarization response appeared after a much shorter (0.8-1 sec) latent period (Fig. 1b). The hyperpolarization response to stimulation of the nerve appeared only when the microelectrode was intracellular in position, i.e., in the presence of a stable RP. When the microelectrode came out of the cell the RP decreased, and this was accompanied by a parallel decrease in the hyperpolarization response, which was replaced by an extracellular positive potential generated after a longer latent period (Fig. 1c, d). Reinsertion of the microelectrode into the cell led to restoration of the RP and the hyperpolarization response (Fig. 1e).

The response to stimulation of the nerve described above was recorded only in the cells of the papillae and was never observed outside the papillae, thus proving its receptor origin. Further proof of this is given by the results of labeling experiments. In the presence of an RP and hyperpolarization response to stimulation of the nerve, dye injected into the cell was found in histological sections as a tag measuring about 4 μ in the region of localization of the receptor cells.

The relationship between the hyperpolarization response and the frequency of stimulation of the lingual nerve is shown on the right in Fig. 1. With an increase in the frequency of stimulation (the numbers on the left of the curves) the magnitude of the response and the steepness of its rise increased, while the latent period was reduced. During prolonged stimulation at low frequency the response was maintained at a stable level throughout the period of stimulation (Fig. 1f).

If the response observed was in fact generated by the gustatory cells, the appearance of a depolarization response to chemical stimuli would be expected. The use of taste stimuli as solutions, as was done in earlier investigations [8, 9, 11, 13] and also in the writers' preliminary experiments, easily leads to the appearance of artefacts. Accordingly, in the subsequent experiments the chemical stimuli were applied in the gaseous state (acetic acid vapor or CO_2 [10], applied in small quantities from a syringe).

If the microelectrode was intracellular in position, i.e., in the presence of a stable RP and hyperpolarization response to stimulation of the nerve (Fig. 2A), application of small quantities of CO_2 evoked a positive potential (Fig. 2B, C). After the microelectrode had left the cell (as shown by disappearance of the RP and hyperpolarization response to nerve stimulation – Fig. 2D), the response to approximately the same dose of CO_2 was sharply reduced (Fig. 2E). Consequently, the response was mainly intracellular and it probably reflected depolarization of the gustatory cell. Similar positive waves of potential when the microelectrode was intracellular in position were frequently observed after application of acetic acid vapor also.

It must be emphasized, however, that neither the results described above nor, more especially, those obtained by Beidler and Sato in their experiments are strict proof of the receptor origin of the responses to CO_2 or to acetic acid vapor, for these agents, in somewhat larger doses, evoked similar waves of potential even with the electrode in the extracellular position.

In a series of experiments, intracellular spikes were recorded in the papillae at a depth of 30-50 μ in response to stimulation of the nerve. Examples of these recordings are given in Fig. 3. Usually the potentials were recorded up to very high frequencies of stimulation (up to 100 Hz or more), indicating the high lability of the corresponding nerve fibers. It is difficult to imagine that such recordings are possible from the fibers themselves, which are less than 1 μ in thickness in the papilla. Recently, Gray [6] has described synaptic swellings, up to 4 μ in diameter, in the taste papillae of the cat. The same synaptic thickenings, which are also evidently present in the frog, could be the source of the impulses recorded in the present experiments. This hypothesis would explain the complex shape of the impulse, consisting of more than one component. The inflection frequently observed on the ascending part of the curve (Fig. 3B) or close to its apex (Fig. 3A; compare frequencies of 10 and 40 Hz) can be explained by delay in the transmission of the impulse from the fiber to the thickening. The unmyelinated thickening probably cannot reproduce the high frequency of stimulation, so that with an increase in the frequency of stimulation the second component disappears (Fig. 3; 40 Hz). Loss of one of the components was sometimes observed in the course of an experiment (Fig. 3B), probably as the result of injury to the membrane of the thickening by the microelectrode.

What is the nature of the synaptic thickenings described by Gray [6]? He does not state whether they belong to afferent or efferent nerve endings. The present observations are more indicative of efferent endings for the following reason: first, slow changes of potential analogous to the hyperpolarization response of the gustatory cells were never observed in this case, and second, stimulation of the tongue by acetic acid vapor never evoked a discharge of these impulses.

It can be concluded from these results that centrifugal regulation can take place at the level of the gustatory cells, the actual receptor structures of the taste papilla. The hyperpolarization potential recorded in the receptor cell in response to stimulation of the nerve is in all probability a postsynaptic response (the analog of the inhibitory postsynaptic potential – the IPSP). The mechanism of the effect of hyperpolarization of the gustatory cell on the spike response in the afferent fibers is not yet clear, and the nature of the inhibitory synapse is likewise unknown. However, there is no doubt about the correlation between the hyperpolarization produced by electrical stimulation of the lingual nerve and the inhibition of spike activity observed previously [1] under the same experimental conditions.

LITERATURE CITED

1. A. I. Esakov, Byull. Éksperim. Biol. i Med., No. 3, 3 (1961).
2. A. I. Esakov, in: Primary Processes in Receptor Elements of the Sense Organs [in Russian], Moscow–Leningrad (1966), p. 102.
3. A. I. Esakov, Fiziol. Zh. SSSR, No. 5, 575 (1967).
4. A. De Lorenzo, in: Olfaction and Taste, Oxford (1962), p. 64.
5. A. T. Farbman, J. Ultrastruct. Res., 12, 328 (1965).
6. E. G. Gray and K. C. Watkins, Z. Zellforsch., 66, 583 (1965).
7. A. Kaneko and H. Hashimoto, Vision Res., 7, 847 (1967).
8. R. Kimura and L. Beidler, Am. J. Physiol., 187, 610 (1956).
9. R. Kimura and L. Beidler, J. Cell. Comp. Physiol., 58, 131 (1961).
10. J. Konishi, J. Hidaka, M. Toyota, et al., Jap. J. Physiol., 19, 327 (1969).
11. A. Nemetschek, Z. Zellforsch., 63, 155 (1964).
12. T. Sato, Experientia, 25, 709 (1969).
13. H. Tateda and L. Beidler, J. Gen. Physiol., 47, 479 (1964).
14. O. Trujillo-Cénoz, Z. Zellforsch., 46, 272 (1957).