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Electrical responses to taste chemicals across the dorsal epithelium of bullfrog tongue

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Summary. Stimulation of the bullfrog tongue with taste chemicals produced a slow change in transepithelial potential difference across the dorsal epithelium. The potential profile was in many respects similar to that of the intracellularly recorded potential changes in taste cells and to the activity of taste fibers in frogs.
Key words. Bullfrog tongue; dorsal epithelium; taste chemicals; transepithelial potential difference.

It has been shown that the dorsal epithelium of the dog tongue immersed in Krebs-Henseleit solution transports Na and Cl ions actively, producing a transepithelial potential difference across the tissue, which is increased by application of hypertonic NaCl solution to the mucosa and is associated with decreased resistance of the tissue[2].

In the present study, we show that the dorsal epithelium of the bullfrog tongue in contact with Ringer's solution does not develop a transepithelial potential difference, but responds to various chemicals on the mucosa in association with changes of the potential difference and of the tissue resistance. The potential change causes a change of passive transport of some ions through the tissue and may play some role in the process of taste reception.

Materials and methods. Bullfrog tongue *(Rana catesbeiana)* was isolated, and the dorsal epithelium was dissected from the underlying muscle. The tissue was mounted vertically on an apparatus designed to isolate the mucosal and the serosal surfaces (each 1 cm^2, elliptic in shape), using a modified Ussing chamber[3]. Both surfaces of the tissue were continuously perfused with aerated Ringer's solution (NaCl 112, KCl 2, $CaCl_2$ 1.8, and $NaHCO_3$ 2 mM per liter). To record the potential difference, agar-1 M KCl electrodes with an Ag-AgCl wire were placed immediately below each surface, and connected to an electrical recording apparatus via a high impedance amplifier. For stimulation, NaCl, acetic acid, quinine hydrochloride or sucrose mixed with Ringer's solution were applied to the mucosa for 50 sec instead of Ringer's solution.

Results and discussion. Only a negligible transepithelial potential difference (-1.3 ± 2.1 mV, serosa grounded) was observed across the tissue in contact with Ringer's solution alone, compared with the frog skin (-76.3 ± 18.9 mV) and the bladder membrane (-84.1 ± 17.3 mV), which were measured using the same apparatus. These findings suggest that active ion transport in this tissue is ineffective compared with transport in frog skin[3], toad bladder[4] and dog tongue[1,2].

After adaptation to Ringer's solution, a stimulation of the mucosa with NaCl, acetic acid or quinine produced a slow change of the potential difference; in the mucosa the change was negative to the grounded serosa in response to NaCl, acetic acid and quinine (NaCl-, acetic acid- and guinine-responses), while with sucrose the mucosa became positive (sucrose-response). Resting potential was restored upon withdrawal of stimulants within 1–10 min as shown in figure 1. These responses, characteristic of the agents and their concentration, resembled those

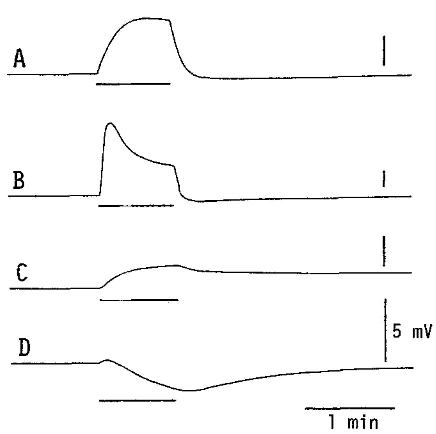

Figure 1. Records showing changes in transepithelial potential difference between the mucosa and the serosa of the same dorsal epithelium in bullfrog tongue, appearing when stimuli were applied to the mucosa during a period presented by a horizontal bar in each record. Stimulating chemicals mixed with Ringer's solution applied to the mucosa were 0.2 M NaCl (A), 5 mM acetic acid (B), 5 mM quinine (C) and 0.5 M sucrose (D). Upward direction indicates the mucosa electrically negative to the serosa, grounded, and vice versa. Vertical bars in all records show 5 mV.

of intracellular potential changes in taste cells[5,6] and in non-taste cells[7,8]. During the experiment, near zero potential was determined by punching a hole into the tissue, both solutions being identical. No potential was elicited when any stimuli were applied to the serosa. Relations between stimuli concentrations and the maximum magnitude of the responses to each stimulus are shown in figure 2. These relations, excluding the sucrose-response, are in approximate agreement with those of the receptor potentials in taste cells, and with those of neural activity in frogs[5]. The responses to each stimulus were reversibly depressed in the presence of 0.05 to 0.5% tetracaine or procaine on the mucosa, as was observed in frog taste cells[9]. The results indicate that the response to chemicals is not simply a physicochemical potential occurring at a fluid interface.

The magnitude of the response to each stimulus was not influenced when the air supply to the Ringer's solution was turned off, or 1 mM ouabain or 1 mM 2,4-dinitrophenol (DNP) was applied to the serosa and/or the mucosa for over 1 h. On the other hand, in the frog skin and bladder, NaCl-responses obtained using the same apparatus rapidly decreased to zero in the presence of ouabain or DNP, being associated with a marked reduction of the resting potential difference. These observations further indicate that the dorsal epithelium, including taste disks, can receive taste chemicals and produce the responses caused by a change of passive transport of some ions through the tissue rather than through active transport. Different results have been reported for the dog tongue[1,2]. This could be due to the fact that an amphibian isolated tissue is generally insensitive to oxygen and temperature and is therefore easier to maintain alive than a mammalian tissue.

The tissue resistance, which was measured by an application of 100-msec pulse currents (10^{-6} to 10^{-5} A, 0.5 Hz) through a Wheatstone bridge, was 710 ± 180 Ω/cm^2 in resting state. This value is similar to that obtained for the dog tongue[1,2]. During NaCl- and acetic acid-responses a marked decrease in the tissue resistance was observed. On the average, it was changed to 22.6% by 0.2 M NaCl and 56.3% by 5 mM acetic acid from 100% in resting state. Also, in the majority of cases, a small decrease in resistance was produced by 5 mM quinine and a small increase was produced by 0.5 M sucrose. This change in resistance gives further support to the explanation of the production of transepithelial potential changes. Thus, lingual epithelial responses to taste chemicals may have some influence on taste reception, as has been suggested for the dog tongue[1,2].

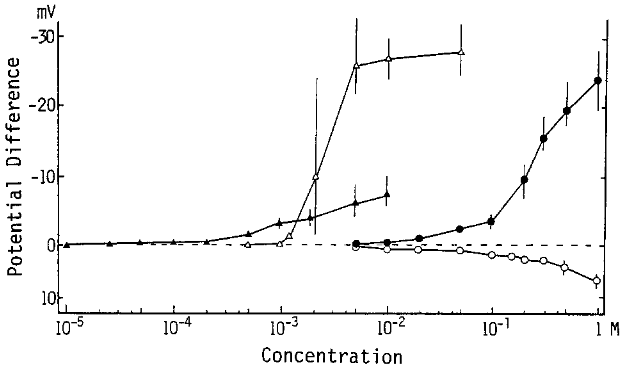

Figure 2. Relations between stimulus concentration and response magnitude, obtained from the dorsal epithelium. Stimuli indicated by solid circles, open circles, open triangles and solid triangles are NaCl, sucrose, acetic acid and quinine, respectively. Each point and its vertical bar in the figure represent the mean values of maximum magnitude of responses and their standard deviations obtained from 7–16 experiments.

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The effect of stimulus frequency on transmission in ganglia of Remak's nerve in the chicken

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Summary. Electrical stimulation of side branches of Remak's nerve evoked a synaptically-mediated spike discharge at the oral end of the nerve which increased in amplitude in response to successive stimuli. The maximum amplitude of the synaptic component was attained at a frequency of about 3 Hz but was almost completely absent at 31 Hz.

Key words. Chicken; ganglionic transmission; Remak's nerve; stimulus frequency.

The organization of Remak's nerve in the chicken is still only poorly understood. The information that is available has come from histochemical[1], pharmacological and electrophysiological studies[2–4]. In a recent study[5,6] evidence was presented suggesting that enteric cholinergic neurones formed part of a pathway which projected orally along Remak's nerve (the intestinal nerve) in the chicken. The function of these cholinergic neurones is not known nor is the identity and projection of the post-ganglionic neurones in Remak's nerve with which they synapse. During the course of the study it was noted that the response recorded from the oral end of the nerve trunk, evoked by stimulation of a side branch between the nerve and the gut, increased in amplitude in response to successive stimuli in a train, although the stimulus intensity remained unchanged. In the present study an attempt has been made to characterize the effect of stimulus frequency on the growth of the post-ganglionic response.

Methods. Nine chickens between 6 and 12 weeks of age were killed by cervical dislocation. About 2–3 cm of Remak's nerve trunk with side branches intact were removed and pinned onto silicone resin (Sylgard, Dow Corning) which lined the base of the organ bath. The bath contained Krebs solution of the fol-