

increase in membrane conductance was unaltered by ouabain. When the membrane was hyperpolarized stepwise, both ouabain-insensitive and ouabain-sensitive ACh-currents were reduced accordingly, and finally reversed their polarities at a potential level between -90 and -120 mV, $n=7$. These results suggest that an increase in potassium permeability is responsible for those ACh-currents. Since the ACh-current was sensitive to ouabain, the contribution of an electrogenic Na-pump to the ACh-current was examined by studying the changes of the K-activated current¹⁰ in the presence or absence of ACh. The K-activated current is a membrane outward current produced by an activation of the electrogenic Na-pump when the perfusate is changed from K-free to Ringer (2 mM K) solution¹⁰⁻¹². In 14 experiments, the K-activated current was markedly depressed (more than 90%, 8 cells) or eliminated (3 cells) and even reversed its direction (3 cells) in the presence of 10^{-5} M ACh (figure 2). Since the K-activated current consists of the net outward current (the electrogenic Na-pump current) and the passive inward K current¹⁰, a

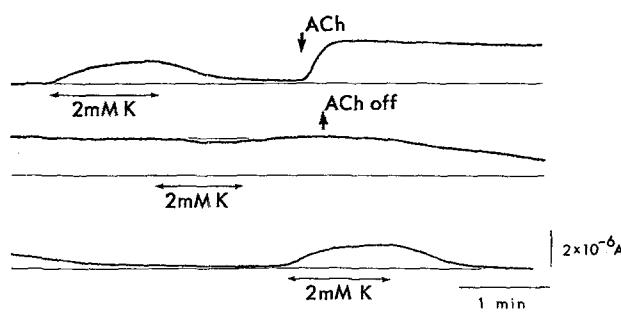


Fig. 2. The effect of 10^{-5} M ACh on the K-activated current; periods of application of 2 mM K and 10^{-5} M ACh are marked by horizontal and vertical arrows, respectively.

reversal of its direction indicates an almost complete loss of the net outward current. These results suggest that the electrogenic Na-pump is already activated in the K-free solution by ACh. A possible reason could be that the increase in K-permeability by ACh increases the net outflux of K⁺ (ouabain-insensitive), and consequently the K⁺ concentration immediately outside of the membrane; similar extracellular K⁺ accumulation was suggested during activity of heart muscles¹³. Such an increase in extracellular potassium ions in the K-free solution could activate the Na-Pump (ouabain-sensitive). According to this concept, the mechanism of ouabain-sensitive ACh-current is similar to that of the K-activated current, and the K-activated current would therefore be depressed during the generation of the ACh-current. To other results presented in this paper could also be reasonably explained by this concept.

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Frog's tongue receptive areas: Neural organization and gustatory function

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Summary. Lateral and medial branch of the frog's IXth nerve innervates rostral third and caudal two-thirds of the tongue surface, respectively. The amounts of gustatory signals in these branches differ in proportion to the area they supply.

Taste organs or the fungiform papillae in the frog tongue receive gustatory as well as somatic fibres only from the glossopharyngeal (IXth) nerve. Neural organization of the tongue receptive area in the frog has thus been considered simple compared with that in the mammalia, whose taste organs in the anterior two-thirds and the posterior third of the tongue are supplied by the chorda tympani and the IXth nerve, respectively. However, because the frog's IXth nerve divides into 2 branches before entering the tongue, it is possible that each of the branches innervates a defined tongue area, as in mammals. We examined this possibility in relation to functional significance on taste reception.

American bullfrogs (*Rana catesbeiana*), weighing between 270 and 450 g, were mainly used besides the common frogs (*Rana nigromaculata*). They were anesthetized with 20% urethane solution (15 ml/kg, i.p.). The IXth nerve and its distal ramifications, the medial (m. br.) and lateral (l. br.) branches, were dissected; the latter 2 being cut at the proximal junction, if required. The nerve was mounted on a pair of silver wire electrodes. For stimulation of the

nerve, brief electric pulses with 1/sec in repetition rate were employed. To record electrical signals from a single fungiform papilla, it was sucked into a glass capillary electrode filled with Ringer's solution. The signals were amplified by c.r. coupled amplifiers, summated by an averaging computer and displayed on a X-Y recorder. When recording taste responses from the nerve, the amplified signals were led to an integrator of a time constant of 0.2 sec, and recorded on a pen-recorder. In some instances, the recording was made from bilateral nerves, simultaneously. Taste stimuli employed were 0.5 M sodium chloride, 0.05 mM quinine hydro-chloride, 0.125 mM hydrochloric acid and 0.1 M sucrose solutions, the latter 3 dissolved in 0.01 M saline. They were applied to, and kept for about 5 sec in, a lucite chamber in which the tongue had been fixed. During this period, one or both sides of the lingual artery was perfused with Ringer's solution, the method being similar to that of other authors^{1,2}. The fungiform papillae were counted in number after injection of 0.05% methylenblue solution into 1 of the lingual arteries. In this study, the tongue attached

to the mandibular symphysis was called the rostral portion, its opposite free end the caudal portion³.

As shown in the left diagram in figure 1, the m. and l. br. of the IXth nerve was stimulated at their proximal end and the resulting antidromic spikes were recorded from different fungiform papillae (numerals from 1 to 5) by moving the suction electrode from the rostral to caudal direction. It illustrates that stimulation of the m. br. can elicit antidromic responses only in the caudal half of the papillae (No. 3-5), whereas the responses of the rostral papillae (No. 1-3) occurred following stimulation of the l. br. A papilla (No. 3) thus receives dual innervation from the both branches. Of 5 frogs examined, the papilla was located within a narrow zone transverse to the longitudinal axis of the tongue. All the sense organs within the field rostral and caudal to this zone were innervated by the l. and m. br., respectively. To express the dimension of the former and the latter field, the longitudinal distance from the zone to the rostral tip, and from that to the caudal end of the

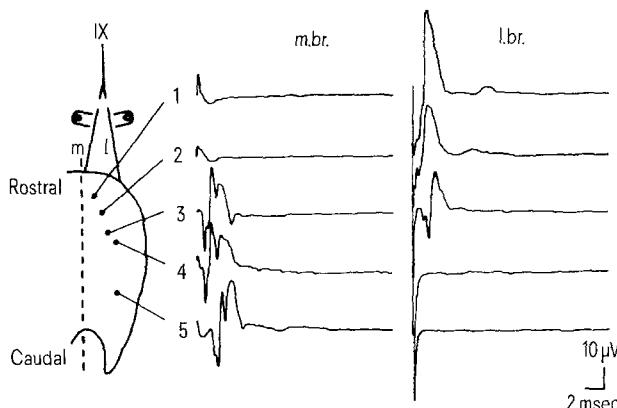


Fig. 1. Records demonstrating electrical responses of 5 fungiform papillae to antidromic stimulation of the IXth nerve branches in a bullfrog. Left and right trace in each row represents the response of a single papilla to successive stimulation of the medial (m. br.) and lateral (l. br.) branch, respectively. In each trace, 30 single responses were averaged in reference to the onset of stimuli. Schematic diagram at the left exhibits the IXth nerve and its branches (m and l) supplying 1 of the tongue halves.

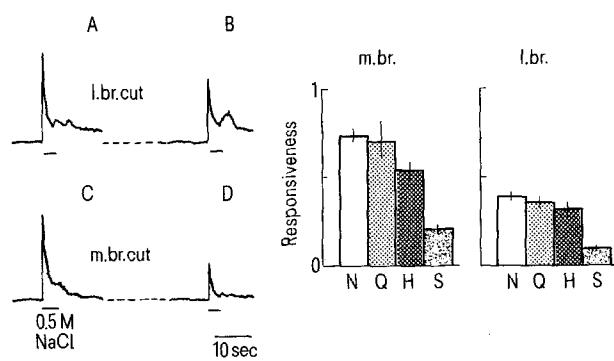


Fig. 2. Left records (A-D) illustrate the effect of transection of the l. and m. br. on the whole IXth nerve response to 0.5 M NaCl. Upper (A and B) and lower (C and D) records were obtained simultaneously from the left and right IXth nerve in a bullfrog, respectively. Right graphs show responsiveness of the field within the m. and l. br. to 4 kinds of taste stimuli. It was expressed relative to the responsiveness of the whole IXth nerve field. N, Q, H and S denotes 0.5 M NaCl, 0.05 mM quinine, 0.125 mM HCl and 0.1 M sucrose solution, respectively. Each vertical bar indicates the mean \pm SE.

tongue relative to the whole tongue length was taken, respectively. The relative dimension of the l. br. field determined in this way was found to be $32.4 \pm 1.3\%$, while that of the m. br. field was $70.8 \pm 1.6\%$, in the mean \pm SE of 10 bullfrogs. Similar results were obtained for the frog. The number of the fungiform papillae in one of the longitudinal halves of the bullfrog tongue was found to be 497 ± 18.3 (mean \pm SE of 10 animals), which was approximately twice that of the frog^{4,5}. Because, in the above experiment, 32.4% of the tongue area was supplied by the l. br. of the IXth nerve, the number of single papillae within this area was also counted. The result showed that, of all the papillae, 31.9% were distributed over the rostral portion, the remaining papillae over the caudal portion of the tongue. The findings indicate that the density of taste organs within the 2 tongue portions is approximately equal. To evaluate taste sensitivity in each of the 2 gustatory areas, the initial maximum magnitudes of taste responses in the m. and l. br. were expressed relative to the response of each branch to 0.5 M NaCl. The relative magnitude of response of the m. br. to quinine, HCl and sucrose was found to be 0.97 ± 0.13 , 0.73 ± 0.08 and 0.28 ± 0.05 , respectively, whereas that of the l. br. to the respective stimulus was 0.92 ± 0.07 , 0.71 ± 0.06 and 0.36 ± 0.06 , in the mean \pm SE of 6-7 bullfrogs. The difference between the magnitudes of responses of the 2 branches to each taste was found to be insignificant ($p < 0.05$), showing that taste organs in the rostral tongue region did not differ in the relative sensitivity from those in the caudal region. For quantitative comparison of taste responsiveness in the m. br. with that in the l. br., the magnitude of response to 0.5 M NaCl relative to one another is necessary. The procedure, similar to that described by Ishiko⁶, is illustrated in the records (A-D) in figure 2 in which a decrease in the intact IXth nerve response (A and C) to the saline following transection of either the l. or the m. br. is indicated. The magnitude of response in B and D thus demonstrates the response of the m. and l. br. to the saline, respectively. Calculation of B/A and D/C in 8 bullfrogs yielded the ratio of 0.74 ± 0.03 and 0.39 ± 0.02 , respectively, the results being attributed in proportion to the difference in the dimension or in the number of fungiform papillae between the 2 tongue regions. Multiplying each ratio by the relative magnitudes of taste responses of the corresponding branch already mentioned, the responsiveness of the m. br. to 4 basic taste stimuli is comparable with that of the l. br. as shown in the right graphs in figure 2.

The present study revealed that the frog tongue can be demarcated into 2 receptive areas, the rostral third and caudal two-thirds. Unlike the result in some mammals⁶⁻⁸, these areas in the frog did not show any local specificity to 4 qualities of taste substances. The l. and m. br. supplying the rostral and caudal region of the tongue thus appears to transmit gustatory signals of different quantities which are proportional to the dimensional difference between the 2 regions.

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