

Interaction among Different Sensory Units within a Single Fungiform Papilla in the Frog Tongue

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ABSTRACT The possible interaction among different sensory units in the frog tongue was studied using several single papillae dually innervated by the medial and lateral branches of the glossopharyngeal (IXth) nerve. The afferent activity in one branch exposed to NaCl stimulation of the papilla revealed marked inhibition after antidromic electrical stimulation (100 Hz, 30 s, and 3 V) of the other branch. The degree of inhibition depended on the number of sensory responses observed in the electrically stimulated branch as well as the nature of the stimulated sensory units. Statistical analysis suggested that antidromic activation of gustatory units conducting the responses to NaCl and quinine and slowly adapting mechanosensitive units produced a large antidromic inhibition amounting to 19–25%, but that of gustatory units conducting the responses to acetic acid and rapidly adapting mechanosensitive units gave rise to only a slight inhibition. To examine the differential effects of these sensory units in antidromic inhibition, antidromic impulses were evoked by chemical stimulation of the adjacent papilla neuronally connected with the dually innervated papilla under study. Antidromic volleys of impulses elicited by NaCl or quinine stimulation produced a large inhibition of the afferent activity in the other branch, as induced by NaCl stimulation of the dually innervated papilla. Plausible mechanisms of synaptic interaction in peripheral gustatory systems are considered.

INTRODUCTION

Afferent impulses conducting gustatory information are modified in the peripheral nervous system as well as in the central nervous system. In the bullfrog (*Rana catesbeiana*), fungiform papillae, which contain taste cells, number ~1,000 on the entire surface of the tongue (Ishiko et al., 1979). These papillae serve as a gustatory organ and each one is innervated by 7–14 nerve fibers including taste, somatic, and autonomic fibers (Chernetski, 1964*a, b*; Rapuzzi and Casella, 1965; Hanamori and Ishiko, 1981). These fibers are supplied from either the medial or lateral branch of the glossopharyngeal (IXth) nerve, which they innervate, depending the location of the papilla on the tongue (Ishiko et al., 1979). Sensory fibers lose their myelin sheaths

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before entering the papilla and form a plexus at the base of the papilla, and some of the terminals synapse with taste cells in the receptor layer. Each fiber gives off three to eight collateral branches to different papillae, and in turn, each papilla is interconnected by two to four fibers (Taglietti et al., 1969), making neuronal connections linking different papillae through collateral branches of a sensory fiber. Therefore, the orthodromic activity arising in one papilla antidromically invades the adjacent papilla and inhibits the afferent activity of the same neuron primarily evoked in the latter papilla (Rapuzzi and Casella, 1965; Taglietti et al., 1969; Taglietti, 1969; Miller, 1971; Macdonald and Brodwick, 1973).

With regard to the mechanism involved in this antidromic inhibition, it was assumed that the sodium channel or ion current is inactivated at the spike-generating site in the terminal of the fiber itself, after antidromic activation (Miller, 1971; Macdonald and Brodwick, 1973). An inhibitory interaction between different sensory units innervating the same papilla was also considered (Taglietti, 1969). This possibility was supported by the finding that antidromic activity in a sensory unit innervating a certain area of the tongue decreased the afferent responses of another unit innervating the neighboring region (Filin and Esakov, 1968; Bernard, 1972). However, with this mutual interaction between different sensory units, the type of sensory unit contributing to depression of the afferent response could not be identified.

In the present study, the type of sensory unit contributing to the antidromic inhibition of the response to NaCl was examined and the extent of the depression produced by each sensory unit was measured quantitatively using single papillae innervated by the two branches of the IXth nerve.

METHODS

American bullfrogs (*Rana catesbeiana*) weighing 250–450 g were anesthetized with an intraperitoneal injection of 20% urethane (15 ml/kg body wt) and placed in a supine position. Both the medial and lateral branches of the IXth nerve were isolated from the connective tissue and cut at the proximal junction. Each branch was suspended with a pair of Ag/AgCl wire electrodes and immersed in paraffin to prevent drying. To avoid tongue movement, the hypoglossal nerves were severed bilaterally. The tongue was pulled out and pinned down with the dorsal surface up in a plastic chamber. The experiment was performed at room temperature (18–25°C).

Stimulation

Electrical pulses (1 Hz and 0.1 ms in duration) were applied to single fungiform papillae through a suction electrode (Rapuzzi and Casella, 1965; Macdonald and Brodwick, 1973). For chemical stimulation of a single papilla, a V-shaped glass tube (Taglietti et al., 1969) was used. As stimulants, 0.5 M NaCl, 2.5 mM quinine, and 10 mM acetic acid solutions (special grade, Wako Pure Chemical Co., Osaka, Japan) were used. The papilla was exposed to the test solution for ~20 s. This solution was pumped from one end of the V-shaped tube to the other end at a rate of 0.25 ml/min, by means of a roller pump. Before and after each application, the papilla was rinsed with 0.01 M NaCl solution for 1 min. About 15 min was allowed to elapse between each successive stimulation. Mechanical stimulation of the papilla was given through a glass rod with a tip diameter of 300 μm (Hanamori and Ishiko, 1981). This rod was attached to an electromagnetic relay and driven by a rectangular electric pulse of 20 ms at 1 Hz. The tip of

the rod was ~1 mm above the surface of the papilla and the papilla was mechanically tapped by the displacement of the tip (2 mm).

Antidromic Stimulation

For antidromic stimulation of the IXth nerve, electrical stimulation with pulses of 0.1 ms in duration was applied to either of the two branches for 30 s at 100 Hz and 3 V. To identify the adjacent papilla neuronally connecting the papilla innervated by the two branches, electrical stimulation was applied to the adjacent papilla with a suction electrode containing Ringer solution, and the antidromic evoked potential was recorded from the papilla innervated by the two branches, using a different suction electrode (Rapuzzi and Casella, 1965). The tongue was immersed in Ringer solution before and during electrical stimulation so that the electrolytes present in the suction electrode would not serve as stimuli. To send naturally evoked antidromic volleys of impulses into the dually innervated papilla, 0.5 M NaCl or 2.5 mM quinine solution was applied to an adjacent papilla, using the V-shaped tube.

Recording and Measurement

The afferent impulses of each branch were amplified using a differential amplifier (AVB-9, Nihon Kohden Co., Tokyo), displayed on an oscilloscope (VC-9, Nihon Kohden Co.), and stored on an FM data recorder (DFR-1907N, Sony Magnescale Inc., Tokyo). The stored data were later played back and photographed, using a kymograph camera (RLG-6101, Nihon Kohden Co.). The frequency of impulses was displayed in the form of a histogram using a laboratory-oriented microcomputer (Signal Processor 7T08, NEC-San-ei Instrument Co., Tokyo).

The number of impulses in the first 5 s during NaCl stimulation was measured with and without electrical antidromic stimulation. The magnitude of the response obtained ~10 s after cessation of electrical stimulation of the one branch or ~3 s after chemical stimulation of an adjacent papilla was determined by the value relative to the control response, i.e., the mean of values obtained before application of, and 15 min after cessation of, the antidromic stimuli. As the manner of stimulations and recordings differed with each section of the experiments, the details are described in the related section of the Results.

RESULTS

Sensory Properties of Single Papillae Dually Innervated by the Medial and Lateral Branches

Several individual fungiform papillae were stimulated with a suction electrode at an intensity of three times the threshold, and orthodromic action potentials were simultaneously recorded from each branch of the IXth nerve (Fig. 1 A). Stimulation of a papilla located rostrally on the tongue (*a* in Fig. 1 A) elicited action potentials in the lateral branch only (*a* in Fig. 1 B). In contrast, stimulation of a papilla located caudally (*c* in Fig. 1 A) elicited action potentials in the medial but not in the lateral branch (*c* in Fig. 1 B). On the other hand, stimulation of a papilla located in the middle of the tongue (*b* in Fig. 1 A) elicited action potentials in both the medial and lateral branches simultaneously (*b* in Fig. 1 B). Thus, a dual innervation from the two branches was identified for papilla *b*. Using this method, 54 single papillae in 10 frogs were found to be dually innervated by these two branches. The distributions on the tongue are shown in Fig. 2. These papillae were distributed within a width of only 5

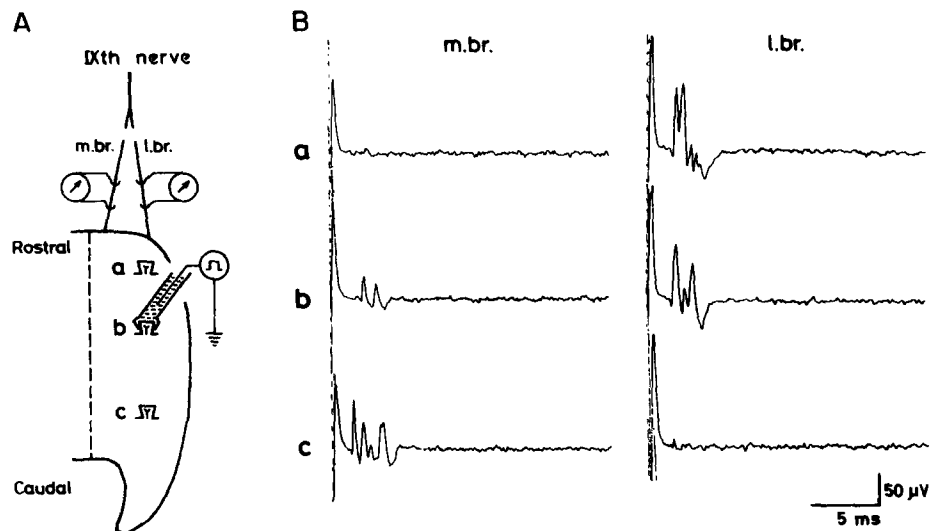


FIGURE 1. Identification of a single fungiform papilla innervated by the two branches of the IXth nerve. (A) Diagram showing the sites of electrical stimulation (at three different individual papillae: *a*, *b*, or *c*) and of recording (at the two branches of the IXth nerve; medial [m.br.] and lateral [l.br.]). (B) Simultaneously recorded responses at the medial and lateral branches after electrical stimulation of individual papillae (*a*, *b*, and *c*). Note that only the stimulation at *b* initiated action potentials in both branches of the IXth nerve.

mm, the center of which was located at the rostral one-third division line of the tongue.

While applying mechanical (*a* in Fig. 3 A) or chemical (*a* in Fig. 3 B) stimulation to these dually innervated fungiform papillae, the responses of the sensory units in each

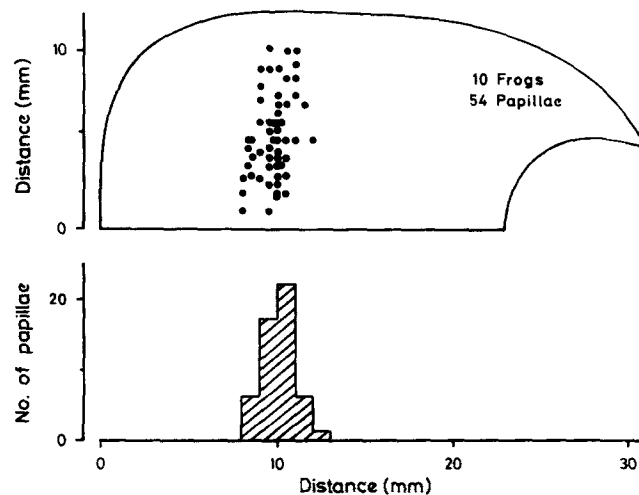


FIGURE 2. Distribution of 54 fungiform papillae innervated by both branches. These papillae were located within a narrow zone transverse to the longitudinal axis of the tongue.

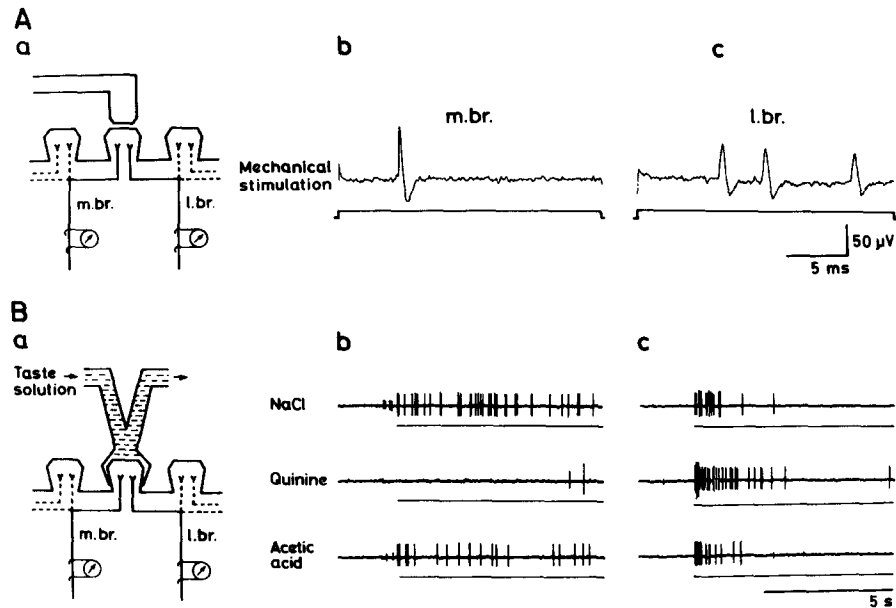


FIGURE 3. Comparison of orthodromic responses between the medial and lateral branches of the IXth nerve after natural stimulation of a dually innervated fungiform papilla from the two branches. (A) Mechanical stimulation. The diagram in *a* shows the sites of stimulation (at a papilla) and of recording (simultaneously at the medial and lateral branches). The traces in *b* and *c* show mechanical responses to the stimulation recorded at the medial and lateral branches, respectively. (B) Chemical stimulation. The traces in *b* and *c* show afferent responses to the three taste stimuli recorded at the medial and lateral branches, respectively. The same papilla was used in A and B. For further explanation, see text.

branch were examined. Fig. 3 A shows an example of simultaneously recorded action potentials in the two branches, after mechanical stimulation (*b* and *c*). In the medial branch, a single large-sized action potential occurred only at the initial phase of stimulation, whereas in the lateral branch, a series of small action potentials was generated during the period of sustained pressure. Thus, this particular fungiform papilla was supplied with different types of mechanosensitive fibers in different

TABLE I
Type of Afferent Responses Recorded from Each Branch to Mechanical Stimulation of Dually Innervated Single Fungiform Papillae

		Response of lateral branch			
		Rm	Sm	Rm and Sm	None
Response of medial branch	Rm	0	16	0	0
	Sm	11	1	0	1
	Rm and Sm	0	0	0	9
	None	1	2	13	0

Each value represents the number of fungiform papillae innervated by both branches. Rm: response from rapidly adapting mechanosensitive units. Sm: response from slowly adapting mechanosensitive units.

TABLE II
Number of Taste Responses Observed in Each Branch or in Both Branches to NaCl, Quinine, and Acetic Acid Stimulation of the Dually Innervated Papilla

Response	Medial	Lateral	Medial and Lateral	Sum
N	21	20	13	54
Q	9	10	32	52
A	17	25	10	52

N: NaCl; Q: quinine; A: acetic acid.

branches. A large action potential in the medial branch was defined as an "Rm" response, since the response might be elicited by the excitation of a rapidly adapting mechanosensitive unit. A series of small action potentials in the lateral branch was defined as an "Sm" response, since the response might be elicited by excitation of a slowly adapting mechanosensitive unit. All of the 54 dually innervated papillae contained terminals of the mechanosensitive units. In 27 of the 54 dually innervated papillae (50%), an Rm response was found in one branch and an Sm response was found in the other one (Table I). In 22 papillae (41%), both Rm and Sm responses were present in one branch, but there were apparently no responses to mechanical stimulation in the other one. In four papillae, there was an Rm or an Sm response in only one of the two branches. In the remaining papilla, each of two Sm responses was recorded from each branch. A vast majority (91%) of dually innervated papillae might be supplied with two mechanosensitive units.

Fig. 3 B (b and c) shows the responses of each branch to three taste stimuli (NaCl, quinine, and acetic acid) applied to the same papilla as for in Fig. 3 A. The afferent responses to NaCl and acetic acid, but not to quinine, were recorded from the medial branch, while those to all of the three taste stimuli were recorded from the lateral branch. The afferent responses to NaCl, acetic acid, and quinine were denoted as "N," "A," and "Q" responses, respectively. Table II summarizes the responsiveness of each branch to chemical stimulation of 54 single papillae innervated by both branches. The rate of N or A responses found simultaneously in both branches was 24 or 19%, values much smaller than that of the N or A responses found in one of the two branches only (76 or 81%). The rate of Q responses simultaneously present in both branches was 63%, a value much larger than that in one of the branches only. In single papillae, the possibility that Q responses can be recorded from both branches simultaneously, but that N and A responses can be recorded from one branch exclusively, was statistically significant ($P < 0.05$; χ^2 test).

TABLE III
Mean Number of Afferent Impulses in Each Branch to NaCl, Quinine, and Acetic Acid Stimulation of the Dually Innervated Papilla

	Medial branch	Lateral branch
N	43.8±3.8 (21)	44.4±4.3 (20)
Q	28.8±3.0 (9)	26.9±4.3 (10)
A	30.0±3.1 (17)	30.6±3.2 (25)

The value in each column represents the mean value ± SEM. Numbers are in parentheses.

Table III shows the mean numbers of afferent impulses in each branch during the first 5 s after the onset of stimulation when 54 dually innervated papillae were selectively stimulated. There were no significant differences ($P < 0.05$; Student's t test) between any pairs of corresponding responses of the medial vs. the lateral branch to taste stimuli.

Inhibitory Effects Produced by Antidromic Electrical Stimulation

A recording electrode was placed on the branch, which produced a greater afferent response to NaCl stimulation of a papilla innervated by the two branches (e.g., the medial in Fig. 3 B), and a stimulating electrode was placed on the other branch (e.g., the lateral in Fig. 3 B). Whether or not the afferent impulses in one branch to NaCl were affected by antidromic impulses in different sensory units produced by electrical stimulation of the other branch was then examined. In the preparation shown in Fig. 4 A, antidromic invasion of the dually innervated papilla by electrical

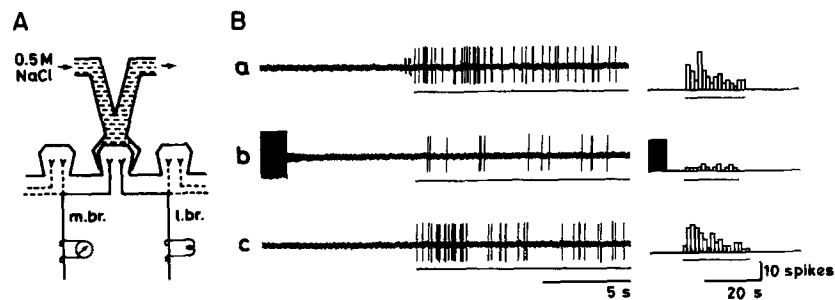


FIGURE 4. Effect of conditioning by antidromic electrical stimulation of one branch of the IXth nerve on responses of the other branch to chemical stimulation of a papilla. (A) Diagram showing the sites of NaCl stimulation (at a dually innervated papilla) and of recording (at the medial branch of the IXth nerve). The lateral branch was used for the site of conditioning. (B) Impulse discharges (left) and histogram of discharge per second (right) in response to NaCl stimulation before (a), 10 s after (b), and 15 min after (c) application of conditioning. Note that the responses to NaCl were inhibited in b but recovered almost completely to the control level in c.

stimulation of the lateral branch with an intensity of 3 V at a rate of 100 Hz for 30 s decreased the afferent response of the medial branch to NaCl stimulation of the same papilla (b in Fig. 4 B). The decreased response was ~20% of the value seen before application of (a in Fig. 4 B), and 15 min after cessation of (c in Fig. 4 B), the antidromic stimulation. The time required for complete recovery was ~5 min. This result indicates that antidromic impulses in the sensory units of the lateral branch can affect the afferent response of the medial branch to NaCl arising from a dually innervated fungiform papilla.

In this manner, using 54 dually innervated papillae, the degree of inhibition of the afferent response of one of the two branches to NaCl was examined, in relation to the number of afferent responses to mechanical and chemical stimulation, recorded from the electrically stimulated branch. The results are summarized in Table IV. According to the number of the sensory responses observed in an antidromically

activated branch, preparations were grouped into five types, I–V. In type I, antidromic stimulation of a branch presenting only an Rm response did not significantly affect the afferent response of the other branch to NaCl, whereas antidromic stimulation of a branch presenting only an Sm response or only a Q response inhibited the afferent response of the other branch to NaCl. The average value of inhibition in type I was $20.8 \pm 4.5\%$ (mean \pm SEM, $n = 17$). Similarly, mean values of inhibition in types II, III, IV, and V were $37.5 \pm 3.3\%$ ($n = 14$), $53.4 \pm 4.1\%$ ($n = 10$), $76.7 \pm 4.0\%$ ($n = 10$), and 82.7% ($n = 3$), respectively. The analysis of variance revealed highly significant differences [$F(4, 49) = 27.4$, $P < 0.001$] in the degree of inhibitory effects across the number of the sensory responses to natural

TABLE IV

Relationship between Sensory Responses Present in the Electrically Stimulated Branch and the Degree of Inhibition of the Response of the Other Branch to NaCl Stimulation of Individual Fungiform Papillae

Types	Variety of Responses	Degree of inhibition (%)	Mean \pm SEM
I	Rm	18, 8, 0, 0, -7, -11	20.8 \pm 4.5 (17)
	Q	24, 35, 44, 11, 24, 26	
	Sm	40, 58, 17, 25, 41	
II	N, Q	33, 48	37.5 \pm 3.3 (14)
	N, A	38, 27	
	Q, A	45, 39	
	Q, Sm	64, 42, 53	
	Q, Rm	18, 19, 33	
III	Sm, Rm	41, 25	53.4 \pm 4.1 (10)
	N, Q, A	48, 45, 53	
	Q, A, Sm	72, 70, 63	
	Q, A, Rm	38, 30	
IV	Q, Sm, Rm	58, 57	76.7 \pm 4.0 (10)
	N, Q, A, Sm	74, 84, 87, 90, 91	
	N, Q, A, Rm	74, 75, 82	
V	Q, A, Sm, Rm	51, 59	82.7 (3)
	N, Q, A, Sm, Rm	72, 82, 94	

Types were defined by the number of sensory responses present in electrically stimulated branches. The degree of inhibition represents the mean value relative to the control response (0%). Negative values indicate a facilitated response.

stimulation, in the branch to which antidromic stimulation had been given. A test for a linear trend was also found to be significant [$F(4, 49) = 77.4$, $P < 0.001$]. These observations indicate that the inhibitory effect increases in proportion to the number of sensory responses observed in the antidromically stimulated branch.

If it is assumed that each sensory response present in the electrically stimulated branch is independent, then the degree of inhibition due to antidromic impulses in the sensory unit conducting an N response alone can be theoretically obtained from the following calculations, using the data in Table IV: 41% (N, Q in type II) – 27% (Q in type I) = 14%. 49% (N, Q, A in type III) – 42% (Q, A in type II) = 7%. 83% (N, Q, A, Sm in type IV) – 68% (Q, A, Sm in type III) = 15%. 77% (N, Q, A, Rm in type IV) – 34% (Q, A, Rm in type III) = 43%. 83% (N, Q, A, Sm, Rm in type V) –

55% (Q, A, Sm, Rm in type IV) = 28%. Accordingly, an overall mean value of $21.4 \pm 5.6\%$ was obtained for the degree of inhibition produced by antidromic impulses in the sensory unit conducting an N response.

Similarly, the inhibitory effects by antidromic impulses in sensory units conducting each of the remaining responses were estimated and the data are summarized in Table V. The order of inhibitory effects was suggested to be: sensory unit conducting an Sm response > N response > Q response > A response > Rm response. Also, there were statistically significant differences between all pairs of inhibitory effects produced by a sensory unit conducting an N response, a Q response, an A response, or an Sm response and the control level, respectively ($P < 0.05$; Student's *t* test). There was no significant difference between the effect produced by the sensory unit conducting an Rm response and the control level ($P > 0.05$; Student's *t* test). These results indicate that the degree of inhibition produced by antidromic impulses in the sensory unit conducting each response ranged from 9.2 to 24.8% and that the antidromic activity in the sensory unit conducting an Rm response did not inhibit the afferent response of the other branch to NaCl.

TABLE V
*Estimated Degree of Inhibition Produced by Activation
of a Sensory Unit Conducting a Sensory Response*

Response	Degree of inhibition (%)	n
N	$21.4 \pm 5.6^*$	5
Q	$19.2 \pm 3.4^*$	5
A	$9.2 \pm 3.0^*$	5
Sm	$24.8 \pm 4.2^*$	8
Rm	0.5 ± 4.1	8

The value in the degree of inhibition represents the mean value \pm SEM.

*Significant difference ($P < 0.05$) when compared with control value (0%).

Inhibitory Effect Produced by Antidromic Chemical Stimulation

To examine the inhibitory effects of taste units even more directly, chemical stimulations were applied to an adjacent papilla neuronally connected with the dually innervated papilla under study. Orthodromic impulses in the branch elicited by NaCl stimulation of a dually innervated papilla were used.

First, it was determined that a particular papilla was dually innervated by taste units in the two branches. One of the fungiform papillae (P.1 in Fig. 5), shown by electrical stimulation to be innervated by the two branches, was selected. The response of each branch to NaCl and quinine solutions applied to this papilla was examined (*a* in Fig. 5 A). In Fig. 5 A, the response to NaCl was observed in the medial branch (*b*), and the response to quinine was observed in both branches (*b* and *c*); that is, this papilla was innervated by each sensory unit simultaneously presenting a Q response in each branch.

Second, while recording the antidromically evoked potential in P.1 and electrically stimulating the adjacent papillae, I searched for an adjacent papilla neuronally connected with P.1. In *b* of Fig. 5 B, stimulation of P.2 elicited an antidromic action

potential in P.1, which suggests that P.2 is neuronally connected with P.1. Quinine solution was then applied to P.2 (*a* in Fig. 5 *B*) and the antidromically evoked impulses were recorded from P.1 (*c* in Fig. 5 *B*). It is evident that P.1 connects neuronally with P.2 through the quinine-sensitive unit.

Third, P. 2 was stimulated with NaCl and quinine solutions (*a* in Fig. 5 *C*). The responses to both of these agents were recorded from only the lateral branch (*b* and *c* in Fig. 5 *C*); thus, P.2 is innervated only by the lateral branch and not by the medial branch.

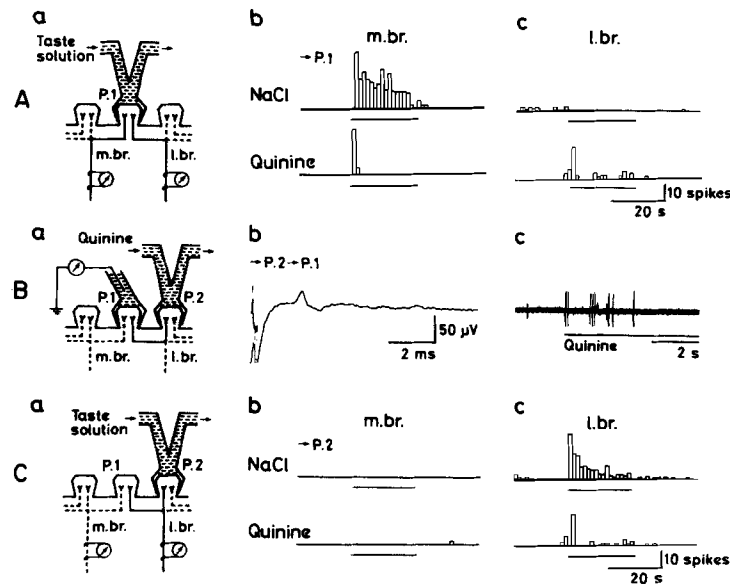


FIGURE 5. Identification of the neuronal connection of a dually innervated fungiform papilla with an adjacent papilla. (A) (*a*) Diagram showing the sites of chemical stimulation (at one particular papilla, P.1), and of simultaneous recordings (at the medial and lateral branches of the IXth nerve). (*b* and *c*) Histogram of discharges per second in the medial and lateral branches, respectively. The horizontal bars indicate application of NaCl and quinine solutions. (B) (*a*) Diagram showing the sites of 2.5 mM quinine stimulation and of recording (P.1). (*b*) An antidromic action potential in P.1 elicited by electrical stimulation of P.2. (*c*) Antidromic discharges in P.1 elicited by quinine stimulation of P.2. (C) (*a*) Diagram showing the sites of chemical stimulation (at P.2) and of simultaneous recording (at both branches). (*b*) Number of discharges per second in the two branches. The horizontal bars show application of NaCl or quinine solution.

On the basis of these results, I investigated whether antidromic impulses in a quinine-sensitive unit of the lateral branch could interfere with the orthodromic activity to NaCl stimulation of the medial branch arising from P.1. of this particular preparation. About 3 s after the stimulation of P.2 with quinine solution, NaCl solution was applied to P.1 (Fig. 6 *A*). The afferent response of the medial branch to NaCl obtained after this conditioning stimulation (*b* in Fig. 6 *B*) decreased, as compared with the control responses obtained in the absence of conditioning

stimulation with quinine (*a* and *c* in Fig. 6 *B*). The depression was greater in the phasic than in the steady state.

This experiment was performed using 20 preparations. Fig. 6 *C* shows the time course of the inhibitory effect produced by stimulation with quinine solution. The mean rates of impulses per second during the first 10-s response period of one branch are expressed as the value relative to the control (100%), which is the mean value obtained in the absence of conditioning stimulation. The afferent impulses obtained after conditioning stimulation decreased to ~60% of the control value in the

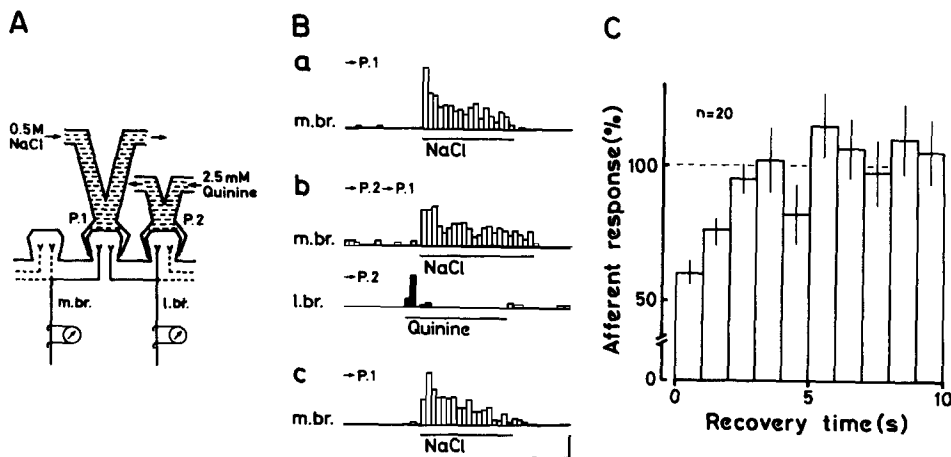


FIGURE 6. Effect of interaction between NaCl- and quinine-sensitive units. The diagram in *A* shows the sites of NaCl (P.1) and quinine (P.2) stimulation and of simultaneous recordings. The dually innervated papilla and the adjacent papilla shown in *A* and *B* are the same papillae as shown in Fig. 5. (*B*) The afferent responses of the medial branch to NaCl stimuli of P.1 before (*a*) and 15 min after (*c*) application of quinine stimuli of P.2. The afferent response of the medial branch to NaCl stimuli of P.1 ~3 s after quinine stimuli of P.2 (*b*). The filled histogram shows the afferent response of the lateral branch to quinine stimuli of P.2. The horizontal and vertical bars under the record denote 10 s and 10 spikes, respectively. *C* shows the time course of depression of the responses to NaCl application to a single papilla innervated by the two branches after antidromic impulses elicited by quinine stimulation of an adjacent papilla. Data are expressed as a percentage of those obtained before and 15 min after application of antidromic stimulation. The dashed line shows the control level (100%). Columns and vertical bars indicate means \pm SEM.

first second and then immediately recovered to the control level. The time required to recover to the control level was 3.9 ± 0.6 s (mean \pm SEM).

Using eight preparations in which an NaCl-sensitive unit innervated both the dually innervated papilla and adjacent ones, NaCl instead of quinine was applied to these adjacent papillae (Fig. 7 *A*) and the inhibitory effects of antidromic impulses in NaCl-sensitive units were examined. The afferent response (*b* in Fig. 7 *B*) of the medial branch to NaCl solution applied to the dually innervated papilla (P.3) decreased after NaCl stimulation of an adjacent one (P.4), as compared with the

control values obtained before (*a* in Fig. 7*B*) and 15 min after (*c* in Fig. 7*B*) conditioning stimulation.

Fig. 7*C* shows the mean time histogram of the recovery from the inhibition produced by NaCl stimulation of the adjacent papilla. At the initial state, the afferent response decreased to ~50% of the control value. The recovery time was 5.6 ± 0.5 s (mean \pm SEM). There was no significant difference in mean inhibitory effects ($P > 0.05$; Student's *t* test) between an NaCl-sensitive unit and a quinine-sensitive unit when the corresponding responses per second were individually compared. However, the recovery time from inhibition by an NaCl-sensitive unit was significantly longer than that by a quinine-sensitive unit ($P < 0.05$; Student's *t* test).

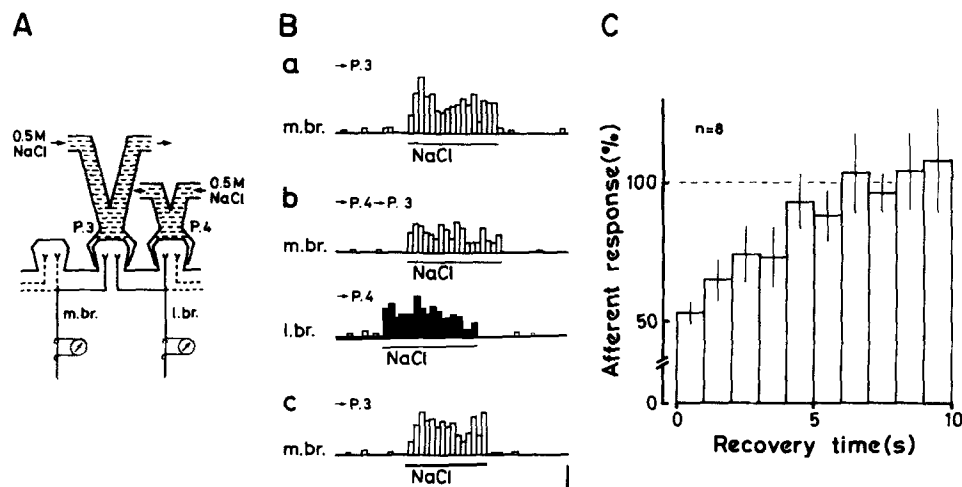


FIGURE 7. Effect of interaction between different NaCl-sensitive units. The diagram in *A* shows the sites of NaCl (P.3 and P.4) stimulation and of simultaneous recordings. (*B*) The response of the medial branch to NaCl stimulation of the papilla innervated by both branches (P.3) before (*a*), 3 s after (*b*), and 15 min after (*c*) application of NaCl solution to an adjacent papilla (P.4). The filled histogram shows the afferent response of the lateral branch to NaCl stimulation of P.4. The horizontal and vertical bars under the record denote 10 s and 10 spikes, respectively. *C* shows the time course of depression of the responses to NaCl application to a single papilla innervated by the two branches after antidromic impulses elicited by NaCl stimulation of an adjacent papilla. Data are expressed as a percentage of those obtained before and 15 min after application of antidromic stimulation. The dashed line shows the control level (100%). Columns and vertical bars indicate means \pm SEM.

DISCUSSION

Sensory Units in the Medial and Lateral Branches Innervating a Single Papilla

The taste organs in the anterior two-thirds and the posterior one-third of the mammalian tongue are supplied by the chorda tympani nerve and the IXth nerve, respectively. The sensitivities of these two nerves to the four basic taste stimuli differ considerably (Pfaffmann et al., 1967; Yamada, 1967; Ishiko, 1974). On the other hand, fungiform papillae in the frog tongue are innervated only by the IXth nerve.

Ishiko et al. (1979) reported that the IXth nerve of the frog divides into two branches before entering the tongue. However, they found that the sensitivities of the two branches for the four basic taste stimuli do not differ, unlike those of the chorda tympani and IXth nerve in the mammal.

When 0.5 M NaCl and 2.5 mM quinine solutions were applied to each of the 20 single papillae innervated by only the medial or the lateral branch, the mean numbers of impulses per 5 s obtained in the medial branch to NaCl and quinine were 50.9 ± 5.3 (\pm SEM) and 36.9 ± 5.0 , respectively, and those of impulses in the lateral branch were 54.1 ± 4.7 and 30.0 ± 3.1 (Murayama and Ishiko, 1979). The present results on dually innervated papillae, as shown in Table III, plus the previous results, show no significant difference ($P > 0.05$; Student's *t* test) in the responsiveness between the medial vs. the lateral branch of the IXth nerve.

Fungiform papillae are sensitive to both mechanical and chemical stimulation, and most of the single papillae so far examined have two types of mechanosensitive units, one being rapidly adapting and other slowly adapting (Hanamori and Ishiko, 1981). The results shown in Table I suggest that the majority of the dually innervated single papillae are also innervated with two types of mechanosensitive fibers.

The results in Table II indicate that the greater number of sensory units presenting Q responses in both the medial and lateral branches simultaneously innervate a single papilla, in comparison with the number of such sensory units presenting an N or an A response in both branches. Thus, information on quinine stimulation is conducted to the central nervous system by a greater number of units.

Inhibitory Effect by Antidromic Electrical or Chemical Stimulation

Murayama and Ishiko (1985, 1986) suggested that the inhibitory effect on the response to NaCl would mainly be mediated by slowly adapting mechanosensitive and taste-sensitive units and not by the rapidly adapting mechanosensitive unit. The present results (Tables IV and V) strongly support this view. In Table V, the inhibitory effect produced by activation of the sensory unit conducting an Rm response was 0.5%. The results in type I (Table IV) (as the antidromically stimulated branch contained only a single sensory unit in this type) also showed that the degree of inhibition by antidromic impulses in a rapidly adapting mechanosensitive unit was 1.3%. Thus, the results indicate that antidromic impulses in the rapidly adapting mechanosensitive unit do not inhibit the afferent response of the other branch. Also, the results in Table IV suggest that the degree of the inhibitory effect depends on the number of sensory responses observed in the branch given antidromic stimulation. Kusano (1960) defined the taste units that respond mainly to each of three taste stimuli (NaCl, quinine, and acetic acid) used here as M, Q, and A units, respectively. He reported that M units and A units hardly respond to any other taste solution, whereas there are some Q units that respond to salts and acid as well as to quinine. Thus, it is considered that the inhibitory effects shown in Table IV may increase with an increase in the number of sensory units in the antidromically stimulated branch. No correlation was found between the types of responses of the 54 single papillae shown in Table IV and the location on the tongue, as shown in Fig. 2.

In antidromic electrical stimulation, the mean values of the inhibitory effects on the afferent responses during 5 s by sensory units conducting an N response and a Q

response, calculated according to the hypothesis, were $21.4 \pm 5.6\%$ (\pm SEM) and $19.2 \pm 3.4\%$, respectively, while those of the inhibitory effects by N and Q units in antidromic chemical stimulation were $28.0 \pm 3.9\%$ and $13.1 \pm 3.8\%$, respectively. There was no significant difference ($P > 0.05$; Student's *t* test) in the inhibitory effects of each corresponding response between the two different antidromic stimulations. Thus, the results suggest that each sensory response in the electrically stimulated branch may be independent to a certain degree.

Although the mean value of the inhibitory effect by the sensory unit conducting the N response was 21.4%, the range of effect varied considerably, as shown in the Results (7–43%). The minimum inhibitory effect (7%) differed greatly from the value. Thus, it is considered that the quinine-sensitive unit in the antidromically stimulated branch may respond to NaCl stimulation of the dually innervated papilla (Kusano, 1960). The maximum inhibitory effect (43%) also differed considerably from the mean value. Thus, antidromic activities conducted by more than one sensory unit contributed to the effect, since the electrically stimulated branch might contain some sensory units responding to taste stimuli other than the stimuli used in the present work.

The duration of the inhibitory effect in antidromic electrical stimulation (data not shown) persisted for ~5 min, whereas the inhibitory effect in antidromic chemical stimulation disappeared within 6 s. These events relate to the number of units conducting antidromic impulses and indicate that the antidromic impulses elicited by chemical stimulation may be less than those elicited by electrical stimulation.

Kutyna and Bernard (1977) showed that electrical stimulation of an adjacent fungiform papilla can also have an effect on the resting potential in taste disk cells of the papilla neuronally connected to the adjacent papilla. Figs. 6 and 7 show that the antidromic impulses in one branch, produced by chemical stimulation of an adjacent papilla, can affect the taste response of the other branch.

The duration of the inhibitory effect elicited by NaCl solution was significantly longer than that seen with quinine solution. The number of antidromic impulses elicited by NaCl apparently exceeds the number of those elicited by quinine, as deduced from the finding that the degree of inhibition depends on the number of antidromic impulses (Taglietti, 1969; Murayama and Ishiko, 1985, 1986). The inhibitory effects with both chemical stimulations only lasted for a period long enough to affect the initial state of the afferent response. The time required for perception of taste quality in the rat and in human is <1 s (Halpern and Tapper, 1971; Yamamoto and Kawamura, 1981). Therefore, such a mutual inhibitory interaction may play an important role in the perception of taste quality.

Mechanism for Inhibition

Taglietti (1969) observed that the depression of the afferent activity in CaCl_2 -sensitive units became more pronounced with an increase in the frequency of the antidromic stimulation of the IXth nerve. He suggested that there was a peripheral interaction among the different sensory units. However, with his technique, there were inhibitory effects produced by inactivation of the sodium conductance of the electrically stimulated fiber itself (Macdonald and Brodwick, 1973). In the present experiment, the nerve branches for recording and stimulation were clearly distinct, as

single papillae innervated by the two branches of the IXth nerve were used. A mutual interaction among that different sensory units, without the threshold change at the spike-generating site, could thus be observed.

With regard to depression of the taste response seen in the present experiment, the following mechanisms have to be considered: (a) inhibition by efferent control of the autonomic fibers, (b) depression by accumulation of extracellular potassium ions, and (c) efferent inhibition by synaptic transmission.

The efferent control of autonomic fibers has been reported (Chernetski, 1964*b*; Brush and Halpern, 1970; Hellekant, 1971; Yamamoto, 1974). However, such a mechanism is probably not involved in the inhibitory effect seen in the present experiment, because the stimulus intensity (3 V, 100 Hz, and 30 s) used was too weak to elicit excitation of autonomic fibers (Chernetski, 1964*b*; Kutyna and Bernard, 1977).

It has been reported that accumulation of extracellular potassium ions elicited by peripheral nerve stimulation resulted in a decreased efficacy of synaptic transmission in the central nervous system of the leech, frog, and cat (Baylor and Nicholls, 1969*a, b*; Kříž et al., 1975; Syková et al., 1976). Filin and Esakov (1968) observed that the afferent response of salt sensory units was depressed for ~12 min after repetitive electrical stimulation (100 Hz and 30 s) of different sensory units of the IXth nerve. Murayama and Ishiko (1986) observed that in tetanic stimulation of the IXth nerve (100 Hz, 3 V, and 30 s), the duration of the inhibitory effect was ~15 min, and the logarithmic increase in the number of antidromic impulses resulted in a linear decrease in the response to quinine, NaCl, and spontaneous discharges. The rates of decrease of these afferent activities did not differ significantly. These authors suggested that the mechanism involved in the generation of antidromic depression was similar with regard to the afferent response. In the present study, the time required for a complete recovery was ~5 min, which is a shorter time than the two inhibitory ones described above (Filin and Esakov, 1968; Murayama and Ishiko, 1986). Thus, there may be an inactivation at the site of pre- or postsynaptic membrane rather than at the spike-generating site in the terminal of the stimulated fiber itself. This slow recovery process suggests a neuronally activated mechanism similar to the electrogenic pumps described by Kutyna and Bernard (1977). After cessation of the tetanic impulses, there may be an increase in the potassium ion permeability and/or an activation of the electrogenic sodium pump in the membrane of taste cells, and the original ionic environment is then gradually restored.

Electron-microscopic studies of synaptic structures of taste cells and nerve terminals in the frog gustatory organ have revealed four types: a sensory cell-to-nerve type, a nerve-to-sensory cell type, a nerve-to-nerve type, and a Merkel cell-to-nerve type (Dehan and Graziadei, 1973; Nomura et al., 1975; Düring and Andres, 1976). Morimoto and Sato (1975) investigated pharmacologically the inhibitory mechanism of efferent synaptic transmission in the frog taste organ and suggested that depression of the taste response might be mediated by cholinergic synapses from the efferent nerve terminals to taste cells. If efferent nerve terminals can be stimulated electrically as well as chemically, efferent synaptic transmission may be involved in the antidromic inhibition seen in the present study. In this case, reciprocal synapses between sensory cells and sensory nerve terminals and/or between two sensory nerve terminals have to be considered.

The inhibitory effect produced by the sensory unit conducting an A response was smaller than that produced by the sensory unit conducting an N, Q, or Sm response (Table V). Sensory units other than those conducting the A response present in the electrically stimulated branch may respond to acetic acid. However, the present findings strongly suggest that synaptic contacts between the terminal site of the sensory unit conducting an A response and salt-sensitive taste cells may be fewer than those between the terminal of sensory unit conducting an N, Q, or Sm response and salt-sensitive taste cells. Antidromic impulses in a rapidly adapting mechanosensitive unit of one branch did not inhibit the afferent response of the other branch to NaCl. The terminal site of a rapidly adapting mechanosensitive unit either may not make a synapse with salt-sensitive taste cells or it may not be located close enough to taste cells to affect their responses, as a result of accumulation of extracellular potassium in the medium.

Whether the release of a transmitter from efferent terminals or the release of potassium from afferent terminals of the antidromically stimulated fibers mediates the inhibitory effect is the subject of ongoing study.

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