Selective Depressant Action of Antidromic Impulses on Gustatory Nerve Signals

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ABSTRACT The depressant action of antidromic volleys of impulses on gustatory nerve signals from the tongues of bullfrogs was studied. Electrical stimulation of the glossopharyngeal nerve at a rate of 100 Hz for 10 s and at supramaximal intensity slightly depressed the integrated glossopharyngeal nerve responses to quinine and to mechanical taps to the tongue. The same antidromic stimuli resulted in a 30–40% reduction in the responses to salt, acid, water, and warmed saline, but depressed >80% of the afferent impulses firing spontaneously. The magnitude of responses to quinine and NaCl and the number of spontaneous discharges decreased gradually with an increase in either the frequency or the duration of antidromic stimuli. Similar results were obtained with intensities above the threshold for exciting gustatory and slowly adapting mechanosensitive fibers. The time required to recover from termination of the antidromic stimuli to two-thirds of the maximal amount of depression ranged between 6 and 7 min, with no significant differences among the depressions. The possible mechanisms involved in the antidromic depression of gustatory nerve signals are discussed.

INTRODUCTION

Gustatory receptors in the frog tongue are buried in the epithelial disk at the top of the fungiform papilla, where they receive synaptic contacts from the glossopharyngeal (IXth) nerve afferents (De Han and Graziadei, 1971; Graziadei and De Han, 1971). A number of receptors scattered over two to several fungiform papillae are linked through branches of the same IXth nerve fiber to form a sensory unit, and receptors belonging to a unit also belong at least in part to adjacent units, i.e., different sensory units overlap (Rapuzzi and Casella, 1965; Taglietti et al., 1969). Thus, afferent impulses arising from the receptors in a given unit conduct antidromically to sensory fiber terminals through connecting fibers (Rapuzzi and Casella, 1965), and hence may alter the spike-generating mechanisms at the sensory nerve terminals (Macdonald, 1971; Taglietti et al., 1971; Miller, 1971) or at the receptor cell itself (Kutyna and Bernard, 1977).

Filin and Esakov (1968) were the first to demonstrate that antidromic stimu-

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lation of a peripheral branch of the IXth nerve could depress the taste receptor discharge recorded from the other branch supplying a neighboring region of the tongue; the period of depression of taste responses varied widely from 1 s to 12 min, depending on the duration of the antidromic stimuli. Depression of the taste receptor discharge from a single papilla also occurred after repetitive stimulation of the IXth nerve; the degree and the duration of the depression became more pronounced as the frequency of the antidromic stimuli was increased from 1 to 100/s (Taglietti, 1969). By recording the electrical activity from a fungiform papilla belonging to a $CaCl_2$-sensitive unit, Taglietti et al. (1969) found that the stimulus-response relationship resulting from chemical stimulation of all the connected papillae in the unit had a lower rate of rise than that obtained by stimulating each individual papilla in the unit. Whether antidromic impulses in the IXth nerve have a selective depressant action on afferent discharges subserving different sensory modalities and qualities and whether they are capable of influencing afferent activities occurring spontaneously have not been elucidated.

We studied the effectiveness of antidromic volleys of impulses elicited by repetitive stimulation of the frog IXth nerve on afferent discharges occurring with and without sensory stimulations of the tongue receptors. The antidromic factors—the frequency, duration, and intensity parameters—were altered over an extensive range.

A preliminary report of this work has been published elsewhere (Murayama and Ishiko, 1985).

METHODS

American bullfrogs (*Rana catesbeiana*), weighing between 250 and 450 g, were used. The animals were anesthetized with intraperitoneal injections of 20% urethane solution (15 ml/kg body wt) and placed in a supine position. One of the glossopharyngeal (IXth) nerves was exposed from the junction of its two peripheral branches (Ishiko et al., 1979) to a point 4–5 cm central to the junction, freed from surrounding tissue, and cut centrally. The dissected nerve was kept in liquid paraffin during the experiment to prevent evaporation of moisture. The caudal tip of the tongue was pulled out of the oral cavity and pinned down in a Lucite chamber. To avoid movements of the tongue, the hypoglossal nerve was severed bilaterally. To maintain the taste response of each animal as constant as possible, the lingual artery was cannulated bilaterally with a polyethylene tube through which the tongue was perfused with a modified Ringer solution (Murayama and Ishiko, 1985). The solution consisted of 121.1 mM NaCl, 2.5 mM KCl, 5 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 10 U/ml heparin sodium (special grade, Wako Pure Chemical Co., Osaka, Japan). The pH was adjusted within the range 7.2–7.5. Two types of experiments were performed.

Experiment 1

Afferent impulses resulting from sensory stimulation of the entire tongue surface and those occurring spontaneously without stimulation were recorded from the IXth nerve (top diagram in Fig. 1) through a pair of Ag/AgCl wires and fed into an amplifier of conventional type (AVB-9, Nihon Kohden Co., Tokyo). The amplified signals of evoked responses were led to an integrator (1305C, San-ei Instrument Co., Tokyo) with a time constant of 0.3 s, and the summed neural responses were displayed on a pen writing

recorder (W-809, San-ei Instrument Co.). To analyze the spontaneous activity in the IXth nerve, the amplified signals were taped using an FM data recorder (DFR-1907N, Sony Magnescale Inc., Tokyo) that had been connected to a computer (Signal Processor 7T08, San-ei Instrument Co.) and through which the spike density histograms were obtained. In both experiments, each histogram was constructed by the number of spikes appearing successively every second. During the experiment, orthodromic and antidromic signals were monitored continuously on an oscilloscope (VC-9, Nihon Kohden Co., Tokyo) and, if necessary, photographed using a kymographic camera (RLG-6101, Nihon Kohden Co.). For chemical stimulation of the taste organs, 0.5 M NaCl, 2.5 mM $CaCl_2$, 0.1 or 1 mM quinine-HCl, 10 mM acetic acid, and distilled water were used. For thermal stimulation, 0.01 M NaCl solution warmed to 35°C was used. About 30 ml of these solutions was poured over the tongue at a flow rate of 2 ml/s. Before and after the application of each test solution, the tongue's surface was rinsed several times with 0.01 M NaCl solution. It was found to be preferable to interpose 15 min between these test stimuli, to avoid deterioration of the taste response of the gustatory organs. All but the thermal solutions applied to the tongue had been adapted to room temperature (18–23°C). For mechanical stimulation of a population of papillae, the tongue's surface was tapped with a Lucite plate with a 1-cm^2 tip and was driven through an arm attached to an electromagnetic relay (Murayama and Ishiko, 1985).

Experiment 2

The experimental arrangements (top diagram in Fig. 5), recording apparatus, and taste stimuli were essentially the same as those used in experiment 1, except that orthodromic signals were produced by stimulating a single fungiform papilla that had been suctioned into a V-shaped glass capillary. Each papilla was stimulated for ~20 s with a taste solution that flowed from one end of the V-shaped tube to the other, at a rate of 0.25 ml/min, with the aid of a roller pump (Morimoto and Sato, 1975). For mechanical stimulation, a single papilla was tapped with a glass rod (300 μm tip diam) driven in a manner similar to that used for experiment 1. The afferent impulses thus produced were amplified and stored in the data recorder. The histograms were obtained as in experiment 1. We found it inappropriate to deal with the spontaneous discharges in this type of experiment, since it was difficult to differentiate the discharges of a particular papilla from others.

Antidromic Stimulation

To produce antidromic volleys of impulses, electric pulses, each 0.1 ms in duration, were delivered to the IXth nerve through a pair of wires placed at a position 1–2 cm proximal to the recording site (Figs. 1 and 5). In this study, the frequency, duration, and intensity of the electric pulses were altered in the range of 1–100 Hz, 2–40 s, and 0.3–3 V, respectively.

Measurements

In experiment 1, the integrated IXth nerve responses to somatic and gustatory stimuli usually consisted of a large phasic response followed by a steady state response of smaller amplitude. Therefore, in an early stage of the experiment, the magnitude of the initial transient and the steady state response at 10 s after the application of taste stimuli were measured. However, because these two values were affected in parallel, owing to antidromic stimulation of the IXth nerve, the magnitude of the former alone was measured in the later stage. To express the number of afferent impulses occurring spontaneously (experiment 1) and those resulting from taste stimulation of a single fungiform papilla (experiment 2), the number of impulses occurring during 5 s in the absence and during

5 s in the presence of taste stimuli was measured. In both experiments 1 and 2, the number of orthodromic signals in the IXth nerve during the period of antidromic stimuli was difficult to measure, because of large artifacts caused by the spread of electrical pulses to the recording site. Therefore, to estimate the effect of antidromic stimuli on afferent signals, the magnitude of the integrated responses or the number of spikes ~10 s after cessation of the antidromic stimuli was measured and expressed relative to the control, i.e., the mean magnitude of each activity obtained before application of, and 30 min after cessation of, the antidromic stimuli.

Statistics

The probability that the mean magnitudes of afferent responses differed significantly was determined using Student's *t* test. Student's *t* test was used for comparison of the slope of regression lines.

RESULTS

Depression of the IXth Nerve Signals

The top diagram in Fig. 1 depicts the experimental set-up in experiment 1, in which afferent discharges from the tongue were recorded from the IXth nerve (R), while the proximal point (S) was stimulated antidromically. In this experiment, the nerve was stimulated repetitively at a rate of 100 Hz for 10 s and at a supramaximal intensity of ~3 V, a stimulation that will excite all somatic and gustatory fibers in the IXth nerve, except autonomic ones (Kutyna and Bernard, 1977). Fig. 1 shows the integrated responses of a frog to seven types of stimuli applied to the tongue obtained before application of (*A*) and 10 s (*B*) and 30 min (*C*) after cessation of the antidromic stimuli. It is apparent that antidromic stimulation of the IXth nerve (thick bars in *B*) resulted in a marked depression in the responses to 0.5 M NaCl, 2.5 mM $CaCl_2$, 10 mM acetic acid, distilled water, and saline solution warmed to 35°C, but in a less marked depression in the responses to both 0.1 mM quinine and mechanical taps to the tongue.

To express the extent of depression of each integrated response in Fig. 1, the magnitudes of the phasic and steady components after the antidromic stimuli were expressed relative to the control values of the respective state. Table I summarizes the mean ± SEM of results obtained from six frogs. The depressant effect of antidromic stimuli on the responses to salt, acid, water, and warmed saline was significantly greater ($P < 0.01$) than that on the responses to quinine and tactile taps. No significant difference ($P > 0.05$) in the relative degree of depression was found between the two groups of responses. Fig. 1 and Table I thus indicate that the depressant action of antidromic stimuli on afferent responses in the IXth nerve is not uniform; rather, it is selective. To extend this observation, two representative types of gustatory signals—the responses to 0.5 M NaCl and 0.1 mM quinine—were chosen for further investigation, since the former was greatly depressed by antidromic stimuli.

Stimulus Factors

In the above experiment, the frequency (100 Hz), duration (10 s), and intensity (3 V) of the antidromic stimuli were unaltered. The effectiveness of each of these

factors on taste responses was investigated by altering the parameters of each. In Fig. 2, the frequency of antidromic stimuli was varied from 10 to 100 Hz while the duration and intensity were held constant at 10 s and 3 V, respectively. The mean ± SEM of the magnitudes of responses of six frogs to 0.1 mM quinine

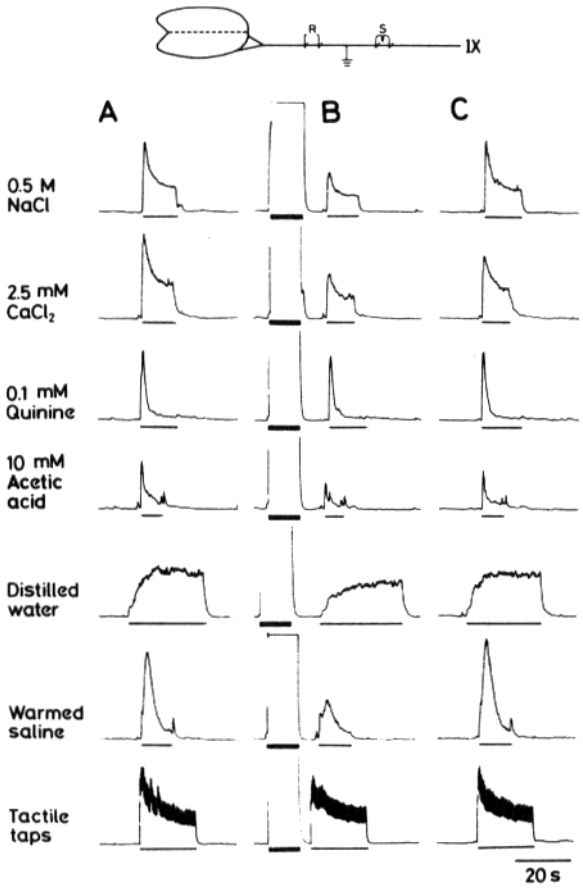

FIGURE 1. The diagram at the top shows the experimental arrangement with recording (R) and stimulating (S) electrodes in position. The records show integrated IXth nerve responses of a frog to various stimuli obtained before the application of (*A*), and 10 s (*B*) and 30 min after (*C*) the end of antidromic stimuli. Note the marked decrease in the magnitude of responses to all stimuli except quinine and tactile taps to the tongue in *B*. The thin and thick bars denote the duration of taste stimulation and antidromic stimuli, respectively.

(triangles) and 0.5 M NaCl (circles) is expressed relative to the control magnitude (100%). The progressive increase in the frequency of antidromic stimuli resulted in a slight depression of the responses to quinine, and in a marked depression of the responses to NaCl, especially at frequencies above 40 Hz. At 100 Hz, the

TABLE I

Antidromic Depression of Integrated Afferent Responses to Chemical, Thermal, and Mechanical Stimulation of Frog Tongue

Stimuli	Degree of depression (%)	
	Phasic state	Steady state
0.5 M NaCl	39.3±5.8	35.5±6.3
2.5 mM $CaCl_2$	33.6±5.0	33.5±4.2
0.1 mM quinine	4.6±5.5*	—
10 mM acetic acid	30.3±5.2	—
Distilled water	—	40.1±4.4
Warmed saline	40.4±6.1	44.9±3.9
Tactile taps	3.2±2.6*	1.6±1.0*

Each entry indicates the mean ± SEM of the degree of depression in six frogs expressed relative to the control (100%).

* Significant difference ($P < 0.01$) when compared with the degree of depression for the NaCl response given at the top of each column.

extent of depression in the responses to quinine and NaCl did not differ significantly ($P > 0.05$) from the corresponding value in Table I.

The duration of the antidromic stimuli was also altered in the range 2–40 s while the frequency and intensity were kept at 100 Hz and 3 V, respectively. In Fig. 2*B*, the mean ± SEM of the magnitude of response to quinine and NaCl is plotted as a function of the stimulus duration. A depression of the IXth nerve responses to NaCl exceeded that observed with quinine at every duration, and a

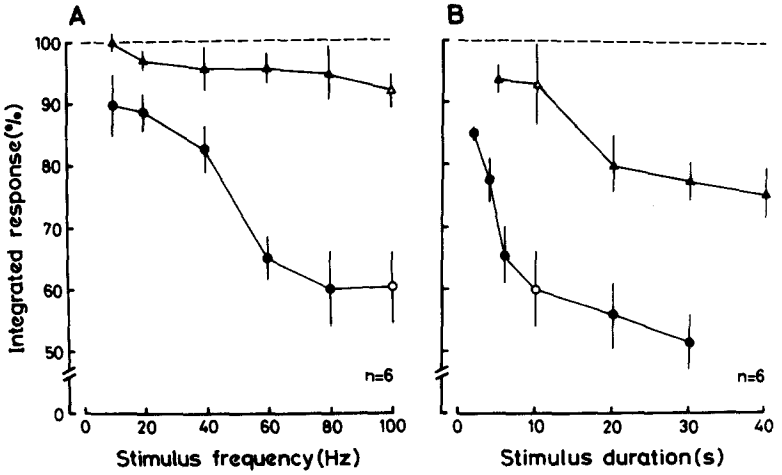

FIGURE 2. Graphs demonstrating the effect of changes in the antidromic stimulus factors on the integrated IXth nerve responses to 0.5 M NaCl (circles) and 0.1 mM quinine (triangles). (*A*) The relationship between the magnitude of taste response and the frequency of antidromic stimuli. (*B*) The same relationship as in *A*, obtained by altering the duration of antidromic stimuli. The open triangle and circle in *A* correspond to the open symbols in *B*, since each pair was obtained under the same conditions of stimulus.

marked decrease in the former response occurred with an increase in the duration from 2 to 10 s, within which period no appreciable change in the latter response was seen. The magnitude of response to quinine decreased sharply when the duration exceeded 10 s, and at 40 s, 76% of the control was attained. This depression was approximately twice that obtained at a frequency of 100 Hz in Fig. 2*A*. When the responses to NaCl in *B* are compared with those in *A*, it is also apparent that prolongation of the stimulus duration for >10 s led to a decrease in the responses to salt.

In the frog IXth nerve, the threshold for exciting mechanosensitive fibers of the rapidly adapting type is lower than that of other types of fibers, such as slowly adapting mechano- and chemosensitive fibers (Hanamori and Ishiko, 1981*a*). Therefore, the intensity factor of antidromic stimuli may also contribute to the depression mentioned above. For elucidation, the IXth nerve was stimulated at a rate of 1 Hz, at various intensities, and the resulting neural activities were recorded from the nerve distal to the site stimulated. The records in Fig. 3*A* demonstrate the IXth nerve action potentials traveling antidromically. The number in each record shows the intensity of stimulus expressed relative to the threshold (1 × T), which elicited the second rather than the first spike component, seen as a small notch (arrow in the second record from the left) at the base of the first spike. Therefore, in *A*, the use of an intensity below 1 × T produced a compound action potential that should reflect excitation of the low-threshold mechanosensitive fibers (Hanamori and Ishiko, 1981*a*). On the other hand, the second spike component, which increased in amplitude as the intensity was increased from 1 to 3.2 × T, is attributed to excitation of high-threshold mechano- and chemosensitive fibers (Hanamori and Ishiko, 1981*a*). The relationship between the amplitude of the respective spike and the relative stimulus intensity is shown in *B*, in which the base-to-peak amplitudes of the first and second spike components were expressed relative to the maximal amplitude of the latter (100%). Thus, the amplitude of the first spike (open circles) increased with a rise in the stimulus intensity from 0.5 to 1 × T; above this point, it reached a saturation level at ~55% of the maximal amplitude of the second spike. On the other hand, the second spike (solid circles) was initiated at an intensity of 1 × T and increased in size to attain a maximum at intensities above 2 × T.

Fig. 3*C* shows the extent of depression in the responses of eight frogs to 0.5 M NaCl, as a function of the intensity of antidromic stimuli, the frequency, and the duration (100 Hz for 10 s) of the antidromic stimuli, which had been left unchanged. It is apparent that the results (open circles) obtained with intensities below 1 × T had little effect on the responses to salt, compared with those (solid circles) obtained with intensities of >1 × T; the depression increased gradually as the intensity was increased to 2 × T, and saturation was noted at around 3 × T. The depression reached ~60% of the control, which is consistent with the corresponding value in Table I and Fig. 2. From the results in Fig. 3, it is reasonable to assume that antidromic impulses mediated through the high-threshold IXth nerve afferents are responsible for the production of most of the depression, the degree of which depends on the stimulus intensity or on the number of the fibers involved in the excitation.

Depression of Spontaneous Discharges

In the 21 bullfrogs used in the experiments, the mean frequency of afferent spikes appearing spontaneously in the whole IXth nerve was 18.9 ± 1.54 (SEM) Hz, within the range 6–33 Hz.

Although the origin of the spontaneous discharges is not fully understood, these events probably reflect excitatory processes at the receptor-axon junction.

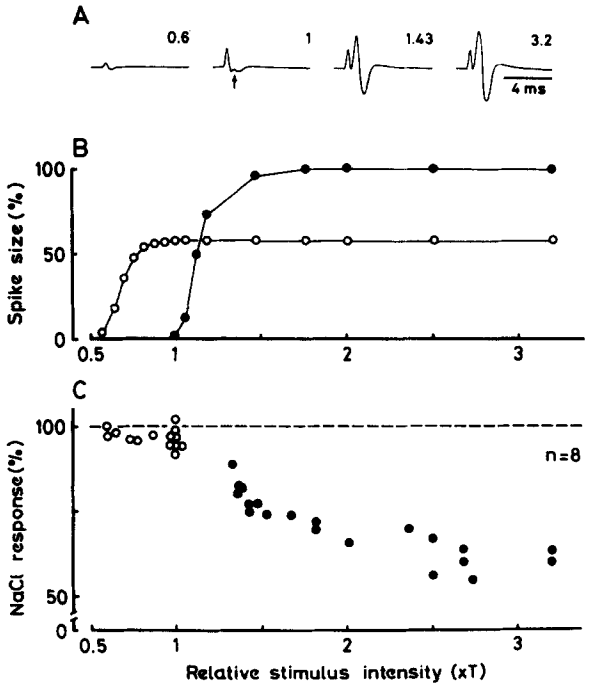

FIGURE 3. The records in *A* demonstrate (from left to right) an increase of the first and second compound action potentials associated with an increase in stimulus intensity given to the IXth nerve. The arrow indicates initiation of the second spike component. In *B*, the amplitudes of the first (open circles) and second (solid circles) spike components are plotted as a function of stimulus intensity. *C* shows the effect of antidromic stimulus intensity on the magnitudes of responses to 0.5 M NaCl. The open and solid circles in *C* show results obtained by the use of intensities below and above 1 × T, respectively.

Therefore, it was of interest to determine whether the rate of spontaneous firings would be affected by antidromic volleys of impulses. Fig. 4 presents the results obtained while the frequency (*A*), duration (*B*), and intensity (*C*) of the antidromic stimuli were altered. The top records (*a–c*) show the spike density histogram to demonstrate changes in the spontaneous activity after stimulation of the IXth nerve at (from left to right) 20, 60, and 100 Hz. The decrease in the number of spontaneous discharges after the antidromic stimuli became more pronounced with further increases in the stimulus frequency. There was a marked decrease in the discharges immediately after the stimuli, but a gradual recovery took place

after cessation of the stimuli. To express these changes, the number of discharges in 5 s (hatched bars) immediately after and 10 s after cessation of the antidromic stimuli were measured and expressed relative to the respective control. In *A*, changes in the former (solid circles) and latter (open circles) values, associated with an increase in the frequency of antidromic stimuli, are presented as the mean ± SEM of eight frogs. A similar relationship, obtained by changing the

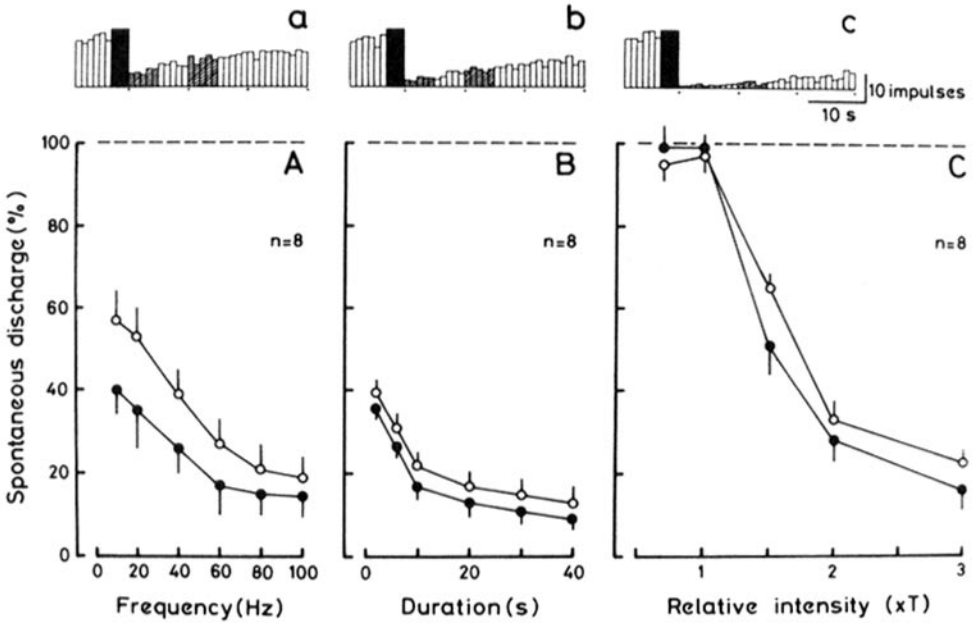

FIGURE 4. The histograms in *a*, *b*, and *c* demonstrate changes in the number of spontaneous discharges before and after antidromic stimulation of the IXth nerve at 20, 60, and 100 Hz, respectively. Each histogram represents the results from a single trial. Solid block; the antidromic stimuli persisted for 10 s but are expressed by one-third of the actual width. Each block represents the number of impulses every second. The two groups of hatched blocks separated from each other were the mode used to measure change in the number of spikes occurring spontaneously during 5 s. *A*, *B*, and *C* show changes in the relative number of spikes, as a function of the stimulus frequency, duration, and intensity, respectively. The solid and open circles denote the mean number of spikes obtained during the first 5 s and between 10 and 15 s after the cessation of antidromic stimuli, respectively. The vertical bar in each symbol indicates the SEM.

duration of antidromic stimuli, is shown in *B*. In *C*, the number of spontaneous discharges, as a function of the stimulus intensity below and above the threshold (1 × T, Fig. 3) for initiating the late spike component in the IXth nerve, is presented. The results in Fig. 4 show that the ability of each antidromic stimulus factor to depress the spontaneous discharges is in general similar to that of the corresponding factor that depresses gustatory impulses (Figs. 2 and 3). It is the spontaneous discharges in the IXth nerve that are depressed to the greatest

extent, the degree of depression being ~80% of the control, which is considerably greater than that shown in Table I.

Effectiveness of Antidromic Impulses

To evaluate the effectiveness of antidromic stimuli on impulses occurring with and without taste stimuli, the extent of depression seen in Figs. 2*B* and 4*B* was plotted against the number of antidromic volleys of impulses, estimated by multiplying the frequency of the antidromic stimuli and the duration.

Fig. 5 illustrates that the logarithmic increase in the number of antidromic impulses resulted in a linear decrease in the responses to quinine (solid triangles) and NaCl (circles), as well as in the spontaneous discharges (squares). The regression lines fitted into the first, second, and last responses were $Y = -22.7 \log x + 157.3$, $Y = -28.8 \log x + 149.8$, and $Y = -21.2 \log x + 88.0$, respectively.

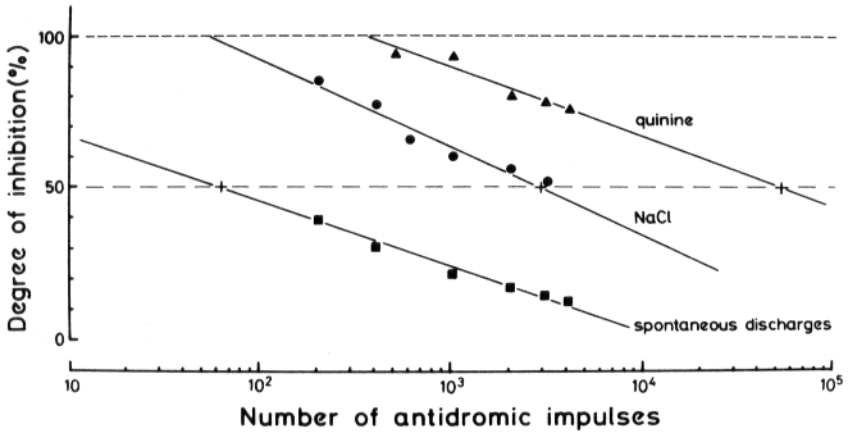

FIGURE 5. Relationship between the degree of inhibition of gustatory nerve signals and the number of antidromic volleys of impulses. Regression lines fitted into changes in the magnitudes of integrated responses to 0.1 mM quinine and 0.5 M NaCl are shown together with the change in the number of spontaneous discharges. Data from the results in Figs. 2*B* and 4*B* were used.

The number of antidromic volleys of impulses required for depressing each afferent response to half of its control magnitude was 63 for the spontaneous discharges, and 3,000 and 51,000 for the responses to NaCl and quinine, respectively. The inverse ratio, 1:0.02:0.001, thus indicates the relative strength of antidromic volleys of impulses in depressing the respective responses.

Depression of Afferent Activity from Single Fungiform Papillae

The top diagram in Fig. 6 demonstrates the experimental arrangement in experiment 2, in which the depressant action of antidromic impulses on the responses of a single fungiform papilla to 0.5 M NaCl and 0.1 mM quinine was studied by changing one of the three stimulus factors, as in experiment 1. In *A*, each of the records exhibits afferent discharges evoked by the salt solution

perfused to a single papilla through the V-shaped glass capillary. The first and fifth traces are control responses obtained in the absence of antidromic stimuli, whereas the second, third, and fourth traces were obtained after antidromic stimulation for 10, 20, and 30 s, respectively. The frequency (100 Hz) and intensity (3 V) were unchanged. The extent of change in these orthodromic responses is shown in *B*, where the afferent impulses shown in *A* are expressed by the spike frequency histogram in the corresponding row. The numbers at the right of the second, third, and fourth traces show the magnitude of the response to salt relative to the control, which indicates that the degree of depression increases as the stimulus duration increases.

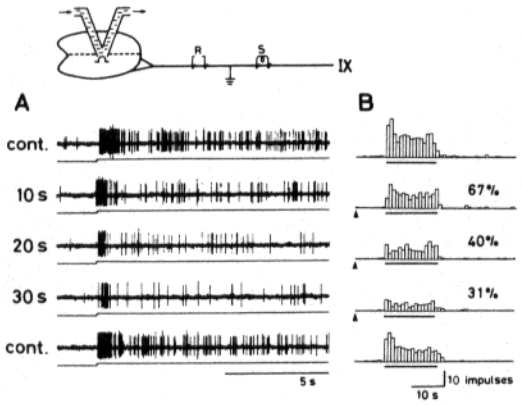

FIGURE 6. The top diagram shows the experimental arrangements in experiment 2. The records in *A* demonstrate part of the afferent impulses resulting from perfusion of 0.5 M NaCl to a single fungiform papilla. The first and fifth traces show the control record obtained before and 30 min after the antidromic stimulation, respectively. The second, third, and fourth traces show records taken 10 s after the cessation of the antidromic stimuli, which were applied for 10, 20, and 30 s, respectively. In *B*, each histogram represents a salt response shown in the corresponding row in *A*. The arrowheads mark the end of the antidromic stimuli.

Fig. 7 shows the results obtained when the frequency, duration, and intensity of the antidromic stimuli were altered. In each graph, the number of afferent impulses obtained in 5 s after repetitive stimulation of the IXth nerve is expressed relative to those obtained in absence of the stimuli. The gradual increase in the frequency (*A*) and duration (*B*) of antidromic stimuli resulted in a marked depression of orthodromic IXth nerve responses to NaCl, but not in the responses to quinine. The degree of depression in the former response with each frequency, and in the latter response at any given duration, did not differ significantly ($P > 0.2$) from the corresponding values in Fig. 2, *A* and *B*. The relationship between the stimulus intensity and the response to NaCl in Fig. 7*C* is similar to that shown in Fig. 3*C*. Thus, the depressant action of antidromic stimuli on gustatory nerve signals originating in a single fungiform papilla did not differ substantially from the action on those from whole taste organs.

Recovery Time

The time required to recover from depression caused by the supramaximal tetanic (100 Hz for 10 s) stimulation of the IXth nerve was studied by measuring the change in the amplitude of afferent signals, in relation to the time after cessation of the antidromic stimuli. The top records in Fig. 8 are the results obtained in experiment 1. They demonstrate the integrated neural responses to 0.5 M NaCl before the application of (*a*), and 10 s (*b*), 10 min (*c*), and 15 min (*d*) after cessation of, the antidromic stimuli. The time course of the magnitudes of these responses (solid circles) is shown in the lower graph (*A*), together with the time courses of the responses to quinine (solid triangles) and tactile taps (solid squares). In this and other cases, the time required for complete recovery was

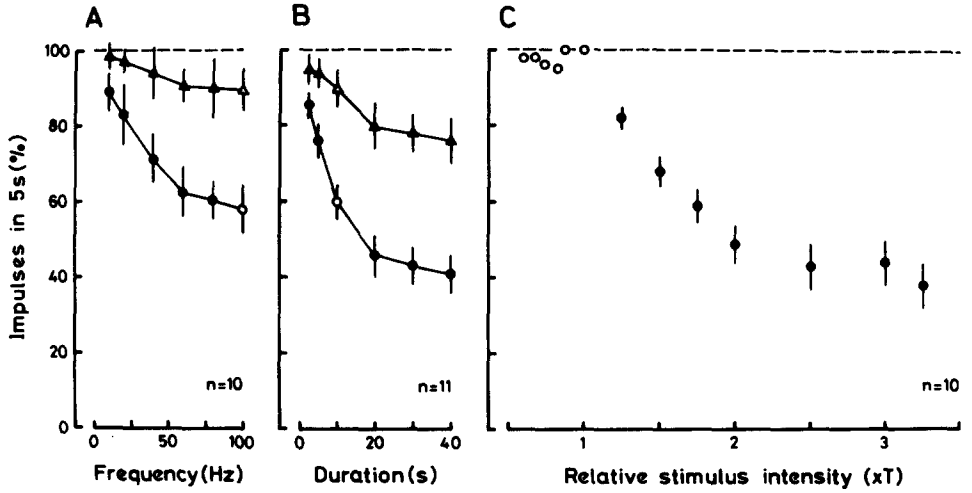

FIGURE 7. Graphs representing the effects of changes in the antidromic stimulus factors on afferent discharges resulting from stimulation of a single papilla by 0.5 M NaCl (circles) and 0.1 mM quinine (triangles). (*A*) Relationship between the number of afferent impulses and the frequency of antidromic stimuli. (*B* and *C*) The same relationship as in *A*, obtained while the duration and intensity were changed. For the open triangle and circle in *A* and *B*, see the explanation in the legend to Fig. 2. For the open and solid circles in *C*, see the legend to Fig. 3*C*.

≥15 min, but was often difficult to determine accurately. Therefore, in the following study, the recovery time was expressed by estimating two-thirds of the decay time, i.e., the time from the end of antidromic stimuli to the time at which the magnitude of initial depression recovered to two-thirds of the original level. The mean recovery times (cross) in four frogs, in the response to NaCl, quinine, and tactile taps were 6.7, 6.3, and 7.0 min, respectively. Fig. 8*B* depicts the mean time course of recovery of the spontaneous discharges obtained from 12 frogs. Each point in the graph represents the number of spontaneous discharges measured every 10 s. The mean ± SEM of the recovery time was 7.3 ± 0.82 min. As seen in Fig. 8*C*, the responses of a single fungiform papilla to NaCl recovered gradually from the maximal depression at the end of the antidromic

stimuli, with a time course similar to that shown in *A* and *B*. The number of afferent impulses in the first 5 s of taste stimulation was measured at the same time intervals as in *A*, and was plotted as a function of the time after cessation of the antidromic stimuli. In the eight cases studied, the mean ± SEM of the recovery time was 7.4 ± 1.05 min, which is not significantly different ($P > 0.5$) from the results in *A* and *B*. Thus, the recovery time from antidromic depression differs little among a variety of afferent signals from the tongue.

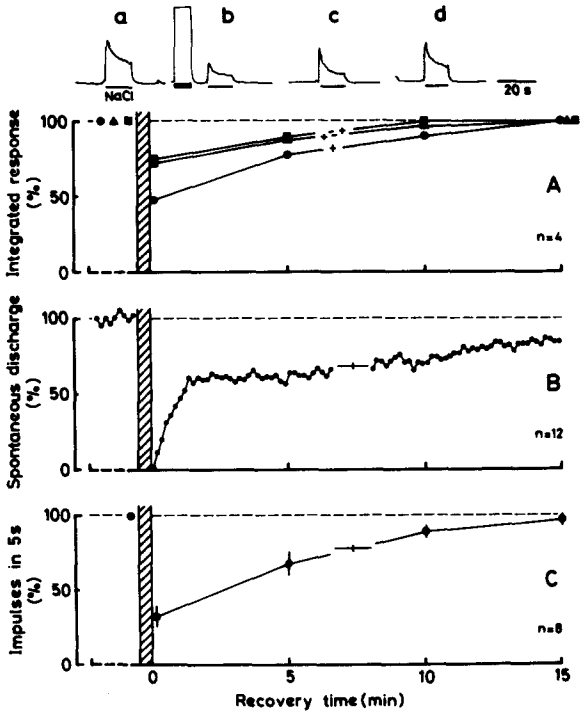

FIGURE 8. Graphs demonstrating the time courses of recovery from antidromic depression. In the top records, *a* and *d* exhibit control responses to 0.5 M NaCl, and *b* and *c* show the responses at 10 s and 10 min after the cessation of the antidromic stimuli (thick bar). (*A*) Changes in the magnitudes of integrated responses to 0.5 M NaCl (●), 0.1 mM quinine (▲), and tactile taps (■) before and after the application of antidromic stimuli (vertical shaded area). The plus denotes the mean recovery time in *A–C*. (*B*) Relationship similar to that in *A* but showing changes in spontaneous discharges. (*C*) Relationship similar to that in *A* and *B*, but showing changes in the number of afferent impulses resulting from stimulation of a single fungiform papilla with 0.5 M NaCl.

DISCUSSION

Our results demonstrate that supramaximal tetanic stimulation (100 Hz for 10 s) delivered to the bullfrog IXth nerve is capable of differentially depressing a variety of orthodromic signals from the tongue (Table I; Figs. 1, 4, and 7). The extent to which the frequency, duration, and intensity of the antidromic stimuli

contributed to the depression was examined by estimating the effect of changing each stimulus parameter on the spontaneous discharges and the responses to 0.5 M NaCl and 0.1 mM quinine. Our results (Figs. 2, 4, and 8) are generally in agreement with those reported by others (Filin and Esakov, 1968; Taglietti, 1969) in that the depression of each afferent activity became more pronounced in both magnitude and duration as the frequency or the duration of the antidromic stimuli was increased. In addition, we showed that the depression became apparent only when the intensity of the antidromic stimuli was above the threshold required to excite high-threshold mechano- and chemosensitive fibers in the IXth nerve (Figs. 3, 4*C*, and 7*C*). Furthermore, regardless of changes in these three stimulus parameters, there was no appreciable change in the relative order of the ability of antidromic stimuli to depress the three afferent responses, i.e., spontaneous discharges > response to salt > response to quinine (Figs. 2 and 7).

It should be pointed out that the decrease in spontaneous activity in the IXth nerve (Fig. 4) does not necessarily affect to an equal extent the magnitude of gustatory, thermal, and tactile fiber activity, because the spontaneous activity may not be evenly distributed over these fibers. This proposal is supported by the result that in some mammals, single chorda tympani fibers responsive to cooling of the tongue, as well as to a particular taste, had a higher rate of spontaneous discharges than did those responsive to other stimuli (Ogawa et al., 1968; Ishiko and Sato, 1973). Although the frog IXth nerve lacks cold-sensitive fibers (Kimura, 1962; Yamashita, 1964), the situation in the frog would be similar to that in mammals, in that the rate of spontaneous discharge would be high in IXth nerve fibers, which are tissues that tend to respond more to some gustatory stimuli than to others. The relation between the spontaneous discharges and the responsiveness of single afferent fibers in the IXth nerve is unknown. Nevertheless, we believe that the tactile fibers in the frog do not possess spontaneous activity, since the solitary tract neurons responsive only to tactile stimulation of the tongue did not discharge spontaneously (Hanamori and Ishiko, 1981*b*). Actually, the magnitude of tactile responses was practically independent of a decrease in the spontaneous activity in the IXth nerve after antidromic stimuli (Fig. 1 and Table I).

The rate of decrease, or the slope of the three regression lines (Fig. 5) drawn along the responses to quinine and NaCl, and the spontaneous discharges did not differ significantly ($P > 0.05$), which suggests that (*a*) the mechanism involved in the generation of antidromic depression is similar in these afferent responses, and (*b*) the degree of depression of each afferent response depends primarily on the number of antidromic volleys of impulses. The relationship between the degree of depression and the frequency of the antidromic stimuli (Fig. 2*A*) did not fit a simple regression line, as in Fig. 5, probably because a change in the stimulus frequency resulted in changes in both the number and interval of antidromic impulses.

If the number of antidromic impulses is, for example, 100, the expected degree of inhibition in the responses to NaCl and quinine would be <10% and 0, respectively, compared with a 55% decrease in the spontaneous discharges.

Therefore, the response to quinine would become more apparent, whereas a decrease in the salt response would in effect be counteracted, because of a lowering of the background noise or the spontaneous discharges. Such a consideration can be extended to events in physiological situations, under which antidromic impulses will be produced not by electrical nerve stimulation but by chemical stimulation of the tongue receptors.

Although the IXth nerve contains efferent fibers implicated in both the enhancement and depression of responses from the taste receptors (cf. Sato, 1976), these fibers may play a minor role in the depression described in this study, in which (*a*) neither the hypoglossal nerve nor the glossopharyngeal nerve remains connected to the central nervous system, (*b*) a depression of the gustatory nerve signals was observed, and (*c*) the high-threshold IXth nerve afferents other than sympathetic efferents contributed to the depression (Fig. 3). Kutyna and Bernard (1977), in their intracellular recording of taste disk cells in the frog, reported that during repetitive stimulation of the IXth nerve, a decrease in gustatory nerve impulses occurred in association with a hyperpolarization of individual cells, whereas an augmentation of the impulses coincided with the cell depolarization at the termination of tetanic stimuli. Although they suggested that antidromic impulses could trans-synaptically alter the membrane potential of the receptor cell itself, this explanation does not seem to apply to our findings since we have dealt with only the post-tetanic depression of afferent neural activity. Macdonald and Brodwick (1973) noted that surgical removal of the taste disk at a single fungiform papilla did not abolish the decrease in excitability at the afferent nerve terminals identified previously (Macdonald, 1971; Taglietti et al., 1971). They therefore claimed that the site of antidromic depression is in the spike-generating membrane of the afferent nerve terminals rather than in the pre- or post-synaptic membranes associated with the receptor cells. Our evidence supports this view because the depression persisting for >15 min (Fig. 8) may represent the cumulative effect of the excitability decrease, which continued for 200–400 ms in their experiment. A similar interpretation could be made of the results reported by others (Filin and Esakov, 1968; Taglietti, 1969; Taglietti et al., 1969).

With regard to the mechanism of antidromic depression, repetitive stimulation of the IXth nerve may produce a post-tetanic hyperpolarization in the sensory nerve terminals, as in the case of the primary afferent nerve terminals in mammalian spinal cord (Křiž et al., 1974), mammaliam motor nerve endings (Gage and Hubbard, 1966), frog myelinated nerve fibers (Connelly, 1959; Straub, 1961), and other neural tissues (cf. Brodwick and Junge, 1972). In these locations, the extracellular $[K^+]$ in the periaxonal space and the intracellular $[Na^+]$ increase in proportion to the number of antidromic action potentials (cf. Nicholl, 1979; Sokolove and Cooke, 1971). However, after the cessation of tetanic impulses, there is an increase in the K ion permeability (Gage and Hubbard, 1966; Brodwick and Junge, 1972) and/or an activation of the electrogenic Na^+ pump (Rang and Ritchie, 1968; Sokolove and Cooke, 1971) and the original ionic environment is restored. The post-tetanic hyperpolarization thus produced then interferes with the initiation of the afferent impulses by counter-

acting the depolarization produced post-synaptically by receptor activity in the tongue (Akaike et al., 1976). In other words, the extent of depression observed in the present study probably represents a consequence of an interaction between excitatory and inhibitory processes occurring at or near the sensory nerve terminals. In these two processes, it is reasonable to suggest that the inhibitory process or the hyperpolarization to be produced by a given number of antidromic impulses may remain at an approximately constant size, since the period of post-tetanic depression determined by the recovery time of gustatory nerve signals was consistent, regardless of the test signals used (Fig. 8). Therefore, the selective depressant action of the antidromic stimuli described in this article can be ascribed largely to a change in the excitatory process. For example, the marked depression seen in the spontaneous discharges (Fig. 4) may reflect the fact that the excitatory process or the membrane depolarization associated with the spontaneous firing fluctuates around the threshold membrane potential for spike initiation and hence may readily be suppressed to a subliminal level at the generation of the inhibitory process or the post-tetanic hyperpolarization caused by antidromic impulses. However, the depression seen in the responses to quinine was smaller than that seen with the responses to salt (Table I; Figs. 1 and 2), probably because the spike-generating membrane of quinine-sensitive fibers was depolarized more intensively than those of salt-sensitive ones, and the post-tetanic inhibitory process was more opposed. In the latter, it is assumed that the nonmyelinated endings of a quinine-sensitive fiber would ramify more extensively than those of a salt-sensitive fiber and would incur a greater depolarization at the spike-generating membrane. According to the model proposed by Miller (1971), the extent of depolarization would depend on the degree of summation of currents at the excitable membrane, into which the current originating in the individual sensory terminals would flow along the pre-nodal axons.

Our experiments revealed that afferent impulses in the frog IXth nerve are subjected to conduction block at the spike-generating site close to the sensory nerve terminals, regardless of whether they were produced by sensory stimulation of the tongue receptors or were spontaneous in origin, in certain types of fibers. The physiological significance of this block, caused by antidromically conducting afferent impulses, may relate to a wide variety of gustatory signal modifications, such as varying degrees of depression in the number of afferent impulses and associated changes in the temporal pattern of impulse trains, depending on the intensity and quality of gustatory stimuli.

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