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Mechanism of Enhancement of the Responses of the Frog Glossopharyngeal Nerve to Electrolytes by Enhancers

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Abstract

In frogs, the responses of the glossopharyngeal nerve (GL) to NaCl are enhanced after treatment of the tongue with 8-anilino-1-naphthalene-sulfonic acid (ANS), a hydrophobic probe for biological membranes. The enhancement by ANS treatment has been explained by removal of Ca^{2+} from the receptor membrane treated with ANS. To explore the mechanism of enhancement by ANS treatment, we recorded neural responses from the frog GL. After ANS treatment, treatment with 10 mM $CaCl_2$ prior to stimulation of NaCl did not affect the enhanced responses to 100 mM NaCl. The response to a relatively high concentration of $CaCl_2$ (50 mM) was enhanced after ANS treatment. It is difficult to interpret these neural events in terms of modulation of the responses by membrane-bound calcium. The presence of $NiCl_2$ in stimulating solution is known as an enhancer. Neural events after ANS treatment were similar to those caused by $NiCl_2$. Our previous studies have demonstrated that enhancement of the responses to electrolytes by $NiCl_2$ is due to modulation of the responses of water fibers in the GL. Water fibers are characterized by sensitivity to water or $CaCl_2$, and they also respond to relatively high concentrations of electrolytes such as NaCl and choline Cl. Using a suction electrode method, we recorded unitary impulses from single water fibers. The ANS treatment led greatly enhanced responses to NaCl or choline Cl in water fibers, suggesting that enhancement by the ANS treatment is due to modulation of the responses of water fibers as well as enhancement by $NiCl_2$. It appears that distinct receptors for each separate cation responsible for the neural responses in water fibers interact with a membrane element that is affected by ANS or Ni^{2+}.

Key words: enhancer, frog, glossopharyngeal nerve, taste neural response, water fiber

Introduction

In frogs, taste receptors on almost the entire tongue are innervated by the glossopharyngeal nerve (GL). The frog GL responds to various electrolytes (Kusano and Sato 1957; Kusano 1960; Yamashita 1963; Kashiwagura et al. 1976; Hanamori et al. 1990; Herness 1991). Water fibers in the frog GL that are excited by application of distilled water to the tongue are very sensitive to $CaCl_2$ (Zotterman 1949; Kusano and Sato 1957; Nomura and Sakada 1965; Junge and Brodwick 1970; Kitada 1978). Threshold concentrations of $CaCl_2$ are below 0.01 mM (Nomura and Sakada 1965; Kitada 1978). Other salts, such as $MgCl_2$ and NaCl, are also effective stimuli for eliciting the response of water fibers, but relatively high concentrations of $MgCl_2$ (>10 mM, Nomura and Sakada 1965; Kitada 1978, 1989) and of NaCl (>100 mM, Nomura and Sakada 1965; Kitada 1991) are required to elicit neural responses. Competitive antagonism between cations in taste responses to electrolytes has been quantitatively demonstrated in water fibers of the frog GL. For example, in the response to a mixture of $CaCl_2$ and $MgCl_2$, Ca^{2+} competitively inhibits the response to Mg^{2+}, whereas Mg^{2+} competitively inhibits the response to Ca^{2+} (Kitada 1989). As a consequence, the net response to a mixture of $CaCl_2$ and $MgCl_2$ is small. Similar mutual competition between Ca^{2+} and Na^+ occurs in the response of water fibers to a mixture of $CaCl_2$ and NaCl (Kitada 1991). Treatment of the frog tongue surface with pronase, a proteolytic enzyme, reduces the responses to Ca^{2+}, Mg^{2+}, and Na^+ to different extents (Kitada 1984, 1986a, 1986b). From mutual competition between cations and the results of treatment with pronase, Kitada (1991) proposed that at least 3 specific receptor sites (receptors or channels) for cations (Ca^{2+}, Mg^{2+}, and Na^+) are involved in salt taste reception: a calcium receptor site (X_{Ca}), a magnesium receptor site (X_{Mg}), and a sodium receptor site (X_{Na}).

One strategy for studying the initial process of salt taste reception is through the use of modulators. In the frog GL, it has been reported that treatment of the tongue surface with 8-anilino-1-naphthalene-sulfonic acid (ANS), a hydrophobic probe for biological membranes, for several minutes led to a great enhancement of the response to NaCl (Kashiwagura et al. 1977). The enhanced responses to NaCl stayed at the enhanced level even after the ANS-treated tongue was thoroughly washed out. The enhanced response to 100 mM NaCl-stimulating solution was reduced to the original level when 1 mM $CaCl_2$ was added to the 100 mM NaCl-stimulating solution. Kashiwagura et al. (1977) proposed that treatment of the frog tongue with ANS removes Ca^{2+} from the receptor membrane and removal of Ca^{2+} from the receptor membrane is responsible for the enhancement of the responses to NaCl. Their explanation for reduction of the enhanced responses to 100 mM NaCl by the presence of 1 mM $CaCl_2$ is that $CaCl_2$ contained in NaCl-stimulating solution increases the amount of membrane-bound calcium and thereby responses to NaCl are reduced to the original level. Addition of $NiCl_2$ to the NaCl-stimulating solution also enhanced the response to NaCl (Kashiwagura et al. 1978). The enhanced response immediately returned to the original level when Ni^{2+} was removed from stimulating solutions. The enhancing effect of $NiCl_2$ was reversible. Hence, it is unlikely that enhancement of the response to NaCl by $NiCl_2$ is associated with removal of Ca^{2+} from the receptor membrane (Kashiwagura et al. 1978).

Kitada (1994d) found that water fibers of the frog GL exhibit an enhancement of the responses to $CaCl_2$, $MgCl_2$, and NaCl by the presence of Ni^{2+} to different extents. Mutual competition between Ca^{2+} and Na^+ and between Ca^{2+} and Mg^{2+} in water fibers remained even in the presence of Ni^{2+} (Kitada and Mitoh 1996, 1997). These findings lead to the idea that diminution of the enhanced response of the ANS-treated tongue to NaCl by the presence of Ca^{2+} may be due to inhibition of the responses to NaCl by competitive antagonism between Ca^{2+} and Na^+ for X_{Na}, but not associated with binding of Ca^{2+} to membrane components other than X_{Na}. The present study was undertaken to determine whether mechanism of enhancement in ANS treatment is similar to that in Ni^{2+} treatment. We report here that enhancement of the responses of the frog GL to electrolytes by enhancers is attributed to modulation of the responses of water fibers.

Materials and methods

Whole-nerve recording

Bullfrogs (*Rana catesbeiana*), weighing 200–400 g, were anesthetized with urethane (3 g/kg body weight). The experiments were performed in accordance with the Guidelines for Animal Experiments at Iwate Medical University. Each animal was put in the supine position, and the tongue was pulled out from the mouth and fixed on the plate of an experimental chamber with pins. The hypoglossal nerve was transected bilaterally to prevent tongue movements. The GL on one side was dissected free from surrounding connective tissues and cut centrally. The nerve was placed on a silver recording electrode. Multifiber neural activity was differentially recorded in reference to a stainless steel needle electrode placed in nearby tissue. The activities were displayed on an oscilloscope and passed through an integrator with a time constant of 0.5 s. The integrated neural activity was then displayed on a rectilinear pen recorder for analyses of response magnitudes.

Single-unit recording

A single fungiform papilla was drawn into a suction electrode. Antidromic nerve impulses, caused by the stimulation of adjacent papillae, were recorded with the suction electrode. The experimental procedures and the methods for neural activity were similar to those described in previous papers (Kitada 1978, 1989). Because distilled water or $CaCl_2$ exclusively excites the water fibers, water fibers are characterized by sensitivity to distilled water or $CaCl_2$ (Kitada 1978). Salts such as $MgCl_2$, NaCl, KCl, NH_4Cl, and choline Cl excite both water fibers and other fibers when their concentrations are relatively high. The impulses generated by water fibers were readily distinguishable from those that originated in other fibers because of the large amplitudes of impulses from water fibers. Stimulation with 1 or 2.5 mM $CaCl_2$ was used to identify a water fiber. In most cases, unitary impulses from a single water fiber were elicited by stimulation with electrolytes.

Treatment of the tongue with ANS

Kashiwagura et al. (1977) and Kashiwayanagi et al. (1981) reported that the enhancing effect of ANS on responses to NaCl after treatment of the tongue surface with 1 mM ANS below 10 °C for 2 min was much larger than that obtained with ANS at 20 °C for 2 min. In our pilot experiments in which effects of ANS treatment were tested at room temperature (20–25 °C), considerably enhanced responses for several minutes after ANS treatment were obtained by longer exposure (4 min) of the tongue to 1 mM ANS solution. Thus, in the present study, ANS treatment of the tongue was carried out as follows. A solution of 1 mM ANS (Eastman Kodak Co., Rochester, NY) dissolved in distilled water was flowed over the tongue surface at a flow rate 15 ml/min for 4 min at room temperature. The tongue was rinsed with 10 mM NaCl solution at 15 ml/min for 1 min, and then stimulating solution was applied to the tongue at the same flow rate. The pH value of 1 mM ANS dissolved in distilled water was 3.1. Because low pH has been reported to enhance the response to NaCl (Kumai and Nomura 1980), a solution of HCl dissolved in distilled water at pH 3.1 was prepared and the HCl solution was used to determine whether

enhancement of the response to NaCl by treatment with 1 mM ANS solution is due to ANS itself or protons.

Stimulation

The experiments were performed at 20–25 °C. Because water fibers of the frog GL are sensitive to distilled water and the water response is inhibited by low concentrations of NaCl (Zotterman 1949), 10 mM NaCl solution was used as an adapting solution. Stimulating solutions of 20–500 mM NaCl, 1–50 mM $CaCl_2$, and 500 mM choline Cl from Kanto Chemical Co. (Tokyo, Japan) were prepared with distilled water. Mixtures of 2.5 mM $CaCl_2$ and 200–500 mM NaCl were also used. To study enhancement by $NiCl_2$, 1 mM $NiCl_2$ was chosen because the maximum enhanced response to NaCl was obtained at this concentration (Kitada 1994d).

Data analysis

The height of the pen recorder deflection at 25 s after stimulus application was used as the measure of tonic response of the GL. The response magnitudes were normalized relative to the magnitude of the standard response. We used 500 mM NaCl for stimulation by NaCl and 50 mM $CaCl_2$ for stimulation by $CaCl_2$ as the respective standard solutions. For single-unit recordings, only the number of unitary impulses from a single water fiber was counted with a spike counter.

Data are expressed as means ± standard errors of the mean. We used Student's *t*-tests. The level of significance was set at $P < 0.05$.

Results

ANS treatment

Figure 1 shows the enhancing effect of ANS treatment on the response to NaCl. Before the ANS treatment, the threshold concentration of NaCl for eliciting tonic response was around 100 mM when taste receptors on the tongue were adapted to 10 mM NaCl. A solution of 1 mM ANS was applied to the tongue surface for 4 min. The tongue was washed out by a rinsing solution (10 mM NaCl) for 1 min. Subsequent application of 100 mM NaCl gave rise to a large enhancement of the response (Figure 1A). The pH value of 1 mM ANS solution dissolved in distilled water was 3.1. We examined whether the enhancing effect of the ANS treatment was due to low pH. The tongue was treated with HCl solution of pH 3.1 for 4 min, and then the tongue was washed out by the rinsing solution (10 mM NaCl) for 1 min. The transient response (off response) was elicited by the rinsing solution after treatment with HCl solution of pH 3.1 and returned to the resting level (Figure 1B). As shown in Figure 1B, treatment of the tongue with HCl solution of pH 3.1 did not give rise to an enhanced response to 100 mM NaCl. The magnitude of the response to 100 mM NaCl after ANS treatment was significantly larger

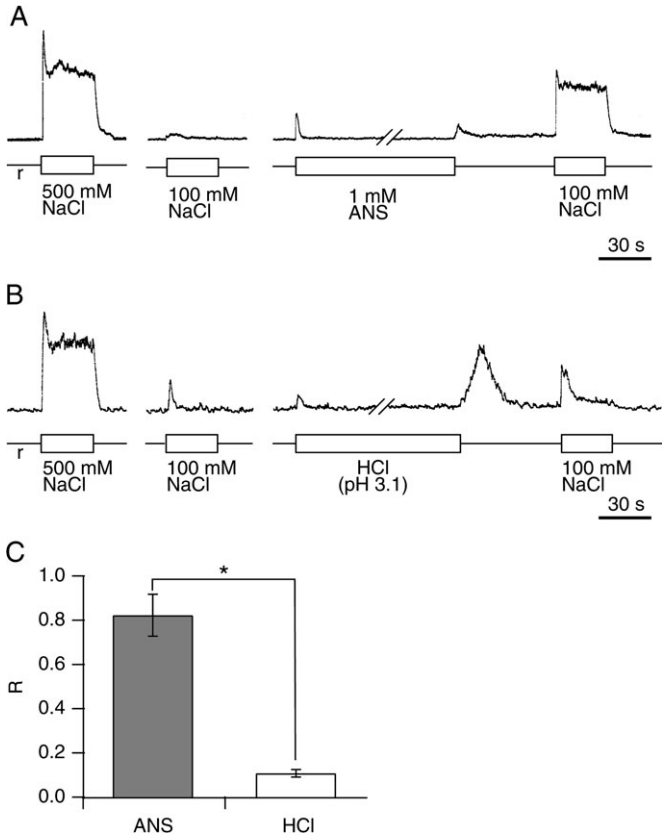

Figure 1 Enhancement of the responses to NaCl by ANS treatment. **(A)** Records represent integrated responses. The tongue surface was treated with 1 mM ANS for 4 min and then rinsed with 10 mM NaCl for 1 min. Subsequent application of a solution of 100 mM NaCl (around the threshold concentration for tonic responses before ANS treatment) to the tongue gave rise to a greatly enhanced response. **(B)** Records represent integrated responses. The tongue surface was treated with HCl solution at pH 3.1, the same pH as that of 1 mM ANS in distilled water, for 4 min and then rinsed with 10 mM NaCl for 1 min. Subsequent application of a solution of 100 mM NaCl to the tongue did not give rise to an enhanced response to 100 mM NaCl. **(C)** Average response ratios of the tonic responses to 100 mM NaCl with ANS (pH 3.1) treatment (n = 16) or HCl (pH 3.1) treatment (n = 11). The magnitude of the response to 500 mM NaCl before ANS treatment or HCl treatment is taken as unity on the ordinate. Values are means ± standard errors of the mean. r, rinsing solution (10 mM NaCl). *$P < 0.0001$ (unpaired Student's *t*-test).

than that after low pH (pH 3.1) treatment (Figure 1C), suggesting that ANS itself brings about the enhancement of the response to NaCl. Figure 2 shows concentration–response (C–R) curves for NaCl before and after ANS treatment. The ANS treatment shifted the curve toward lower concentrations of NaCl, and the threshold concentration after the ANS treatment was reduced to around 20 mM. The ANS treatment enhanced the response to NaCl at any concentration of NaCl.

In the ANS-treated tongue, Kashiwagura et al. (1977) showed that the inhibitory effect of Ca^{2+} on the enhanced response to NaCl promptly appeared when $CaCl_2$ was added to NaCl-stimulating solution. If removal of Ca^{2+} from the

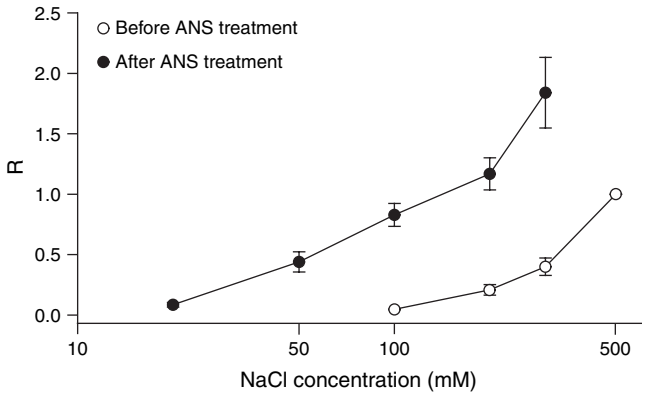

Figure 2 Concentration–response curves for NaCl before and after ANS treatment. The relative magnitude of the responses (*R*) is plotted against the logarithm of the concentration of NaCl. The magnitude of the response to 500 mM NaCl before 1 mM ANS treatment is taken as unity on the ordinate. Values are means ± standard errors of the mean, n = 8–16.

receptor membrane by the ANS treatment caused the enhanced response to NaCl as they proposed, it seems reasonable to assume that exposure of the ANS-treated tongue to a solution containing Ca^{2+} would restore the amount of membrane-bound calcium and would reduce the enhanced response. Thus, a relatively high concentration of $CaCl_2$ (10 mM) solution was applied to the ANS-treated tongue for 30 s (Figure 3A). The solution of 10 mM $CaCl_2$ led to a large tonic response. The tongue was washed out with the rinsing solution. Subsequent application of 100 mM NaCl still brought about an enhanced response to 100 mM NaCl. The average magnitude of the responses to 100 mM NaCl with exposure to 10 mM $CaCl_2$ for 30 s was not statistically different from that without exposure to 10 mM $CaCl_2$ (Figure 3B).

Ni^{2+} effect

Although $NiCl_2$ in the NaCl-stimulating solution has an enhancing effect on the response to NaCl, the effect of long exposure (4 min) of the receptor membrane to $NiCl_2$ on the response to NaCl has not been tested. As shown in Figure 4, $NiCl_2$ at 1 mM was barely effective in producing impulses from the frog GL. Pretreatment with $NiCl_2$ for 4 min did not affect the response to 100 mM NaCl alone. The NaCl-stimulating solution containing 1 mM $NiCl_2$ induced an enhanced response. Thus, we confirmed that the enhanced responses to NaCl appeared only when $NiCl_2$ was present in the NaCl-stimulating solution.

Similarities between responses with the ANS treatment and the presence of Ni^{2+}

ANS treatment (Kashiwagura et al. 1977) and $NiCl_2$ in stimulating solutions (Kashiwagura et al. 1978) did not affect the responses to $CaCl_2$. However, the responses of water fibers to Ca-salts ($CaCl_2$ and $CaSO_4$) were enhanced by Ni-salts ($NiCl_2$ and $NiSO_4$) (Kitada 1994a, 1994d). Thus, we examined in multifiber recordings whether ANS treatment or the presence of $NiCl_2$ in $CaCl_2$-stimulating solution can enhance the response to $CaCl_2$ or not. It has been demonstrated that Ni^{2+} has a dual action on the response to Ca^{2+}, inhibition, and enhancement (Kitada 1994a). The effect of Ni^{2+} on the response to Ca^{2+} was explained by the hypothesis that Ni^{2+}, as well as other cations, inhibits the responses to Ca^{2+} by competing with Ca^{2+} for X_{Ca}, whereas it enhances them by acting on a membrane molecule that interacts with X_{Ca} (Kitada 1994a). To avoid the competitive inhibition of the response to Ca^{2+} by Ni^{2+}, a relatively high concentration of $CaCl_2$ (50 mM) was used in this study. At this concentration of $CaCl_2$, Ca^{2+} would occupy most of X_{Ca} even in the presence of 1 mM Ni^{2+}. The results presented in Figure 5 show that both pretreatment with ANS and the presence of $NiCl_2$ in $CaCl_2$-stimulating solution enhanced the response to 50 mM $CaCl_2$. The magnitude of the enhanced response to 50 mM $CaCl_2$ in the ANS-treated tongue was not statistically different from that in the presence of $NiCl_2$ (Figure 5C).

The C–R curve for NaCl after the ANS treatment shown in Figure 2 is replotted in Figure 6. The C–R curves for NaCl in the presence of $NiCl_2$ and in both treatment with $NiCl_2$ and ANS are plotted in Figure 6. The 3 C–R curves are nearly superposed.

Enhancement of responses of single water fibers to NaCl and choline Cl by ANS treatment

Because the enhanced responses to NaCl (Kitada 1994d; Kitada and Mitoh 1996) and to choline Cl (Kitada 1994b, 1994d) induced by the presence of $NiCl_2$ were observed in water fibers, we examined whether 1 mM ANS treatment can induce enhanced responses of water fibers to NaCl and choline Cl. Figure 7 shows the effects of ANS treatment on water fibers responding to $CaCl_2$. Response to NaCl (Figure 7A) and that to choline Cl (Figure 7B) of water fibers were enhanced by ANS treatment. The frequency of impulses elicited by 200 mM NaCl or by 500 mM choline Cl after ANS treatment was significantly higher than that before ANS treatment (Figure 7C).

Discussion

In the frog GL, Kashiwagura et al. (1977) found that ANS treatment induced enhanced responses to salts such as NaCl, NH_4Cl, KCl, LiCl, and $MgCl_2$, whereas it did not affect the responses to distilled water, $CaCl_2$, D-galactose, and quinine. Similar enhancing effects were observed when a small amount of $NiCl_2$ was present in stimulating solutions (Kashiwagura et al. 1978). Kashiwagura et al. (1977) speculated that the enhanced responses to salt stimuli were due to removal of Ca^{2+} from the receptor membrane. On the other hand, enhancement of the responses by $NiCl_2$

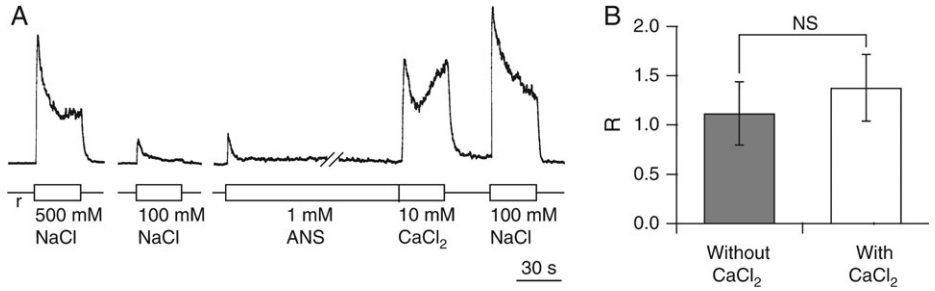

Figure 3 No influence of exposure of the ANS-treated tongue to 10 mM $CaCl_2$ solution on the enhanced response to 100 mM NaCl. **(A)** Records represent integrated responses. After 1 mM ANS treatment, solution of 10 mM $CaCl_2$ was applied to the tongue for 30 s, and then the tongue was rinsed with 10 mM NaCl. Exposure of the ANS-treated tongue to 10 mM $CaCl_2$ did not affect the enhanced response to 100 mM NaCl. **(B)** Average response ratios (*R*) of the tonic responses to 100 mM NaCl in 1 mM ANS-treated tongue with and without pretreatment of 10 mM $CaCl_2$ ($n = 5$). The magnitude of the response to 500 mM NaCl before ANS treatment is taken as unity on the ordinate. r, rinsing solution (10 mM NaCl); NS, $P > 0.05$ (paired Student's *t*-test).

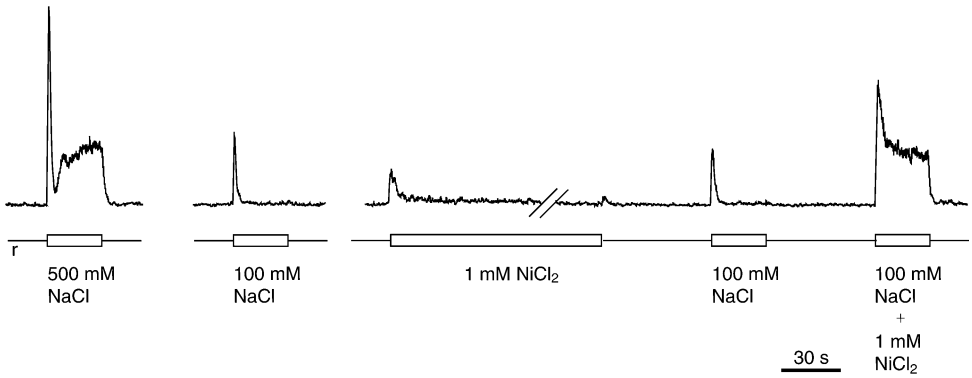

Figure 4 Enhancement of the response to NaCl by the presence of $NiCl_2$. Records represent integrated responses. The tongue surface was treated with 1 mM $NiCl_2$ for 4 min and then rinsed with 10 mM NaCl for 1 min. Subsequent application of a solution of 100 mM NaCl alone to the tongue did not give rise to an enhanced response. Only NaCl-stimulating solution containing 1 mM $NiCl_2$ gave rise to a greatly enhanced response. r, rinsing solution (10 mM NaCl).

was not brought about by removal of Ca^{2+} from the receptor membrane (Kashiwagura et al. 1978). Despite different characteristics of actions of the 2 treatments, there are many similarities between the ANS treatment and action of $NiCl_2$. In the present study, we found that both treatments with ANS and $NiCl_2$ enhanced the response to $CaCl_2$ (Figure 5). The C–R curve after the ANS treatment was similar to that in the presence of $NiCl_2$, and the C–R curve with $NiCl_2$ and ANS was identical to that with $NiCl_2$ or with ANS (Figure 6), suggesting that the presence of 1 mM $NiCl_2$ or 1 mM ANS treatment had a saturated effect on the responses to NaCl. As shown in Figure 7, ANS treatment induced enhanced responses of water fibers to electrolytes as did $NiCl_2$ treatment (Kitada 1994c, 1994d). From these results, it is likely that the mechanism of enhancement by the ANS treatment is similar to that by the presence of $NiCl_2$.

Exposure of the ANS-treated tongue to relatively high Ca^{2+} did not affect the enhanced response (Figure 3). Furthermore, an enhanced response to a relatively high concentration of $CaCl_2$ (50 mM) was induced by ANS treatment (Figure 5), suggesting that Ca^{2+} does not act as an inhibitor during stimulation by Ca^{2+}. Therefore, it is difficult to interpret these results in terms of modulation of the responses by membrane-bound calcium. It appears that ANS molecules can combine with membrane components for several minutes after the tongue has been washed out and can affect the response to salt stimuli.

Because enhancement of the responses to electrolytes induced by treatment with ANS and $NiCl_2$ is thought to be due to modulation of the responses of water fibers, it is likely that the enhanced responses of the GL to various salt stimuli induced by ANS treatment or $NiCl_2$ reflect those of water fibers. As described in the Introduction, there are at least 3 distinct receptor sites (X_{Ca}, X_{Mg}, and X_{Na}) in water fibers of the frog GL. In mixtures of $CaCl_2$ and NaCl, Na^+ inhibited the response to Ca^{2+} by competing with Ca^{2+} for X_{Ca}, whereas Ca^{2+} inhibited the response to Na^+ by competing with Na^+ for X_{Na} (Kitada 1991). The antagonism remained in the presence of $NiCl_2$ (Kitada and Mitoh 1996) or after ANS treatment (Kashiwagura et al. 1977). These findings suggest that only the binding of each separate cation (agonist) to its appropriate receptor sites leads to the initiation of impulses in water fibers and that enhancers (presence of Ni^{2+} and ANS treatment) can enhance the activation of

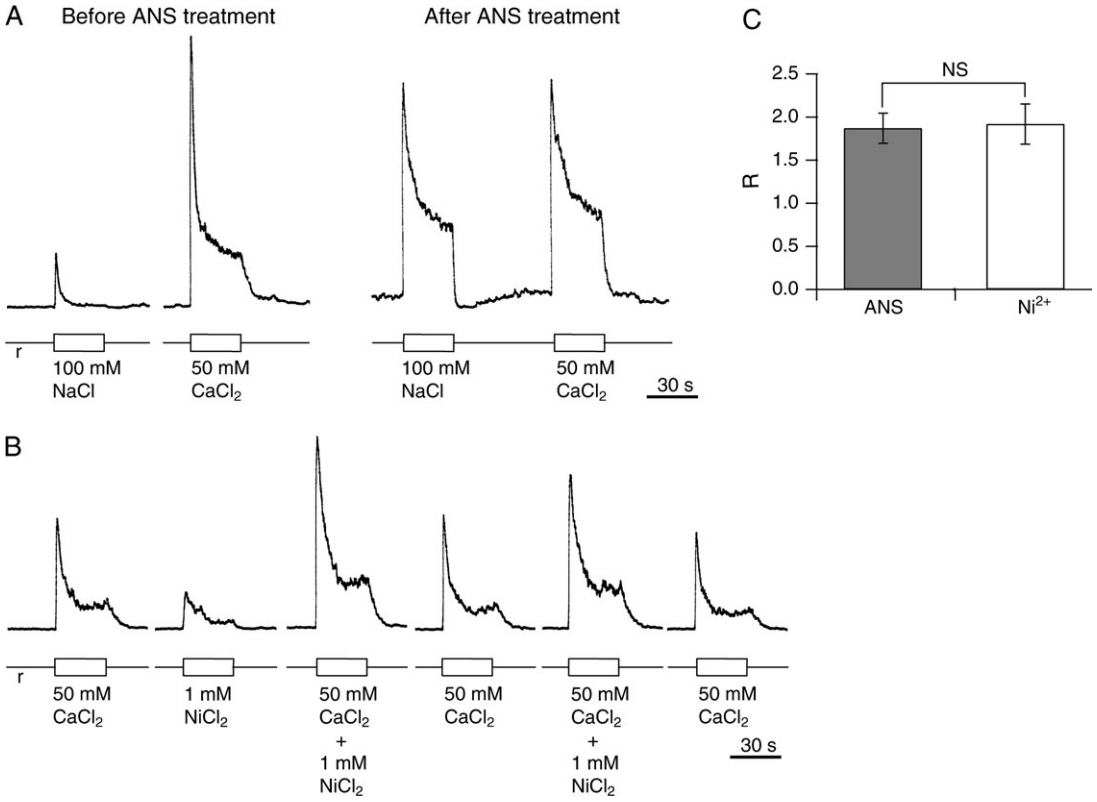

Figure 5 Enhancement of the response to $CaCl_2$ by ANS treatment or presence of $NiCl_2$. **(A** and **B)** Records represent integrated responses. The response to 50 mM $CaCl_2$ was enhanced after 1 mM ANS treatment (A) or in the presence of 1 mM $NiCl_2$ added to 50 mM $CaCl_2$-stimulating solution (B). The effect of $NiCl_2$ was reversible (B). **(C)** Average response ratios (*R*) of the tonic responses to 50 mM $CaCl_2$ after 1 mM ANS treatment ($n = 6$) and in the presence of 1 mM $NiCl_2$ ($n = 5$). The magnitude of the response to 50 mM $CaCl_2$ before ANS treatment or in the absence of $NiCl_2$ is taken as unity on the ordinate. r, rinsing solution (10 mM NaCl); ANS, stimulation with 50 mM $CaCl_2$ after 1 mM ANS treatment; Ni^{2+}, stimulation with 50 mM $CaCl_2$ with the presence of 1 mM $NiCl_2$; NS, $P > 0.05$ (unpaired Student's *t*-test).

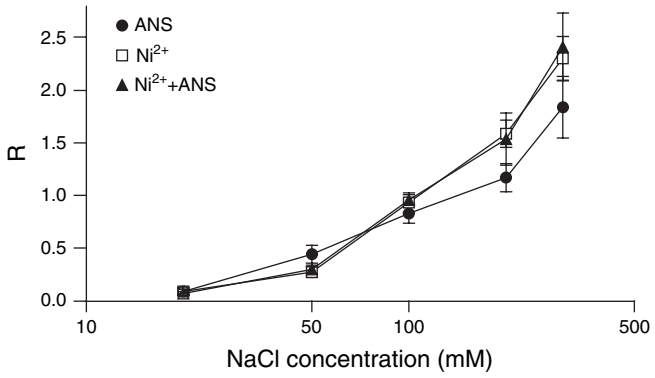

Figure 6 Concentration–response curves for NaCl in the presence of $NiCl_2$ and after ANS treatment. The relative magnitude of the responses (*R*) is plotted against the logarithm of the concentration of NaCl. The magnitude of the response to 500 mM NaCl before ANS treatment is taken as unity on the ordinate. ANS, after 1 mM ANS treatment ($n = 8$–16); Ni^{2+}, in the presence of 1 mM $NiCl_2$ ($n = 6$); Ni^{2+} + ANS, in the presence of 1 mM $NiCl_2$ and 1 mM ANS treatment ($n = 6$). Note that the 3 curves are nearly superposed.

a receptor–agonist complex and they cannot affect a receptor–antagonist complex. Despite different receptor sites for cations, the concentration of Ni^{2+} effective to enhance responses to salts was almost the same among the responses to Ca^{2+}, Mg^{2+}, and Na^{+} (Kitada 1994c). Therefore, it appears that a common mechanism is involved in enhancement of the responses to Ca^{2+}, Mg^{2+}, and Na^{+}. In the mechanism of enhancement by Ni^{2+}, Kitada and Mitoh (1996, 1997) proposed the hypothesis that each receptor site interacts with a membrane element (T) that is affected by Ni^{2+}. In the case of ANS treatment, it is thought that ANS is adsorbed in the hydrophobic region of the receptor membrane and secondarily affects T. Enhancers can enhance the efficacy of cation transduction by affecting T. Affinities of receptors including X_{Na} for monovalent cations can become high by activation of T so that enhancers can reduce the threshold concentrations for monovalent cation salts. Because enhancers did not affect the threshold concentrations for $CaCl_2$ and $MgCl_2$ (Kashiwagura et al. 1977, 1978), activation of T cannot affect the affinities of X_{Ca} for Ca^{2+} and X_{Mg} for Mg^{2+}. Choline Cl also excites water fibers (Kitada 1994b, 1994d). Because $NiCl_2$ did not reduce the threshold concentration for choline Cl, $choline^{+}$ was thought to act on receptors (X_{Ch}) other than X_{Na} (Kitada 1994d). In the present study, we found that the ANS treatment induced an enhanced response of water fibers to choline Cl. It seems that X_{Ch} also interacts with T.

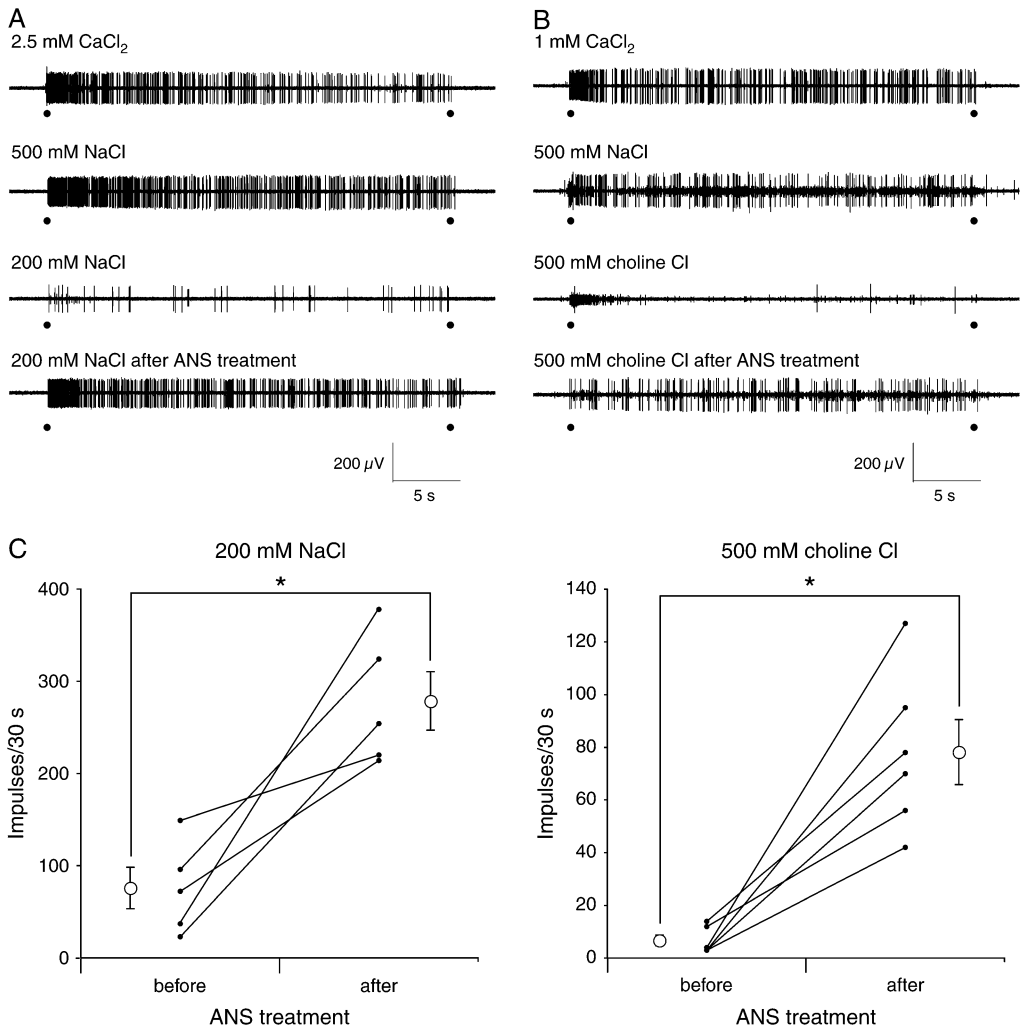

Figure 7 Enhanced responses of water units to NaCl and to choline Cl induced by ANS treatment. The impulses were recorded using a suction electrode method. Two dots below each record indicate the 30-s duration of application of the stimulus. The records are presented in the order of stimulation, from top to bottom. A solution of 1 or 2.5 mM $CaCl_2$ elicited unitary impulses from a water fiber. Treatment of the tongue with 1 mM ANS for 4 min induced an enhanced response to 200 mM NaCl **(A)** and to 500 mM choline Cl **(B)** with impulses of the same shape as those elicited upon stimulation by Ca^{2+}. (A and B) are responses recorded from different preparations. **(C)** Enhancement of the responses of single water fibers to NaCl or choline Cl by ANS treatment. The ordinate represents the number of impulses elicited during stimulation by 200 mM NaCl ($n = 5$) or 500 mM choline Cl ($n = 6$) for 30 s before and after 1 mM ANS treatment. Each line represents the results from a single fiber. Open circle and bar: means ± standard errors of the mean. $*P < 0.01$ (paired Student's *t*-test).

There is another enhancer besides $NiCl_2$ and ANS treatment that enhances the response to salt stimuli (Kamo et al. 1978). The responses of the frog GL to various salts including $CaCl_2$ and distilled water are greatly enhanced after the tongue is treated with an alkaline solution above pH 7.5. Incubation of the alkali-treated tongue in solutions containing Ca^{2+} of low pH (pH 5.3) restores the responses to the original responses before the alkali treatment. In addition, one piece of tongue incubated in a solution of pH 5.3 containing ^{45}Ca released a larger amount of ^{45}Ca by alkali treatment than another piece incubated in pH 7.0. From these findings, Kamo et al. (1978) suggested that the magnitude of the responses of the frog GL to salt stimuli is controlled by the amount of membrane-bound Ca^{2+}. However, the treatment of the tongue surface with ethylenediaminetetraacetic acid, in the attempt to remove membrane-bound Ca^{2+}, brought about only small enhancement of the salt response (Kashiwagura et al. 1977). Hence, it is uncertain whether amount of membrane-bound Ca^{2+} modulates the magnitude of the frog taste responses.

It has been demonstrated that amiloride, an epithelial sodium channel blocker, partially reduces the neural responses to NaCl of the chorda tympani of the rat. The amiloride-sensitive pathway is mediated by the epithelial Na^+ channel (ENaC), a highly Na^+-selective channel (Lindemann 1996), whereas amiloride-insensitive pathway is mediated by

a variant of the nonselective cation channel transient receptor potential V1 (TRPV1), which is a member of the vanilloid class of transient receptor potential channels (Lyall et al. 2004). In the frog GL, amiloride did not affect the response to NaCl (Kitada et al. 2001). Therefore, the responses to NaCl in the frog GL use the amiloride-insensitive pathway. Because Ca^{2+} and Na^{+} were mutually antagonized in responses to mixtures of $CaCl_2$ and NaCl, nonselective cation channels are not thought to be involved in salt taste transduction in water fibers of the frog GL. Kitada (1984, 1986a) has found that treatment of the tongue surface with 0.1% pronase E inhibits the response to $CaCl_2$ but does not inhibit the response to NaCl and suggests that X_{Ca} may be a protein that is distinct from X_{Na}. However, it is unclear whether responses of the frog GL to various salt stimuli are mediated by different specific cation-receptors or by ionic channels. As mentioned above, Na^{+} and choline^{+} act on different receptor sites. Because choline^{+} is a large ion, it seems unlikely that the responses to choline Cl are mediated by an ionic channel.

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