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A Quantitative Study of the Enhancing Effect of Nickel Ions on the Taste Response to Sodium Ions of Single Fibers of the Frog Glossopharyngeal Nerve: Competitive Inhibition by Calcium Ions of the Nickel-Enhanced Response to Sodium Ions

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Abstract

Single water fibers of the frog glossopharyngeal nerve respond to relatively high concentrations of NaCl (>80 mM). $NiCl_2$ at 1 mM enhanced the Na^+ response and reduced the threshold concentration for NaCl to 20 mM. $CaCl_2$ at 0.5–1 mM induced an inhibition of the Ni^{2+}-enhanced response to Na^+ ions. A quantitative explanations for these results is provided by the hypothesis that Ni^{2+} ions secondarily affect a sodium receptor or channel (designated X^*_{Na}) that is responsible for the Na^+ response and that Ca^{2+} ions inhibit the Ni^{2+}-enhanced response to Na^+ ions by competing with Na^+ ions for X^*_{Na}. Double-reciprocal plots of the experimental data indicate that the affinity of X^*_{Na} for both Na^+ ions (agonist) and Ca^{2+} ions (competitive antagonist) in the presence of 1 mM $NiCl_2$ was five times higher than the previously reported values obtained in the absence of $NiCl_2$ (Kitada, 1991). Ni^{2+} ions at 1 mM enhanced the maximal response to Na^+ ions by 190%. It appears that a sodium receptor (or channel) interacts with a Ni^{2+}-binding element that is affected by Ni^{2+} ions and, thus, Ni^{2+} ions can induce both an increase in the affinity of the sodium receptor for the respective cations and an enhancement of the Na^+ response. **Chem Senses 21: 65–73, 1996.**

Introduction

The water fibers of the frog glossopharyngeal nerve respond to distilled water applied to the tongue (Zotterman, 1949; Kusano and Sato, 1957). They also respond to salts, such as calcium (Kusano, 1960; Nomura and Sakada, 1965; Junge and Brodwick, 1970; Kitada, 1978), magnesium and sodium salts (Kusano, 1960; Nomura and Sakada, 1965). However, the responses to a mixture of the salts of two different cations are not always as large as might be anticipated. It has been demonstrated that the response to Ca^{2+} ions (the Ca^{2+} response) is competitively inhibited by Mg^{2+} ions and by Na^+ ions (Kitada and Shimada, 1980) and that the responses to Mg^{2+} ions (the Mg^{2+} response) and to Na^+ ions (the Na^+ response) are competitively inhibited by Ca^{2+} ions (the Mg^{2+} response; Kitada, 1989; the Na^+ response;

Kitada, 1991). As a consequence, the net response to a mixture of $CaCl_2$ plus $MgCl_2$ or of $CaCl_2$ plus NaCl is small. From analysis of the mutual competition between cations and other results (Kitada, 1984, 1986a, b, 1995), Kitada (1990, 1991, 1995) proposed that at least three specific receptors for cations are involved in salt taste reception: a calcium receptor, a magnesium receptor and a sodium receptor. In the present study, the term 'receptor' for a cation is used to indicate a cation-discriminative element or a selective cation-channel that is responsible for the response to the cation.

Transition metal ions, such as Ni^{2+}, Co^{2+} and Mn^{2+} ions, at concentrations below 5 mM are barely effective in producing nerve impulses in the water fibers of the frog glossopharyngeal nerve (Kitada, 1990, 1994b). However, they have enhancing effects on the responses to Ca^{2+} (Kitada, 1994b, c), Mg^{2+} (Kashiwagura *et al.*, 1978; Kitada, 1994b) and Na^+ (Kashiwagura *et al.*, 1978; Herness, 1987, 1991; Kitada, 1994b) ions. Transition metal ions do not affect antagonism between Ca^{2+} and Mg^{2+} ions and between Ca^{2+} and Na^+ ions in the neural responses to a mixture of two salts (Kitada, 1994b). This result suggests that transition metal ions might secondarily affect each of the receptors responsible for various responses to cations via a membrane element other than the receptors (Kitada, 1994b, c). Transition metal ions are, therefore, useful as tools in attempts to study the initial events associated with transduction of salt signals in this nerve. Since the receptors responsible for the responses to Ca^{2+}, Mg^{2+} and Na^+ ions are different from one another (Kitada, 1990, 1991) and since each class of receptors has its own specific properties (Kitada, 1989, 1990, 1991), it is important to characterize the effects of transition metal ions on the responses to each of these three cations.

The characteristics of the effects of transition metal ions on the Na^+ response are unknown. Among transition metal ions, Ni^{2+} ions are the most effective in the enhancement of the responses to Ca^{2+}, Mg^{2+} and Na^+ ions (Kitada, 1994b). In the present study, the enhancement of the Na^+ response by Ni^{2+} ions and the inhibition of the Ni^{2+}-enhanced response to Na^+ ions by Ca^{2+} ions in single water fibers of the frog glossopharyngeal nerve were investigated quantitatively.

Materials and methods

Bullfrogs (*Rana catesbeiana*), weighing 200–400 g, were rapidly decapitated and pithed. Each isolated tongue was placed in a test chamber. The experimental procedures and the methods for recording neural activities were similar to those described in a previous paper (Kitada, 1978, 1989). Single gustatory fibers innervating the frog tongue extend several peripheral branches and each branch innervates a separate fungiform papilla (Rapuzzi and Casella, 1965). Antidromic impulses of single gustatory nerve fibers were recorded from a single fungiform papilla that had been drawn into a suction electrode during stimulation of adjacent papillae by chemical stimuli (Taglietti *et al.*, 1969; Kitada, 1978). Stimulation with 1–2 mM $CaCl_2$ was used to identify individual water fibers, since Ca^{2+} ions at low concentrations stimulate water fibers exclusively (Casella and Rapuzzi, 1957; Nomura and Sakada, 1965; Junge and Brodwick, 1970; Kitada, 1978). In most cases, unitary discharges from a single water fiber were recorded in response to stimulation by solutions of calcium, magnesium and sodium salts. Since each gustatory fiber innervates many taste cells, the results obtained with a suction electrode from the frog tongue reflect integrative cellular properties.

Stimulating solutions of 10–500 mM NaCl, 1–2 mM $CaCl_2$ and 1 mM $NiCl_2$ were prepared in distilled water. To examine the effects of $NiCl_2$ on the responses to NaCl, to $CaCl_2$ and to a mixture of NaCl plus $CaCl_2$, mixtures of 1 mM $CaCl_2$ plus 1 mM $NiCl_2$, of 20–500 mM NaCl plus 1 mM $NiCl_2$, and of 5–500 mM NaCl plus 0.5–1.0 mM $CaCl_2$ plus 1 mM $NiCl_2$ were also used. A solution of 50 mM NaCl was used as the adapting solution. Since the excitatory effect of water on water fibers is suppressed by the presence of electrolytes at low concentrations (Zotterman, 1949; Kusano and Sato, 1957), there was no impulse activity during this adaptation. The tongue was exposed to the adapting solution for at least 5 min before each application of a stimulating solution. Each stimulating solution was applied to the surface of the tongue, near the recording electrode, at a flow rate of 5–8 ml/min for 30 s. All the experiments were performed at 20–25°C.

The number of impulses elicited during the tonic component of the response (from 5 to 30 s after the onset of the stimulus) was measured with a spike counter. The reasons for deleting the initial component of the response have been explained elsewhere (Kitada, 1989). The magnitude of the response to each stimulating solution varied from one unit to another. The neural response to a stimulating solution was normalized by comparison to the magnitude of the response to a standard solution (500 mM NaCl alone) of each fiber. The magnitude of the standard response was taken as the mean value of two responses to the standard

solution, which was applied twice, once before and once after each application of the stimulating solution.

Results

Ni^{2+} ions increase the affinity of the sodium receptor for Na^+ ions

The threshold concentration for a solution of NaCl alone is about 80–100 mM (Kitada, 1991). Since an adapting solution to 10 mM NaCl did not alter the threshold concentration for NaCl, the high threshold concentration for NaCl was not due to the relatively high concentration (50 mM) of the salt in the adapting solution. By contrast to the high threshold concentration of NaCl, a response to $CaCl_2$ can be detected at concentrations below 0.01 mM and the maximal response to $CaCl_2$ is obtained at 1 mM (Kitada, 1978). Figure 1 shows unitary discharges from a single water fiber elicited by stimulation with 1 mM $CaCl_2$, with 1 mM $NiCl_2$, with 50 mM NaCl and with 50 mM NaCl plus 1 mM $NiCl_2$. As shown in Figure 1, $CaCl_2$ at 1 mM elicited a large response while $NiCl_2$ at 1 mM elicited only a few impulses. Application of a solution of 50 mM NaCl, the same solution as the rinsing solution, did not elicit any neural response. However, addition of 1 mM $NiCl_2$ to a stimulating solution of 50 mM NaCl induced a large response. This effect of $NiCl_2$ on the Na^+ response was reversible.

Figure 2 shows concentration–response (C–R) curves for

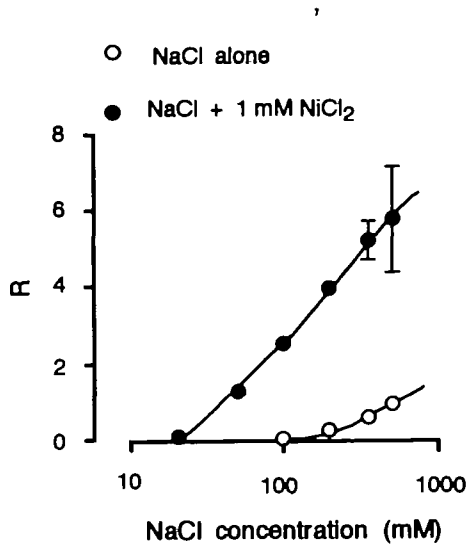

Figure 2 Concentration–response curves for NaCl obtained in the absence and in the presence of 1 mM $NiCl_2$ The relative magnitude of the responses (R) is plotted against the logarithm of the concentration of NaCl. The magnitude of the neural response to 500 mM NaCl alone (the standard solution) is taken as unity on the ordinate. Each point and bar represent a mean ± SEM, n = 3–5 units.

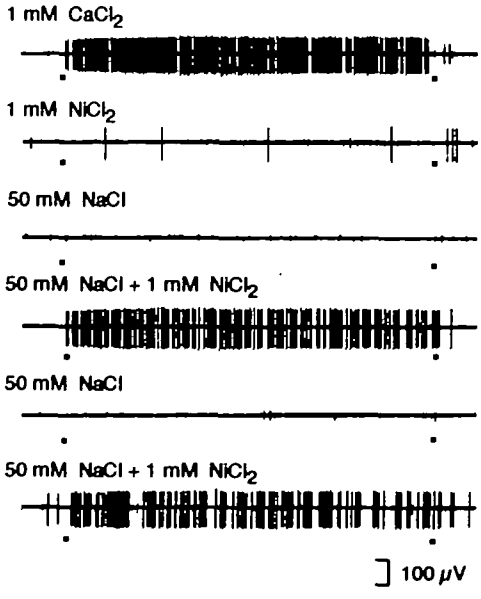

Figure 1 Responses of a single water unit to 1 mM $CaCl_2$, to 1 mM $NiCl_2$, to 50 mM NaCl, to a mixture of 50 mM NaCl plus 1 mM $NiCl_2$, to 50 mM NaCl and to a mixture of 50 mM NaCl plus 1 mM $NiCl_2$. The two dots below each record indicate the 30 s duration of application of the stimulus. The records are arranged in order of stimulation, from top to bottom.

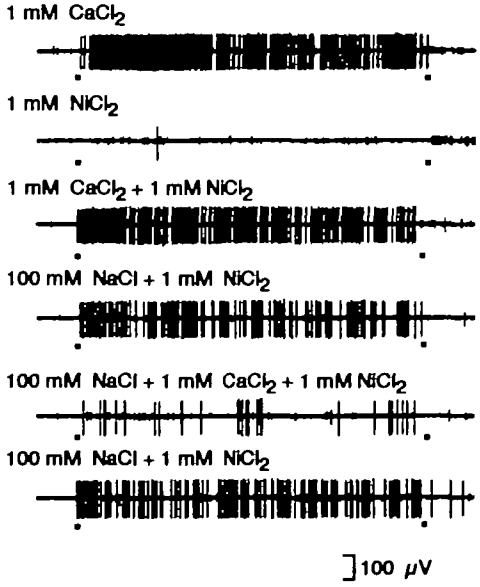

Figure 3 Responses of a single water unit to 1 mM $CaCl_2$, to 1 mM $NiCl_2$, to a mixture of 1 mM $CaCl_2$ plus 1 mM $NiCl_2$, to a mixture of 100 mM NaCl plus 1 mM $CaCl_2$, to a mixture of 100 mM NaCl plus 1 mM $CaCl_2$ plus 1 mM $NiCl_2$, and to a mixture of 100 mM NaCl plus 1 mM $NiCl_2$. The two dots below each record indicate the 30 s duration of application of the stimulus. The records are arranged in order of stimulation, from top to bottom.

NaCl obtained in the absence and in the presence of 1 mM $NiCl_2$. The neural response (R) was normalized by comparing it to the magnitude of the standard response (the response to 500 mM NaCl alone) of each fiber. $NiCl_2$ at 1 mM reduced the threshold concentration for NaCl to 20 mM and increased the response to 500 mM NaCl to about 600% of the original response. The C–R curve for NaCl in the presence of 1 mM $NiCl_2$ had a much steeper slope than that for NaCl in the absence of $NiCl_2$. The results in Figure 2 indicate that $NiCl_2$ both induced an increase in the affinity of a sodium receptor for Na^+ ions and enhanced the Na^+ response at any given concentration of NaCl.

Ca^{2+} ions inhibit the Ni^{2+}-enhanced response to Na^+ ions

Figure 3 shows an example of the mutual antagonism between Ca^{2+} and Na^+ ions in the presence of $NiCl_2$. As seen in Figure 3, the frequency of impulses elicited by the mixture of 100 mM NaCl, 1 mM $CaCl_2$ and 1 mM $NiCl_2$ together was much lower than that of impulses elicited by a solution of 100 mM NaCl plus 1 mM $NiCl_2$ or by a solution of 1 mM $CaCl_2$ plus 1 mM $NiCl_2$. Since mutual antagonism between Ca^{2+} and Na^+ ions occurs in the absence (Kitada, 1991) and in the presence of $NiCl_2$ (Kitada, 1994b), the decrease in the magnitude of the response to a mixture of the sodium, calcium and nickel salts is not due to antagonism between Ni^{2+} ions and other cations, but is due to antagonism between Ca^{2+} and Na^+ ions (Kitada, 1994b).

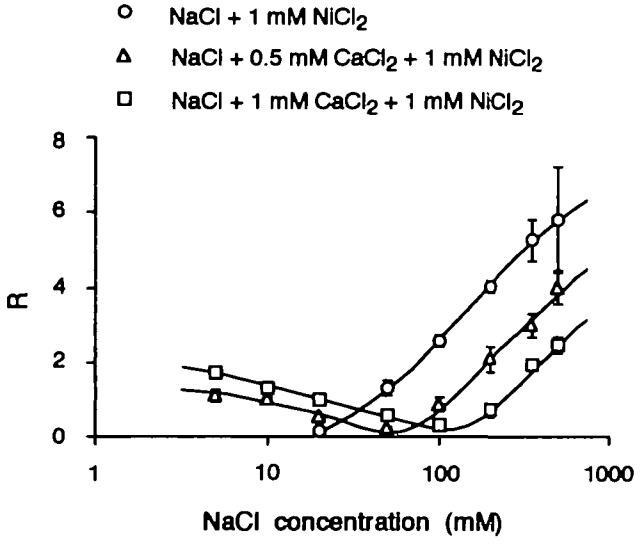

Figure 4 Concentration–response curves for NaCl obtained in the presence of 1 mM $NiCl_2$ and in the absence and the presence of $CaCl_2$. The relative magnitude of the neural responses (*R*) is plotted against the logarithm of the concentration of NaCl. The magnitude of the response to 500 mM NaCl alone (the standard solution) is taken as unity on the ordinate. Each point and bar represent a mean ± SEM, n = 3–5 units.

Figure 4 shows the C–R curves for NaCl obtained in the absence and in the presence of $CaCl_2$. All solutions used in these experiments contained 1 mM $NiCl_2$. The C–R curve for NaCl in the presence of 1 mM $NiCl_2$, shown in Figure 2, is reproduced here. In the presence of 0.5–1.0 mM $CaCl_2$, the magnitude of the responses generated by Ca^{2+} ions was reduced with increasing concentrations of NaCl (see C–R curves with a negative slope with respect to the logarithm of the concentration of NaCl). Further increases in the concentration of NaCl elicited a response that was generated by Na^+ ions. Thus, semilogarithmic C–R curves for NaCl in the presence of $CaCl_2$ were V-shaped, as shown in Figure 4. $CaCl_2$ raised the threshold concentration of NaCl and shifted the semilogarithmic C–R curve for NaCl to the right in a graded and parallel manner as the concentration of $CaCl_2$ was increased (see C–R curves with a positive slope with respect to the logarithm of the concentration of NaCl). Since the response generated by Ca^{2+} ions was markedly inhibited by NaCl (note the minimum values of the responses on V-shaped curves), it is likely that curves with a positive slope resulted from the excitatory action of Na^+ ions.

Competition between Ca^{2+} and Na^+ ions for the sodium receptor

Figure 5 shows C–R curves for NaCl, obtained in the absence and in the presence of $CaCl_2$, plotted with a linear scale. In Figure 5, only those values that gave a positive slope with respect to the logarithm of the concentration of NaCl in Figure 4 are replotted as responses generated by Na^+ ions. Since the number of impulses was taken as a measure of the response, a threshold concentration for stimulation by NaCl should be recognizable. A threshold phenomenon associated with the C–R relationship for stimulation by salts was discussed previously for stimulation by $MgCl_2$ (Kitada, 1989) and NaCl (Kitada, 1991). The curves in Figure 5 were fitted by eye to the points. As shown in Figure 5, the curves could be extrapolated below the abscissa to give a common intercept, $-r$, on the ordinate. Thus, r can be considered to be the magnitude of the response at the threshold that is necessary to just barely elicit a neural response (Kitada, 1989, 1991). The value of r, as determined graphically from Figure 5, was 0.4.

In a previous report (Kitada, 1991), an analysis of double-reciprocal plots demonstrated that Ca^{2+} ions act as a competitive inhibitor of the Na^+ response in the absence of $NiCl_2$. A similar analysis was performed of responses elicited

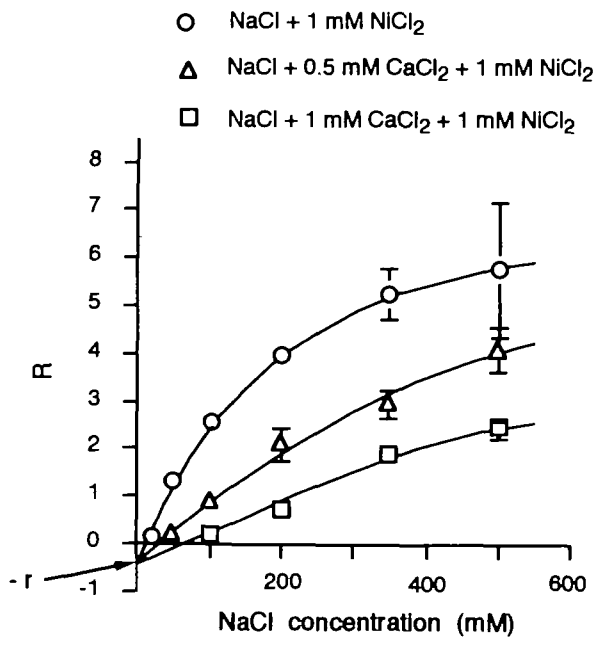

Figure 5 Effects of Ca^{2+} ions on the Ni^{2+}-enhanced response to Na^+ ions. Only the data from curves with a positive slope with respect to the logarithm of the concentration of NaCl shown in Figure 4 are plotted on a linear scale Each point and bar represent a mean ± SEM, n = 3–5 units. The curves were fitted by eye to the points and they were extended below the abscissa. Extrapolation of the curves gives a common intercept on the ordinate. The intercept on the ordinate at a point below zero gives $-r$. The value of r obtained from this Figure was 0.4 (see text for details).

in the presence of 1 mM $NiCl_2$. It was assumed that binding of a Na^+ ion to a sodium receptor (X_{Na}) leads to a neural response and that Ni^{2+} ions secondarily affect X_{Na} via a Ni^{2+}-binding element. Moreover, a receptor, X_{Na}, that is affected by a complex between a Ni^{2+}-binding element and a Ni^{2+} ion is indicated as X^*_{Na}. Since the enhancing effect of Ni^{2+} ions was saturated at 1 mM (Kashiwagura *et al.*, 1978; Kitada, 1994b), it is likely that most X_{Na} is changed to X^*_{Na} in the presence of 1 mM $NiCl_2$. In the present analysis, we also assumed that the magnitude of the neural response (R) in the presence of 1 mM $NiCl_2$ is proportional to the amount of NaX^*_{Na} complex minus a constant value (r; the threshold concentration of the NaX^*_{Na} complex). Thus, in the presence of both 1 mM $NiCl_2$, the enhancer, and Ca^{2+} ions, which are competitive inhibitors, the following equation can be applied (see Kitada, 1989, 1991):

$$\frac{1}{R+r} = \frac{K^*_{Na}}{R^*_{max\text{-}Na}}\left(1 + \frac{[Ca]}{K^*_{Na\text{-}Ca}}\right)\frac{1}{[Na]} + \frac{1}{R^*_{max\text{-}Na}} \quad (1)$$

where K^*_{Na}, $K^*_{Na\text{-}Ca}$ and $R^*_{max\text{-}Na}$ are the dissociation constant of the NaX^*_{Na} complex, the dissociation constant of the CaX^*_{Na} complex and the maximal response to Na^+ ions in the presence of 1 mM $NiCl_2$, respectively.

If the apparent dissociation constant for the NaX^*_{Na} com-

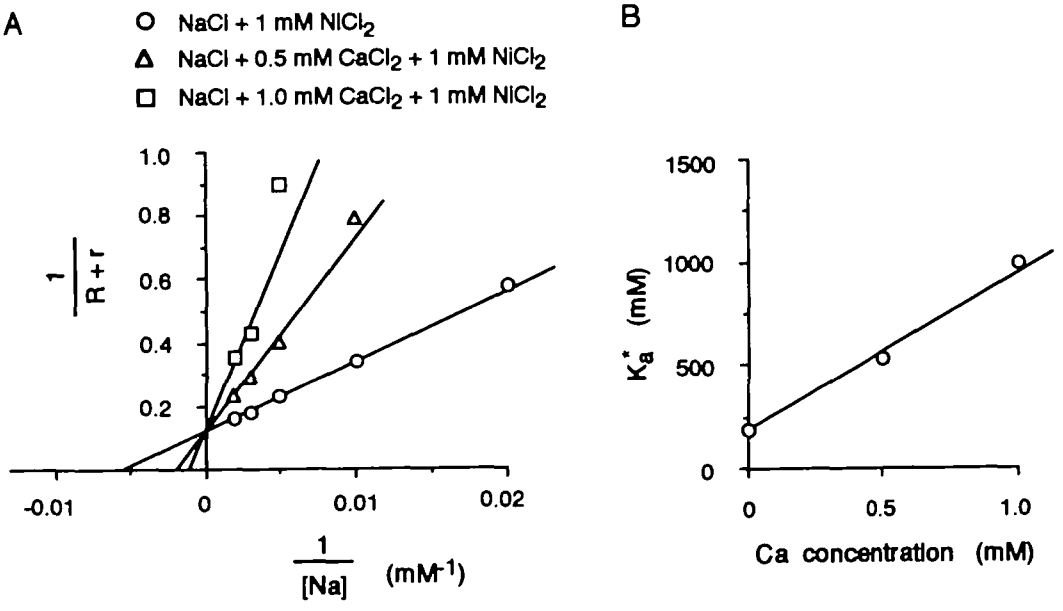

Figure 6 Competitive inhibition of the Ni^{2+}-enhanced response to Na^+ ions by Ca^{2+} ions. **(A)** Double-reciprocal plots of the results shown in Figure 5. The ordinate represents the reciprocal of the magnitude of the response [$1/(R + r)$] and the abscissa represents the reciprocal of the concentration of Na^+ ions (mM^{-1}); r = 0.4 **(B)** The apparent dissociation constant, K^*_a, is plotted against the concentration of Ca^{2+} ions. The values of K^*_a were calculated from the intercepts on the abscissa of the lines in (A). $K^*_a = K^*_{Na}(1+[Ca]/K^*_{Na\text{-}Ca})$ (see text for further details).

plex in the presence of Ca^{2+} ions is given as K_a^*, then, from equation (1):

$$K_a^* = K_{Na}^* + \frac{K_{Na}^*}{K_{Na-Ca}^*}[Ca]. \quad (2)$$

The C–R curves shown in Figure 5 were replotted as the relationship between the reciprocal of the magnitude of the response (R + 0.4) and the reciprocal of the concentration of NaCl (Figure 6A). As shown in Figure 6A, three straight lines were obtained and the three lines had a common intercept on the ordinate, as expected from equation (1). The results in Figure 6A are consistent with a model in which Ca^{2+} ions inhibit the Ni^{2+}-enhanced response to Na^+ ions in competitive manner. The value of R_{max-Na}^* calculated from the intercept on the ordinate in Figure 6A was 7.7. The intercept of the line on the abscissa gives the value of equations 1 and 2. Thus, the values of $-1/K_a^*$ in the absence and in the presence of Ca^{2+} ions can be calculated from the intercepts of the lines on the abscissa in Figure 6A. The value of K_{Na}^*, K_a^* in the absence of Ca^{2+} ions, was 1.9×10^{-1} M and the values of K_a^* in the presence of 0.5 and 1 mM Ca^{2+} ions were 5.3×10^{-1} M and 1.0 M, respectively. As seen in Figure 6B, the relationship between K_a^* and the concentration of Ca^{2+} ions was linear. The slope of the line in Figure 6B gives the value of K_{Na}^*/K_{Na-Ca}^* (equation 2). Moreover, the value of K_{Na}^* was obtained above. Thus, the value of K_{Na-Ca}^* was calculated to be 2.3×10^{-4} M.

Discussion

Transition metal ions enhance the Ca^{2+} response, the Mg^{2+} response and the Na^+ response in a similar manner (Kitada, 1994b). In other words, the concentration at which the transition metal ions effectively enhance the response to cations is almost the same for the responses to Ca^{2+}, Mg^{2+} and Na^+ ions, respectively (Kitada, 1994b). The common enhancing effect of transition metal ions on the responses to the three different cations suggested that the mechanism by which the transition metal ions exert their enhancing effect on the cation-induced responses might be common to the receptors for each of the three separate cations. The effect of Ni^{2+} ions on the Na^+ response was reversible (Figures 1 and 3). Furthermore, Ni^{2+} ions induced a response to choline chloride which, by itself, is barely able to produce a neural response in water fibers (Kitada, 1994a, d). The response to choline chloride, a salt with a large cation, induced by Ni^{2+} ions suggested that the effect of transition metal ions might be exerted at the surface of the apical membrane (Kitada, 1994a, d). Ni^{2+} ions did not always induce an enhancement of the response to Ca^{2+} or Na^+ ions when $CaCl_2$ and NaCl were applied together during stimulation with salts: the minimum value of a V-shaped C–R curve in Figure 4 was very close to zero. This suggests that Ni^{2+} ions do not affect the receptor-antagonist complex, but affect only the receptor-agonist complex. Moreover, an increase in the affinity of the sodium receptor for Na^+ ions caused by Ni^{2+} ions (Figure 2) suggests that Ni^{2+} ions affect the sodium receptor. Therefore, the effect of Ni^{2+} ions are representative not of a general effect on the receptor membrane but of a specific effect on the receptors that are responsible for the responses to salts.

Nickel ions have a dual action on the Ca^{2+} response: they can both inhibit and enhance it (Kitada, 1994b, c). Kitada (1994c) demonstrated that Ni^{2+} ions inhibit the Ca^{2+} response by competing with Ca^{2+} ions for a calcium receptor, as do other cations (Mg^{2+} and Na^+ ions) and that Ni^{2+} ions secondarily enhance the Ca^{2+} response via a membrane element other than the calcium receptor. In the case of stimulation by Na^+ ions, Ni^{2+} ions at 1 mM shifted the C–R curve for NaCl in the low-concentration direction and increased the maximal response to Na^+ ions (Figure 2). It is likely that Ni^{2+} ions do not have any inhibitory effect on the Na^+ response. Since $NiCl_2$ at 1 mM was barely effective in producing impulses, Ni^{2+} ions appear unable to bind directly to the sodium receptor. In previous reports (Kitada, 1978, 1989, 1990, 1991; Kitada and Shimada, 1980), it was suggested that the affinity of the calcium receptor might be charge-specific while that of the sodium receptor might be chemically specific. Therefore, it seems likely that the inhibition of the Ca^{2+} response and the absence of inhibition of the Na^+ response by Ni^{2+} ions might be due to the different affinities of the two types of receptor for Ni^{2+} ions.

Double-reciprocal plots revealed that Ca^{2+} ions serve as competitive inhibitors of the Na^+ response (Figure 6A). A schematic model consistent with both the present results and previous findings is shown in Figure 7. The model includes a sodium receptor (or channel) that is responsible for the Na^+ response in the apical membrane. Na^+ ions (agonistic cations) and Ca^{2+} ions (antagonistic cations) compete for the same sodium receptor. The sodium receptor interacts with a Ni^{2+}-binding element that is affected by Ni^{2+} ions. A complex between a Ni^{2+}-binding element and a Ni^{2+} ion induces a conformational change in a sodium receptor and, in this way, Ni^{2+} ions influence the sodium receptor. Alternatively, via their association with Ni^{2+}-binding elements, Ni^{2+} ions might expose receptors (or channels)

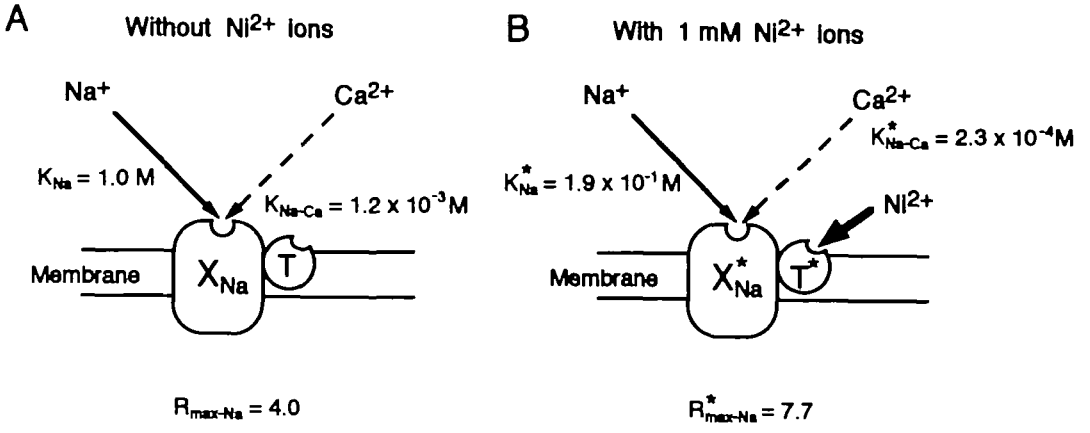

Figure 7 A schematic representation of the modulation of the Na^+ taste response by Ni^{2+} ions in the frog. **(A)** without Ni^{2+} ions; **(B)** with 1 mM Ni^{2+} ions. Thin arrows represent the actions of Na^+ ions (agonists) and dotted arrows represent the actions of Ca^{2+} ions (competitive antagonists). Na^+ and Ca^{2+} ions compete for a sodium receptor or channel (X_{Na}). T represents a Ni^{2+}-binding element. Ni^{2+} ions induce a conformational change in the sodium receptor X^*_{Na} via a Ni^{2+}-binding element (T^*) that is affected by Ni^{2+} ions (thick arrow). In (A), K_{Na}, $K_{Na\text{-}Ca}$ and $R_{max\text{-}Na}$ represent dissociation constants of NaX_{Na}, CaX_{Na} and the maximal response to Na^+ ions in the absence of Ni^{2+} ions, respectively. The values indicated were obtained in a previous study (Kitada, 1991). In (B), K^*_{Na}, $K^*_{Na\text{-}Ca}$ and $R^*_{max\text{-}Na}$ represent the dissociation constants of NaX^*_{Na}, CaX^*_{Na} and the maximal response to Na^+ ions in the presence of 1 mM Ni^{2+} ions, respectively. The values indicated were obtained in the present study. Ni^{2+} ions can modulate both the efficacy of Na^+ taste transduction and the affinity of the sodium receptor for Na^+ and Ca^{2+} ions. Further details can be found in the text.

that are deeply embedded in the receptor membrane to the outside medium and, in this way, Ni^{2+} ions might induce an increase in the number of sodium receptors available for binding of Na^+ ions, with a resultant increase in the maximal response.

The dissociation constants of the putative NaX^*_{Na} complex and the putative CaX^*_{Na} complex in the presence of 1 mM $NiCl_2$ were calculated in the present study and were compared with dissociation constants obtained in the absence of $NiCl_2$ in a previous report (Kitada, 1991; see Figure 7A). The values of K^*_{Na}/K_{Na} and of $K^*_{Na\text{-}Ca}/K_{Na\text{-}Ca}$ were 0.2 and 0.21, respectively. These results imply that the affinities of a sodium receptor for both Na^+ ions (agonists) and Ca^{2+} ions (competitive antagonists) in the presence of 1 mM Ni^{2+} ions are five times higher than those in the absence of Ni^{2+} ions. With respect to the affinity of the calcium receptor, the dissociation constants of a calcium receptor-Ca^{2+} ion complex in the absence and the presence of Ni^{2+} ions and that of a calcium receptor-antagonistic divalent cation (Mg^{2+} or Ni^{2+} ions) complex were almost the same (Kitada, 1994c). The results suggested that Ni^{2+} ions might enhance the Ca^{2+} response without altering the affinity of a calcium receptor for Ca^{2+} ions (Kitada, 1994c). Consequently, Ni^{2+} ions have different effect on the affinity of each of the distinct receptors for its respective cation.

The extent of the enhancement by Ni^{2+} ions at 1 mM of the maximal response to cations was almost the same for the Na^+ response and the Ca^{2+} response. In the case of stimulation by NaCl, the maximal response to Na^+ ions in the absence of $NiCl_2$ was reported to be 4.0 (Kitada, 1991) and that in the presense of 1 mM $NiCl_2$, obtained in the present study, was 7.7. These values were obtained as a relative magnitude, by reference to the response to the same standard solution (500 mM NaCl alone). Thus, the ratio of the relative value (7.7) of the maximal response to Na^+ ions in the presence of 1 mM Ni^{2+} ions to the relative value (4.0) of the maximal response to Na^+ ions in the absence of Ni^{2+} ions was 1.9. This ratio is very close to the ratio (1.8) of the maximal response to Ca^{2+} ions in the presence of 1 mM $NiCl_2$ to that in the absence of $NiCl_2$ (Kitada, 1994c). Therefore, Ni^{2+} ions almost doubled both the maximal response to Na^+ ions and that to Ca^{2+} ions. The identity of the relative increases in the maximal responses to Ca^{2+} ions and to Na^+ ions caused by Ni^{2+} ions suggests that a common transduction mechanism might be involved in reception of Na^+ and Ca^{2+} ions. However, the details of such a mechanism remain to be determined.

The above discussion is based on neural recordings from gustatory afferent fibers. Such recordings provide an indirect measure of the activity of a large group of taste receptors. Intracellular or patch recordings from taste cells provide a more direct measure of membrane events in taste cells than do with neural recordings. In salt taste transduction, intracellular or patch recording studies have revealed that an influx of Na^+ ions through ion channels in the apical membrane of taste cells is responsible for receptor potentials

in the frog (for review, see Schiffman, 1990; Sato *et al.*, 1994), and Ca^{2+} ions elicit depolarizing receptor potentials by modulation of the potassium conductance of the apical membrane in the mudpuppy (Bigiani and Roper, 1991). Thus, Na^+ and Ca^{2+} ions seem to be associated with different transduction pathways when data from intracellular or patch recordings in taste cells are analysed. Moreover, a receptor-related second messenger has been suggested to contribute to the activation of taste cells (for review, see Kinnamon and Cummings, 1992; Roper, 1992; Margolskee, 1993; Sato *et al.*, 1994). Thus, considerable diversity seems to exist in transduction mechanisms related to taste. It is generally thought that the activation of taste cells by chemical stimuli synaptically initiates impulses at the gustatory nerve terminals. Herness (1991) attempted to explain the effects of Co^{2+} ions, which are transition metal ions, on the neural responses of the frog glossopharyngeal nerve in terms of changes in receptor potential and membrane conductance in taste cells. Co^{2+} ions, resembling Ni^{2+} ions, inhibit the Ca^{2+} response and enhance the Na^+ response of the frog glossopharyngeal nerve. Herness (1991) found that a mixture of $CaCl_2$ and $CoCl_2$ produced large receptor potentials that occurred when neural activity had been almost completely inhibited. This result implies that the depolarization of the receptor cell alone may not necessarily activate the synapse. He showed that Co^{2+} ions caused the input resistance of taste cells to increase for stimulation by Ca^{2+} ions and to decrease for stimulation by Na^+ ions. Thus, he suggested that the glossopharyngeal nerve response is not a simple reflection of the magnitude of the receptor potential, but must be considered in conjuction with membrane resistance as an indicator of synaptic transmission. However, it remains unclear how the changes in membrane resistance induced by Co^{2+} ions might be associated with activation of synapses. Although Co^{2+} ions also have an enhancing effect on the neural response to Ca^{2+} ions (Kitada, 1994b, c), Herness (1991) failed to find such an enhancement of the Ca^{2+} response by Co^{2+} ions. Therefore, many unsolved problems remain with respect to the effects of transition metal ions on the responses to cations at the intracellular level.

Further studies, including direct intracellular or patch recordings from receptor cells, are needed to confirm the model proposed herein.

REFERENCES

Bigiani, A.R. and Roper, S.D. (1991) Mediation of responses to calcium in taste cells by modulation of a potassium conductance. *Science*, **252**, 126–128.

Casella, C. and Rapuzzi, G. (1957) Azione dell'acqua del $CaCl_2$ e del NaCl sui ricettori linguali nella rana. *Arch. Sci Biol., Bologna*, **41**, 191–203.

Herness, M.S. (1987) Are apical ion channels involved in frog taste transduction? Neural and intracellular evidence. In Roper, S. and Atema, J. (eds), *Olfaction and Taste IX, Ann. NY Acad. Sci.*, **510**, 362–365.

Herness, M. S. (1991) Specificity of mono-and divalent salt transduction mechanisms in frog gustation evidenced by cobalt chloride treatment. *J. Neurophysiol.*, **66**, 580–589.

Junge, D. and Brodwick, M.S. (1970) Stimulation of frog gustatory units by calcium. *Comp. Biochem. Physiol.*, **35**, 623–630.

Kashiwagura, T., Kamo, N., Kurihara, K. and Kobatake, Y. (1978) Enhancement of frog gustatory response by transition metal ions. *Brain Res.*, **142**, 570–575.

Kinnamon, S.C. and Cummings, T.A. (1992) Chemosensory transduction mechanism in taste. *Ann. Rev. Physiol.*, **54**, 715–731.

Kitada, Y. (1978) Inhibitory effects of cations on the Ca^{2+} response of water fibers in the frog tongue. *Jap. J. Physiol.*, **28**, 413–422.

Kitada, Y. (1984) Two different receptor sites for Ca^{2+} and Na^+ in frog taste responses. *Neurosci. Lett.*, **47**, 63–68.

Kitada, Y. (1986a) Different receptor sites for Ca^{2+} and Na^+ in single water fibers of the frog glossopharyngeal nerve. *Brain Res.*, **377**, 211–215.

Kitada, Y. (1986b) Salt taste responses in the frog glossopharyngeal nerve: different receptor sites for Mg^{2+} and Na^+. *Brain res.*, **380**, 172–175.

Kitada, Y. (1989) Taste responses to divalent cations in the frog glossopharyngeal nerve: competitive inhibition of the Mg^{2+} response by Ca^{2+}. *Chem. Senses*, **14**, 487–502.

Kitada, Y (1990) Taste responses to electrolytes in the frog glossopharyngeal nerve: initial process of taste reception. *Brain Res.*, **535**, 305–312.

Kitada, Y. (1991) Competitive inhibition of the response to Na^+ by Ca^{2+} in water fibers of the frog glossopharyngeal nerve. *Chem. Senses*, **16**, 95–104.

Kitada, Y. (1994a) Competitive inhibition of the nickel-induced response to choline by calcium ions in single water fibers of the frog glossopharyngeal nerve. *Chem. Senses*, **19**, 641–650.

Kitada, Y. (1994b) Enhancing effects of transition metals on the salt taste responses of single fibers of the frog glossopharyngeal nerve: specificity of and similarities among Ca^{2+}, Mg^{2+} and

Na^+ taste responses. *Chem. Senses*, **19**, 265–277.

Kitada, Y. (1994c) A quantitative study of dual action of nickel ions on the taste response to calcium ions of single fibers of the frog glossopharyngeal nerve: inhibition and enhancement by nickel ions. *Chem. Senses*, **19**, 401–411.

Kitada, Y. (1994d) The responses to choline ions induced by transition metal ions in single water fibers of the frog glossopharyngeal nerve. *Chem. Senses*, **19**, 627–640.

Kitada, Y. (1995) Anions modulate cation-induced responses of single units of the frog glossopharyngeal nerve. *Brain Res.*, **694**, 253–263.

Kitada, Y. and Shimada, K. (1980) A quantitative study of the inhibitory effect of Na^+ and Mg^{2+} on the Ca^{2+} response of water fibers in the frog tongue. *Jap. J. Physiol.*, **30**, 219–230.

Kusano, K. (1960) Analysis of the single unit activity of gustatory receptors in the frog tongue. *Jap. J. Physiol.*, **10**, 620–633.

Kusano, K. and Sato, M. (1957) Properties of fungiform papillae in frog's tongue. *Jap. J. Physiol.*, **7**, 324–338.

Margolskee, R.F. (1993) The molecular biology of taste transduction. *BioEssays*, **15**, 645–650.

Nomura, H. and Sakada, S. (1965) On the 'water response' of frog's tongue. *Jap. J. Physiol.*, **15**, 433–443.

Rapuzzi, G. and Casella, C. (1965) Innervation of the fungiform papillae in the frog tongue. *J. Neurophysiol.*, **28**, 154–165.

Roper, S.D. (1992) The microphysiology of peripheral taste organs. *J. Neurosci.*, **12**, 1127–1134.

Sato, T., Miyamoto, T. and Okada, Y. (1994) Comparison of gustatory transduction mechanisms in vertebrate taste cells. *Zool Sci.*, **11**, 767–780.

Schiffman, S.S. (1990) The role of sodium and potassium transport pathways in transduction: an overview. *Chem. Senses*, **15**, 129–135.

Taglietti, V., Casella, C. and Ferrari, E. (1969) Interactions between taste receptors in the frog tongue. *Pflügers Arch.*, **312**, 139–148.

Zotterman, Y. (1949) The response of the frog's fibres to the application of pure water. *Acta Physiol. Scand.*, **18**, 181–189.

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