Enhancing Effect of Nickel Ions on the Response to Magnesium Ions of Single Fibers of the Frog Glossopharyngeal Nerve: Competitive Inhibition by Calcium Ions of the Nickel-enhanced Response to Magnesium Ions

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Abstract

Single fibers of the frog glossopharyngeal nerve respond to MgCl₂ at concentrations exceeding 10 mM. NiCl₂ at 1 mM enhanced the Mg²⁺ response. CaCl₂ at 0.5–2 mM induced an inhibition of the Ni²⁺-enhanced response to Mg²⁺ ions. A quantitative explanation for these results is provided by the hypothesis that Ni ions secondarily affect a magnesium receptor (designated X*Mg) that is responsible for the Mg²⁺ response and that Ca²⁺ ions inhibit the Ni²⁺-enhanced response to Mg²⁺ ions by competing with Mg²⁺ ions for X*Mg. Double-reciprocal plots of the experimental data indicate that Ni²⁺ ions do not affect the affinities of X*Mg for both Mg²⁺ ions (agonist) and Ca²⁺ ions (competitive antagonist) appreciably, and that Ni²⁺ ions at 1 mM enhanced the maximal response to Mg²⁺ ions by 270%. It appears that a magnesium receptor interacts with an Ni²⁺-binding element that is affected by Ni²⁺ ions and, thus, Ni²⁺ ions can induce an enhancement of the Mg²⁺ response. Chem. Senses 22: 613–622, 1997.

Introduction

The frog glossopharyngeal nerve (GL) innervates taste receptors housed in the fungiform papillae of the tongue. The frog GL is very sensitive to calcium salts (Casella and Rapuzzi, 1957; Nomura and Sakada, 1965; Junge and Brodwick, 1970; Kitada, 1978). Threshold concentrations of calcium salts are <0.01 mM (Nomura and Sakada, 1965; Kitada, 1978). The single fibers of the frog GL that respond to calcium salts also respond to magnesium and sodium salts (Kusano, 1960; Nomura and Sakada, 1965; Kitada, 1989, 1991). The threshold concentrations of MgCl₂ and NaCl are 10 mM (Kitada, 1989) and 100 mM (Kitada, 1990, 1991) respectively. Since threshold concentrations of CaCl₂ and MgCl₂ are relatively low, and since both Mg²⁺ and Ca²⁺ ions are divalent cations, it was thought that the response to Mg²⁺ ions (the Mg²⁺ response) was similar to that to Ca²⁺ ions (the Ca²⁺ response) in the mechanism of excitation.
(Kusano and Sato, 1957). However, the responses to a mixture of MgCl\(_2\) plus CaCl\(_2\) are not as large as might be anticipated. It has been demonstrated that the Ca\(^{2+}\) response is competitively inhibited by Mg\(^{2+}\) ions (Kitada and Shimada, 1980) and that the Mg\(^{2+}\) response is competitively inhibited by Ca\(^{2+}\) ions (Kitada, 1989). As a consequence, the net response to a mixture of CaCl\(_2\) plus MgCl\(_2\) is small. Similar antagonism between Ca\(^{2+}\) and Na\(^{+}\) ions in response to a mixture of CaCl\(_2\) plus NaCl was found: the Ca\(^{2+}\) response is competitively inhibited by Na\(^{+}\) ions (Kitada and Shimada, 1980) and the response to Na\(^{+}\) ions (the Na\(^{+}\) response) is competitively inhibited by Ca\(^{2+}\) ions (Kitada, 1991). However, Na\(^{+}\) ions do not affect the Mg\(^{2+}\) response (Kitada, 1989), and Mg\(^{2+}\) ions do not affect the Na\(^{+}\) response (Kitada, 1990). From the mutual antagonism between cations and other evidence (Kitada, 1984, 1986a,b, 1990, 1995), it has been proposed that at least three specific receptors (discriminating elements) for cations are involved in salt taste reception: a calcium receptor (X\(_{Ca}\)), a magnesium receptor (X\(_{Mg}\)) and a sodium receptor (X\(_{Na}\)).

Transition metal ions, such as Ni\(^{2+}\), Co\(^{2+}\) and Mn\(^{2+}\) ions, have a variety of actions on excitable membranes. For example, transition metal ions inhibit passive Ca\(^{2+}\) influx associated with the action potentials in the barnacle giant muscle fiber (Hagiwara and Takahashi, 1967) and the slow Ca\(^{2+}\) inward current of mammalian cardiac muscle fibers (Kohlhardt et al., 1973). They induce a prolongation of the duration of action potentials in the myelinated nerve fiber of the toad (Takahashi et al., 1958). In gustation, transition metal ions slightly inhibited responses of the mouse chorda tympani nerve to NaCl and quinine hydrochloride, but not HCl (Iwasaki and Sato, 1984). However, it was found that transition metal ions have enhancing effects on the responses of the frog GL to Ca\(^{2+}\) (Kitada, 1994b,c), Mg\(^{2+}\) (Kashiwagura et al., 1978; Kitada, 1994b) and Na\(^{+}\) ions (Kashiwagura et al., 1978; Herness, 1987, 1991; Kitada, 1994b). 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). Since mutual antagonism exists between Ca\(^{2+}\) and Mg\(^{2+}\) ions and between Ca\(^{2+}\) and Na\(^{+}\) ions in the neural responses to a mixture of two salts in the presence of Ni\(^{2+}\) ions, it was suggested that Ni\(^{2+}\) ions might secondarily affect each of the receptors responsible for generation of the response to cations via a membrane element other than the receptors (Kitada, 1994b). Although Ni\(^{2+}\) ions have the common enhancing effect on the responses to Ca\(^{2+}\), Mg\(^{2+}\) and Na\(^{+}\) ions, they affect the responses to the cations differently. For example, Ni\(^{2+}\) ions have a dual action on the Ca\(^{2+}\) response: they cause both inhibition and enhancement of the Ca\(^{2+}\) response (Kitada, 1994c). On the other hand, Ni\(^{2+}\) ions have only an enhancing effect on the Na\(^{+}\) response and reduce the threshold concentration of NaCl to 20 mM (Kitada and Mitoh, 1996). A quantitative analysis of the effects of Ni\(^{2+}\) ions on the Ca\(^{2+}\) and Na\(^{+}\) responses has been carried out. The dual action of Ni\(^{2+}\) ions on the Ca\(^{2+}\) response was explained by the hypothesis that Ni\(^{2+}\) ions inhibit the Ca\(^{2+}\) response by competing with Ca\(^{2+}\) ions for X\(_{Ca}\) as do Mg\(^{2+}\) and Na\(^{+}\) ions, and that Ni\(^{2+}\) ions enhance the Ca\(^{2+}\) response by affecting the CaX\(_{Ca}\) complex (Kitada, 1994c). For the Na\(^{+}\) response, Ni\(^{2+}\) ions enhance the Na\(^{+}\) response by affecting the NaX\(_{Na}\) complex and increase the affinity of X\(_{Na}\) for Na\(^{+}\) ions by affecting X\(_{Na}\) (Kitada and Mitoh, 1996). Since each class of receptors has its own specific properties, it is important to characterize the effects of Ni\(^{2+}\) ions on the responses to each of these three cations. However, the characteristics of the effects of Ni\(^{2+}\) ions on the Mg\(^{2+}\) response are unknown. In the present study, the enhancement of the Mg\(^{2+}\) response by Ni\(^{2+}\) ions and the inhibition of the Ni\(^{2+}\)-enhanced response to Mg\(^{2+}\) ions by Ca\(^{2+}\) ions in single calcium-sensitive fibers of the frog GL were investigated quantitatively. We will discuss specificity of and similarities among responses to Ca\(^{2+}\), Mg\(^{2+}\) and Na\(^{+}\) ions.

**Materials and methods**

**Preparation and recording**

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, 1989). In brief, 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. Fibers of the frog GL that are sensitive to water stimulation (water fiber; Zotterman, 1949) also respond to calcium, magnesium and sodium salts (Kusano, 1960; Nomura and Sakada, 1965; Kitada, 1978). Since CaCl\(_2\) at low concentrations stimulates water fibers exclusively (Nomura and Sakada, 1965; Taglietti et al., 1969; Kitada, 1978), calcium-sensitive fibers are identical to water fibers (Casella and Rapuzzi, 1957;
Nomura and Sakada, 1965; Kitada, 1978). In the present experiments, stimulation with 1–2 mM CaCl\(_2\) was used to identify a calcium-sensitive fiber. In most cases, unitary discharges from a single calcium-sensitive fiber were recorded in response to stimulation by solutions of calcium, magnesium and sodium salts.

### Stimulation

Stimulating solutions of 5–200 mM MgCl\(_2\), 1–2 mM CaCl\(_2\) and 1–5 mM NiCl\(_2\) were prepared in distilled water. Mixtures of 2 mM CaCl\(_2\) plus 1 mM NiCl\(_2\), of 5–100 mM MgCl\(_2\) plus 1 mM NiCl\(_2\), of 100 mM MgCl\(_2\) plus 5 mM NiCl\(_2\), of 2–200 mM MgCl\(_2\) plus 0.5–2 mM CaCl\(_2\) plus 1 mM NiCl\(_2\) and of 5–200 mM MgCl\(_2\) plus 0.5–2 mM CaCl\(_2\) plus 1 mM NiCl\(_2\) plus 50–100 mM NaCl were also used. The reasons for choosing the concentrations of the salts are as follows. (i) The threshold concentration of MgCl\(_2\) was ~10 mM (Kitada, 1989). (ii) In the absence of NiCl\(_2\), the Mg\(^{2+}\) response was reduced by the addition of 0.2–5 mM CaCl\(_2\) to the MgCl\(_2\) stimulating solutions (Kitada, 1989). (iii) NiCl\(_2\) was barely effective for producing nerve impulses in calcium-sensitive fibers at concentrations of <5 mM and the enhancing effect of NiCl\(_2\) on the cation-induced response was saturated at 1 mM of NiCl\(_2\) (Kashiwagura et al., 1978; Kitada, 1994b). (iv) Since Ni\(^{2+}\)-enhanced responses to 0.5–1 mM CaCl\(_2\) were greatly inhibited by 50–100 mM NaCl (Kitada and Mitoh, 1996), 50–100 mM NaCl was used to eliminate selectively the component of the Ca\(^{2+}\) response from the responses to the MgCl\(_2\) stimulating solutions containing 0.5–2 mM CaCl\(_2\) and 1 mM NiCl\(_2\).

A solution of 50 mM NaCl was used as the adapting solution. 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.

### Analysis of data

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. A total of 35 calcium-sensitive fibers that yielded a large response (impulse frequency >120 impulses/25 s) to stimulation by the standard solution (100 mM MgCl\(_2\)) was tested: the standard solution elicited 120–169 impulses/25 s in 12 fibers, 170–219 impulses/25 s in eight fibers, 220–269 impulses/25 s in five fibers, 270–319 impulses/25 s in seven fibers, 320–369 impulses/25 s in one fiber, 370–419 impulses/25 s in one fiber and 420–469 impulses/25 s in one fiber. The sensitivity of calcium-sensitive fibers to the chemical stimuli tends to wane with repeated applications of stimulating solution to the tongue. In each unit, the magnitude of the response was normalized to the standard response, which was taken as the mean of measurements made prior to and after application of the stimulating solution. Only data obtained from calcium-sensitive fibers that yielded a large response (impulse frequency >120 impulses/25 s) to the standard solution were included in data analysis.

### Results

#### Effect of NiCl\(_2\) on the Mg\(^{2+}\) response

Figure 1 shows enhancement of the Mg\(^{2+}\) response by Ni\(^{2+}\) ions. In Figure 1A, unitary discharges from a single fiber elicited by stimulation with 1 mM CaCl\(_2\), with 1 mM NiCl\(_2\), with 100 mM MgCl\(_2\) and with 100 mM MgCl\(_2\) plus 1 mM NiCl\(_2\) were included in data analysis.

![Figure 1](image)
NiCl₂ are shown. CaCl₂ at 1 mM elicited a large response while NiCl₂ at 1 mM barely elicited impulses from a calcium-sensitive fiber. MgCl₂ at 100 mM elicited a large response. Addition of 1 mM NiCl₂ to a solution of 100 mM MgCl₂ brought about enhancement of the Mg²⁺ response. The effects of NiCl₂ were always reversible. Figure 1B shows concentration–response (C-R) curves for MgCl₂ obtained in the absence and in the presence of 1 mM NiCl₂. The neural response (R) was normalized by comparing it with the magnitude of the standard response of each fiber to 100 mM MgCl₂ alone. It is evident that NiCl₂ at 1 mM enhanced the Mg²⁺ response but the threshold concentration of MgCl₂ was not changed appreciably by the presence of 1 mM NiCl₂ (Figure 1B). Since Ni²⁺ ions caused both enhancement and inhibition of the Ca²⁺ response (Kitada, 1994c), we examined whether or not Ni²⁺ ions have an inhibitory effect on the Mg²⁺ response. The relative magnitudes of the response to a mixture of 100 mM MgCl₂ plus 1 mM NiCl₂ and that to 100 mM MgCl₂ plus 5 mM NiCl₂ were 2.69 ± 0.15 (mean ± SEM, n = 5) and 2.62 ± 0.21 (mean ± SEM, n = 5), respectively, indicating that Ni²⁺ ions do not inhibit the Mg²⁺ response (Student’s t-test, P > 0.05).

Inhibition by Ca²⁺ ions of the Ni²⁺-enhanced response to Mg²⁺ ions

An example of the mutual antagonism between Ca²⁺ and Mg²⁺ ions in the presence of 1 mM NiCl₂ is shown in Figure 2. As seen in Figure 2, the frequency of impulses elicited by a mixture of 100 mM MgCl₂ plus 2 mM CaCl₂ plus 1 mM NiCl₂ (third trace) was much lower than that of impulses elicited by a solution of 2 mM CaCl₂ plus 1 mM NiCl₂ (first trace) or by a solution of 100 mM MgCl₂ plus 1 mM NiCl₂ (second trace or fourth trace). Since mutual antagonism between Ca²⁺ and Mg²⁺ ions occurs in the absence of NiCl₂ (Kitada, 1989), the decrease of the response to a mixture of magnesium, calcium and nickel salts together is due not to antagonism between Ni²⁺ ions and other cations but to antagonism between Ca²⁺ and Mg²⁺ ions.

Figure 3 shows the C-R curves for MgCl₂ in the presence of 0.5 mM CaCl₂ (Figure 3A) and of 2 mM CaCl₂ (Figure 3B). All solutions used in these experiments contained 1 mM NiCl₂. As seen in Figure 3, the magnitude of the response decreased and then increased with increasing concentrations of MgCl₂. Thus, semilogarithmic C-R curves for MgCl₂ were V-shaped. A V-shaped C-R curve shown in Figure 3 reveals that the response to the mixture of MgCl₂ plus CaCl₂ in the presence of 1 mM NiCl₂ is composed of two components: the response generated by Ca²⁺ ions and that by Mg²⁺ ions. That is, a negative slope with respect to the logarithm of the concentration of MgCl₂ is due to inhibition of the Ca²⁺ response by Mg²⁺ ions and a positive slope with respect to the logarithm of the concentration of MgCl₂ is due to excitatory action of Mg²⁺ ions. It has been shown that the responses to 0.5–1 mM CaCl₂ in the presence of 1 mM NiCl₂ are greatly inhibited by the presence of 50–100 mM NaCl and the responses to 50–100 mM NaCl in the presence of 1 mM NiCl₂ are greatly inhibited by the
Concentrations of MgCl\(_2\) were relatively high. These results agree with a positive slope obtained in the absence of NaCl when the magnitude of the residual response coincided with curves that were added to the mixture. As seen in Figure 3, it is evident that the response generated by Ca\(^{2+}\) ions was selectively eliminated or suppressed by addition of NaCl and the presence of 0.5-1 mM CaCl\(_2\) (Kitada and Mitoh, 1996). Moreover, addition of 50-100 mM NaCl to a solution of MgCl\(_2\) did not affect the Mg\(^{2+}\) response (Kitada, 1989, 1990). Hence, addition of 50-100 mM NaCl to a mixture of MgCl\(_2\) plus 0.5-2 mM CaCl\(_2\) plus 1 mM NiCl\(_2\) may selectively eliminate the component of the Ca\(^{2+}\) response from the mixture. Thus, 50-100 mM NaCl was added to the mixture. As seen in Figure 3, it is evident that the response generated by Ca\(^{2+}\) ions was selectively eliminated or suppressed by addition of NaCl and the magnitude of the residual response coincided with curves with a positive slope obtained in the absence of NaCl when concentrations of MgCl\(_2\) were relatively high. These results indicate that NaCl does not affect the Ni\(^{2+}\)-enhanced response to Mg\(^{2+}\) ions even when Ca\(^{2+}\) ions antagonize the effect of Mg\(^{2+}\) ions. Selective elimination of the Ca\(^{2+}\) response by NaCl suggests that the excitatory effect of Mg\(^{2+}\) ions is independent of that of Ca\(^{2+}\) ions. The results in Figure 3 suggest that curves with a positive slope obtained in the presence of 50-100 mM NaCl result from the excitatory action of Mg\(^{2+}\) ions and that the excitatory effect of 0.5 or 2 mM Ca\(^{2+}\) ions cannot be exerted in the region of relatively high concentrations of Mg\(^{2+}\) ions when 50-100 mM Na\(^{+}\) ions are present.

**Figure 4** Competitive inhibition by Ca\(^{2+}\) ions of the Ni\(^{2+}\)-enhanced response to Mg\(^{2+}\) ions. (A) The concentration-response curves for MgCl\(_2\) in the presence of 1 mM NiCl\(_2\) (shown in Figure 1B; filled circles), in the presence of 0.5 mM CaCl\(_2\), 1 mM NiCl\(_2\) and 50 mM NaCl (shown in Figure 3A; filled triangles), and in the presence of 2 mM CaCl\(_2\), 1 mM NiCl\(_2\) and 100 mM NaCl (shown in Figure 3B; filled squares) are reproduced together. (B) Only the data from curves with a positive slope with respect to the logarithm of the concentration of MgCl\(_2\) shown in (A), which result from excitatory action of Mg\(^{2+}\) ions, are plotted on a linear scale. Symbols are the same as in (A). The curves were fitted by eye to the points and 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 0.3 (see text for details). (C) Double-reciprocal plots of the results shown in (A). The ordinate represents the reciprocal of the magnitude of the logarithm of MgCl\(_2\) shown in (A), which result from excitatory action of Mg\(^{2+}\) ions, are replotted on a linear scale. Symbols are the same as in (A). (D) The apparent dissociation constant, \(K_a^{*}\), is plotted against the concentration of Ca\(^{2+}\) ions. The values of \(K_a^{*}\) were calculated from equation (3). See text for further details.

**Competition between Ca\(^{2+}\) and Mg\(^{2+}\) ions for the magnesium receptor**

The aim of this study was to investigate the characteristics of the effect of Ni\(^{2+}\) ions on the Mg\(^{2+}\) response. Thus, only those values on the C-R curves for MgCl\(_2\) that result from the excitatory action of Mg\(^{2+}\) ions were analyzed. The C-R curve for MgCl\(_2\) obtained in the absence of CaCl\(_2\) and the presence of 1 mM NiCl\(_2\) (filled circles in Figure 1B) is replotted in Figure 4A. The curves for MgCl\(_2\) obtained in the presence of 0.5 mM CaCl\(_2\), 1 mM NiCl\(_2\) and 50 mM NaCl (filled triangles in Figure 3A) and in the presence of 2 mM CaCl\(_2\), 1 mM NiCl\(_2\) and 100 mM NaCl (filled squares in Figure 3B) are also replotted in the same figure. As seen in Figure 4A, addition of CaCl\(_2\) to a solution of MgCl\(_2\) in the presence of 1 mM NiCl\(_2\) shifts the semilogarithmic C-R curve to the right in a graded and parallel manner, as the concentration of CaCl\(_2\) is increased (see curves with a positive slope with respect to the logarithm of the concentration of MgCl\(_2\)). This result suggests the possibility that Ca\(^{2+}\) ions competitively inhibit the Mg\(^{2+}\) response even in the presence of Ni\(^{2+}\) ions, as observed in the absence of Ni\(^{2+}\) ions (Kitada, 1989).

Only those values on the curves with a positive slope shown in Figure 4A, which result from the excitatory action of Mg\(^{2+}\) ions, are replotted with a linear scale in Figure 4B. Since we took the impulse frequency as a measure of the response, a threshold concentration for stimulation by MgCl\(_2\) should be recognizable. A threshold phenomenon associated with the C-R relationship for stimulation by salts was discussed previously (Kitada, 1989, 1991, 1994; Kitada and Mitoh, 1996). The curves in Figure 4B were fitted by eye to the point. The curves could be extrapolated below the abscissa and had a common intercept \((-r)\) on the ordinate. Thus, the value of \(r\) in Figure 4B gives the magnitude of the response at the threshold that is necessary for elicitation of a
neural response and it was determined graphically by extrapolation of curves. The value of $r$ was 0.3.

Double-reciprocal plots have been used to examine the nature of the competition between agonistic and antagonistic cations in the taste responses of fibers in the frog GL (Kitada and Shimada, 1980; Kitada, 1989, 1991, 1994a,c; Kitada and Mitoh, 1996). In the present study, a similar analysis was performed of the Mg$^{2+}$ response enhanced by 1 mM NiCl$_2$. It was assumed that binding of a Mg$^{2+}$ ion to $X_{Mg}$ leads to a neural response and that Ni$^{2+}$ ions secondarily affect $X_{Mg}$ via an Ni$^{2+}$-binding element. Moreover, a receptor that is affected by a complex between an Ni$^{2+}$-binding element and an Ni$^{2+}$ ion is indicated as $X^{*}_{Mg}$. Since the enhancing effect of Ni$^{2+}$ ions was saturated at 1 mM (Kashiwagura et al., 1978; Kitada, 1994b), it is likely that most $X_{Mg}$ are changed to $X^{*}_{Mg}$ 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 Mg$^{2+}$ complex minus a constant value (the threshold concentration of the Mg$^{2+}$ complex necessary for just eliciting a neural response). Thus, the sum of $R + r$ is taken as the 'true' magnitude of the response that includes a subthreshold response. In the presence of both 1 mM NiCl$_2$ (which is the enhancer) and Ca$^{2+}$ ions (which are competitive inhibitors) the following equation can be applied (see Kitada, 1989):

$$\frac{1}{R + r} = \frac{K^{*}_{Mg}}{R_{max-Mg}} \left(1 + \frac{[Ca]}{K^{*}_{Mg-Ca}}\right) \frac{1}{[Mg]} + \frac{1}{R_{max-Mg}}$$  \tag{1}

where $K^{*}_{Mg}$, $K^{*}_{Mg-Ca}$ and $R_{max-Mg}$ are the dissociation constant of the Mg$^{2+}$ complex, the dissociation constant of the Ca$^{2+}$ complex, and the maximal response to Mg$^{2+}$ ions in the presence of 1 mM NiCl$_2$ respectively.

If the apparent dissociation constant for the Mg$^{2+}$ complex in the presence of Ca$^{2+}$ ions is given as $K^{*}_{a}$, then, from equation (1):

$$\frac{1}{R + r} = \frac{K^{*}_{a}}{R_{max-Mg}} \times \frac{1}{[Mg]} + \frac{1}{R_{max-Mg}}$$  \tag{2}

Since the slope ($S^{*}_{Mg}$) of the lines in the double-reciprocal plot gives $K^{*}_{a}/R_{max-Mg}$, $K^{*}_{a}$ is represented by

$$K^{*}_{a} = R_{max-Mg} \times S^{*}_{Mg}$$  \tag{3}

The C-R curves shown in Figure 4B were replotted as the relationship between the reciprocal of the magnitude of the response ($R + 0.3$) and the reciprocal of the concentration of MgCl$_2$ (Figure 4C). As shown in Figure 4C, three straight lines were obtained and the three lines had a common intercept on the ordinate, as expected from equation (1). Therefore, the results in Figure 4C are consistent with a model in which Ca$^{2+}$ ions inhibit the Ni$^{2+}$-enhanced response to Mg$^{2+}$ ions in competitive manner. The value of $R_{max-Mg}$ calculated from the intercept on the ordinate in Figure 4C was 6.7. The values of were obtained from three lines in Figure 4C. Given the values of $R_{max-Mg}$ and $S^{*}_{Mg}$, the values of $K^{*}_{a}$ in 0, 0.5 and 2 mM Ca$^{2+}$ ions were calculated to be $1.4 \times 10^{-1}$, $2.7 \times 10^{-1}$ and $5.5 \times 10^{-1}$ M, respectively (see equation 3). From equations (1) and (2), $K^{*}_{a}$ gives $K^{*}_{Mg} + K^{*}_{Mg-Ca}/K^{*}_{Mg-Ca}$. Thus, a relationship between $K^{*}_{a}$ and the concentration of Ca$^{2+}$ ions is shown in Figure 4D. The relationship was almost linear. Since the slope of the line in Figure 4D gives $K^{*}_{Mg}/K^{*}_{Mg-Ca}$ and the value of $K^{*}_{Mg}$ was obtained above, the value of $K^{*}_{Mg-Ca}$ was calculated to be $6.5 \times 10^{-4}$ M.

**Discussion**

As noted earlier, mutual antagonism exists between Ca$^{2+}$ and Mg$^{2+}$ ions (Kitada, 1989) and between Ca$^{2+}$ and Na$^+$ ions (Kitada, 1991) in the responses to mixtures of various salts in calcium-sensitive fibers of the frog GL. However, Na$^+$ ions do not affect the Mg$^{2+}$ response and Mg$^{2+}$ ions do not affect the Na$^+$ response (Kitada, 1989, 1990). Although Ni$^{2+}$ ions have an enhancing effect on the separate responses to Ca$^{2+}$, Mg$^{2+}$ and Na$^+$ ions, the present results clearly showed that Ni$^{2+}$ ions did not affect the mutual interaction among the three cations in the responses to mixtures of the three salts. As seen in Figure 3, the components of the Ca$^{2+}$ response were selectively eliminated from the responses to the MgCl$_2$ stimulating solutions containing 0.5–2 mM CaCl$_2$ and 1 mM NiCl$_2$ by addition of 50–100 mM NaCl to the stimulating solutions. Although NaCl alone at concentrations of <100 mM does not elicit a neural response, addition of 1 mM NiCl$_2$ to a solution of 50–100 mM NaCl induced a large response generated by Na$^+$ ions (Kitada and Mitoh, 1996). Hence, reduction in the magnitude of the response by addition of Na$^+$ ions (shown in Figure 3) results from mutual antagonism between Ca$^{2+}$ and Na$^+$ ions. The magnitude of the residual response in the
In the present study, the magnitude of the response to 100 mM MgCl$_2$ in the presence of NaCl (curves with a positive slope) almost coincided with that of the Ni$^{2+}$-enhanced Mg$^{2+}$ response in the absence of NaCl (see curves with a positive slope with respect to the logarithm of the concentration of Mg$^{2+}$ ions) when concentrations of Mg$^{2+}$ ions were relatively high (Figure 3). These results indicate that the excitatory effect of Mg$^{2+}$ ions is independent of that of Ca$^{2+}$ ions or Na$^+$ ions, even though Ni$^{2+}$ ions have an enhancing effect on the response to cations. Since mutual antagonism between cations occurs in the responses to mixtures of different salts, it is likely that Ni$^{2+}$ ions do not affect the receptor–antagonist complex but affect the receptor–agonist complex for enhancing the response to cations. Ni$^{2+}$ ions themselves were barely effective for producing nerve impulses in calcium-sensitive fibers at concentrations of <5 mM. Therefore, the effects 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 response to cations.

In a previous report (Kitada, 1994c), it was found that Ni$^{2+}$ ions at 0.2–2 mM shifted the semilogarithmic C-R curves for Ca$^{2+}$ ions towards high concentrations of Ca$^{2+}$ ions because of competitive inhibition of the Ca$^{2+}$ response by Ni$^{2+}$ ions, even though the maximal response to Ca$^{2+}$ ions was increased by Ni$^{2+}$ ions. In the present study, the magnitude of the response to 100 mM MgCl$_2$ in the presence of 5 mM NiCl$_2$ was almost the same as that in the presence of 1 mM NiCl$_2$. This indicates that Ni$^{2+}$ ions had no inhibitory effect on the Mg$^{2+}$ response but, rather, enhanced effect on it.

The Ni$^{2+}$-enhanced response to Mg$^{2+}$ ions was inhibited by the presence of Ca$^{2+}$ ions (Figure 2). A double-reciprocal plot revealed that Ca$^{2+}$ ions serve as competitive inhibitors of the Mg$^{2+}$ response (Figure 4C). A schematic model consistent with the present results is shown in Figure 5. In this model, X$_{Mg}$ is responsible for the Mg$^{2+}$ response in the apical membrane. Ca$^{2+}$ ions (antagonistic cations) compete with Mg$^{2+}$ ions for the common X$_{Mg}$. Ni$^{2+}$ ions interact reversibly with some Ni$^{2+}$-binding element (T) that interacts with X$_{Mg}$. A conformational change is induced by a complex between an Ni$^{2+}$ ion and its Ni$^{2+}$-binding element (T*) that interacts with Mg$^{2+}$ ions. With respect to the effects of Ni$^{2+}$ ions on the Ca$^{2+}$ response (Kitada, 1994c) and on the Na$^+$ response (Kitada and Mitoh, 1996), a similar model has been proposed. The model is shown schematically in Figure 6. In this figure, Ca$^{2+}$ and Na$^+$ ions affect X$^*$ via a T*-binding element (T*). X$^*$ is responsible for the Mg$^{2+}$ response in the apical membrane. Ca$^{2+}$ ions (antagonistic cations) compete with Mg$^{2+}$ ions for the common X$_{Mg}$. Ni$^{2+}$ ions interact reversibly with some Ni$^{2+}$-binding element (T) that interacts with X$_{Mg}$ and Na$^+$ ions. This interaction is consistent with the present results.
as competitive antagonists, but Ni$^{2+}$ and Na$^+$ ions do not interact with X$^*$ alone. For X$^*$, Na$^+$ ions serve as agonists and Ca$^{2+}$ ions serve as competitive antagonists, but Ni$^{2+}$ and Mg$^{2+}$ ions do not interact with X$^*$ directly (Kitada and Mitoh, 1996). Therefore, the affinities of X$^{*}$ for cations might be chemically specific. The dissociation constants of the putative MgX$^{*}$ complex and the putative CaX$^{*}$ 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 (see figure 5 in Kitada, 1989). The values of $K^{*}$/K$_{Mg}$ and of $K^{*}$/K$_{Mg-Ca}$/K$_{Mg-Ca}$ were 1.7 and 0.9 respectively. Hence, the affinity of X$_{Na}$ for cations without Ni$^{2+}$ ions and that of K$^*$ for cations with Ni$^{2+}$ ions seem not to be very different. For the Ca$^{2+}$ response, it was suggested that Ni$^{2+}$ ions might enhance the Ca$^{2+}$ response without altering the affinity of X$_{Ca}$ for both Ca$^{2+}$ ions (agonists) and Ni$^{2+}$ ions (competitive antagonists) (Kitada, 1994c). However, Ni$^{2+}$ ions greatly affected the affinity of X$_{Na}$ for cations. That is, the affinity of X$^{*}$ for both Na$^+$ ions (agonists) and Ca$^{2+}$ ions (competitive antagonists) in the presence of 1 mM Ni$^{2+}$ ions was reported to be five times higher than that of X$_{Na}$ in the absence of Ni$^{2+}$ ions (Kitada and Mitoh, 1996). Consequently, Ni$^{2+}$ ions have different effect on the affinity of the distinct receptors for its respective cation.

The maximal response to Mg$^{2+}$ ions in the presence of 1 mM Ni$^{2+}$ ions ($R^{*}_{max-Mg}$) was calculated to be 6.7 and that in the absence of Ni$^{2+}$ ions ($R_{max-Mg}$) was reported to be 2.5 (see figure 5 in Kitada, 1989). These values were obtained as a relative magnitude, by reference to the response to the same standard solution (100 mM MgCl$_2$ alone). The ratio of $R^{*}_{max-Mg}$ to $R_{max-Mg}$ was 2.7. With respect to the Ca$^{2+}$ (Kitada, 1994c) and the Na$^+$ (Kitada, 1996) responses, the ratios of the maximal response to Ca$^{2+}$ ions in the presence of 1 mM Ni$^{2+}$ ions to that in the absence of Ni$^{2+}$ ions and of the maximal response to Na$^+$ ions in the presence of 1 mM Ni$^{2+}$ ions to that in the absence of Ni$^{2+}$ ions were 1.8 and 1.9 respectively. Therefore, Ni$^{2+}$ ions doubled or tripled the maximal response to each of the three cations. The identity of the relative increases in the maximal responses to Ca$^{2+}$ ions, Mg$^{2+}$ ions and Na$^+$ ions caused by Ni$^{2+}$ ions suggests that the mechanism by which Ni$^{2+}$ ions exert their enhancing effect on the cation-induced responses might be common to the receptors for each of the three separate cations.

Studies with intracellular microelectrodes revealed that frog taste receptor cells produce receptor potentials with a large decrease in membrane resistance to monovalent stimuli (NaCl and KCl), whereas divalent stimuli (CaCl$_2$ and MgCl$_2$) generally produce depolarizations without large resistance changes (Akaike et al., 1976; Herness, 1991). The results suggested that Na$^+$ and K$^+$ flux through cation channels in the apical membrane of taste cells are responsible for the taste cell depolarization and that adsorption of Ca$^{2+}$ or Mg$^{2+}$ ions on the receptor membrane is involved in the initial event of transduction in frog taste cells. Amiloride, which blocks Na$^+$-transepithelial currents in many species (for review see Schiffman, 1990), reduces the influx of Na$^+$ and K$^+$ ions into frog taste cells (Avenet and Lindemann, 1988). However, Miyamoto et al. (1989) failed to observe amiloride-sensitive channels in intracellular recordings from frog taste cells. Miyamoto et al. (1989, 1993) also showed that Na$^+$ ions permeate cation channels, but not amiloride-sensitive channels, at the apical membrane of the taste cells. Non-selective monovalent cation channels in receptive membrane and basolateral membrane of frog taste cells were reported using single channel recordings of the patch clamp method (Fujiyama et al., 1993). 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, monovalent (Na$^+$ and K$^+$ ions) and divalent cations (Ca$^{2+}$ and Mg$^{2+}$ ions) seem to be associated with different transduction pathways when data from intracellular or patch recording in taste cells are analyzed. Moreover, it has been suggested that a receptor-related second messenger may contribute to the activation of taste cells (for review see Kinnamon and Cummings, 1992; Roper, 1992; Sato et al., 1994; Lindemann, 1996). Therefore, considerable diversity seems to exist in transduction mechanisms related to taste.

In the frog GL, Co$^{2+}$ ions (transition metal ions) inhibit the Ca$^{2+}$ response (Kitada, 1978) and enhance the Na$^+$ response (Kashiwagura et al., 1978). Herness (1991) attempted to explain the effects of Co$^{2+}$ ions on the neural responses of the frog GL in terms of changes in receptor potential and membrane conductance in taste cells. However, he found that a mixture of CaCl$_2$ plus CoCl$_2$ produced large receptor potentials that occurred when neural activity had been almost completely inhibited. Therefore, the frog GL response is not a simple reflection of the magnitude of the receptor potential. Only membrane resistance change during salt stimulation with cobalt treatment followed the qualitative pattern observed with the neural response. However, it remains unclear how the
changes in membrane resistance induced by Co$^{2+}$ ions might be associated with activation of taste cells. Since Co$^{2+}$ ions, resembling Ni$^{2+}$ ions, have the inhibitory and enhancing effects on the neural response to Ca$^{2+}$ ions (Kitada, 1996b), effects of Co$^{2+}$ ions appear to be complicated. Thus, many unsolved problems remain with respect to the effects of transition metal ions on the response to cations at the intracellular level.

From analysis of neural responses in single calcium-sensitive fibers of the frog GL, it has been shown that the receptors responsible for the responses to Ca$^{2+}$, Mg$^{2+}$ and Na$^+$ ions are entirely distinct from one another. Nevertheless, Ni$^{2+}$ ions enhance the responses to Ca$^{2+}$, Mg$^{2+}$ and Na$^+$ ions in a similar manner. Transition metal ions are, therefore, useful tools for attempts to study initial events of the transduction mechanism for salts in calcium-sensitive fibers of the frog GL. The results of treatment with Ni$^{2+}$ ions lead to the proposal that the mechanism of enhancement of the response by Ni$^{2+}$ ions is common to all of the three different cation receptors. Moreover, Ni$^{2+}$ ions at 1 mM induced a large neural response to 500 mM choline chloride which, by itself, is barely able to produce a neural response in calcium-sensitive fibers (Kitada, 1994d). Ni$^{2+}$-induced response to choline$^+$ ions was competitively inhibited by the presence of Ca$^{2+}$ ions (Kitada, 1994a). Other organic salts, such as tris(hydroxymethyl)aminomethane–HCl, triethanolamine–HCl and tetraethyl-

ammonium chloride, elicited no response or only a very small response from calcium-sensitive fibers, and NiCl$_2$ did not affect these responses. Kitada (1994d) suggested that there exists a choline receptor for the response to choline$^+$ ions in the apical membrane of frog taste cells and the choline receptors also interact with the Ni$^{2+}$-binding element that affects the choline receptors. Since choline$^+$ ions (which are large in size) barely permeate excitable membranes and since the mechanism of the enhancement of the response by Ni$^{2+}$ ions is common to all of the cation receptors, it appears that specific cation channels responsible for the responses to each of the cations might not be involved in reception of the cations in calcium-sensitive fibers. It is probable that binding of an Ni$^{2+}$ ion to its Ni$^{2+}$-binding element increases the efficacies of agonistic cation–receptor complexes that produce second messengers. Alternatively, via their association with Ni$^{2+}$-binding elements, Ni$^{2+}$ ions might expose receptors which are deeply embedded in the receptor membrane to the outside medium, and in this way might induce an increase in the number of receptors available for binding of agonistic cations, with a resultant increase in the maximal response.

Neural recordings from afferent taste fibers provide an indirect measure of the activity of a large group of taste receptors. The model described above should be confirmed by more direct experiments that include intracellular or patch recording studies from receptor cells.

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Received on May 26, 1997; accepted on August 4, 1997