J. Physiol. (1986), **373**, *pp.* 463–479
With 12 *text-figures*
Printed in Great Britain

BLOCKING EFFECTS OF COBALT AND RELATED IONS ON THE γ-AMINOBUTYRIC ACID-INDUCED CURRENT IN TURTLE RETINAL CONES

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(*Received 19 June 1985*)

SUMMARY

1. Red-sensitive cone photoreceptors were isolated from the turtle retina, and GABA-induced currents were recorded under voltage clamp. The effect of Co^{2+}, widely used as a blocker of chemical synapses, on the GABA-induced current was studied.

2. Co^{2+} blocked the GABA-induced current evoked by local application either at the synaptic region (cone pedicle) or at the extra-synaptic region (cell body). 5 μM-Co^{2+} suppressed the GABA-induced current by 50 %, and a few hundred μM-Co^{2+} blocked it almost completely.

3. Co^{2+} suppressed the GABA-induced current non-competitively: the saturating response amplitude decreased without a change in the threshold or saturating dose of GABA. The blocking was not voltage dependent in the physiological range of the membrane potential.

4. Ni^{2+} and Cd^{2+} also blocked the GABA-induced current non-competitively, and were as effective as Co^{2+}. Tetraethylammonium (25 mM) showed a similar but weaker blocking effect. On the other hand, Mg^{2+} (20 mM), Mn^{2+}, Sr^{2+}, Ba^{2+} (10–100 μM each), D-600 (10 μM) or Cs^{+} (10 mM) did not affect the GABA-induced current.

5. The Ca current in the turtle cones was blocked almost completely by 20 mM-Mg^{2+} or 4 mM-Co^{2+}, or strongly suppressed by 10 μM-D-600. However, Cd^{2+} and Ni^{2+} (10 μM each) blocked the Ca current by *ca.* 50 %, and Co^{2+} and Mn^{2+} (10 μM each) suppressed it only partially.

6. The blocking of the GABA-induced current by these agents was, therefore, not directly related to the blocking of the Ca current and/or Ca-mediated currents.

7. These observations present a warning on the use of some divalent cations, such as Co^{2+}, Ni^{2+} or Cd^{2+}, as a presynaptic blocker at the GABAergic synapse. High concentrations of Mg^{2+} are recommended as a more appropriate blocker.

INTRODUCTION

In studies to identify the chemical transmitter substance at a particular synapse, it is important to determine whether a candidate substance applied exogenously acts directly on the target cell. A popular method of obtaining the direct effect is to record from the post-synaptic neurone deprived of endogenous inputs by an application of presynaptic blocker, such as Co^{2+} or Mg^{2+}. The underlying idea of this procedure is that the transmitter release from the presynaptic terminal requires Ca influx (Katz & Miledi, 1969), which is suppressed by these divalent cations (Weakly, 1973; see also Hagiwara & Byerly, 1981). However, when Ca-channel antagonists are used as a presynaptic blocker, it is also necessary to examine whether they affect post-synaptic cells either, (1) by interfering with synaptic receptors or transmitter-activated channels, or (2) by modifying other ionic channels related with Ca influx. Furthermore, a component of release of some chemical transmitters has been reported to be Ca-independent (Schwartz, 1982).

Isolated cells dissociated from the retina have provided a good opportunity to examine voltage-, Ca-, and transmitter-activated conductances without interference from other retinal cells (Bader, MacLeish & Schwartz, 1978; Bader, Bertrand & Schwartz, 1982; Tachibana, 1981, 1983; Johnston & Lam, 1981; Lasater & Dowling, 1982; Shingai & Christensen, 1983; Kaneko & Tachibana, 1985*a*, *b*). For example, the present authors found that only red-sensitive and green-sensitive cones among turtle photoreceptors are sensitive to γ-aminobutyric acid (GABA) (Tachibana & Kaneko, 1984; Kaneko & Tachibana, 1986), and supported the hypothesis that there exists a negative feed-back synapse from monophasic horizontal cells to cones (Baylor, Fuortes & O'Bryan, 1971), which is mediated by GABA (Lam, 1972; Marc, Stell, Bok & Lam, 1978; Lam, Su, Swain, Marc, Brandon & Wu, 1979; Schwartz, 1982; Murakami, Shimoda, Nakatani, Miyachi & Watanabe, 1982). The present study was made to examine whether Ca-channel blockers, such as Co^{2+} or other pharmacological agents, interact with GABA receptor-channel complexes (see Olsen, 1982 for review). We report here that Co^{2+} and other cations block the GABA-induced current in turtle cones.

METHODS

Preparations

Photoreceptors were isolated from the turtle (*Geoclemys reevesii*) retina as described in detail in the previous papers (Tachibana & Kaneko, 1984; Kaneko & Tachibana, 1986). The cells were kept in an incubator at 10 °C for 1 h to 2 days before use. In this study, single cones with red oil droplet were used, since they were highly sensitive to GABA (Tachibana & Kaneko, 1984).

Recording procedures

Membrane currents were recorded by using patch pipettes in the whole-cell clamp configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The methods of recording and data processing have been described in detail elsewhere (Kaneko & Tachibana, 1985*a*, 1986). A control pipette solution consisted of (in mM): KCl, 120; EGTA, 5 and HEPES, 10, and the pH was adjusted to 7·3 with KOH (final concentration 18 mM). In the series of experiments about Ca current, the recording pipette was filled with a solution containing (in mM): CsCl, 120; EGTA, 5; HEPES, 10, and NaOH, 13; (pH 7·3). The cells were continuously superfused with a solution maintained at 15 °C. The rate of superfusion was 0·6 ml/min and it took about 1 min to exchange the solution in the bath. A standard bath solution contained (in mM): NaCl, 79; KCl, 10; $CaCl_2$, 2·5; $MgCl_2$, 1; glucose, 16; HEPES, 2; choline Cl, 37; (pH 7·4). The junction potentials were measured as described in the preceding paper and the membrane potentials in this paper are the values corrected for these junction potentials by calculation.

Application of drugs

Divalent cations and other pharmacological agents were either simply added to the superfusate when the final concentration was less than 100 μM, or substituted for choline Cl in the standard solution in an equimolar ratio when the concentration was higher than 1 mM. They were bath-applied or ejected by pressure (0·4 kg/cm^2, 5–20 s in duration) from a 20 μm-tip pipette positioned approximately 20 μm away from the recorded cell (Ishida, Kaneko & Tachibana, 1984; Tachibana, 1985). Recovery from the blocking by bath-applied agents was usually partial, partly due to a deterioration of the recorded cell and partly due to an insufficient wash-out of the agents. Complete recovery was always observed after a brief application of agents by pressure.

GABA was applied by ionophoresis or by pressure ejection. For ionophoresis, a glass micropipette was filled with 1 M-GABA (pH adjusted to 4·0 with HCl), and placed at the cone pedicle under visual control. For pressure ejection, GABA was dissolved in the same solution as the superfusate and ejected by the method mentioned above. As a control experiment, the standard solution was pressure applied to a voltage-clamped cell bathed in the same solution. There was neither a significant change in peak amplitude of the responses evoked by ionophoresis of GABA, nor was there any detectable mechanical artifact.

$CoCl_2$, $NiCl_2$, $CdCl_2$, $MnCl_2$, $BaCl_2$, $SrCl_2$, and CsCl were purchased from Katayama Chemical Industries Co. (Osaka), tetraethylammonium (TEA) from Tokyo Chemical Industry Co. (Tokyo), and muscimol from Sigma Chemical Co. (St. Louis, MO, U.S.A.). D-600 (AG chemische Fabriken) was a generous gift from Dr. A. Noma in the National Institute for Physiological Sciences, Okazaki.

RESULTS

Co^{2+} block the GABA-induced currents

Isolated cones with red oil droplet (red-sensitive cones) were voltage clamped at −66 mV and GABA was repetitively applied at cone pedicles by ionophoresis. When the cone was superfused with the standard solution, GABA evoked an inward current (Fig. 1*A* and *B*), which is carried by Cl^- (Kaneko & Tachibana, 1986). As soon as 100 μM-Co^{2+} was introduced into the bath, the peak amplitude of the GABA-induced current markedly decreased (Fig. 1*A* and *C*): the peak amplitude was suppressed by approximately 90 %. Even a saturating dose of GABA could not evoke a response comparable to that in the standard solution (Fig. 1*A*, asterisk). The blocking effect of Co^{2+} was reversible (Fig. 1*A*).

The effect of Co^{2+} on the dose–response relationship of the GABA-induced current. To examine the blocking effect of Co^{2+} more closely, the dose–response relationship of the GABA-induced current was compared with and without 3 μM-Co^{2+} (Fig. 2*A*). Under both conditions, the dose(GABA)–response curves were sigmoidal: when the GABA dose was increased, the peak amplitude of the GABA-induced current became larger and finally reached a saturating level. An obvious effect of Co^{2+} was to suppress the maximum response amplitude: 3 μM-Co^{2+} reduced the maximum amplitude by about 30 % (27 $\pm$ 7 %, mean $\pm$ S.D., $n = 5$, number of cells examined). On the other hand, the dose which produced a half saturating response (K_D) in the Co solution (300 $\pm$ 140 pC, $n = 5$) was almost identical to that in the standard solution (310 $\pm$ 80 pC, $n = 5$). The Hill coefficient was not affected significantly by Co^{2+} (2·04 $\pm$ 0·29, $n = 5$, in the standard solution; 1·86 $\pm$ 0·43, $n = 5$, in the Co solution). These data indicate that the dose–response curve in the presence of Co^{2+} and that in the standard solution are superimposable by normalization of the amplitude.

The blocking effect of Co^{2+} was further studied by plotting the reciprocal of response amplitudes against the reciprocal of the square of dose (a modified Lineweaver plot; see Kaneko & Tachibana, 1986), as illustrated in Fig. 2*B*. The dose which evoked a half saturating response (the intercept on the abscissa) did not change, but the maximum response amplitude (the intercept on the ordinate) decreased in the presence of Co^{2+}. Thus, the blockade of GABA-induced responses by Co^{2+} seems to operate in a non-competitive manner.

The blocking effect as a function of Co^{2+} concentration. We examined how the suppression of the GABA-induced responses depended on the concentration of Co^{2+}. Since the GABA-induced current in Co^{2+} was suppressed by a fixed ratio to its control amplitude at every dose of GABA (Fig. 2), we measured the peak amplitudes of the

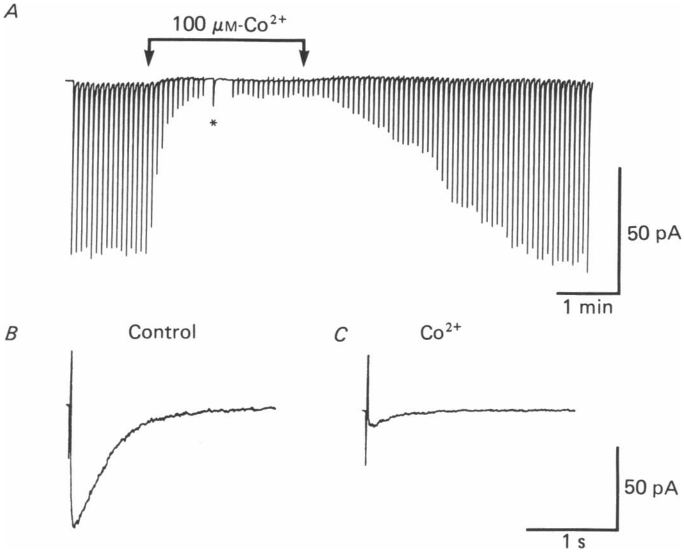

Fig. 1. Blocking of the GABA-induced current by Co^{2+}. An isolated cone was voltage-clamped at -66 mV, and GABA was applied ionophoretically to the cone pedicle repetitively. The ionophoretic pulse (intensity, 40 nA; duration 10 ms; brake current, -4 nA) induced 70% of the saturating response (100 pA) in the standard solution. *A*, 100 μM-Co^{2+} was bath-applied at the period indicated. At * the duration of ionophoretic pulse was increased to 100 ms, which induced a saturating response in the standard solution. *B* and *C*, GABA-induced currents on a faster time scale. *B*, in the standard solution and *C*, in the solution containing 100 μM-Co^{2+}. A transient biphasic deflexion is an artifact due to the application of the ionophoretic pulse. Inward currents are shown as a downward deflexion.

GABA-induced current evoked by identical doses of GABA before and during the bath-application of a known concentration of Co^{2+} (see Fig. 1*A*). The normalized response amplitudes are plotted in Fig. 3 as a function of the concentration of Co^{2+}. A few μM of Co^{2+} were effective in reducing the GABA-induced current and 800 μM of Co^{2+} almost completely blocked it. 50% suppression was seen with approximately 5 μM-Co^{2+}. Since mM-order concentation of Co^{2+} is usually used to block chemical synapses in *in situ* experiments, GABA-induced responses would have been blocked in these experiments almost completely.

Voltage dependence of blocking by Co^{2+}. We examined whether the blocking of the GABA-induced current by Co^{2+} was voltage dependent. It is known that some chemically-induced currents are blocked in a voltage-dependent manner. For example, in mouse central neurones Mg^{2+} blocks the current through *N*-methyl-D-aspartic acid-(NMDA)-activated channels, a subtype of glutamate channels, more strongly as the membrane potential is hyperpolarized (Nowak, Bregestovsky, Ascher, Herbert & Prochiantz, 1984).

Fig. 4*A* shows an example of the GABA-induced current (*I*) *versus* membrane potential (*V*) relationship. Voltage dependence of the blocking was not obvious in the voltage range we examined: the reversal potential was the same under both

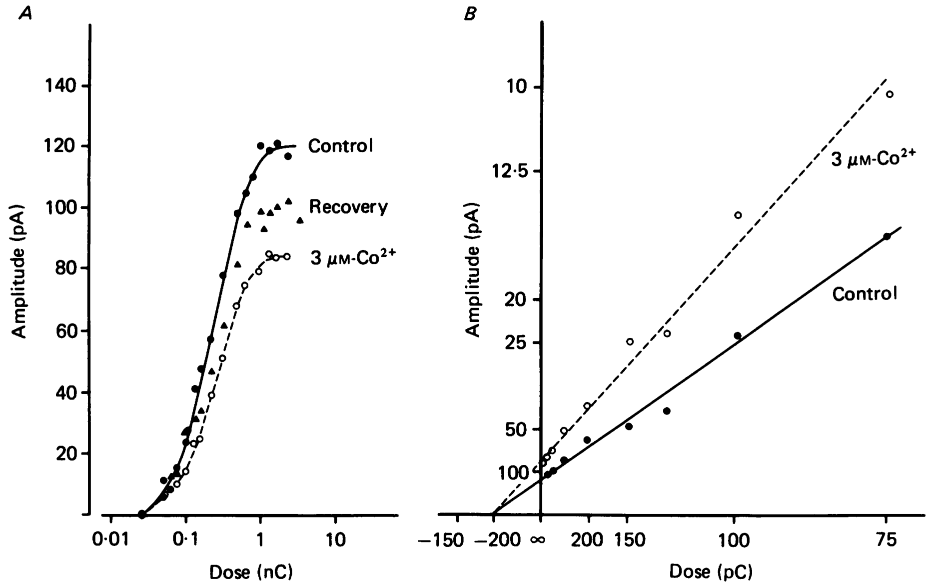

Fig. 2. Effect of 3 μM-Co^{2+} on dose–response relationship. GABA was ionophoretically applied at the cone pedicle. Holding potential −66 mV. Dose (*D*)–response (*I*) relationship was examined in the standard solution (control; ●) and in the solution containing 3 μM-Co^{2+} (○). A partial recovery was observed (▲). The dose of GABA is the product of intensity (5–30 nA) and duration (5–70 ms) of ionophoretic pulses (*A* in nC and *B* in pC). *A*, a plot of log *D versus I* (peak response amplitude; absolute value). The Hill coefficient was 2·1 (control), 1·9 (Co) and 1·9 (recovery). Fitting curves drawn by eye. *B*, a modified Lineweaver plot in the standard solution (●) and in the Co solution (○): a plot of $1/D^2$ *versus* $1/I$. The reciprocal of squared dose was used, since the Hill coefficient was close to 2. The K_D value, the dose to evoke a half saturating response, was 192 pC in the standard solution and 194 pC in the Co solution. Fitting curves drawn by least squares method.

conditions and the *I–V* curve in the standard solution could be fitted to that in the Co solution by multiplying by a constant factor ($0{\cdot}45 \pm 0{\cdot}13$, $n = 7$). Response wave forms obtained under both conditions were superimposable after a current scale adjustment (Fig. 4*B* and *C*). Thus, the blocking by Co^{2+} seems independent of membrane voltage at least within the physiological range.

The effect of Co^{2+} on the GABA-induced current evoked in the extra-synaptic region. The responses evoked in the extra-synaptic region were also affected by Co^{2+}. In this experiment, cones whose pedicles had been lost during dissociation were voltage clamped and GABA was applied to the cell bodies by ionophoresis. As has been reported previously (Tachibana & Kaneko, 1984), the amplitude of the GABA-induced current was small (Fig. 5*A* and *B*). Pressure-applied Co^{2+} depressed the peak amplitude of the GABA-induced current reversibly (Fig. 5*A* and *C*). The extent of the blocking was comparable to that observed in the synaptic region (Fig. 3).

Effects of other Ca-current blockers and K-current blockers on the GABA-induced current

Co^{2+} blocks voltage-activated Ca currents and thereby suppresses Ca-mediated currents (see Hagiwara & Byerly, 1981). It has been reported that photoreceptors

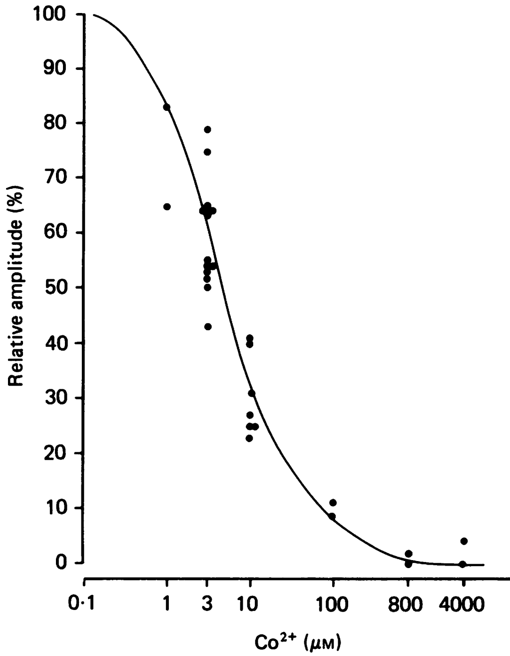

Fig. 3. Relationship between the relative amplitude of GABA-induced currents and the concentration of Co^{2+}. Each isolated cone was voltage clamped at −66 mV and a fixed amount of GABA, which induced 50–70 % of the saturating response, was applied at its pedicle by ionophoresis. The peak amplitude of the evoked response in the standard solution was defined as 100 %. Then, various concentrations of Co^{2+} were bath applied. The relative amplitudes in the Co solutions are calculated for each cell and plotted here. Data from twenty-seven cells. The curve was drawn by eye.

have a voltage-activated Ca conductance and Ca-mediated conductances (cones: Piccolino & Gerschenfeld, 1978, 1980; rods: Fain, Quandt & Gerschenfeld, 1977; Bader *et al.* 1982). One might argue, therefore, that the blocking of the GABA-induced current by Co^{2+} would be somehow related to the Ca current and/or the Ca-mediated currents, although GABA selectively increases a Cl conductance and does not affect a Ca conductance in turtle cones (Kaneko & Tachibana, 1986). To examine this possibility, we tested whether the GABA-induced responses were also affected by some other divalent cations and pharmacological agents known to affect the Ca current and/or Ca-mediated currents.

Effects of Ni^{2+}, Cd^{2+}, Mn^{2+} and Mg^{2+} on the GABA-induced current. Besides Co^{2+}, both Ni^{2+} and Cd^{2+} were found to be strong blockers of the GABA-induced current (Figs. 6 and 7). Ni^{2+} and Cd^{2+} (10 μM each) decreased the responses to 11 ± 3 % ($n = 4$) and 26 ± 3 % ($n = 6$), respectively. The mechanism of blocking by Ni^{2+} and Cd^{2+} seems similar to that by Co^{2+}: both Ni^{2+} and Cd^{2+} reduced the maximum response

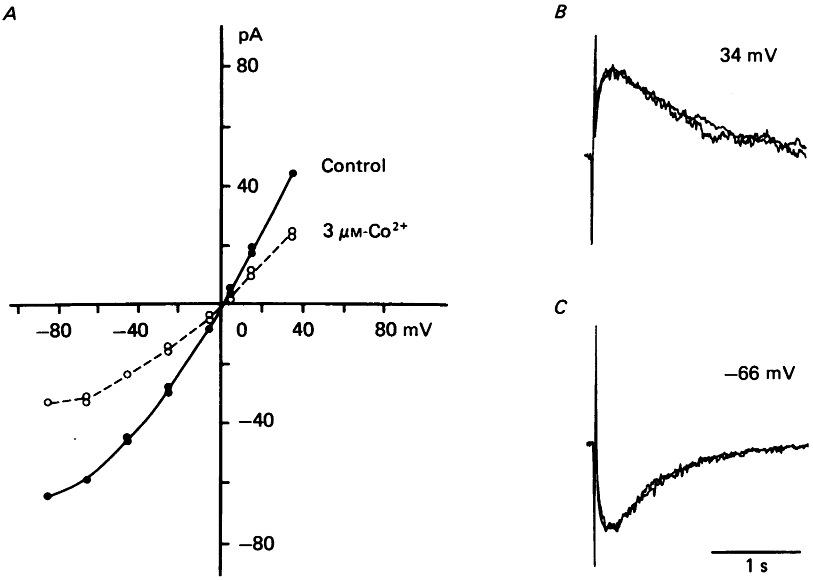

Fig. 4. *A*, the relationship between the GABA-induced current and the membrane potential in the presence or absence of Co^{2+}. A cone was voltage clamped in the standard solution (●) or in the solution containing 3 μM-Co^{2+} (○), and the same amount of GABA, which evoked 70 % of the saturating response in the standard solution, was ionophoretically applied (intensity, 30 nA; duration, 10 ms; brake current, −3 nA). Co^{2+} reduced the response amplitude to 53 % irrespective of the membrane potential. The reversal potential was the same in either solution (+1 mV). *B* and *C*, wave forms of the GABA-induced current in the presence or absence of Co^{2+}. The current trace in the Co solution was superimposed on the current trace in the standard solution, the peak amplitude of which was multiplied by 0·53. The membrane potential was +34 mV (*B*) and −66 mV (*C*). Records obtained from the same cell as in *A*.

amplitude without causing a change in the threshold or saturating dose (Cd^{2+}, Fig. 6*A*; Ni^{2+}, not illustrated). The modified Lineweaver plot indicates that these divalent cations also behaved as a non-competitive blocker (Fig. 6*B*).

On the other hand, 10 μM-Mn^{2+} or 10–20 mM-Mg^{2+} (in the presence of either 0·5 mM-Ca^{2+} or 2·5 mM-Ca^{2+}) caused no significant changes either in the amplitude of the GABA-induced current (Figs. 7 and 8) or in the dose(GABA)–response relationship (not illustrated). These results seem very important, since solutions containing a high concentration of Mg^{2+} and a low concentration of Ca^{2+} are also widely used to block chemical synaptic transmissions in various neuronal tissues.

Effects of Sr^{2+}, Ba^{2+} and Ca^{2+} on the GABA-induced current. Since Sr^{2+} and Ba^{2+}

are known to carry a larger amount of current through Ca channels than Ca^{2+} (see Hagiwara & Byerly, 1981), the effects of these divalent cations were examined. The results are summarized in Fig. 7. Neither Sr^{2+} nor Ba^{2+} affected the GABA-induced current significantly, even when their concentration was increased to 100 μM. A high concentration of Ca^{2+} (10 mM) weakly suppressed the GABA-induced current. These results suggest that the amount of current through the Ca channels is not directly related to the suppression of the GABA-induced current.

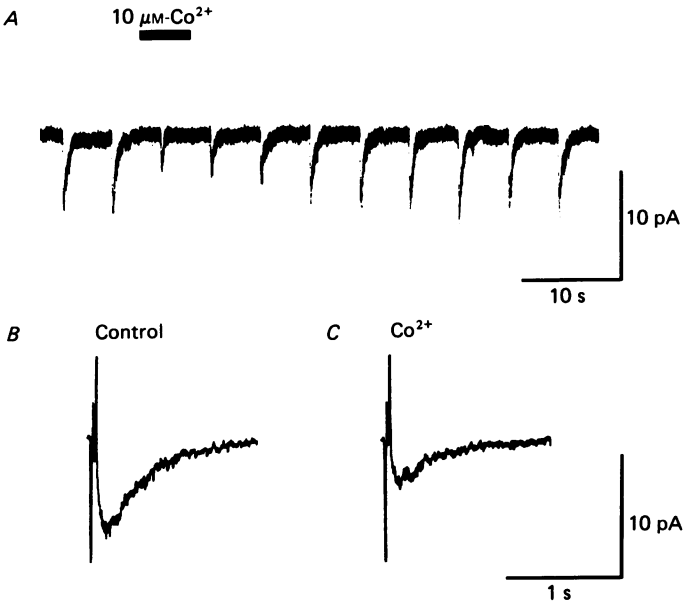

Fig. 5. Effect of Co^{2+} on the GABA-induced current evoked at the extra-synaptic region. An isolated cone, whose pedicle had been lost during the dissociation, was superfused with the standard solution and GABA was applied at the nuclear region by ionophoresis (intensity, 30 nA; duration, 40 ms; braking current, −3 nA). The sensitivity to GABA was very low and the maximum response amplitude was 13 pA. Holding potential −66 mV. *A*, 10 μM-Co^{2+} was pressure applied for 5 s, as indicated by a thick bar. *B* and *C*, averaged current traces on a faster time scale. Data obtained before (*B*) and during (*C*) the application of Co^{2+}. Four responses were averaged. The transient biphasic deflexion is an artifact due to the ionophoretic pulse.

Effects of some pharmacological agents on the GABA-induced current. D-600 (10 μM), an organic antagonist of a Ca current, did not suppress the GABA-induced current, and even enhanced it in five of eight cells (Fig. 7). Tetraethylammonium (TEA; 25 mM), which is known to block some types of K currents, reduced the amplitude of the GABA-induced current. As shown in Fig. 9, TEA suppressed the maximum response amplitude but did not shift the dose–response relationship along the abscissa. The modified Lineweaver plot indicates that TEA was also a non-competitive blocker of the GABA-induced current, suggesting a similar blocking mechanism to that of Co^{2+}. On the other hand, Cs^+ (10 mM), another K-current blocker, induced no significant blocking effect on the GABA-induced current (Fig. 7).

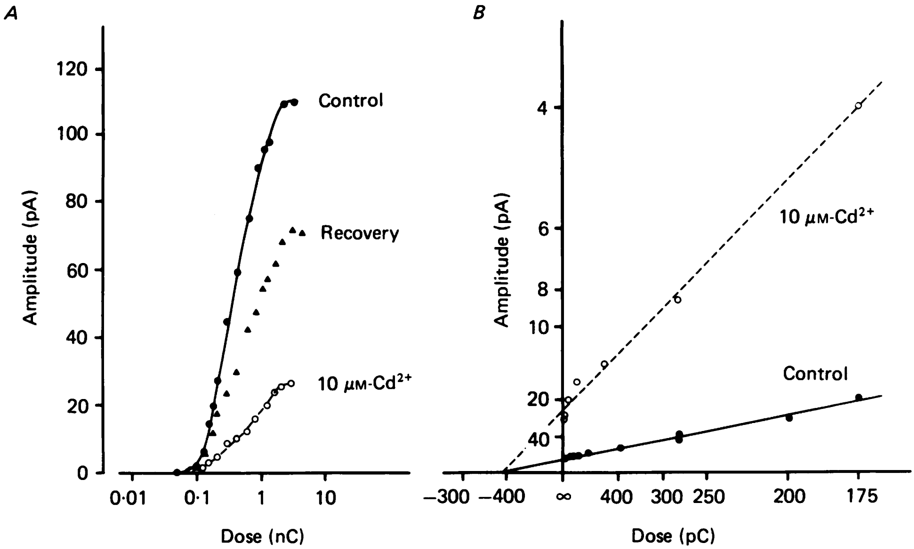

Fig. 6. Effect of Cd^{2+} on the GABA-induced current. Dose(D)–response (I) relationship was examined in an isolated cone bathed in the standard solution (●) or in a solution containing 10 µM-Cd^{2+} (○). A partial recovery was observed after the wash-out of Cd^{2+} (▲). Holding potential −66 mV. *A*, a plot of log D *versus* I (peak response amplitude; absolute value). Curves drawn by eye. *B*, a modified Lineweaver plot: a plot of $1/D^2$ *versus* $1/I$. Both lines intercept at a similar K_D value (approximately 370 pC) on the abscissa. Lines fitted by least squares method.

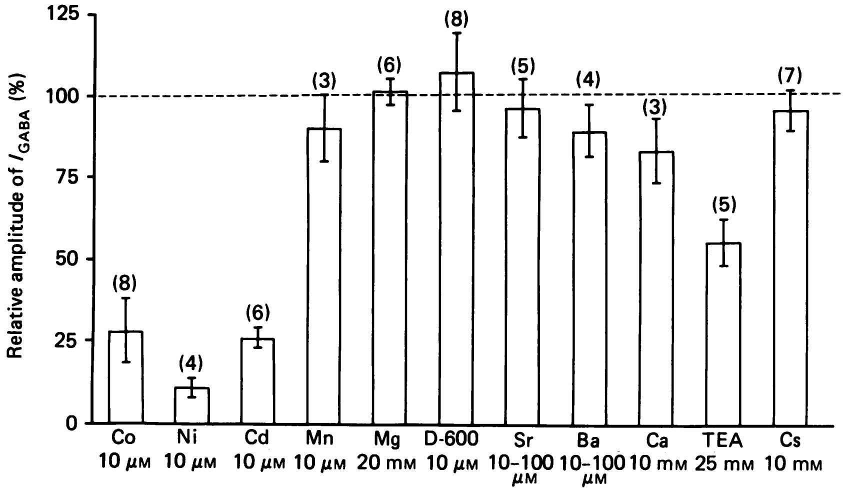

Fig. 7. Comparison of the effects of divalent cations and some pharmacological agents on the GABA-induced current. The peak amplitude of the GABA-induced current was measured in the standard solution for each cell and defined as 100%. The peak amplitude of the current evoked by identical doses of GABA was then measured in the presence of each agent and relative amplitude was calculated. Means and standard deviations are illustrated. Numbers of cells examined are shown in parentheses.

Muscimol is a potent agonist of GABA in turtle cones (Kaneko & Tachibana, 1986). We found that the muscimol-induced current was similarly suppressed by Co^{2+} (not illustrated).

Effects of divalent cations and D-600 on the Ca current in turtle cones

Co^{2+}, Ni^{2+} and Cd^{2+} drastically suppressed the GABA-induced current at a 10 μM dose in turtle cones, while Mn^{2+} and Mg^{2+} and D-600, which are also known as Ca-current blockers, showed little effect on it. These results, therefore, lead to the conclusion that the blocking of the GABA-induced current is not related to the Ca

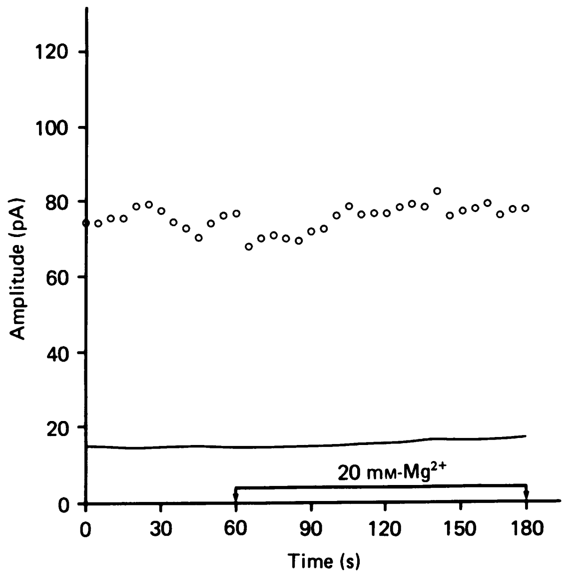

Fig. 8. Mg^{2+} has no effect on the GABA-induced current. GABA was repetitively applied by ionophoresis (intensity, 30 nA; duration, 10 ms; brake current, −2 nA; pulse interval, 5 s) and evoked a half-saturating response. The ordinate plots the peak amplitude of the GABA-induced current (○) and the amplitude of the steady current required to hold the membrane potential at −66 mV (continuous line); both currents were inward-going but absolute values were plotted. A high Mg solution (20 mM-Mg^{2+}, 0·5 mM-Ca^{2+}) was bath applied during the period indicated by the arrows. Fluctuations of the peak amplitude were mainly due to the displacement of the ionophoretic electrode, which was caused by the ripple of the meniscus of superfusates.

current or the Ca-mediated currents. However, it is important to examine the relative potency of these agents in blocking the Ca current in turtle cones, since it has been reported that the sequence for rating the blocking potencies of various divalent cations somewhat varies depending on the kind of cells (see Hagiwara & Byerly, 1981).

The Ca current in turtle cones. To isolate the Ca current, most of the outward currents were suppressed by superfusing cells with a solution containing 25 mM-TEA and 10 mM-Cs, and by applying Cs intracellularly from the recording patch pipette filled with 120 mM-CsCl (see Bader *et al.* 1982).

A net inward current was evoked when the membrane potential was depolarized from −66 mV (holding potential) to −16 mV (Fig. 10*A*). During the pressure application of 4 mM-Co^{2+} (the solution containing Co, TEA and Cs), the identical voltage shift induced a net outward current, which showed little time dependence (Fig. 10*B*). The blocking effect of Co^{2+} was reversible (Fig. 10*C*). The relationship between the membrane current (I_m) and the membrane potential (V) was examined by applying voltage pulses with various intensity (Fig. 10*D*). In the absence of Co^{2+}, the I_m–V relationship was N-shaped and the slope resistance was negative between

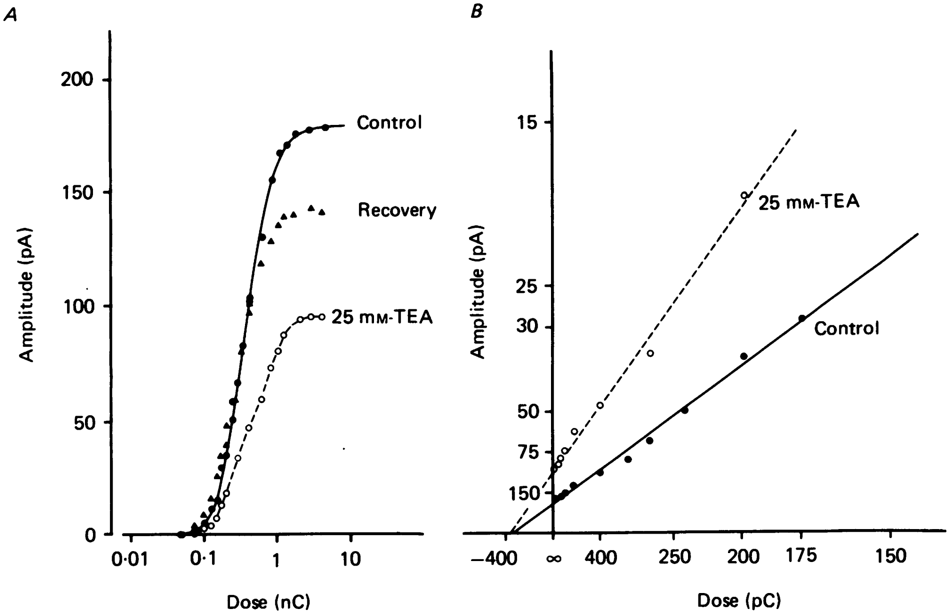

Fig. 9. Effect of TEA on the GABA-induced current. Dose(D)–response (I) relationship. A cone was voltage clamped at −66 mV and GABA was applied ionophoretically. The cell was bathed initially in the standard solution (●) and then in a solution containing 25 mM-TEA (○). Partial recovery was observed (▲). *A*, a plot of log D *versus* I (peak response amplitude; absolute value). Curves drawn by eye. *B*, a modified Lineweaver plot, i.e. plot of $1/D^2$ *versus* $1/I$. Lines fitted by least squares method.

approximately −45 and −15 mV (filled circles and filled triangles). In the presence of Co^{2+}, the I_m–V relationship was nearly linear and the slope resistance of the cell was very high (a few GΩ). It seems reasonable to suppose that 4 mM-Co^{2+} suppressed the Ca current almost completely in turtle cones and that the difference between the two I_m–V curves corresponded to the total amount of the Ca current. The Ca current was activated at potentials positive to approximately −45 mV, reached a maximum amplitude at around −15 mV, and decreased in amplitude by further depolarization, similar to the Ca current found in isolated rods of the tiger salamander retina (Bader *et al.* 1982). The maximum amplitude of the Ca current was 21 ± 9 pA ($n = 42$).

Blocking effects of divalent cations and D-600 on the Ca current. Relative potencies of various divalent cations and D-600 in blocking the Ca current were examined under the voltage-clamp condition mentioned above. 4 mM-Co^{2+} and one of these agents were pressure or bath applied to each cell (Fig. 11 *A–D*), and the ratio of the response amplitude of the Ca current in the presence of the agent (Fig. 11 *E*) to that of the maximal Ca current (Fig. 11 *F*; the difference between the evoked current in the TEA, Cs solution and that in the presence of 4 mM-Co^{2+}) was calculated. Means and standard deviations are illustrated in Fig. 12. 20 mM-Mg^{2+} (Ca^{2+} was reduced to 0·5 mM) blocked the Ca current almost as completely as 4 mM-Co^{2+}. At a 10 μM dose, D-600 was a stronger antagonist than Ni^{2+} and Cd^{2+}: D-600 suppressed the response

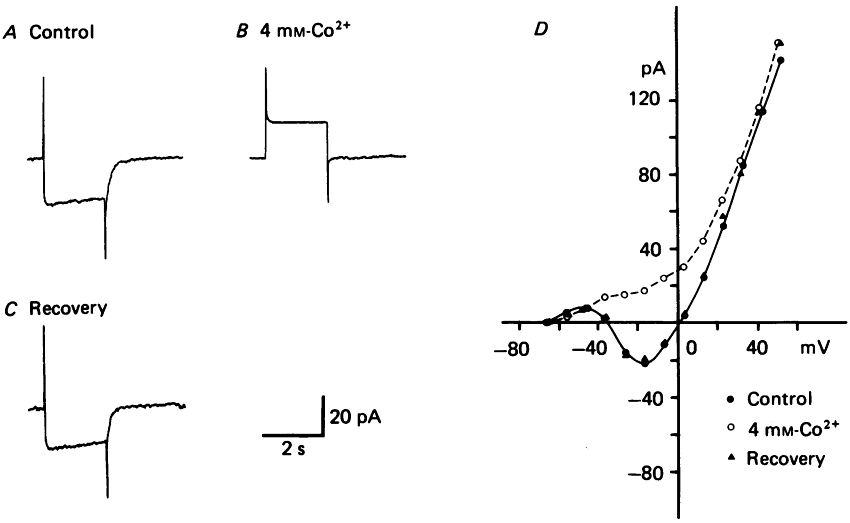

Fig. 10. Voltage-dependent Ca current. An isolated cone was superfused with a solution containing 25 mM-TEA and 10 mM-Cs, and voltage clamped by a patch pipette containing 120 mM-CsCl (the control condition). A Co solution (4 mM-Co, 25 mM-TEA and 10 mM-Cs) was pressure applied to block the Ca current. *A–C*, membrane currents evoked by a 2 s voltage pulse from −66 mV (holding potential) to −16 mV. *A*, control condition. *B*, effect of Co^{2+}. Co^{2+} was pressure ejected for 4 s, during which the voltage pulse was applied. *C*, recovery observed 30 s after the termination of the pressure pulse. *D*, membrane current *versus* membrane potential relationship examined in the control solution (●) and in the presence of Co^{2+} (○). The current amplitude was measured at 150 ms after the onset of voltage pulses. The effect of Co^{2+} was reversible (▲). The relationship in the Co solution showed outward rectification at potentials more positive than 0 mV, perhaps due to an incomplete suppression of K currents. Data obtained from the same cell as shown in *A–C*.

amplitude to *ca.* 35 %, while Ni^{2+} and Cd^{2+} reduced it to *ca.* 50 %. Co^{2+} and Mn^{2+} (10 μM each) suppressed the Ca current partly. Thus, it is clear that these agents showed different potencies in blocking the Ca current and in blocking the GABA-induced current (see Fig. 7).

DISCUSSION

On mechanisms of the blocking of the GABA-induced current by Co^{2+}, Ni^{2+}, Cd^{2+} and TEA

The present study has shown that Co^{2+}, Ni^{2+} and Cd^{2+}, as well as TEA, blocked the GABA-induced current in turtle cones. The blocking of the GABA-induced current was not related to the suppression of the Ca current, either directly or indirectly. We came to this conclusion for the following reasons. First, the GABA-induced current in turtle cones was carried by Cl^- selectively and not by Ca^{2+}, and the cells had $GABA_A$ receptors (Kaneko & Tachibana, 1986). Secondly, the relative potencies of various agents in blocking the GABA-induced current were very different from those in blocking the Ca current: $Ni^{2+} > Cd^{2+} \simeq Co^{2+} \gg Mn^{2+} \simeq$ D-600 as the

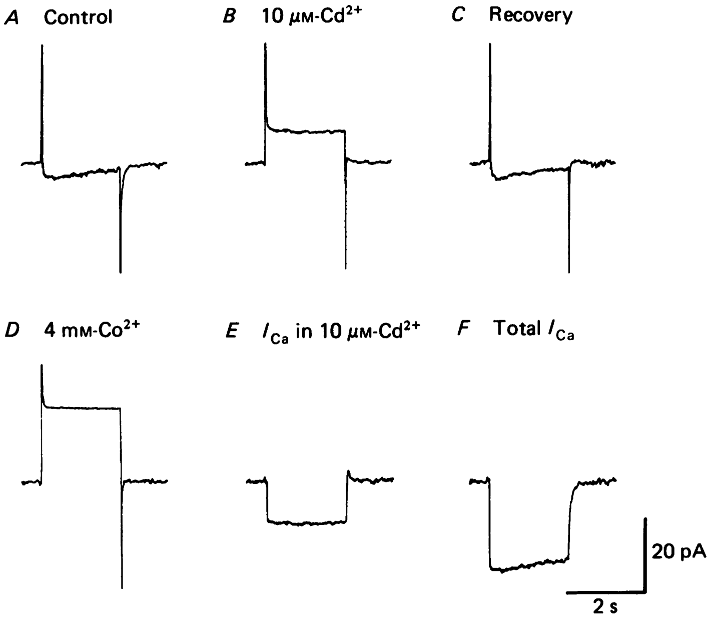

Fig. 11. Effect of Cd^{2+} and Co^{2+} on the Ca current. An isolated cone was voltage clamped at −66 mV with a patch pipette filled with a solution containing 120 mM-CsCl. The membrane potential was shifted to −16 mV by a 2 s command pulse. *A*, control condition. The cell was superfused with a solution containing 25 mM-TEA and 10 mM-Cs. *B*, effect of Cd^{2+}. The command pulse was applied while a solution containing 10 μM-Cd, 25 mM-TEA and 10 mM-Cs was pressure ejected to the cell. *C*, recovery observed 40 s after the termination of the pressure pulse. *D*, effect of Co^{2+}. The cell was superfused with a solution containing 4 mM-Co, 25 mM-TEA and 10 mM-Cs. *E*, Ca current in the presence of 10 μM-Cd, which was obtained by subtracting *D* from *B*. *F*, total Ca current; the difference between *A* and *D*.

blocker of the GABA-induced current (10 μM each), while D-600 > $Cd^{2+} \simeq Ni^{2+} > Mn^{2+} \simeq Co^{2+}$ as the Ca-current blocker (10 μM each). Thirdly, the blocking effect of these agents was observed at membrane potentials more negative than −50 mV, at which the Ca current was not activated. These results also suggest that the binding selectivity of divalent cations to the GABA receptor-channel complex is quite different from that to the Ca channel.

Co^{2+}, Ni^{2+}, Cd^{2+} and TEA caused a reduction of the saturating response amplitude without shifting the dose–response relationship for GABA along the abscissa, and the blocking occurred in a non-competitive manner. This rejects the possibilities that these agents compete with GABA molecules in the binding site at the GABA receptor of the GABA receptor–channel complex, like bicuculline (Zukin, Young & Snyder, 1974; Kaneko & Tachibana, 1986), and that GABA molecules decrease in amount by producing ineffective complexes with these agents: if either of these had been the case, these agents would have caused a parallel shift of the dose–response curve for GABA to the right along the abscissa.

The blocking by Co^{2+} is similar to that by picrotoxin in that both agents behaved as a non-competitive blocker. At present it is not clear whether Co^{2+} behaves as a

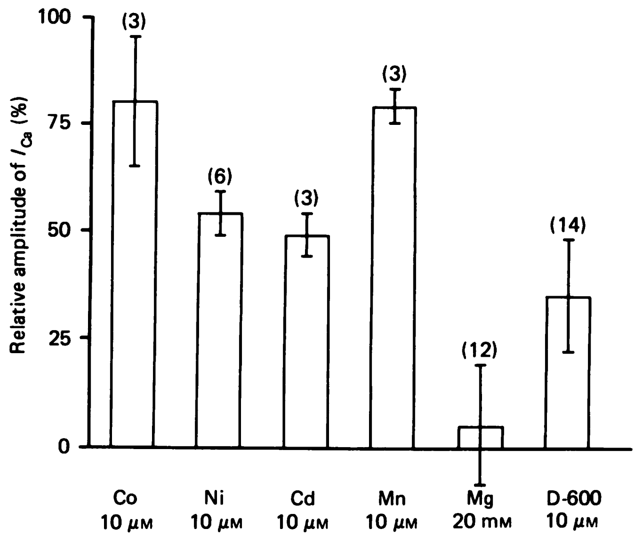

Fig. 12. Effect of divalent cations and D-600 on the Ca current. The Ca current was separated from outward currents by superfusing isolated cones with a solution containing 25 mM-TEA and 10 mM-Cs, and by intracellularly applying Cs through the patch pipette (the control condition). Membrane current was evoked by a 2 s command pulse (from −66 mV to −16 mV) in various solutions. The relative amplitude of the Ca current was defined by $(I_X - I_{4\,mM\text{-}Co})/(I_{control} - I_{4\,mM\text{-}Co}) \times 100\,\%$. $I_{control}$ was the current evoked under the control condition, I_X the current in the presence of a pharmacological agent X, and $I_{4\,mM\text{-}Co}$ the current in the presence of 4 mM-Co^{2+}, which was assumed to block the Ca current completely. Means and standard deviations of the relative amplitude were calculated. Sample size given in parentheses.

channel blocker like picrotoxin (Akaike, Hattori, Inomata & Oomura, 1985) or modifies the GABA molecule–GABA receptor interaction by binding at either a barbiturate receptor or a benzodiazepine receptor in the GABA receptor–channel complex (Study & Barker, 1981; Akaike *et al.* 1985). Further studies are required to identify the binding site of Co^{2+} in the GABA receptor–channel complex. Single-channel current recordings from GABA-activated channels (Hamill, Bormann & Sakmann, 1983; Ozawa & Yuzaki, 1984) would also help elucidate the mechanisms underlying the reduction of the GABA-induced current.

Caution should be exercised in applying divalent cations as a presynaptic blocker

Ca antagonists are widely used as blockers of chemical synapses, and their action is assumed to be limited to the presynaptic terminals. However, the present results demonstrate that some of the divalent cations, commonly accepted as Ca antagonists, also interfere with the GABA-induced responses in turtle cone photoreceptors. A similar observation was presented recently in an abstract (Yakushiji, Akaike & Oomura, 1985) reporting that some divalent cations suppressed the GABA-induced current in frog dorsal root ganglia. Since such observations are limited to a few preparations at present, it is not obvious whether the blocking of GABA-induced responses by Co^{2+}, Ni^{2+}, and Cd^{2+} is generally found in any preparation. However, these observations present the possibility that the disappearance of GABA-induced responses during the application of divalent cations can be accounted for partly by a post-synaptic effect of 'presynaptic' blockers. The present study suggests that Mg^{2+} might be more appropriate than Co^{2+} when one wishes to block the transmission presynaptically in a nervous tissue containing GABAergic synapses: in turtle cones, Mg^{2+} blocked the Ca current without affecting the GABA-induced current. Neither Ni^{2+} nor Cd^{2+} seems appropriate.

The glutamate-induced current in isolated horizontal cells dissociated from the goldfish retina was unaffected by Mg^{2+} (Tachibana, 1985). On the other hand, the current through NMDA-activated channels is suppressed by various divalent cations including Co^{2+} and Mg^{2+} (sequence of the inhibitory potency: Ni^{2+}, $Co^{2+} > Mg^{2+} > Mn^{2+}$, Cd^{2+}) (Ault, Evans, Francis, Oakes & Watkins, 1980; Nowak *et al.* 1984; Mayer & Westbrook, 1985). It is therefore important to examine whether other types of transmitter-activated currents are also affected by these divalent cations. While such knowledge remains uncertain, it would be better to use several kinds of divalent cations and pharmacological agents as presynaptic blockers and to examine carefully whether they also have effects on the transmitter-activated channels.

We thank Miss Michi Hosono for her excellent technical assistance in preparing isolated photoreceptors. This research was supported in part by the Grants in Aid for Scientific Research from the Ministry of Education, Science and Culture (No. 60226021 to A.K. and 60770133 to M.T.).

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