Modulation of $GABA_C$ Response by Ca^{2+} and Other Divalent Cations in Horizontal Cells of the Catfish Retina

M. Kaneda,* M. Mochizuki,‡ K. Aoki,* and A. Kaneko*

From the *Department of Physiology, Keio University School of Medicine, Tokyo 160, Japan; and ‡Department of Biomedical Engineering, Faculty of Science and Technology, Keio University, Yokohama 223, Japan

ABSTRACT $GABA_C$ responses were recorded in cultured cone-driven horizontal cells from the catfish retina using the patch clamp technique. At a holding potential of −49 mV, a bicuculline-resistant inward current (I_{GABA}) was observed when 10 μM GABA was applied. The amplitude of I_{GABA} increased as the extracellular Ca^{2+} ($[Ca^{2+}]_o$) was increased. Concentration–response curves of I_{GABA} at 2.5 and 10 mM $[Ca^{2+}]_o$ had similar EC_{50} (3.0 and 3.1 μM) and Hill coefficients (1.54 and 1.24). However, the maximal response estimated at 10 mM $[Ca^{2+}]_o$ was larger than the maximal response at 2.5 mM $[Ca^{2+}]_o$. Increasing Ca influx through voltage-gated Ca channels and the resulting rise in the intracellular Ca^{2+} concentration had no effects on I_{GABA}. However, I_{GABA} was inhibited by extracellular divalent cations, with the following order of the inhibitory potency: $Zn^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+}$. The inhibitory action of Zn^{2+} on the $[Ca^{2+}]_o$-dependent I_{GABA} increase was noncompetitive. The action of $[Ca^{2+}]_o$ on I_{GABA} was mimicked by Ba^{2+} or Sr^{2+}. These results demonstrate that the extracellular domain of $GABA_C$ receptors has two functionally distinct binding sites represented by Ca^{2+} (facilitation) and Zn^{2+} (inhibition). Since $[Ca^{2+}]_o$ and $[Zn^{2+}]_o$ change into the opposite direction by light, it seems likely that they modify cooperatively the efficacy of the positive feedback consisting of the $GABA_C$ receptor.

KEY WORDS: GABA • horizontal cell • retina • Ca • catfish

INTRODUCTION

The outer plexiform layer, the first synaptic layer of the vertebrate retina, is where signals transduced in photoreceptors are transmitted to bipolar cells. Photoreceptor-bipolar transmission is modulated by the feedback synapse from horizontal cells to photoreceptors. The input–output relation of these synapses has been studied in the tiger salamander and other animals (see Wu, 1994). The transmission at these synapses may be more complex than originally thought, based on the modulatory actions of divalent cations. For example, Zn^{2+} has been shown to be coreleased with transmitters from the synaptic vesicles of photoreceptors (Wu et al., 1993; Dong and Werblin, 1995).

It has been reported that the Ca^{2+} concentrations in the inner and outer plexiform layers are changed by illumination (Livsey et al., 1990; Gallemore et al., 1994). These Ca^{2+} concentration changes are thought to result primarily from Ca influx accompanying transmitter release. We have shown that the extracellular Ca^{2+} concentration in the inner plexiform layer is important for determining the activities of nicotinic ACh receptors in retinal ganglion cells (Kaneda et al., 1995). Modulation of known chemical receptors by the extracellular Ca^{2+} concentration in the outer plexiform layer has not been reported.

The $GABA_C$ receptor was initially identified in horizontal cells of the catfish retina (Qian and Dowling, 1993). Since the resting potential of the horizontal cell is ∼−80 mV ($[K^+]_o$ = 5 mM), and the reversal potential of the $GABA_C$ response (E_{Cl}) of the intact horizontal cell is ∼−30 mV (Takahashi et al., 1995*a*), GABA induces a depolarization that enhances GABA release from horizontal cells (Schwartz, 1987). In this sense, $GABA_C$ receptors in catfish horizontal cells make a positive auto-feedback loop, the concept proposed by Stockton and Slaughter (1991) and Kamermans and Werblin (1992) for $GABA_A$ receptors of the amphibian horizontal cells. These $GABA_C$ auto-receptors are thought to contribute to accelerating light-evoked responses (Takahashi et al., 1995*a*). In the present experiments, we examined the actions of extracellular Ca^{2+} and other divalent cations on the $GABA_C$ receptor. We found that the extracellular domain of the $GABA_C$ receptor had two divalent cation binding sites with contrasting functions; i.e., a facilitative Ca^{2+} binding site and an inhibitory Zn^{2+} binding site. Based on the present findings, we speculate as to the functional role of $GABA_C$ receptors in horizontal cells.

Address correspondence to M. Kaneda, MD, Ph.D., Department of Physiology, Keio University School of Medicine, Tokyo 160, Japan. Fax: 81-3-3359-0437; E-mail: mkaneda@mc.med.keio.ac.jp

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MATERIALS AND METHODS

Cell Preparation

Our methods were previously described in detail (Takahashi et al., 1995*a*). Briefly, preparations were made from a catfish that had been dark adapted for more than 3 h. Retinas were incubated for 40 min at room temperature in a solution containing (mM): 125 NaCl, 1 NaH_2PO_4, 2.6 KCl, 1 Na pyruvate, 10 Glucose, 5 EGTA, 10 HEPES, and 3–4 U/ml papain (Worthington Biochemical Co., Freehold, NJ) together with 5 μM of its activator, L-cysteine (pH adjusted to 7.0 with NaOH). Isolated cell preparations contained both rod- and cone-driven horizontal cells, easily distinguished by their characteristic morphology under an inverted microscope (TMD; Nikon, Tokyo, Japan). Dissociated cells were kept in a one-to-one mixture of L-15 medium (Life Technologies, Inc., Grand Island, NY) and a culture medium containing (mM): 56.5 NaCl, 0.5 $MgCl_2$, 0.3 $MgSO_4$, 1.5 $CaCl_2$, 5 glucose, 10 HEPES, and 10 mg/liter BSA, pH adjusted to 7.6 with NaOH, for 4–7 d at 10°C. As the rod-driven horizontal cells died in 2–3 d, only the cone-driven horizontal cells were used for recordings.

Current Recordings

Patch pipettes were fabricated from borosilicate capillaries (GC-150F-10; Clark Electromedical Instruments, Reading, UK) using a two-stage electrode puller (PP-83; Narishige, Tokyo, Japan). To block the voltage-gated K conductances, pipettes were filled with a Cs-rich internal solution containing (mM): 120 CsCl, 1 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, pH adjusted to 7.4 with CsOH. The resistance of the electrode filled with the Cs-rich internal solution was 8–12 MΩ. To reduce stray capacitance, the outer wall of the pipette except for the tip was coated with Apiezon wax (Apiezon Products Ltd., London, UK) and the residual capacitance was compensated for electrically. The reference electrode was a Ag-AgCl wire connected to the dish by a 140 mM NaCl agar bridge. Recordings were made using a patch clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo, Japan). The series resistance was not compensated, but the error due to the series resistance was less than a few millivolts, as the peak current amplitude did not exceed 100 pA. Current and voltage signals were monitored on an oscilloscope, recorded on a chart recorder (WR7700; Graphtech, Yokohama, Japan) and stored on a video tape recorder (HV-S21; Mitsubishi, Tokyo, Japan) through a PCM processor (frequency characteristics 0–20 kHz ± 1 dB, PCM-501ES; Sony, Tokyo, Japan). Signals were digitized directly or from the tape recorder by a 12 bit A/D converter (ADX-98H; Canopus, Kobe, Japan) attached to a personal computer (PC9801RX; NEC, Tokyo, Japan). Command voltages were generated by a D/A converter (DAX-98; Canopus) driven by the same computer. The holding potential was corrected for the liquid junction potentials measured by a previously reported method (Kaneko and Tachibana, 1986*a*).

Drug Application

To block voltage-gated cation conductance and the current carried via GABA transport (Haugh-Scheidt et al., 1995; Takahashi et al., 1995*b*), cells were superfused with Na-free solution containing (mM): 127.6 *N*-methyl-D-glucamine (NMDG), 1 $MgCl_2$, 2.5 $CaCl_2$, 15 glucose, and 10 HEPES, adjusted to pH 7.8 with 1 N HCl (final Cl^- concentration of 124.6 mM was calculated from the amount of 1 N HCl, $MgCl_2$, and $CaCl_2$). The Y-tube system (Suzuki et al., 1990) was used for GABA application to expedite (100–200 ms) solution changes. Repeated application of 10 μM GABA every 3 min induced a response augmentation that reached a steady level in ~10 min. We therefore used the data collected after I_{GABA} had reached the steady state (>10 min). All experiments were carried out at room temperature. Unless otherwise specified, experiments were carried out in at least three different cells.

RESULTS

Effects of the Extracellular Ca Concentration on I_{GABA}

When 10 μM GABA was applied to a cone-driven horizontal cell at a holding potential (Vh) of −49 mV, an inward current (I_{GABA}) was induced. The current began to flow without a detectable delay and peaked within 2 s (Fig. 1 *A, left*). The GABA-induced response showed a biphasic decay during prolonged application (~2 min). Within 30 s, the amplitude of I_{GABA} was reduced to 55% of the peak value, while only a slight amplitude reduction was seen after 30 s. I_{GABA} recorded in the present experiment consisted exclusively of current passing through $GABA_C$ channels, since the response blocked by 100 μM picrotoxin (not shown) was not affected by addition of 100 μM bicuculline (Fig. 1 *D*) as reported previously (Takahashi et al., 1995*a*).

The I_{GABA} amplitude increased monotonically when the extracellular Ca concentration ($[Ca^{2+}]_o$) was increased from 0.1 to 10 mM (Fig. 1 *C*). At 0.1 mM $[Ca^{2+}]_o$, the amplitude of I_{GABA} was 69 ± 8% (mean ± SD, $n = 11$) that of the control ($[Ca^{2+}]_o$ = 2.5 mM; Fig. 1 *A*). I_{GABA} recorded in a nominally Ca^{2+}-free solution was identical to that recorded at 0.1 mM $[Ca^{2+}]_o$. However, such a solution may contain Ca^{2+} in the micromolar range (Kurahashi, 1990). Because of the large increase in holding current and the decrease in the I_{GABA} amplitude, $[Ca^{2+}]_o$ lower than that in the nominally Ca^{2+}-free solution was not assessed in this study. On the other hand, when $[Ca^{2+}]_o$ was increased from 2.5 to 10 mM, the amplitude of I_{GABA} rose to 129 ± 12% (n = 11) that of the control (Fig. 1 *B*). The response time course was the same under these two conditions. The $[Ca^{2+}]_o$-dependent I_{GABA} modulation was reversible; I_{GABA} returned to the control value 30–40 s after reverting to the control medium.

The $[Ca^{2+}]_o$-modulated current represented I_{GABA} through the $GABA_C$ receptor channel, since the amplitude of I_{GABA} was unaffected by 100 μM bicuculline (Fig. 1 *D*), confirming that the $GABA_A$ receptor was not involved.

Concentration–Response Relationships for I_{GABA} at 2.5 and 10 mM $[Ca^{2+}]_o$

To understand the mechanism of the Ca^{2+} and GABA receptor interaction, the GABA concentration–response relation was examined in both 2.5 and 10 mM $[Ca^{2+}]_o$ (Fig. 2). Under both $[Ca^{2+}]_o$ conditions, the least effective concentration of GABA was ~500 nM. With an in-

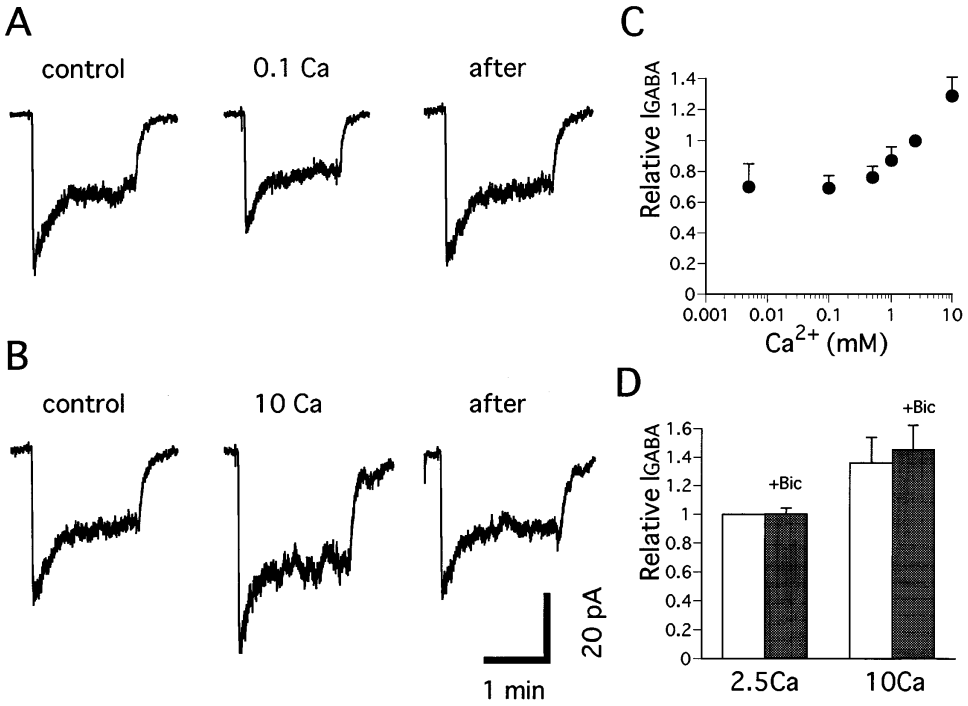

FIGURE 1. Effects of $[Ca^{2+}]_o$ on $GABA_C$ response. Whole cell currents recorded after application of 10 μM GABA in control ($[Ca^{2+}]_o$ = 2.5 mM), low (*A*), and high (*B*) Ca solutions. Currents were evoked by 10 μM GABA applied every 3 min. (*C*) Relationship between $[Ca^{2+}]_o$ and I_{GABA}. Peak current amplitude of I_{GABA} plotted against $[Ca^{2+}]_o$. Peak current amplitude of each response was normalized to control response ($[Ca^{2+}]_o$ = 2.5 mM). Each point represents mean of 4–15 cells, and bars indicate SD. The left-most point represents I_{GABA} in a nominally Ca^{2+}-free solution. (*D*) Effects of bicuculline on I_{GABA} at 2.5 and 10 mM $[Ca^{2+}]_o$. Peak current amplitude of each response was normalized to control response (recorded in $[Ca^{2+}]_o$ = 2.5 mM and no added bicuculline). Each point represents the mean of three to eight cells, and bars indicate SD. Open column, GABA alone; shaded column, GABA response recorded at 100 μM bicuculline (*Bic*). Vh = −49 mV.

crease in the GABA concentration, the response amplitude increased sigmoidally and reached an apparent saturation at 100 μM. The two curves were similar except that the saturated amplitude at 10 mM $[Ca^{2+}]_o$ was 36% larger than that at 2.5 mM $[Ca^{2+}]_o$. These curves were fitted by the equation $I = \text{Imax}/(1 + [EC_{50}/C]^n)$, where Imax is the maximal response, C is the concentration of GABA, n is the Hill coefficient, and EC_{50} is the concentration of GABA that produced a half-maximal I_{GABA}. The two curves were described by a similar

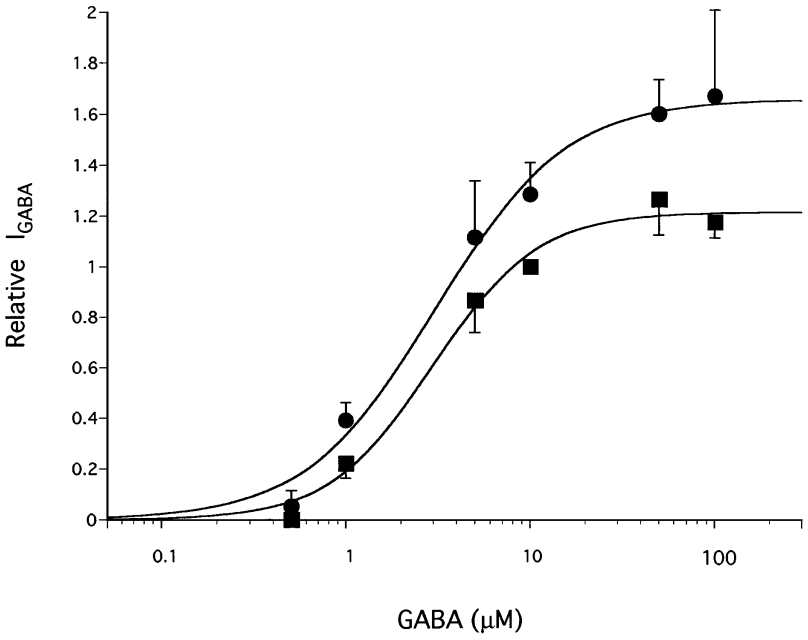

FIGURE 2. Concentration–response curve of I_{GABA} at 2.5 (■) and 10 (●) mM $[Ca^{2+}]_o$. Peak current amplitude of I_{GABA} at 2.5 and 10 mM $[Ca^{2+}]_o$ plotted against GABA concentration. Currents were evoked by GABA applied every 3 min. Each response was normalized to control response evoked by application of 10 μM GABA at 2.5 mM $[Ca^{2+}]_o$. Each point represents the mean of three to seven cells. Bars indicate SD. Data points were fitted to a Hill equation with parameters shown in the text. Vh = −49 mV.

Hill coefficient and EC_{50}; at 2.5 mM $[Ca^{2+}]_o$, the Hill coefficient was 1.54, EC_{50} 3.0 μM (n = 7), while at 10 mM $[Ca^{2+}]_o$ the Hill coefficient was 1.24, EC_{50} 3.1 μM (n = 11). As described above, the only difference between the two curves was in the Imax values; 1.22 for 2.5 mM $[Ca^{2+}]_o$ and 1.66 for 10 mM $[Ca^{2+}]_o$.

Effects of Ca Influx on I_{GABA}

There is a possibility that $GABA_C$ receptor activity is modulated via changes in the intracellular Ca^{2+} concentration. Therefore, in the present experiments, Ca influx produced by the L-type Ca current (step to −9 mV, Vh = −49 mV, 10 s in duration) (Shingai and Christensen, 1983) was increased to study the effects of the intracellular Ca^{2+} concentration on the amplitude of I_{GABA}. The I_{GABA} amplitude was not augmented when additional Ca influx was induced before or during an application of 10 μM GABA, indicating that the action of $[Ca^{2+}]_o$ on I_{GABA} is due to Ca^{2+} binding to an extracellular allosteric site on the $GABA_C$ receptor.

To clarify the mechanism underlying the facilitation of I_{GABA} by Ca^{2+}, we attempted to record single channel activity in outside-out patches excised from horizontal cells. In 12 successfully excised patches, however, we were unable to detect any single-channel activity. This failure is probably attributable to the extremely low density of $GABA_C$ receptor channels. According to a reported noise analysis, the single channel conductance of the $GABA_C$ receptor channel is ~8 pS (Takahashi et al., 1995*a*), which generates a single channel current of 0.4 pA at −50 mV. The maximal I_{GABA} was ~100 pA, which is consistent with 250 channels. The surface area of an average horizontal cell (60–100 μm in diameter) is ~8,000 μm^2 (assuming a flat disk 100 μm in diameter), and has an estimated density of 0.03 channels/μm^2. This value suggests that the channel density is too low for detection with a patch pipette that has a 1–2 μm opening.

Effects of Divalent Cations on I_{GABA}

It has been shown that $[Ca^{2+}]_o$ modulates the activity of the nicotinic ACh receptor in a concentration-dependent manner, and that various divalent cations also affect the activity of this receptor (Mulle et al., 1992; Kaneda et al., 1995). Furthermore, an inhibitory action of divalent cations on the $GABA_A$ receptor was also reported in the retina (Kaneko and Tachibana, 1986*b*). As $GABA_C$ receptor activity was found to be dependent upon $[Ca^{2+}]_o$, we also studied the effects of other divalent cations on I_{GABA}. The response to 10 μM GABA was completely blocked when 4 mM $CoCl_2$ was added to the external solution (Fig. 3 *A*). This inhibitory effect appeared immediately and was reversible. The magnitude of inhibition was sigmoidally dependent upon the Co^{2+} concentration (Fig. 3 *B*); the inhibitory effect of Co^{2+} became apparent at 40 μM and the response to GABA was completely blocked at 4 mM. The concentration–inhibition curve was fitted by the equation $I = 1 - \text{Imax}/(1 + [IC_{50}/C]^n)$, where I represents the normalized response amplitude in the presence of Co^{2+}, Imax is the control response amplitude (1.0) in the absence of Co^{2+}, C is the concentration of Co^{2+}, n is the Hill coefficient, and IC_{50} is the concentration of Co^{2+} that in-

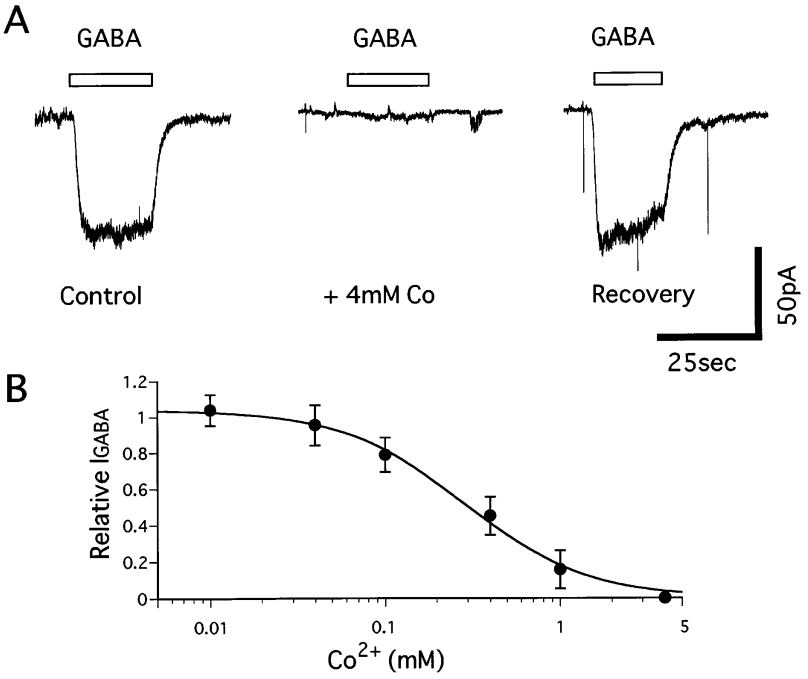

FIGURE 3. Inhibitory effects of Co^{2+} on I_{GABA}. (*A*) Whole cell currents recorded after application of 10 μM GABA (*open bar*) in the presence and absence of 4 mM Co^{2+}. (*B*) Concentration inhibition curve of Co^{2+} on I_{GABA}. Peak current amplitude of I_{GABA} plotted against extracellular Co^{2+} concentration. Currents were evoked by 10 μM GABA applied every 3 min. Each response was normalized to a control response evoked by application of 10 μM GABA without Co^{2+}. Each point represents the mean of three to five cells. Bars indicate SD. Data points were fitted to a Hill equation with parameters shown in the text. Vh = −49 mV.

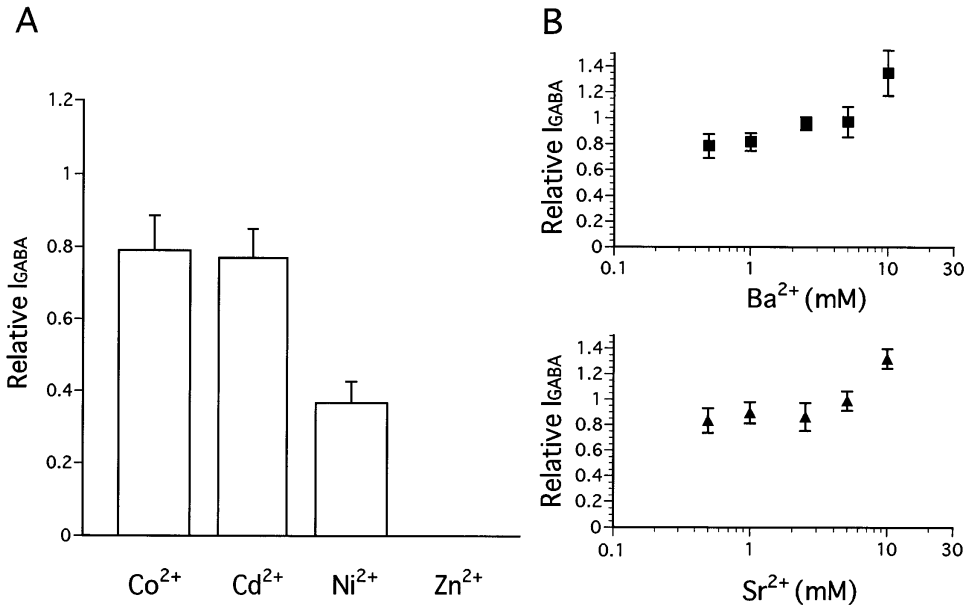

FIGURE 4. Actions of various divalent cations on I_{GABA}. (*A*) Inhibitory effects of inorganic Ca^{2+} channel blockers on I_{GABA}. Currents were evoked by 10 μM GABA applied every 3 min in the presence and absence of 0.1 mM divalent cations. Peak current amplitude of each response was normalized to a control response evoked by application of 10 μM GABA at 2.5 mM $[Ca^{2+}]_o$ alone. Solution containing 2.5 mM $[Ca^{2+}]_o$ plus one of the divalent cations was perfused during GABA application. Each point represents mean of two to five cells. Bars indicate SD. Vh = −49 mV. (*B*) Effects of $[Ba^{2+}]_o$ and $[Sr^{2+}]_o$ on I_{GABA}. Peak amplitude of I_{GABA} plotted against $[Ba^{2+}]_o$ (*top*) or $[Sr^{2+}]_o$ (*bottom*). Peak current amplitude of each response was normalized to a control response evoked in the control solution ($[Ca^{2+}]_o$ = 2.5 mM, 10 μM GABA). Each point represents the mean of three to five Ba^{2+} and three to six Sr^{2+} cells. Bars indicate SD.

hibited I_{GABA} to 0.5. Based on the data obtained from 11 cells, we estimated the Hill coefficient to be 1.24 and IC_{50} to be 284 μM.

We examined the effects of various divalent cations on I_{GABA} and classified the tested divalent cations into three groups: those showing an inhibitory effect like Co^{2+}, those showing a facilitatory action like Ca^{2+}, and those that had no effect. To the first group belonged Zn^{2+}, Ni^{2+}, Cd^{2+}, and Co^{2+}. The order of the inhibitory potency of these divalent cations (at 100 μM) on I_{GABA} was $Zn^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+}$ (Fig. 4 *A*). The second group included Ba^{2+} and Sr^{2+}. When extracellular Ca^{2+} was replaced with Ba^{2+} or Sr^{2+}, the amplitude of I_{GABA} was increased in a concentration-dependent manner (Fig. 4 *B*). Mg^{2+} and Mn^{2+} were neither facilitatory nor inhibitory; the amplitude of I_{GABA} did not change when

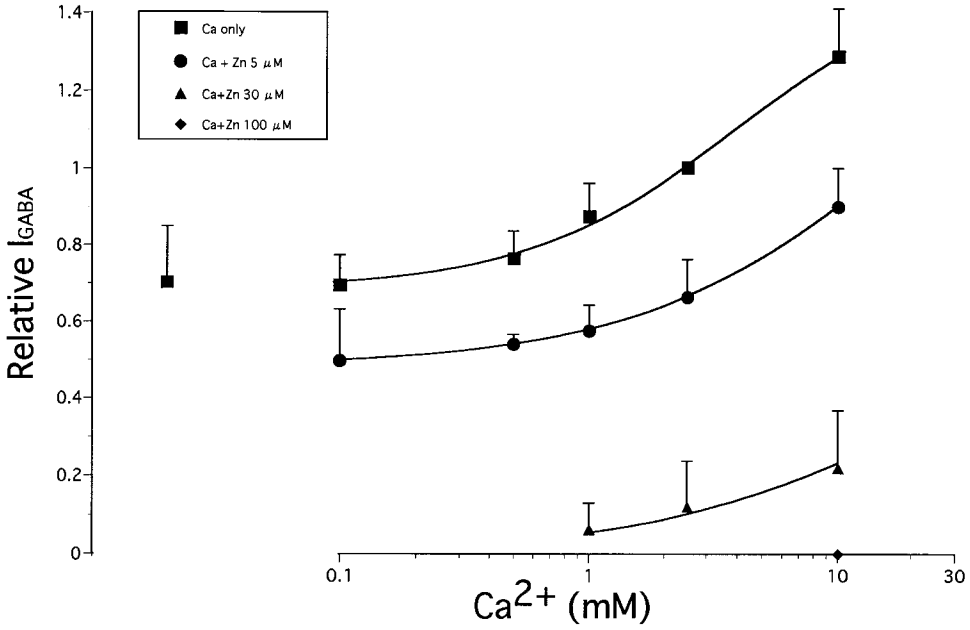

FIGURE 5. Inhibitory effects of Zn^{2+} on the relationship between $[Ca^{2+}]_o$ and I_{GABA}. Peak current amplitude of I_{GABA} is plotted against $[Ca^{2+}]_o$. Currents were evoked by 10 μM GABA applied every 3 min in the presence and absence of Zn^{2+}. Peak current amplitude of each response was normalized to a control response evoked by application of 10 μM GABA at 2.5 mM $[Ca^{2+}]_o$. Each point represents mean of 4–10 cells. Bars indicate SD. Curves were fitted by visual inspection. All experiments were carried out at 2.5 mM $[Ca^{2+}]_o$. The left-most point (■) represents I_{GABA} in a nominally Ca^{2+}-free solution. Vh = −49 mV.

Mg^{2+} was excluded from the solution or 1 mM Mn^{2+} was added to the perfusate.

Inhibitory Actions of Zn^{2+} on $[Ca^{2+}]_o$-dependent Facilitation of I_{GABA}

Of the divalent cations affecting I_{GABA}, Ca^{2+} and Zn^{2+} have the physiological potential to affect the outer plexiform layer via changes in their extracellular concentrations (Gallemore et al., 1994; Wu et al., 1993). It has also been demonstrated that the activity of $GABA_C$ receptors is inhibited by Zn^{2+} (Dong and Werblin, 1995). We attempted to determine whether Ca^{2+} and Zn^{2+} compete for the same binding site on the $GABA_C$ receptor and found that $[Ca^{2+}]_o$-dependent I_{GABA} modulation was observed in the presence of 5 μM Zn^{2+}. However, the I_{GABA} amplitude was reduced by ~30% at all $[Ca^{2+}]_o$ (Fig. 5). When 30 μM Zn^{2+} was added to the perfusate, the minimal amplitude of I_{GABA} was greatly reduced and became nearly undetectable even in the presence of 1 mM $[Ca^{2+}]_o$. At 100 μM Zn^{2+}, there was no current even at 10 mM $[Ca^{2+}]_o$. These observations demonstrate that the inhibitory action of Zn^{2+} on the $[Ca^{2+}]_o$-dependent facilitation of $GABA_C$ receptors is noncompetitive.

DISCUSSION

Mechanisms of Ca-dependent $GABA_C$ Receptor Modulation

Our experiments demonstrate the activity of $GABA_C$ receptors to be regulated by extracellular Ca^{2+}. The concentration–response curve revealed an increase in Imax with no apparent change in either EC_{50} or the Hill coefficient when $[Ca^{2+}]_o$ was increased from 2.5 to 10 mM. Thus, Ca binding to an extracellular site on $GABA_C$ receptors increases the number of active $GABA_C$ receptors but does not change the affinity of GABA for $GABA_C$ receptors. A $[Ca^{2+}]_o$-dependent augmentation of the ACh response in neuronal nicotinic ACh receptors was explained by an increase in the maximal probability of channel opening (Mulle et al., 1992). These investigators showed that Ca binding to an extracellular site increased the opening frequency of ACh channels without affecting the actual channel kinetics. In addition, the effects of extracellular Mg^{2+}, Ba^{2+}, and Sr^{2+} in $GABA_C$ receptors are similar to those exerted by these ions on neuronal nicotinic ACh receptors. Therefore, the increase in active $GABA_C$ receptors produced by $[Ca^{2+}]_o$ may be explained by an increase in the opening frequency, as proposed for neuronal nicotinic ACh receptors.

Differentiation of Ca^{2+}-binding Site from Zn^{2+}-binding Site

In the present experiments, I_{GABA} was modulated by divalent cations in two different ways. An apparent inhibition of I_{GABA} was observed with Zn^{2+}, Ni^{2+}, Cd^{2+}, and Co^{2+} while Ba^{2+} and Sr^{2+} mimicked the action of Ca^{2+}. We observed that the action of Zn^{2+} on the $[Ca^{2+}]_o$-dependent facilitation of $GABA_C$ receptors was noncompetitive, indicating that the masking action of Zn^{2+} on the $[Ca^{2+}]_o$-dependent facilitation of $GABA_C$ receptors is not due to occupation of the Ca^{2+}-binding site by Zn^{2+}. In $GABA_A$ receptors, there are also two distinct types of $GABA_A$ receptor modulation, the inhibitory actions of Zn^{2+} and other divalent cations (Kaneko and Tachibana, 1986*b*) and the facilitatory actions of lanthanides (Ma and Narahashi, 1993). Based on detailed studies, lanthanides are thought to exert their facilitatory action through a lanthanide-binding site different from the Zn^{2+}-binding site on $GABA_A$ receptors. Therefore, it is probable that $GABA_C$ receptors have two functionally contrasting divalent cation binding sites within their extracellular domain, as proposed for $GABA_A$ receptors.

Physiological Significance of $GABA_C$ Receptor Modulation by $[Ca^{2+}]_o$

The activity of $GABA_C$ receptors is augmented at a high $[Ca^{2+}]_o$, as shown in the present experiments, but inhibited in the presence of extracellular Zn^{2+} (Dong and Werblin, 1995). In light of the potential physiological relevance, we have described herein the distribution and dynamics of Ca^{2+} and Zn^{2+} in the outer plexiform layer. It has been shown that a long-lasting increase in $[Ca^{2+}]_o$ occurs in the outer plexiform layer (~0.5 mM), when the feline retina is illuminated (Gallemore et al., 1994). In addition, in the tiger salamander retina, Zn^{2+} reportedly accumulates in photoreceptor terminals, strongly suggesting corelease of glutamate and Zn^{2+} (Wu et al., 1993). Since the reversal potential of the $GABA_C$ response of the intact horizontal cell is ~−30 mV (Takahashi et al., 1995*a*), GABA induces a depolarization of horizontal cells that makes a positive auto-feedback loop (Stockton and Slaughter, 1991; Kamermans and Werblin, 1992). To summarize, there are three events going on in the dark, an increase in GABA release, an increase in $[Zn^{2+}]_o$, and a decrease in $[Ca^{2+}]_o$. These events interact in such a way that the increase in $[Zn^{2+}]_o$ and the decrease in $[Ca^{2+}]_o$ both work to reduce the effect of GABA release on auto-receptors on horizontal cells.

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