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ACCELERATED COMMUNICATION

Noncompetitive Inhibition of γ-Aminobutyric Acid$_A$ Channels by Zn

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SUMMARY

The action of zinc on chloride currents evoked by γ-aminobutyric acid (GABA) was examined on cultured hippocampal neurons using whole cell voltage clamp and outside-out patch recording. Zn (5–30 μM) noncompetitively blocked responses evoked by GABA (0.5–100 μM), but did not affect either the time-to-peak or desensitization of the macroscopic current. In outside-out patches, Zn had no effect on the mean conductance or lifetime of the 19 or 30 pS openings of the GABA channel; however, the frequency of channel opening was markedly decreased in a voltage-independent manner. Zn inhibition of GABA responses appeared to be independent of the benzodiazepine binding site as Zn was effective in the presence of either diazepam or Ro15-1788, a competitive antagonist of benzodiazepine agonists and inverse agonists. In contrast to prior reports, Zn also inhibited GABA currents in a similar manner on cultured superior cervical ganglion neurons. These results suggest that Zn acts at an extracellular site on the GABA$_A$ receptor complex, which is distinct from either the GABA or benzodiazepine binding sites. The structural similarity of the Cys-Cys loop of the α and γ GABA$_A$ receptor subunits to some Zn-binding proteins suggests one possible region for a Zn binding site.

The GABA$_A$ receptor is a multisubunit complex which incorporates a chloride ionophore as well as binding sites for several classes of drugs which interact allosterically to either potentiate or inhibit receptor function (1, 2). Electrophysiological studies of neuronal GABA$_A$ receptors have quite similar features, although molecular studies have revealed a surprising degree of structural heterogeneity (3). Divalent cations have also recently been shown to modulate GABA$_A$ receptors in binding and electrophysiological assays (4–9), although more than one mechanism appears to be involved. For example, Cd, Co, or Ni noncompetitively antagonizes GABA receptors on turtle retinal cone cell (8), whereas increases in intracellular calcium have been reported to decrease GABA$_A$ receptor affinity (6). On hippocampal neurons, Zn potently inhibits GABA responses with an IC$_{50}$ of 11 μM (10). Studies of these interactions may be valuable in revealing structural homologies between ligand-gated ion channels, as Zn can also inhibit *N*-methyl-D-aspartate receptors, but not kainate/(α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, on central neurons (11, 12).

Previous studies of Zn modulation of GABA responses have given widely varying results. Zn inhibits GABA responses on lobster muscle (13) and spinal and hippocampal neurons (10, 12), but has been reported to have no effect on rat sympathetic neurons (13) while potentiating GABA responses on prepyriform neurons (14). Both competitive inhibition by Cd and Zn of GABA responses in frog dorsal root ganglion neurons (7) and noncompetitive block of GABA responses by Cd and Co on turtle retinal cone neurons have been reported (8). It is possible that these differences could reflect the structural heterogeneity of GABA$_A$ receptors.

To further explore the mechanism of Zn action on GABA$_A$ receptors, whole cell and single channel recordings were used on cultured hippocampal and superior cervical ganglion neurons. Our results suggest that Zn inhibits GABA receptors by binding to an extracellular site which is distinct from either the GABA recognition site or the benzodiazepine binding site.

Methods

Cell culture. Cultures of hippocampal neurons were prepared as previously described (15). Hippocampi from postnatal day 1 Sprague-Dawley rat pups were incubated in a low calcium/papain (5–20 U/ml, Worthington Biochemicals) solution for 1 hr, mechanically dissociated, and plated onto a confluent layer of hippocampal astrocytes. The culture medium contained minimum essential medium with 5% heat-inactivated horse serum and a supplement containing 200 μg/ml of transferrin, 200 μM putrescine, 60 nM sodium selenite, 40 nM proges-

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ABBREVIATIONS: GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

terone, 40 ng/ml of corticosterone, 20 ng/ml of triiodothyronine, 10 μg/ml of insulin, and 0.6% glucose. Superior cervical ganglia from newborn rats were digested using collagenase and dispase (2 mg/ml); neurons were dissociated mechanically and plated on poly-L-lysine-coated culture dishes. Culture medium was the same as for hippocampal neurons except that nerve growth factor (1 μ/ml, a gift from Dr. Felix Eckenstein, Oregon Health Sciences University) was added.

Electrophysiology and drug delivery. Outside-out and whole cell patch clamp recordings were performed as described by Hamill *et al.* (16). The extracellular solution contained (in mM): Na, 162; K, 2.4; Ca, 1.3; Cl, 167; HEPES, 10; pH adjusted to 7.3; osmolarity was adjusted to 325 mOsM with sucrose. Tetrodotoxin (500 nM) and strychnine (2 μM) were added to block spontaneous electrical activity and glycine channels, respectively. Patch pipettes were pulled from borosilicate glass (TWF 150, WPI), coated with Sylgard and fire-polished; DC resistance were 2–20 MΩ. Pipette solutions contained in (mM): Cs, 140; Ca, 1; Mg, 1; Cl, 126; Fl, 14; EGTA, 10; HEPES, 10; pH adjusted to 7.2 with CsOH; osmolarity was adjusted to 295 mOsM with sucrose. NaATP (2 mM) was added to the intracellular solution to reduce GABA current rundown (17).

Neurons were placed in a continuously perfused chamber (1–2 ml/min) at room temperature (≈20°C) on the microscope stage. Single channel currents in the outside-out patch configuration and macroscopic whole cell currents were recorded using an Axopatch 1B (Axon Instruments) with the current filtered at 10 kHz. An agar bridge connected the chamber to a side well containing the same solution as the patch electrode, thus neutralizing liquid junction potentials. Membrane current records were displayed on a chart recorder and stored on videotape for later analysis using an analog/digital converter (VR10, Instrutech Corp.). GABA (0.5–100 μM) and Zn (10–30 μM) were dissolved in the extracellular solution and applied via an array of flow pipes (300 μm i.d.) positioned within 100–200 μm of the cell. Diazepam and Ro15-1788 were dissolved as stock solutions in absolute ethanol and then diluted in extracellular medium with a final ethanol concentration of <0.1%. In experiments with diazepam and Ro15-1788, the control flow also contained the same concentration of ethanol. Each flow pipe was controlled by solenoid values; solutions were changed by simultaneously closing one valve and opening another. The solution exchange time constant was less than 20 msec as measured by change in membrane current evoked by kainic acid in two concentrations of sodium (18). Low concentrations of GABA (2 μM) were used during single channel recordings to minimize inactivation of the GABA receptors.

GABA or Zn concentrations were not corrected for the possible formation of inactive Zn-GABA complexes since no association constant for this complex is available. However, as noted by Smart and Constanti (13), the stability constant for biliganded glycine-zinc complexes ($K_1 \approx 10^{5.5}$ at 25°C) (19) should be similar to that for GABA. For a fixed concentration of Zn, complex formation would be expected to be most significant at low GABA concentrations, but should not affect maximal response at high GABA concentrations.

Single channel analysis. Single channel currents were replayed, filtered at 2 kHz (8 pole Bessel), digitized at 5 times the filter cutoff frequency, and analyzed on an IBM AT clone using pClamp software (v. 5.5). Opening and closing transitions were detected using a 50% threshold criterion. Events briefer than 200 μsec were deleted from the events list when the filter cutoff frequency was 2 kHz. Open time histograms were fitted with the sum of exponentials using a least-squares method. All patches contained multiple channel openings; therefore, we did not attempt a complete analysis of closed times. We also observed the marked rundown of GABA channel activity noted by prior investigators (17, 20). Application of 2 μM GABA to the outside-out patch initially evoked the opening of a large number of channels. This response declined rapidly and was followed by single openings with only a few overlapping events. Even in the presence of 2 mM ATP in the patch electrode, the frequency of channel opening declined with time, and usually disappeared after 2–5 min of continuous GABA application. Therefore, analysis was performed during the first 2 min of GABA application. Epochs of channel activity before and after Zn application were used to control for the effects of channel rundown.

Results are presented as mean ± standard deviation.

Results

The action of Zn on GABA-evoked responses was investigated on hippocampal neurons after 7–14 days in culture. Under whole cell voltage clamp at −60 mV, rapid application of GABA evoked an inward current in all neurons which remained stable during the 10–20 min of recording. The observed reversal potential was near 0 mV, consistent with the calculated chloride equilibrium potential. At concentrations above 2–5 μM GABA responses peaked rapidly then decayed to a steady state level after 10–20 sec, indicative of macroscopic desensitization (Fig. 1).

Zn inhibition of macroscopic GABA currents is noncompetitive. The action of Zn was initially tested when GABA-evoked currents had decayed to a steady state (Fig. 1A). Application of Zn reduced the current to a similar degree over a range of GABA concentrations from 2–100 μM (Fig. 1B). The potency of Zn inhibition was similar to that observed on cultured spinal cord neurons (10). Zn (30 μM) decreased the response by 60 ± 7% (n = 13) at 2 μM GABA, and by 49 ± 8%

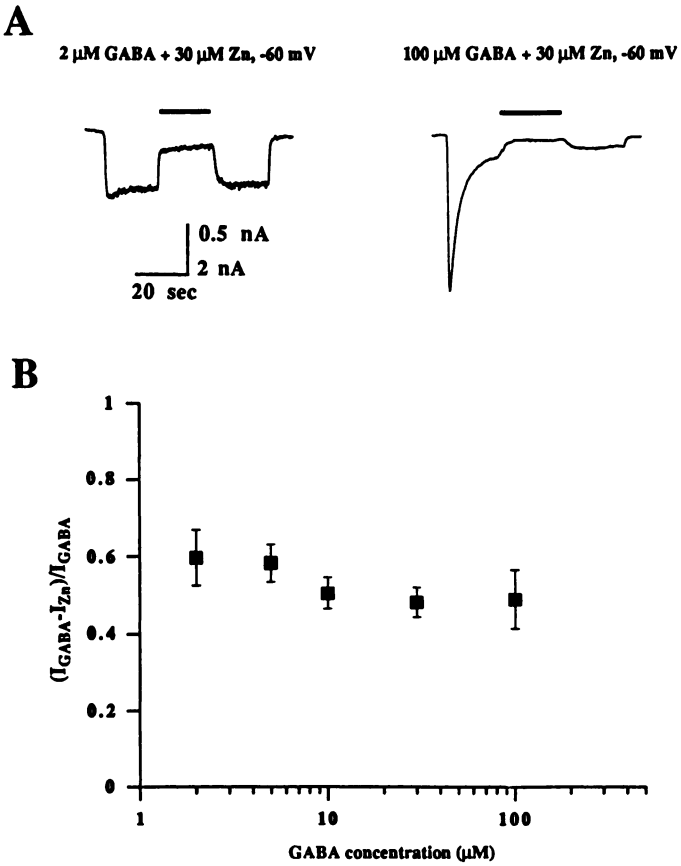

Fig. 1. Zn reduced the $GABA_A$ response in a noncompetitive manner. A, Zn inhibition of the $GABA_A$ responses evoked by 2 μM (*left*) and 100 μM (*right*) GABA (V_h = −50 mV). *Bar* indicates duration of Zn application. Desensitization was prominent for the response evoked by 100 μM GABA. B, Plot of the percent block by Zn as a function of the GABA concentration. Zn was applied during the plateau (steady state) phase of the response; inhibition was not strongly dependent on the GABA concentration (see text). Points represent the mean of 5–13 experiments.

($n = 5$) at 100 μM GABA. Both the onset of Zn inhibition and recovery were rapid, limited only by the speed of drug application. The inability to overcome Zn inhibition at high GABA concentrations is consistent with a noncompetitive mechanism. There appeared to be a slight decrease in Zn inhibition between 2 and 100 μM GABA, but this is likely to be due to Zn-GABA complex formation at low GABA concentrations. This is supported by the apparent increase in Zn inhibition of responses evoked by 0.5 μM GABA (77 ± 4%, $n = 4$). However, complex formation cannot explain Zn inhibition at higher concentrations of GABA (see "Methods").

Activation and desensitization. The onset of GABA current was unaffected by Zn. GABA or GABA + Zn were applied using a rapid flow pipe technique with solution exchange time of ≤20 msec which allowed adequate resolution of the onset and peak of the GABA response. As shown in Fig. 2A, the onset of the response in the presence and absence of Zn was superimposable. For this neuron the onset was well fitted in both cases with a single exponential ($\tau = 47$ msec). Similar values were obtained in 9 other neurons. As the activation time constant is dependent on the agonist concentration (27), the lack of a slower activation rate in the presence of Zn is additional evidence against complex formation as the primary explanation for the inhibition.

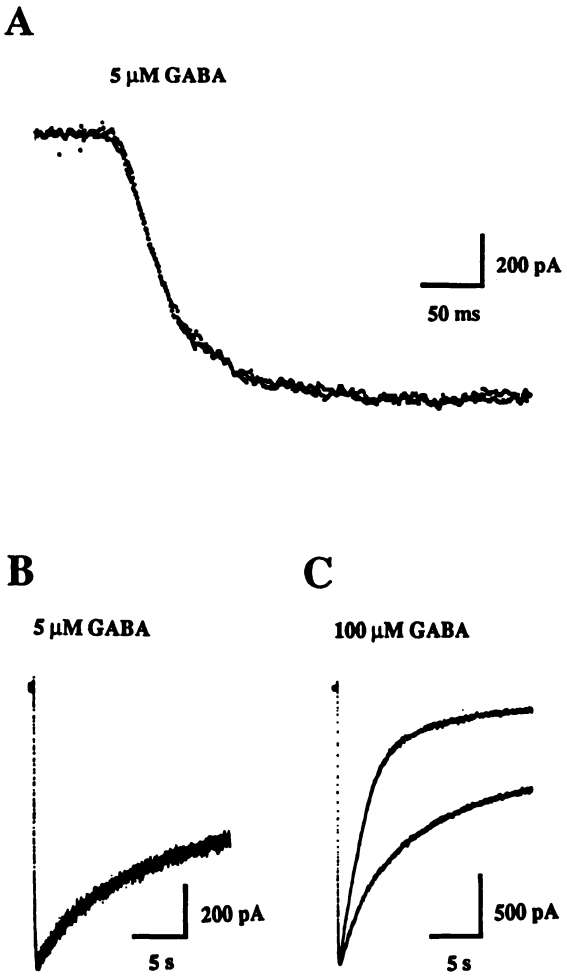

Fig. 2. The onset and slow desensitization were unaltered by Zn. A, Activation of the response evoked by GABA (5 μM) at a faster time base for the same responses shown in B. The peak responses were scaled to the peak of the control response. The activation rate was unaffected by Zn (see text). B, Coapplication of Zn (30 μM) had no effect on the decay time constant of the current during a 40-sec exposure to GABA (5 μM). Currents are superimposed for the first 20 sec of exposure and scaled to the peak of the control response. C, At high concentrations of GABA (100 μM) an additional fast component of desensitization was present. The proportion of fast desensitizing current increased in Zn, while the decay time constants were unaffected. Responses scaled to the peak of the control response. A holding potential of −20 mV was used to minimize chloride redistribution.

The decay of the GABA current during prolonged agonist exposure has been primarily attributed to receptor desensitization (21–24), although changes in the chloride equilibrium potential may also contribute in some cases to fading of the response (25, 26). Responses evoked by low concentrations of GABA (5 μM) slowly decayed to approximately 60% of the initial value with a time constant of 12.6 ± 0.9 sec ($n = 5$). Both the peak and steady state response were reduced to a similar degree by coapplication of Zn as illustrated by the scaled records in Fig. 2B. For 10 neurons the control steady state/peak current ratio was 0.60 ± 0.12 and 0.58 ± 0.08 in GABA + Zn. This suggests that the kinetics of the slow desensitizing state are unaffected by Zn.

The decay of GABA current was altered with agonist concentration. At high concentrations of GABA, a fast component of desensitization (τf) was present in addition to slow desensitization (τs) as described by Akaike *et al.* (27). The current decay at 100 μM GABA was fitted with 2 exponentials (τf = 2.0 ± 0.4 sec; τs = 10.1 ± 0.8 sec; $n = 6$). Coapplication of Zn with 100 μM GABA resulted in a decrease in the steady state current (Figs. 1 and 2C); but the steady state/peak ratio decreased from 0.24 ± 0.04 to 0.10 ± 0.05 ($n = 6$). When currents were scaled (Fig. 2C), this appeared to reflect enhanced desensitization; however, the decay time constants were unaffected by Zn (τf = 2.2 ± 0.4 sec; τs = 10.0 ± 1.5 sec; $n = 6$). The reduction in the peak current by Zn at high concentrations of GABA was less than at steady state (Zn/control = 0.16 ± .06, $n = 6$). Using the coefficients of the fast and slow exponentials at $t = 0$, the inhibition of the peak current was due to a decrease of the slow desensitizing component (0.54 ± 0.16) and an increase in the component of current which can rapidly desensitize (1.77 ± 0.62). This biphasic action accounts for the apparent enhanced desensitization seen in Fig. 2C and suggests that Zn has no direct effect on desensitization kinetics.

Zn reduces the frequency of GABA channel openings. To examine the mechanism of Zn action of GABA-activated channels, single channel currents were recorded in the outside-out configuration. Application of GABA evoked well resolved single channel currents in all neurons tested. As shown in Fig. 3 in a patch containing at least two channels, the most prominent effect of Zn was a marked decrease in the number of channel openings. The inhibition was similar at holding potentials of +60 and −60 mV and was similar in magnitude to the inhibition of the macroscopic current as calculated from the probability density of point-by-point amplitude histograms (not shown).

In all patches tested, GABA-activated channels showed at least three conductance levels: 19, 30, and 45 pS. The 30 pS conductance level was most frequently seen as in prior reports (28, 29). The extrapolated reversal potentials of all three levels was near 0 mV consistent with the calculated chloride equilibrium of −4 mV in our experiments. Zn reduced the number of 19 and 30 pS openings (Fig. 4A), but had no effect on the single

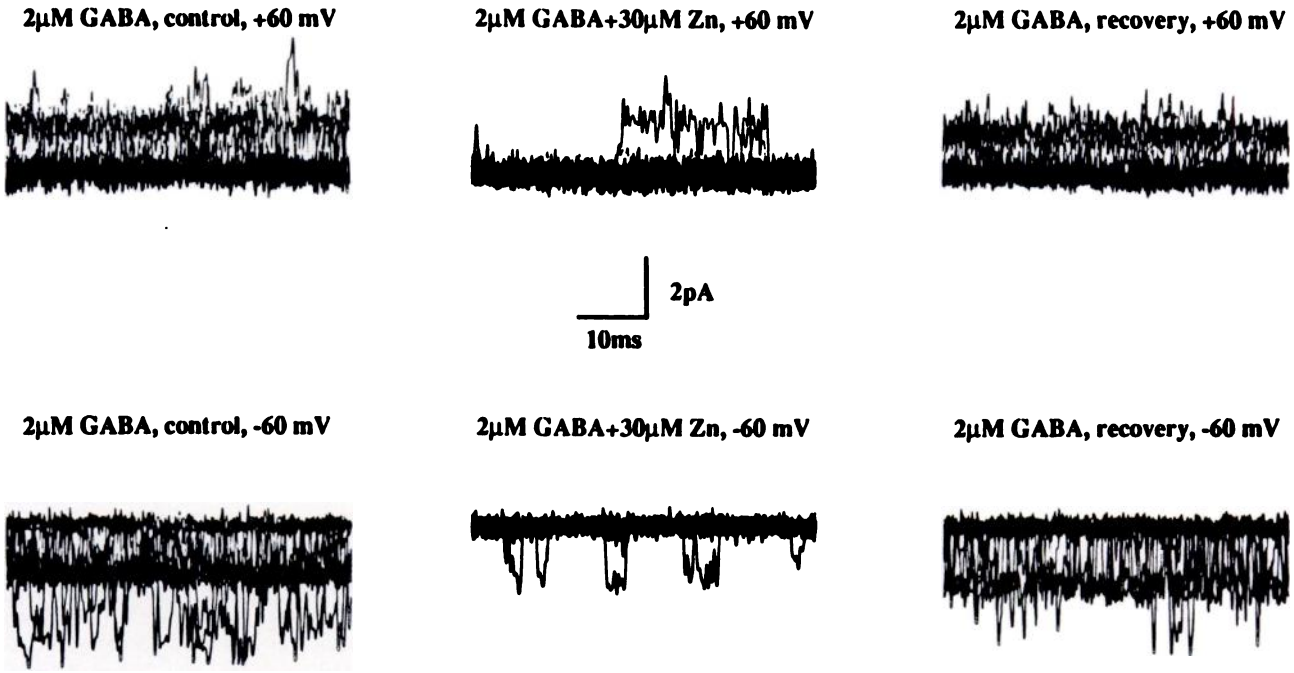

Fig. 3. Zinc reduced the GABA-induced channel activity in a voltage-independent manner. *Left* and *right* panels, Single channel activity evoked by 2 μM GABA was recorded at a holding potential of −60 and +60 mV. Channel openings were markedly reduced during application of Zn (*middle panel*). For each trace 16 continuous 50-msec epochs are superimposed. Data at −60 mV and +60 mV were recorded from two different patches. Both patches contained at least two channels.

channel conductance of these substates (Fig. 4B). The 45 pS level was only rarely observed, and thus the effect of Zn on this level could not be assessed.

The analysis of the gating properties of GABA-activated channels was complicated by the multiple conductance levels as well as transitions between levels (28–30). Thus, for open time analysis, thresholds were set to exclude the low conductance level. In the presence of 2 μM GABA, open time histograms could be fitted with the sum of three exponentials (Fig. 4C). Mean values were $\tau 1 = 0.4 \pm 0.3$ msec, $\tau 2 = 2.0 \pm 0.8$ msec, and $\tau 3 = 10.1 \pm 2.0$ msec ($V_m = -60$ mV, $n = 5$), and remained stable during the period of recording. These values are quite similar to those observed in cultured spinal neurons (29). Zn had no effect on the mean open times of the 30 pS level. In the presence of 10 μM Zn, $\tau 1 = 0.4 \pm 0.2$ msec, $\tau 2 = 2.2 \pm 0.5$ msec, and $\tau 3 = 9.8 \pm 0.4$ msec ($n = 5$). The relative exponential areas were also unaffected by Zn. In 2 μM GABA the areas were 0.44 ± 0.10, 0.43 ± 0.10, and 0.13 ± 0.11 ($n = 5$) compared to 0.43 ± 0.02, 0.43 ± 0.04, and 0.14 ± 0.02 ($n = 3$) in 10 μM Zn. The longest open time disappeared in the presence of 30 μM Zn probably because of the large decrease of the number of events and the small proportion of long openings. However, $\tau 1$ (0.3 ± 0.2 msec) and $\tau 2$ (2.1 ± 0.6 msec) were unaffected.

Does Zn act as an inverse agonist at the benzodiazepine binding site? Diazepam and β-carbolines bind to the benzodiazepine site on the $GABA_A$ receptor and either increase or decrease GABA-activated chloride currents (31). This has led to the nomenclature of benzodiazepine agonists and inverse agonists. Ro15-1788 is a competitive benzodiazepine antagonist which is equipotent in blocking potentiation by diazepam and inhibition by the β-carbolines (32, 33). Single channel studies have confirmed that the primary effect of the benzodiazepine agonists and inverse agonists is on the frequency of channel opening (34, 35). As Zn and inverse benzodiazepine agonists reduce single GABA channel activity, it was important to determine whether Zn inhibits channel activity via the benzodiazepine binding site.

As shown for one neuron in Fig. 5A, diazepam (1 μM) potentiated the inward current evoked by flow pipe application of 2 μM GABA. The enhancement of the GABA response by 1 μM diazepam (136 ± 26%; $n = 6$) was almost completely eliminated by Ro15-1788 (1 μM, 27 ± 12%, $n = 6$). However, Zn was equally effective in blocking GABA responses in the absence or presence of diazepam. For 30 μM Zn, the GABA response was reduced by 56 ± 3% compared to 59 ± 8% in the presence of 1 μM diazepam. GABA responses were also potently inhibited by Zn in the presence of Ro15-1788 (48 ± 4%; $n = 6$), although there was a consistent slowing of the activation rate of the response. The results for 6 neurons are summarized in Fig. 5B and indicate that effects of Zn are independent of drugs acting on the benzodiazepine binding site.

GABA responses on sympathetic neurons are also blocked by Zn. Previous data had suggested that Zn does not antagonize GABA responses on rat sympathetic neurons (13) while molecular cloning has demonstrated a high degree of structural heterogeneity in $GABA_A$ subunits (3). This suggested the possibility that $GABA_A$ receptors on sympathetic neurons had no Zn binding site. To address this issue, we tested the effect of zinc on GABA responses using cultured superior cervical ganglion neurons. As shown in Fig. 6A, zinc antagonized GABA-induced current on superior cervical ganglion neurons with fast on/off kinetics similar to that observed on cultured hippocampal neurons (10, 12). The degree of inhibition by 30 μM Zn (54 ± 6%, $n = 8$) was also indistinguishable from cultured hippocampal neurons (see Fig. 1). As shown in Fig. 6B, Zn did not alter outward rectification of the GABA current. Zn block was voltage-independent at holding potentials between −70 and +70 mV (Fig. 6, B and C).

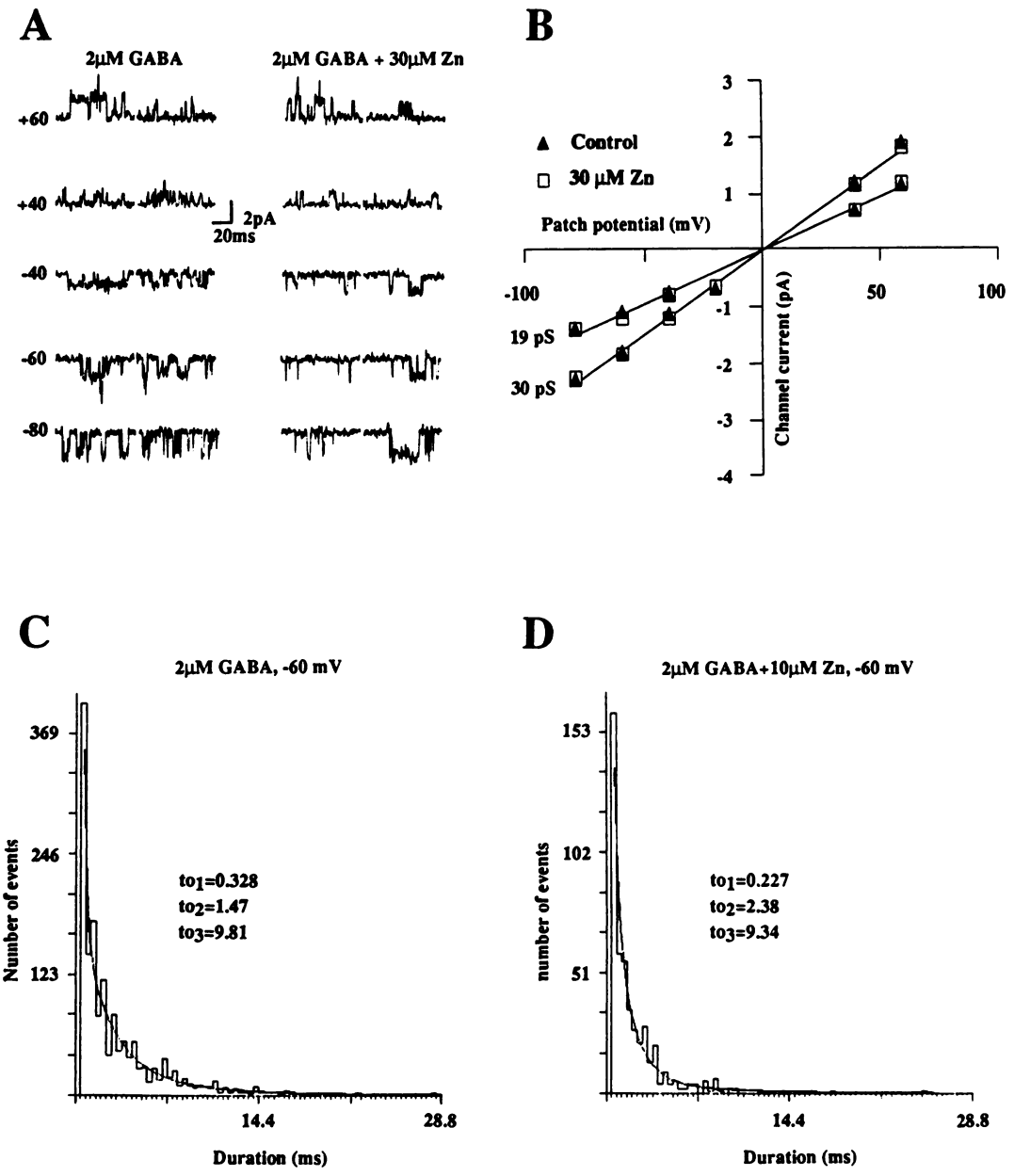

Fig. 4. Zn effect on $GABA_A$ channel kinetics. A, Single GABA-activated channel openings recorded at different holding potentials in the presence of GABA (*left*) and in GABA + Zn (*right*). B, Current-voltage plot demonstrates that the single channel conductance of the 30 and 19 pS levels was unaffected by Zn. Mean conductances were measured from the slope of the regression lines. *C* and *D*, Open-time histograms of the 30 pS conductance events exposed to GABA (*C*) and to GABA + Zn (*D*). Both histograms were fitted with the sum of three exponentials. There was no significant difference in the open durations in the presence of Zn (see text). The bin width of the two histograms is 0.4 msec.

Discussion

Our results demonstrate that Zn is a noncompetitive inhibitor of $GABA_A$ receptors on both cultured hippocampal and superior cervical ganglion neurons. The inhibition can be explained by a reduction in the frequency of channel opening. As the inhibition is not voltage-sensitive and independent of drugs acting on the benzodiazepine binding site, Zn appears to bind to a distinct site on the extracellular domain of the receptor complex. Whether physiological release of Zn from excitatory nerve terminals in the central nervous system (36, 37) may act on this site is still unclear.

Divalent effects on GABA channels. Divalent cations have multiple sites of action on ion channels. Several of these can be easily excluded in considering the action of Zn on GABA channels. It is unlikely that Zn acts as an open channel blocker for an anion channel where multiple positively charged residues surround the probable entry into the pore (38). Likewise the voltage insensitivity of Zn inhibition contrasts with the cationic open channel blockers which are more effective at hyperpolarized membrane potentials. Zn has been shown to affect the resting chloride conductance in skeletal muscle (39, 40); this effect occurred only at high Zn concentrations and was not reproduced by Ni or Co (39). The noncompetitive inhibition by Zn seen in our experiments may be similar to the action of Ni and Co on GABA responses in turtle retinal cones (8), but contrasts with the competitive inhibition by high concentrations of Zn on GABA responses seen on frog dorsal root ganglion neurons (9). Although we cannot definitely explain these differences, the formation of Zn-GABA complexes might result in apparent competitive inhibition (13). On the other hand, complex formation cannot explain the noncompetitive inhibition seen on cultured hippocampal neurons. The effects of Zn were not produced by millimolar concentrations of other endogenous divalent cations such as Ca and Mg, making it

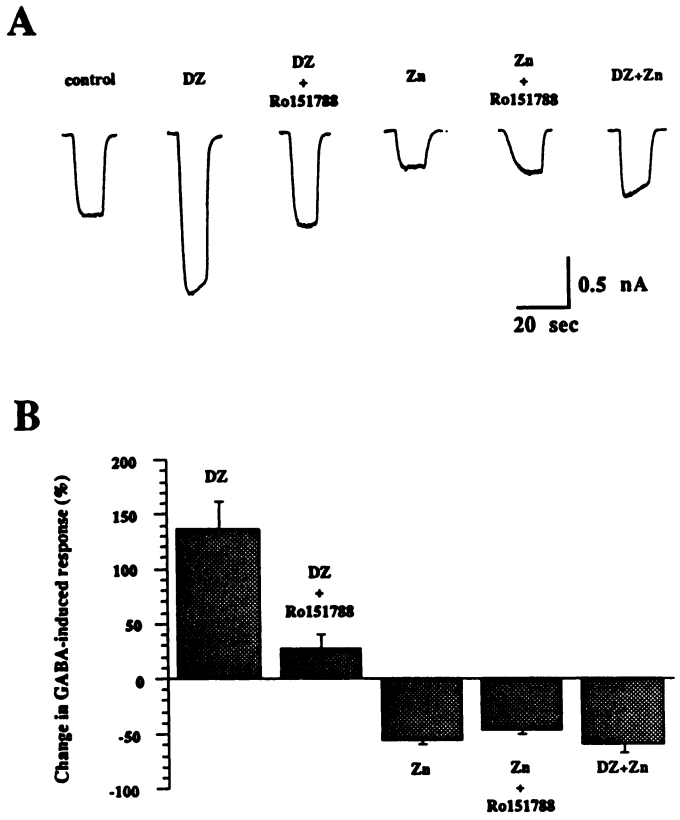

Fig. 5. Zn binding is independent of benzodiazepine action. A, Inward currents were evoked on a cultured hippocampal neuron (V_h = −60 mV) by 2 μM GABA and in the presence of 1 μM diazepam (DZ), 1 μM diazepam + 1 μM Ro15-1788, 30 μM Zn + 1 μM Ro15-1788, and 1 μM diazepam + 30 μM Zn. B, Summary of data for 6 neurons expressed as percent control response to GABA. Zn + diazepam response was calculated as percent inhibition of the response evoked by GABA + diazepam. Zn produced a similar degree of inhibition in the presence of either diazepam or Ro15-1788, although Zn was slightly less effective in the presence of Ro15-1788 ($p < .02$). Application of Ro15-1788 alone (1 μM) had no effect on the GABA response (not shown, n = 2).

unlikely that screening effects near the channel are responsible for the inhibition. Overall the evidence is most consistent with a direct action of Zn on the receptor/channel complex.

Mechanism of Zn action. The effect of Zn on single GABA-activated channels under steady state conditions was mainly characterized by a decrease in the opening frequency. Zn (10–30 μM) did not alter the microscopic conductances, the mean open times, or the relative proportion of the short and long events. This pattern of inhibition most closely resembles the action of the β-carbolines on GABA-activated channels; however, our results demonstrate that Zn and benzodiazepines act independently. In general terms Zn must act on kinetic steps leading up to channel opening. The onset of Zn action appears to be more rapid than channel opening kinetics, as even with fast applications of GABA the peak current was reduced. It has been suggested that brief channel openings represented the monoliganded state (22, 29); if true, this suggests that Zn affects the opening frequency of both mono-liganded and doubly liganded state. This could occur either as a true decrease in opening probability or as enhanced desensitization.

Although we saw no effect on macroscopic slow desensitization, Zn could promote direct transitions from the closed state to a new desensitized state. At low GABA concentrations,

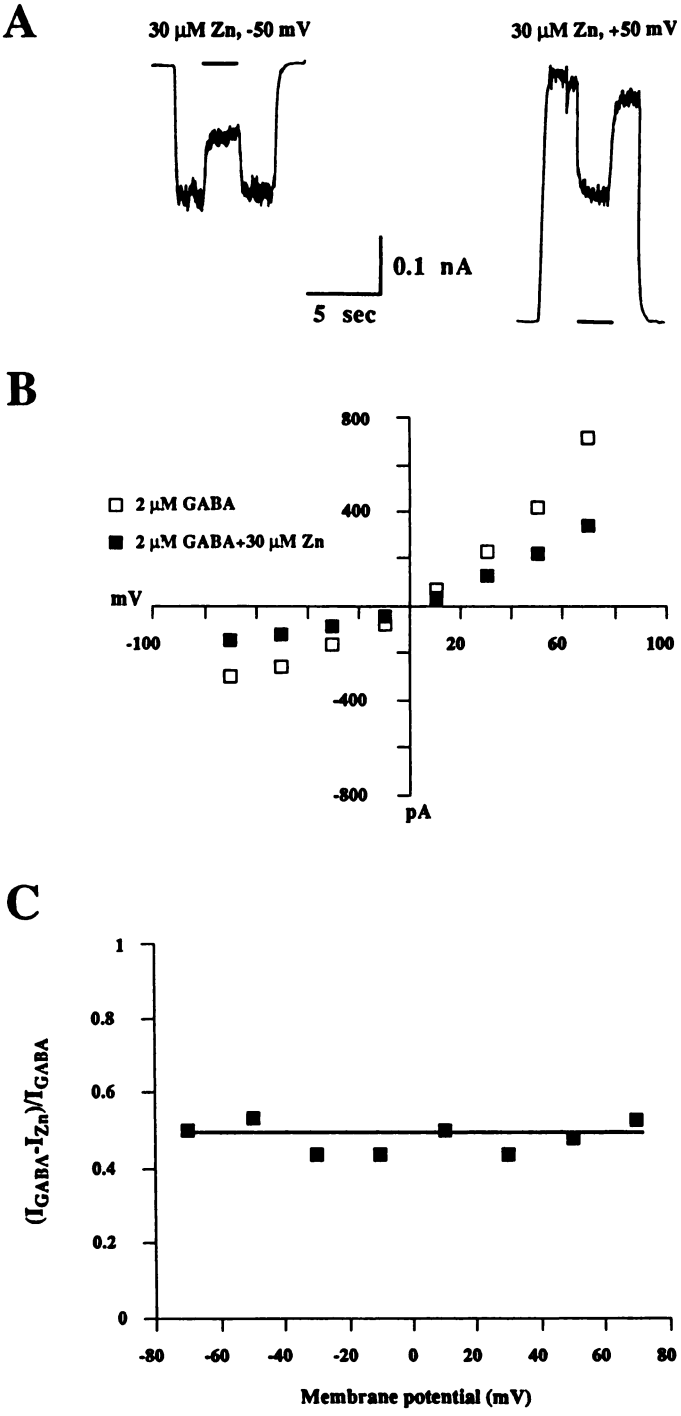

Fig. 6. Zn also inhibited GABA currents on sympathetic neurons. A, Whole cell recording from a cultured superior cervical ganglion neuron at holding potentials of −50 mV and +50 mV. Zn inhibited both the inward and outward GABA-activated chloride current. B, Current-voltage plot for responses to GABA and GABA + Zn from holding potentials of −70 mV to +70 mV. C, Data from the same cell as B plotted as percent inhibition shows voltage-independent action of Zn. Line fitted by eye.

control responses decayed to approximately 0.6 of the peak current even in the presence of Zn, consistent with the approximately equal on and off rates for slow desensitization previously observed (27). The effects of Zn on the fast desensitization seen at high GABA concentrations are more complex. Since recovery from fast desensitization is apparently slow (90 sec)

(27), this component of the current is nearly completely desensitized at steady state. This is supported by the fact that the steady state current in our experiments did not increase at concentrations above 5 μM GABA (27). Thus, Zn inhibition reflects an action on receptors which can slowly desensitize. It has been suggested that the fast desensitizing current reflects a different receptor-channel complex (27); our results at least suggest a different action of Zn on these channels. This may be resolved as more detailed kinetic models are developed (41).

Possible sites of Zn binding on the GABA receptor. Studies of Zn-dependent enzymes serve as a guide to the amino acid residues that might be involved in Zn binding, in particular cysteine and histidine residues. Several possible locations of the binding site of Zn can be envisaged. The rapid reversibility of the Zn inhibition of GABA channels makes a reaction with –SH group on cysteine residues unlikely, although such effects have been observed for some receptors (42). On the frog muscle chloride channel and on the lobster muscle GABA receptor, histidine residues were proposed as zinc binding sites, partly based on pH sensitivity (13, 39). Previous structural data on the bovine brain $GABA_A$ receptor indicate the presence of 6 and 3 histidine residues in the postulated extracellular N-terminal sequence of the α1 subunit and the β1 subunit, respectively (38). The recent sequencing of the γ2 subunit of the $GABA_A$ receptor revealed a "Cys-loop" in the N-terminal region with histidine residues included (43) which is also observed on the α1 but not β1 subunit. These loops resemble the C2H2 structure of some "Zn finger" DNA-binding proteins (44). Homomeric receptors expressed from α1, β1, or γ2 clones generate GABA-induced chloride currents; however, co-expression of γ2 with α1 and β1 appears to be required for benzodiazepine action (43, 45). If a histidine-containing structural loop is involved in Zn binding, then homomeric β1 receptors might be expected to be Zn insensitive.

Acknowledgments

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