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VOLTAGE-DEPENDENT BLOCK BY ZINC OF SINGLE CALCIUM CHANNELS IN MOUSE MYOTUBES

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SUMMARY

1. The blocking actions of Zn^{2+} on currents carried by Ba^{2+} through single dihydropyridine-sensitive Ca^{2+} channels were recorded from cell-attached patches on myotubes from the mouse C2 cell line.

2. Adding 100 μM-Zn^{2+} to the patch electrode containing 110 mM-$BaCl_2$ produced an increase in the open channel noise, presumably arising from unresolved blocking and unblocking of the open channel by Zn^{2+}. Adding between 200 and 1000 μM-Zn^{2+} to the electrode reduced the amplitude of the unitary current in a concentration-dependent manner.

3. The single-channel current–voltage (i–V) relations showed that Zn^{2+} reduced the amplitude of the unitary Ba^{2+} currents at all potentials more negative than 0 mV. A plot of the amplitude of the unitary current in the presence of Zn^{2+}, normalized to the amplitude in its absence, showed that block of the current depended on voltage, decreasing as the patch potential was made more negative.

4. The normalized amplitudes of the unitary currents were plotted as a function of the logarithm of $[Zn^{2+}]$ in the electrode. The relation for currents recorded at different potentials were fitted to an expression for binding to a single site with a K_D at 0 mV of $\sim$500 μM. The K_D changed $\sim$ e-fold per 83 mV with hyperpolarization. The results suggest Zn^{2+} binds to a site located at $\sim$ 15% of the potential drop from the surface membrane.

5. Reducing the concentration of Ba^{2+} in the patch electrode enhanced the steady-state block of unitary currents by Zn^{2+}. The inverse of the unitary current was plotted as a function of $[Ba^{2+}]_o$ in the presence and absence of Zn^{2+}; both were linear and intersected at the ordinate, indicating Ba^{2+} and Zn^{2+} compete for a channel site.

6. The kinetics of Zn^{2+} block of unitary Ba^{2+} currents were studied by amplitude distribution analysis. As expected for a simple reaction between blocking ion and open channel, the blocking rate depended linearly on the concentration of Zn^{2+}, while the exit rate was independent of concentration. The second-order rate coefficient for Zn^{2+} entry in the presence of 110 mM-$BaCl_2$ at 0 mV was $\sim 2{\cdot}0 \times 10^7$ M^{-1} s^{-1}, while the exit rate was $\sim$ 16000 s^{-1}.

7. Both entry and exit rates increased as the membrane potential was made more negative. The entry rate increased $\sim$ e-fold per 66 mV, while the exit rate increased

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~e-fold per 41 mV. The steeper increase in the exit rate with voltage can account for the reduction of steady-state block with hyperpolarization.

8. The results support the idea that Zn^{2+} blocks current through Ca^{2+} channels by binding to a site within the pore. The results are discussed in terms of the molecular mechanisms governing the interaction of Zn^{2+} with the channel pore.

INTRODUCTION

The use of analogues of physiological ions which vary in size, shape or charge to probe the permeation pathway of ionic channels has helped our understanding of the mechanisms of selective ion transport. This approach has helped define the nature and location of the chemical groups within the pore that allow it to distinguish among various ions, as well as placing restrictions on the size and shape of the permeation pathway (Armstrong, 1975; Hille, 1975; Latorre & Miller, 1983). Probes of ion permeation mechanisms fall into two broad classes: those which enter the channel, but cannot pass through, and those which can traverse the channel, although do so with varying degrees of ease. Multivalent metal cations of the transition elements and lanthanides strongly inhibit currents through voltage-gated Ca^{2+} channels (Hagiwara & Takahashi, 1967; Hagiwara, 1975; Hagiwara & Byerly, 1981), but the mechanisms which allow some to pass through the channel while excluding others have only recently been investigated (Lansman, Hess & Tsien, 1986; Lansman, 1990).

Zn^{2+} is an important co-factor in many enzymatic reactions and is present at high concentrations in the central nervous system. Zn^{2+} reversibly inhibits the Ca^{2+} action potential of the barnacle muscle fibre (Hagiwara & Takahashi, 1967), but when present as the only divalent cation in the extracellular solution, supports full regenerative action potentials in snail neurons (Kawa, 1979). That Zn^{2+} both blocks Ca^{2+} channel current as well as carries charge through the channel suggests that its interaction with the channel pore may be similar to that of Ca^{2+}, which also blocks current through the channel carried by divalent or monovalent cations (Kostyuk, Mironov & Shuba, 1983; Almers & McCleskey, 1984; Hess & Tsien, 1984; Fukushima & Hagiwara, 1985; Lansman *et al.* 1986; Matsuda, 1986).

The physical and chemical properties of Zn^{2+}, however, differ fundamentally from those of Ca^{2+}. Zinc is much smaller, having an ionic radius of about 0·06 nm at a favoured co-ordination number of four, while Ca^{2+} has ionic radii of 0·106 and 0·112 nm at co-ordination numbers of 8 and 9, respectively (Shannon, 1976). Zn^{2+} also prefers to form complexes with nitrogen donor groups, unlike Ca^{2+} which binds with greater affinity to oxygen donors. In the light of these differences, it is interesting to consider the mechanisms of ion–pore interaction which allow these divalent cations to both permeate and block the channel.

We have analysed the mechanism of block produced by Zn^{2+} on currents carried by single dihydropyridine-sensitive Ca^{2+} channels. We find Zn^{2+} produces flickery block of the open channel in the presence of Ba^{2+} as the charge carrier in which transitions between the open and blocked states are too fast to resolve as discrete events. We have used the method of amplitude distribution analysis (Yellen, 1984) to obtain the elementary rates of Zn^{2+} entry into and exit from the pore. The results

suggest Zn^{2+} blocks by binding to a site closer to the surface membrane than the Ca^{2+} binding site. Both entry and exit rates increase with hyperpolarization; a more steeply voltage-dependent exit rate can account for the relief of steady-state block at negative membrane potentials.

METHODS

Preparation of cells

C2 cells (Yaffe & Saxel, 1977) were cultured following the procedure of Inestrosa, Miller, Silberstein, Ziskind-Conheim & Hall (1983). Most experiments were done on myotubes less than a week old (see Lansman, 1990 for details).

Electrophysiological methods

Unitary Ca^{2+} channel activity was recorded from cell-attached patches with the technique described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Patch electrodes were pulled from Boralex haematocrit pipettes (Rochester Scientific), the shanks coated with Sylgard (Dow Corning), and the tips polished with a heated filament. Patch electrodes had resistances of 2–4 MΩ when filled with 110 mM-$BaCl_2$ and immersed in an isotonic potassium aspartate bathing solution. All experiments were done at room temperature (~ 21–23 °C).

Current signals were recorded with a List EPC-7 patch clamp amplifier, filtered with an eight-pole Bessel filter (−3 dB at 2 kHz) and digitized at 10 kHz. Unitary Ca^{2+} channel currents evoked by test pulses were stored directly on a laboratory computer (PDP 11/73, Indec Systems, Sunnyvale, CA, USA). At the time of recording, analog capacity compensation was used. Before analysis, the remaining capacity and leakage current was removed by subtracting a template formed by averaging current records lacking channel openings from individual records with channel activity.

We used the dihydropyridine agonist (+)-202-791 (Sandoz) to increase the duration of channel openings so that open channel block could be detected more easily (Lansman *et al.* 1986; Lansman, 1989, 1990). Strong depolarizing pre-pulses were applied to the membrane to activate channels and unitary events were detected after repolarizing the membrane to the desired test potential (0 to −60 mV) as single-channel tail currents (cf. Hoshi & Smith, 1987; Lansman, 1990).

Solutions

The patch electrode-filling solution contained 110 mM-$BaCl_2$, 10 mM-glucose and 10 mM-HEPES. The pH was adjusted to 7·5 by adding tetraethylammonium hydroxide (TEA-OH). Zinc (Aldrich Chemical, > 99·9% purity) was added to the electrode filling solution directly as the chloride salt. Hydrolysis of Zn^{2+} is negligible at pH 7·5 and the divalent ion is the predominant species in solution (Smith & Martell, 1976).

The bathing solution contained 150 mM-potassium aspartate, 5 mM-$MgCl_2$, 10 mM-K-EGTA, 10 mM-HEPES and the pH was adjusted to 7·5 with TEA-OH. An isotonic K^+ bathing solution was used to zero the cell's resting potential so that the patch membrane potential would be identical to the command voltage applied to the amplifier (Hess, Lansman & Tsien, 1986). We tested this assumption by excising the patch at the end of the experiment and measuring the i-V relation. In some experiments, the i–V relation was shifted to more negative potentials after patch excision and the maximum shift indicated a voltage error of ~ 10 mV. Indicated patch potentials could be in error by this amount.

Analysis of channel blockade

The blocking actions of Zn^{2+} on unitary Ca^{2+} channel currents were analysed by assuming a simple two-state blocking model (cf. Armstrong, 1969; Neher & Steinbach, 1978; Lansman *et al.* 1986). Because the unitary current in the presence of Zn^{2+} showed flickery block in which the transitions between the open and blocked channel were unresolved, we analysed the distribution of current amplitudes following the method of FitzHugh (1983) and Yellen (1984). This method assumes that the excess noise in the open channel represents unresolved transitions between the open and blocked channel.

We measured the amplitude distribution of the open channel noise in recordings obtained with

Zn^{2+} in the electrode and compared it with a theoretical distribution obtained by specifying the transition rates of a two-state random process and the cut-off frequency of a single-pole filter. A two-state random process with transition rates α and β that is filtered with a single-pole filter having a cut-off frequency τ will give an amplitude distribution which can be described by a β-distribution with a probability density function

$$f(y) = y^{a-1}(1-y)^{b-1}/B(a, b),$$

where $a = \alpha\tau$, $b = \beta\tau$ and

$$B(a, b) = \int_0^1 y^{a-1}(1-y)^{b-1}dy,$$

as described in detail by Yellen (1984). If the blocking and unblocking rates are equal, the β-distribution should be a symmetrical Gaussian function. A larger blocking rate produces a distribution that is skewed toward the blocked level; a larger unblocking rate skews the distribution toward the open channel level.

We fitted amplitude distributions by choosing transition rates which, with the cut-off frequency of the filter, were used to generate a β-distribution. We then convolved a Gaussian function fitted to the closed channel noise with the numerically generated β-distribution. The rate constants were changed in increments of 5–10% to obtain the best fit by eye.

To test the fitting procedure, we simulated records of channel opening and closing by specifying transition rates of a stochastic two-state process, added Gaussian noise to the records and filtered at one-fifth the sampling rate. The amplitude distribution of the simulated open channel noise was then fitted to obtain the best fit by eye. The transition rates we obtained by analysing the simulated records were within $\sim \pm 5\%$ of the original rates specified for simulating the underlying idealized stochastic process. The indicated rates obtained from fitting real records could be in error by at least this amount. The amplitude distribution analysis does not distinguish between excess noise in the open channel due to unresolved blocking and unblocking transitions and that arising from other sources (see Ogden & Colquhoun, 1985 for discussion).

RESULTS

Steady-state block of unitary Ba^{2+} currents by Zn^{2+}

Low concentrations of Zn^{2+} (~ 100 μM) added to the electrode solution containing 110 mM-$BaCl_2$ increased the open channel noise. As the concentration of Zn^{2+} was raised, the amplitude of the unitary current decreased. We interpreted these changes as a concentration-dependent blockade by Zn^{2+} of the open channel currents carried by Ba^{2+}. The experiments that follow analysed steady-state block as the reduction in the amplitude of the unitary Ba^{2+} current produced by adding different concentrations of Zn^{2+} to the electrode.

Figure 1 shows the effects of increasing the concentration of Zn^{2+} in the patch electrode on the unitary currents carried by Ba^{2+}. The records show the effects of 200, 600 and 1000 μM-Zn^{2+}. Increasing the concentration of Zn^{2+} reduced the amplitude of the unitary current at all patch potentials. The single channel i–V relations are shown in Fig. 2*A*. The slope conductance in 110 mM-$BaCl_2$ was ~ 15 pS. As the concentration of Zn^{2+} in the electrode was raised, the slope of the single channel i–V relation was reduced in a concentration-dependent manner.

To determine whether the block of the single-channel current produced by Zn^{2+} depends on membrane potential, we plotted the amplitude of the unitary current in the presence of Zn^{2+}, normalized to the amplitude of the current in the absence of Zn^{2+}, as a function of the patch potential. When the normalized current is one, there is no inhibition of the unitary current; when it is zero, the unitary current is

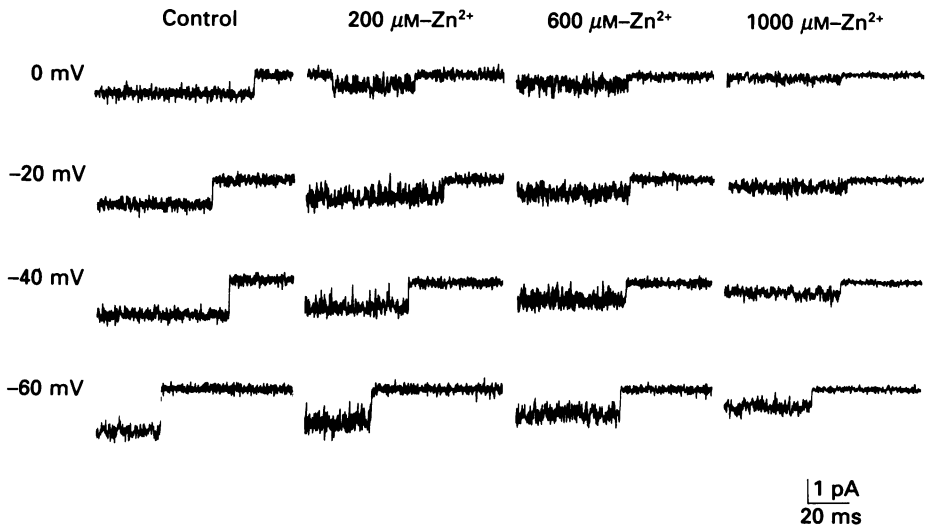

Fig. 1. Block of unitary Ba^{2+} currents by Zn^{2+}. The patch electrode contained 110 mM-$BaCl_2$ and the concentration of Zn^{2+} indicated at the top. Unitary currents were recorded at 0, −20, −40 and −60 mV following a strong depolarizing pre-pulse (+30 to +70 mV).

completely blocked. Figure 2*B* shows the normalized amplitude of the unitary current in the presence of three concentrations of Zn^{2+} plotted as a function of the patch potential. The points were fitted to a straight line with similar slopes at each concentration. Figure 2*B* shows that, at any given concentration of Zn^{2+}, making the membrane potential more negative reduces the block of the single-channel current.

The lessening of channel blockade with hyperpolarization suggests the blocking site lies within the membrane field. To quantify the voltage dependence of channel blockade, we plotted the unitary current in the presence of Zn^{2+} normalized to the current in the absence of Zn^{2+} as a function of the logarithm of the concentration of Zn^{2+} in the patch electrode as shown in Fig. 3*A* for currents recorded at 0, −40 and −60 mV.

The normalized currents at each potential were fitted with an expression which assumes binding to a single site (details in figure legend). The single site approximation is assumed for simplicity and we do not exclude the existence of multiple sites. At each membrane potential, the curve fitted the experimental results with dissociation constants of 502, 801 and 1067 μM at 0, −40 and −60 mV, respectively. Figure 3*A* shows that the dose–response curve is shifted along the concentration axis as the membrane potential was made more negative, indicating that the K_D depends on membrane potential.

The logarithm of the K_D measured at 0, −20, −40 and −60 mV is plotted as a function of voltage in Fig. 3*B*. The results suggest the voltage dependence of the steady-state block produced by Zn^{2+} can be described by a relation of the form

$$i/i_{max} = \{1 + [Zn^{2+}]/K_D \exp(-z\partial FV/RT)\}^{-1}, \quad (1)$$

in which the K_D is a function of voltage (Woodhull, 1973) and where z is the valency

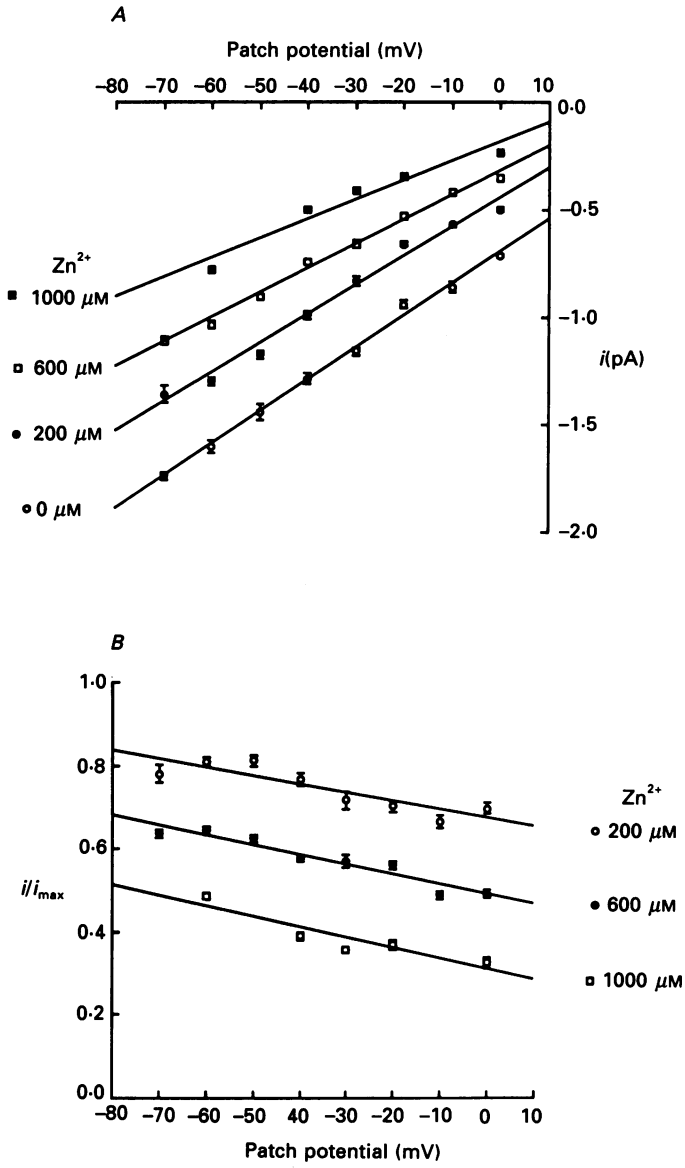

Fig. 2. Voltage dependence of the block produced by Zn^{2+}. *A*, single channel *i–V* relation for currents carried by 110 mM-Ba^{2+} in the absence of Zn^{2+} and in the presence of 200, 600 and 1000 μM-Zn^{2+}. The unitary current in the presence of Zn^{2+} was measured as the amplitude of the current from the closed channel level to a cursor positioned midway in the open channel noise. *B*, the amplitude of the unitary current in the presence of the indicated concentration of Zn^{2+} normalized to the amplitude of the control current in the absence of Zn^{2+} plotted as a function of the patch potential. The control current was taken as the mean of recordings from six different patches.

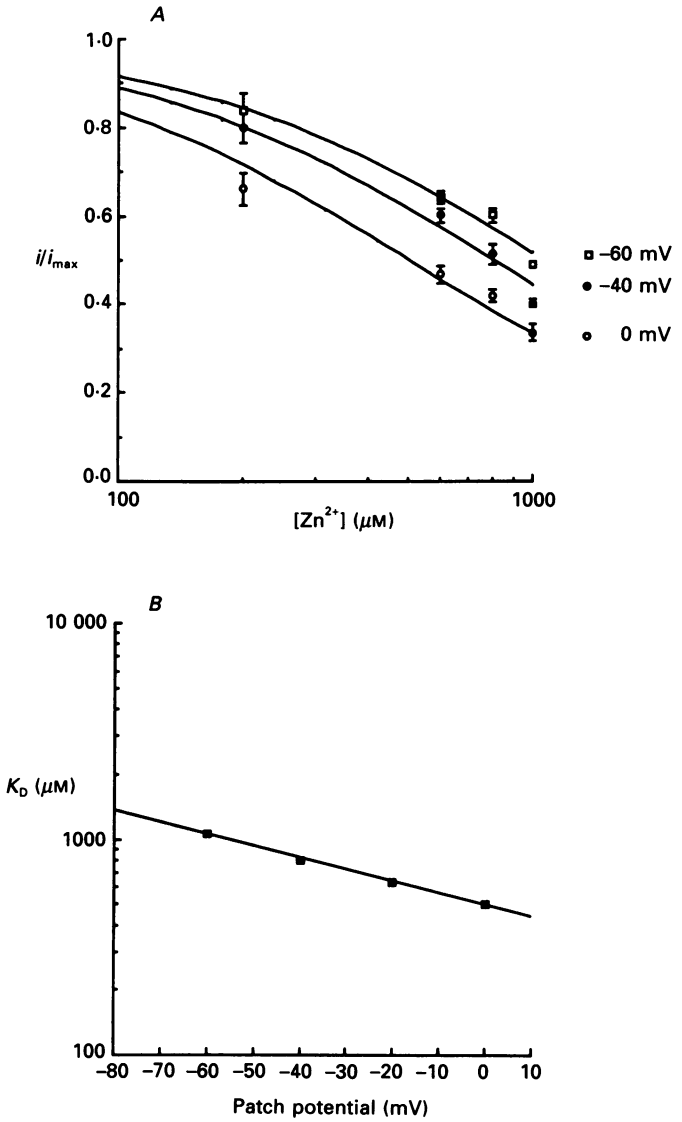

Fig. 3. *A*, dose dependence of the block of Ba^{2+} currents by Zn^{2+}. The normalized currents obtained at 0, −40 and −60 mV are plotted as a function of the logarithm of the Zn^{2+} concentration in the electrode. The smooth curve was drawn to an equation for binding to a single site of the form $i/i_{max} = 1/1 + [Zn^{2+}]/K_D$. *B*, the logarithm of the K_D plotted as a function of the patch potential. The experimental points are fitted with a straight line $K_D = 503 \times 10^{-0.0054V}$ ($r^2 = 0.93$).

of the blocking particle, δ is the effective electrical distance to the blocking site, and F, R and T have their usual meanings. The fit to this relation suggests the blocking site is located at ~ 15% of the potential drop from the membrane surface.

Because the chemical properties of Zn^{2+} are different from Ba^{2+}, the permeant ion used in the single-channel recordings, we asked whether the ions compete for a site

within the channel pore. To answer this question we determined the dependence of the single-channel current on the concentration of Ba^{2+} in the pipette in the presence and absence of a fixed concentration (600 μM) of Zn^{2+}. Figure 4 shows the results of this experiment.

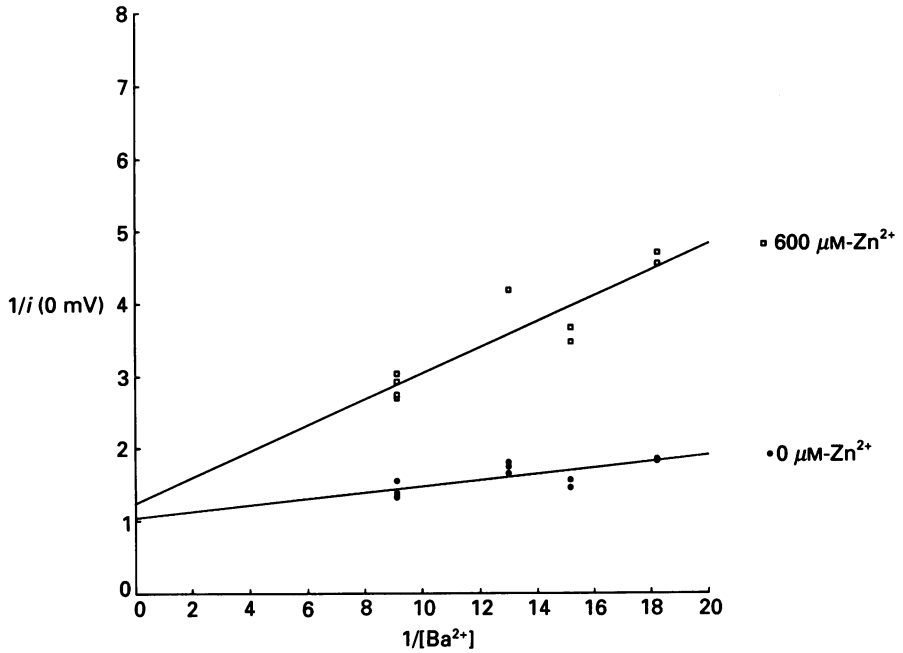

Fig. 4. Competition between Ba^{2+} and Zn^{2+}. Plot of $1/[Ba^{2+}]_o$ *versus* the inverse of unitary current at 0 mV in the absence (●) and presence of 600 μM-Zn^{2+} (□). The least-squares regression line in the absence of Zn^{2+} is $1/i = 1{\cdot}04 + 0{\cdot}043x$ ($r^2 = 0{\cdot}83$) with the intercept at the abscissa ($-1/K_D$) giving an affinity constant for Ba^{2+} of ~ 24 mM. In the presence of 600 μM-Zn^{2+}, the regression line is $1/i = 1{\cdot}24 + 0{\cdot}179x$ ($r^2 = 0{\cdot}56$).

Assuming that the dependence of the unitary current on the permeant ion concentration can be described by a rectangular hyperbola of the form (Hagiwara & Takahasi, 1967; Hagiwara, 1975; Hess *et al.* 1986)

$$i = 1/(1 + K_{Ba}/[Ba^{2+}]_o, \quad (2)$$

with K_{Ba} the dissociation constant for Ba^{2+}, a plot of the reciprocal of the unitary current as a function of $[Ba^{2+}]_o$ should be linear. The results give $K_{Ba} = \sim 24$ mM (details in figure legend), close to the value of ~28 mM reported by Hess *et al.* (1986) from measurements of unitary currents of the larger conductance cardiac Ca^{2+} channel.

If Zn^{2+} competes with Ba^{2+} for a channel site, eqn (2) becomes

$$i^{-1} = 1 + [Zn^{2+}]/K_{Zn})K_{Ba}/[Ba^{2+}]_o. \quad (3)$$

Figure 4 shows the double reciprocal plots for the concentration dependence of the unitary current in the absence and presence of Zn^{2+}. The relations are linear and intersect the ordinate at roughly the same value, indicating that eqn (3) holds and the ions compete for a channel site, although the results do not specify the location

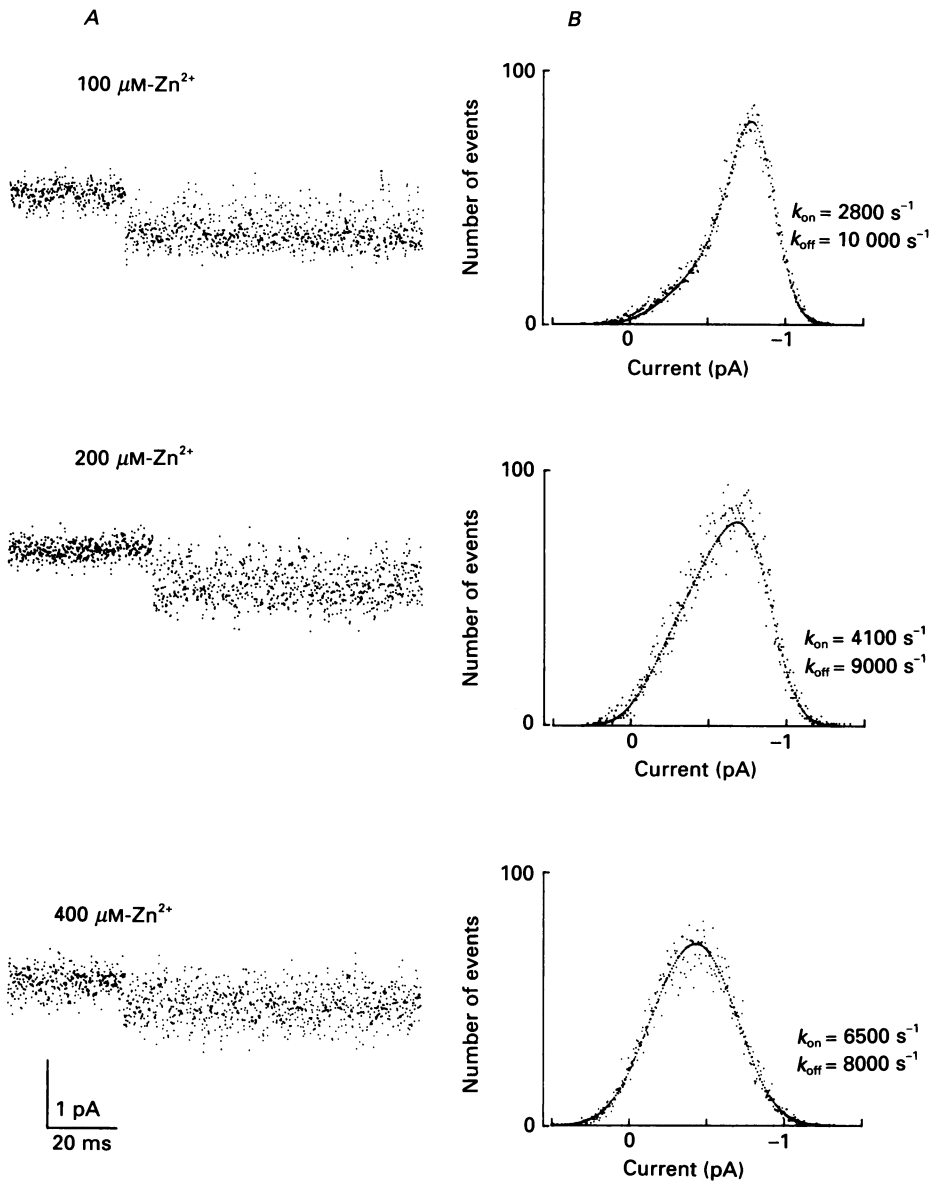

Fig. 5. Dependence of the kinetics of block on the concentration of Zn^{2+}. *A*, records of open channel current noise in the presence of 100, 200 and 400 μM-Zn^{2+} at 0 mV. *B*, amplitude distributions of open channel current. Dotted lines represent the fit to a β-distribution.

of the site or whether it is in the permeation pathway. This issue is taken up in the Discussion.

Kinetics

We could not resolve discrete blockages produced by Zn^{2+} because the transitions were faster than the bandwidth of our recording conditions. Because the unitary conductance of the skeletal muscle channel is small, we could not increase the

bandwidth without seriously reducing the signal-to-noise ratio. The limited bandwidth did not allow us to analyse the frequency composition of the excess variance in the open channel (cf. Ogden & Colquhoun, 1985; Quayle, Standen & Stanfield, 1988). Instead, we used the method of amplitude distribution analysis (Yellen, 1984) to obtain the rate constants for blocking and unblocking in the presence of Ba^{2+}.

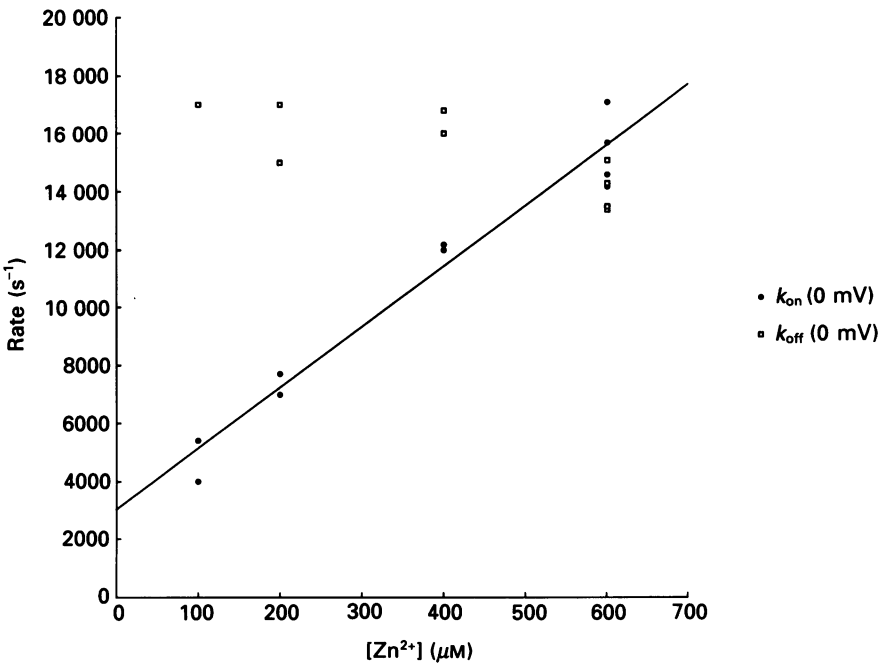

Fig. 6. Dependence of the entry (blocking) and exit (unblocking) rates obtained from the amplitude distribution analysis on the concentration of Zn^{2+} in the electrode. ●, entry rates; □, exit rates. The line drawn through the entry rates is the least-squares regression line, $y = 2990 + 21{\cdot}1x[Zn^{2+}]$ ($r^2 = 0{\cdot}96$), which gives a second-order rate coefficient of $\sim 2 \times 10^7$ M^{-1} s^{-1}.

We analysed the kinetics of the block produced by 100–600 μM-Zn^{2+}. In this range of concentrations, the blocking/unblocking transitions produced obvious excess open channel noise in the single-channel records. Figure 5*A* shows on an expanded time scale digitized records of the open and closed channel current noise for events in the presence of either 100 μM (top), 200 μM (middle) or 400 μM (bottom)-Zn^{2+}. With 100 μM-Zn^{2+} in the electrode, the separation of open and closed channel levels can be seen. At higher concentrations of Zn^{2+}, the peak-to-peak amplitude of the open channel noise increased with more points falling towards the closed channel level which are presumed to represent brief, unresolved blockages.

Figure 5*B* shows examples of the amplitude distribution for only the open channel noise obtained from segments of channel activity in which the channel was open for an extended period of time. As the concentration of Zn^{2+} was increased from 100 to 400 μM, the distribution of current amplitudes shifted towards the closed channel level, consistent with an increase in the rate of blocking as expected for a simple

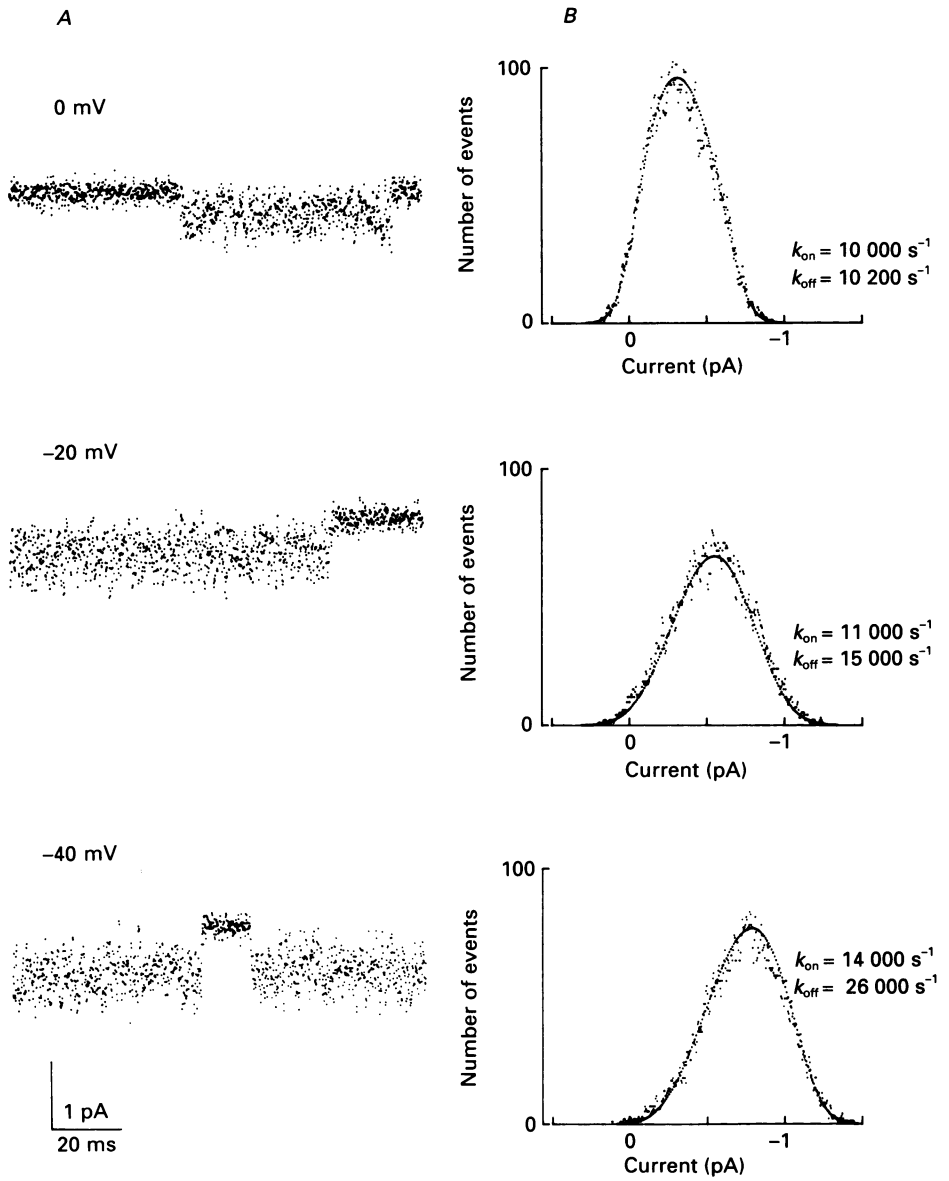

Fig. 7. Analysis of the voltage dependence of the entry and exit rates. Experiment in which the patch electrode contained 110 mM-$BaCl_2$ + 600 μM-Zn^{2+}. *A*, unitary currents recorded at 0, −20 and −40 mV. Records sampled at 10 kHz and filtered at 2 kHz. *B*, amplitude distributions formed from segments of open channel current like those shown in *A*. The dotted line is the theoretical β-distribution.

model of open channel block. The fit to a β-distribution is shown with the dotted line through the data points.

The predictions of the open channel block model are quantitatively tested in Fig. 6 in which the transition rates obtained from the fit of the amplitude histogram to a β-distribution are plotted as a function of the concentration of Zn^{2+} in the electrode. As expected for a simple reaction between the open channel and blocker, the entry

rate increased linearly with the concentration of blocker, while the exit rate did not depend strongly on concentration. The slope of the best-fit regression line to the entry rates gives a second-order rate coefficient of $2{\cdot}1 \times 10^7$ M^{-1} s^{-1}. The exit rates varied between extreme values of $\sim$ 14000–17000 s^{-1}. These values give a steady-state dissociation constant at 0 mV of between $\sim$600–700 μM compared with

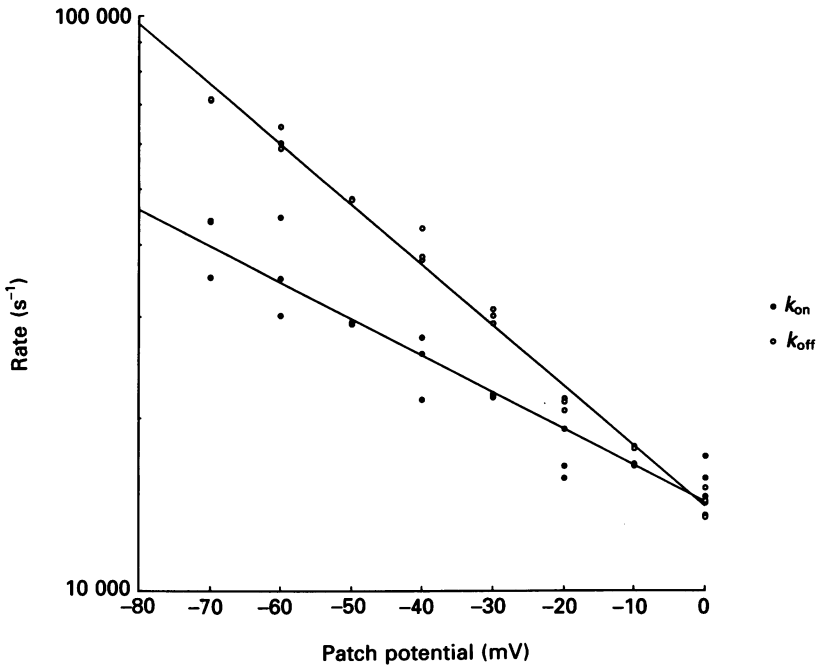

Fig. 8. Effect of patch potential on the entry and exit rates. Collected results from four patches in which the electrode contained 110 mM-$BaCl_2$ with 600 μM-Zn^{2+}. The entry rate is fitted to $k_{on} = 14110 \times \exp^{-0{\cdot}015V}(r^2 = 0{\cdot}92)$; the exit rate is fitted to $k_{off} = 14051 \times \exp^{-0{\cdot}024V}(r^2 = 0{\cdot}99)$.

$\sim$ 500 μM obtained from the fit to the reduction of the unitary current in the presence of different Zn^{2+} concentrations (Fig. 3*A*), indicating reasonable agreement between the two methods.

The steady-state voltage dependence of block indicated that hyperpolarizing the membrane reduces the amount of block. Previous studies have shown that hyperpolarizing relief of block by Cd^{2+} and La^{3+} results entirely from a strongly voltage-dependent increase in the rate of exit from the channel (Lansman *et al.* 1986; Lansman, 1989). We obtained the individual rate constants for Zn^{2+} blocking and unblocking at different patch potentials to determine whether a similar mechanism can account for the relief of steady-state block at negative membrane potentials (Fig. 2*B*).

Figure 7*A* shows records of the block produced by Zn^{2+} at 0, -20 and -40 mV obtained from a single experiment in which the patch electrode contained 110 mM-$BaCl_2$ + 600 μM Zn^{2+}. The corresponding amplitude distributions are shown in Fig. 7*B*. At 0 mV the symmetrical amplitude distribution indicates transition rates are similar, the expected result because the K_D is close to 600 μM. As the membrane

potential was made more negative (Fig. 7*B*, bottom left, −40 mV), the amplitude distribution became more skewed toward the open channel level, indicating an increase in the exit rate relative to the entry rate.

The dependence of the entry and exit rates on membrane potential obtained from an analysis of amplitude distribution from several experiments in which the patch electrode contained 600 μM-Zn^{2+} is shown in Fig. 8. The entry and exit rates for individual experiments are plotted on a semilogarithmic scale. Both entry and exit rates increased with hyperpolarization. The voltage dependence of the rate constants was fitted to an exponential function. The results show the entry rate increased ~e-fold per 66 mV, while the exit rate increased more steeply, ~e-fold per 41 mV. That the exit rate increased more steeply with hyperpolarization is consistent with the results shown in Fig. 2*B* showing that steady-state block is reduced with hyperpolarization.

DISCUSSION

The main finding of this study is that block by Zn^{2+} of unitary Ba^{2+} currents carried by dihydropyridine-sensitive Ca^{2+} channels is voltage dependent. Hyperpolarizing the membrane was found to reduce steady-state block. The kinetic analysis showed that the rates of both entry and exit increase with hyperpolarization. A greater increase in exit rate with hyperpolarization can account for the reduction in steady-state block at negative membrane potentials.

The rate of Zn^{2+} exit from the Ca^{2+} channel pore is very rapid compared with other blockers: ~ 16000 s^{-1} compared with ~ 3000 s^{-1} for Mg^{2+}, ~ 1000 s^{-1} for Cd^{2+}, and ~ 300 s^{-1} for La^{3+} at 0 mV in the presence of 110 mM-$BaCl_2$ (Lansman *et al.* 1986; Lansman, 1989, 1990). Zinc falls within the category of fast channel blockers. It therefore differs from Cd^{2+} and the lanthanides which produce blockages which can be resolved as discrete excursions to the closed channel level (Lansman *et al.* 1986; Lansman, 1989, 1990). The short dwell time of Zn^{2+} within the pore may account for its ability to produce action potentials when present as the only divalent cation in the external solution (Kawa, 1979).

The rate of exit of Zn^{2+} from the channel increases with hyperpolarization ~ e-fold per 41 mV. This can be compared with the rates of exit of Ca^{2+}, Cd^{2+} or the lanthanides from the pore which increased somewhat more steeply with hyperpolarization, ~e-fold per 20–25 mV (Lansman *et al.* 1986; Lansman, 1990). The results suggest that Zn^{2+} is less effectively driven from its binding site to the cell interior by hyperpolarizing the membrane than Ca^{2+}.

The absolute rate of entry of Zn^{2+} into the channel (~ 2×10^7 M^{-1} s^{-1} at 0 mV) is somewhat slower than the rate of Ca^{2+} entry (~ 5×10^8 M^{-1} s^{-1}, Lansman *et al.* 1986). The slower rate of Zn^{2+} entry is consistent with its slower rate of dehydration (~ 5×10^7 M^{-1} s^{-1} compared with ~ 5×10^8 M^{-1} s^{-1} for Ca^{2+}; Diebler, Eigen, Ilgenfritz, Maas & Winkler, 1969; Hague, 1977). The results support the idea that entry rates may be explained in terms of an Eigen–Diebler mechanism in which water loss at the ion's inner hydration sphere is the rate limiting step for complex formation.

Unlike Ca^{2+} whose rate of entry into the channel is insensitive to membrane potential, entry of Zn^{2+} into the channel increases strongly with hyperpolarization. This difference may arise from the necessity of removing bound water molecules from

the inner co-ordination sphere before Zn^{2+} can interact with pore ligands. According to this view, entry of pore ligands into the inner co-ordination sphere may be required to catalyse the loss of bound water molecules and this process occurs within the membrane field (cf. Lansman *et al.* 1986). In this respect, Zn^{2+} resembles Mg^{2+} in having both a slow entry rate which is also voltage dependent and stands in contrast to Ca^{2+}, Cd^{2+} and the lanthanides which have diffusion-limited entry rates which are voltage insensitive (Lansman *et al.* 1986; Lansman, 1990).

Analysis of steady-state block indicated that Zn^{2+} binds to a site located at $\sim 15\%$ of the potential drop from the membrane surface. Fukushima & Hagiwara (1985) found that block by Ca^{2+} of monovalent currents through Ca^{2+} channels in lymphocytes occurred at a site located $\sim 60\%$ of the potential drop from the membrane surface. This difference could reflect the fact that blocking ions interact at different apparent electrical distances depending upon whether divalent or monovalent cations carry charge through the channel, particularly if more than one ion at a time occupies the pore (Hille & Schwarz, 1978; Almers & McCleskey, 1984; Hess & Tsien, 1984).

An alternative interpretation is that Zn^{2+} interacts with a more superficial site in the pore. Different binding sites within the pore may reflect the different complexation requirements of Ca^{2+} and Zn^{2+}. Calcium prefers oxygen donor groups and has relatively flexible steric requirements; Zn^{2+} complexes involve nitrogen donor groups and have a more rigid fourfold co-ordination geometry. This interpretation would have a complexation site for Zn^{2+} composed of nitrogen donor groups close to the external membrane surface. The Ca^{2+} co-ordination site with its carboxyl and carbonyl donor groups (cf. Lansman, 1990) would lie more deeply within the pore.

Physiological significance

Zinc is a co-factor for many enzymes and is present in the central nervous system at high concentrations in excitatory nerve terminals (Charton, Rovira, Ben-Ari & Leviel, 1985; Choi, Yokoyama & Koh, 1986) and is released during neuronal activity (Assaf & Chung, 1984; Howell, Welch & Frederickson, 1984; Charton *et al.* 1985). The physiological role of synaptically released Zn^{2+} is not well understood, although its postsynaptic actions include blocking *N*-methyl-D-aspartate (NMDA) receptor channels (Peters, Koh & Choi, 1987; Mayer, Vyklicky & Westbrook, 1989*a*, *b*) and modifying inhibitory GABA responses (Westbrook & Mayer, 1987). Less attention has focused on possible presynaptic actions of Zn^{2+} (Smart & Constanti, 1983) even though its ability to block Ca^{2+} action potentials has been known for some time (Hagiwara & Takahashi, 1967). The results presented here show that in the presence of 110 mM-$BaCl_2$, Zn^{2+} blocks current through dihydropyridine-sensitive Ca^{2+} channels at concentrations greater than $\sim 50\ \mu$M. In physiological solution containing several millimolar Ca^{2+}, Zn^{2+} would be expected to produce strong block of presynaptic Ca^{2+} current thereby inhibiting neurotransmitter release.

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