Zinc (Zn\(^{2+}\)) blocks voltage gated calcium channels in cultured rat dorsal root ganglion cells

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Dorsal root ganglion cells (DRGs) exhibit 3 types of voltage-dependent calcium channels. We have cultured DRGs from 2- to 4-day-old rat pups and obtained whole-cell patch-clamp recordings of calcium-channel currents after 1–5 days in culture. The calcium-channel currents (carried by barium) were recorded with tetrodotoxin (TTX) in the external solution. A cesium-based solution containing Na-ATP, HEPES and EGTA was used in the recording pipette. Cells were held at -80 mV and calcium channel currents were evoked by stepping to depolarized voltages. The divalent cation zinc (Zn\(^{2+}\)) blocked sustained and transient voltage sensitive calcium channel currents. Onset of the blockade was fast and a steady-state was reached within 5–15 min, depending upon the concentration used. The IC\(_{50}\) for inhibition of the peak current evoked by a step depolarization from -80 mV to 0 mV (N plus L channels) for 80 ms was 69 \(\mu\)M Zn\(^{2+}\) and the Hill slope about 1. The calcium current evoked by a voltage step from -80 mV to voltages between -40 mV and -15 mV (T-type current) was more sensitive (>80% block with 21 \(\mu\)M Zn\(^{2+}\)). During wash the effect was only partly reversible in 50% of the neurons. Thus, Zn\(^{2+}\) is a potent blocker of voltage dependent calcium currents in mammalian neurons, especially of T-type currents.

INTRODUCTION

Zinc is an essential element for all mammals, and zinc ions (Zn\(^{2+}\)) have a variety of actions on nerve cells. It has been reported that even small amounts of this heavy metal cation are toxic to neurons\(^{20}\) but also that high concentrations of Zn\(^{2+}\) are naturally attached to cell membranes, especially in the nervous system\(^{1}\). Zn\(^{2+}\) is a reversible blocker of calcium influx in synaptosomes\(^{14}\), and also alters the kinetics of sodium and potassium currents through nerve axons, probably by binding at a negatively charged site close to the gating apparatus\(^{8}\). Zn\(^{2+}\) blocks N-methyl-D-aspartate-operated channels in mammalian neurons\(^{12,17}\).

Tsiens et al.\(^{18}\) have shown that chick dorsal root ganglion (DRG) neurons express 3 different types of voltage gated calcium currents: a rapidly inactivating, low-threshold activated (T) current, a slowly inactivating high-threshold activated current (L) and a high-threshold rapidly inactivating current (N). Numerous studies have shown that other di- and trivalent cations (like Co\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\) and La\(^{3+}\)) are potent antagonists of voltage-dependent calcium currents on a variety of nerve cells\(^{2,11,13-15}\). Ni\(^{2+}\) is most effective in blocking the low-threshold (T) calcium channel current at low concentrations but affects other channels at higher concentrations\(^{7,10}\). In previous studies\(^{3,4}\) we have shown that Zn\(^{2+}\) is effective in blocking the voltage-activated calcium currents of invertebrate neurons. There has, however, been little study of the effect of Zn\(^{2+}\) on the voltage-activated calcium channels of mammals, despite the fact that, unlike many other divalent cations, Zn\(^{2+}\) is generally present and has a physiologic function in the nervous system. Therefore, we have used the rat DRG preparation in order to investigate the effects of Zn\(^{2+}\) on the 3 different types of voltage activated calcium channels.

MATERIALS AND METHODS

DRG neurons were cultured from 2- to 4-day-old rat pups as described previously by Wood et al.\(^{10}\). Records from these cells were taken between day 1 and 5 in culture, to avoid space clamping.
problems arising from long axons and dendrites in older cultures. The cells were whole-cell patch-clamped using an HEKA EPC-9 or a List EPC-7 amplifier. Electrode resistances were between 2 and 5 MΩ. The pipette solution contained (in mM): CsCl 135, HEPES 10, MgCl₂ 1.2, Na-ATP 4 and pH was adjusted to 7.2 using CsOH. An external solution of the following composition was used (in mM): TEA-Cl 135, HEPES 10, glucose 10, BaCl₂ 10, MgCl₂ 1, tetrodotoxin (TTX) 0.002 (adjusted to pH 7.4 with TEA-OH). Zinc (II) chloride (Aldrich, 99.9995% purity) was made up in a 10 mM stock solution, which was added to the external Ringer solution just before the experiment. Zn²⁺ (5–10 ml) was applied by a bath perfusion system (bath volume about 0.5 ml) over a period of 15–45 s. All experiments were done at room temperature (20–22°C).

Cells were routinely patch-clamped at −80 mV. Voltage-clamp command pulses were delivered by the EPC-9 unit controlled by the ATARI ST computer or by the EPC-7 unit using P-clamp software and IBM-hardware. Raw data were filtered at 10 kHz, leak data and corrected data at 3 kHz. All data (raw data, leak data from the P/4 protocol, and corrected data) were stored on hard disc and analyzed off line using the M2-lab software package on an ATARI ST computer or P-clamp software on IBM computers.

Calcium channel currents (carried by Ba²⁺) were evoked, at a rate of one per 15 s, by stepping from the holding potential of −80 mV to depolarized voltages usually to 0 mV for 80 ms and then back to the holding potential. All data were leak corrected by a P/4-protocol as described by Chad and Eckert. Adding Cd²⁺ (50 μM) resulted in a total blockade of all currents recorded under these circumstances, indicating adequate isolation of the calcium current.

Although Na-ATP was used in the pipette solution, a rundown of the calcium-channel current occurred. We excluded all experiments in which the calcium-channel currents showed a rundown of more than 10% of the total current in 5 min. The data presented are not corrected for rundown.

RESULTS

Addition of Zn²⁺ (up to 200 μM) while holding the membrane at −80 mV had no effect on the resting membrane current but did block the voltage-activated calcium-channel current completely. Fig. 1 shows averaged data of 3 traces of the voltage-activated calcium-channel current evoked by a voltage step from −80 mV to 0 mV for 80 ms. Superimposed is the effect of 50 μM Zn²⁺ on this current. While the time to peak (5–10 ms) did not change in the presence of Zn²⁺, there was a reduction in current caused by Zn²⁺. This voltage step should activate both N and L channels. Differential effects on these two types of channels may be detected by comparing reduction of the transient and maintained components, since N but not L channels show partial inactivation during the voltage jump. The peak current was reduced more (58%) than the sustained current, measured at the end of the voltage step (51%) at 75 ms. Since the N current is usually small relative to the L current in these neurons, these observations suggest a greater sensitivity of N than L currents to the effects of Zn²⁺.

Fig. 2 demonstrates the onset of the blockade of the peak calcium channel current during application of 50 μM Zn²⁺ and the effect of the following wash. The apparent increase in current while starting the application of Zn²⁺ is an artefact of the application system, resulting from a slight rise of the bath level before the suction started). A steady state of blockade was reached within 10 min (between 5 and 15 min in n = 35), while there was no recovery upon wash in this cell. In about 50% of the neurons (for example Fig. 3A,B) the blockade reversed with washing to about 40% of control. If the currents were corrected for rundown, this would represent a recovery of up to 50 or 60%. We have never seen a complete recovery after application of Zn²⁺ (10–200 μM) for ten or more minutes.

The current–voltage relation of peak voltages activation.
Fig. 3. A: current-voltage relation of voltage-activated calcium current of DRG cells. After application of 50 μM Zn²⁺, the current is greatly reduced at all voltages, but the calcium current, which is activated with small voltage jumps (between −35 mV and −20 mV; T-current), is nearly abolished. B: upon wash there is a recovery of about 25% over the whole voltage range.

Fig. 4. Dose-response relationship for the inhibitory effect of Zn²⁺. Half of the peak calcium current was inhibited by 69 μM Zn²⁺. Threshold concentration for action was below 2 μM Zn²⁺ and for total blockade concentrations over 200 μM Zn²⁺ were needed. The calculated Hill coefficient was about 1.

Fig. 5. Concentrations that do not affect the peak calcium current evoked by a voltage step from −80 mV to 0 mV (≤ 20 μM Zn²⁺) (A,B) do have a clear effect on the fast inactivating calcium current evoked by voltage steps from the holding potential of −80 mV to −30 mV (C) and −40 mV (D), respectively, but had no effect on the sustained current with the same voltage jumps.
Upon wash there is about 25% recovery (Fig. 3B) over the whole voltage range.

A blockade of half of the peak calcium channel current evoked on a jump from −80 to 0 mV was achieved with 69 μM Zn$^{2+}$. The Hill coefficient was about 1. The threshold concentration was below 2 μM and nearly complete inhibition (<80%) of the current was reached with concentrations of 200 μM Zn$^{2+}$ or higher. The dose–response relationship for the inhibitory effect of Zn$^{2+}$ on total currents is shown in Fig. 4.

Relatively low concentrations of Zn$^{2+}$ (≤ 20 μM), which have only a slight or no effect on the peak calcium channel current evoked by a voltage step from −80 mV to 0 mV (Fig. 5A,B), do clearly reduce the fast inactivating calcium-channel current (T) evoked by voltage steps from the holding potential of −80 mV to −30 mV (Fig. 5C) or −40 mV (Fig. 5D), respectively. Since this voltage should not activate the N channels, the current that inactivates with time can be ascribed to the T channels. Consistent with this conclusion is the observation that this transient is larger with the jump to −30 mV than to only −40 mV. The small sustained current at the end of the voltage jump, which is a result of slight activation of L channels, is basically unchanged. In this neuron the T-type current recovered partly (up to 40%) upon wash for several minutes.

**DISCUSSION**

Zn$^{2+}$ blocks voltage activated calcium currents in mammalian neurons at micromolar concentrations. N, L, and T currents are all blocked (<80%) at concentrations over 200 μM Zn$^{2+}$. At a voltage jump from −80 mV to 0 mV 50 μM Zn$^{2+}$ blocks the peak current (due to N and L channels) more effectively than the sustained current (due primarily to L channels, indicating that N channels are more sensitive to Zn$^{2+}$ blockade than are L channels). At lower concentrations (≤ 20 μM) there is a relatively selective blockade of the transient T-current. In both cases the effect appears in the absence of channel opening, as indicated by the observation that the block proceeds without channel activation. Thus, the sensitivity to blockade by Zn$^{2+}$ is in the order of T > N > L.

Zn$^{2+}$ is unlikely to have a large charge screening effect in this concentration range. The fast rate of onset and the stability of degree of current depression suggest that a direct blockade of the calcium channel by Zn$^{2+}$ is the most likely mechanism. Zn$^{2+}$ presumably competes with Ca$^{2+}$ for a binding site within the channel or close to its mouth. There are two possible explanations for the greater sensitivity of T channels to Zn$^{2+}$ blockade: (1) Zn$^{2+}$ binds more tightly to one of the calcium binding sites or (2) the binding sites are easier to access. The observation that the block of the fast inactivating T-current was, at least partly, reversible suggests that the second possibility is more likely.

The total lack of reversibility upon wash in some other cells might indicate that Zn$^{2+}$ binds very tightly to a site within the channel or acts from inside the cell membrane; however, this would require Zn$^{2+}$ to cross the channel. That Zn$^{2+}$ and other divalent cations do pass through voltage activated calcium channels in the absence of Ca$^{2+}$ was shown in invertebrate preparations by Oyama et al. and Byerly et al. We cannot exclude such a mechanism, but so far we do not have any proof that Zn$^{2+}$ passes the membrane in rat DRG neurons.

Fox et al. and Hagiwara et al. have shown that Ni$^{2+}$ is more effective in blocking the low-threshold fast inactivating (T) calcium current in chick sensory neurons or in sino-atrial node cells respectively than the N or L currents. Concentrations of 100 μM blocked the T-current completely but the other channels were altered only at higher concentrations. Our results suggest that Zn$^{2+}$ is also a selective blocker of the T-type calcium channel at even lower concentrations in rat DRG cells.

These results demonstrate that Zn$^{2+}$ blocks all types of calcium channel currents, most effectively the fast inactivating T channels at relatively low concentrations. This suggests that Zn$^{2+}$ might be a selective T-channel antagonist at low doses.

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