Pb²⁺ blocks calcium currents of cultured dorsal root ganglion cells

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The divalent cation lead (Pb²⁺) blocks sustained and transient voltage sensitive calcium channel currents of cultured rat dorsal root ganglion cells. The IC₅₀ for inhibition of the total peak current evoked by a step depolarization from −80 to 0 mV was 0.6 μM, compared to an IC₅₀ of 2.2 μM for Cd²⁺. The current activated by a depolarization from −40 to 0 mV was inhibited by 50% by 1.0 μM Pb²⁺. Low threshold currents activated by a step from −100 to −30 mV were blocked by Pb²⁺ at higher concentrations (IC₅₀=6 μM). The block progressed in the absence of channel activation and showed little voltage dependence. Peak sodium current was reduced by 6.6% at 1 μM Pb²⁺ while at 20 μM the peak current was reduced by 40% with marked slowing of the time course of activation. The potassium rectifier current was reduced by 4.1% at 1 μM Pb²⁺. Thus, Pb²⁺ selectively blocks calcium currents at concentrations in the range of those causing toxicity in man.

Three neuronal Ca²⁺ currents have been distinguished when chick or mammalian dorsal root ganglion (DRG) cells are depolarized after being held at hyperpolarized voltages under voltage clamp: a rapidly inactivating low-threshold activated (T) current, a slowly inactivating high-threshold activated current (L) and a high-threshold rapidly inactivating current (N) [18]. A variety of divalent metal cations are known to block these calcium currents [4, 5, 12], and some have differential effects on these calcium currents. Ni²⁺ is most effective at blocking the low threshold calcium current, but will affect the others at higher concentrations [9]. Cd²⁺ blocks all three currents at 50 μM [12]. The site of action is assumed to be close to or within the lumen of the ion channel [17].

Lead ions (Pb²⁺) are highly toxic, causing well characterized acute and chronic intoxication syndromes, both of which produce symptoms in the central and peripheral nervous systems [19]. Children are particularly sensitive to exposure to lead, and a variety of cognitive deficits and behavioral disturbances have been reported in children with blood lead levels in the range of 10–60 μg/dL [1, 2, 7, 14]. A previous study has demonstrated that Pb²⁺ is a potent blocking agent of voltage-dependent calcium currents of neurons of the marine mollusc, Aplysia californica [5]. The calcium current of Aplysia, while having several features in common with the high-threshold activated calcium currents of mammalian neurons, is not directly comparable to either the N or L type currents described in mammalian neurons. Thus, we have investigated the effect of Pb²⁺ on the calcium currents of cultured rat DRG cells, which express all three types of calcium currents and which have been well characterized [3].

DRG cells were cultured from 2- to 4-day-old rats as described by Wood et al. [20]. Cells were used for electrophysiological experiments several hours after isolation or for up to 8 days in culture. Standard whole cell patch clamp techniques [10] were employed to hold the cells under voltage clamp using a List EPC-7 amplifier. The pipette solution contained (in mM): CsCl 135; HEPES 10; EGTA 10; MgCl₂ 2; Na-ATP 4; pH 7.2. The external solution contained (in mM): TEA-Cl 135, HEPES 10, MgCl₂ 2.6, BaCl₂ 10, TTX 0.002 (adjusted to pH 7.4 with TEA-OH). Lead (II) chloride (99.99%, Aldrich) was dissolved in distilled water at 10 mM and diluted into the external solution immediately prior to an experiment. Drug application was by a rapid perfusion of 5 ml within 5–10 s through a bath volume of 300 μl. Voltage-clamp command pulses were derived from a CED laboratory interface controlled by an IBM PC/AT computer; the resulting membrane currents were stored on hard disk for off-line analysis using CED software.

Calcium channel currents (carried by Ba²⁺) were evoked at a rate of 1 per 15 s by holding the membrane
Fig. 1. Effects of Pb$^{2+}$ on calcium channel currents (A), voltage-dependent sodium currents (B) and delayed rectifier potassium currents (C). Both the peak and maintained components of the calcium channel currents produced by a voltage step from -80 to 0 mV were reduced by 0.5 and 2 nM Pb$^{2+}$. At 1 nM Pb$^{2+}$ had little effect on either the sodium or delayed rectifier potassium current.

When cells under voltage clamp were held at a holding potential of -80 mV, Pb$^{2+}$ (0.1-20 nM) had no effect on the resting membrane current. When cells were depolarized to 0 mV for 100 ms, the resulting inward current (N plus L) inactivated by 10 to 15% of the peak during the duration of the current. This high threshold current was blocked by Pb$^{2+}$ in the range of 0.1–20 μM (Fig. 1A). The time course of the block was rapid, reaching a steady level within 2–3 min, but was rarely completely reversed by wash for up to 10 min. The IC$_{50}$ for inhibition by Pb$^{2+}$ was 0.6 μM and the Hill slope 1.2 (Fig. 2A). The dose response curve for Cd$^{2+}$ was determined in the same manner. Cd$^{2+}$ was less potent, with an IC$_{50}$ of 2.2 μM and a Hill slope of 0.9 (Fig. 2A). The block produced by Cd$^{2+}$ was rapidly and nearly completely (>90%) reversible on wash.

If Pb$^{2+}$ was applied to a neuron for 3 min without evoking currents, the amplitude relative to control upon resuming stimulation was reduced to almost the same degree as when stimulation was maintained at the usual rate of one per 15 s. We did not detect differences in the current–voltage relations or activation parameters in the presence of 0.5 μM Pb$^{2+}$ as compared to control. The slope factor in the Boltzmann distribution, $k_f$, had a control value of 2.6, and a value of 3.6 in the presence of 0.5 μM Pb$^{2+}$, while $V_f$, the potential at which half-maximal currents are generated, was 18.8 mV in control and 19.0 in the presence of Pb$^{2+}$ (n = 4). Neither of these parameters was significantly different at the 99% level. The degree of block at a given test potential did not reveal an obvious voltage dependence of the peak current (Fig. 3), in that in both the absence and presence of Pb$^{2+}$ the peak current was generated at about -10 mV, and the current was reduced with either hyperpolarized or depolarized holding potentials. When voltage commands were applied to 0 mV from a holding potential of -40 mV (mostly L current), Pb$^{2+}$ was found to be slightly less effective at blocking the calcium channel current, with an IC$_{50}$ of 1.0 μM and a Hill slope of 1.2 (Fig. 2B).

Treating cells with 10 μM ω-conotoxin (CTX) (which should selectively block the N current) applied from a puffer pipette positioned close to the cell produced a rapid onset block of the inactivating component of the current evoked by a voltage step from -80 to 0 mV.
This effect was usually maximal after five to ten 1 s puffs and could not be washed out over 10–20 min. CTX inhibited 42% of the original peak current (n = 6), but did not cause a shift in sensitivity to block by Pb2+.

The dihydropyridine, nimodipine, is most effective at blocking the L type calcium channel at depolarized holding potentials. Thus, 10 μM nimodipine was applied at −40 mV while depolarizing to 0 mV to monitor the progress of block until a steady state was reached. The holding potential was then returned to −80 mV and the effect of Pb2+ assessed. The dose-response curve for nimodipine treated cells lay between those for holding potentials of −80 and −40 mV. This result is consistent with the conclusion that the N current is slightly more sensitive to blockade by Pb2+ than the L current.

The low threshold (T) current was recorded using command pulses to −30 mV from a holding potential of −100 mV. T currents were less sensitive to Pb2+, with an IC50 of 6 μM (Fig. 2B).

The voltage-dependent sodium current of DRG neurons, recorded in the presence of 50 μM CdCl2 to block all calcium currents, was reduced by 6.6 ± 0.6% in 1 μM Pb2+ (Fig. 1B). At 20 μM, Pb2+ reduced the amplitude of the Na+ current by 40%. This does not appear to be due to direct channel block, but rather to a slowing of the kinetics of activation. This effect was rapid in onset and fully and rapidly reversible on washout. The delayed rectifier potassium channel, also recorded in the presence of Cd2+, was reduced by 4.1 ± 0.9% in 1 μM Pb2+ (Fig. 1C).

These results demonstrate that Pb2+ is a potent blocker of voltage dependent calcium currents in mammalian sensory neurons. Pb2+ appears, on the basis of the results obtained from holding potentials of −80 and −40 mV as well as those from CTX and nimodipine treated cells, to be almost equipotent in blocking high threshold currents (N and L). The T currents are an order of magnitude less sensitive. Na+ and K+ currents are much less sensitive to Pb2+.

The mechanism of Pb2+ block appears to be independent of channel opening, as indicated by the lack of voltage dependence revealed by the current-voltage curves and the observation that block of currents proceeds in the absence of channel activation (Fig. 3). The site of action is thus probably not the same site bound by Cd2+, which blocks by binding to the Ca2+ or Ba2+ binding sites within the channel [12]. However, the lack of effect of Pb2+ on the parameters of activation indicates that the site of action is not a general charge screening, and suggests the possibility of an external binding site near the mouth of the channel.

An intracellular site of Pb2+ action must also be considered, although our results do not provide evidence for such action. Pb2+ activates protein kinase C at submicromolar concentrations [13]. Protein kinase C activators have been demonstrated to reduce the amplitude of calcium currents in both peripheral [15] and central [8] neurons. Pb2+ may enter the neurons during the period of channel opening [16] and activate internal protein kinase C. Alternatively, it has been shown that diacylglycerol and phorbol esters may depress calcium currents even when protein kinase C is blocked [11], and Pb2+ might also interact at this site.

Whatever the precise mechanism of action, the concentration of Pb2+ that we find to block voltage-dependent calcium channels is of the order of magnitude of serum concentrations found in children documented as showing cognitive and behavioral effects of lead toxicity [1]. Block of calcium channels by Pb2+ may contribute to this neurologic damage.

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