Novel Type of Ion Channel Activated By Pb$^{2+}$, Cd$^{2+}$, and Al$^{3+}$ in Cultured Mouse Neuroblastoma Cells

M. Oortgiesen, R.G.D.M. van Kleef, and H.P.M. Vijverberg
Research Institute of Toxicology, University of Utrecht, NL-3508 TD Utrecht, The Netherlands

Summary. Superfusion with Pb$^{2+}$ induces a slow, noninactivating and reversible inward current in voltage-clamped N1E-115 neuroblastoma cells. The amplitude of this inward current increases in the range of 1-200 $\mu$M Pb$^{2+}$. Single-channel patch-clamp experiments have revealed that this inward current is mediated by discrete ion channels. Reversal potentials from linear $I$-$V$ relationships are close to 0 mV for whole-cell and single-channel currents and the single-channel conductance amounts to 24 pS. The Pb$^{2+}$-induced membrane current is not mediated by various known types of ion channels, since it is not blocked by external tetrodotoxin, tetraethylammonium, d-tubocurarine, atropine, ICS 205-930 and by internal EGTA. In Na$^+$-free solutions superfusion with Pb$^{2+}$ neither evokes a whole-cell inward current, nor single-channel openings. At -80 mV the open-time distribution of the single channels activated by 1 $\mu$M Pb$^{2+}$ is dual exponential with time constants of 17 and 194 msec. When the Pb$^{2+}$ concentration is increased from 1 to 20 $\mu$M these time constants decrease to 2 and 13 msec, but the amplitude of single-channel currents remains -1.9 nA. Cd$^{2+}$ and Al$^{3+}$ induce inward currents and single-channel openings similar to Pb$^{2+}$. Time constants fitted to the open-time distribution of single channels are 14 and 135 msec in the presence of 1 $\mu$M Cd$^{2+}$ and 15 and 99 msec in the presence of 50 $\mu$M Al$^{3+}$. Conversely, Cu$^{2+}$ induces an irreversible inward current in neuroblastoma cells. Single-channel openings are undetected in the presence of Cu$^{2+}$ and in Na$^+$-free solutions Cu$^{2+}$ is still able to induce an inward current. It is concluded that Pb$^{2+}$, Cd$^{2+}$ and possibly Al$^{3+}$ activate a novel type of metal ion-activated (MIA) channel in N1E-115 cells.

Key Words: neuroblastoma cell · voltage clamp · single-channel current · heavy metal · lead · cadmium · aluminum · copper

Introduction

Various metal ions interfere with membrane functions that are normally regulated by Ca$^{2+}$. Elevation of the internal Ca$^{2+}$ concentration may cause activation of K$^+$- and Cl$^-$-selective, as well as nonselective cation channels in different cell types (for reviews see, Owen, Segal & Barker, 1986; Blatz & Magleby, 1987; Partridge & Swandulla, 1988). Some metal ion species are known to permeate through voltage-dependent Ca$^{2+}$ channels, whereas others block Ca$^{2+}$ currents and are widely used as inorganic Ca$^{2+}$ antagonists (Hagiwara & Byerly, 1981). Injection of a range of metal ions into molluscan neurones has demonstrated that certain ion species only are able to induce an outward K$^+$ current, which is very similar to the Ca$^{2+}$-activated K$^+$ current (Gorman & Hermann, 1979). In addition, an inward current, which is supposed to be mediated by nonselective cation channels, has been observed in molluscan neurones after external application of high concentrations of Cu$^{2+}$ (Weinreich & Wonderlin, 1987). In human erythrocytes, internal Pb$^{2+}$ has been shown to activate Ca$^{2+}$-dependent K$^+$ channels, but also to block these channels at high concentrations (Shields et al., 1985).

In N1E-115 neuroblastoma cells a Ca$^{2+}$-activated, nonspecific cation channel and two types of Ca$^{2+}$-activated K$^+$ current have been characterized (Yellen, 1982; Romey et al., 1984; Quandt, 1988). Blocking effects of metal ions on two types of voltage-dependent Ca$^{2+}$ current have been described in detail (Narahashi, Tsunoo & Yoshii, 1987). Recently, we have reported that in N1E-115 cells nanomolar concentrations of Pb$^{2+}$ block the nicotinic receptor-mediated inward current, whereas voltage-dependent Ca$^{2+}$ channels are blocked in the micromolar range. In the course of this study an inward current induced by concentrations of Pb$^{2+}$ higher than 1 $\mu$M was discovered (Oortgiesen et al., 1989).

The present study provides a more detailed description of the Pb$^{2+}$-induced inward current in voltage-clamped neuroblastoma cells and outside-out membrane patches. In addition, the effects of Pb$^{2+}$ and those of Cd$^{2+}$, Al$^{3+}$ and Cu$^{2+}$ are compared.

Materials and Methods

Mouse neuroblastoma cells of the clone N1E-115 (Amano, Richelson & Nirenberg, 1972) were grown in Dubbecco's modi-
fied Eagle medium supplemented with 7.5% fetal calf serum and the following amino acids (in mM): L-cysteine, HCl 0.3, L-alanine 0.4, L-asparagine 0.45, L-aspartic acid 0.4, L-proline 0.4 and L-glutamic acid 0.4. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. Cells of passages 30-45 were subcultured in 35-mm plastic tissue culture dishes. Cell differentiation was initiated 2-3 days later by adding 1 mM N6, 2′-O-dibutyryladenosine 3′:5′-cyclic monophosphate and 1 mM 3-isobutyl-1-methylxanthine to the culture medium. This medium was refreshed every 2-3 days. Cells were used for experiments 6-12 days after induction of differentiation.

Experiments were carried out using whole-cell voltage clamp and single-channel patch-clamp techniques (Hamill et al., 1981). The resistance of fire polished glass pipettes was 3-5 MΩ in whole-cell voltage-clamp experiments and 5-8 MΩ in patch-clamp experiments. The liquid junction potential at the tip of the electrode was compensated before each experiment and remained constant within 1 mV. During whole-cell voltage-clamp experiments the series conductance of approximately 0.15 μS was compensated for 60-70%. The membrane potential was held at -80 mV unless otherwise stated. The recordings were low-pass filtered (-3 dB at 1 kHz; 12 dB/octave), digitized by a transient recorder (8 bits; 1024 points/record) and stored on magnetic disc for off-line computer analysis.

Transitions between open and closed states of the single ion channels were identified using 50% of the open-channel amplitude as threshold criterion. The probability of channels being open was determined as the ratio between the time spent in the open configuration by all channels and the total recording time:

\[ P_{\text{on}} = \frac{\text{time open}}{\text{time total}}. \]  

(1)

Open-time histograms were obtained from records containing only single channel openings. When more than three channels were open simultaneously, the patch was excluded from kinetic analysis. The channel open times were divided into classes of approximately equal frequency and are presented in frequency density histograms (Bendat & Piersol, 1971). Time constants and SD's were estimated by a nonlinear least-squares exponential algorithm (Marquardt, 1963). Results were compared using a two-tailed Student’s t test (Diem & Lentner, 1968).

The control external solution contained (in mM): NaCl 125, KCl 5.5, CaCl2 1.8, MgCl2 0.8, HEPES 20, glucose 25 and sucrose 36.5. The pH was adjusted to 7.3 with approximately 10 mM NaOH. In some experiments nitrate salts instead of chloride salts were used. To compensate for osmolality changes that occurred when high concentrations of metal salts were added, the sucrose concentration of the external solution was reduced. The pipette solution contained (in mM): KCl 150, NaCl 10, HEPES 10 and MgCl2 1. The pH was adjusted to 7.2 with approximately 3 mM KOH. In whole-cell voltage-clamp experiments ion channels were activated by direct, whole-cell superfusion with known concentrations of the metal for adjustable periods using a servomotor operated valve (Neijt, te Duits & Vijverberg, 1988). In patch-clamp experiments ion channels were activated by addition of the metals to the bathing solution. The concentration of heavy metal ions contaminating the control external solution was less than 130 nM as calculated from the data supplied with the chemicals used. All experiments were carried out at room temperature (20-24°C).

Pb(NO3)2, Cu(NO3)2, Cd(NO3)2 and Al(NO3)3 were obtained from Fluka Chemie AG, Buchs, Switzerland; tetraethylammonium chloride (TEA); tetrodotoxin (TTX) and ouabain from Sigma, St. Louis, MO; D-tubocurarine chloride (DTC) from Sigma, St. Louis, MO; D-tubocurarine chloride (DTC) from Sigma, St. Louis, MO.

During whole-cell superfusion with Pb2+ a noninactivating inward current was observed in cells voltage clamped at a membrane potential of -80 mV. During washing with control external solution the inward current decayed to the control level. The amplitude of the Pb2+-induced inward current increased between 1 and 200 μM Pb2+ in a concentration-dependent way (Fig. 1). Further elevation of the Pb2+ concentration was impossible, due to the limited solubility of Pb(NO3)2 in the external solution. The amplitude of the inward current induced by 200 μM Pb2+ varied from 0.8-14 nA (n = 4). Membrane potential was held at -80 mV.

![Fig. 1. Concentration dependence of the inward current induced by superfusion of Pb2+ in whole-cell voltage-clamped neuroblastoma cells. During superfusion with 100 μM Pb2+ a noninactivating inward current appeared that reversed upon washing with control external solution (see inset, superfusion period is indicated by bar). The relative inward current (ordinate) was calculated by normalizing for each cell the amplitude of the inward current at various concentrations of Pb2+ to the value obtained at 200 μM Pb2+. The amplitude of the 200 μM Pb2+-induced inward current varied between cells and ranged from 0.8-14 nA (n = 4). Membrane potential was held at -80 mV.](image)

**Results**

**INWARD CURRENTS INDUCED BY Pb2+, Cd2+ AND Al3+**

During whole-cell superfusion with Pb2+ a noninactivating inward current was observed in cells voltage clamped at a membrane potential of -80 mV. During washing with control external solution the inward current decayed to the control level. The amplitude of the Pb2+-induced inward current increased between 1 and 200 μM Pb2+ in a concentration-dependent way (Fig. 1). Further elevation of the Pb2+ concentration was impossible, due to the limited solubility of Pb(NO3)2 in the external solution. The amplitude of the inward current induced by 200 μM Pb2+ varied from 0.8-14 nA in four different cells.

In order to elucidate the nature of the Pb2+-induced inward current, the effects of antagonists of various known types of ion channels were examined. The inward current induced by 100 μM Pb2+ was neither blocked by 1 μM of the Na+ channel...