Multiple, distinct actions of metal ions on cellular signaling processes

Wirkungen von Metallionen auf Zelluläre Signalprozesse

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From a neurotoxicological perspective, we have investigated effects of Pb^{2+} and other divalent metal ions on membrane signaling by voltage-, Ca^{2+}- and ligand-gated ion channels using voltage clamp and patch clamp techniques. The results from our and other studies of the effects of metal ions reported in the literature are summarized here and demonstrate that metal ions cause multiple, distinct effects on a variety of membrane signaling processes. A situation similar to that with membrane ion channels applies to the effects of metal ions on intracellular signaling. Effects on exocytosis, a resultant of highly integrated and complex intracellular signaling, indicate that Pb^{2+} is a substitute for intracellular Ca^{2+} in the triggering the fusion and release of exocytotic vesicles. Despite the relatively detailed knowledge of metal effects at the molecular level, few specific cellular targets associated with specific toxic effects of metals have been identified. Such knowledge is essential for the understanding of metal toxicity.

Key words: heavy metal ions; ion channels; patch clamp; Ca2+ substitution; neurotransmitter release

Mit voltage clamp- und patch clamp- Technik wurden Wirkungen von Pb^{2+} und anderen zweiwertigen Metallionen auf Membranpotentiale untersucht, die durch Spannungs-, Ca^{2+} -und Liganden-gesteuerte Kanäle entstehen. Unsere Befunde und die anderer Gruppen zeigen, dass Metallionen unterschiedliche Effekte auf verschiedene Signalprozesse an der Zellmembran entfalten. In ähnlicher Weise trifft das für intrazelluläre Effekte zu. Wirkungen auf die Exozytose, die auf einem komplexen intrazellulären Signalprozess beruht, weisen darauf hin, dass Pb^{2+} als Ersatz für intrazelluläres Ca^{2+} die Fusion und die Freisetzung von exozytotischen Vesikeln triggert. Trotz der recht detaillierten Kenntnisse von Metalleffekten auf molekularer Ebene sind bislang nur wenige spezifische zelluläre Angriffsmechanismen von Metallionen identifiziert worden. Solches Wissen ist aber erforderlich, um zu verstehen, wie Metallionentoxizität entsteht.

Schlagworte: Schwere Metallionen; Ionkanäle; Patch Clamp; Ca2+ Ersatz; Neurotransmitter Freisetzung

1 Introduction

Divalent metal ions are known to exert multiple toxic effects in the nervous system, kidney, and blood, and various metals are selectively deposited in bone tissue [9, 35]. A basic and general adverse effect of many divalent metal ions is their ability to substitute to some degree for Ca^{2+} in the allosteric regulation of protein activity. By specific competition for Ca^{2+}-binding proteins metal ions may mimic calcium effects and act as agonists or may block Ca^{2+} effects and act as antagonists of specific protein functions. In addition, non-specific binding of metal ions to proteins and other molecules may occur.

Ion channels, present in the membranes of excitable and non-excitable cells, are large membrane-spanning proteins containing multiple sites which are able to bind Ca^{2+} ions [10, 15]. Voltage-, Ca^{2+}-, and ligand-gated ion channels are at the basis of the generation and transmission of electrical signals in the nervous system, but are also expressed in non-excitable cells where they may be involved in the regulation of membrane potential, ion homeostasis and cell volume [11]. Effects of Ca^{2+} and other divalent metal ions on ion channels are reflected in changes in channel function, which are readily studied using voltage clamp and patch clamp techniques [32].

From a neurotoxicological perspective, we have investigated effects of Pb^{2+} and several other divalent metal ions on membrane signaling by various types of ion channels using voltage clamp and patch clamp techniques. In addition, the ability of Pb^{2+} to substitute for Ca^{2+} in the triggering of exocytosis was investigated. The results from our and other studies of the effects of metal ions reported in the literature are summarized here and demonstrate that metal ions cause multiple, distinct effects on a variety of signal transduction processes.

2 Extracellular effects of metal ions on membrane ion channels

At the extracellular face of the cell membrane multiple effects of metal ions on membrane proteins occur over a wide range of metal ion concentrations. An example are the in vitro effects of Pb^{2+} on ion channels of cultured mouse neuroblastoma cells of the clone N1E-115 [1]. These cells express multiple types of ion channels, which are sensitive to extracellular metal ions. Depending on the metal concentration a range of effects of Pb^{2+} is observed. Each of these effects appears to be

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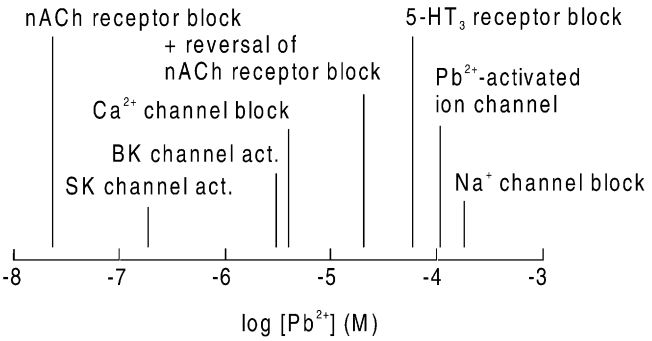

Fig. 1. The various effects of Pb^{2+} on ion channels and receptors observed in mouse N1E-115 neuroblastoma cells when exposed to Pb^{2+} at concentrations ranging from nanomolar to the solubility limit of Pb^{2+} in physiological saline solution. Note that related subtypes of ion channels (e. g., voltage-gated Na^+ and Ca^{2+} channels; SK and BK types Ca^{2+}-activated K^+ channels; and nicotinic acetylcholine type and serotonin 5-HT_3 type ligand-gated ion channels) are selectively affected by distinct concentrations of Pb^{2+} (for references see text)

selective for subtypes of related members of ion channels families, i. e., micromolar concentrations of Pb^{2+} block voltage-gated Ca^{2+} channels and not voltage-gated Na^+ channels in mouse N1E-115 neuroblastoma cells [28]. Similar differences are observed for the effects of Pb^{2+} on ligand-gated ion channels in N1E-115 cells, i. e., nicotinic acetylcholine receptor-operated ion channels are potently blocked by nanomolar concentrations of Pb^{2+}, whereas the closely related 5-HT_3 receptor-operated ion channels expressed in the same cells are more than three orders of magnitude less sensitive to block by Pb^{2+} [28]. In this respect it is also remarkable that for related subtypes of neuronal nicotinic ACh receptors, heterologously expressed in Xenopus oocytes from cDNAs coding for rat receptor subunits, the action of acetylcholine is either strongly potentiated, e. g., for α3β2 nicotinic receptors, or blocked by Pb^{2+}, e. g., for α3β4 and for α4β2 subtypes of nicotinic receptors [45]. In addition to these effects several metal ions (e. g., Pb^{2+}, Cd^{2+}, Al^{3+}) appear to be able to activate a specific type of metal ion-activated ion channels in mouse N1E-115 neuroblastoma cells by their action at the extracellular face of the cell membrane [29]. The multiple effects of Pb^{2+} observed when mouse N1E-115 neuroblastoma cells are exposed to Pb^{2+} concentrations ranging from nanomolar up to the solubility limit in physiological saline are depicted in *Fig. 1*.

Ion channels on which the effects of metal ions have been studied in sufficient detail to establish a potency order are the voltage-gated Ca^{2+} channels and Ca^{2+}-activated K^+ channels of mouse neuroblastoma N1E-115 cells. These cells express distinct subtypes of voltage-gated Ca^{2+} channels, both of which are blocked by metal ions but with different potency orders. These potency orders are $La^{3+} > Pb^{2+} >> Ni^{2+} > Cd^{2+}$, Co^{2+} for T-type or low voltage-gated Ca^{2+} channels and $La^{3+} > Pb^{2+} > Cd^{2+} >> Ni^{2+}$, Co^{2+} for L-type or high voltage-gated Ca^{2+} channels [27, 28]. Another study of voltage-gated Ca^{2+} current rat dorsal root ganglion cells indicates that Pb^{2+} is a much more potent blocker than Zn^{2+} and Al^{3+} [5]. It is of interest that metal ions may permeate the plasma membrane through voltage-gated Ca^{2+} channels [21, 34, 38] or through channels involved in Ca^{2+} release activated Ca^{2+} influx [20, 43]. Although the blocking effects of metal ions on these ion channels are in apparent contradiction with the permeation of the metal ions through the channels, a slow and sustained permeation through the channels competitive with and obstructing the permeation of Ca^{2+} ions may occur [38].

3 Intracellular effects of metal ions on membrane ion channels

Several types of Ca^{2+}-activated K^+ channels, i. e., K^+ channels which require intracellular Ca^{2+} to trigger channel opening, are distinguished by their single channel conductance into small (SK), intermediate (IK) and big (BK) Ca^{2+}-activated K^+ channels [41]. Mouse neuroblastoma (N1E-115) cells express both SK and BK channels. SK channels are highly sensitive to block by the bee venom peptide apamin and insensitive to the non-selective K^+ channel blocking tetraethylammonium ions (TEA), whereas BK channels are sensitive to TEA and insensitive to apamin [16]. In addition, the Ca^{2+} sensitivity of the BK channel depends to some degree on membrane potential and the internal Ca^{2+} concentration required to activate BK channels is higher than that required to activate SK channels [2, 24, 30]. The functional properties of IK channels, which are expressed in human erythrocytes, have also been characterized in detail [e. g., ref. 13]. Recent evidence indicates that BK channels are directly gated by Ca^{2+} interacting with the channel protein [33], whereas SK and IK channels may use calmodulin bound to the channel protein as a Ca^{2+} sensor [8, 17, 44].

The effects of metal ions on Ca^{2+}-activated K^+ channels have been investigated in excised membrane patches of mouse N1E-115 neuroblastoma cells containing SK and BK channels [22] and of human erythrocytes containing IK channels [23]. The comparison of the potency of Ca^{2+} and other metal ions, tested at 1 μM and 100 μM concentrations, to induce Ca^{2+}-activated K^+ channel opening showed that some metals mimic the effects of Ca^{2+}, whereas others fail to cause Ca^{2+}-activated K^+ channel opening. Pb^{2+} appears to be the more potent metal ion to mimic the effect of Ca^{2+} in activating Ca^{2+}-activated K^+ channels (see *Fig. 2*). Like with the distinct types of voltage-gated Ca^{2+} channels, the potency orders for the effects of the various metal ions to activate SK and BK channels in mouse neuroblastoma cells and IK channels in human erythrocytes appear to be distinct, i. e., for SK channels: $Pb^{2+} \approx Cd^{2+} > Ca^{2+} > Co^{2+} >> Fe^{2+}$, Mg^{2+}; for BK channels: $Pb^{2+} > Ca^{2+} > Co^{2+} >> Cd^{2+}$, Fe^{2+}, Mg^{2+} [22]; and for IK channels: $Pb^{2+} \approx Cd^{2+} > Ca^{2+} \geq Co^{2+} >> Fe^{2+}$, Mg^{2+} [23]. The more pronounced difference is that Cd^{2+} potently activates SK and IK channels, but fails to activate BK channels at concentrations up to 100 μM. The potency orders for the blocking effects of metal ions on voltage-gated Ca^{2+} channels and for the activating effects on Ca^{2+}-activated K^+ channels are summarized in *Tab. 1*.

4 Effects of metal ions on intracellular signaling

It has been established that, apart from the activation of Ca^{2+}-activated K^+ channels, intracellular Pb^{2+} interferes more generally with cellular signaling. Pb^{2+} has been demonstrated to bind to and activate protein kinase C (PKC) at low

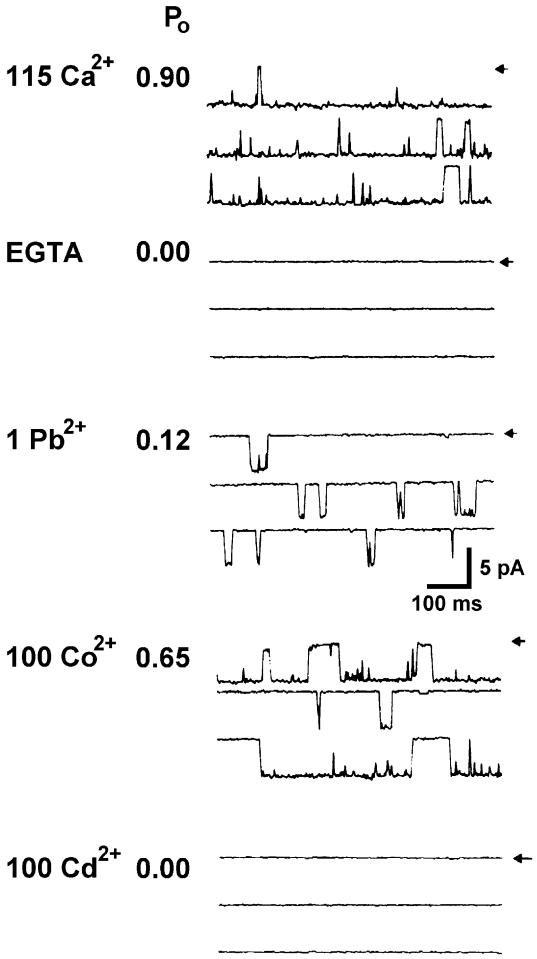

Fig. 2. An example of metal effects on ion channels. The internal face of a membrane patch of a mouse neuroblastoma cell was consecutively exposed to buffer solutions containing a high concentration of Ca^{2+}, 10 mM EGTA, and various metal ions at the free metal ion concentrations indicated (μM) and the single channel activity of large (BK) Ca^{2+}-activated K^+ channels was recorded (the closed level of the channels is indicated by arrows). BK channels are activated by Ca^{2+}, Pb^{2+}, and Co^{2+} but not by Cd^{2+} up to 100 μM, and not by 1 μM Co^{2+} (not shown). The free Ca^{2+} concentration in the presence of other metal ions was < 1 μM, which is below the threshold concentration of Ca^{2+} required for activating BK channels. P_o is the open probability of the channels normalized to that in the presence of the maximum-effective concentration of 115 μM Ca^{2+} (after Leinders et al., 1992 [22]). Different potencies of metal ions were obtained for other types of Ca^{2+}-dependent K^+ channels

concentrations and to inhibit PKC at high concentrations [26, 37]. The activation of PKC by Pb^{2+} occurs in the pico- to nanomolar concentration range, whereas the Ca^{2+} concentrations required for PKC activation are in the range of 0.1–100 micromolar [37]. Conversely, Cd^{2+} and Zn^{2+}, which promote phorbol ester-induced translocation of PKC to the nucleus in mouse fibroblasts, are unable to stimulate PKC-mediated phosphorylation and inhibit the PKC activity in vitro at concentrations > 1 μM [3]. A systematic investigation of the effects of a wider range of divalent metal ions on PKC activity appears to be lacking thus far.

Several metal ions have been demonstrated to bind to the Ca^{2+} binding protein calmodulin with different potencies. Pb^{2+}, which binds to the Ca^{2+} recognition sites of calmodulin, is not only the more potent metal ion, but also the more effective in triggering calmodulin-mediated phosphorylation and phosphodiesterase activity. The effectiveness of other metal ions, which are less potent than Pb^{2+}, e. g., Cd^{2+}, Ba^{2+}, Mg^{2+}, Ni^{2+}, and Co^{2+}, in triggering calmodulin activity appears to be complex and depends on the specific endpoint investigated [6, 14, 31].

Calmodulin, PKC and several other proteins sensitive to divalent metal ions have a modulatory role in exocytosis. Exocytosis involves the mutual interactions of a variety of proteins present on the plasma membrane, on the membrane of secretory vesicles, and in the cytosol. In addition, Ca^{2+} ions trigger the actual fusion of vesicles with the plasma membrane leading to the actual exocytosis of the vesicle contents [36]. Pb^{2+} is more potent than Ca^{2+} as an activator of calmodulin [14, 19], of PKC [37, 39], of calcineurin [18], and of the presumed Ca^{2+} sensor of exocytosis synaptotagmin [4]. Effects of Pb^{2+} on neurotransmitter release are well-established. After acute inhibition of evoked release, presumably associated with block of voltage-gated Ca^{2+} channels, Pb^{2+} causes an enhancement of the basal release of the neurotransmitter ACh at the neuromuscular synapse [25]. A similar enhancement of the release of catecholamines from bovine chromaffin cells has been reported [39, 40]. The latter is caused by an intracellular effect of Pb^{2+} and is also observed for a number of other metal ions with the potency order: $Pb^{2+} >> Cd^{2+}$, Ca^{2+}, $La^{3+} > Ba^{2+}$, $Sr^{2+} >> Zn^{2+}$, Co^{2+}, Mn^{2+} [40]. This study on permeabilized cells once more demonstrates that with respect to evoking exocytosis Pb^{2+}, with a half maximum effect at 4 nM, is also much more potent than Ca^{2+}, with a half maximum effect at 2.4 μM. Using rat phaeochromocytoma PC12 cells [12] we have recently demonstrated that the neurotransmitter release is caused by Pb^{2+} acting as a high-affinity substitute for Ca^{2+} to trigger essential steps leading to the

Table 1. Potency orders for the blocking effects of metal ions on T-type and L-type voltage-gated Ca^{2+} channels in mouse N1E-115 neuroblastoma cells and for the activation of SK and BK type Ca^{2+}-activated K^+ channels in the same cells as well as for the activation of IK type Ca^{2+}-activated K^+ channels in human erythrocytes (for references see text)

	active/potent	inactive/weak
Ca^{2+} channel block		
T type channel	$La^{3+} > Pb^{2+}$	$>> Ni^{2+} > Cd^{2+}$, Co^{2+}
L type channel	$La^{3+} > Pb^{2+} > Cd^{2+}$	$>> Ni^{2+}$, Co^{2+}
K^+ (Ca^{2+}) channel activation		
SK type channel	$Pb^{2+} \approx Cd^{2+} > Ca^{2+} > Co^{2+}$	$>> Fe^{2+}$, Mg^{2+}
BK type channel	$Pb^{2+} > Ca^{2+} > Co^{2+}$	$>> Cd^{2+}$, Fe^{2+}, Mg^{2+}
IK type channel	$Pb^{2+} \approx Cd^{2+} > Ca^{2+} \geq Co^{2+}$	$>> Fe^{2+}$, Mg^{2+}

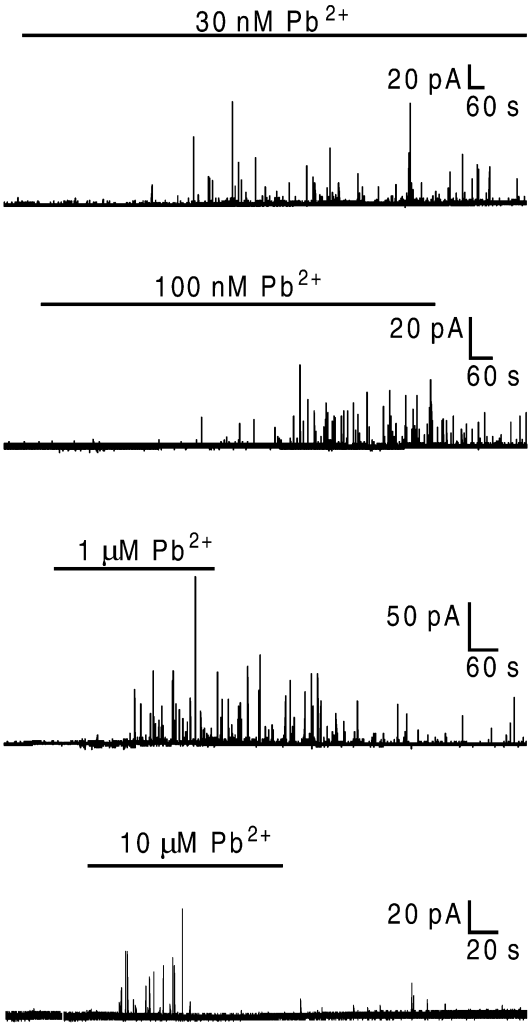

Fig. 3. Concentration dependence of Pb^{2+}-induced exocytosis in PC12 cells permeabilized with ionomycin. In the amperometric recordings each peak represents the exocytosis of a single neurotransmitter vesicle. The catecholamines released from the vesicles are oxidized at the surface of a carbon fiber microelectrode on the cell surface. The permeabilized PC12 cells are superfused with Ca^{2+}-free saline containing 5 µM ionomycin Ca^{2+}-salt and 0.03, 0.1, 1 and 10 µM Pb^{2+}, as indicated by the bars on top of the recordings. Pb^{2+}-induced exocytosis was observed from a threshold concentration of 30 nM Pb^{2+} added to the extracellular saline solution. The delay to onset of Pb^{2+}-induced exocytosis decreases with increasing Pb^{2+} concentration. At the highest concentration, Pb^{2+} blocks release following a transient stimulatory effect (after Westerink and Vijverberg, 2002 [42])

exocytosis of catecholamine containing vesicles [42]. In PC12 cells permeabilized with ionomycin, which is permeable to Ca^{2+} and Pb^{2+} [7], exocytosis is triggered when the cells are exposed to external Pb^{2+} concentrations ≥ 30 nM (*Fig. 3*). Using various chelators it was shown that the effect of Pb^{2+} occurs independent of the presence of intracellular Ca^{2+}. However, Pb^{2+}-induced exocytosis starts before intracellular Pb^{2+} is detected with the fluorescent dye indo-1, indicating that high-affinity components in the cytosol buffer intracellular Pb^{2+}. By extrapolation it was estimated that, independent of the extracellular Pb^{2+} concentration, partial saturation ($\sim 20\%$) of this intracellular high-affinity binding capacity for Pb^{2+} (~ 2 attomol/cell) was required for the triggering of exocytosis. Thus it appears that the binding of Pb^{2+} to some of the high-affinity cytoplasmic components causes exocytosis [42]. The results indicate that the amount of Pb^{2+} sequestered intracellularly over time, rather than the acute extracellular concentration of Pb^{2+}, is a determinant of adverse effects on exocytosis.

5 Conclusion

Available evidence indicates that metal ions may exert multiple effects on cellular proteins involved in signal transduction. Metal ions selectively affect distinct types of ion channels. In addition, metal ion interaction sites of ion channels may be highly selective for particular metals. Thus, it appears that the specificity of the metal effects partly originates from the metal ion and partly from the protein involved. Despite the multitude of molecular effects, cellular targets for the specific toxic effects of metals remain to be established. The absence of knowledge on which specific targets are involved in which specific adverse effects of metal ions is an important factor in the uncertainty on the risk for metal toxicity.

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Received in final form: 11/1/02 [T 550]

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Nach einem typischen Einführungskapitel über die Materialstrukturen wird im Kapitel 2 auf die Produktion/Herstellung nicht nur von Pulvern für das Sintern, sondern auch von Whiskern und Fasern eingegangen. Kapitel 3 handelt technologische Aspekte mit den üblichen, aber auch modernen Verfahren zur Herstellung ab. Wertvoll erscheint mir die ausführliche Beschreibung der Eigenschaften und vor allem der Gebrauchseigenschaften, hier mechanische, thermische, optische, elektrische und magnetische Eigenschaften und das Verhalten bei Korrosion. Im Kapitel 5 werden die üblichen Methoden zur Charakterisierung von Werkstoffen mit zu recht einem gewissen Schwerpunkt auf die Untersuchung keramischer Gefüge behandelt. Dass das Kapitel über Anwendungen etwas schwach ist, halte ich nicht für einen wesentlichen Nachteil, es hätte den Rahmen des Buches gesprengt.

Extrem wertvoll sind die Kapitel 7 und der Anhang mit der Beschreibung des Eigenschaftsprofils von Werkstoffgruppen und Tabellen mit Daten aller Art. Hierfür sind die mehr als 100 Seiten sinnvoll genutzt. Ein Index zum Nachschlagen rundet den Charakter des Buches als Review und Nachschlagewerk ab.

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