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Toxic Effects of Heavy Metals on Ionic Channels*

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I. Introduction

Heavy metal ions are utilized by a large number of cellular enzymes. It is important, therefore, for cells to regulate and maintain appropriate levels of essential heavy metal ions. However, low levels of nonessential, excess concentrations of essential, or the presence of toxic metal ions can harm cells in a variety of ways, including inactivation of enzymes and catalysis of the oxidative damage of lipids, proteins, and nucleic acids (Ralston and O'Halloran, 1990). To understand the mode of neurotoxic action of heavy metals, it is important to know the precise cellular site of their action.

Metals with a density greater than 5 kg/m^3 are usually termed "heavy metals." This definition is based on an arbitrary physical parameter and not on a chemical properties of the metals. Recently, the term of transition metal is favoured, because the latter is more correct chemically. Here we would like to use the former term, because it comprises more elements than transition metals (e.g., Pb^{2+}, Sn^{2+}, Al^{3+}). The only exception is aluminum, which does not belong to the group of transition

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metals or to the group of the heavy metals. Effects of the elements of groups Ia and IIa are also discussed throughout this article to compare them with those of heavy metals. The basic chemical property of metals is that they are all electron donors. Therefore, they are capable of interacting with ligands (-OH, -SH, $-NH_2$, -COOH, $-PO_3H_2$, etc.), which form integral parts of any molecule of biological significance in aqueous solution (Passow et al., 1961). At neutral pH, for example, Pb^{2+} forms significant quantities of $PbOH^+$ complex with OH^- and $PbCl^+$ if Cl^- is present (Simons, 1993). Because chloride ions are always present in cells and also form complexes with other metals, it is difficult to establish the metal concentration in solution. This causes a serious problem in studies of the metal ion effects, because metals at low concentrations (nM or μM) may bind to the specific or sensitive sites, whereas at high concentrations they bind to the nonspecific or insensitive sites (Passow et al., 1961).

A second serious problem in interpreting data could be caused by metal-metal interaction. They may interact through synergism, antagonism, or joint independent action, they can compete for metal-binding proteins, and they can induce formation of metal-binding proteins. Mechanisms through which metals may interact have been reviewed (Vallee and Ulner, 1972). As mentioned above, metals interact with anions. In the present review, no attempt has been made to describe and discuss in detail the consequences of the counter-ion interaction. The chemistry and the chemical interaction with biochemical substances have been reviewed (Passow et al., 1961).

Because metal ions can interfere with most biological processes, theoretically, as many kinds of effects can be found as there are cellular activities. Although the hazardous effects of exposure to heavy metals have long been recognized, relatively little is known regarding the cellular and subcellular mechanisms involved, especially in nerve and muscle cell membranes. Therefore, in this review, we pay particular attention to the effects of metal ions on the permeability of the surface membrane of excitable cells.

The surface membrane, as a diffusion barrier, protects the cell interior from environmental changes. In addition, sensitive ligands located within the membrane structure or on the outer cell surface can aid in transmitting information to the cell interior. Consequently, functions associated with the cell membrane are particularly susceptible to the action of heavy metals (Passow et al., 1961). It has been assumed that heavy metals react nonspecifically with ubiquitous sulfhydryl, carboxyl, or phosphate groups or through a loose interaction with macromolecular structures in proteins or polynucleotides. However, a study of poisoning by metals revealed some toxic specificity, an observation that justifies a search for individual modes of heavy metal action (Haberman and Richardt, 1986).

The purpose of this review is to summarize recent data indicating that membrane functions, such as permeability and gating, are affected by different metal ions. Furthermore, we present evidence in favour of the idea that such actions cannot be generalized. Selected examples of metal ion effects on excitable membranes are presented that indicate different, likely specific, mechanisms of their action. During the last few years, evidence has accumulated suggesting that heavy metal ions are capable of interacting with ionic channels, receptors, and/or ionic-channel-receptor complexes. Furthermore, most of the heavy metal ions themselves are capable of inducing new ionic conductances in the cell membrane.

The mechanisms of metal ion interaction could be quite diverse. Metal ions may compete with essential ions such as Ca^{2+} for binding sites or they may react with sulfhydryl groups of enzymes, thereby altering the enzyme and membrane functions. Metal ions may catalyse the formation of oxygen radicals which, in turn, depolarize the cell membrane (Scott and Rabito, 1988; Wetterhahn-Jenette, 1981; Clarkson, 1993). Although several types of signal transduction pathways operate in the cell for a whole series of stimuli, signal transduction pathways for heavy metals appear to involve metalloregulatory proteins. These proteins are multifunctional heavy metal receptors that mediate cellular responses to heavy metal ions by modulating the activity of biological components involved in different cellular functions, including those involved in gene expression (Ralston and O'Halloran, 1990).

No attempt has been made to provide a comprehensive survey of the literature, considering all of the available knowledge in the field. For this reason, this contribution should be considered as a source of ideas that might stimulate other scientists in their efforts to understand the molecular mechanisms underlying the effects produced by heavy metals.

II. Effect on Voltage-activated Ionic Channels

Voltage-dependent Ca^{2+} channels found in a variety of cells are blocked more or less effectively by di- or trivalent cations (Tsien et al., 1989). However, it was also found that these metal ions affect the functioning of other voltage-activated channel types. Divalent and trivalent cations have strong effects on the gating properties of voltage-dependent ionic channels. The effects are usually explained in terms of surface charge theory, according to which cations are attracted by fixed negative charges on the membrane surface. The adsorption of cations locally changes the membrane field without altering the membrane potential, as measured by electrodes in the bulk solution. Because the gating of voltage-dependent channels are confined to the membrane, there is no distinction between an electric field change caused

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by an altered membrane voltage and that caused by altered cation composition (Green and Andersen, 1991).

A prediction of the theory is that all properties of channel gating should be affected equally, i.e., the curves relating opening and closing kinetics, the conductance-voltage curves, should all be shifted along the voltage axis by equal amounts and increases in cation concentration of the extracellular medium should shift all of the curves to the right. An alternative mechanism of divalent cation action would be a direct interaction of the cation with the channel protein or channel-gating charges.

In the sections that follow, we will pay particular attention to the studies in which the effects of metal ions were determined rather than those in which the metal ions were used as tools for the isolation of different current components.

A. Sodium Channel

Externally applied metal ions such as Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+}, at concentrations of 0.1 to 10 mM affect the Na^+ system of the nerve membrane; the observed effects are complex and reversible, except for the actions of Hg^{2+}. All of the metal ions studied decreased Na^+ permeability. It is important to emphasize that efficiency sequences for permeability parameters differ in different species. The relative efficiencies decreasing Na^+ permeability for two amphibians are as follows: *Xenopus laevis*: $Cu^{2+} > Zn^{2+} > Cd^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+}$; *Rana pipiens*: $Co^{2+} > Zn^{2+} > Ni^{2+} > Mn^{2+}$. All of the metal ions studied shifted the activation-variable curve to the right along the potential axis and increased both activation and inactivation time constants. The effect was found to differ in magnitude for different ions (Arhem, 1980).

Hg^{2+} affected Na^+ currents of the nerve membrane irreversibly at the lowest concentration (1 to 10 μM). This observation seems reasonable, considering the qualitative differences that exist between the acute effects of Hg^{2+} and those of the other heavy metal ions, because the mechanism of the membrane actions of Hg^{2+} is substantially different from that of the other metal ions (Arhem, 1980).

The actions of Cd^{2+}, Zn^{2+}, and Hg^{2+} were investigated in calf Purkinje fibres and in isolated guinea pig ventricular cells (Visentin et al., 1990). The Na^+ channels of the heart muscle differ substantially from those of nerve and skeletal muscle. It was observed that on cardiac cells Cd^{2+}, Zn^{2+}, and Hg^{2+} reduced the inward sodium current already at μM concentrations and that this reduction occurred via a voltage-independent blockade. For both cardiac cell types, the blocking efficiency is $Hg^{2+} > Cd^{2+} > Zn^{2+}$. The position of the inactivation curve on the voltage axis was unaltered at metal ion concentrations causing substantial current inhibition, and the position moved to the right only at mM concentrations. This effect can be explained by a channel-blocking rather than a charge-screening effect on the external membrane surface.

On single canine cardiac Purkinje cells the divalent cations Ba^{2+}, Mg^{2+}, Co^{2+}, and Mn^{2+} produced voltage-dependent open channel blockade with the relative blocking efficacy of $Co^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+} > Ba^{2+}$. However, Cd^{2+}, Zn^{2+}, and La^{3+} blocked the I_{Na}§ at low concentrations in a voltage-independent way. It was concluded that di- and trivalent cations bind to at least two different blocking sites on cardiac Na^+ channels. It is believed that the voltage-dependent and -independent sites are distinct from each other (Sheets and Hauck, 1992). It was suggested that binding to the sulfhydryl groups may be responsible for the voltage-independent blockade produced by divalent cations (Begenisich and Lynch, 1984; Schild and Moczydlowski, 1991). A second, distinct binding site, a negatively charged carboxyl group, may be responsible for a voltage-dependent block (Woodhull, 1973; Begenisich and Danko, 1983).

Mn^{2+}, Co^{2+}, and Ni^{2+} also decreased the Na^+ currents in squid axons but had no effect on K^+ channels (Baker et al., 1973). The interaction of externally applied Zn^{2+} ions on Na^+ channels was studied by Gilly and Armstrong (1982a) using squid giant axon. They found that at a concentration of 30 mM Zn^{2+} slows opening kinetics of Na^+ channels with almost no effect on closing kinetics and reduces peak Na^+ current by 50%.

Zn^{2+} (1 mM applied internally) blocked Na^+ currents almost completely in the squid axon with little affect on the voltage dependence of the current. Large concentrations of Zn^{2+} (10 mM) caused nonspecific, but reversible, effects. Conditioning hyperpolarizations reduced the Zn^{2+} effect. The effects of Co^{2+}, Cd^{2+}, and Ni^{2+} were qualitatively similar to those of Zn^{2+} (Begenisich and Lynch, 1974). It is interesting to note that Begenisich and Lynch (1974) did not find any effect of increased intracellular Ca^{2+} on either Na^+ or K^+ conductances. Tanguy and Yeh (1988) and Green et al. (1987) measured the voltage-dependent block of Zn^{2+}, and Cd^{2+} on nerve Na^+ channels. The effects were similar to those observed with Ca^{2+} in different preparations (Pusch, 1990).

Using snail neuronal soma membranes, external application of Cu^{2+} and Pb^{2+} at micromolar concentrations, and Zn^{2+} at mM concentrations, depressed the Na^+ current by about 50 and 80%, respectively. The blocking effects of the metal ions were found to be voltage independent, and the sequence of the blocking effectiveness was $Zn^{2+} > Cu^{2+} > Pb^{2+}$ (Osipenko et al., 1992a).

§ Abbreviations: I_{Na}, sodium current; I_{Ca}, calcium current; I_K, potassium current; I_A, transient outward current; I_{Cl}, chloride current; I_X, current induced by a given cation (*x*); I_{in} or I_{out}, inward or outward directed current; I_w, washout current; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; NMDA, N-methyl-D-aspartic acid; 5-HT, 5-hydroxytryptamine (serotonin); ACh, acetylcholine; E_{rev}, reversal potential; I_{Cuin} (I_{Pbin}), inward current of copper (lead); I_{Pbout}, outward current of lead; $I_{Pb,w}$, current during lead washout; $I_{MeHg,out}$, outward methyl mercury chloride current.

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The effect of an organic tin compound (triphenyltin) was investigated on the kinetics of voltage-dependent Na^+ inward current in acutely dissociated pyramidal cells of rat hippocampus. Triphenyltin, at a concentration of 10^{-6} M, decreased both the time-to-peak and the half-decay time of the I_{Na}, without affecting the current-voltage (I-V) relationship (Oyama, 1992). Triphenyltin also increased the peak amplitude of the I_{Na} irreversibly (Oyama and Akaike, 1990). It was suggested that triphenyltin increased the excitability of mammalian central nervous system neurons by affecting the kinetics of I_{Na}.

Triphenyltin had no effect on voltage-dependent Ca^{2+} channels; however, it did dose-dependently increase the intracellular Ca^{2+} concentration. This sustained increase in the intracellular Ca^{2+} could explain the toxicity of organotin compounds (Oyama et al., 1992).

The interaction of heavy metal ions with sulfhydryl groups of channel proteins has been suggested as a possible reason for the reducing action of these ions on nerve and muscle Na^+ current. The stability constants for the complex formation were $Mn^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Arhem, 1980). This sequence, however, is not in accordance with any of the sequences shown above. In the case of Zn^{2+}, for example, it is more realistic to consider a specific interaction of the divalent cation with the voltage sensor/gating apparatus of the Na^+ channel. The interaction proposed is an electrostatic stabilization of exposed negative charges at the external membrane surface when the channel is closed. Although fixed surface charges undoubtedly exist in nerve (Neumcke and Stämpfli, 1984), it is clearly not necessary to postulate a significant role of such charges in mediating the action of extracellular Zn^{2+} ions on Na^+ channel gating (Gilly and Armstrong, 1982a).

Frankenhauser and Hodgkin (1957) showed that for a given depolarization fewer channels opened, and opened more slowly in the presence of high Ca^{2+}, than occurred in control solution. To explain the mechanism of calcium's action, it was postulated that Ca^{2+} (and perhaps many other di- or trivalent cations) tends to accumulate near negative surface charges, thereby neutralizing them. Divalent cations are attracted more strongly than monovalent ones. If the fixed surface charges were uniformly distributed, changing the surface potential by adding Ca^{2+} (or any kind of divalent cation) would be identical with the hyperpolarization. This criterion, however, has not been satisfied for any biological membrane system.

It was shown that Ni^{2+} or La^{3+} decreased the Na^+ current activation and shifted the conductance-voltage relationship along the voltage axis very little (Dodge, 1961; Takata et al., 1966; Hille, 1968; Hille et al., 1975; Arhem, 1980). Sodium channel gating was studied on rat pituitary cells (GH3) in solution in which Ca^{2+} was substituted for La^{3+}. In the presence of 2 mM La^{3+} in the extracellular solution, the kinetics parameters of the gating were shifted unequally. The main finding was, however, that changing from 2 mM Ca^{2+} to 10 μM La^{3+} induces a positive shift of the opening rate and fraction of open curves but a negative shift of the closing rate curve. The opposite effect on the kinetics parameters caused by La^{3+} cannot be explained in terms of surface charge theory (Armstrong and Cota, 1990). An alternative mechanism of divalent cation action would be a direct interaction with the channel protein or channel-gating charge.

B. Potassium Channels

It is well known that increasing the concentration of extracellular Ca^{2+} decreases the I_K (potassium channel) by the mechanism of surface charge screening.

On *Xenopus* axon, it was found that Zn^{2+} (1 to 10 mM), Ni^{2+} (1 to 10 mM), Cd^{2+} (1 to 34 mM), Co^{2+}, Mn^{2+} (10 mM), and Cu^{2+} (100 μM) decreased the permeability constant, shifted the activation curve in a positive direction along the voltage axis ($Zn^{2+} > Cd^{2+}$), and increased the inactivation time constant. In contrast, Cd^{2+} did not affect the time constant and decreased the permeability constant about equally as did Zn^{2+} (Arhem, 1980). On the same preparation, Vogel (1974) found that, in high extracellular Ca^{2+} (10 mM), La^{3+} (0.1–0.5 mM), and Tb^{3+} (0.1 mM) solutions, the K^+ permeability and the leakage conductance were reduced. The activation curve of the permeability constant was shifted to the right along the voltage axis. It was assumed that the observed shift reflected a change in membrane surface potential because of electrostatic screening by the cations in the external solution.

On squid giant axon, externally applied Ca^{2+} (12.5 to 37.5 mM) Zn^{2+} (2 to 40 mM), Cd^{2+} (2 to 10 mM), Hg^{2+} (0.02 to 1 mM), Mn^{2+} (5 to 25 mM), Ni^{2+} (2 to 10 mM), and Cu^{2+} (2.5 to 10 mM) ions slowed the opening kinetics of K^+ channels and had no effect on closing kinetics. Zn^{2+} reduced potassium conductance in a voltage-independent manner. This effect clearly cannot be explained by an alteration of a diffuse surface charge. Instead, a more specific interaction with the gating apparatus or with channel proteins was suggested (Gilly and Armstrong, 1982b).

Varying La^{3+} concentrations within a 0.01 to 0.5 mM range shifted the conductance-voltage curves of the node of Ranvier along the voltage axis in the direction of depolarization. In contrast, Zn^{2+}, at a concentration lower than 0.5 mM, shifted the conductance-voltage curve in a hyperpolarizing direction. At higher concentrations (>0.5 mM) Zn^{2+} and Cu^{2+} had effects similar to La^{3+}. Cu^{2+} and Zn^{2+}, however, decreased the steepness of the conductance-voltage curve. It was suggested that the La^{3+} effect reflects a change in electrostatic screening. The effect of Zn^{2+} and Cu^{2+}, on the other hand, cannot be explained solely by this mechanism (Mozhaeva and Naumov, 1973).

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Outward currents in snail neurons were decreased by Cu^{2+}, whereas the effects produced by Pb^{2+} and Zn^{2+} were either depression or enhancement, depending on the neuron being studied. Cu^{2+} decreased I_K by 80% at μM concentrations that had no effect on kinetics parameters (Osipenko et al., 1992a).

On *Lymnaea* neurons, 10 μM Hg^{2+} depressed the transient potassium current (I_A) as well as the inactivation rate. Hg^{2+} markedly influenced the steady-state inactivation; however, it did not change the rate of recovery from inactivation (Alekseev, 1992). Spigelman and Carlen (1991) and Harrison et al. (1993) demonstrated the depressant effect of Zn^{2+} on I_A in rat and guinea pig hippocampal neurons. Zn^{2+} (1 to 1000 μM) shifted both activation and inactivation curves in the depolarizing direction. Agus et al. (1991) described a similar modulation of I_A in cardiac myocytes by Zn^{2+}. It was concluded that Zn^{2+} ions have a complex effect on hippocampal neurons and that these actions are likely to delay repolarization and promote spontaneous activity, resulting in action potential prolongation and hyperexitability of the neurons.

Divalent cations produced positive shifts of the steady-state activation and inactivation curves for I_A in rat DRG cells; the order of potency was $Cd^{2+} > Mn^{2+} = Co^{2+} > Ca^{2+} > Mg^{2+}$. These data showed that divalent cations can evoke a depolarizing shift of both the activation and inactivation gates controlling I_A. The effect produced was either depression or augmentation of I_A, depending on the species, concentration of the divalent cation, and the pre-pulse potential used to deinactivate I_A. The modulatory effect of divalent cations on the gating of I_A was suggested to reflect binding to a specific, saturable site, either on the channel protein itself or on the phospholipids close to the gating apparatus (Mayer and Sugiyama, 1988).

Intracellular application of low (1 μM) concentrations of Zn^{2+}, Ni^{2+}, Cd^{2+}, or Co^{2+} substantially slowed the potassium channel kinetics in voltage-clamped squid axon. A 1 mM internal Zn^{2+} concentration selectively and reversibly slowed the kinetics of the I_K with little effect on its voltage dependence. At high concentrations (10 mM), a nonspecific reversible reduction of the ionic currents was observed. The effect of internal Co^{2+}, Cd^{2+}, and Ni^{2+} are qualitatively similar to those of Zn^{2+}. Application of Ca^{2+}, up to 10 mM intracellularly, had no effect. Consequently, it was suggested that divalent cations bind to the channel-forming proteins (Begenisich and Lynch, 1974). In contrast to these data, an activation of Ca^{2+}-dependent K^+ currents could be obtained on molluscan neurons (Müller et al., 1989).

Spires and Begenisich (1992) described the effects of Zn^{2+} on K^+ channels of neurons isolated from stellate ganglion of *Loligo pealei*. The authors examined some chemical properties of the external divalent cation binding sites on K^+ channels. They found that channel activation kinetics were greatly (3- to 4-fold) slowed by 2 to 5 mM Zn^{2+}; however, the deactivation kinetics were only slightly affected. These effects of Zn^{2+} were inhibited by a low solution pH in a manner consistent with competition between Zn^{2+} and H^+ ions for a single site. The apparent inhibitory permeability constant for this site was approximately 7.2. Treatment of the neurons with specific amino acid reagents implicated amino, but not histidyl or sulfhydryl, residues in divalent cation binding. Divalent cations have a substantial influence on the function of potassium channels, they may stabilize the channel in a closed state, or their lack may remove the selectivity of the channel (Begenisich, 1988).

Harrison et al. (1992) demonstrated a potent modulatory action of Zn^{2+} on the gating of cloned rat and human K^+ channels. Two delayed rectifier currents and an inactivating (I_A) current were studied. Low concentrations of Zn^{2+} (K_D = 50 μM) shifted the kinetics parameters of these currents, whereas concentrations greater than 200 μM blocked the K^+ channels. The action of Zn^{2+} on these different K^+ channels suggests the existence of a common Zn^{2+}-binding domain, the occupation of which influences the voltage sensor. The amino acid located at position 369 is a key determinant of the selectivity of the voltage-gated K^+ channel (2.1 kV). Replacing isoleucine for basic histidine at this position produced a Cs^+-selective channel with a Cs^+:K^+ permeability ratio of 4:0.1. External protons and Zn^{2+}, which are known to interact with the imidazole ring of histidine, blocked the mutant channel effectively and in a voltage-independent manner (De Biasi et al., 1993).

C. Ca^{2+} Channel

1. Blocking of Ca^{2+} channel by metal ions. Voltage-dependent Ca^{2+} channels are found in most cells and are sensitive to blockade by a variety of di- and trivalent metal ions, including Co^{2+}, Cd^{2+}, La^{3+}, Mg^{2+}, and Ni^{2+} (Hagiwara and Byerly, 1981). It was also reported that different metal ions can discriminate between Ca^{2+} channel types. In neurons, metal ions at mM concentrations cause an unselective block of I_{Ca} (Ca^{2+} channel). Their selectivity, however, increases at μM concentrations. For example, in most neurons 20 μM Cd^{2+} blocks high-threshold (high-voltage activated) I_{Ca} leaving low-voltage-activated I_{Ca} unaffected. Alternatively, in a number of neurons, 40 μM Ni^{2+} blocks low-voltage-activated currents and has no effect on high-voltage-activated currents. However, exceptions exist that argue against the use of low concentrations of Ni^{2+} as a criterion for selective blockade of different types of I_{Ca} (Carbone and Swandulla, 1989).

The divalent cation Zn^{2+} blocked all types of Ca^{2+} channel currents in rat DRG neurons. The IC_{50} for inhibition of the peak current was 69 μM for N- and L-type channels and 20 μM for the T-type channel. The effect was partly reversible. The sensitivity of blockade,

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therefore, was T > N > L. It was suggested, because of the fast onset and stability of blockade, that Zn^{2+} competes with Ca^{2+} for binding site(s) within the Ca^{2+} channel or close to it. The slight selectivity of blocking T-type channels over other types was explained by (*a*) Zn^{2+} binding more tightly to T-channel site(s) or (*b*) the binding sites being easier to access (Büsselberg et al., 1992).

The blockade of Ca^{2+} channel currents in *Aplysia* neurons requires mM concentration of Zn^{2+}. A 50% reduction was observed at 3.75 mM, and full blockade occurred at 20 mM concentrations of Zn^{2+} (Büsselberg et al., 1990a). The current-voltage curve and the activation and inactivation curves were shifted to the right along the voltage axis, an observation that could be explained by a charge-screening effect combined with the binding of Zn^{2+} near the channel mouth (Büsselberg et al., 1990a, 1991a). In mouse myotubes, Zn^{2+} blocks unitary currents similarly to Cd^{2+}; however, Zn^{2+} causes a fast-flickering block of the open channel (Winegard and Lansman, 1990).

Similarly to the rat DRG cells, and in contrast to *Aplysia* on *Helix pomatia* neurons, no shift of the current-voltage curve was observed in the presence of 3 mM Zn^{2+} (Osipenko et al., 1992a). In contrast to the Zn^{2+} effect, extracellular application of 1 μM Pb^{2+} shifted the current-voltage curve to hyperpolarized voltages on *Aplysia* neurons. The effect was similar to that caused by a reduction of the Ca^{2+} concentration in the absence of Pb^{2+}. Pb^{2+} did not significantly change inactivation but shifted the voltage dependence of activation to the left. Application of Pb^{2+} decreased Ca^{2+} current within 3 to 7 min at IC_{50} = 61 μM. Extracellular perfusion with either triethyl lead (5 to 50 μM) or Hg^{2+} (5 to 200 μM) resulted in only a small reduction of the Ca^{2+} current within 2 min. The decrease continued for the total duration of application; the effect, however, was irreversible. A direct effect on the Ca^{2+} channel was postulated. The blockade of Ca^{2+} currents by Pb^{2+}, but not Zn^{2+}, was voltage dependent and increased with depolarization. In both cases, the block was concentration dependent. It was suggested that Pb^{2+} competes with Ca^{2+} at a binding site within the channel (Büsselberg et al., 1990b, 1991b).

Pb^{2+} blocked sustained and transient voltage-activated Ca^{2+} channel currents of cultured rat DRG cells. The IC_{50} for inhibition of the peak current induced by depolarization from −80 to 0 mV was 0.64 μM, compared to an IC_{50} of 2.23 μM for Cd^{2+}. Low threshold currents (T-type) activated by depolarization from −100 to −30 mV were blocked by Pb^{2+} at IC_{50} 6 μM. The block progressed in the absence of channel activation and showed little voltage dependence. Therefore, the site of action of Pb^{2+} is probably not the same site bound by Cd^{2+}; the latter ion blocks the Ca^{2+}-channel binding at the site located within the channel. It is suggested that the site of Pb^{2+} action is intracellular, where protein kinase C could be activated (Evans et al., 1992a).

Neither Pb^{2+} nor Cu^{2+} shifted the current-voltage relationship on *Helix* neurons. Pb^{2+} at 50 μM blocked fully I_{Ca}, whereas 5 μM Cu^{2+} caused only a 50% block. Moreover, Cu^{2+} shifted the steady-state inactivation curve to the right along the voltage axis (Osipenko et al., 1992a).

On *Lymnaea* neurons, Pb^{2+} irreversibly inhibited the Ba^{2+} current by 60% between 0.25 and 14 μM, in a manner that was independent of the Pb^{2+} concentration. The voltage dependence of the current, similar to the observations on *Helix*, appeared to be unaffected by Pb^{2+}. At sufficient concentrations Cd^{2+} and Co^{2+} completely blocked Ca^{2+} channels and the residual outward current that remains in Ba-tetraethylammonium saline. This implies that Pb^{2+} affects Ca^{2+} channels in a fundamentally different way, perhaps by binding to a different site on the channel (Audesirk, 1987).

Pb^{2+} (1 to 300 μmol/liter) blocked all types of contractile responses and as well as calcium current on single muscle fibres of the crayfish. The effect of Pb^{2+} was concentration and time dependent. The blocking effect was more pronounced on the fast-inactivating Ca^{2+} current component at lower concentrations (100 μmol/liter). The Pb^{2+} ions prolonged the time constant of inactivations of the slow channel but left that of the fast channel unchanged (Zacharova et al., 1993).

In vivo exposure of *Lymnaea* neurons to Pb^{2+} for 6 to 12 weeks caused a 2-fold increase in Ca^{2+} currents. The probable explanation is an upregulation of Ca^{2+} channel density (Audesirk, 1987). Chronic exposure to Pb^{2+} had an overall effect on electrical parameters of the neurons, i.e., a significant interaction between Pb^{2+} and neuron type was observed, increasing excitability in some neuron types and depressing excitability in others (Audesirk and Audesirk, 1983, 1984).

The chronic Pb^{2+} exposure of cell cultures of mouse DRG neurons caused a decreased excitability, an increased resting membrane potential, and a decreased membrane time constant. The effect was explained either by a direct Pb^{2+} effect at membrane channels or by an indirect effect, namely, by augmentation of intracellular Ca^{2+} concentration (Scott and Lew, 1985).

Thévenod and Jones (1992) showed that Cd^{2+} blocked Ca^{2+} currents in frog sympathetic neurons by 50% at 300 nM concentrations. Previous studies have shown that blockade of open Ca^{2+} channels by Cd^{2+} was voltage dependent and that this was reversed at negative voltages (Brown et al., 1983). This is expressed as a decrease in mean closed times in single-channel records (Lansmann et al., 1986) and as a "hook" in tail currents (Swandulla and Armstrong, 1989; Chow, 1991). Many of the effects of Cd^{2+} can be explained by a binding to sites in the ion permeation pathway of the Ca^{2+} channel, but additional actions at the orifice of the channel also are possible (Almers and McCleskey, 1984; Taylor, 1988).

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The trivalent cation, gadolinium (Gd^{3+}), was reported to be a selective blocker of an N-type I_{Ca} in neuroblastoma × glioma hybrid cells. The current blocked by 0.5 to 5 μM Gd^{3+} was activated at potentials more positive than −35 mV. The effect was different from that caused by Cd^{2+} or other trivalent ions tested (Docherty, 1988). This selectivity was not shown on rat DRG cells, frog peripheral neurons, rat cardiac myocytes, or mouse skeletal muscle cells using Gd^{3+}, Lu^{3+}, and La^{3+} as inorganic blockers (Biagi and Enyeart, 1990; Lansman, 1990; Boland et al., 1991). In rat DRG neurons, however, a fraction of the Ba^{2+} current appeared to be resistant to saturating concentrations of Gd^{3+} (50 to 100 μM) in the presence of bicarbonate. Bicarbonate modification of Gd^{3+} block occurred both before and after ω-conotoxin block of N-type currents, suggesting that Gd^{3+} was not selective for N-type I_{Ca} (Boland et al., 1991).

On C2 myotubes (mouse skeletal muscle) in patch-clamp experiments, a series of trivalent lanthanide cations (La^{3+}, Ce^{3+}, Nd^{3+}, Gd^{3+}, Dy^{3+}, and Yb^{3+}) at 5 to 100 μM caused the unitary Ba^{2+} current to fluctuate between fully open and shut states. The kinetics of channel blockade followed the predictions of a simple open channel block model in which the fluctuations of the single-channel currents arose from the entry and exit of blocking ions from the pore. Channel blockade produced by the lanthanides depends on membrane potential and can be relieved by hyperpolarizing the membrane (Lansman, 1990).

Dakin and Ruddock (1990) reported that Tb^{3+} ions mimic Ca^{2+} ions in suppression of the electroretinogram and hyperpolarization in fish retinal horizontal cells. The effects of Tb^{3+} are analogous to those of Ca^{2+} on retina; however, they differ from those of the synaptic blocker, Co^{2+}, which did not block the photoreceptor response (Cervetto and Piccolino, 1974).

Kasai and Neher (1992), using neuroblastoma glioma cells, found the following sequence of blocking Ca^{2+} channel currents (high-voltage activated): Gd^{3+} (39 nM) > La^{3+} (92 nM) > Cd^{2+} (1.4 μM) > Cu^{2+} (7.1 μM) > Mn^{2+} (85 μM) > Ni^{2+} (230 μM).

It was found that Al^{3+} blocks both the sustained and transient components of the Ca^{2+} current in rat DRG neurons. The effect was slowly developing (2 to 7 min) and the recovery was never complete. (It is interesting that the Al^{3+} effect on Na^{+} current is completely and easily reversed by washing with normal physiological saline.) A 50 to 100 μM Al^{3+} concentration was necessary to block 50% of the Ca^{2+} current. The pH of the external saline greatly influences the Al^{3+} effect. At higher pH values the blocking effect of Al^{3+} was facilitated. Frequently, a shift of the I-V curve was observed after Al^{3+} was applied (Büsselberg et al., 1993; Platt et al., 1993). Blocking by Al^{3+} of voltage-gated Ca^{2+} channels was found in brain synaptosomes (Nachsen, 1984; Koening and Jope, 1987) and in isolated atrial cells (Meiri and Shimoni, 1991). The blocking effect is pH, concentration, and use dependent in the micromolar range. The effect is rather specific but irreversible.

Comparison between data shown above and the values of the permeability constant for divalent cation complexes with different anionic groups of amino acids suggests that one of the possible binding sites of Ca ions, and therefore for divalent metal ions, contains a carboxylic group. This is supported by the fact that the sequence of binding of different ions to a carboxylic group in aqueous solution (Ni^{2+} > Co^{2+} > La^{3+} > Cd^{2+} > Mn^{2+} > Ba^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+}) resembles, with some species-specific variations, the analogous sequence for the Ca channel (Martell and Smith, 1977; Akaike et al., 1978; Kostyuk and Mironov, 1982).

2. Substitution of Ca^{2+} for other divalent cations. Fukuda and Kawa (1977) demonstrated that several divalent cations are able to carry inward current in nominally Ca^{2+}-free saline. On larval muscle fibre of a beetle, they found that Cd^{2+}, Zn^{2+}, Mn^{2+}, or Be^{2+} could be a current carrier, whereas Co^{2+}, Ni^{2+}, and Mg^{2+} did not appear to penetrate the membrane. These interactions partly reflect the physicochemical properties of the cations in the aqueous solution and the membrane.

In snail neuronal membranes placed in a Na^{+}-, Mg^{2+}-, and Ca^{2+}-free solution, Zn^{2+} could be a charge carrier through Ca^{2+} channels (Kawa, 1979; Oyama et al., 1982; Osipenko et al., 1992a), as could Ba^{2+}, Sr^{2+}, and Mn^{2+} (Byerly et al., 1985; Chesnoy-Marchais, 1985; Hess et al., 1986). The I-V relationships for each ionic current were shifted, according to the different stabilizing effects on the membrane. The order of effectiveness of the above mentioned ions on surface charge modification was Mn^{2+} = Zn^{2+} > Cu^{2+} > Ba^{2+} = Sr^{2+}.

In mammalian myocardial cells, Sr^{2+}, Ba^{2+}, and Mg^{2+} can carry the slow inward current. In contrast, Ni^{2+}, Co^{2+}, and Mn^{2+} appeared strongly to block this current between 2.0 and 10 mM (Kohlhardt et al., 1973).

Inward currents in several different cells can be carried by monovalent cations through the Ca^{2+} channel (Kostyuk and Kristhal, 1977; Kostyuk et al., 1983; Hess and Tsien, 1984; Almers et al., 1984; Fukushima and Hagiwara, 1985). The selectivity sequence of monovalent cations through the Ca^{2+}-free Ca^{2+} channel was Na^{+} > K^{+} > Rb^{+} > Cs^{+}.

Recently, it was observed, however, that in *Helix aspersa* neurons Co^{2+} (3 mM) and Ni^{2+} (0.5 mM) increased the Ca^{2+} current density (Kim and Woodruff, 1991). This effect was explained by the decreasing action of divalent cations on the inactivation of Ca^{2+} channels or by the blocking of the remaining outward currents.

The data suggest that the Ca^{2+} channel does not make a distinction between "blocking" and "permeant" ions but only identifies quantitative differences among inorganic ions in the rates at which they enter and leave the channel pore (Lansman et al., 1986; Lansman, 1990).

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D. Proton-induced Channel

Voltage-dependent hydrogen ion movements have been detected in a number of tissues (Thomas and Meech, 1982). Rapid increases in extracellular proton (H^+ ion) concentrations induced a transient inward current carried by Na^+ ions in different nerve cells (Kristhal and Pidoplichko, 1980; Gruol et al., 1980; Grantyn and Lux, 1988; Davies et al, 1988). This current, in chick DRG neurons, was blocked by divalent cations, and it was suggested that proton channels appear prior to voltage-dependent Na^+ or Ca^{2+} channels during development (Gottmann et al., 1989). A number of agents block H^+ currents, but the most effective are heavy metal cations, such as Cd^{2+}, La^{3+}, and Zn^{2+}, which also block Ca^{2+} currents, although at higher concentrations. Because proton-induced Na^+ currents were blocked not only by inorganic but also by organic Ca^{2+} channel blockers, it was suggested that sodium current flows through the Ca^{2+} channel (Konnerth et al., 1987). In *Helix* neurons, H^+ currents were blocked by 16 μM Zn^{2+}. The rapid effect of Zn^{2+} on the H^+ current suggests that the receptor for Zn^{2+} is situated on the extracellular membrane surface. In addition, the lack of voltage dependence of the block supports a surface action for Zn^{2+} rather than an effect in the voltage field (Mahaut-Smith, 1989).

III. Heavy Metal Effects on Ca^{2+}-activated Currents

A. Ca^{2+}-activated K^+ Channel

There are a number of currents that are activated by an increase in intracellular Ca^{2+} concentration: $I_{K(Ca)}$, $I_{Cl(Ca)}$, $I_{Ca(Ca)}$, etc. As originally found by Gorman and Herman (1979), Ca^{2+} evokes $I_{K(Ca)}$, as do other metal ions as well (table 1).

The effect of intracellularly injected heavy metal ions was studied on molluscan (*Aplysia californica*) pacemaker neurons, R_{15} and L_6. Ca^{2+} was the most effective in the activation of $I_{K(Ca)}$, followed by Cd^{2+}, Hg^{2+}, Sr^{2+}, Mn^{2+}, and Fe^{2+}. Injections of Ba^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, and Zn^{2+} were ineffective. The average intracellular divalent cation concentration produced following injection was about 370 μM. The effectiveness of a divalent cation in activating $I_{K(Ca)}$ was partly related to its ionic radius. These findings were supported by data of Müller et al. (1989) on a similar preparation (pacemaker neurons RPa1 or F_1 and U- or D-neurons from *Helix pomatia* brain). Divalent cations were injected intracellularly using a technique allowing large amounts to be injected, i. e., up to 5 mM based on cell volume calculation. Ca^{2+} was also most effective in activating a nonspecific (inward) cation current and two types of (outward) K^+ currents ($I_{K(Ca)}$) found in these cells. Inward and outward currents were parallelled by reversible increases in membrane conductance. Nonspecific current was carried by many cations, including the larger species, such as choline, tetraethylammonium, and tris(hydroxymethyl)-aminomethane, whereas Cl^- did not contribute significantly to these currents. One type of $I_{K(Ca)}$ was quickly activated following injections with increasing effectiveness for divalent cations of ionic radii that were close to the radius of Ca^{2+} ($Ca^{2+} > Cd^{2+} > Hg^{2+} > Mn^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+} > Pb^{2+} > Sr^{2+} > Mg^{2+} > Ba^{2+}$).

TABLE 1
Summary of metal actions on membrane ionic permeability

Channel type	Metals	Metal concentration	Effect on current	Proposed binding site
Voltage-activated channels				
I_{Na}	Mn, Co, Ni, Cd, Zn, La,	0.1–10 mM	Decrease	Binding to channel protein sulfhydryl or carboxyl group
	Mg, Ba, Ca, Hg	1–10 μM	Decrease	
I_K	Zn, Ni, Cd, Mn, Cu, La, Ca, Hg	0.1–10 mM	Decrease	Surface charge screening or channel protein amino group
I_A	Hg, Zn, Cd, Mn, Co, Ca, Mg	0.01–40 mM	Decrease	Channel protein sulfhydryl group or phospholipid
I_{Ca}	Co, Cd, La, Mg, Ni, Zn, Pb, La	0.01–40 mM	Decrease	Competition with Ca at the binding site
Ca-activated channels				
I_K	Cd, Hg, Mn, Fe, Pb, Zn	100 μM	Activation	Binding to channel protein
I_{Cl}	Cd, Ni, Zn, Co, Mn, Cr, Sr, Ba, Mg	5–10 μM	Activation	Cyclic nucleotide (?)
I_{Ca}	Hg, Cu, Cd, Ag, Ni			Binding to channel protein sulfhydryl group
Ligand-gated channels				
GABA	Zn, Cd, Co, Cu, Mn, Ni,	1–100 μM	Decrease	Two different binding sites
	Pb, La	1–100 μM	Increase	
NMDA	Mg, Zn, Cd, Ni, Co, Mn, Hg, Al, La	1–100 μM	Decrease	Receptor or channel protein imidazole or histidine group
5-HT	Cd, Zn	1–100 μM	Decrease	?
Dopamine	Pb, Cd, Hg	1–5 μM	Decrease	Adenyl cyclase
ACh	Cu, Zn, Hg, Pb, Mg, Ca, Sr, Mn, Ni	1–1000 μM	Decrease	Receptor protein sulfhydryl group
ATP	Zn	10 μM	Decrease	

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The other type of $I_{K(Ca)}$ was activated with a delay by $Ca^{2+} > Sr^{2+} > Hg^{2+} > Pb^{2+}$; Mg^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} were ineffective.

A comparison of the properties of Ca^{2+}-sensitive proteins related to the binding of divalent cations suggests that a Ca^{2+}-binding protein of the calmodulin/troponin C type was involved in Ca^{2+}-dependent activation of the fast-activated type of $I_{K(Ca)}$. The sequence obtained for the slowly activated type is compatible with the effectiveness of different cations in activating protein kinase C. The nonspecific cation current was activated by $Ca^{2+} > Hg^{2+} > Ba^{2+} > Pb^{2+} > Sr^{2+}$, a sequence unlike sequences for known Ca^{2+}-binding proteins (Müller et al., 1989).

Similar observations for metal ion activation of $I_{K(Ca)}$ were made on the human red blood cell (Shields et al., 1985) for Pb^{2+} (10 μM) and on cultured canine kidney epithelioid cells (Jungwirth et al., 1991) for Hg^{2+} (0.2 μM). Furthermore, Shields et al. (1985) found that the activity of single $I_{K(Ca)}$ can also be inhibited by high Pb^{2+} concentrations (100 μM), and it was concluded that Ca^{2+} and Pb^{2+} independently activate the same $I_{K(Ca)}$ channel. Jungwirth et al. (1991) showed that higher concentrations of Hg^{2+} (>10 μM) eventually depolarized the cell membrane, and it was proposed that Hg^{2+} activates $I_{K(Ca)}$ by a mechanism independent of an increase of intracellular calcium activity and of cholera or pertussis toxin-sensitive G-proteins. This Hg^{2+} effect was abolished in the presence of dithiothreitol, a compound that is (sulfhydryl) reducing and is a mercury-chelating agent. Using mouse DRG neurons, Scott and Lew (1985) found that Pb^{2+} (μM) increased the resting membrane potential through a Pb^{2+}-induced augmentation of intracellular Ca^{2+}.

Leinders et al. (1992a) recently studied the effects of Cd^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+} (1 to 100 μM), and Pb^{2+} (1 to 90 μM) on single-channel properties of the small conductance and high conductance $I_{K(Ca)}$ channels using the inside-out membrane patches of N1E-115 mouse neuroblastoma cells. The potency of the metals in stimulating small conductance channel opening probability follows the sequence $Cd^{2+} \geq Pb^{2+} > Ca^{2+} > Co^{2+} \gg Mg^{2+}$, and Fe^{2+}. In the case of the high conductance channel, $Pb^{2+} > Ca^{2+} > Co^{2+} \gg Cd^{2+}$, Mg^{2+}, and Fe^{2+}. They also described distinct metal-binding sites on $I_{K(Ca)}$ in human erythrocytes using the same method (Leinders et al., 1992b). The potency of metals was the following: $Pb^{2+} > Cd^{2+} > Ca^{2+} > Co^{2+} \gg Mg^{2+}$, Fe^{2+} (1 to 10 μM Cd^{2+}, and Co^{2+}; 10 μM Fe^{2+} and Mg^{2+}; 1 μM Pb^{2+}). At the higher (90 to 100 μM) concentrations, Pb^{2+}, Cd^{2+}, and Co^{2+} also blocked $I_{K(Ca)}$ by reducing the opening frequency (with potency $Co^{2+} \geq Cd^{2+} > Pb^{2+} \gg Ca^{2+}$) and the single-channel current amplitude ($Cd^{2+} > Pb^{2+} > Co^{2+}$). Fe^{2+} altered the channel-opening frequency and the two open times, whereas Mg^{2+} had no effect on any of the single-channel parameters. They concluded that various metal ions bind to the same regulatory site(s) at which Ca^{2+} activates the $I_{K(Ca)}$ under physiological conditions. Zn^{2+} ions, at submicromolar concentrations, however, irreversibly reduced the calcium-dependent potassium current in rat hippocampal neurons in vitro (Sim and Cherubini, 1990).

In excised membrane patches from human red blood cells, it was shown that low concentrations of Pb^{2+} (10 μM) evoke that same single-channel current as does Ca^{2+}. High concentrations of Pb^{2+} (100 μM) block the Ca^{2+}-activated K^{+} current. It was concluded that both Ca^{2+} and Pb^{2+} independently activate the same K^{+}-selective channels in the erythrocyte membrane (Shields et al., 1985).

B. Ca^{2+}-activated Cl^{-} Channel

$I_{Cl(Ca)}$ could also be activated by metal ions. Miledi et al. (1989) showed on the oocytes from frog *Xenopus laevis* that $Cd^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Cr^{2+} > Sr^{2+} = Ba^{2+} = Ca^{2+}$: Mg^{2+} (thresholds of 5 to 10 μM) interact with the cell surface to increase cytosolic levels of inositol phosphates. This causes mobilization of intracellular Ca^{2+}, in turn activating Ca^{2+}-gated Cl^{-} channels in the plasma membrane. Although extracellular application of metal ions consistently elicited currents, in the same oocytes intracellular injections either gave no responses or activated very small currents, which might occur as a result of the leakage from the cell. In addition to these currents, divalent cations generated maintained currents associated with decreases in membrane conductance; however, size and the ionic basis of these currents varied between oocytes. Moreover, Zn^{2+} also elicited current associated with an increase in membrane conductance which was carried predominantly by K^{+} ions. Likely, Zn^{2+} interacted with a cyclic nucleotide-activated K^{+}-gating mechanism.

C. Ca^{2+}-activated Ca^{2+} Channel

$I_{Ca(Ca)}$ was originally found in the sarcoplasmic and endoplasmic reticulum of muscular cells. The caffeine-sensitive Ca^{2+} release channel (ryanodine receptor) is homologous to the inositol (1,4,5)-trisphosphate receptor, both proteins have the same subunit structure, and both bind ATP and calmodulin and conduct Ca^{2+} as well as Na^{+}. However, it was shown that inositol (1,4,5)-trisphosphate not only activates $I_{Ca(Ca)}$ in the endoplasmic reticulum but also activates Ca^{2+} entry pathways in the plasma membrane (Tepikin and Peterson, 1992). Furthermore, in sarcoplasmic reticulum from rabbit psoas fibres, for example, Salama et al. (1992) described Ca^{2+} release induced by metal ions and mercaptans (cysteine, cysteamine, and homocysteine). Metal ions (at 2 to 5 μM) elicited phasic contractions by triggering Ca^{2+} release from sarcoplasmic reticulum and had the following order of potency: $Hg^{2+} > Cu^{2+} > Cd^{2+} > Ag^{+} > Ni^{2+}$. Contractions induced by metal ions were blocked by ruthenium red and were inhibited by free Mg^{2+} and

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sulfhydryl-reducing agents such as dithiothreitol or reduced glutathione which reduced the newly formed disulphide bonds.

These results were consistent with the notion that Ca^{2+} release channels, in the closed state, contain sulfhydryl group(s) which would not be expected to react with exogenously added mercaptans unless a catalyst (metal ions) was available to promote the oxidation of the fixed sulfhydryl sites on the proteins with the exogenously added sulfhydryl sites on cysteine molecules. The formation of mixed disulphide bonds resulted in the opening of the channel and Ca^{2+} release. Similar findings were shown at plasma membranes of bovine eye rod outer segments (Schnetkamp and Szerencsei, 1989) in the presence of Ag^+ (50 μM). The Ag^+-induced Ca^{2+} release pathway proved to be broad with little discrimination existing between Li^+, Na^+, K^+, and Cs^+ or between Ca^{2+} and Mg^{2+}. The properties of this pathway likely suggested that it may reflect the cyclic guanosine 3′,5′-monophosphate-dependent conductance opened in the absence of cyclic guanosine 3′,5′-monophosphate by Ag^+.

IV. Metal Ion Effects on Ionic Pumps

It is known that pumps could also be a target of metal ion action. The Na^+-K^+-ATPase of avian (chick) and mammalian (bovine) brain was inhibited by Cu^{2+} (Ting-Bell et al., 1973; Prakash et al., 1974), and inhibition of the Na^+-K^+-ATPase has been suggested as an important factor in the depolarizing action of Cu^{2+} on cat central neurons (Dreifuss et al., 1969). The active form of vanadium (VO_4^{3-}) was also a well-known inhibitor of Na^+-K^+-ATPase (μM) in many preparations (Chasteen, 1983). Brain microsomal Na^+-K^+-ATPase activity was also specifically inhibited by cations in the order $Zn^{2+} > Cu^{2+} > Fe^{2+} > Mn^{2+}$ in vitro as well as in vivo (Chvapil et al., 1974).

Anner et al. (1992) described the blocking effect of Hg^{2+} (IC_{50} 200 nM) on Na^+-K^+-ATPase obtained from the outer medulla of rabbit, rat, or rat kidneys. The IC_{50} was modulated by the presence of ethylenediaminetetraacetic acid (IC_{50} 20 μM) as well as by the pump ligands Mg^{2+}, Na^+, K^+, and ATP. The Hg^{2+} chelator 2,3-dimercapto-1-propanesulfonic acid was able to reactivate approximately 70% of the blocked enzyme, whereas cysteine blocked only approximately 10%. Taken together, these data indicate that the stability of the enzyme-mercury complex lies between the stability of monothiol-mercury and dithiol-mercury complexes (Anner and Moosmayer, 1992). It was shown that Hg^{2+} weakens the membrane anchoring of the α-subunit of Na^+-K^+-ATPase (Imesch et al., 1992). A dilution assay, combined with sided ATP addition and flux measurement in the symmetrically reconstituted liposomes, indicated that Hg^{2+} acted primarily at the cytoplasmic side (Anner and Moosmayer, 1992).

Similar data were published with respect to Ca^{2+}-Mg^{2+}-ATPase. Chao et al. (1990), using rabbit back and leg muscle myofibrils, showed that activation of myofibrillar Ca^{2+}-Mg^{2+}-ATPase by Cd^{2+} and Pb^{2+} (100 μM) is mediated through troponin C. Abramson et al. (1983) found that in sarcoplasmic reticulum vesicles from rabbit white skeletal muscle Cu^{2+} (IC_{50} 2 μM), Cd^{2+} (15 μM), Zn^{2+} (20 μM), Hg^{2+} (4 μM), Ag^+ (10 μM), and CH_3Hg^+ (30 μM), but not Ba^{2+}, stimulated Ca^{2+}-Mg^{2+}-ATPase activity and inhibited the active Ca^{2+} uptake. In the same preparation, N-ethylmaleimide, a relatively specific sulfhydryl reagent, caused a release of Ca^{2+} at a somewhat higher concentration (40 μM). Ca^{2+} release could also be triggered by oxidation of a sulfhydryl group to a disulfide group. Incubation with cupric phenanthroline, which catalyses air oxidation of sulfhydryl groups to disulfides, leads to a large increase in Ca^{2+} permeability of the membrane.

Vanadate is a well-known inhibitor of Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase in many preparations (Cantley et al., 1978; Chasteen, 1983; Benaim and Romero, 1990; Kostyuk et al., 1989). Hechtenberg and Beyersmann (1991) studied the effect of Cd^{2+}, Pb^{2+}, and Hg^{2+} on the Ca^{2+}-ATPase activity present in the sarcoplasmic reticulum from rabbit muscle. As a result, ATP hydrolysis was found to be inhibited with an IC_{50} of 950 nM free Cd^{2+} or 95 nM free Pb^{2+}. Although calculation of the free Hg^{2+} was difficult, the comparison of the IC_{50} values for total metal ions showed that Hg^{2+} is the strongest inhibitor of enzyme activity. The inhibition by Cd^{2+} seems to be independent of substrate concentration, whereas the inhibitory effect of Pb^{2+} is decreased in the presence of higher Mg^{2+}-ATP concentrations. The data illustrate that the three heavy metals are potent inhibitors of the Ca^{2+} pump. Low concentrations of these metal ions, therefore, may disturb intracellular Ca^{2+} homoeostasis and act on Ca^{2+}-mediated cell functions.

On *Helix* neurons, it was observed that lowering the temperature slowed the activation of I_{Cu} (copper-activated current; see section VI) and the activation and inactivation of I_W (washout current, see section VI). Furthermore, at 7°C the transient component of I_W was blocked, which suggested the participation of the pump mechanism in generating both the I_{Cu} and I_W. The cardiac steroid ouabain, a known electrogenic Na^+/K^+ pump blocker, induced an increase of the Cu^{2+}-activated current amplitude. Verapamil, ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and Ca-free saline, however, which block the Na^+/Ca^{2+} exchange mechanisms, decreased the transient component of I_W and increased the inward I_{Cu}. The results suggest the participation of both pumps (Na^+/K^+ and Na^+/Ca^{2+}) in Cu^{2+}-induced permeability changes (Kiss et al., 1991).

Hg^{2+} (*p*-chloromercurobenzene sulfonate, mM) (Gerencser, 1990a), as well as V^{2+} (Gerencser, 1990b), blocked active electrogenic Cl^- transport in *Aplysia* gut; surface (sulfhydryl) groups also appeared to be affected, because

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dithiothreitol (a specific thiol-reducing agent) reversed this inhibition (Gerencser 1990a, 1990c).

Taken together, all of these experiments suggests that the ionic pumps have metal-binding properties with particularly high affinity for Hg^{2+}, Pb^{2+}, and V^{2+}. Targets of heavy metal actions are presumably sulfhydryl groups located predominantly on the cytoplasmic side of the membrane, at least in the case of the Hg^{2+}.

V. Effects on Agonist-operated Channels

A. γ-Aminobutyric Acid-activated Conductances

It is well known that Zn^{2+} is present in synaptic vesicles in the central nervous system; there also is evidence that it can be released together with transmitters (Draguhn et al., 1990; Anikszteјn et al., 1987; Slovifer, 1985; Assaf and Chung, 1984; Howell et al., 1984; Frederickson, 1989). In the mammalian brain, Zn^{2+} is distributed unequally; the highest amounts are in the neocortex and hippocampus. The highest concentrations of Zn^{2+} are encountered in the giant boutons of the mossy fiber system (Vallee and Falchuk, 1993). Therefore, it can be expected that, in addition to the physiologically occurring cations, other ones are also able to modify transmitter receptor function (Yakushiji et al., 1987).

The effect of Zn^{2+} was studied on cultured rat sympathetic and cerebellar neurons (Smart, 1992), on cultured rat hippocampal neurons (Legendre and Westbrook, 1991), and on DRG neurons of vertebrates (Akaike, 1989; Ma and Narahashi, 1993a). The antagonism of GABA-induced membrane effects by Zn^{2+} is subject to developmental influence. Embryonic neurons are more sensitive to the depression by Zn^{2+} compared to adult neurons. This developmentally sensitive aspect of $GABA_A$ receptor pharmacology may be partly dependent on expression of the α-subunit. Similarly, GABA-induced chloride current in frog DRG neurons was blocked not only by Zn^{2+} but also by Cd^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, and Ni^{2+}. As observed in lobster muscle (Akaike et al., 1987), the inhibition is noncompetitive with picrotoxin.

There are at least two GABA receptor subtypes composed of combinations of three different subunits, α, β, or γ (reviewed by Barnard et al., 1987). Receptors composed of α- and β-subunits are very sensitive to Zn^{2+}, whereas those containing α-subunit are insensitive (Draguhn et al., 1990). Recent studies have clearly demonstrated that polyvalent cations modulate the GABA receptor channel complex in rat DRG neurons. La^{3+} augmented the GABA-induced current, whereas Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, and Pb^{2+} decreased the current. However, the suppression effect of La^{3+} was observed in dorsal horn cells of the rat (Reichling and MacDermott, 1991).

Experiments with agonist compounds revealed that at least La^{3+}, Cu^{2+}, and Zn^{2+} did not act on any of the GABA, barbiturate, benzodiazepine, and picrotoxin sites. They appeared to act on novel sites at or near the external orifice of the chloride channel. It was suggested that Cu^{2+} and Zn^{2+} share a common binding site, whereas La^{3+} binds to a separate site (Ma and Narahashi, 1993a, 1993b). Similarly, Co^{2+}, Ni^{2+}, and Cd^{2+} noncompetitively blocked the GABA-induced chloride current, recorded from turtle retina, whereas Mg^{2+}, Mn^{2+}, Sr^{2+}, and Ba^{2+} had no effect (Kaneko and Tachibana, 1986). Hg^{2+} at 1 to 10 μM greatly enhanced the GABA-activated current of rat DRG neuron. The effect was similar to that caused by La^{3+} except that the Hg^{2+} effect was irreversible (Arakawa et al., 1991).

On rat DRG neurons, the effect of a series of lanthanides (Lu^{3+}, Er^{3+}, Tb^{3+}, Eu^{3+}, Nd^{3+}, Ce^{3+}, and La^{3+}) was studied on the GABA receptor channel complex. All of the lanthanides tested have been found to augment the GABA induced Cl^- current, an action that correlated inversely with the hydrated ionic radii of these ions (Ma and Narahashi, 1993b).

The competitive character of Zn^{2+} (100 μM) action was also described in the DRG of the bullfrog (*Rana catesbiana*). This effect was accompanied by relatively little alteration in both the rising and the falling phases of the response (Akaike, 1989). In CA1 and CA3 pyramidal neurons in adult rat hippocampal slices, Zn^{2+} hyperpolarized pyramidal neurons, increased the membrane excitability, and evoked giant depolarizing potentials. It was concluded that giant depolarizing potentials were the result of a Zn^{2+}-induced synchronized GABA release (Xie and Smart, 1993).

There are several possible explanations for the modulation of GABA-induced responses by metal ions. First, formation of a metal ion-GABA complex decreases the available concentration of GABA, thereby reducing the GABA-induced response. However, the noncompetitive inhibition of GABA-induced responses by metal ions do not support this view (Smart and Constanti, 1990; Kilic et al., 1993). Second, metal ions might occlude the channel at its external orifice, an observation supported by high potency, voltage independence, and ineffectiveness when applied intracellularly (Celentano et al., 1991).

To elucidate the structural requirements for the modulation of $GABA_A$ receptors by Zn^{2+}, recombinant experiments were conducted using different $GABA_A$ receptor subunits, and subsequently the effect of Zn^{2+} was determined on GABA-activated currents (Draguhn et al., 1990). For example, Smart et al. (1991), using human kidney cells (A293), found that $GABA_A$ receptors that lacked the α-subunit were inhibited by Zn^{2+} (threshold 0.3 μM and K_D 1 μM) in a noncompetitive manner. It was found that Zn^{2+} reversibily inhibited the GABA response in the lobster muscle, depressing the GABA-induced chloride-dependent conductance increase. The metal-binding site differs from that of picrotoxin, because the effects of the two antagonists were additive (Smart and Constanti, 1982). The presence of an α-subunit in any

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combination with the other subunits led to the formation of GABA receptors that were almost insensitive to Zn^{2+}.

Although much of the research concerning Zn^{2+} and GABA transmission has focused on the $GABA_A$ receptor, there is also some evidence for Zn^{2+} modulation of $GABA_B$ receptor activity (Xie and Smart, 1991; Drew et al., 1984). Binding experiments showed that Zn^{2+}, at concentrations exceeding 100 μM, noncompetitively inhibited $GABA_B$ binding. Increased Ca^{2+} concentrations did not prevent Zn^{2+}-induced inhibition, indicating a separate site of action for these cations (Turgeon and Albin, 1992). At low concentrations, however, Zn^{2+} enhanced $GABA_B$ receptor binding. Therefore, the biphasic effect of Zn^{2+} on $GABA_B$ binding suggested that here may be two modulatory sites for Zn^{2+}. This suggestion is supported by observations that one group of divalent cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, and Ni^{2+}) enhanced [^{3}H]baclofen binding (Kato et al., 1983), whereas others (Hg^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}) inhibited it (Drew et al., 1984).

B. Glutamate-activated Conductances

The NMDA-selective subtype of glutamate receptor is a ligand-gated ion channel that conducts cations into and out of the neurons. Studies during the last decade have revealed that, in addition to the binding site that recognizes agonists, there are a variety of additional ligand-binding sites that serve to recognize endogenous modulators and xenobiotics (Collingridge and Lester, 1989; Reynolds, 1993; Seeburg, 1993).

Mg^{2+}, at physiological concentrations, produced a voltage-dependent inhibition of NMDA receptor activity of vertebrate neurons (Nowak et al., 1984; Mayer et al., 1984). Similar properties of Zn^{2+} (5 μM) and Pb^{2+} (5 μM) were described on the glutamate-evoked (NMDA) responses in hippocampal neurons (Westbrook and Mayer, 1987; Mayer and Vyklicky, 1989; Alkondon et al., 1990). Zn^{2+} is a noncompetitive NMDA antagonist with a K_D of approximately 5 μM on hippocampal neurons from mouse embryos in culture (Westbrook and Mayer, 1987; Peters et al., 1987; Yeh et al., 1990). At 50 μM Zn^{2+} blocked the NMDA current, whereas responses to kainic acid and quisqualic acid were slightly potentiated.

Mg^{2+} binds to a site within the NMDA receptor channel, producing a voltage-dependent block. This behaviour is consistent with an action as a positively charged channel blocker. Similar effects against NMDA were also observed with Cd^{2+}, Ni^{2+}, Co^{2+}, and Mn^{2+} (10 μM). This contrasts with the irreversible reduction of the response of acutely dissociated hippocampal neurons to kainate by micromolar quantities of Hg^{2+} or 5 to 10 mM Zn^{2+}.

Neither glycine (10 to 100 μM) nor Ca^{2+} (10 mM) reversed the effect of Pb^{2+}. The actions of Pb^{2+} on NMDA channel currents of acutely dissociated hippocampal neurons (CA1, CA3) from rats activated by aspartate plus glycine were examined by Uteshev et al. (1993). Two different stages of Pb^{2+} modulation were observed: a fast and reversible phase and a slowly developing irreversible decrease of aspartic acid/glycine-activated inward current. The decrease showed no voltage dependence. It was suggested that Pb^{2+} binds to the NMDA-glycine receptor complex, modulating in this way the NMDA channel activity. Pb^{2+} also inhibited the [^{3}H] MK-801 binding to rat hippocampal membranes in vitro. These data suggested the presence of a third regulatory site on the NMDA receptor channel complex in addition to those found for Mg^{2+} and glycine. Although Zn^{2+}, Cd^{2+}, and Hg^{2+} all react with sulfhydryl bonds, this seems unlikely to underlie the heavy metal ion action on the NMDA receptor channel. Zn^{2+} is known to coordinate with other side chain residues in proteins, including the imidazole ring in histidine, but further work will be required to resolve its site of action at a molecular level. Results of Baba et al. (1991) suggested that intracellular Zn^{2+} is essential for the agonist-induced translocation of protein kinase C in guinea pig synaptosomes.

In cultured rat hippocampal neurons, responses to NMDA, L-aspartate, and glutamate, recorded with glycine in the extracellular fluid, were antagonised by 50 μM Zn^{2+}. Responses to kainate, quisqualate, and glutamate (in glycine-free saline) were potentiated by 50 μM Zn^{2+} but were partially antagonized in the presence of 1 mM Zn^{2+}. Cd^{2+} had a similar, although less potent, action. It antagonized the NMDA responses but potentiated those to 50 μM kainate and quisqualate. Hg^{2+} (5 μM) increased the leakage current with no reduction of the NMDA response. The data indicate that the NMDA receptor channel complex contains at least two distinct binding sites for divalent cations: one with selectivity for Mg^{2+} and related ions and another at which Cd^{2+} and Zn^{2+} act as noncompetitive receptor antagonists (Mayer et al., 1989).

Koening and Jope (1987) proposed that Al^{3+} interacts competitively with the Ca^{2+} channel-binding sites that influence either the pore structure itself or the phospholipid domain in the immediate vicinity of the channel. The latter effect may allow Al^{3+} to modify an intramembranal G-protein, which regulates Ca^{2+} channel function or protein kinase C activity, resulting in a glutamate release. Provan and Yokel (1992) reported that Al^{3+} decreases Ca^{2+} channel function in the neurons of rat hippocampus, thereby affecting learning and memory. The inhibition of the release of glutamate by Al^{3+} is attained by more than one mechanism involving Ca^{2+} and other signal transduction mechanisms of neuronal activity.

The effects of La^{3+} on excitatory amino acid-evoked currents were characterized by Reichling and McDermott (1991) using cultured or acutely dissociated neurons from the dorsal horn of the rat spinal cord. The La^{3+} antagonism of currents evoked by NMDA was potent, with an EC_{50} of 2 μM. The block of NMDA currents was voltage independent and noncompetitive with re-

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spect to activation of the NMDA receptor. La^{3+} had both enhancing and blocking actions on currents evoked by kainate or by quisqualate; concentrations of La^{3+} between 1 and 100 μM enhanced kainate and quisqualate currents, whereas the currents were blocked by concentrations of La^{3+} >100 μM. An enhancing dose of La^{3+} shifted the dose-response curve for kainate to lower concentrations of agonist without changing the maximum evoked current, and a similar leftward shift of the quisqualate dose-response curve occurred at nonsaturating concentrations of quisqualate. This enhancement might occur because of either an increased affinity of the receptor for the ligand or to an increased concentration of ligand at the membrane surface; the latter effect could result from a reduction in the membrane surface charge. Zn^{2+} mimicked the effects of La^{3+} on excitatory amino acid-evoked currents in dorsal horn neurons but was less potent both as a blocker and as an enhancer. This suggests that La^{3+} and Zn^{2+} could act with different potencies at the same site.

When studying *Helix pomatia* neurons (RPa1, LPa3) Belan and Osipenko (1991) observed strong and fast inhibitory effects of extracellular La^{3+} application (50 to 1000 μM) on cyclic AMP-induced currents as well effects on the increasing of intracellular Ca^{2+} by cyclic AMP injection. The cyclic AMP-induced current is a good model of a postsynaptic transmitter effect (Kononenko et al., 1986; Kononenko and Shcherbatko, 1985), because it is independent from extracellular events (transmitter release, binding, etc); Cd^{2+}, however, only partially blocked cyclic AMP-induced current.

Long-term potentiation consists of an activity-dependent increase in synaptic efficiency and is thought to be a good model of learning and memory at the synaptic level. Activation of the NMDA subtype of the glutamate receptor is a primary means of triggering this potentiation by Ca^{2+} influx through the receptor-operated ion channel (Collingridge and Bliss, 1987; Malenka et al., 1988). It was found that acute as well as chronic exposure of hippocampal neurons to Pb^{2+} ions impaired long-term potentiation (Altmann et al., 1991; Lasley et al., 1993; Hori et al., 1993). One explanation for this could be the ability of Pb^{2+} ions to block presynaptic Ca^{2+} channels or, alternatively, the receptor-operated Ca^{2+} channels. This explanation is probably valid in acute experiments; however, in the case of chronic exposure to Pb^{2+}, further investigation is required to delineate the mechanism underlying the Pb^{2+}-induced effect on long-term potentiation (Lasley et al., 1993).

C. 5-Hydroxytryptamine-activated Conductances

The effect of micromolar concentrations of divalent metal cations on 5-HT-induced current was investigated on NCB-20 mouse neuroblastoma cells using the whole-cell configuration of the patch-clamp technique (Lovinger, 1991). The group IIb metal cations Cd^{2+} and Zn^{2+} and the group Ib cation Cu^{2+} inhibited 5-HT-induced current in a concentration-dependent manner over micromolar concentrations of the ions. The order of potency of the ions for inhibiting 5-HT current was Zn^{2+} (IC_{50} = 20 μM) ≥ Cu^{2+} (IC_{50} = 25 μM) > Cd^{2+} (IC_{50} = 75 μM) at −50 mV. The other divalent metal cations tested (Ba^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, and Ni^{2+}) produced little or no inhibition of the 5-HT-response at concentrations up to 200 μM. Inhibition of 5-HT responses by Cd^{2+} and Zn^{2+} was dependent on membrane potential with the K_D increasing e-fold per 72 and 52 mV, respectively. Inhibition by Cu^{2+} was much less voltage dependent with the K_D increasing e-fold per 233 mV. Inhibition by all three cations decreased with increasing concentration of agonist over a range of 5-HT concentrations from 1 to 10 μM.

Data suggest that metal ions may inhibit ion current through 5-HT receptor-linked ion channels in different ways. Cd^{2+} and Zn^{2+}, but not Cu^{2+}, enter the electric field of the 5-HT receptor-coupled ionophore (Lovinger, 1991). Ca^{2+} and Mg^{2+} were found to inhibit the 5-HT-activated current in a voltage-independent way, and thus, the inhibition differed from that caused by Zn^{2+} and Cd^{2+} (Nash and Wallis, 1981; Peters et al., 1988). The inhibition by Ca^{2+} and Mg^{2+} could result from the surface charge screening which might alter the gating of the receptor-coupled ion channel.

D. Dopamine-activated Conductances

Dopamine-sensitive adenyl cyclase is inhibited by Pb^{2+}, Cd^{2+}, and Hg^{2+} at the submicromolar range (Ewers and Erbe, 1980). Muneoka et al. (1979) showed that 100 μM Zn^{2+} potentiated relaxation of the anterior byssus retractor muscle of *Mytilus edulis* in response to the 5-HT but not to dopamine. Studies of Cory-Slechta and Widzowski (1991) demonstrated that Pb^{2+} exposure produced a functional dopaminergic supersensitivity that involved both the D_1 and D_2 receptor subtypes.

On neurons of *Lymnaea stagnalis*, Cd^{2+} and Hg^{2+}, at micromolar concentrations, blocked the inhibitory effect of dopamine (S.-Rózsa and Salánki, 1987).

E. Acetylcholine-activated Conductances

ACh-induced responses have also been affected by extracellular application of Cu^{2+}, Zn^{2+}, Hg^{2+}, and Pb^{2+}. For example, Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, and Ni^{2+} decreased the current induced by ACh at the neuromuscular junction of the frog. Ni^{2+} and Sr^{2+} markedly lengthened the decay of the end-plate current, whereas Mg^{2+}, Ca^{2+}, and Mn^{2+} had little effect on it (Magleby and Weinstock, 1980). Chiarandini et al. (1967) reported that Cu^{2+} (100 μM) practically abolished the ACh inhibitory postsynaptic potential and ACh-evoked change of membrane potential in molluscan (*Cryptomphallus aspersa*) neurons. Muneoka at al. (1979) found that ACh-induced contraction was potentiated by 100 μM Zn^{2+}. Eldefrawi et al. (1975) found that Zn^{2+} binds to an sulfhydryl group

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on the ACh receptor of the *Torpedo* electric organ. Del Castillo et al. (1971) reported that sulfhydryl reagents, including organic mercurials, depolarized the frog neuromuscular junction by activating ACh receptors and then blocking the combination of ACh with its receptor.

Miledi et al. (1989), studying *Xenopus* oocytes, observed a potentiating effect of divalent cations on ACh responses (e.g., 1 mM Co^{2+}). Alternatively, Oortgiesen et al. (1990b) showed an inhibitory Pb^{2+} effect (1 μM) on ACh currents in the perfused mouse hemidiaphragm preparation. On frog neuromuscular junction, Pb^{2+} blocked the nicotinic receptor in a competitive manner (Manalis et al., 1984; Kostial and Lentner, 1957; Silbergeld et al., 1974; Atchison and Narahashi, 1984). However, on mouse N1E-115 neuroblastoma cells, Oortgiesen et al. (1990a) observed that the inhibitory Pb^{2+} effect (nM) is independent of ACh concentration. Pb^{2+}, in the micromolar range, directly blocked the postsynaptic response (Oortgiesen et al., 1990c).

It is accepted that the release of ACh elicited by membrane depolarization strongly depends on the extracellular Ca^{2+} concentration, whereas the spontaneous release of ACh presumably depends on the intracellular concentration of Ca^{2+} ions (Silinsky, 1985). The modulation by Zn^{2+} (0.1 to 5 mM) of ACh-activated ion channels at the neuromuscular junction of toad end-plate is similar to the effect produced by high concentrations of extracellular Ca^{2+}, in that both ions increase the decay time constant of the end-plate current and increase the mean open time.

Metal ions block depolarization-evoked release of ACh by blocking presynaptic Ca^{2+} currents (Cooper and Manalis, 1983). In contrast, in mouse motor nerve terminals, thallium (a monovalent cation) failed to block presynaptic Ca^{2+} or K^+ currents (Wiegand et al., 1990). Because the Th^+ ions produced an enhancement of the spontaneous release of transmitter, the Th^+-induced reduction of phasic transmitter release at the neuromuscular synapse must act through a separate mechanism (Wiegand, 1988).

The influence of Al^{3+} on the presynaptic release of ACh was examined using an isolated preparation of the frog neuromuscular junction. It was found that, following the addition of $AlCl_3$, no postsynaptic changes were encountered in the presence of Al^{3+} (Banin and Meiri, 1987). Prolonged exposure of the isolated neuromuscular junction to Al^{3+} revealed another effect of this substance—by reducing the life span of synaptic activity (Meiri et al., 1993).

F. Adenosine Triphosphate-activated Conductances

ATP is a mediator of sympathetic transmission in various smooth muscle tissues. Recently, it was shown that ATP can act as a fast excitatory transmitter at neuro-neuronal synapses both in the central nervous system and in the peripheral nervous system (Edwards et al., 1992; Evans et al., 1992b).

The ATP-activated current in cultured rat superior cervical ganglion (Cloues et al., 1993), as well as in mammalian nodose ganglion cells (Chaoying et al., 1993), was modulated by extracellular Zn^{2+}. ATP activated a nonspecific cation conductance that was potentiated by low (10 μM) concentrations of Zn^{2+}. Higher concentrations of Zn^{2+} reduced and prolonged the ATP-activated current, an observation consistent with open channel block. Several lines of evidence suggest that the stimulatory effect of Zn^{2+} on ATP-activated currents is attributable to an interaction with a modulatory site on the receptor ion channel complex. Two sites of action of Zn^{2+} were suggested: a positively adding allosteric site that enhances current amplitude and a site within the pore that blocks conductance through the channel.

Decavanadate applied to the cytoplasmic face of the membrane in mouse flexor digitorum muscles was a potent activator of ATP-sensitive K^+ channels (K_D 250 μM). Divalent cations (Mg^{2+}, Ca^{2+}) were a prerequisite for the activating process. The maximal effect was achieved using 1 mM vanadate, increasing the open-state probability about 9-fold. Vanadate shifted the dose-response curve of the channels for ATP towards higher ATP concentrations without affecting their slopes (K_D 4 and 65 μM, respectively; Weik and Neumcke, 1989; Neumcke and Weik, 1991). In skeletal muscle, these channels can also be inhibited by the intracellular application of reagents that modify sulfhydryl groups (Weik and Neumcke, 1989). Thus, vanadate and modifiers of sulfhydryl groups have antagonistic interactions. The active form of vanadate inhibiting Na-K-ATPase is orthovanadate, whereas stimulation of the ATP-sensitive K-channels was induced by decavanadate. However, although micromolar concentrations of vanadate inhibit the pump activity, millimolar concentrations of the total vanadate are necessary to stimulate the ATP-sensitive K channel (Chasteen, 1983; Neumcke and Weik, 1989). Pai et al. (1977) showed that vanadate binds to the phosphate-binding region of the ATP-binding site of adenylate cyclase.

VI. Metal Ion-activated Channels

A. Cu^{2+}-activated Conductances

Chiarandini et al. (1967) discovered that Cu^{2+} (100 μM) depolarises molluscan (*Cryptomphallus aspersa*) neurons by reducing the steady-state permeability to chloride ions. Furthermore, data indicated that this action was not achieved through a modification of the chloride pump. Cu^{2+} (100 μM) also depolarized cat cortical neurons (Dreifuss et al., 1969), and bathing the outside surface of frog skin completely inhibited chloride transport without changing sodium transport (Ussing and Zerahn, 1951).

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Weinreich and Wonderlin (1987) reported that 1 to 100 μM of Cu^{2+} produced a rapid and reversible steady-state depolarization of the *Aplysia* neuronal membrane by increasing the membrane permeability for sodium. This Cu^{2+}-activated (I_{Cu}) Na^+ current was not inhibited by voltage-activated Na^+ current blockers, such as tetrodotoxin (10 to 100 μM) and procaine (1 mM), whereas lidocaine (1 mM) reduced I_{Cu} by 68 ± 15%. Decreasing the pH from 7.8 to 5.8 did not affect I_{Cu}. Perfusion with 5 times normal Ca^{2+} concentrations (55 mM) reduced I_{Cu} by 65 ± 6% in a voltage-independent manner. The E_{rev} was approximately −40 to +35 mV with a mean of −3.3 ± 2.1 mV. Weinreich and Wonderlin (1987) examined the Cu^{2+} effect on the axonal membrane of the same neuron. Axons isolated from the soma were insensitive to bath-applied Cu^{2+} (100 μM), even after 20 to 60 min of exposure. The insensitivity of isolated axons to Cu^{2+} indicated that the site of action of Cu^{2+} was restricted to the soma membrane. I_{Cu} was not blocked by Ni^{2+} (5.5 mM, an effective inhibitor of the presynaptic Ca^{2+} current) or *d*-tubocurarine (1 mM, an antagonist of the increased Na^+ conductance gated by ACh, dopamine, octopamine, phenylethanolamine, histamine, GABA, and aspartic and glutamic acids). Similar data were obtained with hexamethonium (1 mM, ACh) and strychnine (500 μM, ACh, dopamine, 5-HT).

One mechanism that might produce a nonspecific conductance increase is the generalized membrane breakdown resulting from lipid peroxidation. Reduced Cu^{2+}, i.e., Cu^+ formed by oxidation of membrane sulfhydryls, can be oxidized to form an oxygen radical, superoxide anion. This anion is capable of initiating lipid peroxidation with a concomitant generalized breakdown of the membrane integrity and an increase in membrane permeability (Kumar et al., 1978). Cell membranes can be protected from lipid peroxidation by the enzyme superoxide dismutase, which scavenges and detoxifies superoxide anion, with the production of oxygen and hydrogen peroxide. Because bath application of superoxide dismutase (100 μg/ml) did not prevent activation of I_{Cu}, the Kumar et al. (1978), therefore, concluded that I_{Cu} did not result from (*a*) activation of known chemically or voltage-gated Na^+ conductances, (*b*) inhibition of the Na^+-K^+-ATPase, or (*c*) a generalized increase in membrane permeability resulting from lipid peroxidation. An intracellular Cu^{2+} injection failed to activate I_{Cu} but, rather, hyperpolarized the soma membrane potential and decreased the membrane conductance. Furthermore, Weinreich and Wonderlin (1987) observed that application of Cu^{2+} into synaptic regions of the neuropil could activate conductances that resembled synaptically activated K^+ conductances ($I_{Cu,K}$). They concluded that the apparent lack of specificity of the ionic selectivity could be explained by a concurrent activation of multiple ionic conductances rather than by an activation of a single, nonspecific conductance.

Oortgiesen et al. (1990c), using mouse neuroblastoma cell culture (clone NIE-115), also found I_{Cu}. The I_{Cu} slowly increased during continued application of Cu^{2+} (10 to 500 μM) without reaching a steady level. This effect was also observed in Cl^-- and Na^+-free saline and could not be washed out. Addition of 30 μM Cu^{2+} to the external solution bathing excised membrane patches failed to cause discrete single-channel openings but did produce noisy burst-like fluctuations of the membrane current. Similar effects were seen when Ag^+ was applied onto the external surface of *Helix* neurons in cell-attached patch-clamp conditions (Gola and Kiss unpublished observations).

In isolated, nonidentified, intracellularly perfused *Helix* neurons, extracellular application of Cu^{2+} induced more complex permeability changes. On *Helix* neurons three types of Cu^{2+}-activated currents were observed (Kiss et al., 1991; Salánki et al., 1991). Following cell dialysis with 130 mM tris(hydroxymethyl)aminomethane-aspartate after extracellular Cu^{2+} application, an inward current developed (I_{Cuin}) which was carried mainly by Na^+ ions. The I_{Cuin} was pH independent and was decreased by increasing the extracellular Ca^{2+} concentration in a manner similar to that observed on *Aplysia* neurons. The amplitude of the inward component of I_{Cu} was not influenced by replacing Na^+ ions for Cs^+ in the extracellular saline, which suggested that the inward component of I_{Cu} was carried through Ca^{2+}-dependent nonspecific cationic channels (K_D = 500 ± 50 μM, E_{rev} = −38 ± 20 mV). When the intracellular solution was 120 mM KCl, or when part of the tris(hydroxymethyl)aminomethane-aspartate was replaced by KCl, an outward component appeared that was followed by an inward current when Cu^{2+} was applied. The outward component (E_{rev} = −20 ± 10 mV) resulted from the blockade by Cu^{2+} of the steady-state Cl permeability.

The main difference between *Helix* and *Aplysia* neurons was observed during the washout procedure. When the cells were washed with physiological saline, a large, slowly inactivating inward current developed, which was mainly due to the outward movement of Cl^- ions accumulated in the cell during Cu^{2+} application. This current was designated I_w, which was also observed when Pb^{2+} and Cd^{2+} were applied in snail and mouse neuroblastoma cells, respectively (Salánki et al., 1991; Oortgiesen et al., 1990c).

B. Pb^{2+}-activated Conductances

Extracellular application of Pb^{2+} (0.1 μM) to rabbit red blood cells evoked a rapid net loss of K^+, and only a very small fraction of the effect could be related to the deficiency of cellular ATP, which is necessary as substrate for active transport (Joyce et al., 1954). This effect was reproduced in isolated red blood cell membranes ("ghosts"), where Pb^{2+} produced a drastic and specific increase in membrane permeability, which was inde-

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pendent from active transport and intracellular second-messenger systems of the cell (Passow et al., 1961). Audesirk and Audesirk (1983, 1984) showed that Pb^{2+} (μM) slightly reduced input resistance and greatly reduced the junctional resistance between electrically coupled neurons (*L. stagnalis*). Furthermore, in most neurons, chronic exposure to Pb^{2+} caused an increase in the resting potential.

The following studies have examined the ionic mechanisms involved in Pb^{2+}-induced changes in membrane permeability. Oortgiesen et al. (1990c), using mouse neuroblastoma cell culture (clone NIE-115), found that during superfusion with Pb^{2+} (1 to 200 μM) a noninactivating inward current (I_{Pb}) was observed. E_{rev} was 0.0 ± 7.3 mV. This current was independent of Ca^{2+}, K^+, and Cl^- and was not blocked by nicotinic, muscarinic, 5-HT, or Na^+/K^+ pump antagonists. However, in the absence of Na^+, it was abolished. In excised outside-out membrane patches, 10 μM Pb^{2+} caused the opening of discrete ion channels. The amplitude of these channels at −80 mV was 1.85 ± 0.12 pA at either 1 or 10 μM Pb^{2+} with a single-channel conductance of 24 pS. Open probability increased 20- to 30-fold between these concentrations. The frequency-density histograms showed dual exponential open time distributions. These constants were reduced with increasing Pb^{2+} concentrations. Because the I_{Pb} was carried not only by Na^+ but also by Cs^+ ions, showed unit conductance and E_{rev} values similar to that of Ca^{2+}-activated nonselective cation channels, and was independent of intracellular Ca^{2+}, Oortgiesen et al. (1990c) concluded that this channel can be distinguished clearly from ion channels previously characterized. They proposed that Pb^{2+} ions activated a new channel type, a metal ion activated channel, a conclusion similar to one made by Weinreich and Wonderlin (1987).

Pb^{2+}-activated changes in membrane permeability were reported in *Helix* neurons (Salánki et al., 1991; Osipenko et al., 1992b). In most of the cells, Pb^{2+} induced an outward current (I_{Pbout}), less frequently an inward current (I_{Pbin}), and, rarely, an inward current produced during washout ($I_{Pb,w}$). The reason for this multiplicity of effects is difficult to explain, however the most plausible explanation could be that different neurons respond differently upon Pb^{2+} application (Osipenko et al., 1992b; Osipenko and Kiss 1991, 1992; Salánki et al., 1991). Both $I_{Pb,in}$ and $I_{Pb,w}$ were associated with the increased membrane conductance, whereas $I_{Pb,out}$ was due to the blocking of the resting Na^+ conductance. The negative slope of the current-voltage relationship of the $I_{Pb,out}$ supported this conclusion. Patch-clamp data showed that $I_{Pb,out}$ is the result of blockade by Pb^{2+} of the Na^+ channel with 12 pS (150 μM Pb^{2+}) and 7 pS (100 μM) conductance. Open and closed time histograms were fitted by one exponential. Both probabilities of the channel were voltage dependent (Osipenko and Kiss, 1991, 1992).

C. Ag^+-activated Conductances

In frog muscle fibres application of extracellular Ag^+ (10 μM) produced a dose-dependent inward current at a holding potential of −90 mV. In Na^+ and divalent cation-free solution, the inward current through the Ag^+-activated channel was blocked completely. This current was also blocked by 1 mM Cd^{2+} and partially blocked by nifedipine. It was concluded that the Ag^+-induced current is Ca^{2+} dependent (Oba et al., 1993) and that these L-type channels are located on the sarcoplasmic reticulum (Salama and Abramson, 1984).

In *Aplysia* neurons, Ag^+ induced an inward current response of similar magnitude and reversal potential, which suggests that current, at least partly, is carried by Na^+ ions (Weinreich and Wonderlin, 1987).

On isolated nonidentified neurons of *Helix pomatia* it was observed that application of Ag^+ (1 to 100 μM) activates an inward current, which is concentration and potential dependent. The Ag^+-activated current in saline has essentially the same reversal potential, suggesting that Ag^+ activates Ca^{2+}-dependent nonspecific cationic conductances (Gyôri et al., 1991). Furthermore, it was clearly demonstrated that Ag^+ induced an increase of the intracellular Ca^{2+}-concentration, even in Ca^{2+}-free external saline (measurements with fura-2, Gyôri et al., 1991). It seems, therefore, that Ag^+ does not activate a new type of conductance in *Helix* neurons and in this respect differs from the current activated by Cu^{2+} and Ag^+ ions in *Aplysia* neurons (Weinreich and Wonderlin, 1987).

Ag^+ ions at μM concentrations have been reported to activate Ca^{2+} fluxes across the plasma membrane of intact red outer segments isolated from bovine retinas. It was suggested that Ag^+ binds to a sulfhydryl group on the extracellular side of multiple subunits of the cyclic guanosine 3′,5′-monophosphate-dependent conductances, opening the channel. The ion selectivity of the Ag^+-induced channel proved to be broad with little discrimination among Li^+, Na^+, K^+, and Cs^+ or between Ca^{2+} and Mg^{2+} (Schnetkamp and Szerencsei, 1989).

D. Hg^{2+}-activated Conductances

At the motor nerve synapse, Hg^{2+} causes an irreversible depolarization, increased transmitter release, and subsequent block of transmitter release (Juang, 1976; Manalis and Cooper, 1975). All of the effects are antagonized by tetrodotoxin and Co^{2+} but not when either blocker is used alone. The effects are not antagonized by tetrodotoxin plus Co^{2+} when the mercurial is applied in lipid-soluble form (methylmercury). This shows that Hg^{2+} acts at an intracellular site and that entry is gained through Na^+ and Ca^{2+} channels. The inhibitory effect on transmitter release could be the result of binding of the Hg^{2+} to the Ca^{2+} channel (divalent cation-binding site), but the irreversible toxicity is due to an intracellular action involving sulfhydryl groups (Miyamoto, 1983).

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On *Aplysia* neurons, 100 μM extracellular Hg^{2+} also caused depolarization and induced a current similar to I_{Cu} (Weinreich and Wonderlin, 1987). The Hg^{2+}-activated current is mainly Na^+ dependent and in this respect differs from earlier observations (Cooper and Manalis, 1983; Miyamoto, 1983). On rat DRG neurons, Arakawa et al. (1991) observed Hg^{2+}-activated, slow inward current. The current is carried largely by cations through nonspecific cationic channels.

The membrane effect of Hg^{2+}, usually irreversible, is explained by the tight binding of Hg^{2+} to sulfhydryl groups. However, in a model membrane composed of a series of different phospholipids, it was shown that Hg^{2+} binds to the amino groups of phospholipids (Delnomdedieu et al., 1989). Some observations also indicate the involvement of nitrogen atoms in amino acids as binding sites for Hg^{2+} compounds.

Bohme et al. (1992) recently studied the actions of two mercury compounds, $HgCl_2$ and methyl mercury chloride, and of $CdCl_2$ on the epithelium of the rat colon. Methyl mercury chloride (50 μM) induced an increase of outward current ($I_{MeHg,out}$) in enterocytes of isolated crypts patched from the basolateral side. This action was inhibited by a Cl^- channel blocker and a K^+ channel blocker, indicating an increase of both the Cl^- and the K^+ conductance. In contrast, $HgCl_2$ (50 μM) did not affect $I_{MeHg,out}$, whereas $CdCl_2$ (50 μM) decreased it slightly. In mucosal preparations, all three compounds induced a concentration-dependent increase in short-circuit current when administered to the serosal, i. e., contraluminal, side. Sensitivity to chloride transport blockers and anion replacement experiments revealed that the increase in short-circuit current represented Cl^- secretion. In contrast to the actions of luminally applied mercury compounds, the increase of tissue conductance was only small. Tetrodotoxin and indomethacin suppressed the effect of the metal compounds on short-circuit current and tissue conductance, whereas atropine diminished it only partly. This indicates that the secretory action of these heavy metals not only has a direct effect on epithelial cells but also is mediated by prostaglandins and cholinergic and noncholinergic neurons.

E. Other Di- and Trivalent Ion-induced Conductances

Superfusion with Al^{3+} (100 μM) or Cd^{2+} (100 μM) induced a noninactivating current in mouse neuroblastoma cells. The amplitude of I_{Al} was smaller than that of I_{Pb} or I_{Cd}, but the single-channel amplitude induced by Al^{3+} was significantly greater (2.19 ± 0.03 pA) than that induced by Pb^{2+} or Cd^{2+} (1.75 ± 0.9 pA). These results indicated than Pb^{2+}, Cd^{2+}, and Al^{3+} activate a new type of conductance, i.e., metal ion-activated channels (Oortgiesen et al., 1990c). Properties of the Pb^{2+}-activated channels are distinct from those of presently known types of ion channels, possibly constituting a nonselective cation channel. Nonselective cation channels activated by internal Ca^{2+} in N1E-115 cells (Yellen, 1982) and Ca^{2+}-activated cation channels in various cell types (Partridge and Swandulla, 1988) have unit conductances and reversal potentials similar to those of the Pb^{2+}-activated ion channels presently described. However, the Ca^{2+}-activated ion channels are blocked by internal ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, whereas Pb^{2+}-activated ion channels are not. Therefore, the Na^+-dependent, Pb^{2+}-activated ion channel can be distinguished clearly from ion channels previously characterized in N1E-115 and other types of cells.

In *Helix* neurons, extracellular Cd^{2+} at μM concentrations evoked an inward current that appeared either during washing or during Cd^{2+} exposure. Similarly, Cd^{2+} induced an inward current in rat zona glomerulosa and fasciculata cells (Kiss and Osipenko, 1994). In contrast, Cd^{2+} (5 mM) elicited an outward current in stomach smooth muscle cells in guinea pig. This current was more pronounced, substituting intracellular K^+ ions for Cs^+ ions (see fig. 13 of Noack et al., 1992). The phenomenon, however, was not discussed by the authors.

Zn^{2+} may induce either inward or outward currents, depending on the cell type in *Helix* brain. However, this occurs at much higher concentrations than required for the other heavy metal ions. It is interesting that the Zn^{2+}-induced depolarization was paralleled by a decreased concentration of intracellular Ca^{2+}, as was revealed by fura-2 measurements (Kiss and Osipenko, 1994).

It was also observed on chicken cortical neurons that La^{3+}, at μM concentrations, evoked an inward current, which showed a similarity to the I_{Pb} (Kiss and Osipenko, 1994). A series of lanthanides were capable of generating inward currents themselves when applied extracellularly on rat DRG neurons. The effectiveness of inducing current is $Lu^{3+} > Er^{3+} > Tb^{3+} > Eu^{3+} > Nd^{3+} >= Ce^{3+} > La^{3+}$. The effectiveness increased with the atomic number of the metals (Ma and Narahashi, 1993b; table 2).

VII. Conclusions

Data presented in this review clearly demonstrate the great variety of the heavy metal action on ionic channels and receptors of excitable cell membranes. This is the reason for the lack of a general hypothesis that can explain how cellular functions are influenced by metal ions.

We did not specially discuss the intracellular effects of heavy metals. However, their actions could also be a result of penetration into the cytoplasm. Metal ions, for example, may exert their action through an intracellular messenger system like protein kinase C (Evans et al., 1992a; Murakami et al., 1993) or adenylate cyclase (Doroshenko et al., 1982). We also have shown that metal ions can penetrate the membrane (Gyôri et al., 1991), causing additional (secondary) changes in cell functions

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TABLE 2
Metal-induced conductance changes

	Concentration	Ion dependence	Direction of the current	Cell type	Proposed site of action	Reference
Cu^{2+}	1–100 μM	Na^+	Inward	*Aplysia* neuron	MIA* channel	Weinreich and Wonderlin, 1987
	50–500 μM	Na^+	Inward	*Helix* neuron	Activation of nonspecific cation channels	Kiss et al., 1991; Salánki et al., 1991
		Cl^-	Outward	*Helix* neuron	Blocking of steady-state Cl^- permeability	Kiss et al., 1991; Salánki et al., 1991
	10–500 μM		Inward	Neuroblastoma	MIA channel	Oortgiesen et al., 1990c
Pb^{2+}	1–200 μM	Na^+	Inward	Neuroblastoma	MIA channel	Oortgiesen et al., 1990c
		Na^+	Outward	*Helix*	Blocking of resting sodium conductance	Salánki et al., 1991; Osipenko et al., 1992b
Ag^+		?	Inward		?	Salánki et al., 1991; Osipenko et al., 1992b
	10 μM	Ca^{2+}	Inward	Frog muscle	Sarcoplasmic L-type Ca channel	Oba et al., 1993
		Na^{2+}	Inward	*Aplysia* neurone	MIA channel	Weinreich and Wonderlin, 1987
Hg^{2+}	1–100 μM	Cations	Inward	*Helix* neuron	Ca-dependent nonspecific cationic conductance	Gyôri et al., 1991
Cd^{2+}	100 μM	Na^+	Inward	*Aplysia* neuron	MIA channel	Weinreich and Wonderlin, 1987
	100 μM	Na^+	Inward	Neuroblastoma	MIA channel	Oortgiesen et al., 1990c
	1–100 μM	?	Inward	*Helix* neuron	?	Kiss and Osipenko, 1994
		?	Inward	Rat zona glomerulosa cells	?	Kiss and Osipenko, 1994
		?	Inward	Rat zona fasciculata cells	?	Kiss and Osipenko, 1994
	5 mM		Outward	Smooth muscle		Noack et al., 1992
Zn^{2+}	1–5 mM	Na^+?	Outward	*Helix* neuron	?	Kiss and Osipenko, 1994
La^{3+}	1–100 μM	?	Inward	Chicken cortical neuron	MIA channel	Ma and Narahashi, 1993b
Al^{3+}	100 μM	Na^+	Inward	*Aplysia* neuron	MIA channel	Oortgiesen et al., 1990c

* MIA, metal ion activated.

as, for example, in the case of Ag^+- or Pb^{2+}-associated increases of intracellular Ca^{2+} concentrations (Kiss and Osipenko, 1994). Because intracellular metal ions substantially influence the Ca^{2+} homeostasis of the cell (Silbergeld, 1992), it is important to make a clear-cut distinction between surface and intracellular binding sites.

Effects of heavy metals could also be due to the formation of free radicals (Wetterhahn-Jennette, 1981; Scott and Rabito, 1988). It was observed that H_2O_2-mediated depolarization was also cell-type dependent; this could be related to differences in membrane lipid or sulfhydryl content (Scott and Rabito, 1988).

To explain membrane effects of metal ions based on their physical-chemical properties or chemical reactivity, several explanations were given, namely, that metal ions (*a*) bind electrostatically to the membrane proteins or lipids or (*b*) bind chemically to different groups of channel proteins or transmitter receptors. A third possibility, the existence of specific metal receptors, also was suggested.

One of the most plausible explanations of metal ion effects on voltage-activated ionic channels would involve their surface charge-screening effect. A number of studies support this explanation. Di- and trivalent cations have strong effects on the gating properties of voltage-dependent ionic channels. These effects are usually explained in terms of surface charge theory, according to which cations are attracted by fixed negative charges on the membrane surface. A prediction of the theory is that all properties of channel gating should be affected equally, i.e., the curves relating opening and closing kinetics, the conductance-voltage curves, should all be shifted along the voltage axis by an equal amount and cation concentrations in the extracellular medium should shift all of the curves to the right.

The metal ion-induced screening of membrane surface charges cannot easily explain the wide cation concentration range required to shift the kinetic parameters of the gating. Some metal ions produced shift of the gating parameters at millimolar concentrations. Others had effects at micromolar concentrations, making it highly unlikely that the observed effects were due to the changes in surface charge.

Evidence presented here indicated that metal ions bind specifically to the channel proteins (Büsselberg et al.,

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1992; Osipenko et al., 1992b; Audesirk, 1987; Thevenod and Jones, 1992; Lansman, 1990; Visentin et al., 1990; Arhem, 1980; Gilly and Armstrong, 1982a,b; Begenisich and Lynch, 1974; De Biasi et al., 1993). The mode of action of metal ions on transmitter gated channels, as was shown earlier, is even more complex. Results can be explained by proposing a novel site of metal ion binding, at least on the GABA, NMDA, and ATP receptors, by competition for Ca-binding sites or binding to ligands of the membrane proteins or phospholipids.

Although Chiarandini et al. (1967) described the depolarizing effects of Cu^{2+} on molluscan neurons, the so-called metal ion-activated currents (Oortgiesen et al., 1990c) were only recently investigated in detail. It was found that a number of well-known Ca^{2+} channel antagonists, as well as other metal ions, including lanthanides, induced metal ion-activated currents in different preparations (Weinreich and Wonderlin, 1987; Oortgiesen et al., 1990c; Kiss et al., 1991; Gyôri et al., 1991; Ma and Narahashi, 1993b). The observed currents were the result of nonspecific cation channel activation, a steady-state Na^{+} channel inhibition, or induction of the novel type of conductances.

The above-mentioned results strongly support the presence of specific "receptor" sites for metal ions on the extracellular side of biological membranes. These receptors can have different distributions among the cells in tissues and can couple to different ionic channels, receptors, and pumps in a manner resembling that of classical transmitters.

At the present time, however, it cannot be unequivocally decided whether or not alterations of surface potential or binding to channel proteins, to receptor-ionophore complex, or to the specific "metal" receptors plays a significant role in metal toxicity. It seems that the effect of metal ions on cell membrane conductances can be explained sufficiently by any one of the above mechanisms depending on the cell type investigated.

Little information is available concerning the relationship between metal ion concentrations in blood and those in the central nervous system. The penetration of metal ions into brain tissue depends on the function of the blood-brain barrier. It looks like that, instead of a simple threshold for metal entry into the brain, there may be a dose- and equilibrium-dependent saturation level of the blood-brain barrier beyond which the metal penetrates into brain tissue more freely. For example, an important factor in penetration of Pb^{2+} into the brain is the age at which the metal is administered. Young animals are more susceptible to Pb^{2+} exposition than adults. On the other hand, Al^{3+} is more effective on adult neurons. Furthermore, cations of metals rarely exist as free cations but combine with numerous endogenous ligands, and their effects can be extrapolated to the in vivo situation with more confidence for oxyanions (Clarkson, 1993).

The pathogenetic mechanism of metal intoxication is not a simple one. Various toxic actions may occur simultaneously, leading to multifaceted pathological consequences. It must be emphasized that the toxicological effect and pathological impact of metals on the central nervous system may be influenced by numerous factors, such as the metal compound involved, the route of entry, the chronicity and duration of exposure, the species, age and sex of the animal, and the presence of other elements. Thus, a simple extrapolation of the in vitro data to understand all aspects of metal toxicity in vivo is questionable. This statement is probably true for all metals described in this review or for the metals representing a possible hazard for humans.

VIII. Perspectives

Because metal ions have an influence on a number of biological activities, such as growth, peptide synthesis, and gene expression, it could be expected that metal ions may affect cellular functions in a different way. The reviewed data suggest that many of the metals may have a specific effect in changing the membrane permeability, selectivity, and gating. These effects could be realized through specific metal receptors, although metal-receptor ligands have not been clearly identified. It is not clear whether these receptors are exclusively metallo-metal-activated or are metalloregulatory proteins (O'Halloran, 1991). Ionic channel or receptor proteins also possess special binding sites for metal ions. The diverse membrane effects induced by metal ions, however, may suggest the presence of such metal receptors, because the interactions of metal ions with surface charges can only partially explain the effects produced by these cations. The decoding of the chemical nature of these receptors is faced with great difficulties because, unlike a number of chemicals, heavy metal ions do not have the highest specificity in physiological or biochemical processes. Although some physiological processes could be more sensitive than others with respect to a particular metal ion, the metal ion-induced effect, even in a single system, is, as a rule, complex. Nevertheless, it can be expected that investigations of the heavy metal actions might contribute to exploring the molecular structure of the ionic channels and receptors. The present and future investigations in this field may contribute to an understanding of the mechanisms of neurotoxicity as well.

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