Metal Interactions with Voltage- and Receptor-activated Ion Channels

Henk P.M. Vijverberg, Marga Oortgiesen, Trese Leinders, and Regina G.D.M. van Kleef

Research Institute of Toxicology, Utrecht University, Utrecht, The Netherlands

Effects of Pb and several other metal ions on various distinct types of voltage-, receptor- and Ca-activated ion channels have been investigated in cultured N1E-115 mouse neuroblastoma cells. Experiments were performed using the whole-cell voltage clamp and single-channel patch clamp techniques. External superfusion of nanomolar to submillimolar concentrations of Pb causes multiple effects on ion channels. Barium current through voltage-activated Ca channels is blocked by micromolar concentrations of Pb, whereas voltage-activated Na current appears insensitive. Neuronal type nicotinic acetylcholine receptor-activated ion current is blocked by nanomolar concentrations of Pb and this block is reversed at micromolar concentrations. Serotonin 5-HT3 receptor-activated ion current is much less sensitive to Pb. In addition, external superfusion with micromolar concentrations of Pb as well as of Cd and aluminum induces inward current, associated with the direct activation of nonselective cation channels by these metal ions. In excised inside-out membrane patches of neuroblastoma cells, micromolar concentrations of Ca activate small (SK) and big (BK) Ca-activated K channels. Internally applied Pb activates SK and BK channels more potently than Ca, whereas Cd is approximately equipotent to Pb with respect to SK channel activation, but fails to activate BK channels. The results show that metal ions cause distinct, selective effects on the various types of ion channels and that metal ion interaction sites of ion channels may be highly selective for particular metal ions. — Environ Health Perspect 102(Suppl 3):153-158 (1994).

Key words: lead, cadmium, aluminum, ion channels, neuroblastoma, acetylcholine, serotonin, calcium, potassium, patch clamp, voltage clamp

Introduction

In excitable cells ion channels are directly responsible for the rapid electric signaling and are also involved in the modulation of excitability. Calcium ions play a prominent role in the modulation of excitability, because these ions permeate through various ion channels and activate K channels at the internal face of the membrane (1). In addition, internal Ca ions trigger a range of biochemical processes involved in signal transduction (2). Various metal ions may substitute for Ca. These metal ions activate or block Ca-dependent processes, which may lead to altered excitability and may disturb the intracellular Ca homeostasis, ultimately leading to cell death (3).

In mouse neuroblastoma cells of the clone N1E-115, a range of distinct types of ion channels and receptors have been identified. These cells express voltage-activated sodium channels, at least two types of voltage-activated Ca channels and both voltage-activated and Ca-activated K channels (4-7). Recently, serotonin 5-HT3 receptors and neuronal type nicotinic acetylcholine (ACh) receptors, which are directly coupled to distinct cation channels, have been characterized in N1E-115 cells (8,9). The detailed knowledge of the properties of ion channels in neuroblastoma cells and the suitability of these cultured cells for intracellular electrophysiology permit the separation of the various types of ion channels in voltage clamp experiments and thereby the investigation of effects of metal ions on each type of ion channel.

The results reviewed below demonstrate that metal ions interact with multiple sites on ion channels in the mammalian nervous system.

Materials and Methods

Mouse neuroblastoma cells of the clone N1E-115 (10) were grown as described previously (9). Experiments were carried out using the whole-cell voltage clamp or the single-channel patch-clamp technique (11). Fire-polished glass pipettes had an internal tip diameter of 1 to 1.5 μm and a resistance of 3 to 5 MΩ. Membrane currents recorded under voltage clamp were low-pass filtered, digitized (8 bits; 1024 points per record) and stored on magnetic disc for off-line computer analysis.

Voltage-activated ion currents were evoked by step depolarizations of the membrane. Receptor-activated ion currents were evoked by whole-cell superfusion with external solution containing known concentrations of agonist and/or metal ions for adjustable periods (≥1 sec). In between agonist-induced responses, evoked at intervals of 3-4 min, desensitization was completely reversed by continuous superfusion of the cell with external solution. Single Ca-activated K channels were recorded by the single channel patch clamp technique from inside-out membrane patches of N1E-115 cells. Patches were superfused with buffered Ca-free and with internal solutions in which Ca or other metal ions were buffered with citric acid. All experiments were carried out at room temperature (20-24°C).

Different external and pipette solutions for optimum recording of the various types of ion currents independently were prepared from ultrapure chemicals and double glass-distilled water. The ionic compositions of the solutions are presented in Table 1, which includes the total contamination by Pb as calculated from the data supplied with the chemicals. Indicated metal-ion concentrations refer to the
Table 1. Ionic compositions of external and pipette solutions used to record ion currents through different types of ion channels indicated. All concentrations are in mM except the Pb values, which are in nM and represent total Pb contamination as calculated from data supplied with the chemicals used.

| Calcium channels | Sodium channels | Receptor-activated channels | Calcium-activated potassium channels |
|------------------|-----------------|-----------------------------|-------------------------------------|
| External         | Pipette         | External                    | Pipette                             | Outside | Inside |
| NaCl             | 30              | NaCl                        | 120                                 | NaCl    | 10     |
| NaCl             | 5               | NaOH                        | 10                                  | NaCl    | 10     |
| CsCl             | 5               | MgCl₂                       | 0.4                                 | MgNO₃   | 0.6    |
| CaCl₂            | 2               | CaCl₂                       | 15                                  | KCl     | 1      |
| BaCl₂            | 50              | Gluac                       | 125                                 | CaCl₂   | 1.8    |
| TTX              | 0.0005          | MgCl₂                       | 25                                  | MgCl₂   | 1      |
| TEA              | 25              | Sucrose                     | 15                                  | MgCl₂   | 0.8    |
| HEPES            | 5               | HEPES                       | 25                                  | Glucose | 25     |
| pH 7.4           | 5               | HEPES                       | 25                                  | Glucose | 30     |
| TEA OH           | 5               | HEPES                       | 25                                  | Glucose | 10     |
| Pb               | <130nM          | Pb                          | 1.8 nM                              | KOH     | Pb     |

*Used to superfuse the inside face of excised inside-out membrane patches. Citric acid was used to buffer divalent metal ions (see Materials and Methods). Free metal ion concentrations are indicated for experiments using this solution. For all other experiments total Pb²⁺ is indicated. Abbreviations: EGTA, ethylene glycol bis-(β-aminoethyl ether) N,N'-tetraacetic acid; gluac, L-glutamic acid; HEPES, N-2-hydroxyethyl)piperazine-N'(2-ethanesulfonic acid); Pipes, piperazine-N,N'-bis(2-ethanesulfonic acid); TEA, tetraethylammonium; TMA, tetramethylammonium; TTX, tetrodotoxin.

---

Results

Effects on Voltage-activated Ion Channels

In external solution containing 50 mM of Ba ions membrane depolarization from a holding potential of -80 mV to +10 mV evokes a fast transient as well as a nonactivating inward Ba current in whole cell voltage-clamped neuroblastoma cells. These Ba current components are carried by distinct transient and sustained types of voltage-activated Ca channels (5). Superfusion with Pb²⁺ causes a reduction of the amplitude of both types of Ba current within 5 to 10 min and the blocking effects are reversed after 5 to 10 min of washing with control external solution. The blocking effect of Pb²⁺ on the transient Ba current component is concentration-dependent with an IC₅₀ value of 4.8 ± 0.8 µM and a slope factor of -0.88 ± 0.14 (Figure 1). The transient and sustained Ba current components appear to have a similar sensitivity to Pb²⁺. In contrast, 10 to 100 µM Pb²⁺ does not affect voltage-activated sodium current, evoked by a step depolarization to 0 mV for 20 msec preceded by a conditioning membrane hyperpolarization to -120 mV for 100 msec (not shown).

Effects on Receptor-Activated Ion Channels

Whole-cell superfusion of voltage-clamped N1E-115 cells with external solution containing acetylcholine (ACh) evokes a transient inward current. The ACh-induced inward current is mediated by neuronal type nicotinic ACh receptors, which are selectively blocked by K-bungarotoxin and

Figure 1. Effects of Pb²⁺ on voltage-activated Ca channels. Upper panel: Barium currents evoked by step depolarizations from -80 mV to +10 mV in control external solution, after 5 min of superfusion with 10 µM Pb²⁺ and recovery after 5 min of washing with control external solution. The peak amplitude of the transient inward Ba current was reduced to 34% of the control value by 10 µM Pb²⁺. This peak amplitude was calculated by subtracting the steady inward current at the end of the depolarization from the peak inward current. Lower panel: Concentration-effect curve of block by Pb²⁺ of voltage-activated transient Ba current. Ordinate represents the peak amplitude of the transient inward current normalized to control value. The estimated IC₅₀ value and the slope factor of the concentration-effect curve of block of the transient component of the Ba current by Pb²⁺ are 4.8 ± 0.8 µM and -0.88 ± 0.14, respectively. Modified after Oortgiesen et al. (14).
are insensitive to α-bungarotoxin (9). Superfusion with Pb²⁺ rapidly reduces the amplitude of the 1 mM ACh-induced inward current. This effect is partially reversed during a 5 to 10 min period of washing with control external solution. Figure 2 shows that after 14 min of superfusion with 1 μM Pb²⁺, the peak amplitude of the ACh-induced inward current is reduced to 18% of the control value. In addition, the decay of the remaining ACh-induced inward current is delayed. These effects remain steady during continued superfusion with Pb²⁺. The superfusion of cells with external solution in which the concentration of Pb²⁺ was varied revealed that Pb²⁺ has a biphatic, concentration-dependent effect on the amplitude of the ACh-induced inward current. On superfusion with 1 nM Pb²⁺, the peak amplitude of the ACh-induced inward current is reduced to 74 ± 7% (n=3) of the control value and the amplitude gradually further reduces to 15 ± 4% (n=3) for Pb²⁺ concentrations up to 1 μM. However, a concentration-dependent reduction of block is observed on superfusion of concentrations >3 μM Pb²⁺, and in the presence of 100 μM Pb²⁺ the peak inward current amplitude amounts to 69 ± 11% (n=3) of the control value. An effect on the rate of decay of the ACh-induced inward current is observed at concentrations beyond 0.1 μM Pb²⁺. Concentration-dependent effects of Pb²⁺ on the peak amplitude of the ACh-induced inward current are well fitted by the sum of an ascending and a descending concentration-effect curve (Figure 2) with an IC₅₀ value of 19 ± 6 nM for block and an EC₅₀ of 21 ± 5 μM for the reversal of the blocking effect. The slope factors of the two curves are -0.45 ± 0.08 and 0.84 ± 0.15, respectively.

Superfusion of neuroblastoma cells with 3 μM serotonin (5-HT) activates a transient inward current, mediated by an independent population of serotonin 5-HT₃ receptor-activated ion channels (8). Pb²⁺ reversibly reduces the amplitude of the 5-HT-induced inward current within 4 to 8 min, without changing the kinetics of the inward current. During superfusion with 1 μM Pb²⁺, the peak amplitude of the 5-HT-induced inward current is reduced to 74 ± 5% (n=3) of the control value. The estimated values of the IC₅₀ and the slope factor of the concentration-effect curve of block of the 5-HT-induced inward current by Pb²⁺ are 49 ± 18 μM and -0.32 ± 0.04, respectively (14).

**Table 2.** Blocking and unblocking or activating effects of Pb²⁺ on various types of ion channels in cultured mouse neuroblastoma cells of the clone N1E-115.

| Voltage-activated ion channels | Block (IC₅₀) | Activation (EC₅₀) |
|-------------------------------|-------------|-----------------|
| Calcium channels              | 5 μM        |                 |
| Sodium channels               | > 100 μM    |                 |
| Receptor-activated ion channels |            |                 |
| Neuronal nicotinic ACh receptor | 20 nM | 21 μM |
| Serotonin 5-HT₃ receptor      | 50 μM       |                 |
| Metal ion-activated ion channels |        |                 |
| SK channels                   | > 100 μM    | < 1 μM²         |
| BK channels                   | > 100 μM    | > 1 μM²         |

IC₅₀ values are the external concentrations of Pb²⁺ required to inhibit 50% of the ion current. EC₅₀ values denote the concentrations for 50% reversal of block or 50% activation of the ion currents. *Buffered free Pb²⁺ concentrations. All others indicate total Pb.

**Effects on Calcium-Activated Potassium Channels**

Two types of Ca-activated K channels can be identified in excised membrane patches of N1E-115 neuroblastoma cells. SK channels, which have a low single-channel conductance of 5 pS, are potently blocked by the bee venom peptide apamin and show a relatively high sensitivity to Ca. BK channels, which have a high single-channel conductance of 98 pS, are sensitive to block by...
tetraethylammonium ions (TEA) and are less sensitive to Ca (7). Figure 3 shows representative traces of single SK and BK channel recordings from two inside-out excised patches of N1E-115 membrane at a holding potential of 0 mV. In these experiments, SK and BK channels were maximally activated by superfusion of the inside of the patches with solutions containing 14.4 and 115.2 μM buffered free Ca, respectively. During subsequent superfusion with Ca-free, EGTA-containing solution no single-channel openings are observed. In the same membrane patches, Pb²⁺ activates the SK and the BK channel. At 1 μM free Pb²⁺ the open probability of the SK channel is the same as during superfusion with a maximally activating concentration of Ca. Conversely, BK-channel open probability in the presence of 1 μM Pb²⁺ is only a fraction of the maximum attainable with Ca. Effects of several other metal ions on SK and BK channels in N1E-115 cells have been investigated. In the presence of the various metal ions the open probability of the channels, as related to the maximum obtained with Ca, varies. Potency orders derived from effects measured at metal ion concentrations between 1 and 100 μM are for the SK channel: Cd²⁺ = Pb²⁺ > Ca²⁺ > Co²⁺ > Mg²⁺ and for the BK channel: Pb²⁺ > Ca²⁺ > Co²⁺ > Cd²⁺, Mg²⁺. The sequences show that Pb²⁺ is more potent than Ca²⁺ in activating both SK and BK channels. Cd²⁺ is also a very potent activator of SK channels, but is unable to activate BK channels even at a concentration of 100 μM. Mg²⁺ is completely inactive at concentrations up to 100 μM (15).

**Effects on Metal Ion-activated Ion Channels**

Superfusion with Pb²⁺ also induces a slow, noninactivating and reversible inward current in N1E-115 cells. The amplitude of this inward current increases in the range of 1 to 200 μM Pb²⁺. Exposure of excised outside-out membrane patches to Pb²⁺ revealed that the slow inward current is mediated by the opening of discrete ion channels (Figure 4a) with a single-channel conductance of 24 pS. Single-channel events can be detected at Pb²⁺ concentrations ≥ 0.1 μM. Chelation of external Pb²⁺ by superfusion with EGTA-containing solution fully abolished single-channel activity as illustrated in Figure 4b for a patch containing multiple channels. The reversal of the whole-cell membrane current and of the single-channel currents at approximately 0 mV (not shown) suggests that the current is carried by nonselective cation channels. The Pb²⁺-induced membrane current appears not to be mediated by various known types of ion channels, since it can neither be blocked by external tetrodotoxin, TEA, d-tubocurarine, atropine, the potent 5-HT₃ antagonist ICS 205-930, nor by internal EGTA. In N1E-115 neuroblastoma cells Cd and Al activate ion channels similar to those activated by Pb²⁺ (16).

**Discussion**

Evaluation of direct effects of Pb²⁺ on ion channels in cultured N1E-115 neuroblastoma cells demonstrates differential sensitivities of various types of receptor-activated and voltage-activated ion channels to this heavy metal. The results, summarized in Table 2, show that the neuronal nicotinic receptor-activated ion current is the more sensitive target, and that it is selectively blocked by nanomolar concentrations of Pb²⁺. Inhibitory as well as activating effects of Pb²⁺ on ion channels are observed. The reversal of block of nicotinic ion currents at high Pb²⁺ concentrations suggest that besides the blocking effect at low concentrations, micromolar concentrations of Pb²⁺ are able to enhance activation of the nicotinic receptor-activated ion channel. Further, the two types of Ca-activated K channels and cation channels are activated by micromolar concentrations of Pb²⁺. Within the class of receptor-activated ion channels Pb²⁺ selectively affects the neuronal nicotinic receptor, because the serotonin 5-HT₃ receptor-activated ion current is affected only by Pb²⁺ at micromolar concentrations. In addition, glutamate NMDA receptor-activated ion channels in rat hippocampal neurons are also blocked by Pb²⁺ only at concentrations in the high micromolar range (17).

Voltage-activated Ca channels in N1E-115 cells are blocked by Pb²⁺ in the micromolar concentration range with an IC₅₀ value for block of the transient current of 5 μM. The noninactivating Ca channels were blocked in the same concentration range. Pb²⁺ appears to be nearly as potent as La³⁺, the most effective inorganic Ca antagonist in N1E-115 cells (5). Recently, relating the blocking effects on both types of Ca channels in N1E-115 cells to the measured free Pb²⁺ concentration yielded even slightly lower IC₅₀ values (18). Very similar IC₅₀ values for block of Ca channels by Pb²⁺ have been obtained from experiments on rat dorsal root ganglion cells and on human neuroblastoma cells (19,20). The results show that, despite the close relation between voltage-activated sodium and Ca channels (21), Pb²⁺ selectively blocks Ca channels.

**Figure 3.** Effects of Pb²⁺ on Ca-activated K channels. Maximum open probability of single SK (left) and BK (right) channels in two inside-out excised patches by superfusion with 14.4 and 115.2 μM buffered free Ca, respectively. Subsequent superfusion with Ca-free EGTA-containing solution abolished single channel activity. In the same membrane patches superfusion with 1 μM buffered free Pb²⁺ evoked full activation of the SK and partial (10%) of maximum open probability activation of the BK channel. Membrane potential was held at 0 mV.
The results on Ca channel block in N1E-115 cells are consistent with reported blocking effects of Pb\(^{2+}\) on various presynaptic functions in \textit{ex vivo} preparations and support the generally accepted hypothesis that voltage-activated Ca channels are the major target site for presynaptic block of cholinergic neurotransmission by Pb\(^{2+}\). (22–25). An additional delayed enhancement of spontaneous ACh release by Pb\(^{2+}\) is thought to be a consequence of Pb\(^{2+}\) entry into the presynaptic terminal. The latter effect is supposed to be caused either by a direct enhancement of the release of ACh containing vesicles by Pb\(^{2+}\) substituting for Ca, or by the mobilization of Ca from intracellular stores by Pb\(^{2+}\). NMR studies have demonstrated an increase of intracellular Ca and a very slow influx of Pb\(^{2+}\) during incubation of NG108-15 cells with micromolar concentrations of Pb\(^{2+}\) (26). On the other hand, results on ACh release from intact and digitonin-permeabilized rat cerebrocortical synaptosomes suggest that nanomolar concentrations of intracellular free Pb\(^{2+}\) directly trigger the excytosis of synaptic vesicles (27). Presynaptic effects of Pb\(^{2+}\) are not restricted to a particular type of synapse. In cholinergic, GABA-ergic, dopaminergic, and serotonergic synaptosome preparations, blocking and stimulating effects of Pb\(^{2+}\) on K-evoked and spontaneous neurotransmitter release, respectively, have been reported (22,23,28–30). A continuous spontaneous release of low amounts of neurotransmitters and block of nerve-evoked neurotransmitter release may disturb neural networks particularly during development (31).

SK and BK channels, two distinct types of Ca-activated K channels of N1E-115, are directly activated by intracellular Pb\(^{2+}\) applied to excised membrane patches. Voltage-activated K channels have been reported to be insensitive to Pb\(^{2+}\) (19,20). In particular, the SK channel appears sensitive to submicromolar Pb\(^{2+}\) concentrations. The SK channel is responsible for the afterhyperpolarization that follows the action potential (6) and is involved in the regulation of neuronal firing frequency. Activation of SK channels by Pb\(^{2+}\) may cause hyperpolarization, increase the excitation threshold and reduce action potential duration. These effects would contribute to a reduction of neurotransmitter release when occurring in the presynaptic terminal. Of various metal ions, Pb\(^{2+}\) is the more potent to activate SK and BK channels, whereas Cd\(^{2+}\) is a potent activator of SK channels, but does not activate BK channels at concentrations below 100 \(\mu\)M. The distinct potency sequences for activation of subtypes of Ca-activated K channels deviate from the potency sequence to block voltage-activated Ca channels in N1E-115 cells (5). Although Pb\(^{2+}\) is also the more potent Ca channel blocker, Cd\(^{2+}\) blocks sustained Ca current more potently than Co\(^{2+}\) and the two metal ions are equipotent in blocking transient Ca current (5). This suggests that metal ions interact with ion channel proteins in a highly selective manner.

At high concentrations, external Pb\(^{2+}\) directly activates a slow inward current in N1E-115 cells. Results of experiments with channel blockers, receptor antagonists, and chelated internal Ca indicate that this slow inward current is not mediated by a previously described type of neurotransmitter receptor-activated ion channel, voltage-activated ion channel or Ca-activated ion channel (16). Pilot experiments on various other cell lines and on primary cultured mammalian neurons have not confirmed the presence of a similar metal ion-activated ion channel thus far (R. Zwart and M. Oortgiesen, unpublished).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Single-channel openings induced by Pb\(^{2+}\). (a) In control solution no channel openings were recorded. After addition of Pb\(^{2+}\) to the external solution at a final concentration of 10 \(\mu\)M discrete single channel openings occurred. (b) Multiple single channel openings induced by 10 \(\mu\)M Pb\(^{2+}\) disappear within 3 to 4 min after addition of 2 mM Ca-EGTA to the bathing solution. Calibration: horizontal 100 ms, vertical 2 pA. Membrane potential was held at -80 mV. Modified after Oortgiesen et al. (18).}
\end{figure}
The comparison of effects on subsets of ion channels that are functionally and structurally related shows that Pb^{2+} selectively interacts with specific membrane proteins. In addition, different metal ions selectively modify distinct target sites. This implies that for any particular neuron the effects of metal ions on electrical activity may vary, depending on metal ion species, the extra- and intracellular concentrations, and on the presence, availability, and density of specific types of ion channels.

REFERENCES

1. Hille B. Ionic channels of excitable membranes. Sunderland, MA: Sinauer Associates Inc, 1992.
2. Orrenius SW, McConkey DJ, Jones DP, and Nicotera, P. Ca^{2+}-activated mechanisms in toxinity and programmed cell death. ISI Atlas Sci Pharmacol 2:319–324 (1988).
3. Schanne FAX, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death: a final common pathway. Science 206:700–702 (1979).
4. Moolenaar WH, Specter I. Ionic currents in cultured mouse neuroblastoma cells under voltage-clamp conditions. J Physiol (Lond) 278:265–286 (1978).
5. Narahashi T, Tsunoo A, Yoshii M. Characterization of two types of calcium channels in mouse neuroblastoma cells. J Physiol (Lond) 383:231–249 (1987).
6. Hugues M, Roney G, Duval D, Vincent JP, Lazdunski M. Apamin as a selective blocker of the calcium-dependent potassium channel in neuroblastoma cells: voltage-clamp and biochemical characterization of the toxin receptor. Proc Natl Acad Sci USA 79:1308–1312 (1982).
7. Leinders T, Vijverberg HPM. Ca^{2+} dependence of small Ca^{2+}-activated K^{+} channels in cultured N1E-115 mouse neuroblastoma cells. Pfliigers Arch 422:223–232 (1992).
8. Neijt HC, Plomp JJ, Vijverberg HPM. Kinetics of the membrane current mediated by serotonin 5-HT_{3} receptors in cultured mouse neuroblastoma cells. J Physiol (Lond) 411:257–269 (1989).
9. Oortgiesen M, Vijverber, HPM. Properties of neuronal type acetylcholine receptors in voltage-clamped mouse neuroblastoma cells. Neurosciences 31:169–179 (1989).
10. Amano T, Richelson E, Nirenberg PG. Neurotransmitter synthesis by neuroblastoma clones. Proc Natl Acad Sci USA 6:258–263 (1972).
11. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfliigers Arch 391:85–100 (1981).
12. van Heeswijk MPE, Geertjen JAM, van Os CH. Kinetic properties of the ATP-dependent Ca^{2+} pump and the Na^{+}/Ca^{2+} exchange system in basolateral membranes from rat kidney cortex. J Membr Biol 79:19–31 (1984).
13. Sillen LG, Martell AE. Stability constants of metal-ion complexes, Suppl No. 1, Special Publication No 25, London: The Chemical Society, 1971.
14. Oortgiesen M, van Kleef RGDM, Bajnath RB, Vijverberg HPM. Nanomolar concentrations of lead selectively block neuronal nictinic responses in mouse neuroblastoma cells. Toxicol Appl Pharmacol 103:165–174 (1989).
15. Vijverberg HPM. Divalent cations activate SK and BK channels in mouse neuroblastoma cells: selective activation of SK channels by cadmium. Pfliigers Arch 422:217–222 (1992).
16. Oortgiesen M, van Kleef RGDM, Vijverberg HPM. Novel type of ion channel activated by Pb^{2+}, Cd^{2+}, and Al^{3+} in cultured mouse neuroblastoma cells. J Membr Biol 113:261–268 (1990).
17. Alkondon M, Costa ACS, Radhakrishnan V, Aronstam RS, Albuquerque EX. Selective blockade of NMDA-activated channel currents may be implicated in learning deficits caused by lead. FEBS Lett 261:124–130 (1990).
18. Audesirk G, Audesirk T. Effects of inorganic lead on voltage-sensitive calcium channels in N1E-115 neuroblastoma cells. Neurotoxicology 12:519–528 (1991).
19. Evans ML, Büsselberg D, Carpenter DO. Pb^{2+} blocks calcium currents of cultured dorsal root ganglion cells. Neurosci Lett 129:103–106 (1991).
20. Reuveny E, Narahashi T. Potent blocking action of lead on voltage-activated calcium channels in human neuroblastoma cells SH-SYSY. Brain Res 545:312–314 (1991).
21. Miller C. Genetic manipulation of ion channels: a new approach to structure and mechanism. Neuron 2:1195–1205 (1989).
22. Szuszkiw J, Toth G, Murawska M, Cooper GP. Effects of Pb^{2+} and Cd^{2+} on acetylcholine release and Ca^{2+} movements in synaptosomes and subcellular fractions from rat brain and torpedo electric organ. Brain Res 323:31–46 (1984).
23. Minnema DJ, Michaelson IA, Cooper GP. Calcium efflux and neurotransmitter release from rat hippocampal synaptosomes exposed to lead. Toxicol Appl Pharmacol 92:351–357 (1988).
24. Manalis RS, Cooper GP, Pomeroy SL. Effects of lead on neuro-muscular transmission in the frog. Brain Res 294:95–109 (1984).
25. Pickett JB, Borstein JC. Some effects of lead at mammalian neuromuscular junction. Am J Physiol 246:C271–C276 (1984).
26. Schanne FAX, Moskal JR, Gupta RK. Effect of lead on intracellular free calcium ion concentration in a presynaptic model: 19F-NMR study of NG108-15 cells. Brain Res 503:308–311 (1989).
27. Shao Z, Szuszkiw J. Ca^{2+}-sustrogate action of Pb^{2+} on acetylcholine release from rat brain synaptosomes. J Neurochem 56:568–574 (1991).
28. Minnema DJ, Greenland RD, Michaelson IA. Effect of in vitro inorganic lead on dopamine release from superfused rat striatal synaptosomes. Toxicol Appl Pharmacol 84:400–411 (1986).
29. Minnema DJ, Michaelson IA. Differential effects of inorganic lead and 8-aminocaprylic acid in vitro on synaptosomal 8-aminobutyric acid release. Toxicol Appl Pharmacol 86:437–447 (1986).
30. Oudar P, Caillard L, Fillion G. The effects of inorganic lead on the spontaneous and potassium-evoked release of [3H]–5-HT from rat cortical synaptosome: Interaction with calcium. Pharmacol Toxicol 64:459–463 (1989).
31. Bresler JP, and Goldstein GW. Mechanisms of lead neurotoxicity. Biochem Pharmacol 41:479–484 (1991).