Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 924549, 12 pages
doi:10.1155/2012/924549

Review Article

Mercury Toxicity on Sodium Pump and Organoseleniums Intervention: A Paradox

Ige Joseph Kade[1,2]

[1] *Department of Biochemistry, Federal University of Technology, FUTA Road, PMB 704, Akure, Ondo State, Nigeria*
[2] *Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados, Sede Sur, Calzada de los Tenorios 235, Colonia Granjas Coapa, DF, 14330 México, Mexico*

Correspondence should be addressed to Ige Joseph Kade, ijkade@yahoo.com

Received 9 April 2012; Accepted 1 July 2012

Academic Editor: João B. T. Rocha

Mercury is an environmental poison, and the damage to living system is generally severe. The severity of mercury poisoning is consequent from the fact that it targets the thiol-containing enzymes, irreversibly oxidizing their critical thiol groups, consequently leading to an inactivation of the enzyme. The Na^+/K^+-ATPase is a sulfhydryl protein that is sensitive to Hg^{2+} assault. On the other hand, organoseleniums are a class of pharmacologically promising compounds with potent antioxidant effects. While Hg^{2+} oxidizes sulfhydryl groups of Na^+/K^+-ATPase under *in vitro* and *in vivo* conditions, the organoselenium compounds inhibit Na^+/K^+-ATPase *in vitro* but enhance its activities under *in vivo* conditions with concomitant increase in the level of endogenous thiols. Paradoxically, it appears that these two thiol oxidants can be used to counteract one another under *in vivo* conditions, and this hypothesis serves as the basis for this paper.

1. Introduction

There are several earlier and more contemporary reviews on the toxicology of mercury and its compounds available to readers that cover a spectrum of findings ranging between types and classifications, sources of mercury exposure to the toxicity of different species and biological outcomes [1–7]. Yearly, data and reviews on mercury and its toxic effects are emerging on an astronomical scale. Hence, the objective of this paper is not to duplicate the earlier reviews but to sensitize readers on intervention strategies that recent authors are pointing to. In this regard, this paper will focus on the toxicity of mercury on a key transmembrane enzyme transporter, Na^+/K^+-ATPase (otherwise known as sodium pump), and the possible intervention of an emerging class of selenium-based compounds—the organoselenium compounds to ameliorate the toxic effect of mercury on this important transmembrane enzyme. Consequently, this paper will present recent findings in the last few years on the observed effect of mercurial compounds on sodium pump and the paradox surrounding the use of organoselenium as possible intervention in mercury toxicity on the activities of sodium pump.

2. Sodium Pump: A Brief Overview

In 1957, Skou described an ATPase preparation from crab nerve membranes that was stimulated by addition of Na^+ and K^+ to the incubation medium [8], and he proposed that this enzymatic activity was associated with the physiological mechanism for active transport of monovalent cations, across the plasma membrane, and eventually this enzymic system was christened Na^+/K^+-ATPase or sodium pump. Na^+/K^+-ATPase transforms chemical energy in ATP to osmotic work and maintains electrochemical Na^+ and K^+ gradients across cell membranes. At rest, Na^+/K^+-ATPase converts 20–30% of the current ATP production in mammals to active Na^+ and K^+ transport in kidney, central nervous system, and other cells of the body where Na^+ and K^+ gradients are required for maintaining membrane potential and volume of animal cells and organelles bounded by flexible lipid

bilayers. Lowering the intracellular Na^+ concentration helps to prevent cell lysis. The electrochemical gradient generated by the unequal exchange of Na^+ for K^+ is used to drive a variety of fundamental physiological processes. The electrical gradient contributes to the resting membrane potential, thereby affecting channel-regulated electrical stimulation of nerve and muscle cells. The Na^+ gradient is the energy source for facilitated transport of ions (e.g., Na^+/Ca^{2+} exchange) and metabolites (e.g., Na^+/glucose cotransport) by carriers. Presumably because the pump is essential for cell viability, Na^+/K^+-ATPase has also been indirectly implicated in the etiology of diseases like essential hypertension and diabetes. Sodium pump is the receptor for cardiac glycosides, like digitalis, used to treat patients with congestive heart failure. Several excellent reviews have described in details the structure and function of the Na^+/K^+-ATPase, and I encourage the readers to consult these references [9–12].

3. Transmembrane Topology

Of importance in this paper is a brief description of the Na^+/K^+-ATPase especially with emphasis on the amino acid composition of the active sites of the pump. Generally, Na^+/K^+-ATPase belongs to the P-type ATPases, and like the H^+/K^+-ATPase, it is composed of majorly two subunits. The larger constituent of the heterodimer is the α-subunit with a molecular mass of about 112000 Da which is responsible for both catalytic and transport function [11]. The α-subunit comprises an acceptor for the γ-phosphate of ATP, phosphorylation of the aspartic residue Asp-369 [13] which leads to formation of the phosphorylated intermediate during the reaction cycle [14]. In addition to the cation binding sites, also the receptor for cardiac glycosides is located on the α-subunit [15]. The smaller constituent is the glycosylated β-subunit with a molecular mass of about 55 000 Da. This subunit was for a long time believed not to participate directly in the catalytic cycle or the binding of cardiac glycosides. Recently, it was demonstrated that the assembly of an (α/β) heterodimer is necessary for a stable and functionally competent configuration of the pump; in particular, the β subunit is needed for the α-subunit to exit from the endoplasmic reticulum and to acquire the correct configuration [16–18]. A third subunit with a molecular mass of about 12000 Da has been detected as a part of the native enzyme assembly [19]. However, the importance of this so-called γ-subunit for neither ATPase or phosphatase activity nor ion transport characteristics has been demonstrated so far [20]. All these subunits have been cloned and sequenced from a variety of tissues and animal species [11].

4. Binding Sites of the Na^+/K^+-ATPase

By definition as P-type ATPase, the sodium pump molecule is temporarily phosphorylated during the reaction cycle. Demonstration that an aspartate residue accepts the γ-phosphate from ATP during its hydrolysis was provided by Post and Kume [14]. Localization of the phosphorylation site has been achieved by means of controlled tryptic digestion [21]. After sequence analysis of the sheep kidney enzyme, Asp-369 has been identified as the target for catalytic phosphorylation [13]. The consensus sequence Cys-Ser-Asp-376-Lys-Thr-GIy-Thr-Leu-Thr around the phosphorylation site of the α-subunit is highly conserved among α-subunit isoforms and even with respect to other P-type ATPases like H^+, K^+-ATPase, sarcoplasmic reticulum Ca^{2+}-ATPase, and the yeast H^+-ATPase [22]. Mutations of Asp-376 α-subunit to Asn, Glu, or Thr resulted in loss of enzyme function [23]. Seven amino acid residues Asp-376, Lys-487, Lys-507, Cys-663, Asp-716, Asp-720, and Lys-725 are suggested to participate in the formation of the ATP-binding pocket [24, 25].

With respect to the cationic binding sites, the role of transmembrane glutamic acid residues in cation binding was investigated by the technique of site-directed mutagenesis. Mutations of Glu-955 and Glu-956 of rat α-subunit (corresponding to Glu-959 and Glu-960 of the *Torpedo* pump) to glutamine or aspartic acid revealed no differences in cation stimulation of ATPase activity compared to the wild type if measured in open membrane fragments. This has been taken as evidence that the carboxyl groups of these glutamic acid residues are not essential for cation binding or occlusion. In addition to these glutamic amino acid residues, Pro-333 and Leu-337 (Pro-328 and Leu-332 of the α-subunit) of the Pro-Glu-334-Gly-Leu-Leu range have been suggested to be important for the Na^+ and K^+ affinities [26]. It has been discussed that particularly the proline is involved in optimizing cation binding to the glutamic acid residue Glu-334 in the E_2 conformation [27]. A diagrammatic representation of the operation of the sodium pump is presented in Figure 1.

5. Interaction of Mercurial Compounds with Sodium Pump

Excellent findings and reviews have shown that Hg^{2+} has very strong affinity for thiols on proteins. And considering the topology of Na^+/K^+-ATPase, the ATP and cationic binding sites are potential targets for Hg^{2+} assaults. For example, the nucleotide binding site contains SH group that favors the binding of Hg^{2+}. Herein, it suffices to say that since inorganic mercury salts and other nonorganic forms of mercury only poorly penetrate the cerebral microvascular endothelial cells comprising the blood-brain barrier (BBB), their neurotoxicity may be predicted to result from interference with this transport enzyme in other tissues. In addition, it is of note that specific binding of Hg^{2+} to ouabain-sensitive Na^+/K^+-ATPase of rat liver plasma membrane was demonstrated and observed that the binding of mercury to the enzyme also causes significant inhibition of the enzyme, which is greater than its ouabain sensitivity. Also it has been established that in the cytosol Hg^{2+} binding to reduced glutathione (GSH) is stimulated by GSH-S-transferase (GST). It is proposed that the transport of Hg^{2+} inside the cell takes place by increased dissociation of Hg^{2+} from the membrane due to greater avidity of Hg^{2+} towards cytosolar GSH binding. The GSH-Hg complex enters the nucleus where it dissociates to bind the metal response element (MRE) of the metallothionein (MT) gene to induce MT transcription. In fact, a schematic

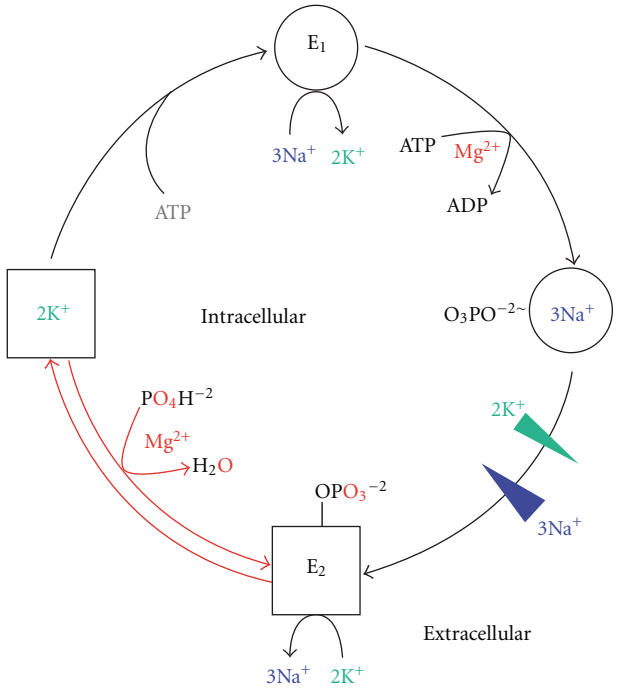
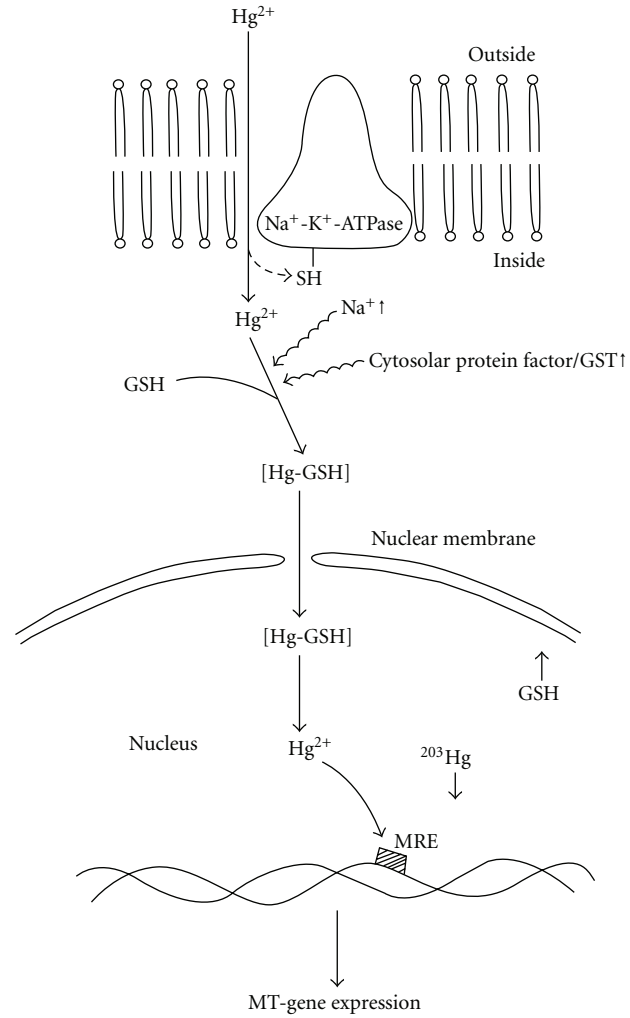


Figure 1: Reaction cycle. The minimal steps in the catalysis-transport cycle of sodium pump are shown schematically. Black arrows indicate the normal, clockwise direction of the reaction sequence. Oxygen isotope (red) exchange may occur in the reversible step colored red. The cycle represents the sidedness, as well as repetition, of the reactions. For example, the 3 Na^+ (blue) that displace 2 K^+ (green) intracellularly are subsequently displaced by 2 K^+ extracellularly, generating the inward Na^+ (larger) and outward K^+ (smaller) gradients indicated by wedge shapes. A circle and a square symbolize the limiting protein conformations (E_1 and E_2, resp.) with enclosed ions indicating occlusion. Different colors denote ATP acting as an effector (gray) or substrate (black) [9].



Scheme 1: The model proposes a mechanism of signal transduction by inorganic mercury in the rat hepatocyte, inducing metallothionein synthesis. Hg first binds to the Na^+-K^+-ATPase at the inner surface of the membrane and then dissociates to bind to GSH which is preferred due to higher affinity of the metal for GSH. The Hg-GSH complex enters the nucleus through the 9 nm aqueous channel of the nuclear membrane where Hg induces the expression of the metallothionein gene by binding to the metal responsive element [28].

illustration of the possible differential avidity of Hg^{2+} towards different thiols in cellular compartment is illustrated in the excellent work described by Bhattacharya and his coworkers (Scheme 1, [28]). At this point, information will be abstracted from available findings by various authors with respect to binding and subsequent toxicity of Hg^{2+} on the activity of the sodium pump under *in vitro* and *in vivo* conditions.

6. Mercury Toxicity on Sodium Pump *In Vitro*

In 1986, Kramer et al. [29] observed that among other heavy metals, Hg^{2+} ranked the highest in their inhibition of the Na^+/K^+-ATPase. Therefore, this finding showed that mercury toxicity on sodium pump is of significance and deserved critical attention. In that paper [29], they observed that the degree of inhibition of sodium pump by heavy metals is in the order: $Hg^{2+} > Pb^{2+} > Cd^{2+} > Ur^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+} > Ba^{2+} > Ni^{2+}Sr^{2+}$. Enzyme kinetic studies showed that Hg^{2+} and other heavy metals such as Pb^{2+}, and Cd^{2+} competitively, whereas Cu^{2+} noncompetitively, inhibited the enzyme. Similarly, in the same year, Magour [30] showed that mercurial compounds inhibited the brain synaptosomal Na^+/K^+-ATPase *in vitro*. In the paper [30], he observed that $HgCl_2$ is a more powerful inhibitor than methylmercury (MeHg). But in contrast to Kramer et al., they reported that both mercurials exhibited a noncompetitive type of inhibition. He observed however that the nonessential lipids do not play a significant role in the inhibitory effect of MeHg and that the potency of these mercurials to inhibit brain synaptosomal Na^+/K^+-ATPase largely depends on their capacity to block sulfhydryl groups. In the following year, Magour and his collaborators [31] revealed that the nonionic detergent Lubrol potentiated the inhibitory effect of mercurial compounds on the Na^+/K^+-ATPase, and they concluded that Lubrol removes the bulk lipids present

outside the catalytic center of the enzyme. Consequently, they concluded that the enzyme was more sensitive to the inhibition by both mercurials. However, in my opinion, this argument is not plausible since such lipid moiety that possibly shields the sodium pump as proposed by Magour and his associates can equally serve as a hindrance for the entry of the substrates (Na^+ and K^+) of the sodium pump as well as interaction of Mg^{2+} with the ATP. It is reasonable to conclude that Lubrol could possibly alter the fluid mosaic bilayer structure anchoring the sodium pump consequently leading to an altered three-dimensional structure of the transmembrane pump under consideration. Apparently, the emerging transmembrane protein structure could be such that the partially embedded thiols on the protein are more exposed and consequently affording a more direct interaction of Hg^{2+} with these exposed thiols, thus leading to a potentiated inhibitory effect of Hg^{2+} on the sodium pump. Alternatively, experimental evidence in our laboratory (data not yet published) has revealed that prooxidants that induce lipid peroxidation can also cause a diminished activity of the pump. With this preliminary observation, it is rational to conclude that lubrol may per se have a direct inhibitory effect on the pump by disrupting the lipids anchoring the sodium pump ultimately leading to a loss of activity which was potentiated by Hg^{2+}. Furthermore, in that same year that Magour and his associates evaluated the effect of lubrol on the pump, Ahammad Sahib and his coworkers [32] further observed that dithiothreitol (DTT) and the monothiols, glutathione (GSH), and cysteine (CYS) can protect against inhibition of sodium pump caused by mercury MeHg. Both monothiols and dithiols have the same ability in regenerating sulfhydryl (-SH) groups or chelating the metals. In addition, they also reported that Hg^{2+} inhibited K^+-p-nitrophenyl phosphatase (K^+-PNPPase), the component enzyme catalyzing the K^+-dependent dephosphorylation in the overall Na^+-K^+-ATPase reaction. They partially concluded that the mechanism of inhibition of the pump by mercurials involves binding to this phosphatase. Interestingly, they also found that the inhibition imposed by Hg^{2+} on the activity of K^+-PNPPase could be reversed by dithiothreitol (DTT), glutathione (GSH), and cysteine (CYS) suggesting the presence of sulfhydryl groups at the binding sites. Binding of ouabain, a cardiac glycoside and inhibitor of both phosphorylation and dephosphorylation, to brain fraction was significantly decreased by MeHg, and this inhibition was reversed by the three thiol compounds, suggesting presence of -SH group(s) in the ouabain receptor site. They concluded that the critical conformational property of enzyme common to both kinase (E_1) and phosphatase (E_2) is susceptible to MeHg and that MeHg decreased the catalytic velocity of dephosphorylation of the enzyme-phosphoryl complex and inhibits K^+-PNPPase by binding at two different sites [33]. Interestingly, Kumar et al. [34] also reported that mercury binds specifically to thiol groups present in the platelet membrane Na^+/K^+-ATPase, consequently inhibiting the enzyme, and induces changes in platelet function, namely, platelet aggregation by interfering with the sodium pump. In the work of Wang and Horisberger [35], they observed that although Hg^{2+} reacts with sulfhydryl groups on proteins to form mercaptides, in Xenopus laevis oocytes expressing wild-type and mutant forms of Na^+/K^+-ATPase, the pump was inhibited with first-order kinetics. Furthermore, they observed that Hg^{2+} binding to C_{113} of the first transmembrane segment of the alpha subunit from the extracellular side is one of the mechanisms by which mercury inhibits Na^+/K^+-ATPase. More recently, in my research group, we observed that Hg^{2+} significantly inhibited the transmembrane enzyme in a concentration-dependent manner. In addition, Hg^{2+} exerts its inhibitory effect on the activity of the enzyme by interacting with groups at the ATP, Na^+, and K^+ binding sites which was prevented but not reversed by the monothiol CYS. Hence, we speculated that the small molecular volume of Hg^{2+} in comparison with the substrates (ATP, Na^+, and K^+) of sodium pump, its possibly high reactivity, and strong affinity for thiols may account for its high toxicity towards the membrane bound ouabain-sensitive electrogenic pump [36]. However, the monothiol GSH also has the same protective effect as CYS. One worrisome finding is the fact that GSH at 4 mM markedly inhibited the activity of the pump. However, since GSH is differentially distributed in various organelles (1–15 mM), we speculate that there is a probable differential sensitivity of the sodium pump in various organelles towards the antioxidant tripeptide suggesting the possibility of a strong dynamics in the regulation of GSH in the various compartments of the organelles. We partially concluded that GSH may possibly be a potential candidate as a preintervention remedy in cases of mammalian exposure to inorganic mercury [37]. It should be emphasized that the inhibition of sodium pump by Hg^{2+} in the absence of ATP as observed in our group [36, 37] elicits a complex and puzzling chemistry. This puzzle is consequent from the fact that the binding of Hg^{2+} to a vicinal thiol not directly participating in ATP binding possibly impose steric hindrance to the overall three-dimensional structure of the pump consequently leading to loss of activity.

7. Mercury Toxicity on Sodium Pump *In Vivo*

Although *in vitro* data clearly showed that Hg^{2+} toxicity can be related to its inhibition of the Na^+/K^+-ATPase, it is desirable to also discuss the effect of Hg on the activities of the sodium pump under *in vivo* conditions. Herein, the focus will shift to a discussion of some findings by authors on the effect of Hg^{2+} on the activities of Na^+/K^+-ATPase under *in vivo* conditions. About a decade ago, Chuu et al. [38] treated mice with MeHg and HgS and observed that the analysis of auditory brainstem response (ABR) indicated that significant elevation of the physiological hearing threshold as well as significant prolongation of interwave latency was observed for MeHg- (2.0 and 0.2 mg/kg per day) or HgS- (1.0 g/kg per day, but not 0.1 g/kg per day) treated mice. Furthermore, both MeHg- and HgS-treated animals demonstrated a significant prolongation of interwave latency that increased with an increasing mean blood-Hg level. The otoneurotoxicity of MeHg (2.0 mg/kg per day) persisted to at least 11 weeks subsequent to the cessation of its administration. The toxic effect of HgS, however, disappeared completely 5 weeks

subsequent to the cessation of its administration. These results suggest that there is a correlation between the Hg^{2+}-elicited hearing dysfunction and the availability of mercury in brain tissue. However, of particular importance to this paper is the relationship of these physiological abnormalities to the activities of the sodium pump. They observed that the inhibition of Na^+/K^+-ATPase activity was accompanied with overproduction of nitric oxide in the brainstem and is consistent with an analysis of the physiological hearing threshold and latencies of ABR waveform at all time points throughout the experimental process. Consequently, they proposed that high-dose HgS or MeHg intoxication is associated with a decrease in functional Na^+/K^+-ATPase activity in the brainstem of affected animals, this presumably arising via excessive nitric oxide production, and suggesting that brainstem damage may play a role in mercury-induced hearing loss. Similarly, while studying the neurobehavioral effect of MetHg, HgS, and cinnabar (HgS-based Chinese medicine), they also observed that MeHg and cinnabar prominently and irreversibly caused a decrease in body weight, prolongation of latency for escape from electric shock, a decrease in the percentage for the conditioned avoidance response (CAR) to electric shock, impairment of spontaneous locomotion and inhibition of Na^+/K^+-ATPase activity of the cerebral cortex. In contrast, these authors also found that HgS reversibly inhibited spontaneous locomotion, and Na^+/K^+-ATPase activity. It was noted that HgS significantly decreased the latency of escape from electric shock during the administration period, which lasted for 33 weeks after discontinuous administration [39]. In fact, further study [40] showed that cinnabar and HgS evoked a significant inhibition of the enzymatic Na^+/K^+-ATPase activity of cerebellum, and this inhibition was associated with increase in the amount of cerebellar nitric oxide (NO) production. Hence, they partly concluded that the increased Hg^{2+} contents in the cerebellum following oral administration of HgS and cinnabar were responsible, at least in part, for the detrimental neurotoxic effect, decreasing Na^+/K^+-ATPase activity and increasing NO production within the cerebellum. About half a decade ago, Chuu et al. [41] also found that MeHg reversibly decreased both of motor nerve conduction velocity (MNCV) and tail flick response, whereas, irreversibly inhibited all of the motor equilibrium performance, recovery of compound muscle action potentials (CMAPs) following exhaustic tetanic stimuli, and Na^+/K^+-ATPase activity of the isolated sciatic nerve. These toxic effects of MeHg were found to correlation well with that of Hg^{2+} contents of various tissues (blood, cerebral cortex, liver, and kidney) in rats. In this paper, it was reported that neurotoxic effects produced by HgS were estimated to be about 1000 of those induced by MeHg. These authors have consistently reported that HgS, the major component of cinnabar as well as other mercurials such as MeHg decreased Na^+/K^+-ATPase activities, and they associated this decrease to production of NO radicals subsequently, suggesting that Hg^{2+} toxicity on the Na^+/K^+-ATPase may be an indirect effect. In fact in some of these papers, increase in lipid peroxidation has been associated with increase in NO and decrease in Na^+/K^+-ATPase [42–46]. In a closely related study [47], Chanez and his colleagues investigated the effects of thimerosal and mercuric chloride on Na^+/K^+-ATPase activity in total brain homogenate, synaptosomes and myelin at weaning, and the reversal effect of serotonin on mercury-inhibited Na^+/K^+-ATPase activity. They reported that the toxicity in terms of inhibition of Na^+/K^+-ATPase activity was greater with mercuric chloride than with thimerosal. In addition, synaptosomes and principally myelin were more sensitive to the metal salts than total homogenate. In addition, they found that serotonin stimulated Na^+/K^+-ATPase activity in total brain homogenate and synaptosomes but inhibited the enzyme in the myelin fraction. Furthermore, serotonin (1 mM) added to total homogenate pretreated with the mercury salts produced variable reversal effects. In the synaptosomal fraction, reverse effect was noted with serotonin. In myelin fraction, added serotonin increased inhibition caused by thimerosal. On the other hand, Verma and his associates [48] studied the effect of sublethal concentrations of $HgCl_2$ Na^+-K^+, Mg^{2+}, and total ATPase activities in brain, gills, kidney and liver of Notopterus notopterus after 30 days exposure. Their findings indicated that Na^+-K^+-ATPase were inhibited maximally and significantly in brain and minimally and insignificantly in liver. Mg^{2+} ATPase was inhibited maximally and significantly (P less than 0.01) in brain and minimally and insignificantly in kidney. The relative inhibition of total, Na^+-K^+ and Mg^{2+} ATPases for the tissues studied was brain > gill > kidney > liver. At the concentration (1/25 fraction) the enzyme activity returned to the normal range.

In the work of Klonne and Johnson [49], they investigate whether DTT, a sulfhydryl-reducing agent, protected renal cortical sulfhydryl status in general, or the activity of various renal enzymes (Mg^{2+} and Na^+/K^+-ATPases, alkaline phosphatase, and glutathione peroxidase) in particular. Additionally, the occurrence of conjugated dienes was used to assess the degree of lipid peroxidation. It was found that $HgCl_2$ produced significant decreases in renal cortical protein-bound sulfhydryl concentration, alkaline phosphatase activity, and Na^+-K^+-ATPase activity within 2.5 h of administration, with no effect observed on glutathione peroxidase activity or the levels of conjugated dienes in rat renal cortex. Furthermore, administration of DTT 60 min after mercury neither provided protection from inhibition nor promoted restoration of the affected enzymes or sulfhydryl status. Consequently, they concluded that the partial protection of renal function offered by DTT in the early stages of mercury toxicity does not result from maintaining the integrity of renal cortical sulfhydryl status or the activity of the enzymes investigated. Furthermore, the early stages of mercury toxicity did not appear to be related to lipid peroxidation. However, using routine histochemical staining in conjunction with light and electron microscopy to evaluate the changes in the Na^+-K^+-ATPase activity in cerebral cortical microvessels of rats who received a single intraperitoneal injection of 6 mg/kg $HgCl_2$, Szumańska and his collaborators [50] observed that at 1 h after $HgCl_2$ administration, light microscopy revealed uniform reduction of the Na^+-K^+-ATPase reaction in all cortical layers. Electron microscopy confirmed that the enzyme reaction is to be very weak to completely absent in both the luminal and abluminal endothelial cell membranes, and the luminal plasmalemma showed invaginations and

pinocytic vesicles indicative of changes in its transport functions. The enzyme inhibition coincided with, and was likely to contribute to, profound perivascular swelling, involving mainly the astrocytic endfeet. The enzyme activity showed a partial recovery 18 h after $HgCl_2$ treatment, mainly in cortical layers II and III. After 5 days, the recovery of the enzyme activity appeared complete as observed by light and electron microscopy. They also reported that the recovery of the microvascular Na^+-K^+-ATPase coincided with the appearance of a strongly positive Na^+-K^+-ATPase reaction in the adjacent astrocytic processes and with the diminution of perivascular swelling. Using genetically hypertensive rats models, Anner and his collaborates [51] investigated putative Na^+-K^+-ATPase alterations associated with the disease. Na^+-K^+-ATPase of two strains of spontaneously hypertensive rats, the Milan hypertensive strain (MHS) and the spontaneously hypertensive rat (SHR), were characterized in comparison with enzymes isolated from their matched normotensive controls; the sensitivity to Na ions and the shape and span of the inhibition curves for ouabain and mercury of the isolated Na^+/K^+-ATPases were compared. They found that no functional changes between the purified "normotensive" and "hypertensive" Na^+-K^+-ATPase from brain and kidney were detected ruling out drastic structural alterations of the transport system in these two organs of diseased animals. Mondal et al. [52] demonstrated that $HgCl_2$ treatment enhanced a remarkably high rate of progesterone synthesis accompanied by a low rate of conversion to 17 beta-estradiol in the oocyte of *Channa punctatus*. On depuration, however, there was a reversal of the steroidogenic scenario with a low progesterone and high estradiol level. They observed that the accumulation of progesterone was positively correlated with the significant increase in 3 beta-hydroxysteroid dehydrogenase activity in the Hg-treated fish. Thus, it was clear that at the early stage of intoxication Hg^{2+} does play a role in the induction of 3 beta-hydroxysteroid dehydrogenase in the oocyte of fish at the spawning stage. The induction of this enzyme was found to be mediated by specific binding of Hg^{2+} to the plasma membrane Na^+-K^+-ATPase and increase in the specific messenger RNA translating 3 beta-hydroxysteroid dehydrogenase. It is concluded that inorganic mercury is able to initiate translatable messenger RNA synthesis in fish oocyte at a low degree of intoxication.

8. Organoseleniums: Promising Intervention in Mercury Toxicity

Excellent reports and reviews have shown that selenium is a promising candidate in the management of mercury toxicity. In recent years, organoselenium compounds have been given considerable attention. These compounds have been well documented as potent antioxidants [53–62] and have a wide range of application in the management of oxidative stress-related diseases such as diabetes [63], cancer [64–66] and atherosclerosis [67] and other oxidative stress-related diseases [68]. Interestingly, since mercury toxicity has been linked to oxidative stress [69–71], consequently organoselenium compounds may be potential agents that can remedy mercury toxicity. Indeed, while the literature has an enormous volume of data on inorganic selenium, only few data have been available on the potential candidacy of organoselenium compounds in ameliorating mercury toxicity. Herein, some key findings on the effect of organoselenium compounds are described.

Although not the first reported organoselenium compound, diphenyl diselenide, a member of the diorganyl diselenide has shown promise as a candidate in the management of degenerative diseases [53–68] including mercury toxicity [69–71]. Diphenyl diselenide (1 mg/kg) has been shown to confer protection against MeHg-induced hepatic, renal, and cerebellum lipid peroxidation and prevented the reduction in hepatic NPSH levels. Of particular importance, diphenyl diselenide decreased the deposition of Hg^{2+} in cerebrum, cerebellum, kidney, and liver. This indicates that DPDS can protect against some toxic effects of MeHg in mice [62]. Furthermore, DPDS protected against the observed reduction in some haematological immunological alterations induced by mercury in mice [73]. In combination with N-acetyl cysteine, (NAC), DPDS was reported to be partially effective in protecting against the effects of mercury. In combination with 2,3-Dimercapto-1-propanesulfonic acid (DMPS), DPDS was reported to be effective in restoring the increment in urea concentration caused by mercury [74]. In the work of Meinerz et al. [75], they found that coincubation with DPDS (100 μM) completely prevented the disruption of mitochondrial activity as well as the increase in TBARS levels caused by MeHg. The compound 3′3-ditrifluoromethyldiphenyl diselenide provided a partial but significant protection against methylmercury-induced mitochondrial dysfunction (45.4 ± 5.8% inhibition of the methylmercury effect). These authors also found that hydrogen peroxide as a vector during methylmercury toxicity and that thiol peroxidase activity of organoselenium compounds accounts for their protective actions against methylmercury-induced oxidative stress. They conclude that DPDS and potentially other organoselenium compounds may represent important molecules in the search for an improved therapy against the deleterious effects of methylmercury as well as other mercury compounds. On the other hand, Brandão et al. [76] showed that for mice treated with a daily dose of $HgCl_2$ (4.6 mg kg (-1), subcutaneously) for three consecutive days followed by treatment with DPDS (31.2 mg kg (-1), subcutaneously), they observed that the combination of Hg^{2+} and DPDS exposure caused a decrease in renal GST and Na^+/K^+-ATPase activities and an increase in renal ascorbic acid and TBARS concentrations when compared with the Hg^{2+} group. DPDS potentiated the increase in plasma urea caused by Hg^{2+}. Similarly, combination of Hg^{2+} and DPDS exposure caused a reduction in plasma protein levels and an increase in hemoglobin and hematocrit contents when compared with the $HgCl_2$ group. There was a significant reduction in hepatic CAT activity and an increase in TBARS levels in mice exposed to Hg^{2+} and DPDS when compared with the Hg^{2+} group. The results demonstrated that DPDS did not modify mercury levels in mice. In conclusion, DPDS potentiated damage caused by Hg^{2+} affecting mainly the renal tissue.

Farina and his collaborates [77] studied the effects of MeHg exposure (subcutaneous injections of methylmercury chloride: 2 mg/kg) on the hepatic levels of thiobarbituric acid reactive substances (TBARS) and nonprotein thiols (NPSH), and on liver glutathione peroxidase (GSHPx) activity, as well as the possible antagonist effect of ebselen (another potent organoselenium compound; (10 mg/kg, subcutaneously)) against MeHg effects during the postnatal period. They observed that ebselen abolished the MeHg-induced inhibition on liver GSHPx activity, but did not prevent the oxidative effects of MeHg on liver lipids and NPSH. MeHg affects the *in vitro* interaction between ebselen and GSH, and this phenomenon seems to be responsible for its inhibitory effect toward thiol-peroxidase activity. Additionally, ebselen presents pro-oxidative effects on rat liver, pointing to thiol depletion as a molecular mechanism related to ebselen-induced hepatotoxicity [77]. However, it has been observed that while Hg^{2+} inhibited renal ALA-D activity, increased TBARS level in kidney, and reduced the hepatic content of nonprotein thiol groups, but organoselenium compounds did not prevent such effects. However, under *in vitro* conditions, renal and hepatic ALA-D activity was inhibited by Hg^{2+} and ebselen. Also Hg^{2+} significantly increased TBARS production in renal and hepatic tissue preparations *in vitro*, and this effect was completely or partially prevented by organoselenium compounds. They concluded that it appears that organoselenium compounds could not prevent mercury toxicity *in vivo*. Consequently, they related the protective effect of ebselen and other organoselenium compounds tested against mercury-induced increase of TBARS production *in vitro* to an antioxidant action rather than to mercury binding [78, 79]. Also Yin et al. [80] found that although MeHg treatment significantly decreased astrocytic [3*H*]-glutamine uptake at all time points and concentrations, ebselen fully reversed MeHg's (1 μM) effect on [3*H*] -glutamine uptake at 1 min. At higher MeHg concentrations, ebselen partially reversed the MeHg-induced astrocytic inhibition of [3*H*] -glutamine uptake. In addition, ebselen inhibited MeHg-induced phosphorylation of ERK ($P < 0.05$) and blocked MeHg-induced activation of caspase-3. These results are consistent with the hypothesis that MeHg exerts its toxic effects via oxidative stress and that the phosphorylation of ERK and the dissipation of the astrocytic mitochondrial membrane potential are involved in MeHg toxicity. In addition, the protective effects elicited by ebselen reinforce the idea that organic selenocompounds represent promising strategies to counteract MeHg-induced neurotoxicity [80].

9. Organoselenium and Na^+/K^+-ATPase

So far, we have seen that Hg^{2+} exerts its inhibitory effect on the sodium pump by oxidizing critical thiols on the enzyme. In like manner, available data shows that organoseleniums also inhibit the sodium pump by oxidizing thiols on the sodium pump. The data presented thereafter illustrates this fact. For example, while investigating the toxic effects of some organoselenium compounds: m-trifluoromethyl-diphenyl diselenide (m-CF(3)-C(6)H(4)Se)(2), p-chloro-diphenyl diselenide (p-Cl-C(6)H(4)Se)(2), and p-methoxyl-diphenyl diselenide (p-CH(3)O-C(6)H(4)Se)(2), Brüning et al. [81] observed that these organoseleniums inhibited $Na(^+)$, $K(^+)$-ATPase activity and that the enzyme is more sensitive to (p-Cl-C(6)H(4)Se)(2), and (m-CF(3)-C(6)H(4)Se)(2) (IC(50) 6 microM) than (p-CH(3)O-C(6)H(4)Se)(2) and (PhSe)(2) (IC(50) 45 and 31 microM, resp.). Furthermore, they observed that the inhibition was reversed by the thiol-protecting agents such as dithiothreitol DTT. Furthermore, they concluded that the effect of diselenides on Na^+/K^+-ATPase is dependent on their substitutions in the aromatic ring and that oxidation of critical thiols on the enzyme is one mechanisms by which these organoselenium compounds inhibit Na^+/K^+-ATPase activities involves the oxidation of thiol groups. Earlier, Borges et al., [82] observed that besides the diroganyl diselenides, other classes or organochalcogens such as ebselen and telluride compounds evoke similar inhibitory pattern as reported in [81]. They concluded that cerebral Na^+/K^+-ATPase is a potential molecular target for the toxic effect of organochalcogens, and the inhibition may occur through a change in the crucial thiol groups of this enzyme.

The above data were obtained under *in vitro* conditions, and clearly it shows that organoselenium compounds are potential target of the sodium pump. However, it is not clear whether the observed *in vitro* effect will be replicated *in vivo*. Findings on the effect of organoseleniums on the activities of the sodium pump consistently show that organoselenium compound significantly improves the activities of the pump under *in vivo* conditions. Some specific data are presented to drive home this fact. Barbosa and her collaborates observed that when organoselenium was administered to diabetic rats, they attenuated both hyperglycemic conditions and oxidative stress indices that were high in the diabetic rats. Interestingly, the low activities of cerebral sodium pump observed in diabetic rats were significantly improved upon after treatment with DPDS [83, 84]. In a related study, I equally observed that although STZ evokes a significant diminution on the antioxidant status and activity of Na^+/K^+-ATPase, DPDS was able to markedly restore the observed imbalance in cerebral antioxidant status and also relieve the inhibition of Na^+/K^+-ATPase caused by streptozotocin [85].

More recently, a comparison of the effect of two organoselenium compounds, DPDS and dicholesteroyl diselenide (DCDS), shows that mice administered these compounds at doses of 0.5 mmol kg^{-1} body weight in soya bean oil for four consecutive days exhibited no alteration in the activities of thiol-containing enzymes such as delta aminolevulinic acid dehydratase (ALA-D), Na^+/K^+-ATPase, and isoforms of lactate dehydrogenase (LDH) and catalase further indicating that cerebral Na^+/K^+-ATPase may not be a molecular target of organodiselenides toxicity [86]. Consequently, the pharmacological and toxicological chemistry of organoselenium compounds is complex and multifactorial and is dependent on delicate equations [86].

In a related experiment, authors have reported that subchronic administration of DPDS (300 micromole/kg body weight once a day for 14 days) significantly increased aspartate aminotransferase (AST) and alanine aminotransferase

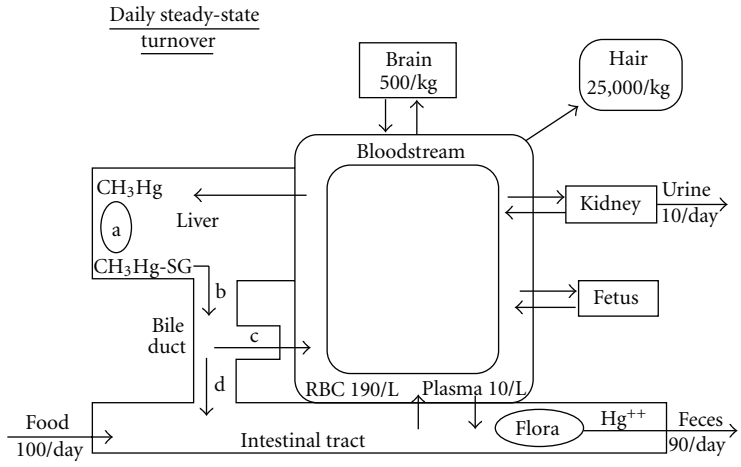


Figure 2: A diagram of the enterohepatic recirculation of methylmercury and its movement to maternal brain, kidney, and hair and to fetal tissues. (a) Methylmercury combines with reduced glutathione to form a complex, (b) which is secreted into bile. This glutathione is hydrolyzed to its constituent amino acids, releasing the methylmercury-cysteine complex. The latter, in part, is reabsorbed in the gallbladder into the bloodstream (c) and, in part, secreted into the intestinal tract along with any unhydrolysed glutathione complex (d). Once in the intestinal tract, it is in part reabsorbed into the portal circulation as the cysteine complex and in part demethylated by intestinal microflora. Most of the inorganic mercury produced in this way is excreted in the feces. The numbers quoted in each body compartment are the relative concentrations of methylmercury and the intakes and excretion rates [72].

(ALT) activities in plasma but did not alter lactate dehydrogenase (LDH) activity, urea and creatinine levels in plasma, and renal Na^+/K^+-ATPase [87]. Conversely, chronic administration of DPDS (500 mg/kg) increased the levels of lipid peroxidation and catalase activity as well as decreased delta-ALA-D (delta-aminolevulinate dehydratase) and Na^+/K^+-ATPase activity in the brain of rat pups [88, 89].

10. Organoselenium and Mercury Toxicity on Na^+/K^+-ATPase: A Paradox

From the foregoing, it is apparent that both mercury and organoselenium compounds inhibit Na^+/K^+-ATPase under *in vitro* conditions, and the mechanism involved in this inhibition mainly involves the oxidation of critical thiols on the pump. However, under *in vivo* conditions, Hg^{2+} inhibits the pump, whereas under *in vivo* and even different pathological conditions, organoselenium improves the activity of the pump that had been hitherto inhibited by the pathologies. This is a paradox, and the mechanisms for the observed protection offered by organoselenium under these conditions are yet to be completely elucidated. However, available data on the protection offered by organoselenium on the pump under consideration are scanty. One striking observation from my lab [66, 85, 86] and my collaborators [83, 84] as case studies is the fact that irrespective of the route of administration, organoseleniums elicit an increase in thiol contents in tissues of rats or mice. This effect of organoselenium points to the fact that the use of organoseleniums holds promise as a preventive measure in the management of mercury toxicity. It has been reported that inhibition of the sodium pump under conditions of oxidative stress *in vivo* can be prevented or reversed by thiols [49, 90] showing that oxidation of the critical thiols in the pump is responsible for its inactivity. Similarly, mercury has also been documented to oxidize these critical thiols on this transmembrane pump [49]. It is rational therefore to speculate that preadministration of organoseleniums may be a first line of defense in occupationally exposed individuals such as those in mines and factories where contact with mercury is generally unavoidable. Interestingly, mercury has been demonstrated to bind thiols such as glutathione with such efficiency, and such thiols have been employed in the management of mercury toxicity. For example, while they are not the first to make this observation, however, as a reference, the work of Khan [91] and his collaborators will underscore this point. In their paper, they observed that decrease in GSH level was dependant on mercuric chloride concentration and time of incubation *in vivo*. They suggested that the decrease in the concentration of reduced state glutathione may be due to the interaction of reduced state glutathione (GSH) and mercuric chloride to form oxidized glutathione (GSSG) or mercuric-glutathione complex. This change in GSH metabolic status provides information regarding the role of GSH in detoxification of mercuric chloride. Their argument is well illustrated in Figure 2.

Consequently, this paper may serve as a drive towards the synthesis as well as biological testing of these emerging selenocompounds on the activities of the sodium pump. Few available data show that this is feasible [53, 54]. Consider for example, a paper by Hassan et al., [92] where he and his collaborators reported on the antioxidant and toxicological profile of an imine (-N) containing organoselenium compound that did not inhibit Na^+/K^+-ATPase activities. The interaction of mercury with sodium pump may be critical in medical assessment and intervention of mercury toxicity.

For example, due to the inaccessibility of human nerve tissue for direct biochemical evaluation, there appears to be a need to identify peripheral markers which will reflect toxicity to the central nervous system by relatively noninvasive means. The erythrocytes Na^+/K^+-ATPase have been postulated to be a possible marker of mercury toxicity even though the first experimental data is not pointing in this direction especially under acute exposure [93].

Acknowledgment

The author is grateful for the financial support of CNPQ-INCT for excitotoxicity and neuroprotection.

References

[1] W. J. Crinnion, "Environmental medicine, part three: long-term effects of chronic low- dose mercury exposure," *Alternative Medicine Review*, vol. 5, no. 3, pp. 209–223, 2000.

[2] T. Y. K. Chan, "Inorganic mercury poisoning associated with skin-lightening cosmetic products," *Clinical Toxicology*, vol. 49, pp. 886–891, 2011.

[3] R. A. Bernhoft, "Mercury toxicity and treatment: a review of the literature," *Journal of Environmental and Public Health*, vol. 2012, Article ID 460508, 10 pages, 2012.

[4] L. C. Masur, "A review of the use of mercury in historic and current ritualistic and spiritual practices," *Alternative Medicine Review*, vol. 16, no. 4, pp. 314–320, 2011.

[5] P. Li, X. Feng, and G. Qiu, "Methylmercury exposure and health effects from rice and fish consumption: a review," *International Journal of Environmental Research and Public Health*, vol. 7, no. 6, pp. 2666–2691, 2010.

[6] D. A. Geier, P. G. King, L. K. Sykes, and M. R. Geier, "A comprehensive review of mercury provoked autism," *Indian Journal of Medical Research*, vol. 128, no. 4, pp. 383–411, 2008.

[7] S. T. Schultz, "Does thimerosal or other mercury exposure increase the risk for autism? A review of current literature," *Acta Neurobiologiae Experimentalis*, vol. 70, no. 2, pp. 187–195, 2010.

[8] J. C. Skou, "The influence of some cations on an adenosine triphosphatase from peripheral nerves," *Biochimica et Biophysica Acta*, vol. 23, pp. 394–401, 1957.

[9] L. D. Faller, "Mechanistic studies of sodium pump," *Archives of Biochemistry and Biophysics*, vol. 476, no. 1, pp. 12–21, 2008.

[10] P. L. Jorgensen and P. A. Pedersen, "Structure-function relationships of Na^+, K^+, ATP, or Mg^{2+} binding and energy transduction in Na,K-ATPase," *Biochimica et Biophysica Acta*, vol. 1505, no. 1, pp. 57–74, 2001.

[11] L. A. Vasilets and W. Schwarz, "Structure-function relationships of cation binding in the Na^+/K^+-ATPase," *Biochimica et Biophysica Acta*, vol. 1154, no. 2, pp. 201–222, 1993.

[12] J. D. Robinson and P. R. Pratap, "Indicators of conformational changes in the Na^+/K^+-ATPase and their interpretation," *Biochimica et Biophysica Acta*, vol. 1154, no. 1, pp. 83–104, 1993.

[13] G. E. Shull, A. Schwartz, and J. B. Lingrel, "Amino-acid sequence of the catalytic subunit of the (Na^+- K^+)ATPase deduced from a complementary DNA," *Nature*, vol. 316, no. 6030, pp. 691–695, 1985.

[14] R. L. Post and S. Kume, "Evidence for an aspartyl phosphate residue at the active site of sodium and potassium ion transport adenosine triphosphatase," *Journal of Biological Chemistry*, vol. 248, no. 20, pp. 6993–7000, 1973.

[15] E. M. Price, D. A. Rice, and J. B. Lingrel, "Structure-function studies of Na,K-ATPase. Site-directed mutagenesis of the border residues from the H1-H2 extracellular domain of the α subunit," *Journal of Biological Chemistry*, vol. 265, no. 12, pp. 6638–6641, 1990.

[16] K. Geering, I. Theulaz, F. Verrey, M. T. Hauptle, and B. C. Rossier, "A role for the β-subunit in the expression of functional Na^+-K^+-ATPase in Xenopus oocytes," *American Journal of Physiology*, vol. 257, no. 5, pp. C851–C858, 1989.

[17] M. Kawamura and S. Noguchi, "Possible role of the β-subunit in the expression of the sodium pump," in *The Sodium Pump: Structure, Mechanism, and Regulation*, J. H. Kaplan and P. DeWeer, Eds., pp. 45–62, The Rockefeller University Press, New York, NY, USA, 1991.

[18] A. A. McDonough, K. Geering, and R. A. Farley, "The sodium pump needs its β subunit," *FASEB Journal*, vol. 4, no. 6, pp. 1598–1605, 1990.

[19] W. E. Harris and W. L. Stahl, "Origin of the γ polypeptide of the Na^+/K^+-ATPase," *Biochimica et Biophysica Acta*, vol. 942, no. 2, pp. 236–244, 1988.

[20] R. W. Mercer, D. Biemesderfer, D. P. Bliss, J. H. Collins, and B. Forbush, "Molecular cloning and immunological characterization of the γ-subunit of the Na, K-ATPase," in *The Sodium Pump: Recent Developments*, P. DeWeer and J. Kaplan, Eds., pp. 37–41, Rockefeller University Press, New York, 1991.

[21] J. Castro and R. A. Farley, "Proteolytic fragmentation of the catalytic subunit of the sodium and potassium adenosine triphosphatase. Alignment of tryptic and chymotryptic fragments and location of sites labeled with ATP and iodoacetate," *Journal of Biological Chemistry*, vol. 254, no. 7, pp. 2221–2228, 1979.

[22] P. L. Jorgensen and J. P. Andersen, "Structural basis for E1-E2 conformational transitions in Na,K-pump and Ca-pump proteins," *Journal of Membrane Biology*, vol. 103, no. 2, pp. 95–120, 1988.

[23] M. Ohtsubo, S. Noguchi, K. Takeda, M. Morohashi, and M. Kawamura, "Site-directed mutagenesis of Asp-376, the catalytic phosphorylation site, and Lys-507, the putative ATP-binding site, of the α-subunit of Torpedo californica Na^+/K^+-ATPase," *Biochimica et Biophysica Acta*, vol. 1021, no. 2, pp. 157–160, 1990.

[24] C. H. Pedemonte and J. H. Kaplan, "Chemical modification as an approach to elucidation of sodium pump structure-function relations," *American Journal of Physiology*, vol. 258, no. 1, pp. C1–C23, 1990.

[25] K. N. Dzhandzhugazyan, S. V. Lutsenko, and N. N. Modyanov, "Target-residues of the active site affinity modification are different in E1 and E2 forms," in *The Na^+, K^+-Pump. Part A: Molecular Aspects*, J. C. Skou, J. G. Norby, A. B. Maunsbach, and M. Esmann, Eds., pp. 181–188, Alan R. Liss, New York, NY, USA, 1988.

[26] B. Vilsen, "Functional consequences of alterations to Pro328 and Leu332 located in the 4th transmembrane segment of the α-subunit of the rat kidney N^+,K^+-ATPase," *FEBS Letters*, vol. 314, no. 3, pp. 301–307, 1992.

[27] J. P. Andersen and B. Vilsen, "Structural basis for the E1/E1P-E2/E2P conformation changes in the sarcoplasmic reticulum Ca^{2+}-ATPase studied by site-specific mutagenesis," *Acta Physiologica Scandinavica, Supplement*, vol. 146, no. 607, pp. 151–159, 1992.

[28] S. Bhattacharya, S. Bose, B. Mukhopadhyay et al., "Specific binding of inorganic mercury to Na^+-K^+-ATPase in rat liver plasma membrane and signal transduction," *BioMetals*, vol. 10, no. 3, pp. 157–162, 1997.

[29] H. J. Kramer, H. C. Gonick, and E. Lu, "*In vitro* inhibition of Na-K-ATPase by trace metals: relation to renal and cardiovascular damage," *Nephron*, vol. 44, no. 4, pp. 329–336, 1986.

[30] S. Magour, "Studies on the inhibition of brain synaptosomal Na^+/K^+-ATPase by mercury chloride and methyl mercury chloride," *Archives of Toxicology*, vol. 9, pp. 393–396, 1986.

[31] S. Magour, H. Maser, and H. Greim, "The effect of mercury chloride and methyl mercury on brain microsomal Na^+-K^+-ATPase after partial delipidisation with Lubrol," *Pharmacology and Toxicology*, vol. 60, no. 3, pp. 184–186, 1987.

[32] K. I. Ahammadsahib, R. Ramamurthi, and D. Dusaiah, "Mechanism of inhibition of rat brain (Na^+-K^+)-stimulated adenosine triphosphatase reaction by cadmium and methyl mercury," *Journal of Biochemical Toxicology*, vol. 2, pp. 169–180, 1987.

[33] K. I. Ahammad Sahib, K. S. Moorthy, and D. Desaiah, "Effects of methyl mercury and cadmium on the kinetics of substrate activation of (K^+)-paranitrophenyl phosphatase," *Journal of Applied Toxicology*, vol. 7, no. 3, pp. 221–226, 1987.

[34] S. V. Kumar, S. Maitra, and S. Bhattacharya, "*In vitro* binding of inorganic mercury to the plasma membrane of rat platelet affects Na^+-K^+-ATPase activity and platelet aggregation," *BioMetals*, vol. 15, no. 1, pp. 51–57, 2002.

[35] X. Wang and J. D. Horisberger, "Mercury binding site on Na^+/K^+-ATPase: a cysteine in the first transmembrane segment," *Molecular Pharmacology*, vol. 50, no. 3, pp. 687–691, 1996.

[36] T. I. Omotayo, J. B. T. Rocha, E. O. Ibukun, and I. J. Kade, "Inorganic mercury interacts with thiols at the nucleotide and cationic binding sites of the ouabain-sensitive cerebral electrogenic sodium pump," *Neurochemistry International*, vol. 58, no. 7, pp. 776–784, 2011.

[37] I. J. Kade, T. I. Omotayo, and J. B. T. Rocha, "Threshold protective effect of glutathione on inorganic mercury inhibition of cerebral sodium pump," *Biochemistry-An Indian Journal*, vol. 6, no. 2, pp. 65–70, 2012.

[38] J. J. Chuu, C. J. Hsu, and S. Y. Lin-Shiau, "Abnormal auditory brainstem responses for mice treated with mercurial compounds: involvement of excessive nitric oxide," *Toxicology*, vol. 162, no. 1, pp. 11–22, 2001.

[39] J. J. Chuu, S. H. Liu, and S. Y. Lin-Shiau, "Effects of methyl mercury, mercuric sulfide and cinnabar on active avoidance responses, Na^+/K^+-ATPase activities and tissue mercury contents in rats," *Proceedings of the National Science Council, Republic of China B*, vol. 25, no. 2, pp. 128–136, 2001.

[40] Y. H. Young, J. J. Chuu, S. H. Liu, and S. Y. Lin-Shiau, "Neurotoxic mechanism of cinnabar and mercuric sulfide on the vestibulo-ocular reflex system of guinea pigs," *Toxicological Sciences*, vol. 67, no. 2, pp. 256–263, 2002.

[41] J. J. Chuu, S. H. Liu, and S. Y. Lin-Shiau, "Differential neurotoxic effects of methylmercury and mercuric sulfide in rats," *Toxicology Letters*, vol. 169, no. 2, pp. 109–120, 2007.

[42] C. F. Huang, S. H. Liu, C. J. Hsu, and S. Y. Lin-Shiau, "Neurotoxicological effects of low-dose methylmercury and mercuric chloride in developing offspring mice," *Toxicology Letters*, vol. 201, pp. 196–204, 2011.

[43] C. F. Huang, C. J. Hsu, S. H. Liu, and S. Y. Lin-Shiau, "Ototoxicity induced by cinnabar (a naturally occurring HgS) in mice through oxidative stress and down-regulated Na^+/K^+-ATPase activities," *NeuroToxicology*, vol. 29, no. 3, pp. 386–396, 2008.

[44] C. F. Huang, C. J. Hsu, S. H. Liu, and S. Y. Lin-Shiau, "Neurotoxicological mechanism of methylmercury induced by low-dose and long-term exposure in mice: oxidative stress and down-regulated Na^+/K^+-ATPase involved," *Toxicology Letters*, vol. 176, no. 3, pp. 188–197, 2008.

[45] J. J. Chuu, Z. N. Huang, H. H. Yu, L. H. Chang, and S. Y. Lin-Shiau, "Attenuation by methyl mercury and mercuric sulfide of pentobarbital induced hypnotic tolerance in mice through inhibition of ATPase activities and nitric oxide production in cerebral cortex," *Archives of Toxicology*, vol. 82, no. 6, pp. 343–353, 2008.

[46] C. F. Huang, S. H. Liu, and S. Y. Lin-Shiau, "Neurotoxicological effects of cinnabar (a Chinese mineral medicine, HgS) in mice," *Toxicology and Applied Pharmacology*, vol. 224, no. 2, pp. 192–201, 2007.

[47] C. Chanez, M. A. Flexor, and J. M. Bourre, "Effect of organic and inorganic mercuric salts on Na^+K^+ATPase in different cerebral fractions in control in intrauterine growth-retarded rats: alterations induced by serotonin," *NeuroToxicology*, vol. 10, no. 4, pp. 699–706, 1989.

[48] S. R. Verma, M. Jain, and I. P. Tonk, "In vivo effect of mercuric chloride on tissue ATPases of Notopterus notopterus," *Toxicology Letters*, vol. 16, no. 3-4, pp. 305–309, 1983.

[49] D. R. Klonne and D. R. Johnson, "Enzyme activity and sulfhydryl status in rat renal cortex following mercuric chloride and dithiothreitol administration," *Toxicology Letters*, vol. 42, no. 2, pp. 199–205, 1988.

[50] G. Szumańska, R. Gadamski, and J. Albrecht, "Changes of the Na/K ATPase activity in the cerebral cortical microvessels of rat after single intraperitoneal administration of mercuric chloride: histochemical demonstration with light and electron microscopy," *Acta Neuropathologica*, vol. 86, no. 1, pp. 65–70, 1993.

[51] B. M. Anner, E. Imesch, and M. Moosmayer, "Normal sensitivity of Na^+/K^+-ATPase isolated from brain and kidney of spontaneously hypertensive rats to sodium, ouabain or mercury," *Biochimica et Biophysica Acta*, vol. 1270, no. 1, pp. 95–99, 1995.

[52] S. Mondal, B. Mukhopadhyay, and S. Bhattacharya, "Inorganic mercury binding to fish oocyte plasma membrane induces steroidogenesis and translatable messenger RNA synthesis," *BioMetals*, vol. 10, no. 4, pp. 285–290, 1997.

[53] C. W. Nogueira, G. Zeni, and J. B. T. Rocha, "Organoselenium and organotellurium compounds: toxicology and pharmacology," *Chemical Reviews*, vol. 104, no. 12, pp. 6255–6285, 2004.

[54] C. W. Nogueira and J. B. T. Rocha, "Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds," *Archives of Toxicology*, vol. 85, no. 11, pp. 1313–1359, 2011.

[55] A. S. Prestes, S. T. Stefanello, S. M. Salman et al., "Antioxidant activity of b-selenoamines and their capacity to mimic different enzymes," *Molecular and Cellular Biochemistry*, vol. 365, no. 1-2, pp. 85–92, 2012.

[56] C. A. Prauchner, A. S. Prestes, and J. B. T. Rocha, "Effects of diphenyl diselenide on oxidative stress induced by sepsis in rats," *Pathology*, vol. 207, pp. 554–558, 2011.

[57] A. S. Freitas and J. B. T. Rocha, "Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: a pathway for their neuroprotective effects," *Neuroscience Letters*, vol. 503, pp. 1–5, 2011.

[58] M. Ibrahim, M. Prigol, W. Hassan, C. W. Nogueira, and J. B. T. Rocha, "Protective effect of binaphthyl diselenide, a synthetic organoselenium compound, on 2-nitropropane-induced hepatotoxicity in rats," *Cell Biochemistry and Function*, vol. 28, no. 4, pp. 258–265, 2010.

[59] M. Ibrahim, C. Luchese, S. Pinton et al., "Involvement of catalase in the protective effect of binaphthyl diselenide against

renal damage induced by glycerol," *Experimental and Toxicologic Pathology*, vol. 63, no. 4, pp. 331–335, 2011.

[60] C. L. D. Corte, R. Fachinetto, R. Puntel et al., "Chronic treatment with fluphenazine alters parameters of oxidative stress in liver and kidney of rats," *Basic and Clinical Pharmacology and Toxicology*, vol. 105, no. 1, pp. 51–57, 2009.

[61] D. H. Roos, R. L. Puntel, M. M. Santos et al., "Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: involvement of oxidative stress and glutamatergic system," *Toxicology in Vitro*, vol. 23, no. 2, pp. 302–307, 2009.

[62] A. S. de Freitas, V. R. Funck, M. D. S. Rotta et al., "Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice," *Brain Research Bulletin*, vol. 79, no. 1, pp. 77–84, 2009.

[63] M. Soriano-García, "Organoselenium compounds as potential therapeutic and chemopreventive agents: a review," *Current Medicinal Chemistry*, vol. 11, no. 12, pp. 1657–1669, 2004.

[64] R. Naithani, "Organoselenium compounds in cancer chemoprevention," *Mini-Reviews in Medicinal Chemistry*, vol. 8, no. 7, pp. 657–668, 2008.

[65] K. El-Bayoumy and R. Sinha, "Mechanisms of mammary cancer chemoprevention by organoselenium compounds," *Mutation Research*, vol. 551, no. 1-2, pp. 181–197, 2004.

[66] N. B. de Vargas Barbosa, C. W. Nogueira, T. N. Guecheva, M. de Lourdes Bellinaso, and J. B. T. Rocha, "Diphenyl diselenide supplementation delays the development of N-nitroso-N-methylurea-induced mammary tumors," *Archives of Toxicology*, vol. 82, no. 9, pp. 655–663, 2008.

[67] A. F. D. Bem, M. Farina, R. D. L. Portella et al., "Diphenyl diselenide, a simple glutathione peroxidase mimetic, inhibits human LDL oxidation *in vitro*," *Atherosclerosis*, vol. 201, no. 1, pp. 92–100, 2008.

[68] F. C. Meotti, E. C. Stangherlin, G. Zeni, C. W. Nogueira, and J. B. T. Rocha, "Protective role of aryl and alkyl diselenides on lipid peroxidation," *Environmental Research*, vol. 94, no. 3, pp. 276–282, 2004.

[69] M. Farina, M. Aschner, and J. B. T. Rocha, "Oxidative stress in MeHg-induced neurotoxicity," *Toxicology and Applied Pharmacology*, vol. 256, no. 3, pp. 405–417, 2011.

[70] M. Farina, J. B. T. Rocha, and M. Aschner, "Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies," *Life Sciences*, vol. 89, no. 15–16, pp. 555–563, 2011.

[71] M. Aschner, T. Syversen, D. O. Souza, J. B. T. Rocha, and M. Farina, "Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity," *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 3, pp. 285–291, 2007.

[72] T. W. Clarkson and L. Magos, "The toxicology of mercury and its chemical compounds," *Critical Reviews in Toxicology*, vol. 36, no. 8, pp. 609–662, 2006.

[73] R. Brandão, L. P. Borges, R. de Oliveira, J. B. T. Rocha, and C. W. Nogueira, "Diphenyl diselenide protects against hematological and immunological alterations induced by mercury in mice," *Journal of Biochemical and Molecular Toxicology*, vol. 22, no. 5, pp. 311–319, 2008.

[74] R. Brandão, F. W. Santos, M. Farina et al., "Antioxidants and metallothionein levels in mercury-treated mice," *Cell Biology and Toxicology*, vol. 22, no. 6, pp. 429–438, 2006.

[75] D. F. Meinerz, M. T. de Paula, B. Comparsi et al., "Protective effects of organoselenium compounds against methylmercury-induced oxidative stress in mouse brain mitochondrial-enriched fractions," *Brazilian Journal of Medical and Biological Research*, vol. 44, pp. 1156–1163, 2011.

[76] R. Brandão, R. N. Moresco, L. P. Bellé et al., "Diphenyl diselenide potentiates nephrotoxicity induced by mercuric chloride in mice," *Journal of Applied Toxicology*, vol. 31, pp. 773–782, 2011.

[77] M. Farina, F. A. A. Soares, G. Zeni, D. O. Souza, and J. B. T. Rocha, "Additive pro-oxidative effects of methylmercury and ebselen in liver from suckling rat pups," *Toxicology Letters*, vol. 146, no. 3, pp. 227–235, 2004.

[78] J. Perottoni, O. E. D. Rodrigues, M. W. Paixão et al., "Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic mercury and organoselenium compounds," *Food and Chemical Toxicology*, vol. 42, no. 1, pp. 17–28, 2004.

[79] J. Perottoni, L. P. Lobato, A. Silveira, J. B. T. Rocha, and T. Emanuelli, "Effects of mercury and selenite on δ-aminolevulinate dehydratase activity and on selected oxidative stress parameters in rats," *Environmental Research*, vol. 95, no. 2, pp. 166–173, 2004.

[80] Z. Yin, E. Lee, M. Ni et al., "Methylmercury-induced alterations in astrocyte functions are attenuated by ebselen," *NeuroToxicology*, vol. 32, no. 3, pp. 291–299, 2011.

[81] C. A. Brüning, M. Prigol, D. A. Barancelli, C. W. Nogueira, and G. Zeni, "Disubstituted diaryl diselenides inhibit δ-ALA-D and Na^+, K^+-ATPase activities in rat brain homogenates *in vitro*," *Molecular and Cellular Biochemistry*, vol. 332, no. 1-2, pp. 17–24, 2009.

[82] V. C. Borges, J. B. T. Rocha, and C. W. Nogueira, "Effect of diphenyl diselenide, diphenyl ditelluride and ebselen on cerebral Na^+, K^+-ATPase activity in rats," *Toxicology*, vol. 215, no. 3, pp. 191–197, 2005.

[83] N. B. V. Barbosa, J. B. T. Rocha, D. C. Wondracek, J. Perottoni, G. Zeni, and C. W. Nogueira, "Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress," *Chemico-Biological Interactions*, vol. 163, no. 3, pp. 230–238, 2006.

[84] N. B. V. Barbosa, J. B. T. Rocha, J. C. M. Soares et al., "Dietary diphenyl diselenide reduces the STZ-induced toxicity," *Food and Chemical Toxicology*, vol. 46, no. 1, pp. 186–194, 2008.

[85] I. J. Kade, C. W. Nogueira, and J. B. T. Rocha, "Diphenyl diselenide and streptozotocin did not alter cerebral glutamatergic and cholinergic systems but modulate antioxidant status and sodium pump in diabetic rats," *Brain Research*, vol. 1284, pp. 202–211, 2009.

[86] I. J. Kade and J. B. T. Rocha, "Comparative study on the influence of subcutaneous administration of diphenyl and dicholesteroyl diselenides on sulphydryl proteins and antioxidant parameters in mice," *Journal of Applied Toxicology*, vol. 30, no. 7, pp. 688–693, 2010.

[87] F. C. Meotti, V. C. Borges, J. Perottoni, and C. W. Nogueira, "Toxicological evaluation of subchronic exposure to diphenyl diselenide in rats," *Journal of Applied Toxicology*, vol. 28, no. 5, pp. 638–644, 2008.

[88] M. Prigol, E. A. Wilhelm, C. C. Schneider, J. B. T. Rocha, C. W. Nogueira, and G. Zeni, "Involvement of oxidative stress in seizures induced by diphenyl diselenide in rat pups," *Brain Research*, vol. 1147, no. 1, pp. 226–232, 2007.

[89] M. Prigol, E. A. Wilhelm, E. C. Stangherlin, D. A. Barancelli, C. W. Nogueira, and G. Zeni, "Diphenyl diselenide-induced seizures in rat pups: possible interaction with glutamatergic system," *Neurochemical Research*, vol. 33, no. 6, pp. 996–1004, 2008.

[90] O. P. Mishra, M. Delivoria-Papadopoulos, G. Cahillane, and L. C. Wagerle, "Lipid peroxidation as the mechanism of modification of the affinity of the Na^+, K^+-ATPase active sites

for ATP, K^+, Na^+, and strophanthidin *in vitro*," *Neurochemical Research*, vol. 14, no. 9, pp. 845–851, 1989.

[91] H. Khan, M. F. Khan, S. U. Jan, M. Mukhtiar, N. Ullah, and N. Anwar, "Role of Glutathione in protection against mercury induced poisoning," *Pakistan Journal of Pharmaceutical Science*, vol. 25, no. 2, pp. 395–400, 2012.

[92] W. Hassan, S. Pinton, J. T. D. Rocha et al., "Hydroxyl containing seleno-imine compound exhibits improved anti-oxidant potential and does not inhibit thiol-containing enzymes," *Chemico-Biological Interactions*, vol. 190, no. 1, pp. 35–44, 2011.

[93] W. E. Maier and L. G. Costa, "Na^+/K^+-ATPase in rat brain and erythrocytes as a possible target and marker, respectively, for neurotoxicity: studies with chlordecone, organotins and mercury compounds," *Toxicology Letters*, vol. 51, no. 2, pp. 175–188, 1990.