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Na^+/K^+-ATPase in rat brain and erythrocytes as a possible target and marker, respectively, for neurotoxicity: studies with chlordecone, organotins and mercury compounds*

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SUMMARY

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 non-invasive means. The aim of this study was to investigate whether the enzyme Na^+/K^+-ATPase in erythrocytes could be used as a marker for effects on the same enzyme in brain tissue. The compounds chosen to test this hypothesis were the pesticide chlordecone, the organotin compounds triethyltin and tributyltin, mercuric chloride and methyl mercury. All compounds were found to inhibit in vitro Na^+/K^+-ATPase activity in rat brain ($IC_{50}s = 0.9–56\ \mu M$) and in rat erythrocytes ($IC_{50}s = 1.2–66\ \mu M$) with similar potencies. However, administration of these compounds in vivo at high doses produced no significant inhibition of either brain or erythrocyte Na^+/K^+-ATPase activity, despite observed symptoms of neurotoxicity. Dialysis experiments indicated that dissociation of the compounds by dilution during tissue preparation was not responsible for the lack of detectable in vivo inhibition. Measurements of metal concentrations in brain by atomic absorption spectrometry after in vivo administration of triethyltin, mercuric chloride and methyl mercury indicated that levels of these compounds were too low to inhibit significantly NA^+/K^+-ATPase activity. These results suggest that inhibition of Na^+/K^+-ATPase activity might not represent the mechanism responsible for the neurotoxicity of these compounds, and that erythrocyte Na^+/K^+-ATPase activity is not a useful marker for neurotoxicity following acute exposures.

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INTRODUCTION

Several hundreds of environmental contaminants and industrial chemicals are toxic to the nervous system [1]. Assessment of their effects on the central nervous system (CNS) is difficult due to the relative inaccessibility of the human CNS for direct biochemical evaluation. There appears therefore to be a need in toxicology to identify peripheral markers, easily and ethically obtained in humans, that will accurately reflect adverse effects on the CNS from occupational exposures to neurotoxic compounds, such as pesticides and metals [2].

Several parameters of neurotransmission have been identified in blood components, whose pharmacological and biochemical characteristics appear to be similar to those of the CNS. Examples include: β-adrenoceptors and muscarinic receptors in lymphocytes [3,4]; Na^+/K^+-ATPase in erythrocytes [5]; and α_2-adrenoceptors and serotonin uptake in platelets [6,7]. These neurotransmitter parameters may perhaps serve as a 'window' to the CNS, by which neurotoxicity can be monitored with relatively non-invasive means.

This approach has gained support in biological psychiatry, where the potential of peripheral tissues (e.g. platelet monoamine oxidase and erythrocyte Na^+/K^+-ATPase) as diagnostic indicators of mental diseases such as schizophrenia and affective disorders is being examined [8]. In neurotoxicology, clinical use of peripheral markers has been utilized only for evaluating the effects of organophosphate pesticides (OP) on cholinergic transmission. Inhibition of red blood cell acetylcholinesterase has been reasonably well correlated with OP exposure and severity of symptoms, and, therefore, is reflective of the degree of inhibition occuring in nervous tissue [9,10].

The general aim of this study was to determine whether other parameters of neurotransmission present in blood cells could serve as peripheral indicators of neurotoxicity. Specifically, the enzyme Na^+/K^+-ATPase, which plays a primary role in regulating membrane function and neurotransmission, was examined for use as a marker following neurotoxic insults. Some examples of known inhibitors of brain Na^+/K^+-ATPase include organochlorine insecticides [11, 12], organotin compounds [13,14], and metals such as cadmium [15] and mercury [16]. Inhibition of the Na^+-pump by these compounds has been suggested to be at least partially responsible for their observed neurotoxic symptoms.

Na^+/K^+-ATPase is a membrane-bound enzyme found in most tissues and is primarily responsible for maintaining ionic membrane potential by actively transporting Na^+ and K^+ across cell membranes [17,18]. The integrity of Na^+/K^+-ATPase is exceptionally critical in excitatory nerve cells which rely on proper membrane potential for propagation of action potentials and subsequent release of neurotransmitters. Synaptic nerve endings are a rich source of Na^+/K^+-ATPase and synaptosomal preparations are frequently used to study brain ATPase activity [19]. The unit activity of Na^+/K^+-ATPase in erythrocytes was found to be of the same order as that observed in cardiac tissue [20]; however, compared to brain tissue the total en-

zyme activity, expressed per mg protein, is considerably lower. Erythrocyte ATPase activity is frequently studied using hemoglobin-free erythrocyte membranes (ghosts) since this preparation leaves the enzyme intact and permits ATP and ouabain (used to determine specific activity) access to their respective extracellular and intracellular binding sites.

To validate the use of measurements of Na^+/K^+-ATPase in erythrocytes as a marker for the same enzyme in brain, the organochlorine pesticide, chlordecone (Kepone), the organotins triethyltin (TET) and tributyltin (TBT), mercuric chloride ($HgCl_2$) and methyl mercury (CH_3Hg) were chosen as chemical probes. Chlordecone has previoulsy been demonstrated to inhibit Na^+/K^+-ATPase activity both in vitro and in vivo and its neurotoxicity was thought to be at least in part due to inhibition of this enzyme [21,22]. TET and TBT were included in this study based principally on reports of in vitro inhibition of brain Na^+/K^+-ATPase activity [13,14], and also based on speculations that an alteration in Na^+/K^+-ATPase function is responsible for organotin-induced brain edema in white matter [23]. Finally, reports of potent in vitro inhibition of brain ATPase by mercuric chloride and methyl mercury warranted their inclusion in this study [16]. Also considered was a report that a small group of mineworkers chronically exposed to mercury had reduced erythrocyte Na^+/K^+-ATPase activities [24].

MATERIALS AND METHODS

Chemicals

Chlordecone (95–99% pure) was purchased from Supelco Chemical Co. (Bellefont, PA). Triethyltin chloride (TET; 95+%), tributyltin bromide (TBT), tetramethylammonium hydroxide and methyl mercury chloride (CH_3Hg; 95+%) were purchased from Alfa Products (Danvers, MA). Mercuric chloride ($HgCl_2$; 99.99%) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Dimethylformamide (DMF) and Ultra-pure nitric acid were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). All other compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and treatments

Male Sprague–Dawley rats (250–350 g) were obtained from Tyler Laboratories (Bellevue, WA) and were housed 4 per cage in the Department of Environmental Health vivarium at the University of Washington. Food (Purina Lab Chow) and water were available ad libitum.

For the in vitro experiments, chlordecone, TET, TBT and CH_3Hg were dissolved in DMF (3–7.5% v/v), while $HgCl_2$ was dissolved in 50 mM Tris buffer (pH 7.5). DMF inhibited 10–25% of enzyme activity, and results were therefore corrected for this vehicle effect. For the in vivo dosing experiments, rats were administered chlordecone, TET, TBT and CH_3Hg dissolved in corn oil, while $HgCl_2$ was dissolved in

0.9% sodium chloride. Volume of injection was 1 ml/kg body wt. Control rats received the same volume of vehicle only. Rats were fasted overnight before oral administration of chlordecone at a dose of 100 mg/kg body wt [21]; rats were sacrificed after 5 h. Organotins were administered by intraperitoneal injection at a dose of 10 mg/kg (free-base weight) and rats were sacrificed after 5 h [25]. $HgCl_2$ and CH_3Hg were administered by intraperitoneal injection at a dose of 6 mg/kg, and rats were sacrificed 12 h after dosing, since at thise dose death from renal failure would be expected within 24 h [26].

Preparation of erythrocyte ghosts

Hemoglobin-free erythrocyte membranes were prepared according to the methods of Hanahan and Ekholm [27] and Costa and Murphy [28], with slight modifications. Briefly, rats were anesthesized with ether and blood collected by cardiac puncture in a heparinized syringe. Whole blood was centrifuged at $1000 \times g$ for 30 min to separate the plasma from the red blood cells (RBC). The packed RBC were then resuspended in 0.172 M, 310 mosmol Tris buffer (adjusted to pH 7.6 with 10 N HCl) and centrifuged two more times at $1000 \times g$ at 4°C for 30 min. A 5 ml aliquot of a 50% erythrocyte suspension was then diluted with 30 ml of 0.172 M, 20 mosmol Tris buffer, shaken vigorously, and centrifuged for 30 min at $35\,000 \times g$. This washing was repeated at least 5 times until all hemoglobin was removed. Erythrocyte membranes were then stored at −80°C until assayed

Brain tissue preparation

A crude synaptosomal preparation was prepared by homogenizing whole rat brains in 20 vol. of ice-cold 0.32 M sucrose containing 1 mM EDTA in a glass mortar with a Teflon pestle (0.004–0.006 in. clearance). The homogenates were then centrifuged at $1000 \times g$ at 4°C for 10 min. The supernatant fraction was retained and diluted with Tris buffer.

Determination of Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity was determined by slight modifications of previous methods [29,30]. Total ATPase activity was measured in a 1 ml reaction medium containing 115 mM Tris-HCl buffer (pH 7.5), 72.5 mM NaCl, 6.25 mM KCl, and 5.0 mM $MgCl_2$. Mg^{2+}-ATPase activity was measured in a K^+-free medium containing 175 mM Tris-HCl buffer, 5.0 mM $MgCl_2$, 14 mM NaCl, and 1 mM ouabain. The difference of the two activities is taken as Na^+/K^+-ATPase activity. The reaction was carried out at 37°C and initiated by the addition of 20 mM ATP (final conc. 2 mM) and terminated after 10 min by the addition of 1 ml ice-cold 12% trichloroacetic acid. The incubation tubes were then cooled to 4°C before centrifuging at $1000 \times g$ for 15 min to precipitate protein. The supernatant was removed for determination of released inorganic phosphorus by the colorimetry assay of Taussky and Shorr [31]. Protein content was determined by the method of Lowry et al. [32] and was 70–90 μg/ml for brain tissue and between 85–120 μg/ml for erythrocyte ghosts.

To evaluate the in vitro potency of each compound, tissue samples were incubated with 4–6 concentrations of each compound, and the percent of control Na^+/K^+-ATPase activity was determined for each concentration. Results are reported as IC_{50} values (the concentration necessary to inhibit 50% of enzyme activity). IC_{50} values were calculated by plotting the percent of control enzyme activity against the negative natural logarithm of the concentration of inhibitor.

Dialysis experiments

To determine whether the compounds administered in vivo rapidly dissociated from the enzyme during tissue preparation, dialysis experiments were conducted. A continuous flow microdialysis system with a semipermeable membrane (pore size

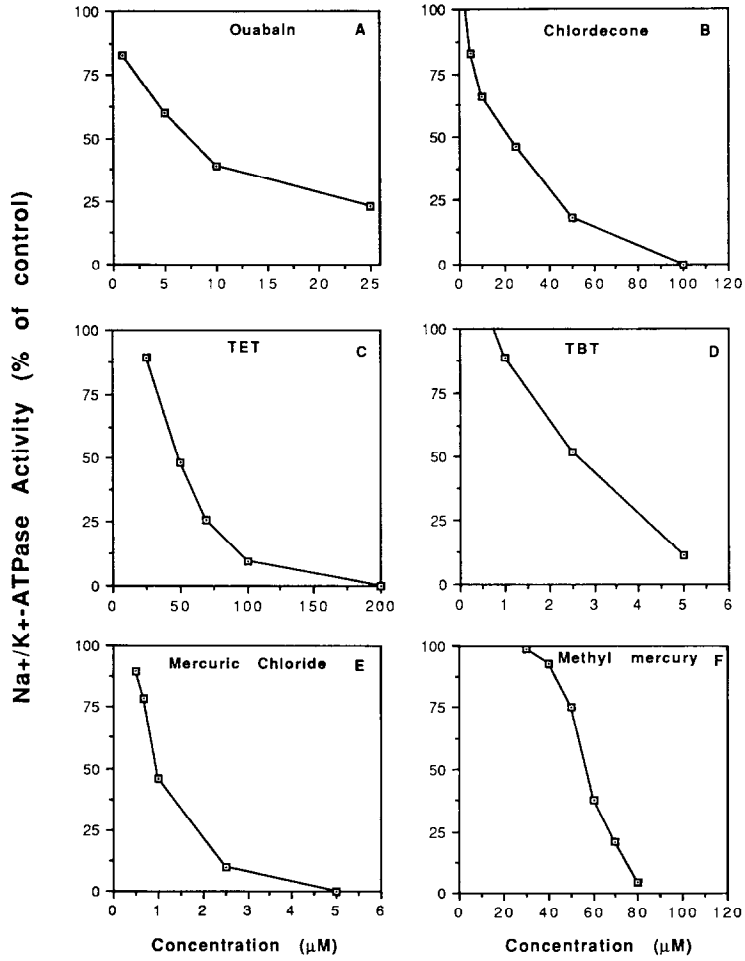

Fig. 1. In vitro inhibition of brain Na^+/K^+-ATPase activity by (A) ouabain, (B) chlordecone, (C) TET, (D) TBT, (E) mercuric chloride, (F) methyl mercury. Results show a representative experiment.

12 000–14 000 D) was utilized. Aliquots (0.5 ml) of synaptosomal preparations were preincubated with and without each inhibitor (present at a concentration equivalent to its IC_{50} value) for 10 min, at 37°C, and then transferred to the dialysis unit (or incubation tubes for undialyzed samples). A 1000-fold excess of Tris buffer flowing at a rate of approximately 40 ml/h was separated from the dialyzed tissue samples by the dialysis membrane. After 24 h at 4°C, dialyzed and undialyzed tissue samples were assayed for Na^+/K^+-ATPase activity.

Tin analysis

Analysis of total tin was conducted to determine the concentration of tin reaching the brain after in vivo administration of TET. Due to higher lipophilicity and a greater dose delivered to the brain, TET was chosen for tin analysis over TBT, de-

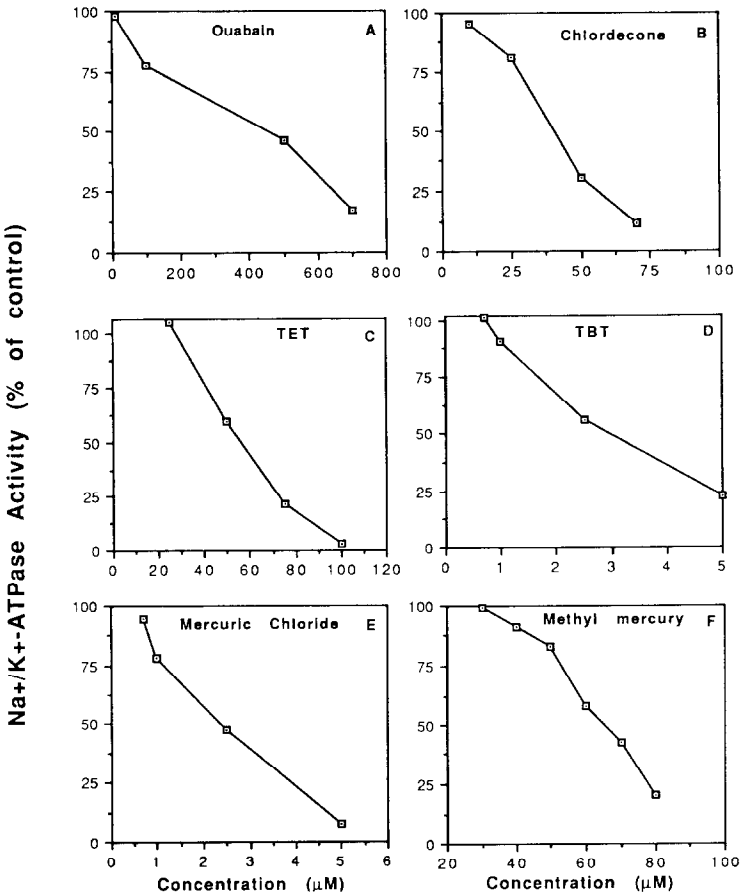

Fig. 2. In vitro inhibition of erythrocyte Na^+/K^+-ATPase activity by (A) ouabain, (B) chlordecone, (C) TET, (D) TBT, (E) mercuric chloride, (F) methyl mercury. Results show a representative experiment.

spite a lower in vitro potency. Whole brains (~1.7 g) were homogenized in 5 vol. 2% nitric acid in a glass tube with a Teflon homogenizer. Aliquots (0.5 ml) of tissue homogenate were digested overnight with 1 ml of tetramethylammonium hydroxide at 34°C in a 5-ml volumetric flask and brought to volume with H_2O prior to analysis. A Perkin-Elmer Zeeman Model 5100 atomic absorption spectrophotometer with a PE HGA-1820 graphite furnace, a PE tin electrodeless discharge lamp (with Zeeman background correction) and an AS-60 auto sampler were employed. Lamp wavelength was 224.6 nm with a slit width of 0.7 nm. During analysis, 10 μl of matrix modifier (0.2 mg $NH_2H_2PO_4$+0.01 mg $Mg(NO_3)_2$) and 20 μl of tissue sample were injected into the graphite furnace. Tin quantification was based on matrix-matched standards obtained by spiking control tissue homogenates with known amounts of TET. These standards were digested, diluted and analyzed along with the ex vivo tissue samples. Reproducibility of analysis was 4% RSD with a 6 ng limit of detection.

Mercury analysis

For mercury analysis, whole brains were homogenized in 2% HNO_3. Aliquots (2.5

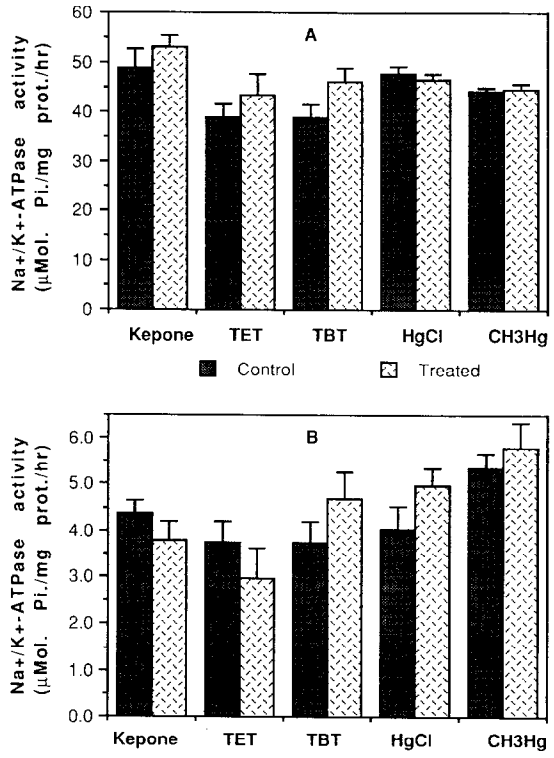

Fig. 3. Na^+/K^+-ATPase activity in (A) brain and (B) erythrocytes, from control and treated animals. Animals were treated with chlordecone (Kepone, 100 mg/kg), TET or TBT (10 mg/kg; free base weight), and $HgCl_2$ or CH_3H (6 mg/kg). Results represent the mean (±SEM) of 5 rats (10 rats for organotins).

ml) of tissue homogenates were then digested in 5.0 ml concentrated nitric acid and 2.5 ml of 6 M HCl. Digestion of tissue samples were conducted by heating in a microwave oven under the following conditions; 10 min at 293 watts; 5 min at 0 watts; 5 min at 345 watts. The vessels were then cooled on ice and solutions transferred to a 25-ml vol. flask and brought to volume with 2% HNO_3 prior to analysis. A Perkin-Elmer model 305B atomic absorption spectrophotometer with a Westinghouse hollow-cathode lamp was utilized with sodium borohydride reduction. Reduced elemental mercury from the samples was concentrated on a silver trap, which was then heated releasing mercury to the spectrophotometer for analysis. As in the tin analysis, quantification of mercury was based on tissue standards obtained by spiking tissue homogenates with known amounts of mercuric chloride or methyl mercury.

Data analysis

Results were analyzed for statistical significance by Student's *t*-test.

RESULTS

In vitro experiments,

Control values for Na^+/K^+-ATPase activities in rat brain and erythrocytes were 39.98 ± 1.6 and 4.58 ± 0.28 μmol P_i/mg prot./h, respectively. Na^+/K^+-ATPase activity comprised 40–60% of total ATPase activity.

All compounds examined inhibited brain and erythrocyte Na^+/K^+-ATPase activity (Figs. 1 and 2). As shown in Table I, IC_{50} values ranged from 0.9 to 56 μM in brain and from 1.4 to 282 μM in erythrocytes. Enzyme sensitivities were not significantly different with potency ratios of approximately one, except for the positive con-

TABLE I

INHIBITION OF NA^+/K^+-ATPase ACTIVITY IN VITRO

Compound	IC_{50} (μM)		Ratio RBC/brain	*n*
	Brain	Erythrocytes		
Ouabain	7.82 ± 2.10	282.10 ± 27.9*	36.0	5
Chlordecone	13.07 ± 1.70	32.42 ± 7.20	2.5	5
TET	56.11 ± 5.50	58.05 ± 7.21	1.0	4
TBT	3.24 ± 0.12	2.80 ± 0.37	0.9	3
$HgCl_2$	0.90 ± 0.10	1.42 ± 0.29	1.6	3
CH_3Hg	55.78 ± 1.50	66.16 ± 0.60	1.2	3

*Significantly different from brain, $P < 0.001$.

IC_{50} values were calculated by plotting the % of control enzyme activity against the negative natural logarithm of the molar concentration. Four to 6 concentrations of inhibitor, in duplicate, were used for each IC_{50} determination. Results are presented as mean values (±S.E.M.).

trol ouabain which was 36-fold less potent in erythrocytes ($P < 0.001$). Based on similar in vitro potencies of these compounds, it appeared that erythrocyte Na^+/K^+-ATPase was a suitable marker for the same enzyme in brain.

In vivo treatments

We then examined the in vivo effect of these compounds on Na^+/K^+-ATPase in the two tissues. Gross symptoms of tremors, muscle spasms, and exaggerated startle response were observed within 2 h after administration of chlordecone. There were no significant differences in enzyme activities between control and treated animals in either brain or erythrocytes. In contrast to chlordecone-treated animals, rats administered TET and TBT became hypoactive and lethargic; analysis of Na^+/K^+-ATPase activity in brain and erythrocytes produced the same results (i.e. no significant inhibition in treated animals, relative to controls). Rats treated with $HgCl_2$ and CH_3Hg were more lethargic than controls, although not as severely as observed following treatment with organotins. Again, there were no differences in Na^+/K^+-ATPase activities between mercury-treated and control animals in either brain or blood. The results of in vivo treatments are summarized in Figure 3.

Dialysis experiments

To determined whether the lack of Na^+/K^+-ATPase inhibition in brain tissue was due to rapid dissociation of the compounds from the enzyme during tissue prepara-

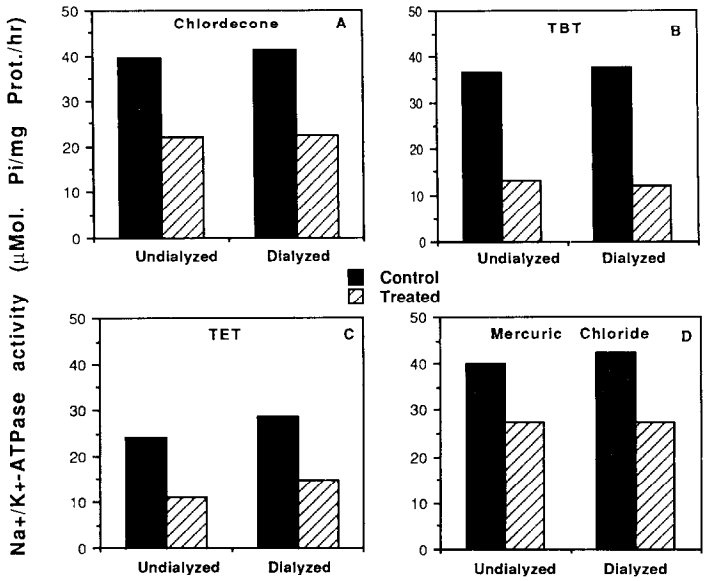

Fig. 4. Effect of dialysis on the in vitro inhibition of Na^+/K^+-ATPase activity by (A) chlordecone, (B), TBT, (C) TET, (D) mercuric chloride. Inhibitors were present at their IC_{50} concentration. Dialyzed and undialyzed samples were preincubated for 24 h at 4°C prior to assay. Each bar represents the average of duplicate experiments.

tion, in vitro dialysis experiments were conducted. If inhibitors easily dissociated from the enzyme complex by dilution, reactivation of enzyme activity would be expected for dialyzed samples. As shown in Figure 4, there were no differences in brain enzyme activities between dialyzed and undialyzed samples in either the presence or absence of inhibitors. This indicates that dilution during tissue preparation and assay was not responsible for the lack of detectable inhibition observed following in vivo administration of these compounds.

Measurement of brain metal concentrations

We then determined whether the concentration of metals reaching the brain after in vivo administration was too low to cause a detectable inhibition of enzyme activity. Using atomic absorption analysis, brain concentrations of metals were measured after administration of TET, $HgCl_2$ and CH_3Hg. Results of atomic absorption analyses are presented in Table II. Comparing the concentrations of tin and mercury measured in the brain of treated animals with the concentrations used in the in vitro studies, it s apparent that an insufficient concentration of metals was present in the brain to significantly inhibit enzyme activity.

DISCUSSION

The present study was initially undertaken to examine the potential of erythrocyte Na^+/K^+-ATPase as a peripheral marker for neurotoxicity. Values of Na^+/K^+-ATPase activity obtained in brain and erythrocytes in the present study are comparable to those reported in literature [15,22,23].

For most compounds, the in vitro potencies toward brain Na^+/K^+-ATPase are similar to those reported in previous studies. $HgCl_2$ was the most potent inhibitor examined with an IC_{50} of 0.9 μM, similar to the value of 0.65 μM previously reported

TABLE II

BRAIN CONCENTRATIONS OF METALS

Compound injected	Metal	Concentration (μM)	n^a
TET	Tin	10.5 ± 3.9	5
$HgCl_2$	Mercury	0.8 ± 0.2	4
CH_3Hg	Mercury	3.0 ± 0.9	5

[a]Number of animals (each analyzed in triplicate).

TET was administered at the dose of 10 mg/kg (free base weight) and rats were sacrificed after 5 h. $HgCl_2$ and CH_3Hg were injected at the dose of 6 mg/kg and animals were sacrificed after 12 h. Tin measurements were conducted using a graphite furnace atomic absorption, while mercury analysis was conducted using cold vapor atomic absorption (see 'Materials and Methods' for more details).

[16]. Interestingly, CH_3Hg was considerable less potent than $HgCl_2$ with an IC_{50} of 55.7 μM, confirming an observation made by another research group [34]. Also, chlordecone, TET and TBT displayed IC_{50} in the micromolar range, as shown earlier [14,22,33]. The effect of these compounds on Na^+/K^+-ATPase activity in erythrocytes had not previously been examined; thus these results cannot be compared with literature values. We found that the in vitro potencies of these compounds were similar in brain and erythrocytes (Table I), with the exception of the positive control, ouabain, which was 36-fold less potent in RBC than in brain. Several studies have reported a lower ouabain affinity in rat cardiac tissues relative to brain [20,36], and it would appear that rat erythrocytes have similar binding characteristics to those observed in cardiac tissues. Indeed, we could not detect any specific binding of [^{3}H]ouabain in rat erythrocytes [Maier and Costa, unpublished observations]. These results suggest that all compounds tested might inhibit Na^+/K^+-ATPase activity by binding at a locus different from the reception site for cardiac glycosides. Their equal in vitro potency toward the enzyme in erythrocytes and brain, however, prompted us to conduct a series of in vivo experiments aimed at comparing Na^+/K^+-ATPase activity in the two tissues.

Within hours after receiving a high dose of chlordecone, rats displayed severe symptoms of neurotoxicity, yet there was no significant inhibition of Na^+/K^+-ATPase activity in brain or erythrocytes. As dialysis experiments indicated, lack of detectable inhibition was not due to rapid dissociation by dilution during tissue preparation. These results differ from those of a previous study [21] where 35% inhibition of whole brain Na^+/K^+-ATPase activity was observed in rats administered 100 mg/kg chlordecone, and might be due to the two different brain preparations used.

Brain edema, often localized in the white matter, is a commonly observed symptom in TET-intoxicated animals. It has been hypothesized that TET alters the function of Na^+/K^+-ATPase resulting in permeability changes in cell membranes, and consequently leading to brain edema [23]. We examined whether an alteration of Na^+/K^+-ATPase activity could be detected following treatment with TET and TBT. After administration of a high dose of TET and TBT, rats became weak and extremely lethargic, yet no inhibition of Na^+/K^+-ATPase activity in brain and erythrocytes was detected. These results are consistent with those of Stine et al. [35], who also were unable to detect inhibition of brain Na^+/K^+-ATPase activity following in vivo administration of 6 mg/kg of TET. The in vivo effect of TBT on ATPases had not previously been examined. Dialysis experiments demonstrated that dilution from tissue preparation was not responsible for the lack of detectable inhibition. The average total tin level in brain following administration of TET at a dose of 10 mg/kg was 10.5 μM. This is consistent with the levels of tin observed in previous studies [24,37]. Using the upper limit of tin detected in brain (23 μM) and comparing this to the concentrations of TET used in the in vitro studies, it is apparent that the concentration of tin reaching the brain is too low to inhibit significantly Na^+/K^+-ATPase activity (Fig. 1). These results, and those of Stine et al. [35], lead us to conclude that inhibition

of whole-brain Na^+/K^+-ATPase might not be the main mechanism responsible for the neurotoxic effects of organotin compounds.

In vivo administration of a high dose of $HgCl_2$ and CH_3Hg also produced no inhibition of Na^+/K^+-ATPase activity in either brain or erythrocytes. This lack of inhibition was not due to decomplexation of mercury during tissue preparation as was determined by dialysis experiments. Atomic absorption analysis demonstrated that mercury levels in the brain of $HgCl_2$ and CH_3Hg-treated animals were 0.2 and 3.0 μM, respectively. Based on the in vitro inhibition studies, it is apparent that these concentrations are below those required to inhibit Na^+/K^+-ATPase. While the in vivo effect of $HgCl_2$ and CH_3Hg on Na^+/K^+-ATPase had not previously been examined, one study reported that a small group of mineworkers chronically exposed to mercury displayed decreased erythrocyte ATPase activities, compared to the general population [24]. This would suggest that perhaps a chronic study is needed in order to detect in vivo inhibition of Na^+/K^+-ATPase following in vivo exposure to mercury.

In summary, all compounds tested were shown to be potent in vitro inhibitors of brain and erythrocyte Na^+/K^+-ATPase, yet administration of these compounds in vivo at high doses produced symptoms of neurotoxicity without a corresponding inhibition of Na^+/K^+-ATPase. The reason for the discrepancy between in vitro inhibition and lack of detectable in vivo inhibition is not always clear. In the case of TET, $HgCl_2$ and CH_3Hg, we have demonstrated that the metal levels present in the brain following administration of neurotoxic doses were not high enough to inhibit Na^+/K^+-ATPase. From these results, one might infer that inhibition of Na^+/K^+-ATPase activity does not appear to be involved in the observed neurotoxicity of these compounds. However, the possibility also exists that Na^+/K^+-ATPase inhibition occurs in specific brain regions or in specific cell types.

An initial aim of this study was to examine erythrocyte Na^+/K^+-ATPase as a potential indicator of the same enzyme in brain. Unfortunately, no consistent pattern of parallel effects of enzyme activity in brain and erythrocytes was observed following in vivo administration of various neurotoxic compounds. If Na^+/K^+-ATPase has no clear relation to neurotoxic symptoms, as suggested by our findings, the erythrocyte enzyme would not be a useful marker for acute CNS toxicity. However, the report of decreased erythrocyte Na^+/K^+-ATPase activities in workers chronically exposed to mercury [24] still suggests that chronic studies might be necessary to infer or rule out conclusively a role for erythrocyte Na^+/K^+-ATPase as a useful peripheral marker for exposure to certain neurotoxicants.

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