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Effects of Lead and Natriuretic Hormone on Kinetics of Sodium-Potassium-Activated Adenosine Triphosphatase: Possible Relevance to Hypertension

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Inhibition of vascular smooth muscle sodium-potassium-activiated adenosine triphosphatase (Na-K-ATPase) has been postulated as a central mechanism in enhancing vascular contractility. In the present study, kinetics of inhibition of Na-K-ATPase by lead, ouabain, and natriuretic hormone (NH) was studied in a purified hog cerebral cortex enzyme preparation. Determination of I_{50} values for lead, ouabain, and NH revealed that NH is the most potent inhibitor of the enzyme system (0.8×10^{-6} M ouabain equivalents). Kinetic analyses indicated that lead and NH exhibited different inhibitory mechanisms. The inhibition by lead was noncompetitive with respect to potassium and competitive with respect to sodium and MgATP. Natriuretic hormone was noncompetitive with respect to potassium, uncompetitive with respect to MgATP, and exhibited no inhibitory effect with respect to sodium. Synergism between lead and NH in the inhibition of Na-K-ATPase raises the possibility that lead may be a contributory factor in hypertension via this mechanism.

Introduction

Epidemiological studies performed both in the United States (*1*) and in Great Britain (*2*) have suggested a correlation between blood pressure and blood lead within the range of blood lead levels seen in the normal population. The prevalence of hypertension in industrially exposed workers has been a matter of controversy. Studies performed by Cramer and Dahlberg in 1966 (*3*) showed no difference in the incidence of hypertension in workers directly exposed to lead as compared to age-matched workers in the same plant who had minimal lead exposure. More recent studies by de Kort et al. (*4*) and Kirby et al. (*5*) demonstrated a positive relationship between blood lead and blood pressure in lead-exposed populations. Thus, the issue has been raised that lead may be a possible causative or contributory factor in hypertension.

Lead, like many other heavy metals (vanadium, mercury, cadmium, uranium) (*6–9*) is a potent inhibitor of the membrane transport enzyme sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase, E. C. 3.6.1.3.). This effect of lead, if exerted on vascular smooth muscle, might produce enhanced vascular contractility. Within the past few years, there has been considerable interest in the possibility that a circulating transport enzyme inhibitor [the putative hypothalamic-renal natriuretic hormone (NH)] might be a major cause of essential hypertension via the same mechanism (*10–12*). A hypothesis advanced by Blaustein et al. (*11*) is that inhibition of the vascular smooth muscle sodium pump by NH leads to increased intracellular calcium concentrations, thus enhancing vascular responsiveness to the vasoconstrictive hormones norepinephrine and angiotensin II.

If lead and NH could be shown to inhibit Na-K-ATPase by different mechanisms, then the possibility exists that lead might act to increase vascular contractility by synergizing with NH in inhibiting the vascular smooth muscle sodium pump. The goals of the present study were first to contrast the kinetics of inhibition of highly purified Na-K-ATPase by lead

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and a semi-purified plasma Na-K-ATPase inhibitor, (NH), with the known specific inhibitor of Na-K-ATPase, ouabain, and second to explore the possibility of synergistic inhibition of the enzyme by lead and NH.

Methods

Purification of Natriuretic Hormone from Human Plasma

Separation of low molecular weight NH from high molecular weight material was achieved by passing the plasma sample from a hypertensive patient through an Amicon YM 2 (1 kilodalton cutoff) membrane at 20 psi. For further purification of the low molecular weight material, the filtrate was passed through a SEP-PAK cartridge and eluted with acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) as described in an earlier publication (*13*). The fraction eluting between 0% ACN and 20% ACN was lyophilized and stored for kinetic studies. Concentration of inhibitor was expressed as ouabain-equivalents (*13*). Lead acetate used for the kinetic studies was purchased from Fisher Scientific (Fair Lawn, NJ).

Na-K-ATPase Inhibition Assay

In these experiments a purified Na-K-ATPase from hog cerebral cortex was employed, purchased from Sigma Chemical (St. Louis, MO). The incubation tubes contained a substrate solution providing final concentrations of 1 mM ATP; 1 mM Mg; 10 mM imidazole-HCl buffer, pH 7.2; 100 mM Na; 20 mM K; and 1 mM ethylene glycol-bis-(β-aminoethyl ether) *N,N'* tetraacetic acid (EGTA). The tubes were incubated at 37°C for 5 min. The reaction was started by adding 0.025 mL of enzyme preparation (25 mg/mL). The incubation was stopped after 10 min by adding ice-cold trichloracetic acid (TCA). After centrifugation (1700*g* × 5 min), 0.5 mL of supernatant was assayed for inorganic phosphate by the method of Fiske and Subbarow (*14*). In all instances, enzyme inhibition was expressed as μmole Pi/mg protein/hr.

For determination of I_{50}, i.e., the amount of inhibitor necessary for 50% enzyme inhibition, serial dilutions of the purified low molecular weight plasma NH were prepared. For comparison, ouabain and lead were dissolved in water to result in final concentrations ranging from 4×10^{-8} M to 4×10^{-3} M ouabain and 5×10^{-5} M to 5×10^{-4} M Pb.

To further characterize the inhibitory activities of lead, ouabain, and NH, kinetic studies were performed with concentrations of potassium ranging from 0.125 mM to 5.0 mM, sodium ranging from 5 mM to 120 mM, and MgATP ranging from 0.25 mM to 3.0 mM, in the presence of constant amounts of lead, ouabain, and NH. For the MgATP studies, the ratio between Mg and ATP molar concentrations was maintained at 1:1. Michaelis-Menten parameters were calculated from the conventional Lineweaver-Burk plot.

Synergy

In order to determine whether lead and NH were synergistic inhibitors of Na-K-ATPase, a lead dose-response curve was first established. The combined effects of lead and NH were assayed in the presence of two different doses of NH. The low dose of NH produced 2% inhibition of the enzyme system, whereas the high dose yielded 12% inhibition.

Results

Lead, ouabain, and NH were found to be potent inhibitors of the Na-K-ATPase enzyme system. The I_{50} values for each substance are shown in Table 1.

Kinetic Studies

Potassium. The effects of varying concentrations of potassium on Na-K-ATPase activity in the presence of lead (5×10^{-5} M), ouabain (4×10^{-9} M), and NH (6.9×10^{-6} M) are shown in Figure 1. The inhibition of Na-K-ATPase by ouabain was found to be competitive with respect to potassium, whereas the inhibitory effects of NH and lead were found to be noncompetitive.

Sodium. With sodium as the variable sustrate, the kinetic analysis revealed that lead (2.5×10^{-5} M) is a competitive inhibitor (Fig. 2), whereas NH exhibited no inhibitory effect.

Magnesium Adenosine Triphosphate (MgATP). With MgATP as the variable substrate, kinetic analysis revealed that lead is a competitive inhibitor; NH (1.3×10^{-7}) exhibited uncompetitive inhibition (Fig. 3). The K_m and V_{max} values are listed in Tables 2 and 3.

Synergy

Figure 4 displays the dose-related inhibition of Na-K-ATPase in the presence of increasing lead concentrations ranging from 0.1×10^{-4} M to 5×10^{-4} M. In the presence of low dose NH, the inhibition curve is shifted to the left, i.e., inhibition is increased. In the presence of high dose NH, a further leftward shift of the Na-K-ATPase inhibition curve was observed. In both instances, the increment of inhibition produced by add-

Table 1. Determination of I_{50} values for inhibitors of Na-K-ATPase.

Substance	I_{50}, M[a]
Ouabain	2×10^{-6}
Lead	80×10^{-6}
NH	0.8×10^{-6}

[a]The substrate concentrations for lead and ouabain are expressed as M, whereas the concentration for NH is expressed as M ouabain equivalent.

ing NH was more than would be predicted by a simple addition effect.

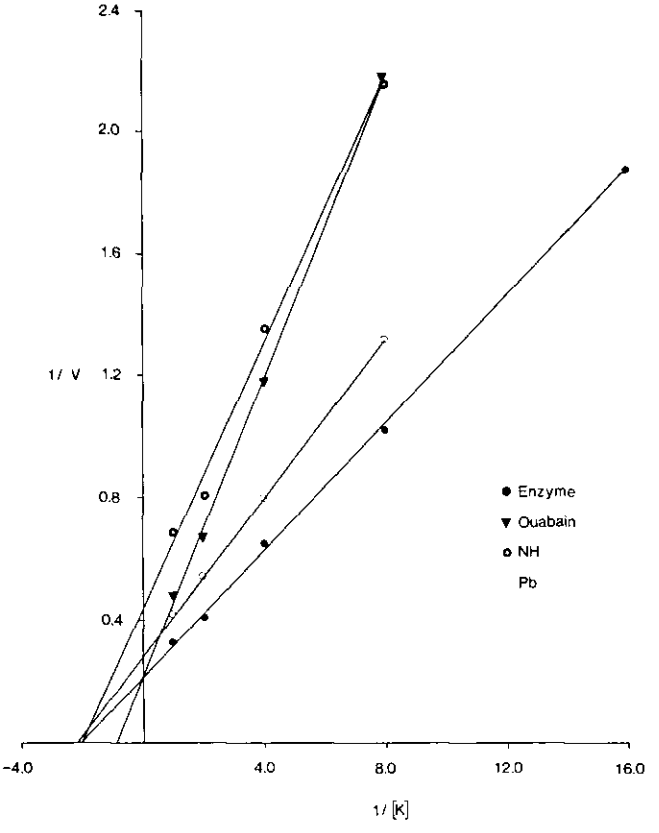

FIGURE 1. Lineweaver-Burk plot of kinetics of inhibition of Na-K-ATPase by lead (5×10^{-5} M), ouabain (4×10^{-9} M), and NH (0.69×10^{-9} M ouabain equivalents) with potassium as the variable substrate.

Discussion

The primary function of the membrane-bound Na-K-ATPase enzyme system is the active transport of sodium and potassium (*15,16*). This enzyme system is also involved in salt and water homeostasis (*17*), the transport of nonelectrolytes (*18*), and the secretion of potassium and hydrogen by kidney tubules (*18*). The activity of the Na-K-ATPase enzyme system is affected by a variety of hormones, including mineralo- and glucocorticoids (*19,20*), thyroid hormone (*21*), insulin (*22,23*), catecholamines (*24*), and NH (*25,26*), as well as trace metals [e.g., lead, vanadium, mercury, uranium, cadmium, silver (*8,9,27*)]. This interaction

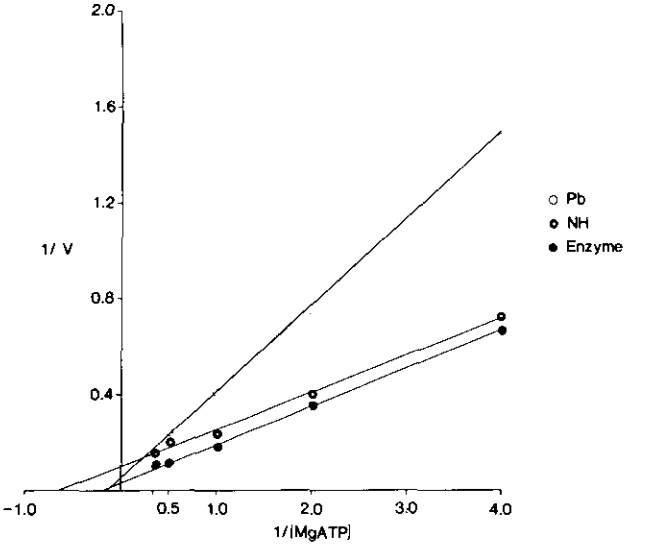

FIGURE 3. Lineweaver-Burk plot of kinetics of inhibition of Na-K-ATPase by lead (5×10^{-5} M) and NH (1.3×10^{-7} M ouabain equivalents) with MgATP as the variable substrate.

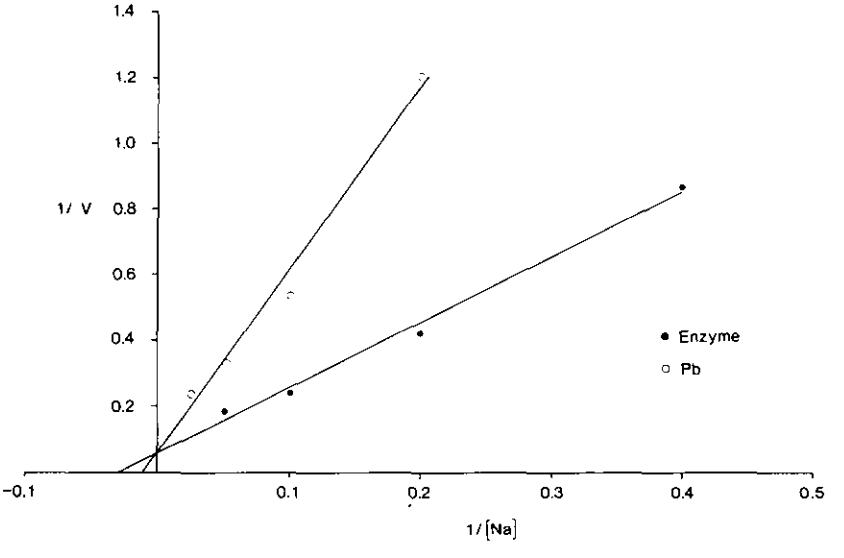

FIGURE 2. Lineweaver-Burk plot of kinetics of inhibition of Na-K-ATPase by lead (25×10^{-6} M) with sodium as the variable substrate.

Table 2. K_m values for inhibitors of Na-K-ATPase.

	K_m, mM		
Substrate	K	Na	MgATP
Enzyme	0.48	34.9	2.69
Lead	0.44	96.8	6.64
NH	1.61	—	—
Ouabain	0.50	—	—

Table 3. V_{max} values for inhibitors of Na-K-ATPase.

	V_{max}, μmole Pi/mg protein/hr		
Substrate	K	Na	MgATP
Enzyme	4.65	17.54	20.16
Lead	3.48	17.30	18.42
NH	2.30	—	10.13
Ouabain	4.77	—	—

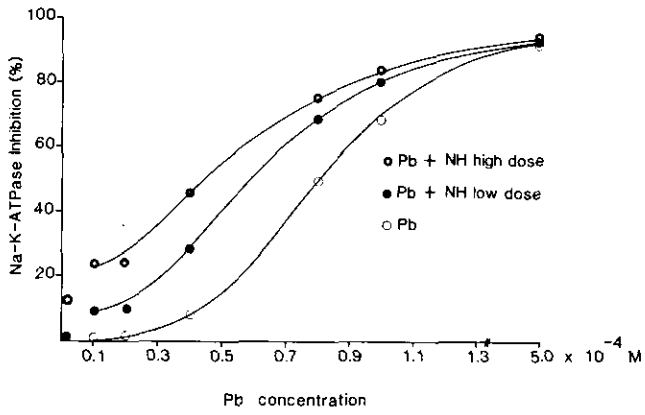

FIGURE 4. Inhibition of Na-K-ATPase by lead in the presence and absence of low dose and high dose NH. The inhibitory effect of low dose NH alone was 2%; the inhibitory effect of high dose NH alone was 12% (plotted adjacent to y-axis).

of metals with Na-K-ATPase may have physiological as well as toxicological significance.

Na-K-ATPase has been classified as a sulfhydryl (SH) enzyme, in part because of its inhibition by mercurials (*28*). Lead, as a metal that reacts with SH groups, would also be predicted to act as a Na-K-ATPase inhibitor. Kramer et al. (*9*) found that in renal cortical tissue homogenates, Na-K-ATPase was inhibited by 50% at a lead concentration of 7×10^{-5} M. Similar results were reported by Nechay and Saunders (*7*). These investigators showed that the concentration for 50% inhibition of Na-K-ATPase ranged from 6 to 8×10^{-5} M in both microsomes and tissue homogenates. The homogenate did not contain any protective substances that ameliorated Na-K-ATPase inhibition, as had been reported for mercury (*8*). Both studies demonstrated that lead caused a reversible inhibition of the enzyme system. Ethylenediaminetetraacetic acid (EDTA) prevents or reverses lead-induced inhibition of Na-K-ATPase (*7*). Kramer et al. (*9*) demonstrated that lead is a competitive inhibitor with respect to its substrate ATP. From earlier studies by Tice (*29*), it would appear that lead competes with magnesium for chelation by ATP. Lead is also known to inhibit Na-K-ATPase at its sodium-sensitive site (*7*). In the present study, which employed a purified hog cerebral cortex Na-K-ATPase enzyme preparation, we also found that 50% inhibition of the Na-K-ATPase enzyme system was achieved at a lead concentration of 8×10^{-5} M. The inhibition of Na-K-ATPase by lead was found to be noncompetitive with respect to potassium, but competitive with respect to sodium and MgATP.

Endogenous inhibitors of Na-K-ATPase have been isolated from both plasma and urine by differing methodologies. Thus, the NH studied by various laboratories may consist of similar or different substances. Kramer et al. (*30*), studying a semi-purified urinary inhibitor, described the NH inhibition of Na-K-ATPase as noncompetitive with respect to potassium. Crabos et al. (*31*) reported that NH, also isolated from urine, inhibited Na-K-ATPase noncompetitively with respect to ATP. Inhibition of the enzyme system was facilitated by sodium. Ouabain, a digitalis glycoside and a specific inhibitor of the Na-K-ATPase system, has been shown to be competitive with respect to potassium (*32*) and noncompetitive with respect to ATP (*31*). In the present study, we employed a semi-purified NH preparation derived from plasma of a patient with essential hypertension to perform kinetic studies. This NH inhibits Na-K-ATPase in a noncompetitive manner with respect to potassium and uncompetitively with respect to magnesium. The semi-purified material exerted no effect on the sodium site of the enzyme system. These results clearly demonstrate that NH and lead differ from each other and also from ouabain in their Na-K-ATPase inhibitory characteristics.

At present, the role of lead as a contributory factor in hypertension remains speculative; however, there are *in vitro* data that support the possibility that lead enhances vascular reactivity. Webb et al. (*33*) found that tail arteries from hypertensive male rats exposed to lead at a dose of 100 ppm showed an increase in maximal contractile force when tested with the α-adrenergic agents, norephinephrine and methoxamine. This finding was attributed to an increase in the intracellular pool of activator calcium in the vascular smooth muscle cell. Piccini et al. (*34*) and Favalli et al. (*35*) found that calcium was necessary in the perfusion solution for lead to exert an effect on vascular contractility of the isolated rat tail artery. Furthermore, tissue calcium was increased in the presence of lead, an effect that could be explained by the inhibitory effect of lead on the vascular smooth muscle membrane Na-K-ATPase system, as predicted by the Blaustein hypothesis (*11*). As lead and NH have a synergistic effect on this enzyme system, one possible action of lead may be to potentiate vascular reactivity in genetically susceptible individuals. Such a hypothesis is supported by observations of Cloix et al. (*36*) that some normotensive individuals with a family history of hypertension have elevated levels of NH.

As hypertension is a multifactorial complex dis-

order, it is unlikely that a single hormone or toxin can account for the entire pathogenesis. A more plausible outcome is that many substances will be found to be contributory factors and the interplay between these substances will determine whether a given individual develops a sustained increase in blood pressure.

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