

EFFECT OF CALCIUM CHELATION BY L-PHENYLALANINE ON (Na⁺ + K⁺)-STIMULATED ATPase OF CHICK BRAIN MICROSOMES

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1. Introduction

Previous work from this laboratory has revealed that chicks showed a reversible narcosis when injected with 1.62 nmole of L-phenylalanine [1]. After 4 hr, the brain phenylalanine level was as high as 3.8 μ mole/g tissue [2]. It has been shown that some amino acids are chelators of divalent cations [3–6]. Since membrane-bound Ca²⁺ is essential for the maintenance of cellular and axonal membrane excitability and low Ca²⁺ leads to depolarization, spontaneous electrical discharges and eventually suppression of membrane excitability [7–15], it was of interest to test the hypothesis that high concentrations of L-phenylalanine (up to 5 mM) might compete for Ca²⁺ and displace it from the membrane surface. This displacement, *in vivo*, would lead to reduction (and ultimate blockage) of the action potential.

This communication reports studies of the effect of L-phenylalanine on membrane-bound (Na⁺ + K⁺)-ATPase as compared with the effects of strong chelators such as EDTA and EGTA on the same system.

2. Methods

Brain microsomes from male white leghorn chicks (1 to 2 days old) were prepared as previously described [16], except that 1 mM EDTA was omitted from the isolation medium. The crude microsomal preparation

Abbreviations:

EDTA: ethylenediaminetetraacetic acid

EGTA: ethylene glycol-bis-(β -aminoethyl ether)-N,N'-tetraacetic acid

was suspended in 0.25 M sucrose and used without further purification. The incubation mixture, in a total volume of 1 ml, contained 90 μ g enzyme protein, 20 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 100 mM NaCl, 20 mM KCl, 3 mM ATP, (with or without 250 μ M ouabain), and various concentrations of CaCl₂, EDTA, EGTA and L-phenylalanine. After 20 min incubation at 37°, the reaction was terminated by adding 1 ml of 10% cold trichloroacetic acid solution. ATPase activity was measured in terms of P_i liberated from ATP by the method of Berenblum and Chain [17]. Protein concentration was determined by the method of Lowry et al. [18].

The following chemicals were purchased from Sigma Chemical Company, St. Louis, Mo., and neutralized with NaOH to pH 7.4: ATP (sodium salt), EGTA and L-phenylalanine. EDTA (sodium salt) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Ouabain was received from Calbiochem, Los Angeles, Calif. All the inorganic salts and organic solvents were reagent grade and were used without further purification. Glass doubly distilled water was used throughout the experiments.

3. Results and discussion

Ca²⁺ inhibited the (Na⁺ + K⁺)-ATPase activity of brain microsomes. Fig. 1 shows that the inhibition of (Na⁺ + K⁺)-ATPase activity increased as the concentration of CaCl₂ increased. At 0.5 mM CaCl₂, the (Na⁺ + K⁺)-ATPase activity was inhibited by 45%. The concentration of calcium is estimated to be 0.5–1.5 mM and 0.001–0.1 mM extracellularly and intracellularly, respectively [19]. The effect of Ca²⁺

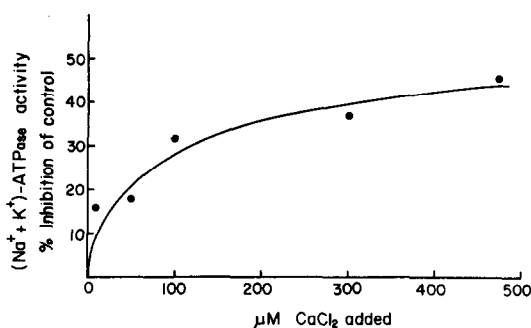


Fig. 1. Inhibition of brain microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase by Ca^{2+} . Samples of microsomes (90 μg protein) were incubated for 20 min at 37° in a medium containing 20 mM Tris-HCl buffer (pH 7.4), 100 mM NaCl, 20 mM KCl, 5 mM MgCl_2 , 3 mM ATP, with or without 250 μM ouabain and various concentrations of CaCl_2 , as indicated. ($\text{Na}^+ + \text{K}^+$)-ATPase activity was calculated from the difference between the total ATPase and ouabain-insensitive component of the ATPase. Each point represents the average values of duplicate samples. Agreement between the duplicates was within the experimental error (5–10%).

on ($\text{Na}^+ + \text{K}^+$)-ATPase is consistent with the report of Whittam and Blond [20] and recent work of Gutman and Katzper-Shamir [21]. The former authors showed that Ca^{2+} inhibited both the ouabain-insensitive and the ouabain-sensitive components of ATPase activity in brain homogenates, but the latter was inhibited to much greater relative extent. Addition of a strong

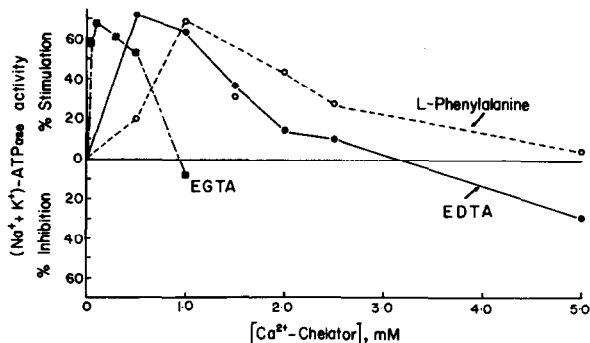


Fig. 2. Effects of EDTA, EGTA and L-phenylalanine on brain microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase. Incubation conditions were the same as described in fig. 1. The abscissa denotes the concentrations of EDTA ●, EGTA ■, and L-phenylalanine ○. Each point represents the average value of two sets of experiments. Agreement between the values was within the experimental error (5–10%).

Ca^{2+} -chelator, such as EDTA, at low concentration stimulated the ($\text{Na}^+ + \text{K}^+$)-ATPase activity. This stimulation was interpreted to be the result of removal of small amounts of Ca^{2+} present in the tissue [20].

Chelators such as EDTA and EGTA at low concentrations also stimulated brain microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase activity (fig. 2). The maximum stimulation (70%) for both chelators was about the same, within the experimental error. A much smaller amount of EGTA, the stronger Ca^{2+} -chelator, was needed for maximum stimulation. Higher concentrations of EDTA and EGTA caused a pronounced decrease in ATPase activity. This inhibition was probably due to gradual chelation of Mg^{2+} , which is required for the formation of the Mg^{2+} -ATP complex, a necessary step for enzymic hydrolysis of ATP. L-phenylalanine mimicked the effects of both EDTA and EGTA (fig. 2). However, the concentration of L-phenylalanine required for maximum stimulation (70%) was significantly higher (1 mM). Again, there was a gradual decrease of ($\text{Na}^+ + \text{K}^+$)-ATPase activity in the presence of high concentrations of L-phenylalanine in the incubation medium. Preliminary studies have shown that naturally occurring amino acids are capable of stimulating brain microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase.

As can be seen in fig. 3, inhibition of ATPase

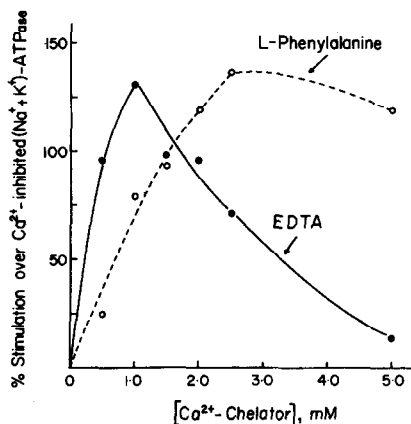


Fig. 3. Stimulation of Ca^{2+} -inhibited brain microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase by EDTA and L-phenylalanine. Incubation conditions were the same as cited in fig. 1, except for the addition of 0.5 mM CaCl_2 to all samples. Symbols are ● EDTA, ○ L-phenylalanine. Each point represents the average value of two sets of experiments. Agreement between the values was within the experimental error (5–10%).

activity by 0.5 mM Ca^{2+} could be reversed by EDTA as well as by L-phenylalanine. The displacement of Ca^{2+} from the membrane has also been reported for local anesthetics such as procaine and chlorpromazine [22–26].

This discussion has been concerned primarily with the effect of L-phenylalanine on the microsomal ATPase system. The fact that the chicks with elevated brain phenylalanine showed a narcotic effect [1] suggests that a chelating of Ca^{2+} may occur, or that there may be competition by L-phenylalanine for the Ca^{2+} -binding sites at the synaptic terminals. Membrane Ca^{2+} is also required for excitation-contraction coupling [27, 28], and stimulussecretion coupling [29, 30]. In addition, we have shown that L-phenylalanine and L-histidine also chelate Cu^{2+} [31]. Cu^{2+} is known to activate such enzymes as cytochrome oxidase, tyrosinase, phenol oxidase, and others [32].

An obvious point of interest stemming from this observation concerns the possible deleterious effect that high levels of L-phenylalanine (1 mM) may have on cerebral ($\text{Na}^+ + \text{K}^+$)-ATPase in man, especially in the developing phenylketonuric infant. For example, it has been estimated that the ($\text{Na}^+ + \text{K}^+$)-ATPase sets the pace for approximately 40% of the respiration and metabolic activity of the cell [33].

While this work was in progress, Specht and Robinson [34] reported similar results on the histidine-stimulation of kidney cortex ($\text{Na}^+ + \text{K}^+$)-ATPase at the meetings of the Federation of American Societies for Experimental Biology in Chicago in April of 1971.

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