Effect of Tissue Specificity of Brain Soluble Fractions on Na⁺, K⁺-ATPase Activity

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(Accepted November 22, 1989)

Previous evidence from this laboratory indicated that catecholamines and brain endogenous factors modulate Na⁺,K⁺-ATPase activity of the synaptosomal membranes. The filtration of a brain total soluble fraction through Sephadex G-50 permitted the separation of two fractions -peaks I and II- which stimulated and inhibited Na⁺,K⁺-ATPase, respectively (Rodríguez de Lores Arnaiz and Antonelli de Gomez de Lima, Neurochem. Res. 11, 1986, 933). In order to study tissue specificity a rat kidney total soluble was fractionated in Sephadex G-50 and kidney peak I and II fractions were separated; as control, a total soluble fraction prepared from rat cerebral cortex was also processed. The UV absorbance profile of the kidney total soluble showed two zones and was similar to the profile of the brain total soluble. Synaptosomal membranes Na⁺,K⁺- and Mg²⁺-ATPases were stimulated 60-100% in the presence of kidney and cerebral cortex peak I; Na⁺,K⁺-ATPase was inhibited 35-65% by kidney peak II and 60-80% by brain peak II. Mg²⁺-ATPase activity was not modified by peak II fractions. ATPases activity of a kidney crude microsomal fraction was not modified by kidney peak I or brain peak II, and was slightly increased by kidney peak II or brain peak I. Kidney purified Na⁺-K⁺-ATPase was increased 16-20% by brain peak I and II fractions. These findings indicate that modulatory factors of ATPase activity are not exclusive to the brain. On the contrary, there might be tissue specificity with respect to the enzyme source.

KEY WORDS: Synaptosomal Na⁺,K⁺-ATPase; synaptosomal Mg²⁺-ATPase; brain soluble fractions; kidney Na⁺-K⁺-ATPase.

INTRODUCTION

The participation of the enzyme Na⁺,K⁺-ATPase (E.C. 3.6.1.3) in the movement of sodium and potassium ions through the membranes has been widely documented (see 1 for references). The modulation of this enzyme by endogenous factors has been demonstrated (see 2 for references). In previous reports from this laboratory it has been shown that Na⁺,K⁺-ATPase activity of synaptosomal membranes may be modified by catecholamines. The enzyme was stimulated or inhibited by noradrenaline and dopamine, depending on the presence or absence of a brain soluble fraction (3-5). From the brain soluble fraction and by means of a Sephadex G-50 column two subfractions, named peak I and II, were separated. Peak I stimulated Na⁺,K⁺- and Mg²⁺-ATPase activities and peak II only inhibited Na⁺,K⁺-ATPase (2,6).

In the present study homologous subfractions from a kidney soluble fraction were prepared and tested on synaptosomal membrane ATPases. To make comparisons, new experiments with brain subfractions were run. Results obtained have shown only slight differences in the characteristics of kidney fractions with respect to those of brain fractions. This suggests that modulatory factors of ATPase activity present in these fractions are not exclusive to the brain. On the other hand experiments...
run with kidney ATPase suggest tissue specificity of the effect of the brain soluble fractions with respect to the enzyme source.

Part of these results were presented during the XII Meeting of the International Society for Neurochemistry, Algarve, Portugal, April 23-28, 1989.

EXPERIMENTAL PROCEDURE

Adult Wistar rats of both sexes were used. All reagents used were of analytical grade. Ouabain, yeast disodium ATP (grade I), dog kidney purified Na+, K+-ATPase and Sephadex G-10 were from Sigma Chemical Co., St. Louis, Mo., U.S.A; Sephadex G-50 (fine grade) was from Pharmacia Fine Chemicals, Uppsala, Sweden.

Preparation of Homogenates and Subcellular Fractions. Throughout these processes the temperature was maintained at 0-4 °C. All homogenization steps were done in a Teflon-glass homogenizer of the Potter-Elvehjem type.

Preparation of Synaptosomal Membranes. Synaptosomal membranes were prepared according to the method described in this laboratory (7). For each preparation, the cerebral cortices of four rats were pooled and suspended in cold 0.32 M sucrose (neutralized to pH 7 with Tris base solution). The tissue was homogenized at 10% (wt/vol) for 2 periods of 1 min each. The homogenate was submitted to differential centrifugation to separate the nuclear (900 g × 10 min, two washings) and the mitochondrial fraction (11,500 g × 20 min, one washing). The crude mitochondrial pellet, which contains synaptosomes and myelin, besides the mitochondria, was homogenized at 10% (wt/vol), original tissue) in bidistilled water (pH 7 with Tris base) for the osmotic shock. A pellet containing mitochondria, synaptosomes and myelin, besides the mitochondria, was homogenized at 10% (wt/vol) in 0.2 M Tris-HCl buffer. This extract was loaded on a Sephadex G-10 column (1 × 20 cm) and a single fraction of 10 ml was collected. This filtrate was applied to a column packed with Sephadex G-50 (1.8 × 2.5 cm). For gel equilibration and elution 0.02 M Tris-HCl buffer pH 7.4 was used.

Preparation of Kidney Soluble Fractions. For each preparation the kidneys of one rat were removed, dropped in bidistilled water and weighed; 1.8-2.0 g of wet tissue were collected. The tissue was minced with scissors and homogenized at 25% (wt/vol) for two periods of one min each. This homogenate was processed as described for the brain homogenate to separate the kidney soluble fraction and the peak I and II kidney subfractions.

ATPase Assay. ATPase activity was measured as described by Albers et al. (8). Total ATPase activity was assayed in a medium containing 100 mM NaCl, 20 mM KCl, 3 mM MgCl₂, 0.16 M Tris-HCl buffer (pH 7.4) and 4 mM ATP. Mg²⁺-ATPase activity was determined in a similar medium, but deficient in Na⁺ and K⁺ and containing 1 mM ouabain. The difference between both activities was taken as that corresponding to Na⁺, K⁺-ATPase activity. When indicated, only total ATPase was assayed.

Before performing the ATPase assays, samples of synaptosomal membranes, kidney microsomal membranes or kidney purified Na⁺, K⁺-ATPase were preincubated with 0.2 M Tris-HCl buffer, pH 7.4, peak I or peak II fractions at 37°C for 10 min. The incubated volume (μl) was 5:25 for membranes: soluble subfractions, and 30:10 for purified Na⁺, K⁺-ATPase: soluble subfractions. In the preincubated mixtures, the pH was neutral. Samples of the preincubated fractions (3 μl) were distributed among two series of microtubes containing 40 μl of each medium for the assay of total and Mg²⁺-ATPase activities. The tubes were incubated at 37°C for 30 min; in each experiment, tubes containing enzyme preparations and assay media maintained at 0°C for 30 min were used as blanks. The reaction was stopped with cold 30% TCA solution.

In all experiments ATPase assay was done in triplicate and microscale. The ATPase activity was monitored by the colorimetric determination of orthophosphate (9).

Protein Determination. Protein concentration was determined in the synaptosomal and microsomal membranes and in the soluble subfractions. The method of Lowry et al. (10) using bovine serum albumin as standard was employed.

In each experiment different preparation of membranes and distinct soluble subfractions were used. Results obtained are presented as mean values ± SD. Data were normalized by means of arcsin √p transformation and the significance of difference between means was determined by Student’s t test.