evidently ensures synaptic contact with a sufficient quantity of the mediator (glutamic acid), and this can be regarded as an adaptive reaction.

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EFFECT OF TRIFLUOPERAZINE, A CALMODULIN INHIBITOR, ON CALCIUM ACTIVATION OF PHOSPHORYLASE IN RABBIT SKELETAL MUSCLE GLYCOSOMES

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KEY WORDS: calmodulin; phosphorylase; muscle; calcium.

Glycogen–protein particles structurally bound with the membranes of the sarcoplasmic reticulum (glycosomes), containing Ca^{++}-sensitive phosphorylase kinase and phosphorylase phosphatase, have been isolated from rabbit skeletal muscle by fractionation in acetone and by acid precipitation and centrifugation [6]. In the presence of ATP, Ca^{++} ions have been shown to induce flash activation of phosphorylase in the same way as activation of Ca,Mg-ATPase [2]. These two systems are closely interconnected in the glycosome, and for that reason calcium activation of phosphorylase is quickly replaced by inhibition because of active Ca^{++} transport inside the sarcoplasmic reticulum (SR) [1]. Various Ca-dependent enzymes, including phosphorylase kinase and Ca,Mg-ATPase, receive Ca^{++} through the Ca-binding low-molecular-weight protein calmodulin [1, 8], which is inhibited by trifluoperazine (TFP) [5].

The object of this investigation was to study the character of calcium activation of phosphorylase in glycosomes isolated from skeletal muscle when calmodulin was inhibited by TFP.

EXPERIMENTAL METHOD

Glycosomes were isolated by the method in [4]. Rabbit muscle proteins (150 g) were homogenized in 2.5 volumes of 4 mM EDTA, pH 7.3, at 0°C. The homogenate was centrifuged at 4000g for 40 min at 0°C. The pH of the supernatant was adjusted to 6.1, and 10 min later it was centrifuged at 4000g for 30 min at 0°C. The residue was resuspended in an equal volume of buffer (100 mM sodium β-glycerophosphate + 4 mM EDTA, pH 8.2) and made up to a final volume of 25 ml with buffer containing 50 mM sodium glycerophosphate + 4 mM EDTA,

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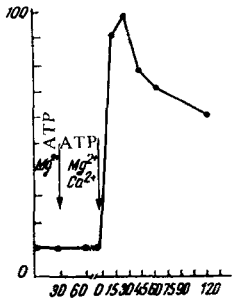

Fig. 1

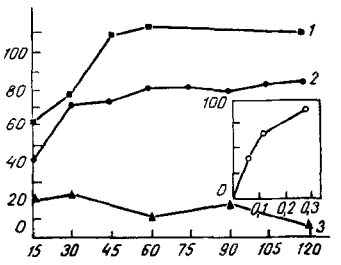

Fig. 2

Fig. 1. Flash activation (after Fisher) in residue of glycosomes diluted 1:5. Abscissa, time (in sec); ordinate, phosphorylase activity (in %). Time of addition of substances indicated by arrow.

Fig. 2. Effect of TFP on flash activation of phosphorylase in residue of glycosomes diluted 1:5. Abscissa, time after addition of activating mixture (in sec); ordinate, phosphorylase activity (in % of control) in the presence of TFP in doses of 65 μM (2) and 300 μM (3). Inset: inhibition of phosphorylase as a function of TFP concentration after 15 sec of flash activation. Abscissa, TFP concentration (in mM); ordinate, percentage inhibition of phosphorylase.

pH 7.0. The suspension was centrifuged at 80,000g for 90 min at 0°C. The residue was resuspended in 4 volumes of solution containing 50 mM sodium β-glycerophosphate + 1 mM EDTA, pH 6.8. In parallel experiments the residue was resuspended in the same buffer containing the stipulated concentration of TFP.

Flash activation was carried out [2] by the addition of activating mixture, consisting of 50 μl of 100 mM $MgCl_2$, 15 μl of 100 mM $CaCl_2$, and 10 μl of 100 mM ATP, to 700 μl of the suspension of glycosomes at 30°C. Samples were taken at different times and diluted in 100 volumes of buffer (50 mM sodium β-glycerophosphate + 30 mM cysteine + 1 mM EDTA + 1 mg/ml albumin, pH 6.8) at 0°C.

Phosphorylase activity was determined by the method in [3] and inorganic phosphorus was determined by the method in [7].

EXPERIMENTAL RESULTS

The time course of Ca-dependent activation and subsequent inactivation of phosphorylase in isolated glycosomes is shown in Fig. 1. Activity reached a maximum 30 sec after the addition of the activating mixture. Activity was reduced to its initial level after 3 min. Further addition of ATP without Ca^{++} could induce a new burst of activity. This indicates that inhibition of phosphorylase activity was due to rapid hydrolysis of ATP.

Preincubation of the glycosomes with TFP substantially modified the time course of phosphorylase activation. The results of this experiment are shown graphically in Fig. 2. Marked inhibition (by 40%) of activation was observed after 15 sec when TFP was used in a concentration of 65 μM, inhibition by 60% developed in the presence of 130 μM TFP, whereas 300 μM TFP inhibited flash activation virtually completely.

When TFP was used in a concentration of 65 μM, phosphorylase activity after 45-120 sec of flash activation was 10-15% higher than the control. This phenomenon can be explained by inhibition of calmodulin-dependent Ca-ATPase activity and, consequently, by the longer period of action of the added Ca^{++} and a high ATP concentration. TFP in concentrations of 130 and 300 μM strongly inhibited phosphorylase kinase, and flash activation is reduced in the first stage.

Both components of Ca-dependent activation — phosphorylase-kinase and Ca-ATPase — in glycosomes of rabbit skeletal muscles can therefore be investigated differentially by means of inhibitor analysis.

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TISSUE UBIQUINONE AND VITAMIN E LEVELS IN RATS WITH EXPERIMENTAL FOCAL MYOCARDITIS AND HYPOXIC HYPOXIA

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KEY WORDS: ubiquinone; vitamin E; hypoxia; focal myocarditis.

Vitamin E not only acts as a biological antioxidant, but it also plays the role of regulator of energy metabolism [1]. Vitamin E and ubiquinine occupy a special place in the normalization of metabolism in the myocardium [9, 10]. In diseases accompanied by disturbance of the circulation, the content of vitamin E and ubiquinone in the myocardium changes [4, 9]. Data on the concentration of these substances in the tissues in various states accompanied by hypoxia are conflicting [8, 12], and this makes it difficult to understand the mechanism of their action.

The object of this investigation was to study the concentrations of vitamin E and ubiquinone in different tissues of rats with acute hypoxic hypoxia and with histotoxic hypoxia arising after administration of adrenalin [6].

EXPERIMENTAL METHOD

Experiments were carried out on inbred male albino rats weighing 160-180 g. Focal myocarditis was studied 24 h after intramuscular injection of 0.4 ml of a 0.1% solution of adrenalin hydrochloride during the period of maximal morphological changes in the myocardium [3]. Hypoxic hypoxia was induced by taking the rats gradually in a pressure chamber to an altitude of 10,000 m (pressure 200 mm Hg). The animals were decapitated. Tissues were removed and homogenates and mitochondria obtained in the cold. Mitochondria were isolated from heart muscle in 0.25 M sucrose made up in 0.05 M Tris-HCl buffer with 0.001 M EDTA. The concentrations of ubiquinone and vitamin E were determined as described previously [2] and protein by Lowry's method [11]. The results were subjected to statistical analysis by Oivin's method [5].

EXPERIMENTAL RESULTS

The concentration of vitamin E in the kidneys of the animals with experimental focal myocarditis was reduced by 44.7%. By contrast, the vitamin E concentration in the myocardium showed a tendency to rise (Table 1).

The ubiquinone concentration in the myocardial tissue was increased by 35.3%, whereas at the same time in the liver tissue it was reduced by 50.7% (Table 1). Changes in the ubiquinone and vitamin E concentrations in the various tissues were in the same direction.

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