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ROLE OF LIPID PEROXIDATION IN INHIBITION OF CARDIAC Na,K-ATP-ase DURING STRESS

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KEY WORDS: emotional-painful stress; lipid peroxidation; Na,K-ATP-ase.

During emotional-painful stress (EPS) lipid peroxidation (LPO) is activated [3] and Na,K-ATPase activity in the heart muscle is depressed [4]. This last effect can be prevented by injecting the β-blocker inderal before exposure to stress [4]. On the basis of these findings it can be posulated that depression of Na,K-ATPase activity in EPS is due to the fact that catecholamines activate LPO [2], with the result that the sarcolemma, the site of Na,K-ATPase, is damaged.

To test this hypothesis, in the first stage of the investigation described below the effect of LPO, induced in rats with EPS *in vivo* was evaluated, and in the second stage the effect of induction of LPO by an Fe^{++} + ascorbate system in the membrane fraction of the heart, rich in sarcolemma, was studied *in vitro*. In both stages the possibility of preventing the inactivating effect of LPO on Na,K-ATPase by 4-methyl-2,6-di-*tert*-butylphenol (ionol), an inhibitor of free-radical oxidation, was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Animals used in the first stage of the experiment were divided into four groups (8 rats in each group): 1) control, 2) EPS, 3) injection of the antioxidant ionol, 4) ionol followed by EPS. Ionol was injected intraperitoneally as a suspension in Tween-60 daily for 3 days before the experiment in a dose of 20 mg/kg body weight. EPS was induced in the form of an anxiety neurosis by the method in [8] in the course of 6 h. The animals were decapitated 2 h after the end of exposure to stress. As a result of EPS, all rats developed gastric ulcers.

The hearts were removed, freed from blood in ice-cold water, suspended, and frozen in liquid nitrogen. To isolate the membrane fraction the heart was homogenized for 30 sec without freezing in medium containing 10 mM imidazole and 1 mM EDTA, pH 7.5, in a homogenizer of "Politron" type, with the ratio of weight of tissue to volume of solution equal to 1:20. The resulting homogenate was passed through two layers of gauze and centrifuged for 20 min at 1000g. The residue was put through a Teflon-glass homogenizer and centrifuged under the same conditions. The last operation was repeated again. The residue was resuspended in the

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TABLE 1. Effect of EPS on Cardiac Na,K- and Mg-ATPases (in nmoles P_i/mg protein/min)

Experimental conditions	Na, K-ATPase	Mg-ATPase
Control	65,0±2,5	181,5±4,8
EPS	47,1±3,5*	174,0±7,3
Control + ionol	58,7±2,3	179,7±5,7
Ionol + EPS	57,5±2,7	169,7±6,1

Legend. *$P < 0.05$, compared with control and with heart with ionol.

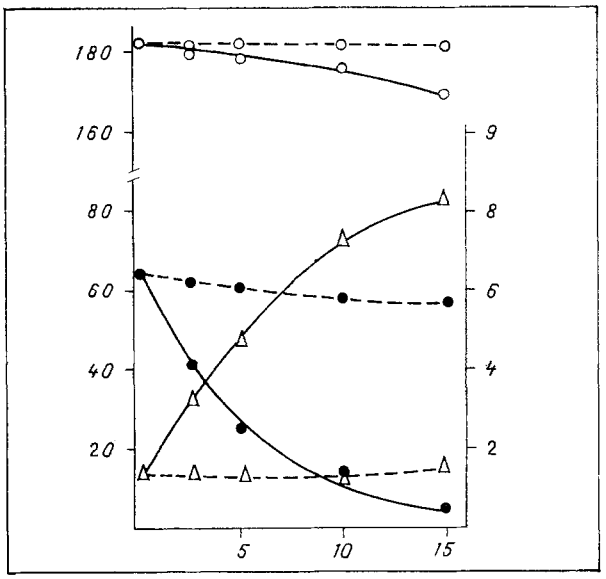
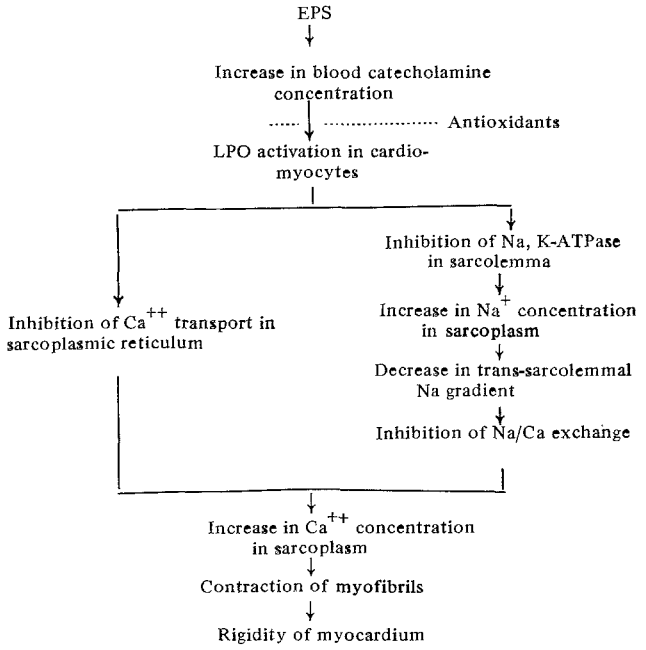


Fig. 1. Changes in cardiac Mg-ATPase (empty circles) and Na,K-ATPase (filled circles) activity and MDA accumulation (triangles) in suspension of sarcolemma vesicles in ascorbate-induced LPO. Abscissa, incubation time (in min); ordinate: left — enzyme activity (in nmoles P_i/mg protein/min), right — MDA concentration (in nmoles/mg protein). Broken line — in presence of ionol (2×10^{-5} M).

original volume of homogenization medium containing 1M KCl and centrifuged for 30 min at 2500g. The residue was reprecipitated in homogenization medium and then washed twice in a medium of 50 mM Tris-HCl, pH 7.4. The resulting membrane suspension was used in the experiments without preliminary cooling. The protein concentration was determined by the biuret method after preliminary incubation of an aliquot of the suspension for 20 min in 1% deoxycholate solution. To determine total ATPase activity 50 μg of protein was incubated in 3 ml of medium containing 3 mM $MgCl_2$, 3 mM ATP, 130 mM NaCl, 20 mM KCl, 30 mM imidazole, pH 7.4, at 37°C. Mg-ATPase was determined in the same medium containing 1 mM ouabain. The reaction was started by addition of ATP. The phosphate concentration was determined as in [9]. Na,K-ATPase activity was determined by subtracting Mg-ATPase activity from total ATPase activity.

LPO *in vitro* was induced in a membrane suspension with protein concentration of 1 mg/ml of a system of 10 μM Fe_2SO_4 + 0.2 mM ascorbate. The concentration of LPO products was determined by the reaction with 2-thiobarbituric acid [7].

Scheme of Order of Mechanisms Leading to Myocardial Injury in EPS



EXPERIMENTAL RESULTS

Table 1 shows that EPS led to a decrease in Na,K-ATPase activity of the heart. Mg-ATPase activity was virtually unchanged under these circumstances. Preliminary administration of antioxidants to rats is known to protect the heart against activation of LPO during EPS [3]. As LPO inhibitor, just as previously, we used ionol, an antioxidant of phenolic nature. Injection of this compound into the rats before EPS prevented the reduction in Na,K-ATPase activity, whereas in the control no increase in activity of this enzyme was observed as a result of injection of ionol. Activation of LPO during EPS can thus be regarded as the key factor in inhibition of the Na-pump, for ionol, a specific inhibitor of LPO, "protected" Na,K-ATPase simultaneously with blocking free-radical oxidation.

In the next series of experiments LPO-induced inhibition of Na,K-ATPase was modeled *in vitro* on a sarcolemma-enriched membrane fraction from hearts of the control animals. Accumulation of LPO products in the membranes was shown to be accompanied by a sharp fall in the Na,K-ATPase activity whereas Mg-ATPase activity remained at its initial level (Fig. 1). Thus both *in vivo* during EPS, and in experiments *in vitro*, LPO-induced inhibition of Na,K-ATPase does not extend to Mg-ATPase. It will be clear from Fig. 1 that both the accumulation of malonic dialdehyde (MDA) and inhibition of Na,K-ATPase were prevented in the presence of ionol. On the whole the results described above confirm the leading role of LPO in damage to the cardiomyocyte Na-pump during stress.

Functioning of Na,K-ATPase is known to depend on preservation of the Na^+ concentration gradient of the cardiomyocytes, and this gradient, in turn, plays a leading role in the working of the Na/Ca exchange mechanism, through which Ca^{++} are removed from the cell into the external medium. This explains why one result of the decrease in activity of the Na-pump described above may be an increase in the Ca^{++} concentration in the cardiomyocytes. Disturbance of the ability of the enzyme system for Ca^{++} transport in the sarcoplasmic reticulum to take up and accumulate Ca^{++}, discovered previously during stress [6], can lead to the same result. In agreement with these data, an increase in Ca^{++} concentration in the myocardial cells during EPS has in fact been demonstrated in experiments with the use of a combination of electron microscopy and histochemistry [5]. Very probably such an increase in the Ca^{++} concentration in the cardiomyocytes lies at the basis of the recently discovered phenomenon of poststress myocardial rigidity, i.e., a disturbance of its relaxation [1]. The sequence of events described above in the heart muscle cell during EPS is summarized in the accompanying general scheme.

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THYROID HORMONES AND PHOSPHOLIPASE ACTIVITY OF RAT LIVER MITOCHONDRIA

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KEY WORDS: thyroid hormones; phospholipase; rat liver mitochondria.

Endogenous mitochondrial phospholipase activity can be controlled by thyroid hormones for a number of reasons. First, thyroid hormones affect the rate of synthesis of phospholipids by the mitochondria [13] and also have a considerable influence on their fatty acid composition [12]. Modification of the phospholipase substrate molecules and, simultaneously, of the lipid environment of the enzyme under study is thus possible. Second, thyroid hormones facilitate transport of Ca^{++} ions through the mitochondrial membranes [11], and this is a matter of undoubted interest in the light of data showing the activating action of these ions on mitochondrial phospholipase [1]. Finally, as was shown previously in the writers' laboratory [3, 4], if thyroxine is added to isolated liver mitochondria it activates the phospholipase of these organelles.

The aim of the present investigation was to study phospholipase activity in liver mitochondria from rats receiving toxic doses of thyroid hormones.

EXPERIMENTAL METHOD

Male Wistar rats weighing 160-200 g were used. Hyperthyroidism was induced by intraperitoneal injection of L-thyroxine in a dose of 150-200 µg/100 g body weight daily for 7-8 days. Thyrotoxicosis was stimulated by injection of the hormone by the same route in a dose of 2 mg/100 g body weight for 5-6 days. Control animals received injections of the solvent (0.05 N KOH). Mitochondria were isolated by the standard method in medium containing 0.3M sucrose, 1 mM EDTA, and 10 mM Tris-HCl buffer, pH 7.4. The mitochondria were washed and kept in a solution of 125 mM KCl, 10 mM Tris-HCl buffer, pH 7.5. Mitochondrial phospholipase activity was estimated from the quantity of free fatty acids (FFA) present at the time of isolation of the mitochondria from the liver or accumulating during incubation, per unit of mitochondrial protein. FFA were determined quantitatively by the method in [9], modified by the writers for use with mitochondria. A calibration graph was plotted for standard solutions of oleic acid. Protein was determined by the biuret reaction.

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