EFFECT OF SODIUM SUCCINATE ON METABOLIC AND MORPHOLOGIC CHANGES IN ACUTE COLD STRESS

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KEY WORDS: exposure to cold; sodium succinate; energy metabolism.

There is an undoubted similarity in the energy expenditure during exposure to cold and to physical exertion. It is therefore interesting to use substances which increase physical endurance in order to increase resistance to cold. One such substance is succinic acid, whose effectiveness has been demonstrated in physical exertion and in a number of pathological and stressed states [1, 4]. This effectiveness is due to the regulating effect of the compound on mitochondrial function, for the switching by the cell to predominant oxidation of endogenous succinic acid under the influence of unfavorable factors in a physiological adaptive mechanism [4]. Enhancing of the role of succinate-dependent reactions in the energy metabolism of the body also has been noted in cold stress [6].

The object of this investigation was to study the action of sodium succinate on metabolic and morphologic changes following acture exposure to cold.

EXPERIMENTAL METHOD

Male tetrahybrid (BALB/c × B10CW × C57BL/6 × CC57W) mice weighing 18 ± 3 g were used. Acute cold stress was induced by keeping the animals in a cold chamber at −16 to −18°C [8]. The length of survival of the animals was recorded. Intact mice were kept at 19°C.

At different stages of exposure to low temperature the blood glucose concentration of the decapitated animals was determined by an enzymic method. The concentration of adenine nucleotides (ATP, ADP, AMP), creatine phosphate [10], and glycogen [9] in the myocardium after exposure to cold for 2.5 h were determined after fixation in liquid nitrogen. For electron-microscopic study tissue from the left ventricle was fixed in 1% OsO_4 solution and embedded in Araldite. Succinate dehydrogenase (SDH) activity was determined by the method in [7]. Electron micrographs were obtained on the JEM-100B microscope. Sodium succinate in a dose of 16 mg/kg was injected intraperitoneally 1 h before exposure to cold.

EXPERIMENTAL RESULTS

Preliminary injection of sodium succinate increased the resistance of the animals to acute cold stress. In the control, mice began to die after exposure to cold for 2 h, and

TABLE 1. Changes in Concentration of Adenine Nucleotides, Creatine Phosphate, and Glycogen in Animals' Heart during Acute Exposure to Cold (n = 6)

Experimental conditions	Creatine phosphate	ATP	ADP	AMP	Total adenine nucleotides	Glycogen, mg/g tissue
		μM/g tissue				
Intact animals (19°C)	3,76±0,31	2,29±0,16*	0,78±0,04*	0,51±0,05	3,58	3,80±0,30*
Acute exposure to cold for 2.5 h (control)	1,28±0,14	1,60±0,17	0,49±0,06	0,45±0,05	2,54	2,20±0,20
Acute exposure to cold (2.5)h after sodium succinate	2,32±0,25*	1,97±0,19*	0,86±0,07*	0,36±0,02	3,19	2,90±0,30*

Legend. *$P \leqslant 0.05$ compared with control.

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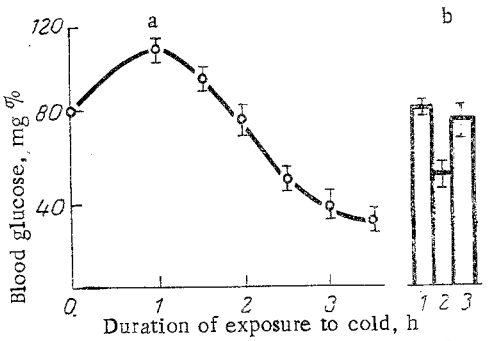

Fig. 1. Time course of blood glucose concentration (in mg %) during acute exposure to cold (a) and effect of sodium succinate on it (b). 1) Intact animals, 2) acute exposure of cold for 2.5 h, 3) acute exposure to cold after injection of sodium succinate.

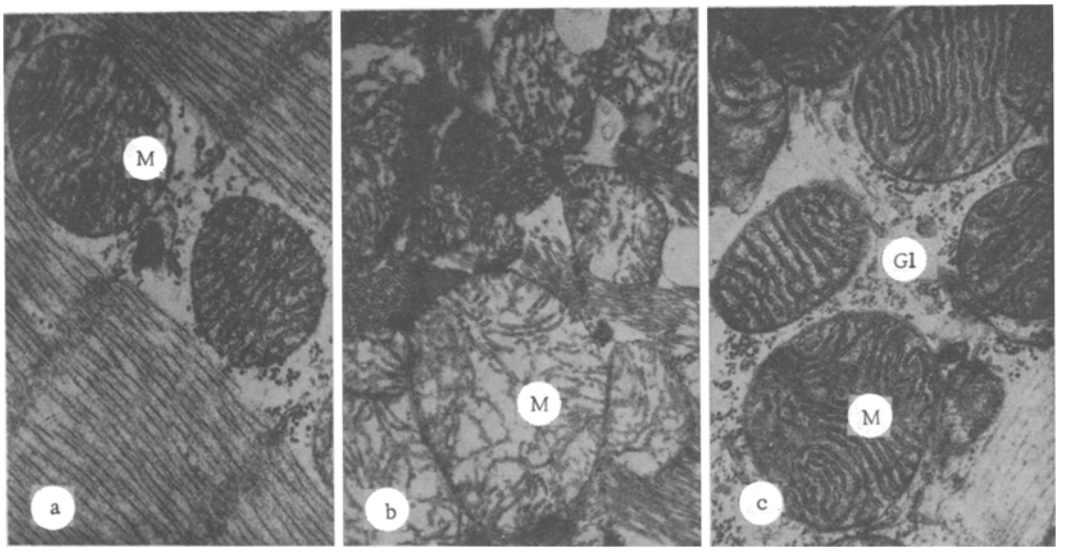

Fig. 2. Cardiomyocytes of left ventricle of mouse heart. a) Intact heart. 30,000 ×; b) acute exposure to cold for 2.5 h. Control. Swelling of mitochondria (M). 26,000 ×; c) acute exposure to cold for 2.5 h after preliminary injection of sodium succinate. Normalization of structure of mitochondria (M) and increase in number of glycogen (Gl) granules. 40,000 ×.

after 5 h the mortality was 100%. After preliminary injection of sodium succinate, 74 ± 9% of the mice still remained alive after exposure for 5 h.

Changes in carbohydrate metabolism were judged from the time course of the blood glucose curve (glucose is the only oxidation substrate whose concentration falls considerably during exposure of animals to cold [5]). It will be clear from Fig. 1a that after exposure for 1 h to cold, hyperglycemia due, evidently, to mobilization of the carbohydrate reserves in response to stress, was observed. Its level then fell and and was somewhat lower than iniitally when the first of the experimental animals began to die (2 h). Further exposure to cold led to a sharp fall in the blood glucose level, which was under 60% of its initial value in the control after exposure to cold for 2.5 h. The effect of sodium succinate on the glucose level was estimated after exposure to cold for 2.5 h, when the metabolic changes in the control as a result of exposure to cold were most marked. By that time, against the background of sodium succinate, the blood glucose concentration was virtually the same as initially (Fig. 1b). Stabilization of the blood glucose was combined with preservation of substrates of energy metabolism in the heart of the experimental animals. As Table 1 shows, exposure to intense cold led to a drastic fall in the creatine phosphate concentration, and to a decrease in the concentrations of ATP, ADP, and glycogen in the myocardium. These changes were evidently due to a decrease in the efficiency of work of the organ in cold stress [3], which led to predominance of ATP breakdown over its synthesis and to exhaustion of the total re-

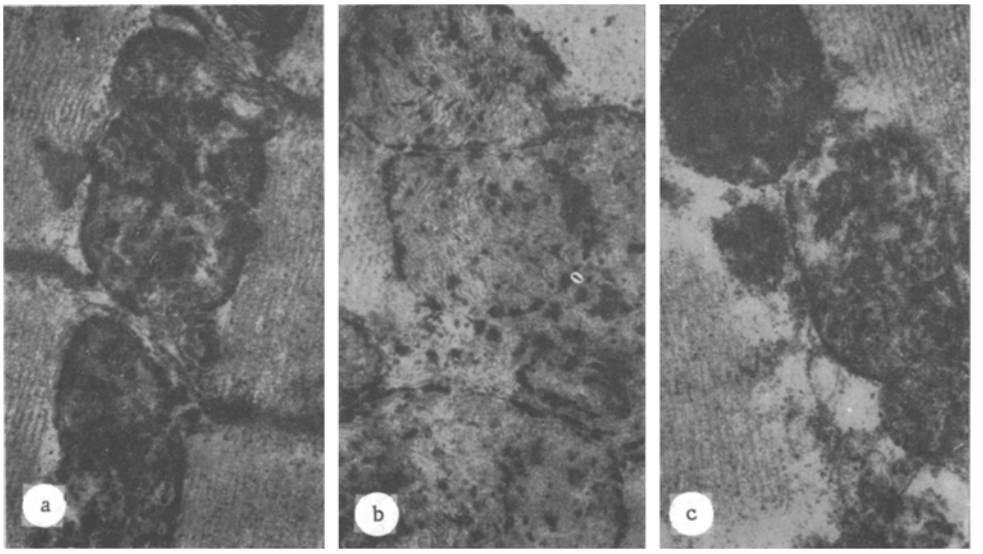

Fig. 3. SDH activity in cardiomyocytes of mouse left ventricle: a) intact heart. 30,000 ×; b) Decrease in mitochondrial SDH activity during acute exposure to cold for 2.5 h. 40,000 ×; c) Increase in SDH activity during acute exposure to cold after injection of sodium succinate. 30,000 ×.

serves of high energy compounds. The more intensive utilization of creatine phosphate was aimed at maintaining a stable ATP concentration, as is observed under the influence of exhausting factors. The increase in the ADP, ATP, and creatine phosphate concentrations after preliminary injection of sodium succinate demonstrated the energized state of the myocardium and activation of oxidative reactions under the conditions of cold stress.

Results of the biochemical study agreed with the electron-microscopic data. Acute exposure to cold in the control led to swelling of the myocardial mitochondria, their matirx was translucent, the cristae had lost their parallel arrangement (Fig. 2b). Against the background of sodium succinate, swelling of the mitochondria was less marked, and glycogen granules in the cytoplasm of the muscle cells were better preserved (Fig. 2c), in agreement with the biochemical data (Table 1). The increase in the glycogen concentration, like that of the blood glucose, was probably due to stimulation of gluconeogenesis, which is observed in certain cases under the influence of succinic acid both at the cellular level and through stimulation of glucocorticoid synthesis (through reduction of NAD^+) in the adrenals [2], or on account of the direct energy contribution of sodium succinate, inducing activation of SDH, which plays an essential role during exposure to intense cold. As will be clear from Fig. 3a, b, SDH activity in the control showed a small decrease after exposure to cold for 2.5 h. Histochemical reaction products were located along the cristae of the mitochondria and their number was considerably reduced. Activity of this enzyme in the liver and kidneys is known to rise adaptively during exposure to intense cold [5]. The opposite effect of cold on the myocardium can evidently be explained by the higher absolute SDH activity and the less reduced state of the respiratory chain of the mitochondria compared with other organs, making a decrease in the activity of this enzyme during exposure to stress more probable [4]. Against the background of sodium succinate an increase was observed in SDH activity (Fig. 3c), substantially greater than that in the control animals.

The results are evidence that a definite role in the mechanism of increased resistance of the animals to cold stress after preliminary injection of sodium succinate is played by stabilization of energy metabolism and of the ultrastructure of the myocardial mitochondria.

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LIPID PEROXIDATION IN CHILDREN WITH DUCHENNE'S HEREDITARY MUSCULAR DYSTROPHY

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One of the most urgent problems in molecular neurobiology is the formation of a clear idea of how membranes really perform their physiological functions, what affects them, and how such knowledge can be utilized in the treatment of severe human neuromuscular diseases.

As a model with which to study the effect of peroxidation (LPO) on membrane permeability in such patients, it was decided to study a hereditary disease, namely Duchenne's progressive hypertrophic muscular dystrophy (PHMD), which has a particularly severe course and semilethal outcome.

In 1958, on the basis of research showing the unusually high activity of certain sarcoplasmic enzymes in the blood serum of patients with PHMD [6], the hypothesis of a lesion of the muscle fiber membranes was first put forward [6]. Subsequent biochemical and morphological studies confirms this hypothesis not only for the sarcolemma, but also for membranes of the sarcoplasmic reticulum (SR) and mitochondria [7, 12, 13]. However, the concrete mechanisms of damage to the muscle cell membranes has not yet been explained.

Considering the universal role of LPO products in membrane lesions of various cells, including muscle cells, it was decided to examine the possible role of LPO in the pathogenesis of PHMD. In the present investigation the content of gaseous LPO products (pentane) was studied in samples of expired air in patients with PHMD.

EXPERIMENTAL METHOD

The subjects studied comprised 18 boys with PHMD, in whom the diagnosis had been made after further clinical, electrophysiological, genealogic, and biochemical analysis. The control group consisted of clincially healthy boys of the same age with no family history of neuromuscular diseases. According to the degree of development of the muscular dystrophy the patients were divided into three groups: 1) minimal clinical manifestations of muscular dystrophy but with maximal activity of sarcoplasmic enzymes in the serum (especially creatine phosphokinase — CPK), 2) with a picture of clinically manifest muscular dystrophy and with a moderately increased CPK activity, 3) maximal clincial manifestations with inability to walk unaided and with low serum CPK activity. The children's mental state was assessed by psychological tests, school reports, and conversations with the child and parents. CPK activity

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