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EFFECT OF SODIUM HYDROXYBUTYRATE ON MYOCYTE ULTRASTRUCTURE IN STRIATED MUSCLE TISSUE DURING PHYSICAL EXERTION

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The ultrastructure of myocytes in the rat myocardium and skeletal muscles was studied during physical exertion in a control group and after preliminary administration of sodium hydroxybutyrate for 2 weeks. A single exposure to maximal physical exertion was shown to cause considerable changes in the fine structure of the cardiomyocytes and, to a rather lesser degree, in the myocytes of skeletal muscles. These changes consisted of masked intermyofibrillary edema, swelling of the mitochondria, and a sharp decrease in the glycogen level. Sodium hydroxybutyrate, if given for 2 weeks beforehand, prevents the changes described above in the myocytes. Normalization of structure observed under the influence of the compound can evidently be attributed to the character of its metabolic conversion and its effect on energy metabolism.

KEY WORDS: sodium hydroxybutyrate; physical exertion; ultrastructure of the myocardium and skeletal muscles.

Sodium hydroxybutyrate has been shown to be capable of increasing the resistance of the body to various types of hypoxia [1, 5, 6] and to accelerate recovery processes under conditions of minimal rest after physical exertion [3]. Sodium hydroxybutyrate prevents the increase in the lactate concentration in the tissues of the brain and heart characteristic of hypoxia and also considerably restricts the increase in the lactate/pyruvate ratio caused by hypoxia [7]. Under the influence of sodium hydroxybutyrate the accumulation of toxic products of nitrogen metabolism is prevented [2]. Since considerable physical exertion causes metabolic changes in muscle tissue, accompanied by accumulation of lactic acid and ammonia and by a decrease in the glycogen concentration, it was decided to study the effect of sodium hydroxybutyrate on the ultrastructure of myocytes of cardiac and skeletal muscles.

EXPERIMENTAL METHOD

Experiments were carried out on 25 noninbred albino rats weighing 170-180 g. The control group, consisting of 10 rats, was compelled to swim carrying a load equal to 6% of the body weight in water at a tempera-

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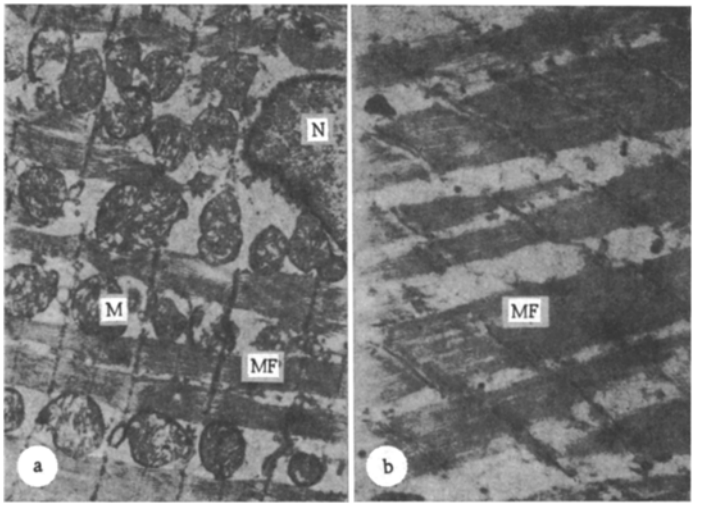

Fig. 1. Edema of cytoplasm of myocytes from cardiac (a) and gastrocnemius (b) muscles after a single period of maximal physical exertion: a) 20,000×, b) 25,000×. Here and in Figs. 2 and 3: M) mitochondria; MF) myofibrils; N) nucleus.

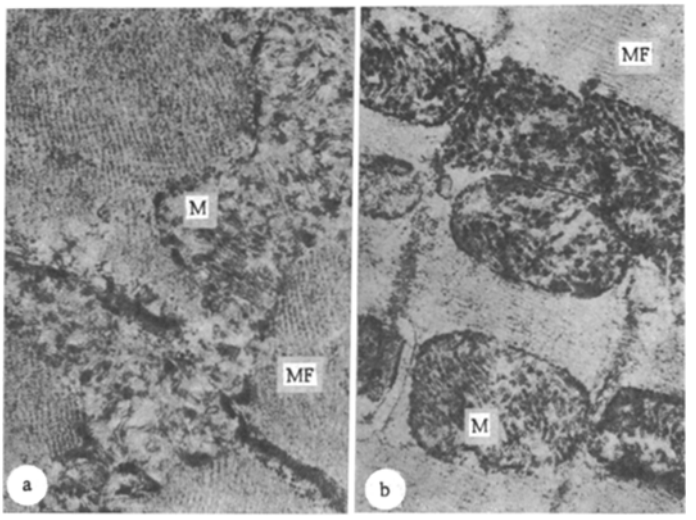

Fig. 2. Succinate-dehydrogenase activity of mitochondria of myocardial myocytes after a single period of physical exertion: a) control; b) after administration of sodium hydroxybutyrate for 14 days. a) 60,000×, b) 600,000×.

ture of 26°C until completely exhausted. The rats of the experimental group were given sodium hydroxybutyrate in a dose of 500 mg/kg by intraperitoneal injection daily for 14 days. On the 15th day after forced swimming for 16 min (the mean duration of swimming by rats of the control group) the animals of the experimental group were decapitated. The myocardium of the left ventricle and the gastrocnemius muscle served as material for electron-microscopic study. The tissue was fixed by Faulfield's method and embedded in Araldite. Sections were stained with uranyl acetate and lead acetate by Reynold's method. The succinate-dehydrogenase activity of the mitochondria also was determined by the method of Kerpel-Fronius and Hajos [11]. Activity of the enzyme was judged from deposition of dense granules of chelate. Electron micrographs were obtained on the JEM-100B microscope with magnification of 15,000-40,000 times.

EXPERIMENTAL RESULTS

The electron-microscopic study of striated muscle tissue of rats after swimming until totally exhausted showed the presence of considerable changes in the myocytes. The most severe structural disturbances were

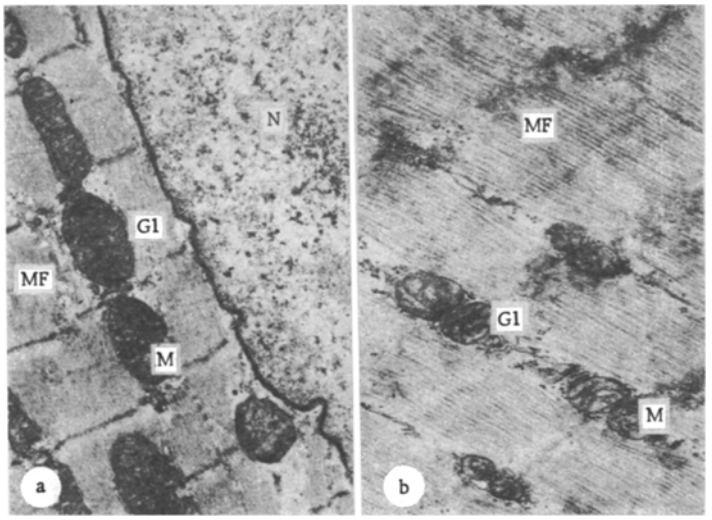

Fig. 3. Normalization of structure of myocytes of cardiac (a) and gastrocnemius (b) muscles on 14th day of sodium hydroxybutyrate administration: a) 25,000×, b) 50,000×. Gl) Glycogen.

found in the muscle cells of the heart. Changes in the fine structure of the cardiomyocytes consisted of marked intracellular edema, the presence of foci of overcontraction of myofibrils, and dystrophic changes in the mitochondria. The sarcolemma frequently formed evaginations filled with liquid and with thread-like structures and granules of low electron density. Myofibrils in most cells were separated and fragmented. The mitochondria were enlarged to 2-2.5 μ, with a pale matrix and with widened or fragmented cristae (Fig. 1a). Often the mitochondria had "lost" their outer membrane and had local regions of complete destruction of the cristae. The cisterns of the sarcoplasmic reticulum were sharply dilated in most cells. Sometimes vacuoles formed by a single membrane and containing two to four reduced and deformed mitochondria were observed in the sarcoplasm. The number of glycogen granules and ribosomes in the cytoplasm was sharply reduced. In some cases foci of overcontraction of the myofibrils was observed, in which the A disks were closer together and the I disks had disappeared. Unlike the mitochondria and myofibrils, the structure of the nucleus was very little different from that of normal cardiomyocytes. The nucleus had distinct outer and inner membranes, clearly distinguishable pores, and a uniformly distributed chromatin. The nucleolus was of the ordinary size and consisted of overlapping loops.

Structural changes in the skeletal muscle myocytes of rats of the control group were less marked and consisted of a decrease in the glycogen content between the myofibrils and well-marked intermyofibrillary edema (Fig. 1b).

The electron-histochemical study of mitochondrial succinate-dehydrogenase activity of the muscle cells of the myocardium and skeletal muscles of the control group of rats, after swimming until totally exhausted, revealed a decrease in activity of the enzyme. The number of granules of chelate was reduced in each mitochondrion, the granules were enlarged, and their distribution on the mitochondrial cristae was irregular (Fig. 2a).

The electron-microscopic study of the muscle cells of rats which had received sodium hydroxybutyrate for 2 weeks showed that this treatment had a distinctly normalizing effect on the structure. The fine structure of the myocytes was virtually indistinguishable from normal.

The mitochondria in the nucleus of the cardiomyocytes were normal in shape, size, and arrangement. The mitochondria contained 15 to 20 parallel cristae and their matrix was of average electron density. By contrast with normal, some degree of hyperplasia of the elements of the sarcotubular system was present. The number of glycogen granules and ribosomes was normal (Fig. 3a).

The protective effect of the compound also was clearly demonstrable in the muscle myocytes of the experimental group. Intermyofibrillary edema was absent and numerous glycogen granules were visible between the myofibrils. The structure of the nucleus and mitochondria was indistinguishable from normal (Fig. 3b).

An electron-histochemical study of succinate-dehydrogenase activity revealed relatively high activity of the enzyme in the mitochondria of rats receiving hydroxybutyrate. Granules of the chelate were uniformly distributed on the inner membrane of the mitochondria and the number of granules was characteristic for mitochondria of normal rat myocytes (Fig. 2b).

The electron-microscopic investigation thus showed that administration of sodium hydroxybutyrate for 14 days in a dose of 500 mg/kg prevents changes otherwise arising in the myocytes after a single period of swimming at maximal intensity.

After administration of sodium hydroxybutyrate for 14 days to rats at rest or engaged in normal motor activity no structural changes were observed in the myocytes.

Structural disturbances were observed in the muscle cells of the myocardium and skeletal muscles after a single period of physical exertion agreed with observations of other workers [4, 8] who noted marked dystrophic changes in the fine structure of the myocytes, indicating a state of relative tissue hypoxia.

The normalization of structure observed under the influence of sodium γ-hydroxybutyrate can evidently be explained by the special character of metabolic conversions of this compound and its effect on energy metabolism. For instance, it has been shown that interconversion of γ-hydroxybutyrate and succinic semialdehyde can take place [10, 16]. Conversion of succinic semialdehyde into succinic acid has also been shown to be possible [14, 15].

Under conditions of complete anoxia (hypoxic or circulatory) the most advantageous reaction is reduction of succinic semialdehyde into γ-hydroxybutyric acid, leading to the formation of reserves of NAD^+, which is in short supply during hypoxia and can also be used to eliminate an excess of lactate. Under conditions of relative hypoxia such as may arise during physical exertion, another conversion of γ-hydroxybutyric acid is evidently advantageous, namely its reduction to succinic acid. The formation of succinic acid, an NAD-independent substrate with a velocity of phosphorylation almost ten times greater than that of NAD-dependent substrates, may enable the level of oxidative processes in the mitochondria to be maintained at a certain level and the mitochondria to be protected against structural changes even during considerable physical exertion. Evidence of the normalizing action of sodium hydroxybutyrate on aerobic tissue metabolism is given by results obtained by various workers. Increased respiratory activity of the brain mitochondria under the influence of sodium hydroxybutyrate has been observed during hypoxia [9], maintaining respiratory control at the characteristic level for intact animals. Experiments on isolated mitochondria [13] showed that the rate of synthesis of mitochondrial ATP is increased by GABA. Because of the existence of interconversions of GABA and γ-hydroxybutyric acid [11], it can be tentatively suggested that the latter may also stimulate ATP.

The results of the electron-microscopic investigations of striated muscle tissue described above indicate that sodium hydroxybutyrate can normalize the structure of the mitochondria during physical exertion, sharply reduce edema of the myocyte cytoplasm, and maintain glycogen at the level characteristic for normal tissue. This action is evidently achieved through the metabolic effects of sodium hydroxybutyrate described above.

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