was related to muscle proteins in process of synthesis (Fig. 3a). The largest polyribosomes are considered [5, 6] to participate in the synthesis of myosin. In some cases ribosome-like particles were seen to escape through the nuclear membrane (Fig. 3b).

The experiments thus showed that during normal activity the cytoplasm of the skeletal-muscle fiber contains functionally active mono- and polyribosomes. Ribosomes participate in intracellular self-renewal processes and, in particular, renewal of the contractile system of the muscle fiber.

It can be concluded from these results that RNA passes periodically from the nucleus into the cytoplasm of the muscle fiber, possibly in connection with resumption of the cycle of intracellular self-renewal.

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CALCIUM TRANSPORT AND ATPase ACTIVITY OF THE SARCOPLASMIC RETICULUM OF NORMAL AND DENERVATED RABBIT MUSCLES

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The properties of the calcium pump of the sarcoplasmic reticulum (SR) from normal and denervated rabbit muscles were studied. The kinetics of transport of Ca^{++} ions in SR from denervated muscles obeys the Michaelis–Menten law. After denervation the rate of fast outflow of Ca^{++} from the vesicles is increased, leading to a decrease in the efficiency of transport and an increase in the activity of "basal" ATPase. Meanwhile the rate of Ca^{++} accumulation and the activity of transport Ca-ATPase are increased by 1.5 times. The kinetic properties of the reticulum from denervated muscles correspond to the pattern of the contraction–relaxation cycle in those muscles.

KEY WORDS: denervation; Ca^{++} transport; transport Ca-ATPase; sarcoplasmic reticulum.

Denervation leads to considerable changes in metabolism and, consequently, to morphological and physiological changes in muscle tissue. After denervation of fast muscles hypertrophy of the sarcoplasmic reticulum (SR), linked with an increase in the synthesis of membrane protein [11], and changes in the phospholipid composition of the membranes [5, 9] are observed. The effect of denervation on the Ca^{++} transport system in SR has received little study.

The object of this investigation was to study the properties of the calcium pump of SR fragments isolated from rabbit skeletal muscles after denervation.

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TABLE 1. Characteristics of SR Preparations (n = 4) Isolated from Normal and Denervated Muscles (M ± m)

SR preparation	V_{acc}	V_{ATP}	V_{bas}	V_{Mg}
Denervated muscles	2,45±0,24	4,55±0,64	1,11±0,14	0,35±0,04
Normal muscles	1,69+0,06	2,92+0,22	0,36±0,24	0,10±0,01

Legend. V_{acc}) Rate of accumulation of Ca^{++}, V_{ATP}) rate of hydrolysis of ATP by Ca-ATPase, V_{bas}) rate of hydrolysis of ATP in state II, V_{Mg}) rate of hydrolysis of ATP by Mg-ATPase. Calculated in μmoles P_i/mg protein/min; n) number of preparations.

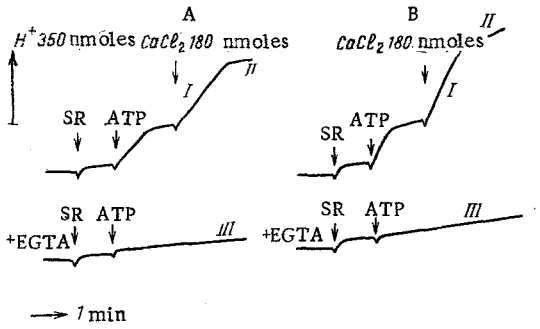

Fig. 1. Acidification of incubation medium as a result of hydrolysis of ATP by SR fragments from normal (A) and denervated (B) muscles: I) hydrolysis of ATP during accumulation of Ca^{++}; II) hydrolysis of ATP by "basal" ATPase; III) hydrolysis of ATP by Mg-ATPase.

EXPERIMENTAL METHOD

Denervation was carried out by dividing a branch of the sciatic nerve in the hind limb of the rabbit in the upper third of the thigh. From 17 to 21 days after denervation the rabbits were decapitated and the plantaris and gastrocnemius muscles removed from the denervated limb. The corresponding muscles of the opposite limb were used as the control. The SR fraction was isolated by the method described previously [2].

Activity of ATPase was measured as acidification of the incubation medium during hydrolysis of ATP [3]. The accumulation of Ca^{++} in the presence of oxalate was recorded continuously at 37°C as the turbidity of a suspension of SR fragments produced by precipitation of Ca oxalate crystals in their internal space. The incubation medium (4 ml) contained (in mmoles): NaCl 100, $MgCl_2$ 2, ATP 2, sodium oxalate 5-6, imidazole 4, pH 7.0; protein 20-60 μg/ml. Protein was determined by Lowry's method [4]. Activity of Mg-ATPase was measured in the presence of 0.3 mM EGTA [ethyleneglycol-bis-(β-aminoethyl ester)-N,N-tetraacetate].

To calculate the velocity of inflow of Ca^{++} (V_{in}) and the velocity of its outflow (V_{out}) the following equations were used [1, 6]:

$$V_{in} = Ca/ATP_{max} \times V_{ATP},$$

where V_{ATP} is the velocity of hydrolysis of ATP by Ca-ATPase, and

$$V_{out} = [Ca/ATP_{max} - Ca/ATP_{exp}] \times V_{ATP},$$

where Ca/ATP_{exp} is the ratio between the rate of accumulation of Ca^{++} and the rate of hydrolysis of ATP determined experimentally.

EXPERIMENTAL RESULTS AND DISCUSSION

The ATPase activity of the SR fragments was made up of the activity of Mg-ATPase and the activity of transport Ca-ATPase. The activity of Mg-ATPase, which has nothing to do with the process of Ca^{++} transport [10], can be measured from the acidification of the incubation medium in the presence of EGTA, a specific complexone for Ca^{++}. Acidification of the incubation medium as a result of hydrolysis of ATP by SR fragments from normal (A) and denervated (B) muscle is shown in Fig. 1. In the presence of ATP, the added Ca^{++} accumulated rapidly in the vesicles on account of hydrolysis of ATP (state I). In this state the velocity of hydrolysis is determined purely by the properties of the enzyme. Activity of Ca-ATPase in state I was thus determined as the difference between total ATPase activity and Mg-ATPase activity. After uptake of Ca^{++} the rate of acidification falls off because the transport system passes into another state. In state II the velocity of hydrolysis depends not only on the properties of the enzyme, but also on the rate of outflow of Ca^{++} from the vesicles.

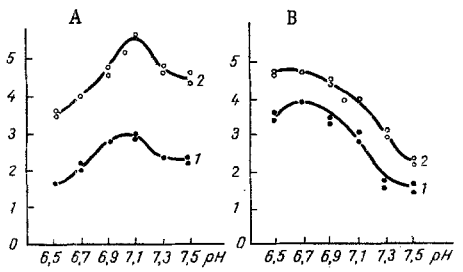

Fig. 2

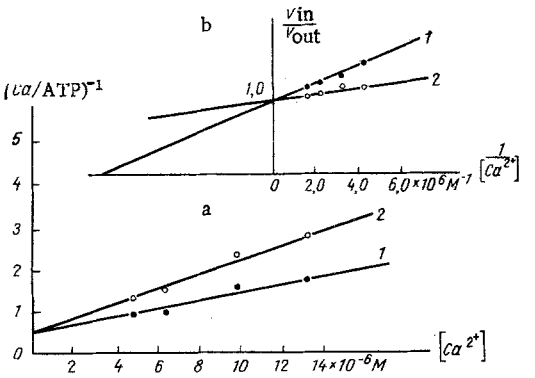

Fig. 3

Fig. 2. Dependence of hydrolysis of ATP by Ca-ATPase and Ca^{++} transport on pH: A) pH-dependence of hydrolysis of ATP by Ca-ATPase for SR from normal (1) and denervated (2) muscles; B) pH-dependence of Ca^{++} transport for SR from normal (1) and denervated muscles (2). Ordinate: A) rate of hydrolysis (in μmoles Ca^{++}/mg protein/min).

Fig. 3. Transport parameters as functions of Ca^{++} concentration in the internal space of SR vesicles: a) (Ca/ATP) as a function of Ca^{++} concentration inside vesicles for SR from normal (1) and denervated (2) muscles; b) V_{in}/V_{out} as a function of $1/[Ca^{+2}]$ for SR from normal (1) and denervated (2) muscles. Ca^{++} concentration in internal space of vesicles calculated from solubility product for calcium oxalate, namely $2 \cdot 10^{-8}$ M^2 [7].

Most authors describe ATPase activity in state II as "basal" ATPase. The rate of ATP hydrolysys in state II can be calculated as the difference between total ATPase activity and Mg-ATPase activity. It characterizes the rate of outflow of Ca^{++} from the vesicles. It was found that on the 17th-21st day after denervation Ca-ATPase activity and the rate of accumulation of Ca^{++} increased on average by 1.5 times (Table 1). Activation was unconnected with any change in the pH-dependence of these processes. It will be clear from Fig. 2 that under normal conditions and after denervation the pH-optimum for Ca-ATPase was 7.1. In both cases Ca^{++} transport took place faster in the region of more acid pH values (6.5-6.7). Although the weight of the muscles after denervation was reduced by half, the outflow of protein of the SR fraction was unchanged at 0.50-0.85 mg/g tissue. The increase in ATPase activity cannot therefore be regarded as the result of better purification of the fraction.

Denervation led to a threefold increase in basal ATPase activity (Table 1), in agreement with other workers' findings [9, 12]. Activation of "basal" ATPase is connected both with activation of Mg-ATPase and with activation of Ca-ATPase, compensating for the outflow of Ca^{++}; this is evidence of an increase in the rate of this process after denervation of the muscle.

During the first 10 days after denervation, Ca-ATPase activity and the rate of Ca^{++} accumulation are unchanged; not until the end of the second week is activation (20%) of both processes observed [9]. The experiments showed that on the 17th-21st day activation of transport and hydrolysis was already 50%. At the same time Mg-ATPase activity also increased. Since the degree of purification of the fraction was unchanged, activation was due either to an increase in the concentration of enzyme in the membrane or to changes in the structure of the membrane or the enzyme itself which led to an increase in the activity of each molecule. To solve this problem further investigations are necessary.

Hydrolysis of one molecule of ATP by Ca-ATPase is accompanied by the transport of two Ca^{++} ions through the membrane [8]. However, with an increase in the Ca^{++} concentration inside the vesicles the opposite process – outflow of Ca^{++} – began. There are two outflow channels: passive diffusion and rapid outflow, which obeys the Michaelis–Menten kinetics [6]. The SR membrane has low permeability for Ca^{++}, and the rate of passive diffusion is therefore very slow. The rate of fast outflow is comparable with the rate of Ca^{++} inflow [6]. To determine the rate of accumulation, the difference between the inflow and outflow of Ca^{++} is found. The value of Ca/ATP, determined as the ratio between the rate of accumulation of Ca^{++} and the rate of ATP hydrolysis is thus always under 2. The rate of outflow is proportional to the Ca^{++} concentration inside the vesicles and, having determined the values of Ca/ATP for different Ca^{++} concentrations, the maximal value of Ca/ATP can be found. This is done by extrapolation of the values of Ca/ATP to an infinitely low concentration of Ca^{++}. It will be clear from Fig. 3a that for normal and denervated muscles the

maximal value of Ca/ATP was 2. This shows simultaneously that the rate of passive diffusion in SR from normal and denervated muscles is much slower than the rate of fast calcium outflow. The observed decrease in efficiency of accumulation in SR from denervated muscles is due to an increase in the rate of the fast Ca^{++} outflow. This possibility was analyzed by calculating the ratio between inflow and outflow at each internal Ca^{++} concentration and the curve of V_{in}/V_{out} as a function of $1/[Ca^{++}]$ was plotted (Fig. 3b). The point of intersection of this line with the abscissa gives the reciprocal of the outflow constant (K_{out}). This is the concentration of Ca^{++} in the internal space of the vesicles at which the outflow reaches 50% of its maximal value. For SR from normal muscles K_{out} on average is twice as high as K_{out} for SR from denervated muscles.

In the modern view Ca^{++} transport in SR is effected by a carrier with cyclically changing affinity for Ca^{++} [8]. The rate of Ca^{++} inflow depends on the dissociation constant of the carrier–Ca^{++} complex on the outer side of the membrane, whereas the rate of outflow depends on the dissociation constant of this complex on the inner side of the membrane. The ratio between the constants determines the Ca^{++} gradient in SR. If the Ca^{++} outflow is proportional to its binding with the carrier, K_{out} is proportional to the dissociation constant of the carrier–Ca^{++} complex on the inner side of the membrane. The decrease in K_{out} after denervation is thus evidence of an increase in affinity of the carrier for Ca^{++} on the inner side of the membrane. If affinity for Ca^{++} on the outer side of the membrane is unchanged, the Ca pump of the denervated muscle can create a gradient only half as low.

Changes taking place in SR on the 17th–21st day after denervation were thus connected mainly with an increase in the Ca^{++} outflow from SR and a reduction in the efficiency of action of the Ca pump. Increased activity of Ca-ATPase under these conditions is possibly a compensatory reaction. Despite this possibility, the Ca^{++} gradient is reduced after denervation and this evidently leads to characteristic changes in the form of contraction of the denervated muscle with a protracted period of contraction and with lengthening of the relaxation time.

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