STATE OF THE CALCIUM PUMP OF THE SARCOPLASMIC RETICULUM IN COMPENSATORY HYPERFUNCTION AND HYPERTROPHY OF SKELETAL MUSCLE

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On the sixth day of compensatory hyperfunction induced by division of the tendon of a synergist, the mass of the rabbit soleus muscle, a slowly contracting red skeletal muscle, is increased. The increase in mass of the muscle is accompanied by a decrease in activity of Ca^{++}- and Mg^{++}- dependent ATPase and in the rate of absorption of Ca^{++} by unit mass of the sarcoplasmic reticulum (SPR). The concentration of SPR protein in the muscle is unchanged. As a result of these changes the power of the calcium pump of the SPR per unit mass of muscle in the hypertrophied skeletal muscle is reduced.

Experiments on heart muscle have shown that the power of the calcium pump of the sarcoplasmic reticulum (SPR), responsible for relaxation of the muscle, falls in the stage of failure [13] and, in all probability, in the stage of relatively stable compensatory hyperfunction and hypertrophy also [3].

The question accordingly arises of how soon the power of the calcium pump decreases during compensatory hypertrophy of a muscle. To answer this question the state of the calcium pump of the SPR was studied in a slowly contracting red skeletal muscle – the rabbit soleus muscle – in the initial stage of the process, on the sixth day of compensatory hypertrophy.

EXPERIMENTAL METHOD

Experiments were carried out on 29 male rabbits weighing 2.5-3 kg. Compensatory hyperfunction and hypertrophy of the soleus muscle were produced in 21 rabbits by removing as much of the tendon of the synergist muscle, the gastrocnemius, as possible. The soleus muscles of the contralateral intact limbs of the same animals were used as the control. Mock operations differing from the ordinary operations simply in the fact that the tendons of the gastrocnemius muscle were not divided, were carried out on eight rabbits.

The rabbits were decapitated on the sixth day after the operation. The intact and hypertrophied soleus muscles of seven rabbits were dried in an incubator at 75°C to constant weight to determine the content of dry substance in the hypertrophied muscle. Samples of SPR were isolated from the soleus muscles of the other animals by a modified Harigaya's technique [6]. Samples of muscles weighing 2 g were homogenized for 80 sec in a Waring blendor with Teflon container at 12,000 rpm in 40 ml medium containing 0.15 M KCl and 0.005 M $NaHCO_3$. The homogenates were centrifuged at 8,000 g for 20 min. The supernatant was passed through several layers of gauze and treated with 3.0 KCl up to a final concentration of 0.6 M. The microsomes were sedimented at 45,000 g for 60 min 20-30 min after addition of the KCl. The residue of micro-

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TABLE 1. Effect of Compensatory Hyperfunction and Hypertrophy for Six Days on SPR of Rabbit Soleus Muscle (M ± m)

Index studied	Intact muscles n = 7(14)	Hypertrophied muscles n = 7 (14)	Differences, %	P
Weight of wet muscles (in mg)	1467±58	1976±60	+34,7	<0,001
Yield of SPR protein from 1 g muscle (in mg)	2,76±0,11	2,54±0,18	—8,0	<0,5
Rate of uptake of Ca^{++} (in nmoles Ca^{++}) calculated:				
per milligram SPR protein	4,79±0,41	4,21±0,41	—12,1	<0,5
per SPR in 1 g muscle	13,2±1,2	10,5±0,9	—20,3	<0,1
Activity of Ca^{++}- and Mg^{++}-dependent ATPase (in nmoles P_{in}/min) calculated:				
per milligram SPR protein	365±21	285±14	—21,9	<0,01
per SPR in 1 g muscle	1007±61	716±38	—28,9	>0,001
Calcium-oxalate capacity (in nmoles Ca^{++}/g SPR protein)	1013±84	1002±66	—1,1	>0,5
Activity of Mg-dependent ATPase (in nmoles P_{in}/min/mg SPR protein)	288±31	403±52	+40,0	>0,05

Note. 1) Number of animals shown in parenthesis, 2) weight of rabbits 2884 ± 73 g.

somes, resuspended in 0.1 M KCl solution with 0.01 M Tris-maleate buffer, pH 7.1, was used as the SPR preparation. Protein was determined by Lowry's method [9].

Calcium and ATP-ase activity of the SPR preparations were determined by the method described by Boldyrev et al. [2, 4]. An incubation medium of the following composition was used (25°C): 0.1 M KCl, 0.002 M $MgCl_2$, 0.002 M ATP, 0.0064 M $Na_2C_2O_4$, 0.01 M Tris-maleate buffer, pH 7.1. The concentration of SPR protein in the reaction mixture was 0.08-0.1 mg/ml and the volume of the sample was 8 ml. Only very small quantities of Ca^{++} ions were added – 140 nmoles $CaCl_2$ in each case.

Statistical analysis of the results was carried out by the usual methods [1].

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results (Table 1) show that the power of the calcium pump of the SPR in the skeletal muscles undergoing compensatory hypertrophy was reduced, as reflected in the rate of absorption of Ca^{++} and the activity of Ca^{++}- and Mg^{++}- dependent ATPase of the SPR per unit mass of muscle. The decrease in power of the calcium pump was due chiefly to a decrease in the rate of uptake of Ca^{++} and activity of Ca^{++}- and Mg^{++}-dependent ATPase per unit mass of SPR because the SPR concentration in the muscle, estimated from the yield of SPR protein from 1 g muscle, was unchanged. A small (8%) decrease in yield of SPR protein per gram muscle, which was not significant ($P > 0.5$), was due to hydration of the hypertrophied muscle – the relative content of dry substance in the muscle was reduced by 12.3% ($P < 0.01$), as a special series of experiments showed. The fact that the decrease in the rate of Ca^{++} uptake per unit mass of SPR and the decrease in SPR per gram muscle was not significant cannot be a decisive factor when the state of the calcium pump of the SPR is assessed, for changes in activity of Ca^{++}- and Mg^{++}-dependent ATPase, a much more reliable index of the Ca^{++}-transporting function of the SPR when ordinary methods of recording are used [7], were considerable and significant (Table 1).

The results in Table 1 also show that the activity of Mg^{++}-dependent ATPase was greatly increased in the SPR samples from the hypertrophied muscles but the calcium–oxalate capacity – the largest quantity of Ca^{++} absorbed per unit mass of SPR in the presence of oxalate ions – was completely unchanged. The functional significance of these findings is not yet clear.

Preparations of SPR isolated from the soleus muscles after mock operations of the limbs were virtually indistinguishable from those of the soleus muscles of intact limbs (Table 2). Taken as a whole, these facts are evidence that the nonspecific effect of the operative trauma on the activity of the SPR preparations was negligible.

The results of these investigations show that the power of the calcium pump of the SPR of skeletal muscle falls very early during compensatory hyperfunction and hypertrophy, in the initial stage of the

TABLE 2. Effect of Mock Operation on SPR of Rabbit Soleus Muscle on Sixth Day After Operation (M ± m)

Index studied	Intact muscles n=4 (8)	Hypertrophied muscles n = 4 (8)	Differences, %	P
Weight of wet muscles (in mg)	1533±39	1580±44	+1,7	>0,5
Yield of SPR protein from 1 g muscle (in mg)	2,94±0,24	3,03±0,10	+3,1	>0,5
Rate of uptake of Ca^{++} (in nmoles Ca^{++}/sec per mg SPR protein)	5,03±0,42	5,33±0,49	+6,0	>0,5
Activity of Ca^{++}- and Mg^{++}-dependent ATPase (in nmoles P_{in}/min per mg SPR protein)	301±32	304±32	+1,0	>0,5
Calcium-oxalate capacity (in nmoles Ca^{++}/g SPR protein)	1281±28	1294±107	+1,0	>0,5
Activity of Mg^{++}-dependent ATPase (in nmoles P_{in}/min/mg SPR protein)	215±21	228±10	+6,0	>0,5

Note. Number of animals shown in parentheses, 2) weight of rabbits 3190 ± 100 g.

process. A decrease in the power of the pump in the later stages of development of the process was demonstrated in experiments on heart muscle [3, 13]. The power of the calcium pump of the SPR in hypertrophied heart muscle, incidentally, falls just as it does in hypertrophied skeletal muscle – despite no change in the concentration of SPR elements [11] or in the calcium-oxolate capacity [5, 13]. These facts, combined with others indicating the considerable similarity between the slowly contracting red skeletal muscle and heart muscle with respect to certain features of function [15], energy supply [12], structure and biochemical properties of the SPR [12, 13], biochemical properties of the myofibrils [10], and changes in the physiological properties of these muscles during compensatory hyperfunction and hypertrophy [8], suggest that the results of the study of the calcium pump of the SPR of hypertrophied skeletal muscle can be applied to heart muscle and vice versa. Hence it follows that the decrease in power of the calcium pump of the SPR discovered in the early (emergency) stage of the process can continue in later stages and can thus be an early manifestation of the general principle that the power of the SPR calcium pump is reduced in muscles undergoing compensatory hypertrophy.

The increase in duration of the single contraction of the hypertrophied muscle could be a direct result of this decrease in power of the calcium pump of SPR [8, 14].

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