Adaptation to Physical Exercise Increases Expression of Ca-ATPase Gene in Myocardial Sarcoplasmic Reticulum

M. G. Pshennikova, G. L. Khaspekov,* A. O. Tatarenko,* I. Yu. Malyshev, and R. Sh. Bibilashvili*

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Gene expression of the key enzyme of the myocardial sarcoplasmic reticulum Ca^{2+}-pump, SERCA-2a isoform was assayed in rats during adaptation to physical exercise (forced swimming). The expression was measured by Northern blot hybridization with subsequent densitometry of the autoradiograms. The signal of mRNA encoding SERCA-2a was referenced to the mRNA signals of marker proteins (S4 and S9 ribosomal proteins, cytoskeletal β-actin and glyceraldehyde-3-phosphate dehydrogenase). The SERCA-2a gene expression gradually increased during adaptation as evidenced by the increased content of SERCA-2a mRNA in particular higher intensity (optical density) of the mRNA signals in autoradiograms. The adaptation-induced increase in the power of the sarcoplasmic reticulum Ca^{2+}-pump can be attributed to activated synthesis and accumulation of SERCA-2a isoform.

Key Words: *myocardium; adaptation to physical exercise; SERCA-2a gene expression*

Adaptation to physical exercise is known to protect the heart against ischemia/reperfusion-induced damage [3]. Protective mechanisms seem to be formed at the cardiac level [9], which is confirmed by high resistance of hearts isolated from trained animals to ischemia and reperfusion [2,10] and toxic concentration of Ca^{2+} and catecholamines [4]. The nature of these local protective mechanisms of adaptation remains unclear. It was shown that contractile dysfunction after acute ischemia [15] or long-term compensatory cardiac hypertrophia and hyperfunction [16] was determined to a great extent by low activity of the Ca^{2+} pump of the myocardial sarcoplasmic reticulum (SR), i. e. SR Ca^{2+}-ATPase and low expression of the corresponding gene [6,15]. It can be suggested that the high resistance of "adapted heart" to adverse factors is due to increased expression of the Ca^{2+}-ATPase gene and increased power of the myocardial SR Ca^{2+} pump. Adaptation to physical exercise increased the mass of membrane SR structures [7] and the number of Ca^{2+} bindings sites [12]. This adaptation increased the rate of Ca^{2+} uptake by the SR and the resistance of Ca^{2+}-ATPase to endogenous damaging factors such as products of free-radical oxidation, high Ca^{2+} concentrations, and thermoinactivation [1,4]. Adaptive changes in the system of Ca^{2+} transport can result from modification of Ca^{2+}-ATPase molecules due to conformational changes and/or their accumulation due to activation of the Ca^{2+}-ATPase gene. These possibilities have not been examined yet. In this study we investigated the dynamics of the Ca^{2+}-ATP gene expression in rat myocardium during adaptation to physical exercise.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 280-350 g. Adaptation to physical exercise consisted of 30 sessions of forced swimming (32°C, 5 times a week for 45 days). During the first 2 weeks

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences; *Russian Cardiology Research and Production Complex, Russian Ministry of Health; Russian State Medical University, Moscow

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the duration of swimming increased from 15 to 60 min by 5 min daily and then remained at this level to the end of training. Myocardial samples were taken after 5, 10, 15, 20 and 30 sessions. The age matched controls were maintained in a vivarium under the same conditions. The experimental animals were decapitated 1 day after the last swimming session, the heart was rapidly removed and ventricles were immersed in liquid nitrogen. Total RNA was extracted with phenol, guanidine isothiocyanate, and chloroform as described previously with modifications [11]. The quality of RNA was tested by electrophoresis in 1% agarose glyoxal-denaturating gel stained with ethidium bromide. RNA samples were stored at -70°C as aqueous solutions. The content of mRNA in these samples was measured by Northern blot hybridization [11]. The standard error of the method determined by the calibration curve for labeled marker DNA mixture was 23%. Quantitative changes in the expression were assessed by the ratio of the Ca^{2+}-ATPase and marker gene mRNA signals to the signal of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA. The genes characterized by conservative expression in different tissues (S4 and S9 ribosomal proteins, cytoskeletal β-actin, and G3PDH [8]) were used as markers. Their expression was measured with oligodeoxyribonucleotide probes constructed according to the algorithms developed at the Russian Cardiology Research-and-Production Complex and synthesized using the corresponding nucleotide sequences from GenBank. The following probes were used:

1) for a slow Ca^{2+}-ATPase, which is analogous to human ATP2A2 gene according to the OMIM classification: 5'-CAG ATT CAC CTG TAA GAA TGG ACT GGT CAA CTC TCA G-3' (724-760), sequence code X15635. This probe is specific for mRNA encoding SERCA-2a and SERCA-2b isoforms [13];

2) for ribosomal S4 protein: 5'-GTG TAA TTC GAT GGA CAG CAA AGC GAC CCT TGG TG-3' (333-368), sequence code X14210;

3) for ribosomal S9 protein: 5'-CAC CTG CTT GAG GAC CCT GAT GTG ACG TTG GC-3' (418-449), sequence code X66370;

4) for cytoskeletal β-actin: 5'-GTG GAC GAC GAG CGC AGC GAT ATC GTC ATC CAT-3' (1247-1279), sequence codes V01217, J00691;

5) for G3PDH: 5'-CAC GGA AGG CCA TGC CAG TGA GCT TCC CGT-3' (734-763), sequence codes M17701, X02231, X00972 (Clontech).

All the probes except No. 5 were synthesized by solid-phase phosphoamidation and purified by electrophoresis in polyacrylamide gel (20%) with subsequent ion-exchange chromatography and gel filtration. Oligonucleotides were labeled with ^{32}P at the 5'-end using T4 fag polynucleotide kinase (Amersham Life Science) and had specific activity no less than 107 cpm/pmol. After fractionation in a glyoxal-denaturating 1% agarose gel [11], the immobilized total myocardial RNA was electrophoretically transferred to membranes. The following control samples were applied to the same gel to test the adequacy of hybridization-produced signals:

- rat brain RNA without matrix poly(A)⁻RNA to exclude the signal of the probe bound to nonpolyadenylated-RNA;
- rat brain poly(A)⁺ RNA to identify the signals of test RNA and to obtain standard tracks revealed by probe for SERCA-2a Ca^{2+}-ATPase;
- total RNA from rat skeletal muscle to differentiate the two isoforms of Ca^{2+}-ATPase (SERCA-1 and SERCA-2a);
- marker DNA and RNA mixture to determine the size of RNA detected by the probe.

Hybridization was performed in a Hybide apparatus. Gene expression was assessed by the intensity of a radioautographic spot on X-ray films (Forma) measured using an Alpha Imaging System (Alpha Innotech. Inc.).

RESULTS

The adaptation increased the content of mRNA encoding SERCA-2a and, therefore, the corresponding gene expression (Fig. 1, *a*). The signals produced by myocardial SERCA-2a mRNA were clearly seen in autoradiograms (Fig. 1, *a*, tracks *1-7*). There were no signals of skeletal muscle mRNA. This indicates that our probe was specific for mRNA encoding myocardial Ca^{2+}-ATPase, SERCA-2a isoform and yielded no cross-hybridization with mRNA encoding Ca^{2+}-ATPase, SERCA-1 isoform typical of fast skeletal muscles. Background hybridization with the nonpolyadenylated fraction of the total RNA was negative. Signals on track *10* (Fig. 1, *a*) indicated that SERCA-2a isoform (short RNA, the lower signal) was expressed in the myocardium and brain, while the SERCA-2b isoform (long RNA, the upper signal) was abundant in rat brain. These results agreed with published data [5] and allowed us to determine the position of studied signals with reference to the background hybridization.

As seen from electrophoregram of the total myocardial RNA (Fig. 1, *b*), the applied RNA samples were equal.

The assessment of marker gene expression revealed that the expression of G3PDH gene underwent only minor changes during adaptation to physical exercise (Table 1). It can be assumed that low variability of G3PDH gene expression is determined by the fact that this enzyme belongs to the system of glycolysis, the basic cell energy production system, which effectively resists to environmental factors. Therefore,

TABLE 1. Changes in Intensity of Signals of Analyzed mRNA (Signals Are Referred to Control Intensity Taken as 1)

Number of swimming sessions	Ribosomal proteins		β-Actin	G3PDH	SERCA-2a
	S4	S9			
5	2.18	2.59	1.08	0.96	1.16
10	2.39	2.91	1.77	1.42	2.74
15	1.81	2.12	1.38	1.11	2.33
20	2.33	3.34	1.71	1.30	3.76
30	3.14	2.75	2.18	1.45	4.82

we used the ratio of SERCA-2a mRNA to G3PDH mRNA for correct estimation of the SERCA-2a gene expression (Table 2). This standardized index increased by 20% after 5 swimming sessions, by 90% after 10 sessions, 2-fold after 15 sessions, 2.8-fold after 20 sessions, and 3.3-fold by the end of training (Table 2, Fig. 2). During the adaptation the intensity of the SERCA-2a mRNA signal significantly increased, while the linear increase in the intensity of β-actin mRNA was insignificant. The signals of S4 and S9 mRNAs underwent more complex changes.

Functional significance of more intense signals from mRNAs encoding ribosomal proteins observed after 5 and 20 swimming sessions cannot be assessed, because of insufficient accuracy and low number of measurements. It can be suggested, that 2.5-fold increase in ribosomal protein mRNA after the first week of training reflects general activation of protein biosynthesis in response to regular exercise.

Thus, our study showed that expression of the SERCA-2a gene encoding the key enzyme of the myocardial Ca^{2+}-transport system increases starting from the first days of adaptation. A significant increase in the content of corresponding mRNA indicates that the expression is activated at the level of transcription. The dynamics of these changes suggests that the in-

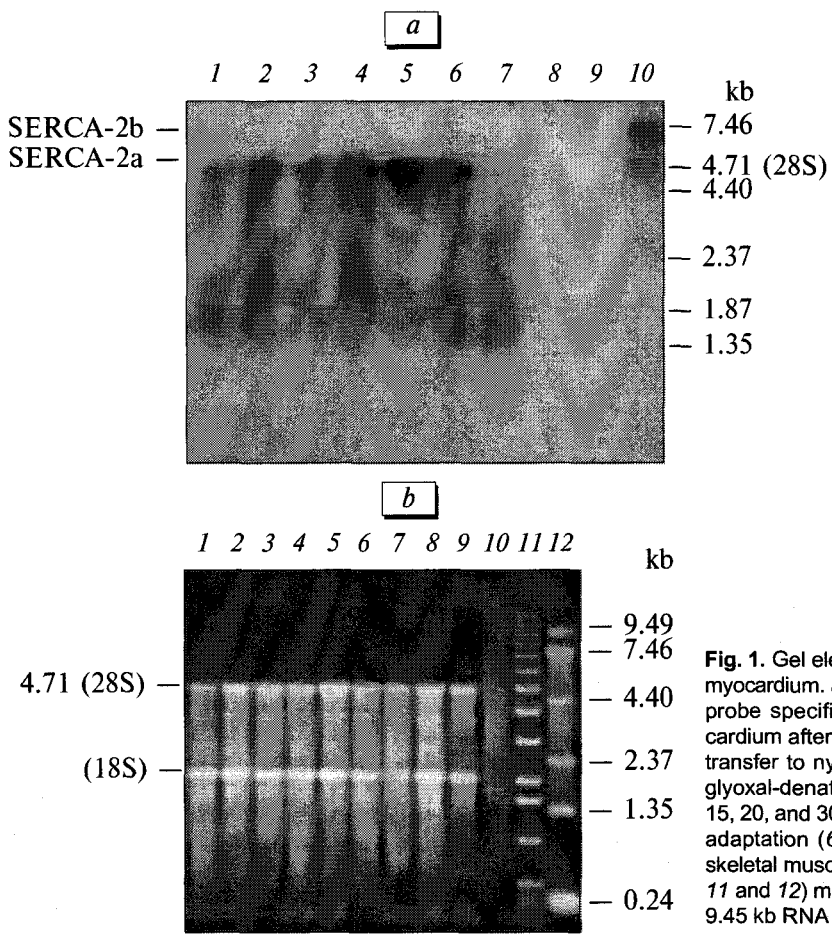

Fig. 1. Gel electrophoresis and hybridization of the total RNA from rat myocardium. *a*) radioautogram of hybridization of oligodeoxynucleotide probe specific for SERCA-2 mRNA with total RNA from rat myocardium after electrophoresis in 1% glyoxal-agarose gel and electrotransfer to nylon membranes; *b*) electrophoregram of total RNA in glyoxal-denaturing 1% agarose gel. *1-7*) rat myocardium after 5, 10, 15, 20, and 30 swimming sessions (*1-5*, respectively), after short-term adaptation (*6*, not discussed in the text), in the control (*7*); *8*) rat skeletal muscle; *9*) rat brain poly(A)$^-$RNA; *10*) rat brain poly(A)$^+$RNA; *11* and *12*) markers (Life Technologies): 1 kb DNA Ladder (*11*); 0.24-9.45 kb RNA Ladder (*12*).

crease in the capacity of the SR Ca^{2+}-transport system after 30 adaptation sessions is associated with the peak increase in the expression of the SERCA-2a gene encoding the key Ca^{2+} pump enzyme, and, therefore, with activation of its synthesis. The mechanism of the Ca^{2+}-ATPase gene activation during adaptation to physical exercise remains to be explored. At the same time, the data on the expression of genes encoding myocardial proteins under conditions of hyperfunction or compensatory hypertrophy of the heart [14] suggest that activation of protein kinase C followed by activation of phospholipase C plays a key role in this process. Protein kinase C can be activated not only by hormones released during physical exercise, but also by changes in mechanical properties of cardiomyocytes in response to increased hemodynamic load. Molecular mechanisms of these processes and the relations between the duration of adaptation and the intensity of ribosomal synthesis need further investigation.

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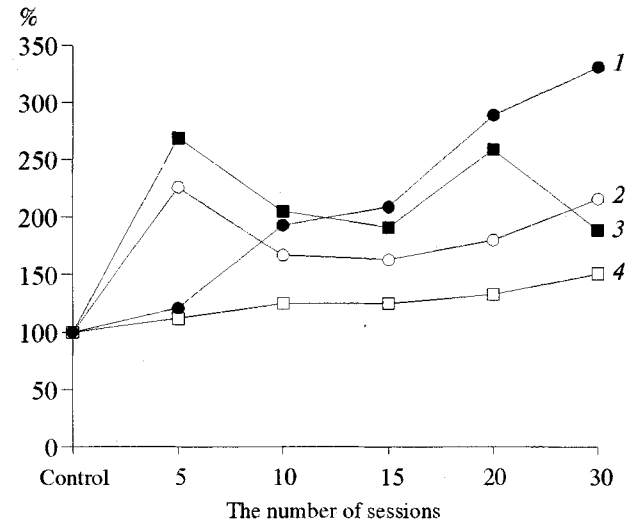

Fig. 2. Adaptation-induced changes in the signal intensity (optical density) of test mRNA normalized to the intensity of glyceraldehide-3-phosphatdehydrogenase (G3PDH) mRNA signal. *1*) SERCA-2a; *2*) S4 ribosomal protein; *3*) S9 ribosomal protein; *4*) cytoskeletal β-actin.

TABLE 2. Adaptation-Induced Changes in Intensity of Signals of mRNAs Encoding Different Proteins (Signals are Standardized to the Intensity of the G3PDH mRNA Signal)

Number of sessions	Ribosomal proteins	S4	S9	β-Actin
Control	0.30	0.56	0.76	1.07
5	0.68	1.51	0.85	1.29
10	0.50	1.15	0.95	2.06
15	0.49	1.07	0.95	2.24
20	0.54	1.45	1.01	3.09
30	0.65	1.06	1.15	3.54

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