α-Skeletal Muscle Actin mRNA’s Accumulate in Hypertrophied Adult Rat Hearts

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Cardiac hypertrophy due to a chronic hemodynamic overload is accompanied by isoformic changes of two proteins of the thick filament of the sarcomere, myosin, and creatine phosphokinase. We have looked for isoactin changes, using deoxyribonucleic acid probes complementary to α-skeletal and α-cardiac actin messenger ribonucleic acids. Three groups of rats were studied at various days after application of a pressure overload (2–4 days, n = 13, 8–15 days, n = 5, and 30–40 days, n = 7) and were compared to control animals (n = 11). Whereas α-skeletal actin messenger ribonucleic acids were hardly detectable in the normal hearts (0.6 ± 0.16%), they accumulated significantly in the first 4 days after the aortic stenosis (4.6 ± 3.1%, p < 0.001 vs. controls) and then slowly declined (8–15 days, 3.2 ± 1.7% and 30–40 days, 1.6 ± 0.6%, p < 0.05 and NS vs. controls). This figure is similar to that observed in 8-day-old rats (2.27 ± 0.3%, p < 0.01 vs. controls). We conclude that, in rat myocardium, the expression of messenger ribonucleic acids encoding the sarcomeric actins is altered at the onset of a pressure overload hypertrophy. Although the physiological significance of isoactin changes is unknown, our results show that the thin filament participates as well as the thick filament in the response of cardiac muscle to new functional requirements. (Circulation Research 1986;59:551-555)

ONE of the most important properties of the mammalian myocardium is to adapt to an increased hemodynamic load. When the overload is long-lasting, as in hypertension, the adaptive response is mainly due to a modified expression of cardiac genes (see Swynghedauw and Delcayre1 for a review) consisting of 1) an overall increase in the rate of protein synthesis, and accumulation resulting in cardi hypertrophy, 2) structural changes, such as an increased number of smaller mitochondria, and 3) modifications in the expression of isoforms for three multigene families of proteins located on the thick filament of the sarcomere and involved in energy metabolism, creatine phosphokinase,2,3 and myosin heavy4–8 and light6–9 chains. Isoomyosin changes lead to a slower10,11 and a more efficient12 contraction of the cardiac fiber, and it is likely that creatine kinase changes would allow a more efficient energy transfer from creatine phosphate to adenosine diphosphate.3

Another major structural component of the sarcomere is actin, which is also encoded by a multigene family in mammals. Two sarcomeric actins exist, the α-cardiac and α-skeletal isoforms. These isoforms are very similar,13 and this makes it difficult to distinguish them at the protein level. The nucleotide sequences, however, are more diverged and the 3' noncoding region of the messenger ribonucleic acids (mRNA’s) can be used as gene specific probes.14–16 In small mammals α-cardiac actin mRNA and protein accumulate as the predominant actin type in adult hearts.14,16–17 whereas in larger mammals α-skeletal actin is also present.15,17 During the development of cardiac muscle in rodents, both α-cardiac and α-skeletal actins and mRNA’s accumulate in fetal and neonatal hearts.16,17

The question naturally arises as to whether such a co-accumulation is associated with hypertrophy of the adult heart. We therefore have looked by Northern blot analysis at the accumulation of α-skeletal and α-cardiac actin mRNA’s in pressure overload hypertrophy of the rat ventricle. Two complementary deoxyribonucleic acid (cDNA) probes were used, one to determine the amount of total striated actin mRNA and the other that of skeletal actin mRNA, a procedure that allowed a precise quantification of the amount of skeletal muscular actin message.

Materials and Methods

Pressure overload of the left ventricle was produced in adult male Wistar rats (body weight 200 g) by coarctation of the upper abdominal aorta using a partially occluded Weck hemoclip. Excision of the hearts and calculation of the degree of ventricular hypertrophy were performed as in Mercadier et al.5 A total of 25
operated, 5 sham-operated, and 6 control unoperated animals kept under identical housing conditions were used. Thirteen operated animals were sacrificed 2–4 days after surgery, 5 between 8 to 15 days, and 7 between 30 to 40 days. For some experiments the liver and the skeletal muscles from the legs were excised from the control animals and immediately frozen in liquid nitrogen. The ventricles from 40 newborn rats (8-day old) were also sampled and handled as the adult ones. They were divided into 3 pools. All tissues were stored at −70°C.

Total cardiac RNA was prepared according to Chirgwin et al14: extraction in guanidinium thiocyanate, isolation by sedimentation through cesium chloride and purification by two precipitations from guanidine hydrochloride solution with acetic acid and ethanol. The RNA samples were routinely stored as a 70% ethanol suspension in the presence of potassium acetate at pH 5, at −20°C. The RNA concentration was determined using a value of 25 absorbance units/1 mg RNA/ml.

The cDNA probes, pAM91-1 and pAF81, were previously described in detail.14,19,30 Both were cloned from poly (A)* RNA isolated from the skeletal muscles of 10-day-old mice. pAM91-1 is a subclone that contains the 185 base pairs (bp) PstI fragment from the 3' noncoding region of α-skeletal actin mRNA. pAF81 contains about 90% of the coding region of α-cardiac actin mRNA. pAF81 hybridized strongly to mRNA extracted from adult rat skeletal muscle (Figure 1A, Lanes 2–7). Under these experimental conditions and assuming 100% α-skeletal actin mRNA as the actin transcript of adult skeletal muscle,14,16 the cardiac actin coding sequence pAF81 cross-hybridized to about 25% with α-skeletal actin mRNA (Figure 1B, Lanes 2–7). Adult cardiac ventricular muscle contains α-cardiac actin mRNA (Figure 1B, Lane 8) and is present in newborn hearts (0.6 ± 0.16% vs. 2.27 ± 0.3%, p < 0.01), which is in agreement with Mayer et al.10 Taken altogether, these data clearly indicated that pAM91-1 was a specific probe for rat α-skeletal mRNA.

The results obtained with 1 sham-operated and 3 operated rats (4 to 8 days after surgery) are illustrated in Figure 2. Accumulation of α-skeletal actin transcripts was detected in cardiac RNA 4 and 8 days after the aortic constriction (Figure 2A, Lanes 2–4). The response, for a given time point, varied between animals (Figure 2A, Lanes 2–3), as expected from the rough reproducibility of the pressure overload induced by our experimental model (see Swynghedauw and Delcayre).1 On this blot, the hybridization signals with animals were normalized for the amount of actin messages measured with probe pAF81, a procedure which circumvented all errors inherent to the RNA transfer. The results were expressed as the percent of α-skeletal actin mRNA. Reproducibility of the whole procedure, calculated according to Henry21 was equal to 23% (14 pairs of duplicate), which is a fairly good value in respect to the small amounts of α-skeletal actin mRNA detected (for example, 0.4, range 0.35–0.45; and 16, range 14–18). Statistical significance between operated and control groups was evaluated by one-way analysis of variance assuming equality of variance between the groups, and group comparison by Scheffe’s test for multiple contrasts. Neonates were compared to adult control rats by Mann and Whitney’s test. Results are expressed as means ± SD.

Results

The probes were derived from mouse mRNAs. DNA sequencing indicates that there is very little difference between the two animal species (see Alonso et al)20, but we have nevertheless checked the specificity of the cDNAs with rat muscles. Figure 1 shows the cross-hybridization of pAF81 and pAM91-1 with various rat tissues. The messengers detected in these experiments had a size of about 1.6 kilobases (kb), as expected for striated muscle actins.14,16 Nonmuscle actin mRNA’s (about 2 kb) were not detected with either of the probes under the hybridization conditions used (Figure 1, A and B, Lane 1). The probe pAM91-1 hybridized strongly to mRNA extracted from adult rat skeletal muscle (Figure 1A, Lanes 2–7). Under these experimental conditions and assuming 100% α-skeletal actin mRNA as the actin transcript of adult skeletal muscle,14,16 the cardiac actin coding sequence pAF81 cross-hybridized to about 25% with α-skeletal actin mRNA (Figure 1B, Lanes 2–7). Adult cardiac ventricular muscle contains α-cardiac actin mRNA (Figure 1B, Lane 8) and very little hybridization to the skeletal actin specific probe, pAM91-1, was detectable (Figure 1A, Lane 8). In contrast, in the ventricles of newborn rats, α-skeletal actin transcripts were detected (Figure 1A, Lane 9). Using RNA from adult skeletal muscle as a standard for α-skeletal actin mRNA, we estimated from the calibration curve (Lanes 2–7) that this mRNA is barely detectable in adult rat hearts and is present in newborn hearts (0.6 ± 0.16% vs. 2.27 ± 0.3%, p < 0.01), which is in agreement with Mayer et al.10 Taken altogether, these data clearly indicated that pAM91-1 was a specific probe for rat α-skeletal mRNA.

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FIGURE 1. RNA blot analysis of \( \alpha \)-skeletal and \( \alpha \)-cardiac actin mRNAs in developing and adult rat skeletal and cardiac muscle.

**Total RNA from adult liver (L, Lane 1, 50 \( \mu \)g RNA), adult skeletal muscles (Sk, Lane 2, 25 \( \mu \)g, Lane 3, 12.5 \( \mu \)g, Lane 4, 6.2 \( \mu \)g, Lane 5, 3.1 \( \mu \)g, Lane 6, 1.5 \( \mu \)g, Lane 7, 0.75 \( \mu \)g RNA), adult (H, Lane 8, 50 \( \mu \)g RNA) and 8-day-old (H, Lane 9, 50 \( \mu \)g RNA) cardiac ventricular muscles.**

Panel A: Hybridization with the skeletal actin probe pAM91-1, specific activity of the nick-translated plasmid: \( 2.2 \times 10^6 \) dpm/\( \mu \)g. Panel B: Hybridization with the cardiac actin probe pAF81, specific activity of the nick-translated TaqI-PstI insert: \( 0.2 \times 10^6 \) dpm/\( \mu \)g. Both autoradiograms were exposed for 3 hours at \(-70^\circ\) C.

**Discussion**

This study demonstrates that transcripts from the \( \alpha \)-skeletal muscle actin gene accumulate in the adult rat heart following a pressure overload. Striated muscle actins are also developmentally regulated. It thus appears that the thin filament of the sarcomere is involved in the response of the myocardial tissue to new functional requirements.

Comparison of the time course for the accumulation of \( \alpha \)-skeletal actin transcripts with that of the rate of total protein synthesis in the same experimental model shows a striking homology. The comparison with probe AF81 are lower with the 4 days samples (Figure 2B, Lanes 2–3). However, this was not reproducibly observed with other samples and we conclude that the amount of cardiac actin mRNA does not fluctuate significantly at this time. A summary of results obtained at different times after aortic stenosis is presented in Figure 3. The degree of cardiac hypertrophy increased consistently with time after surgery (Figure 3A), which is a usual feature in such a model. The amounts of \( \alpha \)-skeletal actin mRNA were not different for control and sham-operated animals, which were combined for statistical analysis (0.6 ± 0.16%). There was a rapid accumulation of \( \alpha \)-skeletal actin mRNA in animals where the cardiac load was increased. This accumulation was maximal at the beginning of the hypertrophic process (2–4 days after aortic constriction), reaching as much as 16-fold in one case with a mean of about a six-fold increase over the basal level (4.6 ± 3.1% vs 0.6 ± 0.16%, \( p < 0.001 \)). The relative amount of \( \alpha \)-skeletal actin mRNA subsequently fell; at 8–15 days, it was 3.2 ± 1.7% (NS vs. 2–4 days), and a month after the aortic constriction, in hearts that were at least 60% hypertrophied, it was only 1.6 ± 0.6% (\( p < 0.01 \) vs. 2–4 days). The accumulation was still above controls at 8–15 days (\( p < 0.05 \)), but the difference was no longer significant at 1 month. There was no correlation between the extent of hypertrophy and the increase in skeletal actin mRNA at any given time.
the modifications in myosin heavy chain is complicated by the fact that the latter have been characterized at the protein rather than mRNA level. β-MHC can be detected by sensitive immunological procedures as soon as 2–3 days after aortic stenosis, suggesting that its expression is also activated immediately after increasing the cardiac load. The amount of β-MHC protein continues to increase in proportion to the degree of hypertrophy and persists as long as the overload is maintained. One might hypothesize that the corresponding mRNA also remains high, in contrast to that of skeletal actin. It would thus appear that in this situation in the same tissue, myosin and actin multigene families are regulated in an uncoordinated fashion.

In terms of increased accumulation of α-skeletal actin mRNA transcripts, this is a striking result, probably implying an increased rate of transcription of this gene, since there is no evidence at present for posttranscriptional regulation of α-skeletal actin in skeletal or cardiac muscle tissue (see Buckingham for a review). It seems likely, by analogy with the situation in developing hearts, that the majority of muscle cells undergoing hypertrophy are involved, rather than a few cells which are producing very high levels of skeletal actin messenger. In newborn rat hearts, experiments designed to look at the DNase I sensitivity of the skeletal actin gene suggested that the transcriptional activity was not restricted to a few cells.

In terms of the contribution of α-skeletal actin transcripts to the total actin mRNA content of hearts subjected to a pressure overload, the result is less striking. In the first week after the aortic stenosis α-skeletal actin mRNA represents about 4–5% of total striated muscle actin transcripts. In humans where the basal level of α-skeletal actin protein is significantly higher (about 20%) in the normal adult ventricle, it is not yet clear whether there is an increase in this isofom under pressure overload. One report on mRNA levels in a diseased human heart suggests this may take place (50% skeletal actin, Gunning et al)35, although in one case of hypertrophic obstructive cardiomyopathy, this is not so. In this context it is noteworthy that in rats it is the predominant myosin heavy chain of fetal hearts which is synthesized in adult hypertrophied hearts, but that this isoform already predominates in the ventricles of adult humans and the change is thus very small on hypertrophy.

Although we did not determine the presence of α-skeletal actin protein in the hypertrophied rat hearts, there is no reason to suppose that it does not also accumulate; mRNA and protein levels seem to be closely correlated for the striated muscle actins. The functional significance of the different actin isoforms is not known. The strength of muscular contraction is generated at the myosin–actin interface and is coupled to Mg2+-ATP hydrolysis catalysed by the actin–myosin–troponin complex. The two sarcomeric actins differ by only four amino acids out of a total of 375. Two of these differences reside in the N-terminal acidic peptide at amino acid residues 2 and 3. Cross-linking experiments demonstrate that the actin residues that interact with the myosin heavy chain are the N-terminal residues at positions 1, 2, 3, 4, and 11. It is thus possible that the modifications between skeletal and cardiac actins may result in a fine-tuning of the actomyosin complex to respond transiently to altered physiological conditions. It is not clear whether the accumulation of skeletal actin transcripts immediately after cardiac overload is in response to a requirement for this isoform, or whether at the onset of the cardiac hypertrophy, there is a requirement for more actin and this is transiently compensated for by the activation of this second striated muscle actin gene.

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References


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