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.

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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 al: extraction in guanidium 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. 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. The probes for hybridization and dehybridization were those recommended by Pall Ultrafine Filtration Corp. After the hybridization, the RNA blots were washed three times in 2 X SSC (150 mM sodium chloride, 0.015 M sodium citrate), 0.1% sodium dodecyl-sulfate (SDS) at room temperature for 5 minutes under vigorous agitation, followed by two washings in 0.1 X SSC, 0.1% SDS at 50°C for 15 minutes. The autoradiograms were exposed at −70°C, using Dupont Quanta III screens, and the intensities of the hybridization signals were determined on a Vernom photometer. Each RNA was analysed in at least two different blotting experiments, and in each experiment, two to three different exposure times chosen to give readable linear intensities were used. For each blot, RNA from control animals was used as the reference and the intensities of the hybridization signals obtained with the RNA from operated 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 Henry 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), 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. 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, 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. 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). On this blot, the hybridization signals with
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 differ-
ence 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.

Discussion
This study demonstrates that transcripts from the
α-skeletal muscle actin gene accumulate in the adult rat
heart following a pressure overload. Striated muscle
actins are also developmentally regulated. It thus ap-
pears that the thin filament of the sarcomere is in-
volved in the response of the myocardial tissue to new
functional requirements.

Comparison of the time course for the accumulation
of α-skeletal actin transcripts with that of the rate of
total protein synthesis in the same experimental mod-
el22 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 sig-
ificantly 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 α-skeletal actin mRNA were not different for con-
trol and sham-operated animals, which were combined
for statistical analysis (0.6 ± 0.16%). There was a
rapid accumulation of α-skeletal actin mRNA in ani-
mals where the cardiac load was increased. This accu-
mulation was maximal at the beginning of the hyper-
trophic 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 α-skeletal actin mRNA subsequently fell; at

FIGURE 1. RNA blot analysis of α-skeletal and α-cardiac actin
mRNAs in developing and adult rat skeletal and cardiac muscles.
Total RNA from adult liver (L, Lane 1, 50 µg RNA), adult skeletal
muscles (Sk, Lane 2, 25 µg, Lane 3, 12.5 µg, Lane 4, 6.2 µg, Lane
5, 3.1 µg, Lane 6, 1.5 µg, Lane 7, 0.75 µg RNA), adult (H, Lane 8,
50 µg RNA) and 8-day-old (H, Lane 9, 50 µg RNA) cardiac ventric-
ular muscles. Panel A: Hybridization with the skeletal actin probe
pAM91-1, specific activity of the nick-translated plasmid: 2.2 ×
10^8 dpm/µg. Panel B: Hybridization with the cardiac actin probe
pAF81, specific activity of the nick translated TaqI-PstI insert: 0.2 ×
10^8 dpm/µg. Both autoradiograms were exposed for 3 hours at
-70°C.

FIGURE 2. Expression of α-skeletal actin mRNA in the adult rat
heart after a hemodynamic overload. Panel A: hybridization with
pAM91-1 (α-skeletal), specific activity of the nick-translated plas-
mid: 0.2 × 10^8 dpm/µg, exposure time 96 hours at -70°C. Panel
B: Hybridization with pAF81 (α-cardiac), specific activity of the
nick-translated plasmid: 4 × 10^8 dpm/µg, exposure time 17 hours
at -70°C. Lane 1: Sham-operated rat. Lanes 2, 3, and 4: Rats
subjected to an aortic stenosis (AS), sacrificed 4, 4, and 8 days (d)
respectively, after surgery. The degrees of hypertrophy for the
operated animals were 21, 36, 48% for samples 2, 3, 4 respective-
ly. All lanes contained 19 µg RNA and the 260/280 absorbance
ratios of the RNA were 1.91, 1.83, 2.09, and 1.97 for Lanes 1, 2, 3,
and 4 respectively.
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). In newborn rat hearts, experiments demonstrating that α-skeletal actin 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.

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KEY WORDS: rat cardiac hypertrophy • pressure overload • coaccumulation of skeletal and cardiac actin mRNA's
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