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EXPRESSION OF ACTIN AND MYOSIN HEAVY CHAIN GENES IN SKELETAL, CARDIAC AND UTERINE MUSCLES OF YOUNG AND OLD RATS

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SUMMARY - The steady-state levels of mRNA and transcription of α-skeletal actin (α-SKA) and adult myosin heavy chain (MHC) genes were measured in the skeletal, cardiac and uterine muscles of young (22-25 week) and old (123-135 week) female rats. The effects of 10^{-8} M 17 β-estradiol/dexamethasone/T_3 on their transcription were also studied. The data show that the α-SKA mRNA level is lower in the old skeletal muscle and uterus, but is higher in the old myocardium. The adult MHC mRNA level is not different in the three muscles of both the ages. The transcription of α-SKA gene is lower in the skeletal muscle and higher in the uterus of old rats. It is unaltered in the myocardium of old rats. The transcription of adult MHC gene is lower in the old uterus. The effects of hormones on transcription of both the genes are different in the three muscles. We show that the expression of α-SKA gene is tissue-specific and age-related. The over-expression of α-SKA gene in the old myocardium is possibly due to derepression of the gene caused by hypertrophy of cardiac myocytes, and continuous hemodynamic pressure overload on the old heart.

INTRODUCTION - The terminal myogenic differentiation in mononucleated myoblasts is characterised by cessation of DNA synthesis, irreversible withdrawal from the cell cycle followed by cell fusion to form multinucleated non-proliferating myotubes. Concomitantly, there appear a number of new muscle specific mRNAs (1) and contractile proteins (2), actins and myosins (3), which are structurally and functionally related. Switching of the expression of different actin and myosin genes occurs during differentiation (4), development (5) and hypertrophy (6) of muscle cells. It has been postulated that actins are coexpressed in pairs in all mammalian cells (7). This results in

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alterations in the levels of corresponding mRNAs and protein isoforms in the cytoplasm leading to alterations in the phenotypes of cells.

We report here that alterations occurring in (i) the steady-state mRNA levels of α-SKA and adult MHC, (ii) the rates of their transcription and (iii) their *in vitro* modulation by steroid and thyroid hormones are tissue-specific and age-related in the skeletal-, cardiac- and uterine muscles of the rat.

MATERIALS AND METHODS - Female albino rats of Wistar strain were used. Skeletal muscle from the fore and hind limbs, cardiac muscle from the entire myocardium and uterine muscle from both the uterine horns from 22-25 week (young) and 123-135 week (old) old rats were used. Tissues from five to seven rats of each age were pooled for each set of experiments, and each set was run in duplicate. All experiments were repeated twice. Precautions were taken against RNase activity.

The following pBR 322 plasmids containing the cDNA of the genes were used: (i) α-SKA (clone-91; pAM 91-1360) - the 1.36 kb cDNA fragment contained the coding sequence, and the 3' untranslated sequence of mouse α-SKA gene cloned into Pst I site of pBR 322 (8); (ii) adult MHC (clone-32) - the 1.25 kb cDNA fragment of mouse cloned into Pst I site of pBR 322 (9). Plasmids containing these genes were nick-translated (11) using 5' (^{32}P) dCTP (3000 Ci/mmole) and *E. coli* DNA polymerase I, and used for hybridization.

Cytoplasmic high m.w. RNA was purified from skeletal, cardiac and uterine muscles by guanidinium isothiocyanate (5.0 M) extraction followed by LiCl (3.5 M) precipitation (11).

RNA samples were denatured by heating at 65°C for 10 min. with 50% formamide, 2.2 M formaldehyde in 10 mM Na_2HPO_4 buffer, pH 7.4, containing 0.5 mM EDTA and applied to nytran membrane (NY 13) filters by a Schleicher and Schuell Minifold II apparatus. Cytoplasmic RNA (1,2 and 4 μg) was slot-blotted, baked at 80°C for 2 hr. in vacuum and prehybridized in 50% formamide, 5X Denhardt's (BSA, Ficoll, PVP, 0.1% each) reagent, 100 μg/ml denatured salmon sperm DNA, 0.1% SDS 5X SSC (20X SSC = 3.0 M NaCl, 0.3 M Na citrate, pH 7.0) with nick-translated ($3-7 \times 10^7$ CPM/μg DNA) α-SKA and MHC 32 probes (2.5×10^6 CPM/ml) (12). The blots were washed under stringent conditions and exposed to X-ray films with intensifying screens for autoradiogrphy at -70°C for 3-7 days.

Nuclei were purified (13-16) for run-on transcription (17). ^{32}P-labelled run-on transcripts were hybridized with α-SKA/adult MHC gene by DNA filter hybridization (17). 1.0 ug of heat-denatured pBR 322 plasmids containing cDNA inserts of the genes were spotted on mini-discs of nitro-cellulose membrane filters (18). pBR 322 plasmids (without any insert), tRNA and the medium alone were used as control. The filter discs were dried, baked, hybridized with ^{32}P-labelled run-on nuclear transcripts (2.5×10^6 CPM) and washed under stringent conditions. The radioactivity retained on the discs were counted and the rate of transcription was expressed as CPM RNA hybridized ug DNA^{-1} $hour^{-1}$. Signals above control values were considered as positive.

RESULTS AND DISCUSSION - Slot-blot hybridization shows that the level of α-skeletal actin mRNA is lower in the skeletal and uterine muscles of old rats, but it is significantly higher in the old myocardium (Fig. 1a). Its rate of run-on transcription is lower in the skeletal muscle and higher in the uterus of old rats. The myocardium does not show any age-related difference (Table I). The 1.36 kb pAM-91 clone, used in this study, is specific for α-SKA (8). It detects homologous α-SKA mRNA in both fetal and adult skeletal muscles (4). Hence, the decrease both in its mRNA level and its transcription in the old skeletal muscle indicates repression of this gene in this tissue in old age. Both the isoforms of sarcomeric actins, α-skeletal and α-cardiac, are extremely homologous in amino acid sequence (19). Hence, it is difficult to distinguish them at the protein level. However, the nucleotide sequences of the two mRNAs at the 3' untranslated regions are divergent (7). Therefore, they can be distinguished by using probes for this region.

The major isoform in the fetal cardiac ventricle is α-SKA, whereas the cardiac isoform is predominant in the adult heart (6,20). Both the isogenes are co-expressed in the adult skeletal and cardiac muscles (7). Under experimental aortic coarctation in the adult, there is a rapid re-expression of the α-SKA mRNA in the adult heart (6). Hypertrophy due to increase in cell size without cell division is a funda-

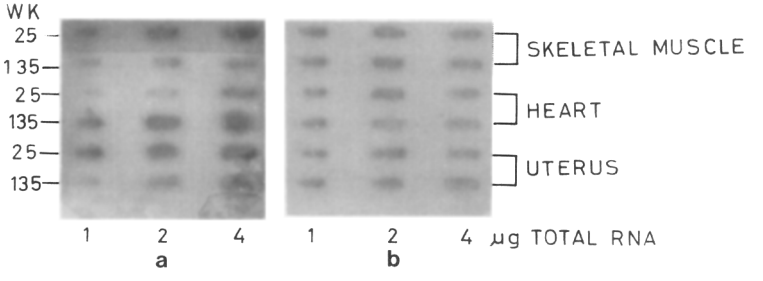

Fig. 1. The levels of (a) α-skeletal actin (α-SKA) and (b) adult myosin heavy chain (MHC) mRNAs in the skeletal muscle, heart and uterus of young (25 week) and old (135 week) female rats by slot-blot hybridization.

TABLE I - Run-on nuclear transcription (CPM RNA hybridized μg DNA^{-1} hr^{-1}) of actin gene in the skeletal muscle, heart and uterus of 22- and 123 week old female rats

	SKELETAL MUSCLE				HEART				UTERUS			
	22 week		123 week		22 week		123 week		22 week		123 week	
	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean
Control	41	42	30	33	51	49	48	49	49	47	58	54
	43		36		47		50		45		50	
Dexamethasone (10^{-8}M)	48	46	51	48	31	31	45	43	60	59	42	41
	44		45		31		41		62		40	
Triiodothyronine (10^{-8}M)	32	33	26	27	32	33	30	31	52	53	51	52
	34		28		34		32		54		53	
17 β-Estradiol (10^{-8}M)	-	-	-	-	-	-	-	-	33	33	38	41
									33		44	

mental adaptive process employed by post-mitotic myocardial cells against the increased hemodynamic pressure overload. The higher level of its mRNA in the old heart without any change in its transcription may be due to its increasing stability. This may increase the synthesis of α-SKA needed for hypertrophy and lead to alterations in the contractile property of cardiac myocytes and affect heart function in old age. This is consistent with the finding that c-*myc*, c-*fos* and *hsp* 70 genes are activated first as an early response to pressure overload, and this is followed by re-expression of α-SKA gene (6). Their protein products may induce other cellular genes. Such changes may be correlated with the age-related myocardial hypertrophy. The re-expression of the fetal α-SKA isogene in the old myocardium may be an adaptation to overcome the effect of continuous hemodynamic load.

The derepression of α-SKA gene in the old myocardium may either be due to prolonged environmental influence or age-related loss of negative regulator(s). The lower levels of α-SKA mRNA in the old skeletal and uterine muscles indicate that the age-related over-

expression of this gene in the old myocardium is not a general feature, but is a special feature of myocardial hypertrophy.

No significant age-related differences in the expression of MHC gene were detected in the three muscles (Fig. 1b). The switching on of normal adult MHC gene to the fetal MHC isoform has been reported during cardiac (21) and skeletal muscle (22) hypertrophy. This has been correlated with the changes in the contractile properties of muscle fibres (23). The mRNA and protein levels of the actin gene seem to be closely related in the striated muscles (24). The decrease in the levels of α-SKA mRNA in the old skeletal muscle and uterus may lead to a lower level of the acto-myosin complexes which are essential for muscle contraction. The situation may become more adverse in the old heart.

The rates of transcription of α-SKA (Table I) and adult MHC (Table II) genes change in the three muscles as a function of age. The effects of hormones on the rate of transcription are tissue-specific and age-related. Run-on transcription *in vitro* elongates the mRNA chains which are already initiated *in vivo* and reflects the levels of

TABLE II - Run-on nuclear transcription (CPM RNA hybridized μg DNA^{-1} hr^{-1}) of the adult myosin heavy chain gene in the skeletal muscle, heart and uterus of 22- and 123 week old female rats

	SKELETAL MUSCLE				HEART				UTERUS			
	22 week		123 week		22 week		123 week		22 week		123 week	
	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean	CPM	Mean
Control	39	38	37	36	50	46	36	41	89	83	37	38
	37		35		42		46		77		39	
Dexametha-sone (10^{-8}M)	43	46	41	44	35	36	40	41	43	41	32	32
	49		47		37		42		39		32	
Triiodothy-ronine (10^{-8}M)	39	37	42	41	31	31	20	26	56	60	40	42
	35		40		31		32		64		44	
17 β-Estra-diol (10^{-8}M)	-	-	-	-	-	-	-	-	52	58	42	40
									64		38	

RNA polymerases. The hormones may act by modulating the efficiency of the elongation reaction through receptors/trans-acting factors. Dexamethasone appears to act as a generalised inducer of α-SKA and adult MHC genes in the skeletal muscle of both the ages. Glucocorticoids are known to cause atrophy of skeletal muscle (25). However, the exact biochemical mechanism of glucocorticoid-mediated muscle atrophy is not known, nor is the molecular basis of atrophy understood. Receptors for estrogen (26) and T_3 (27) are located in the nuclei of target cells. The receptor for dexamethasone is in the cytoplasm, and it is translocated into the nucleus after interacting with the hormone (28). The differential effects of hormones on the transcription of α-SKA and adult MHC genes during aging of the rat may be due to changes in the levels of (i) hormone receptors (29), (ii) transcription and trans-acting factors (30) essential for expression of these genes, and (iii) changes in the accessibility and responsiveness of these genes because of age-related condensation of the chromatin (31,32). The qualitative and quantitative changes in the expression of α-SKA gene in the three muscles of old rats show how alterations in the functional capability of muscle cells may arise due to changes in gene expression. This may contribute to the aging of muscle cells.

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