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PHOSPHORYLASE KINASE ACTIVITY IN I/STRAIN NEONATAL SKELETAL MUSCLE WITH A DEFICIENCY IN α/α' SUBUNIT MRNAS

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In the adult I/LnJ mouse skeletal muscle, phosphorylase kinase activity is 0.2% of that in normal. This deficiency results from a paucity of mRNA's for the phosphorylase kinase regulatory subunit- α and its isoform α'. However, in the I/LnJ neonatal skeletal muscle phosphorylase kinase activity is 20-25% of that in normal. During the first two months of development this activity decreases while in normal tissue it increases. The developmental differences in the magnitude of the I/LnJ deficiency indicate the possibility of stage specific mechanisms regulating the accumulation of α/α' mRNAs. To investigate this possibility, the abundance of α/α' mRNAs and of the catalytic subunit, γ, mRNAs were compared by Northern Blot analysis. The results demonstrate that neonatal and adult I/LnJ skeletal muscle have a similar paucity of α/α' mRNAs whereas accumulation of γ mRNAs is not significantly different from normal.

The multi-subunit phosphorylase kinase enzyme (ATP: phosphorylase-b phosphotransferase, EC 2.7.1.38) activates glycogen phosphorylase in response to the second messengers Ca^{+2} and cAMP. The cAMP response is mediated by the cAMP-dependent protein kinase phosphorylation of the α and β regulatory subunits in phosphorylase kinase (1, 2, reviewed in 3). The calcium response is mediated by the integral calmodulin subunit in phosphorylase kinase (4). These subunits regulate the catalytic activity of the γ subunit (5-7). This regulation makes phosphorylase kinase focal in coupling the hormonal and calcium signals to the regulation of glycogen breakdown.

The multi-subunit composition of phosphorylase kinase complicates our understanding of tissue specific deficiencies in this enzyme. In humans, a skeletal muscle deficiency occurs in both infants and in adults; generally with an autosomal recessive pattern of inheritance (8-11). A liver specific deficiency occurs in infants and is inherited as an X-linked recessive trait (12-14). Recent cases of cardiac specific phosphorylase kinase

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deficiency are reported in infants with an undetermined pattern of inheritance (15,16). The molecular bases of these deficiencies are not known. To account for the tissue specific and stage specific phenotypes of these deficiencies there must be different mechanisms in the various tissues and throughout development that affect the expression of one or more of the subunits. However, not enough is known about the genetics of phosphorylase kinase to distinguish amongst the possible mechanisms. Complementary DNAs encoding the adult skeletal muscle subunits have been isolated, and the respective genes are autosomal for the γ and β subunits and X-linked for α (17-19). It is not known whether these same genes are expressed also in adult liver or in neonatal tissues. Only for the α subunit is there a known isoform designated α' which is expressed in oxidative muscle fibers and cardiac tissue (20).

An animal model for the investigation of phosphorylase kinase deficiencies is the I/LnJ mouse strain[1]. This strain carries an X-linked mutation that results in a marked reduction in the abundance of α and α' encoding mRNAs and less than 0.2 % of normal phosphorylase kinase activity in its adult skeletal muscle (18, 21, 22). The liver tissue exhibits normal phosphorylase kinase activity. Interestingly, the I/LnJ neonatal skeletal muscle does not exhibit so marked a deficiency as does the adult tissue. At birth the I/LnJ skeletal muscle contains 20-25% of the activity of normal tissue. During the first two months of development this activity decreases 10 fold while in normal tissue the activity increases approximately 15 fold (23, 24). This divergence leads to the difference in phosphorylase kinase activity observed between the adult I/LnJ and normal skeletal muscles.

The developmental progression of the I/Lyn deficiency indicates the possibility of stage specific mechanisms regulating α/α' mRNA expression in addition to the tissue specific mechanisms. To investigate this possibility, I determined the relative abundance of α and γ mRNAs in normal and I/LnJ neonatal and adult skeletal muscle by Northern Blot analysis. The results demonstrate that the I/LnJ neonatal skeletal muscle exhibits a paucity of α and α' mRNAs equal to or greater than that found in adult tissue. The abundance of γ mRNAs, however, does not differ significantly from that of normal tissue.

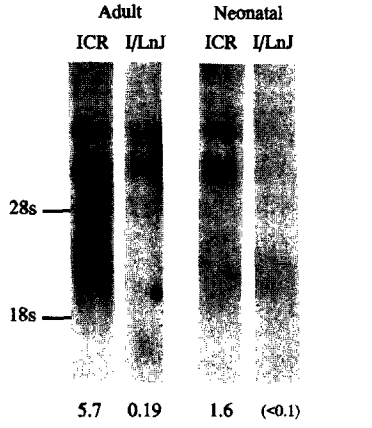
[1]The I/LnJ strain has also been referred to as the I/Lyn strain and is the same stock as the I/St and I/Fn strains. The mutation is thought to be the same as in the ICR/IAn strain. All of these mouse strains are generically referred to as I/Strain.

MATERIALS AND METHODS

RNA Preparation and Northern Blot Analysis. The hind limb skeletal muscle was excised from I/LnJ and ICR (normal) adult and 6-7 day old mice from Jackson Laboratories. Total RNA was isolated from these tissues by extraction in guanidine hydrochloride solution and sedimentation through a CsCl cushion (25). The poly (A+) fraction of RNA was isolated from the total RNA by chromatography on oligo(dT)-cellulose (Pharmacia, type VII). For Northern Blot analysis, poly(A+) RNA was electrophoresed in formaldehyde/agarose gels (26) and electroblotted onto modified nylon membranes (Nytran; Schleicher & Schuell). These membranes were then baked and prehybridized at 42°C in 50% formamide, 6 X SSC (1 X SSC is 150 Mm NaCl/15 mM sodium citrate, pH 7.0), 20 mM NaH_2PO_4, 1 mM EDTA, 0.1% $NaDodSO_4$, 5 X Denhardt's (1 X is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), and 100 ug per ml heat denatured salmon sperm DNA. Hybridization was at 42°C in the same buffer as prehybridization except that 2.5 X Denhardt's was used. Probes for hybridization were an α encoding cDNA containing 1.0 Kb of coding sequence isolated from an adult mouse (ICR) skeletal muscle cDNA library (18) and a γ encoding cDNA isolated from the same library (27). Both probes were labelled with ^{32}P by using [$\alpha^{32}P$]dCTP in a random primed DNA labelling kit (Boehringer Mannheim). Between hybridizations the Northern Blots were incubated twice for 5 minutes at 95°C in fresh 10.0 mM Tris, 0.1% $NaDodSO_4$, 1.0 mM EDTA, to remove the previous probe. The density of the bands on the autoradiographs was determined on a Schimadzu CS9000U scanner and the results normalized to the amount of RNA electrophoresed on the gel.

RESULTS AND DISCUSSION

The I/LnJ adult skeletal muscle has less than 0.2% of normal phosphorylase kinase activity and a near absence of α and α' subunit mRNAs. Yet, in the 5-7 day old mouse, the activity is 10-15% of that in normal (24). To determine whether the adult α/α' and γ mRNAs are expressed in neonatal tissue and whether the abundance of α/α' mRNAs is indicative of the relative phosphorylase kinase activity, abundance of these mRNAs was compared by Northern Blot analysis. The results of this analysis are shown in Figures 1 and 2. Evident in Figure 1 is a near absence in the I/LnJ tissues of the two mRNA species which hybridize to the α cDNA in normal skeletal muscle. These mRNA species have been shown previously to encode the α and α' isoforms (18). The results demonstrate that the adult α/α' mRNAs are expressed in normal 5-7 day old neonates, but they indicate a



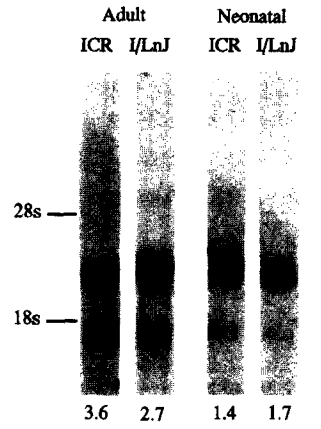


Figure 1. An autoradiograph of a Northern Blot containing poly (A+) RNA from normal (ICR) and phosphorylase kinase deficient (I/LnJ) adult and neonatal skeletal muscle. The approximate amount of RNA loaded for each tissue sample is 6 μg for the ICR adult and 8 μg for the other samples. The I/LnJ adult sample was electrophoresed and processed on a separate membrane. All membranes were hybridized with an α encoding cDNA. The results of the densitometry scans are below each lane and are the sum of both bands which hybridize to this cDNA and then normalized to the total poly (A+) RNA electrophoresed in each lane.

Figure 2. An autoradiograph of the same membranes as shown in Figure 1, but hybridization was with a γ encoding cDNA.

greater paucity of α/α′ mRNAs in the I/LnJ neonatal skeletal muscle than in the adult. Thus, abundance of the α/α′ mRNAs does not correlate with the greater phosphorylase kinase activity in the I/LnJ neonatal tissue. Abundance of the γ mRNAs possibly could account for the activity. However, it is evident in Figure 2 that I/LnJ adult skeletal muscle has more γ mRNA than the neonate and that its abundance in both tissues is not significantly different from that in the corresponding normal tissues. Thus, abundance of the γ mRNAs is not significantly affected by the I/LnJ mutation nor does its abundance correlate with residual phosphorylase kinase activity.

The paucity of α and α′ mRNAs in both the I/LnJ neonatal and adult skeletal muscle raises the question of why I/LnJ neonates exhibit more phosphorylase kinase activity than do adults? One hypothesis is that more γ subunit is present in the neonatal tissue. Early attempts at purifying I/strain neonatal phosphorylase kinase with antibodies have had mixed results. Several investigators (24, 28-30) were able to detect immunological cross-reacting material in I/Strain neonatal skeletal muscle, but the material was not of the same

avidity as that in normal neonatal muscle. At the time these experiments were done immunoblotting was not developed for identifying immunoreactive proteins. Thus, identifying the cross-reactive protein(s) in the I/Strain neonatal muscle was hindered by its low abundance and by the limited sensitivity of the contemporary techniques. As a result, the cross-reactive proteins were not identified. The conclusion from these studies was that either an aberrant phosphorylase kinase or an unknown kinase of limited similarity to phosphorylase kinase is responsible for the activity.

The expression of the γ subunit without the α subunit could give an incomplete phosphorylase kinase enzyme which would likely be recognized by the antibodies with different avidity than would the holoenzyme. Such an enzyme also would exhibit aberrant regulatory properties which are consistent with the biochemical properties of the I/LnJ neonatal phosphorylase kinase. Most notably, this enzyme is not activated by protein kinase A, is not activated by limited trypsin digestion, and has a lower calcium requirement than does the neonatal enzyme from normal skeletal muscle (31). These differences indicate an enzyme with deficient or aberrant α and β regulation. However, because the adult I/LnJ tissue also has γ mRNAs but has a lower phosphorylase kinase activity than the neonatal tissue, one must invoke developmental differences in the post-transcriptional regulation of γ expression. This could involve differences in translational activity of the γ mRNAs, differences in the stability of the γ protein, or differences in post-translational modifications affecting γ activity.

An alternative explanation is that the I/LnJ neonatal activity results from a residual amount of a fetal or a neonatal specific phosphorylase kinase isozyme. If this is so, then this isozyme must not be related closely to the adult form because it cross-reacts poorly with the antibodies to the adult form and none of its subunit mRNAs cross-hybridize with the adult α cDNA used in the Northern Blot of Figure 1.

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