CREATINE TRANSPORTER PROTEIN CONTENT, LOCALIZATION AND GENE EXPRESSION IN RAT SKELETAL MUSCLE

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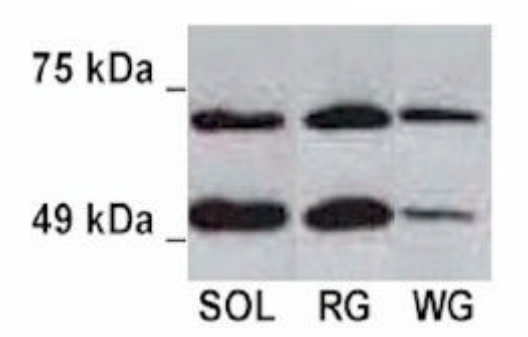
The present study examined the gene expression and cellular localization of the creatine transporter (CreaT) protein in rat skeletal muscle. Tissue was collected from previously killed male Wistar rats (n=6). Soleus (SOL), red (RG) and white gastrocnemius (WG) were analyzed for CreaT mRNA (Real Time RT-PCR), CreaT protein (Western blot) and total creatine (TCr) content (enzymatically). Cellular location of the CreaT protein was visualized using immunohistochemical analysis of muscle cross-sections. TCr was higher (P≤0.05) in WG than both RG and SOL, and RG was higher than SOL. Total CreaT protein content was greater (P≤0.05) in SOL and RG compared with WG. Two bands (55 and 70 kDa) of the CreaT protein were found in all muscle types as seen in the Figure.



Both the 55 kDa (CreaT-55) and the 70 kDa (CreaT-70) bands were present in greater (P≤0.05) amounts in SOL and RG compared with WG. SOL and RG had a greater amount (P≤0.05) of CreaT-55 than CreaT-70. Immunohistochemistry revealed that the CreaT was mainly associated with the plasma membrane in muscle types investigated, although some internal fluorescence was evident. CreaT mRNA expression per μg of total RNA was similar across the three muscle types. As in previous studies, we have identified a difference in the TCr content in different fiber types in rat skeletal muscle. In the present study we have shown that there may be an enhanced potential to transport Cr across the sarcolemma in the predominantly slow twitch (SOL) compared with fast twitch (WG) fibers. A recent study (Opt'Eindje *et al.* 1999) reported that, following Cr loading in rats, TCr content was increased in SOL but not altered in WG muscle. Furthermore, using incubated rat muscle strips, Willott *et al.* (1999) demonstrated that, at normal extracellular Cr concentrations, (e.g. 100 μM) SOL displayed a greater rate of Cr uptake than the extensor digitorum longus muscle. Interestingly, at high extracellular Cr levels (1 mM) the rate of Cr uptake was similar between both muscles. These data support the concept that an elevation in intracellular Cr results in a decreased expression of the CreaT protein. These data indicate that rat SOL and RG have an enhanced potential to transport Cr compared with WG despite a higher TCr in the latter.

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