Staurosporine causes a muscle phenotype dependent atrophy in skeletal muscle of mice via caspase-9 gene induction and downregulation of SUR2A gene

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The ATP-sensitive K channels (KATP) has been proposed as a molecular sensor of atrophy in skeletal muscle. In the present work we evaluated the *"in vitro"* effects of staurosporine (STAU), a well known apoptotic agent, on muscle protein and gene expression of KATP channel subunits (Kir6.2, SUR2A, SUR2B and SUR1) and atrophy genes (caspase-3/9, atrogin-1, MURF1 and BINP3) in slow-twitch Soleus (SOL) and fast-twitch Extensor Digitorum Longus (EDL) and Flexor Digitorum Brevis (FDB) muscles of mice.

The time needed to observe a significant reduction of the muscle proteins in Sol, EDL and FDB muscles was 6 hrs following STAU treatment. The percent reduction of the proteins content/muscle weight observed after 24 hrs of incubation time with STAU was -45%, -25% and -21% for Sol, EDL and FDB, respectively, indicating that STAU induced a phenotype dependent atrophy.

We found that after 3 hrs of incubation time, the treatment with STAU (2 microM) significantly enhanced the caspase-9 expression evaluated by qRT-PCR in both Sol and EDL muscles by 120% and 49% respectively, and this effect was related with the time of incubation time of the muscle with STAU particularly in Sol. The gene expression of caspase-3 increased by 28% in Sol but not in EDL muscles suggesting the activation of caspase 3/9 dependent proteolytic pathway particularly in the slow-twitch muscle. The STAU treatment increased the atrogin-1 expression only in Sol muscles after 3 hrs of incubation time and decreased after 6 hrs of incubation time in the controls and STAU treated muscles suggesting proteolytic effects mediated by ubiquitiun-ligases. In contrast, MURF1 and BINP3 were down regulated or not affected in all muscle phenotypes suggesting a not direct involvement of these factors in the observed atrophy induced by STAU or the activation of a cyto-protective pathway down-regulating these atrophy genes.

The mRNA of channel subunits Kir6.2 increased by 59% in Sol at 3 hrs after treatment, and not changed after 6 and 24 hrs. In EDL and FDB muscles the Kir6.2 gene expression was progressively reduced after 6 hrs in control and treated muscles. A time and muscle-phenotype dependent reduction of the gene expression of the SUR2A subunit was observed in Sol, EDL and FDB muscles in either controls and treated muscles, and it was more pronounced after STAU treatment. The SUR2B expression is upregulated after treatment with STAU in all analyzed muscles and increased of 150%, 20% and 50% at 3 hrs and 200%, 74% and 80% at 6 hrs in Sol, EDL and FDB muscles respectively. The increased of SUR2B was inversely related to the atrophy observed in all muscles. The SUR1 gene is upregulated of 1,5 fold in Sol muscle but not affected in EDL and FDB muscles. The mRNA of SUR1 decreased after 6 hrs of STAU treatment in both control and treated muscles.

Our data indicate that STAU activates atrophic and proteolytic pathways, respectively, via atrogin-1 and caspase-9 particularly in Sol with caspase-9 playing a mayor role. The rate of reduction induced by STAU of SUR2A gene expression in different muscles is correlated with atrophy (velocity of reduction mRNA of SUR2A Sol > > EDL > FDB). The SUR2B subunit of KATP channels may have a protective role in fast-twitch muscles.