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Dual response of the KATP channels to staurosporine: A novel role of SUR2B, SUR1 and Kir6.2 subunits in the regulation of the atrophy in different skeletal muscle phenotypes

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ABSTRACT

We investigated on the role of the genes encoding for the ATP-sensitive K^+-channel (KATP) subunits (SUR1-2A/B, Kir6.2) in the atrophy induced "in vitro" by staurosporine (STS) in different skeletal muscle phenotypes of mouse. Patch-clamp and gene expression experiments showed that the expression/activity of the sarcolemma KATP channel subunits was higher in the fast-twitch than in the slow-twitch fibers. After 1 to 3 h of incubation time, the STS (2.14×10^{-6} M) treatment enhanced the expression/activity of the SUR2B, SUR1 and Kir6.2 subunit genes, but not SUR2A, in the slow-twitch muscle fibers, induced the caspase-3-9, Atrogin-1 and Murf-1 gene expression without affecting protein content. After 3 to 6 h, the STS-related atrophy markedly down-regulated the SUR2B, SUR1 and Kir6.2 genes reducing the KATP currents and reduced the protein content/muscle weight ratio of the slow-twitch muscle by $-36.4 \pm 6\%$ ($p < 0.05$). After 6 to 24 h, no additional changes of the SUR1-2B and Kir6.2 gene expression and muscle protein were observed. In the fast-twitch muscles, STS mildly affected the atrophic genes and protein content, but potentiated the KATP currents down-regulating the Bnip-3 gene. Diazoxide ($250–500 \times 10^{-6}$ M), a SUR1-2B/Kir6.2 channel opener, prevented the protein loss induced by STS in the slow-twitch muscle after 6 h showing an EC50 of 1.35×10^{-7} M and E_{max} of 75%, down-regulated the caspase-9 gene and enhanced the KATP currents. The enhanced expression/activity of the SUR2B, SUR1 and Kir6.2 genes are cytoprotective against STS-induced atrophy in the slow-twitch muscle; their reduced expression/activity is associated with proteolysis and atrophy in skeletal muscle.

1. Introduction

The ATP-sensitive metabolically regulate K^+ channels (KATP) are widely distributed in the tissues [1]. The K_{ATP} channels are hetero-octameric complexes of pore-forming inwardly rectifier K^+ (Kir6) channel-forming subunits associated with regulatory sulphonylureas receptor (SUR) subunits, members of the ATP binding cassette (ABC) family of membrane proteins. Two Kir6-encoding genes, *KCNJ8* (Kir6.1) and *KCNJ11*(Kir6.2), and 2 SUR genes, ABCC8 (SUR1) and ABCC9 (SUR2), encode mammalian KATP subunits, but alternative RNA splicing can give rise to multiple SUR protein variants (*e.g.*, SUR2A and SUR2B) that confer distinct physiological and pharmacological properties on the channel complex [2–5].

In pancreatic beta cells, the main KATP channel complex is formed by the assembly of the Kir6.2 and SUR1 subunits while the Kir6.1/SUR2B channel may represent the predominant vascular K_{ATP}, but other subtypes are also likely to be expressed in specific vascular beds, separately or in combination with Kir6.1/SUR2B subunits [6–8].

The mito-KATP was initially identified into the SUR1/Kir6.1 complex that recapitulated mitoK_{ATP} activity, including diazoxide activation and 5-hydroxydecanoate inhibition [9]. Recently, a role for ROMK2 (Kir1) subunit in generating the mito-KATP channel has been proposed [10].

In striated muscle cells the co-assembly of different sulphonylurea receptor subtypes extends the phenotypic diversity of the sarcolemmal KATP channels (sarco-KATP) [11]. In mouse hearts SUR1 and Kir6.2 are major constituents of the atrial myocyte sarcolemmal K_{ATP}, whereas SUR2A and Kir6.2 generate ventricular K_{ATP} and additional splice variants of SUR1 and SUR2 are expressed

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in the heart [12,13]. In skeletal muscle, hybrid KATP channel complexes composed of SUR1 and SUR2B subunits contribute to functional channels in different muscle phenotypes. A high expression/activity of the Kir6.2-SUR2A and Kir6.2-SUR1 channel subunits is observed in various fast-twitch muscle types, while a low sarco-KATP channel current is observed in the slow-twitch soleus muscle of the rat and the Kir6.2-SUR2B subunits contribute to the functional channel in this muscle phenotype [14].

The KATP channels are involved in genetic diseases associated with insulin/glucose dis-metabolism and dis-function of the cardiovascular apparatus. Gain-of-function and loss-of function mutations in the *KCNJ11* and *ABCC8* genes are now well-understood to underlie neonatal diabetes mellitus and congenital hyperinsulinism, respectively [1,8,13,15–18]. Gain of function and loss of function mutations in the *KCNJ8* gene is associated with the J-wave phenomenon and early repolarization syndrome, and with the sudden infant death syndrome, respectively [19–21]. Loss-of-function mutations of the *ABCC9* gene were found in patients affected by long-standing atrial fibrillation originating in the vein of Marshall and in patients with dilated cardiomyopathy [22,23]. The Cantu' syndrome, a distinctive multi-organ disease characterized by hypertrichosis, osteochondrodysplasia and cardiomegaly has been recently associated with different gain of function mutations in the *ABCC9* gene [24–26].

KATP channel subunits are associated with apoptosis, atrophy and regulation of cell viability. Several reports demonstrated that SUR1- but not SUR2-expressing cells are more susceptible to apoptosis induced by SUR1 inhibitors such as glibenclamide and observed in the SUR1 knock-out mice as determined by monitoring cell detachment, nuclear condensation, DNA fragmentation, and caspase-3-like activity [27–29]. SUR1-induced apoptosis are also associated with activation of extracellular signal-regulated kinase (ERK) [30]. In pancreatic β cells, SUR1-selective blockers of KATP channels leads to apoptotic cell death, while SUR1 openers preserve cell integrity, leading to the idea that down-regulation of the subunits or pharmacological blockade of this channel type is involved in the atrophy of pancreatic tissue [27,30–32].

In skeletal muscle, a down-regulation of the genes encoding for the Kir6.2, SUR1 and SUR2B gene subunits composing the KATP channels in the slow-twitch soleus muscle has been observed in the 14-day-hindlimb-unloaded rat, an animal model of muscle disuse and atrophy. The observed changes in the fiber diameters and KATP channel currents correlate well with the relative expression levels of SUR1 of slow-twitch muscle, suggesting a main role of this subunit in the atrophic process in this *in vivo* model of atrophy and disuse [33,34]. The role of the sarcolemma KATP channels in the muscle atrophy is corroborated by the finding that the incubation of the control muscles *in vitro* with the KATP channel blocker glibenclamide (10^{-6} M) reduced the KATP currents with atrophy and these effects were prevented by the KATP channel opener diazoxide (10^{-4} M) [34]. Furthermore, the incubation of the fibers with an antibody targeting the pyruvate kinase enzyme, which is functionally coupled to the Kir6.2 subunit, reduced the KATP current reducing the diameter with atrophy. Therefore, it seems that the *in vivo* down-regulation of KATP channels or their *in vitro* pharmacological blockade are coupled to atrophic signaling in skeletal muscle [35]. Skeletal muscle atrophy is a debilitating consequence of starvation and many other catabolic conditions, such as aging, immobilization, denervation and chronic disease states in which different molecular pathways are involved [36–38]. However, the role of the SUR2A, SUR1, SUR2B and Kir6.2 gene subunits in the atrophy induced by apoptotic agents in different skeletal muscle phenotypes has never been investigated. Furthermore, the capability of diazoxide to prevent the atrophy induced by an apoptotic agent in skeletal muscle has never been investigated.

In the present work, the role of the genes encoding for the KATP channel subunits and effectors that are important for the development of the muscle protein loss were investigated *in vitro* on cultured slow-twitch *Soleus* (SOL), fast-twitch *Extensor Digitorum Longus* (EDL) and *Flexor Digitorum Brevis* (FDB) muscles of mice in the absence and in the presence of staurosporine (STS), a well known protein kinase C (PKC) inhibitor and ion channel modulator [39–43]. This compound is a potent cytotoxic agent in a variety of tumor cell lines with a fast kinetics of apoptosis inducing caspase activation within 1–2 h [44]. Measurements of muscle protein content used as an index of atrophy and real time RT-PCR experiments for the evaluation of the relative expression of the genes encoding for the effectors involved in the muscle atrophy (MuRF-1, Atrogin-1, Bnip-3, Caspase-3, Caspase-9) and for the channel subunits (SUR1, SUR2A, SURB, Kir6.2) were performed.

The capability of diazoxide to prevent the protein loss induced by the potent apoptotic agent staurosporine and to interfere with the atrophic pathways has been also investigated.

2. Materials and methods

2.1. Animal care

Adult male control mice (25–35 g) were used for the experiments. The animal care was performed in accordance with the *Guide for Care and Use of Laboratory Animals* prepared by the National Academy of Sciences. The approval of the experimental protocol was requested and obtained by the Ethics Committee of the Animal Facility of the University of Bari, Italy.

Soleus (SOL), Extensor Digitorum Longus (EDL) and Flexor Digitorum Brevis (FDB) muscles were removed from the control mice (n = 30 mice) under deep anesthesia induced by intraperitoneal injection of urethane 1.2 g (kg body weight)$^{-1}$. The skeletal muscles were isolated using a normal Ringer solution containing NaCl 145×10^{-3} M, KCl 5×10^{-3} M, $MgCl_2$ 10^{-3} M, $CaCl_2$ 0.5×10^{-3} M, glucose 5×10^{-3} M, and MOPS 10^{-2} M, pH 7.2. After dissection, the animals were rapidly killed with an overdose of urethane according to the EU Directive 2010/63/EU for animal experiments.

Whole muscles were frozen in liquid nitrogen promptly after surgical removal and used for total protein content determination and real time RT-PCR experiments.

Single fibers were prepared from SOL, EDL and FDB muscles by enzymatic dissociation for patch-clamp experiments as previously described [34].

The phenotype distribution of the muscle fibers in this animal specie used in the present work were: FDB muscle is composed of type I, IIA and IIX, the latter two being dominant, while EDL is primarily composed of fibers expressing IIB (60%) and IIX (60%) myosin, total over 100% because of hybrid myosin expression, and SOL mostly type I oxidative fibers.

2.2. Muscle treatments

Intact muscles used for visual and stereo microscope inspection were carefully pinned on Petri disks. Muscles damaged during dissection or showing contraction during the incubation period were discarded. All experiments were performed under 5% CO_2–95% O_2 atmosphere for the maintenance of aerobic conditions, at 37 °C, and the muscles were incubated for 1, 6 and 24 h with the drug solutions under investigation. At the end of the incubation period, all muscle samples were blotted on absorbent paper, carefully weighed and rapidly frozen in liquid nitrogen. Muscles were incubated with Dulbecco's modified Eagle's medium (DMEM+) solution (EuroClone S.p.A., Milano, Italy) composed by 1X antibiotics (1%), L-glutamine (1%), FBS (10%) (EuroClone, S.p.A. Milano, Italy) and enriched with staurosporine at 2.14×10^{-6} M