S. Passamonti, S. Gustincich, T. Lah Turnšek, B. Peterlin, R. Pišot, P. Storici (Eds.)
CONFERENCE PROCEEDINGS with an analysis of innovation management and knowledge transfer potential for a smart specialization strategy
ISBN 978-88-8303-572-2 / e-ISBN 978-88-8303-573-9. EUT, 2014.

IMPROVING THE REGENERATION OF SKELETAL MUSCLE

Marina Sciancalepore, Annalisa Bernareggi, Paola Lorenzon

University of Trieste, Department of Life Sciences, via Fleming, 22, 34127 Trieste

Abstract — Several pathological conditions of the skeletal muscle (myopathies, dystrophies and age-related atrophy) represent a burden for health care system. Satellite cells are postnatal resident myogenic precursors present in the skeletal muscles throughout the entire lifespan. The identification of novel therapeutic strategies able to enhance their regenerative capacity is one of the most promising tools to compensate muscle degeneration and to restore functional muscle performance in patients and elderly. Our research activity is aiming to contribute to this crucial topic.

Index Terms — ATP, ROS, skeletal muscle, regeneration

1 BACKGROUND

ATP is released by skeletal muscle cells during contraction in an activity-dependent manner. Recent evidences reveal that low levels of extracellular ATP have a physiological effect on skeletal myoblasts. The relevance of extracellular ATP for the muscle physiology, is confirmed by the low extracellular ATP-hydrolyzing activity in the niche of proliferating myoblasts, allowing ATP to exert its effect in a peculiar persistent manner compared to other cell models. At the neuromuscular junction, ATP induces potentiation of the cholinergic transmission via the production of reactive oxygen species (ROS). However, the molecular mechanism of the ATP action on resident muscle precursors remains partially unknown.
We have recently demonstrated that the proliferation of satellite cell-derived myoblasts is controlled by extracellular adenosine 5'-triphosphate (ATP) and that low concentrations of ROS work as intermediate signaling molecules in such trophic effect [1].

2 OBJECTIVES

Generation of ROS represents one of the most prominent events during the muscular contractile activity. According to the concept of hormesis, myoblasts would benefit from low doses but would be damaged by high levels of ROS. Our goal is to identify the appropriate electrical stimulation patterns to induce permissive levels of ATP/ROS at the skeletal muscle level, for improving the regenerative potential of the resident muscle precursors.

3 APPROACH & METHODS

General approach

A bioreactor will be designed to stimulate single myoblasts using different electrical stimulation waveforms. The effects induced by electrical stimulation will be analysed in terms of proliferation, differentiation and functional activity of the muscle precursors.

Methods

Primary cultures of satellite cells are obtained from mouse and human biopsies.
Electrical stimulation (ES) will be delivered to mimic muscle activity in vivo and to induce controlled levels of ROS production. Platinum-iridium electrodes emitting bipolar pulses hanging in the bath solution will be programmed by computer-based control software and delivered by the stimulator. Contractile activity will be monitored by a CCD camera (SensiCam®; PCO Computer Optics, Kelheim, Germany), mounted on an inverted phase-contrast microscope (Zeiss Axiovert S100®), and assembled off-line into a movie by using Videomach® software.
Immunoistochemistry will be performed to evaluate proliferative potential and the profile of protein expression.
Patch-clamp technique and calcium imaging will be used to assess the electrical membrane properties and the intracellular calcium handling.

4 RESULTS

Myoblast proliferation is modulated by the extracellular ATP in a dose dependent-manner (Figure 1). The trophic effect is triggered by the activation of metabotropic purinergic receptor and is mediated by ROS acting in the low micromolar range (Figure 2).

Preliminary results were obtained electrically stimulating single multinucleated myotubes in culture. Regular tetanic stimulations at 45 Hz are able to elicit myotube firing and contractions. At high stimulation intensity, fatigue phenomena are often observed indicating that our prototype of bioreactor coupled with electrical field stimulation well mimics the skeletal muscle behavior observed in vivo [2] (Figure 3).

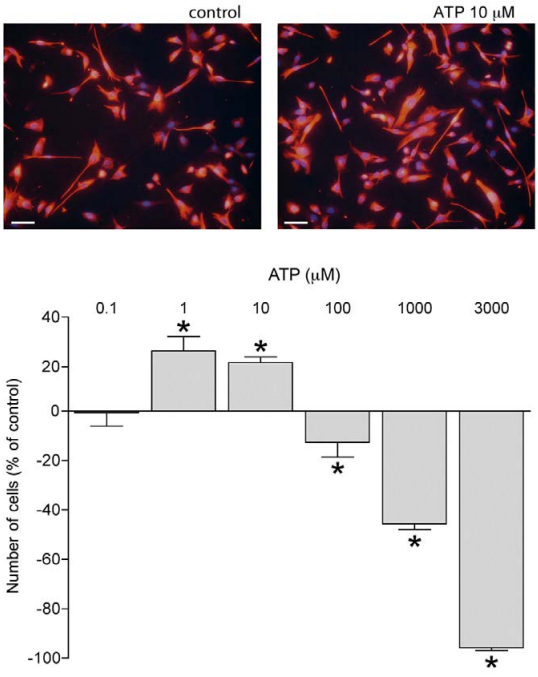

Figure 1: Promotion of myoblast proliferation by ATP. (A) Representative images of fluorescence micrographs of mouse myoblasts stained for desmin (red), grown under control condition and after treatment with 10 μM ATP (nuclei in blue with bis-benzimide). Scale bar 100 μm. (B) Dose-response relationship of the effect of ATP on myoblast proliferation.

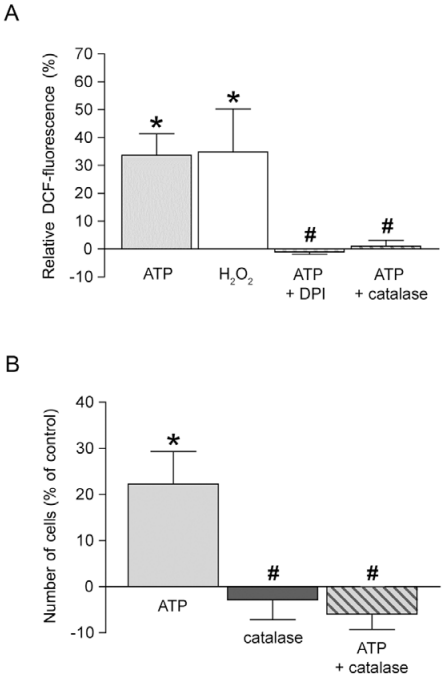

Figure 2: ROS-mediated extracellular effects of ATP. (A) Similar changes in DCF fluorescence in myoblasts over 15 min exposure to 10 μM ATP and to 3 μM H2O2 in cultured mouse myoblasts. Pre-incubation with DPI (25 μM, 60 min) or catalase (1200U/ml, 15 min) blocked the effect of ATP on ROS production. (B) Treatment of proliferating myoblasts with 10 μM ATP significantly increased the number of cells. The effect was prevented by the H2O2 scavenger catalase.

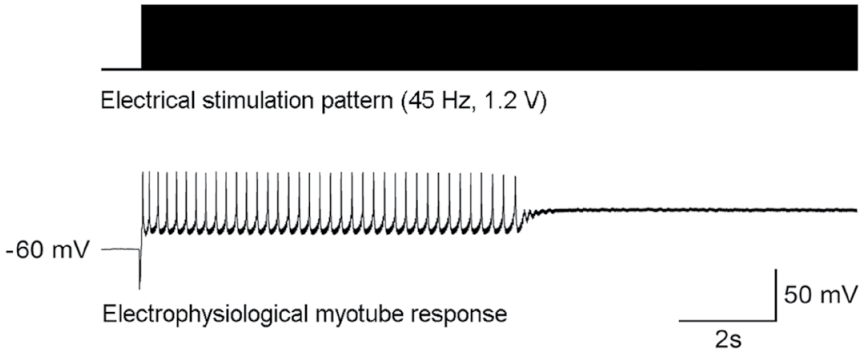

Figure 3: High intensity and frequency regular stimulations (1.2 V, 45 Hz) induce in a single myotube action potential firing detected by patch-clamp whole-cell recordings.

5 POTENTIAL NEW PRODUCTS & SERVICES

FOR SCIENTIFIC KNOWLEDGE
Identification of molecular mechanisms able to control and maintain ROS at beneficial "permissive" levels to foster the intrinsic regenerative capacity of skeletal muscle during aging and, more generally, after an acute tissue injury.

FOR RESEARCH
Bioreactors for electrical stimulation of cultured skeletal muscle cells.

FOR CLINICS
Contribution in planning more efficient stimulation protocols for innovative electrical devices for rehabilitation of weakened or injured muscles.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Institute of Pathophysiology, School of Medicine, University of Ljublana, Slovenia; Department of Neurobiology, University of Eastern Finland, Kuopio, Finland; Neuroscience Department, SISSA, Trieste, Italy.

7 CONTACT OR COLLABORATIONS NEEDED

Future collaboration with biochemical laboratories is needed for detection of ROS levels, oxidative stress and antioxidants' effects.

8 COMMUNICATION TOOLS

The high level of expertise in the field of electrophysiology and videoimaging as well as the high level of knowledge in the biology of muscle cells is disseminated through the high quality scientific publications.
Dissemination of scientific results using media for the general public (local newspaper and events).

9 FUNDS NEEDED

9.1 For basic research (investigation of biolological mechanisms): € 100.000,00

9.2 For pilot & demonstrator activities (to develop a prototype): € 6.000,00

10 CONCLUSION

In light of the dual role of ROS, the identification of molecular mechanisms able to control and maintain ROS at beneficial "permissive" levels could represent one of the novel promising strategies to foster the regenerative capacity of skeletal muscle during aging and, more generally, after an acute tissue injury. Bioreactors generating in situ electrical stimulation of cultured skeletal muscle cells potentially provide excellent new tools to study this aspect in vitro, under controlled conditions and at the single cell level.

ACKNOWLEDGEMENT

This work was supported by: Fondazione Kathleen Foreman Casali, Trieste, FRA2013-University of Trieste, Beneficentia Stiftung-Vaduz (Lichtenstein).

REFERENCES

[1] Marina Sciancalepore, Elisa Luin, Giulia Parato, Elisa Ren, Rashid Giniatullin, Elsa Fabretti, Paola Lorenzon (2012). Reactive oxygen species contribute to promote the ATP-mediated proliferation of mouse skeletal myoblasts. Free Radical Biology and Medicine 53: 1392-1398.
[2] Marina Sciancalepore, Tamara Coslovich, Paola Lorenzon, Giuliano Taccola. "Noisy"-patterned extracellular electrical stimuli facilitate muscle cell contractions in vitro. 64° Congresso della Società italiana di Fisiologia (SIF), Ancona Portonovo, 18-20 Settembre 2013.