estratta

Traccia 1

1) Quali precauzioni si devono adottare per prevenire la contaminazione di colture cellulari?
2) Descrivere il protocollo più adatto per monitorare la produzione di specie ossigeno reattive in una linea cellulare in sospensione
3) Quali componenti devono essere aggiunti ad un sistema di imaging per ottenere un sistema integrato di imaging e patch clamp?

INGLESE

Lettura e traduzione dell'abstract allegato

Absence of physiological Ca2+ transients is an initial trigger for mitochondrial dysfunction in skeletal muscle following denervation

Skeletal muscle 2017, 7 vol 1 pg6 doi 10.1186/s13395-017-0123-0

INFORMATICA

Eseguire il rapporto tra numeri presenti in due colonne contigue in Excel

Karam et al. Skeletal Muscle (2017) 7:6
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Skeletal Muscle

RESEARCH Open Access

Absence of physiological Ca^{2+} transients is an initial trigger for mitochondrial dysfunction in skeletal muscle following denervation

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Abstract

Background: Motor neurons control muscle contraction by initiating action potentials in muscle. Denervation of muscle from motor neurons leads to muscle atrophy, which is linked to mitochondrial dysfunction. It is known that denervation promotes mitochondrial reactive oxygen species (ROS) production in muscle, whereas the initial cause of mitochondrial ROS production in denervated muscle remains elusive. Since denervation isolates muscle from motor neurons and deprives it from any electric stimulation, no action potentials are initiated, and therefore, no physiological Ca^{2+} transients are generated inside denervated muscle fibers. We tested whether loss of physiological Ca^{2+} transients is an initial cause leading to mitochondrial dysfunction in denervated skeletal muscle.

Methods: A transgenic mouse model expressing a mitochondrial targeted biosensor (mt-cpYFP) allowed a real-time measurement of the ROS-related mitochondrial metabolic function following denervation, termed "mitoflash." Using live cell imaging, electrophysiological, pharmacological, and biochemical studies, we examined a potential molecular mechanism that initiates ROS-related mitochondrial dysfunction following denervation.

Results: We found that muscle fibers showed a fourfold increase in mitoflash activity 24 h after denervation. The denervation-induced mitoflash activity was likely associated with an increased activity of mitochondrial permeability transition pore (mPTP), as the mitoflash activity was attenuated by application of cyclosporine A. Electrical stimulation rapidly reduced mitoflash activity in both sham and denervated muscle fibers. We further demonstrated that the Ca^{2+} level inside mitochondria follows the time course of the cytosolic Ca^{2+} transient and that inhibition of mitochondrial Ca^{2+} uptake by Ru360 blocks the effect of electric stimulation on mitoflash activity.

Conclusions: The loss of cytosolic Ca^{2+} transients due to denervation results in the downstream absence of mitochondrial Ca^{2+} uptake. Our studies suggest that this could be an initial trigger for enhanced mPTP-related mitochondrial ROS generation in skeletal muscle.

Keywords: E-C coupling, Calcium imaging, Calcium signaling, Calcium intracellular release, Denervation, Mitochondria

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Background

Skeletal muscle is responsible for voluntary movements of the entire body. Because it comprises around 40% of whole-body lean mass of a human, skeletal muscle is also essential for maintaining the homeostasis of the whole-body metabolism [1]. Skeletal muscle contraction is under the control of motor neurons. In some disease states, the interaction between motor neuron and skeletal muscle is lost, leading to paralysis and muscle atrophy [2]. Muscle atrophy is defined as a decrease in the muscle cell size and the imbalance between protein synthesis and degradation. The molecular mechanisms underlying protein metabolism in muscle atrophy have been extensively evaluated [3–8]. It is believed that skeletal muscle atrophy is caused by the disturbance of signaling networks, in which mitochondria may play a major role. In fact, mitochondria occupy about 10–15% of the muscle fiber volume [9]. They are not only essential for energy supply but also determine the survival or death of muscle fibers. It has been shown that denervation of skeletal muscle induces a dramatic increase in mitochondrial ROS production [10]. However, the initial cause of the mitochondrial ROS production in denervated skeletal muscle remains elusive [11].

Muscle cells use Ca^{2+} as a messenger to control events ranging from activation of contraction to cell death. Defective intracellular Ca^{2+} signaling has been linked to skeletal muscle dysfunction during aging [12, 13] and in muscular dystrophy (mdx) [14–17]. In non-muscle cells, mitochondria dynamically transport Ca^{2+} and modify its flux into the endoplasmic reticulum, nucleus, and across the plasma membrane to such an extent that they have been named "the hub of cellular Ca^{2+} signaling" [18]. There is strong evidence that mitochondria may have a similar role in skeletal muscle. We previously demonstrated that mitochondria take up Ca^{2+} during excitation-contraction (E-C) coupling following rapid calcium transients in skeletal muscle [19]. We also established that malfunction of this mechanism contributes to neuromuscular degeneration in amyotrophic lateral sclerosis [20]. Mitochondrial Ca^{2+} uptake is believed to help regulate mitochondrial metabolism and ATP synthesis, so that the energy demands of muscle contraction are met [21]. However, Ca^{2+} overload in mitochondria is also a pathological stimulus of ROS generation [22]. It has been shown that prolonged muscle denervation leads to an increased resting cytosolic free Ca^{2+} level, that in turn overloads mitochondria, stimulating ROS production [23, 24]. Following denervation, no action potential can be initiated; therefore, physiological Ca^{2+} transient is lost in the denervated muscle fibers. One essential question to ask is how mitochondria respond to the cessation of physiological Ca^{2+} transients. While published studies mainly focused on the effect of a steady state level of intracellular Ca^{2+} on mitochondrial function, it is not known whether the dynamic change of Ca^{2+} level inside mitochondria in response to the intracellular Ca^{2+} transients is also a player in regulating mitochondrial function.

In this study, we used a transgenic mouse model carrying a mitochondrial biosensor mt-cpYFP, which produces mitoflash signal as a functional indication of ROS-related mitochondrial metabolic function [25, 26]. This model allows us to detect the early changes of ROS-related mitochondrial metabolic function in live skeletal muscle in response to the denervation process. Under voltage-clamp condition, we found that the loss of physiological Ca^{2+} transients or mitochondrial Ca^{2+} uptake could be an initial trigger for mitochondrial dysfunction with increased mitochondrial ROS production in skeletal muscle fibers following denervation.

Methods

Generation of transgenic mice

The plasmid (mt-cpYFP/pUCCAGGS) used to generate this transgenic line (mt-cpYFP) is the same as the one developed by Dr. Heping Cheng's Laboratory to generate the original mt-cpYFP transgenic mice [26, 27]. The genetic background for this transgenic mouse model is B6SJL from the Jackson Laboratory. The mt-cpYFP mice at the age of 2.5–3 months were used. All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Protocols on usage of mice were approved by the Institutional Animal Care and Use Committee of Rush University, University of Missouri at Kansas City and Kansas City University of Medicine and Bioscience.

Muscle denervation procedure

Muscle denervation was performed by transection of the sciatic nerve. During a denervation procedure, the mouse was anesthetized with constant-flow isoflurane inhalation and a small incision was made in the mid-posterolateral area of the thigh, and the sciatic nerve was isolated. In one hind limb, the sciatic nerve was severed and a ~5 mm section was removed. The ends of the nerve were sutured to prevent nerve regrowth. For control experiments (sham), the sciatic nerve was exposed in the contralateral hind limb without being severed. The incisions in both legs were closed again with silk sutures, and the animal was euthanized after 24 h for experiments.

Isolation of FDB fibers

The animals were euthanized by CO_2 inhalation followed by cervical dislocation, and the flexor digitorum brevis (FDB) muscles were removed for imaging studies.

NON SORTEGGIATA

Traccia 2

1) Quali condizioni chimico-fisiche si devono adottare per il mantenimento delle colture cellulari?
2) Descrivere il protocollo più idoneo per misurare le concentrazioni di Ca2+ in cellule di tipo nervoso
3) Quali componenti caratterizzano un sistema integrato di imaging e patch clamp?

INGLESE

Lettura e traduzione dell'abstract allegato

Implementing patch clamp and live florescence microscopy to monitor functional properties of freshly isolated PKD epithelium

Journal of Vis Exp 2015 (103) 53035

INFORMATICA

Eseguire la somma di numeri presenti in una Colonna con Excel

Video Article

Implementing Patch Clamp and Live Fluorescence Microscopy to Monitor Functional Properties of Freshly Isolated PKD Epithelium

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URL: http://www.jove.com/video/53035
DOI: doi:10.3791/53035

Keywords: Medicine, Issue 103, Patch-clamp, polycystic kidney disease, ARPKD, ADPKD, kidney, intracellular calcium, Fura-2 AM, nephron, cyst development, polycystin

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Abstract

Cyst initiation and expansion during polycystic kidney disease is a complex process characterized by abnormalities in tubular cell proliferation, luminal fluid accumulation and extracellular matrix formation. Activity of ion channels and intracellular calcium signaling are key physiologic parameters which determine functions of tubular epithelium. We developed a method suitable for real-time observation of ion channels activity with patch-clamp technique and registration of intracellular Ca^{2+} level in epithelial monolayers freshly isolated from renal cysts. PCK rats, a genetic model of autosomal recessive polycystic kidney disease (ARPKD), were used here for *ex vivo* analysis of ion channels and calcium flux. Described here is a detailed step-by-step procedure designed to isolate cystic monolayers and non-dilated tubules from PCK or normal Sprague Dawley (SD) rats, and monitor single channel activity and intracellular Ca^{2+} dynamics. This method does not require enzymatic processing and allows analysis in a native setting of freshly isolated epithelial monolayer. Moreover, this technique is very sensitive to intracellular calcium changes and generates high resolution images for precise measurements. Finally, isolated cystic epithelium can be further used for staining with antibodies or dyes, preparation of primary cultures and purification for various biochemical assays.

Video Link

The video component of this article can be found at http://www.jove.com/video/53035/

Introduction

Ion channels play a significant role in many physiological functions, including cell growth and differentiation. Autosomal dominant and recessive polycystic kidney diseases (ADPKD and ARPKD, respectively) are genetic disorders characterized by the development of renal fluid-filled cysts of the tubular epithelial cell origin. ADPKD is caused by mutations of PKD1 or PKD2 genes encoding polycystins 1 and 2, membrane proteins involved in the regulation of cell proliferation and differentiation. PKD2 by itself or as a complex with PKD1 also function as a Ca^{2+}-permeable cation channel[1]. Mutations of the PKHD1 gene encoding fibrocystin (a cilia-associated receptor-like protein involved in the tubulogenesis and/or maintenance of polarity of epithelium) are the genetic impetus of ARPKD[2]. Cyst growth is a complex phenomenon accompanied with disturbed proliferation[3,4], angiogenesis[5], dedifferentiation and loss of polarity of tubular cells[6-8].

Defective reabsorption and augmented secretion in cystic epithelium contribute to fluid accumulation in the lumen and cyst expansion[9,10]. Impaired flow-dependent $[Ca^{2+}]_i$ signaling has been also linked to cystogenesis during PKD[11-15].

Here, we describe a method suitable for patch-clamp measurements of single channel activity and intracellular Ca^{2+} levels in cystic epithelial monolayers isolated from PCK rats. This method was successfully applied by us to characterize of activity of the epithelial Na^+ channel (ENaC)[10] and $[Ca^{2+}]_i$-dependent processes induced by Ca^{2+}-permeable TRPV4 and purinergic signaling cascade[13].

In these studies we used PCK rats, a model of ARPKD caused by a spontaneous mutation in the PKHD1 gene. The PCK strain was originally derived from Sprague-Dawley (SD) rats[16] thereby SD rats are used as an appropriate control for comparison with the PCK strain. As a result, both SD rat nephron segments and non-dilated collecting ducts isolated from same PCK rats can serve as two different comparison groups for experiments on cystic epithelium.

Protocol

The experimental procedures described below were approved by the Institutional Animal Care and Use Committee at the Medical College of Wisconsin and University of Texas Health Science Center at Houston and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. **Figure 1** demonstrates main steps of the tissue isolation and processing procedure. Briefly, kidneys from

NON SORTEGGIATA

Traccia 3

1) Descrivere il protocollo più efficace per l'isolamento di cellule mononucleate da sangue periferico, e quali test vengono effettuati per verificarne la vitalità
2) Quali tipi di sonde fluorescenti sono più adatte per misurare le oscillazioni spontanee di Ca2+ in cellule di tipo muscolare
3) Quale è la configurazione di patch clamp più adatta per studiare la correlazione tra la variazione del potenziale di membrana e i movimenti di calcio intracellulare in un sistema integrato di imaging e patch clamp

INGLESE

Lettura e traduzione dell'abstract allegato

The effects of storage temperature on PBMC gene expression

BMC Immunol 2016 17: 6 doi: 10.1186/s12865-016-0144-1

INFORMATICA

Eseguire la media di numeri presenti in una Colonna con Excel

BMC Immunol. 2016; 17: 6.
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PMID: 26979060

The effects of storage temperature on PBMC gene expression

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Abstract

Background

Cryopreservation of peripheral blood mononuclear cells (PBMCs) is a common and essential practice in conducting research. There are different reports in the literature as to whether cryopreserved PBMCs need to only be stored ≤ −150 °C or can be stored for a specified time at −80 °C. Therefore, we performed gene expression analysis on cryopreserved PBMCs stored at both temperatures for 14 months and PBMCs that underwent temperature cycling 104 times between these 2 storage temperatures. Real-time RT-PCR was performed to confirm the involvement of specific genes associated with identified cellular pathways. All cryopreserved/stored samples were compared to freshly isolated PBMCs and between storage conditions.

Results

We identified a total of 1,367 genes whose expression after 14 months of storage was affected >3 fold in PBMCs following isolation, cryopreservation and thawing as compared to freshly isolated PBMC aliquots that did not undergo cryopreservation. Sixty-six of these genes were shared among two or more major stress-related cellular pathways (stress responses, immune activation and cell death). Thirteen genes involved in these pathways were tested by real-time RT-PCR and the results agreed with

the corresponding microarray data. There was no significant change on the gene expression if the PBMCs experienced brief but repetitive temperature cycling as compared to those that were constantly kept ≤ −150 °C. However, there were 18 genes identified to be different when PBMCs were stored at −80 °C but did not change when stored < −150 °C. A correlation was also found between the expressions of 2′–5′- oligoadenylate synthetase (OAS2), a known interferon stimulated gene (IFSG), and poor PBMC recovery post-thaw. PBMC recovery and viability were better when the cells were stored ≤ −150 °C as compared to −80 °C.

Conclusions

Not only is the viability and recovery of PBMCs affected during cryopreservation but also their gene expression pattern, as compared to freshly isolated PBMCs. Different storage temperature of PBMCs can activate or suppress different genes, but the cycling between −80 °C and −150 °C did not produce significant alterations in gene expression when compared to PBMCs stored ≤ −150 °C. Further analysis by gene expression of various PBMC processing and cryopreservation procedures is currently underway, as is identifying possible molecular mechanisms.

Electronic supplementary material

The online version of this article (doi:10.1186/s12865-016-0144-1) contains supplementary material, which is available to authorized users.

Keywords: Cryopreservation, Peripheral blood mononuclear cells, Gene expression

Background

Human peripheral blood mononuclear cells (PBMCs) are a critical biological specimen type collected in clinical trials and basic science research. PBMCs are used for the evaluation of various in vitro functional and phenotypic immunological assays, e.g., enzyme-linked immunosorbent spot (ELISPOT) assays [1, 2], proliferation assays [3], flow cytometry [4] and cytometry by time-of-flight (CyTOF) [5] determinations. They also serve as precursors for potential immunotherapy development [6] and are used for biomarker discovery [7, 8] in translational medicine. Many times, PBMC samples need to be collected, processed and cryopreserved at multiple clinical sites. As a result, considerable attention has been given to standardization and/or harmonization [9–11] of the needed assay reagents and assay procedures involved in the testing of these samples that measure the patient's immune response or status. Testing samples in batches is frequently an additional requirement in order to minimize assay variability; therefore, PBMC samples are often cryopreserved and stored at ultra-low temperatures until they are tested. However, the same level of standardization and consistency given to the pre-analytical factors involved in the storage, shipping and general sample handling of frozen cryopreserved PBMCs has not been applied.

According to best practice [12], in order to maintain viability of PBMCs, they need to be stored below −132 °C, the glass transition temperature of water (GTTW). This is the temperature, at or below, that all biological activity stops [13]. Often, biospecimen storage temperature is chosen based on what type of equipment is available at the location where the biospecimen processing and cryopreservation will be performed. Some facilities, both in the US and internationally, cannot easily get liquid nitrogen for specimen storage below −132 °C. It may also be difficult to provide liquid nitrogen to all facilities because of cost or design restrictions in their physical plant. However, samples from these sites may be deemed necessary for the project, e.g., achieving planned participant enrollment numbers or satisfying a demographic requirement. The use of mechanical −140 °C freezers can be an option, but operating procedures need to be in place to avoid repeatedly warming specimens above the GTTW, especially when accessing the freezer for sample retrieval.