Angiotensin II stimulates calcium influx in human muscle satellite cells via activation of AT1 and AT2 receptors and of Transient Receptor Potential Canonical channels

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Skeletal muscle satellite cells (SMC) play a fundamental role in muscle homeostasis during physiological growth, aging and regeneration after muscle injuries (1). In these processes the effects of Angiotensin II (AngII) and cognate receptors (AT1R and AT2R) are divergent since in models of skeletal muscle loss AT1R stimulation leads to inhibition of skeletal muscle regeneration (2), whereas AT2R stimulation promotes myoblast differentiation and potentiates regeneration (3).
Renin angiotensin are a class of voltage independent, nonselective cation channels that upon activation provide Ca2+ entry into the cells, a crucial signal to modulate plasma membrane bioelectricity and promote cell proliferation and differentiation (4). At present, no information is available on the functional expression of TRPC channels in human SMC (hSMC) and whether a functional relation exists between AngII and TRPC channels.
The aim of this study was to characterize the expression pattern and the functional properties of TRPC channels in hSMC and native human skeletal muscle and the modulatory properties of AngII via AT1R and AT2R stimulation.
hSMC were isolated from skeletal muscle specimens obtained from pediatric patients undergoing surgery for pectus excavatum. The investigation conforms with the approval by the local ethical committee. After digestion with 0.2% (wt/vol) collagenase type I/DMEM for 1-2 h at 37°C, hSMC were plated and grown in Promocell Skeletal Muscle Cell Growth Medium and splitted when sub-confluent. All the experiments were performed from passage 2 to 5. TRPC channel protein expression was assessed by western-blot analysis of hSMC and whole muscle extract and by immunofluorescence performed in hSMC. TRPC-mediated Ca2+ influx was measured by epifluorescence microscopy on hSMC loaded with the fluorescent Ca2+indicator FLUOFORTE. TRPC channels were activated as described elsewhere (5): briefly, after sarcoplasmic reticulum Ca2+ depletion with removal of external Ca2+ and exposure to sarcoplasmic reticulum Ca2+-ATPase inhibitor CPA (5μmol/L), external Ca2+ (1.8mmol/L) is reintroduced in the presence of CPA.
Protein expression analysis showed that different TRPC channel isoforms are expressed in hSMC, with a major quantitative predominance of TRPC3/6, and minor expression levels of TRPC4/7. A similar pattern of TRPC isoforms was detected in whole skeletal muscle tissue. Immunofluorescence staining confirmed the plasma membrane localization of TRPC3/4/6/7 proteins, whereas TRPC1 appeared substantially in the perinuclear region. Epifluorescence measurements in hSMC showed that after sarcoplasmic reticulum Ca2+ depletion in the presence of TRPC channel activator OAG, a stable cell-permeable analog to the known TRPC agonist diacylglycerol, reintroduction of Ca2+ elicited a substantial Ca2+ entry into the cells, which was blocked by the pan TRPC antagonist SKF96365 (5μmol/L). In similar experimental conditions, but without OAG, AngII (100nmol/L) stimulated Ca2+ influx into the cells, which was prevented by SKF96365. The effect of AngII is partially abolished by the AT1R or AT2R antagonists irbesartan (1μmol/L) or PD-123319 (1μmol/L), respectively.
In conclusion, in hSMC AngII promotes Ca2+ entrance into the cells via activation of AT1/AT2R and stimulation of TRPC channels. The functional relation between AT1/AT2R and TRPC channels may have important implications for the physiological properties of hSMC, such as self-renewal, proliferation, muscle homeostasis and regeneration and for the adverse modifications induced by muscle diseases, including sarcopenia and wasting disorders.
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1.de Souza GT et al. Stem Cells Int. 2015:487467.
2.Yoshida T et al (2013) J Biol Chem 16;288(33):23823-32.
3.Yoshida T et al (2014) J Biol Chem 19;289(38):26239-48.
4.Shapovalov G et al., Cell Calcium. 2011 Sep;50(3):295-302.
5.Makarewich CA et al. Circ Res. 2014 Aug 29;115(6):567-80.