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Specific profiles of ion channels and ionotropic receptors define adipose- and bone marrow derived stromal cells

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ABSTRACT

Adherent, fibroblastic cells from different tissues are thought to contain subsets of tissue-specific stem/progenitor cells (often called mesenchymal stem cells). These cells display similar cell surface characteristics based on their fibroblastic nature, but also exhibit differences in molecular phenotype, growth rate, and their ability to differentiate into various cell phenotypes. The mechanisms underlying these differences remain poorly understood. We analyzed Ca^{2+} signals and membrane properties in rat adipose-derived stromal cells (ADSCs) and bone marrow stromal cells (BMSCs) in basal conditions, and then following a switch into medium that contains factors known to modify their character. Modified ADSCs (mADSCs) expressed L-type Ca^{2+} channels whereas both L- and P/Q- channels were operational in mBMSCs. Both mADSCs and mBMSCs possessed functional endoplasmic reticulum Ca^{2+} stores, expressed ryanodine receptor-1 and -3, and exhibited spontaneous $[Ca^{2+}]_i$ oscillations. The mBMSCs expressed $P2X_7$ purinoceptors; the mADSCs expressed both P2X (but not $P2X_7$) and P2Y (but not $P2Y_1$) receptors. Both types of stromal cells exhibited $[Ca^{2+}]_i$ responses to vasopressin (AVP) and expressed V_1 type receptors. Functional oxytocin (OT) receptors were, in contrast, expressed only in modified ADSCs and BMSCs. AVP and OT-induced $[Ca^{2+}]_i$ responses were dose-dependent and were blocked by their respective specific receptor antagonists. Electrophysiological data revealed that passive ion currents dominated the membrane conductance in ADSCs and BMSCs. Medium modification led to a significant shift in the reversal potential of passive currents from −40 to −50 mV in cells in basal to −80 mV in modified cells. Hence membrane conductance was mediated by non-selective channels in cells in basal conditions, whereas in modified medium conditions, it was associated with K^+-selective channels. Our results indicate that modification of ADSCs and BMSCs by alteration in medium formulation is associated with significant changes in their Ca^{2+} signaling and membrane properties.

Abbreviations: α,β-MeATP, α,β-Methyleneadenosine 5′-triphosphate lithium salt; aCSF, artificial cerebrospinal fluid; ADSC, adipose derived stromal cell; AVP, arginine vasopressin; bADSC/bBMSC, ADSC/BMSC in basal conditions; bFGF, fibroblast growth factor-basic; BM, bone marrow; BMSC, bone marrow stromal cell; BzATP, 2′(3′)-O-(4-benzoylbenzoyl)adenosine 5′-triphosphate triethylammonium salt; C_m, membrane capacitance; CPA, cyclopiazonic acid; DAPI, diamidino-2-phenylindole; GABA, γ-aminobutyric acid; GVIA, ω-conotoxin GVIA; I_{KCa}, Ca^{2+}-activated K^+ channels; I_{to}, transient outward K^+ current; IK_{DR}, delayed rectifier K^+ current; IR, membrane resistance; mADCS/mBMSC, medium modified ADSC/BMSC; MVIIC, ω-conotoxin MVIIC; NMDA, N-Methyl-D-aspartic acid; OT, oxytocin; PPADS, pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate; RA, retinoic acid; RU, ratio units; V_{rest}, resting membrane potential; VGCC, voltage-gated Ca^{2+} channels.

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1. Introduction

Adherent, fibroblastic cells from different tissues (e.g., from bone marrow, adipose tissue, umbilical cord blood, placenta, Wharton jelly, etc.) are thought to contain subsets of tissue-specific stem/progenitor cells (often called mesenchymal stem cells). These tissue-specific stem/progenitor cells share many biological features. However, they also display differences in molecular phenotype, growth rate, and their ability to differentiate into various phenotypes (Kern et al., 2006; Al-Nbaheen et al., 2013; Choudhery et al., 2013).

Calcium is a ubiquitous intracellular messenger that is a key regulator of the cell cycle, particularly during stem cell proliferation and modification. The Ca^{2+} signaling pathways have been studied in a variety of stem cell types including embryonic (Forostyak et al., 2013; Viero et al., 2014), fetal (Cocks et al., 2013) and adult stromal cells (Resende et al., 2010; Zippel et al., 2012; Kotova et al., 2014; Forostyak et al., 2016). Bone marrow stromal cells (BMSCs) have been shown to express L-type Ca^{2+} channels (Heubach et al., 2004; Li et al., 2006; Wen et al., 2012), glutamate receptors (Fox et al., 2010) and have been reported to generate spontaneous inositol 1,4,5-triphosphate ($InsP_3$)-dependent Ca^{2+} oscillations (Kawano et al., 2002, 2003). Adipose tissue-derived stromal cells (ADSCs), were found to express adrenoceptors, $InsP_3$ receptors ($InsP_3Rs$), purinoceptors and were reported to generate Ca^{2+}-induced Ca^{2+} release (Kotova et al., 2014). BMSCs have also been shown to express specific K^+ channels including Ca^{2+}-activated K^+ channels (I_{KCa}), delayed rectifier K^+ current (IK_{DR}), and transient outward K^+ current (I_{to}) (Li et al., 2006). In human BMSCs in basal conditions (bBMSCs) large conductance voltage- and Ca^{2+}-activated K^+ channels have been identified (Heubach et al., 2004). To the best of our knowledge, an in depth analysis of ion channels and receptors in ADSCs and BMSCs that have been harvested under the same environmental conditions has not been performed. In this study, we compared the functional properties of these two types of cells in basal conditions (bADSCs and bBMSCs) and after their modification (mADSCs and mADSC) induced by switching to a medium containing factors known to alter their characteristics.

2. Experimental procedures

2.1. Animals

All experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regarding the use of animals in research, and were approved by the Ethics Committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic (ASCR), Prague, Czech Republic. The Sprague–Dawley rats were housed under standard laboratory conditions: a 12:12 h dark:light cycle, at 23 °C, with food and water supplied ad libitum. Bone marrow and adipose tissues used for cell isolation were collected from animals that were adequately anesthetized and subsequently euthanized.

2.2. Isolation of ADSCs

The isolation of stromal cells from adipose tissue was performed according to the protocol described previously (Arboleda et al., 2011). Adipose tissue from the inguinal pads was dissected, mechanically minced and treated with 0.2% (w/v) collagenase type I (Worthington Biochemicals, Lakewood, NJ) for 1 h at 37 °C. The isolated cellular fraction was resuspended in a proliferation medium, consisting of Dulbecco's modified Eagle's medium-DMEM/F12 + Glutamax (Gibco) supplemented with 10% fetal bovine serum and 0.2% antibiotics (primocin), and was then plated into culture flasks. Cells were harvested once they reached 90% confluence and re-plated up to the second passage. Cells from the second passage were used in their basal condition (bADSCs) or after growth in modified medium for further studies.

2.3. Isolation of BMSCs

As described previously (Forostyak et al., 2011), bone marrow (BM) was taken from femurs and tibias of 16-day-old rats. After cutting the epiphysis, BM was washed from the bones using a 2-ml syringe with a 21-gauge needle filled with DMEM containing high glucose, Glutamax 15 μl/ml (Gibco), 10% fetal calf serum and primocin 0.2%. The BM was gently dissociated and then plated into Petri dishes. The medium was changed after 24 h. When cells reached 75–90% confluence, they were detached by trypsin/EDTA treatment and transferred into culture flasks. Cells were used in their basal condition (bBMSCs) or after growth in modified medium for further studies.

2.4. Medium-modified ADSCs and BMSCs

Cultured bBMSCs or bADSCs (passage 2), after reaching 75–90% confluence, were plated at a density of 1×10^5 cells on glass bottom Petri dishes. After the attachment of the cells, the culture medium was replaced with medium consisting of a Neurobasal medium with B27 supplements containing retinoic acid (RA), 40 ng/ml, fibroblast growth factor-basic (bFGF) and 1% primocin. The cells were exposed to B27 with RA for 72 h, and then the culture media were replaced with Neurobasal medium containing B27 supplements (without RA), 40 ng/ml, bFGF and 1% primocin, and kept in culture up to 1 week. Growth factors were added every second day. The cells were measured between day 3 and day 5. This process was selected based on previous studies that explored the possibility to differentiate ADSCs into neuronal cells. Although changes in gene expression were noted, differentiation into functional neurons was not achieved (Arboleda et al., 2011).

2.5. Measurements of $[Ca^{2+}]^i$ using the fast fluorescence photometry system

$[Ca^{2+}]_i$ measurements on single cells were performed according to previously reported methods (Dayanithi et al., 1996; Forostyak et al., 2013). The cells were plated on 24 mm glass-bottom dishes (WillCo Dishes BV, Amsterdam, Netherlands) coated with laminin (Sigma-Aldrich), were incubated with 2.5 μM Fura-2 AM (Invitrogen, Carlsbad, CA, USA) with 0.02% Pluronic F-127 (Molecular Probes, Eugene, OR, USA) in culture medium at 37 °C and 5% CO_2 for 40 min. Loaded cells were then washed and the culture medium replaced with Normal Locke's buffer containing (in mM): NaCl, 140; KCl, 5; $MgCl_2$, 1.2; $CaCl_2$, 2.2; glucose, 10; HEPES-Tris, 10; pH 7.25, osmolarity 298–300 mosmol/l^{-1}) and kept at 37 °C throughout the time course of the experiment. Fluorescence measurements of $[Ca^{2+}]_i$ were performed with a fast fluorescence microspectrofluorimetry system based on an inverted microscope (Axiovert, Zeiss-Germany) equipped for epifluorescence (Plan-Neofluar 100×/1.30 oil immersion objective). To achieve fast switching between different excitation wavelengths, a rotating filter wheel was mounted in the excitation light path. The cells were illuminated (200 Hz) alternately at 340 ± 10 and 380 ± 10 nm. In order to minimize the background noise of the Fura-2 signal, successive values were averaged to a final time resolution of 320 ms. The measuring/recording amplifier was synchronized to the filter wheel to measure the fluorescence intensities resulting from different wavelengths. The FFP software controlled the acquisition of the intensity data and provided functions for adjusting the signal values as well as the display and storage of the measured data. A CCD camera was used to visualize the cells. The $[Ca^{2+}]_i$ measurement values are expressed as the ratio units (RU) between the fluorescence obtained with two excitation wavelengths, 340 nm (A) and 380 nm (B). Fura-2 calibration was performed in these cells in vitro following the procedure described previously (Lambert et al., 1994; Komori et al., 2010; Forostyak et al., 2013), which yielded $R_{min} = 0.08$, $R_{max} = 2.02$, $\beta = 1.757$. The dissociation constant for Fura-2 at 37 °C was assumed as $K_D = 224$ nM.