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A comparative study of α-adrenergic receptor mediated Ca^{2+} signals and contraction in intact human and mouse vascular smooth muscle

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ABSTRACT

In many vascular smooth muscle cells, physiological and pharmacological agonists initiate oscillatory fluctuations in intracellular Ca^{2+} to initiate and maintain vasoconstriction. These oscillations are supported by the underlying cellular ultrastructure, particularly the close apposition between the plasma membrane (PM) and superficial sarcoplasmic reticulum (SR), the so-called PM–SR junctions, which are important for SR Ca^{2+} refilling. We hypothesize that the disappearance of PM–SR junctions during aging and/or disease is directly related to the disappearance of agonist-induced Ca^{2+} oscillations. We compared phenylephrine-mediated Ca^{2+} signals and contraction in human and murine smooth muscle cells in small mesenteric arteries and also employed electron microscopy to examine the cytoplasmic distribution of the SR. Phenylephrine elicited tonic contractions in both types of vessels, asynchronous Ca^{2+} oscillations in the mouse mesenteric smooth muscle cells, but only single transient Ca^{2+} signals in the human mesenteric smooth muscle cells. While nifedipine inhibited 90% of the phenylephrine-induced tonic contraction in mouse mesenteric arteries, it only slightly attenuated tonic contraction in human mesenteric arteries, although the nifedipine-resistant component was abolished by the Rho-kinase blocker 1-(5-Isoquinolinyl-sulfonyl)homopiperazine dihydrochloride (HA-1077). Furthermore, superficial SR was found to be abundant in the mouse vessels and many PM–SR junctions were observed, but the smooth muscle of human mesenteric arteries had far less peripheral SR and was almost devoid of PM–SR junctions. As PM–SR junctions are essential for the maintenance of Ca^{2+} oscillations, the change in Ca^{2+} signalling pattern in the relatively old human patients was due to impaired SR refilling.

1. Introduction

The introduction of confocal fluorescence microscopy has led to significant advances in our understanding of Ca^{2+} signalling in vascular smooth muscle of experimental animal models. In this communication we address the important question of whether this newly gained insight can be directly translated to the control of human blood vessels and indirectly to drug therapy of human vascular disease. In virtually all experimental animal studies on intact vascular smooth muscle, both physiological and pharmacological agonists induce oscillatory fluctuations in intracellular calcium ($[Ca^{2+}]_i$) to initiate and maintain vasoconstriction (Iino et al., 1994; Lee et al., 2002; Mauban et al., 2001; Peng et al., 2001; Syyong et al., 2009). Two main types of $[Ca^{2+}]_i$ oscillations have been reported: asynchronous Ca^{2+} waves, underlying tonic contractions of arteries and veins, and synchronous $[Ca^{2+}]_i$ oscillations signalling vasomotion in some resistance arteries (Peng et al., 2001).

Asynchronous Ca^{2+} waves are caused by regenerative Ca^{2+} release from the sarcoplasmic reticulum (SR) and maintained by refilling of the SR by non-specific cation channels and reverse-mode Na^+–Ca^{2+} exchange in the plasmalemma (PM), which is linked to sarco/endoplasmic Ca^{2+}–ATPase (SERCA) within specialized junctions between the PM and SR, termed PM–SR junctions (Fameli et al., 2007). Synchronized $[Ca^{2+}]_i$ oscillations are also paced by periodic Ca^{2+} release from the SR, which however does not directly activate myofilaments, but serves to activate Ca^{2+} dependent Cl^- channels, which depolarize the PM and activate L-type Ca^{2+} channels in electrically coupled smooth muscle cells (Jacobsen et al., 2007).

Although our understanding of $[Ca^{2+}]_i$ oscillations in the regulation of vasoconstriction in animals has seen great recent advances, little is known about Ca^{2+} signalling in intact human vascular smooth muscle. In the only analogous human study to date, no $[Ca^{2+}]_i$ oscillations were

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seen in saphenous veins of patients undergoing coronary artery bypass grafting (Crowley et al., 2002). However, these patients were primarily suffering from vascular disease, which may have affected their smooth muscle regulatory mechanisms.

In this study, we investigated the regulation of vasoconstriction in relation to Ca^{2+} signalling along with a detailed analysis of the membrane ultrastructure in intact human mesenteric arterial smooth muscle obtained from patients undergoing abdominal surgery, but not primarily suffering from vascular diseases and have made critical comparisons with analogous measurements in intact murine smooth muscle. We hypothesize that the disappearance of PM–SR junctions during aging and or disease is directly related to the disappearance of agonist-induced Ca^{2+} oscillations. Herein we report a striking difference in vascular smooth muscle Ca^{2+} signalling, with mice showing asynchronous Ca^{2+} oscillations and human patients single Ca^{2+} transients, which is correlated with an equally striking difference in membrane ultrastructure. Our data further suggest that these functional and morphological differences may be relevant for the treatment of human vascular disease.

2. Methods

2.1. Human tissue preparation

Patients were recruited from those undergoing abdominal surgery at the General Surgery Service (Hospital Clinico Universitario San Carlos, Madrid, Spain). Samples of the mesentery were collected by the surgeons during the surgical procedures, kept in RPMI medium at 4 °C and used within 30 min after the operations. Only tissues devoid of any obvious lesions were used. The tissue was transferred to physiological salt solution (PSS) and small mesenteric arteries were isolated, cleaned from connective tissue and cut into multiple rings that were ~2 mm in length. The study was conducted according to the Declaration of Helsinki and informed consent was obtained from all subjects before sampling. Approval was granted by the institutional Ethics Review Board for the use of human specimen.

2.2. Animal tissue preparation

Male C57BL/6 mice (25–30 g) were sacrificed by stunning followed by cervical dislocation. The second or third-order mesenteric arteries were removed and cleaned from connective tissue and cut into rings 2 mm in length. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Approval was granted by institutional Animal Ethics Review Board.

2.3. Contraction study

Vascular reactivity experiments were conducted according to a method previously described (Syyong et al., 2009). Briefly, the arterial rings (from human and mouse) were mounted in a small vessel Multi Myograph system 610M (Danish Myo Technology, Aarhus, Denmark) using 40 μm tungsten wires (Westinghouse Inc, Pittsburgh, USA). The vessel was set to a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure. Vessels were allowed to equilibrate at 37 ± 0.5 °C in PSS and gassed continuously with a 95%O_2–5%CO_2 gas mixture. The isometric force was digitalized by Myodaq 2.01 program (Danish Myo Technology, Aarhus, Denmark) and stored and displayed on a personal computer. All experiments were performed at 37 °C.

2.4. Confocal $[Ca^{2+}]_i$ imaging

The details of the confocal Ca^{2+} imaging method have been described previously (Dai et al., 2007a). Briefly, the rings of human and mice mesenteric arteries were loaded in PSS with Fluo-4 AM (5 μM, and 5 μM Pluronic F-127) for 120 min at 25 °C and then left to equilibrate for 10 min in normal PSS. They were then inverted and isometrically mounted on a myograph on the stage of a confocal microscope for $[Ca^{2+}]_i$ measurements. The changes in $[Ca^{2+}]_i$ were measured using an inverted Leica TCS SP2 AOBS laser scanning confocal microscope with an air ×10 (numerical aperture 0.3) lens (Leica Microsystems GmbH., Wetzlar, Germany). The tissue was illuminated using the 488-nm line of an argon–krypton laser, and a high-gain photomultiplier tube collected the emission at wavelengths between 505 and 550 nm. The acquisition rate was 3 frames/s. The measured changes in Fluo-4 fluorescence level are proportional to the relative changes in $[Ca^{2+}]_i$. All parameters (laser intensity and gain) were maintained constant during the experiment. All experiments were performed at 37 °C.

2.5. Electron microscopy

Immediately after extraction, both human and mice mesenteric arteries were cut into small rings approximately 1 mm × 0.5 mm × 0.2 mm in dimension and placed in the primary fixative (2.5% glutaraldehyde, in 0.1 M phosphate buffer) for 4 h at 4 °C. The primary fixative solution was then removed and the blocks were placed in 0.1 M phosphate buffer for 10 min followed by three 10 min washes in the same concentration of phosphate buffer. In the process of secondary fixation, the rings were placed in 1% OsO4, 0.1 M phosphate buffer for 2 h. The blocks were then further treated with 1% uranyl acetate for 1 h (en bloc staining). Increasing concentrations of ethanol (50, 70, 80, 90 and 95%) were applied (10 min each) in the process of dehydration. 100% ethanol was used (three 10 min washes) for the final process of dehydration. The rings were left overnight in the resin (TAAB 812 mix, medium hardness) and then embedded in moulds and placed in an oven at 60 °C for 8 h. The embedded blocks were sectioned on a microtome using a diamond knife. The thickness of the sections was 80 nm. The sections were then placed on 400-mesh copper grids, stained with 1% uranyl acetate and Reynolds lead citrate for 4 and 3 min, respectively. Images of the cross-sections of the muscle cells were obtained with a Jeol JEM-1010 high resolution transmission electron microscopy (JEOL, Tokyo, Japan).

2.6. Quantification of the plasmalemma–sarcoplasmic reticulum junctions

Electron micrographs of the human and mice mesenteric arteries were converted into digital images which were analyzed using customized macros in the ImagePro Plus (Media Cybernetics Inc, Silver Spring, USA). The SR membranes and the PM in the individual vascular smooth muscle cell cross-section were manually outlined in ImagePro Plus. For this analysis, we defined a PM–SR junction as an area of the PM that is spatially situated within 30 nm of a peripheral SR membrane. Using the cut-off distance of 30 nm, the software then determined the perimeter of the peripheral SR associated with the PM, the perimeter of the total SR, and the perimeter of total PM in each individual smooth muscle cell. The fraction of peripheral SR perimeter over total PM perimeter gives an estimate of the fraction of the PM associated with the peripheral SR.

2.7. Statistical analysis

All confocal image analyses were performed in ImageProPlus v4.5 (Media Cybernetics Inc, Silver Spring, USA) using customized routines written in Visual Basic. The representative fluorescence traces shown in this report reflect the averaged fluorescence signals from a 3 × 3 pixel region (1.85 μm^2) of the ribbon-shaped vascular smooth muscle cell. The fluorescence intensity derived in each region is linearly proportional to the $[Ca^{2+}]_i$ in that region in such a fashion that any change in $[Ca^{2+}]_i$ would be proportionally reflected in the change