Biochemical and Biophysical Research Communications 445 (2014) 84–88

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Calcium oscillations in human mesenteric vascular smooth muscle

Jorge Navarro-Dorado [a,1], Mauricio Garcia-Alonso [a], Cornelis van Breemen [b], Teresa Tejerina [a], Nicola Fameli [b,*]

[a] Departamento de Farmacología, Universidad Complutense, av. Séneca 2, 28040 Madrid, Spain
[b] Department of Anesthesiology, Pharmacology, and Therapeutics, The University of British Columbia, 2176 Health Sciences Mall, Medical Block C, Vancouver, BC V6T 1Z3, Canada

ARTICLE INFO

Article history:
Received 14 January 2014
Available online 4 February 2014

Keywords:
Calcium oscillations
Human smooth muscle
Mesenteric artery
ER junctions
Sarcoplasmic reticulum
CPA
2-APB
Nifedipine
IP_3 receptors

ABSTRACT

Phenylephrine (PE)-induced oscillatory fluctuations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of vascular smooth muscle have been observed in many blood vessels isolated from a wide variety of mammals. Paradoxically, until recently similar observations in humans have proven elusive. In this study, we report for the first time observations of adrenergically-stimulated $[Ca^{2+}]_i$ oscillations in human mesenteric artery smooth muscle. In arterial segments preloaded with Fluo-4 AM and mounted on a myograph on the stage of a confocal microscope, we observed PE-induced oscillations in $[Ca^{2+}]_i$, which initiated and maintained vasoconstriction. These oscillations present some variability, possibly due to compromised health of the tissue. This view is corroborated by our ultrastructural analysis of the cells, in which we found only (5 ± 2)% plasma membrane-sarcoplasmic reticulum apposition, markedly less than measured in healthy tissue from laboratory animals. We also partially characterized the oscillations by using the inhibitory drugs 2-aminoethoxydiphenyl borate (2-APB), cyclopiazonic acid (CPA) and nifedipine. After PE contraction, all drugs provoked relaxation of the vessel segments, sometimes only partial, and reduced or inhibited oscillations, except CPA, which rarely caused relaxation. These preliminary results point to a potential involvement of the sarcoplasmic reticulum Ca^{2+} and inositol 1,4,5-trisphosphate receptor (IP3R) in the maintenance of the Ca^{2+} oscillations observed in human blood vessels.

1. Introduction

Calcium oscillations and waves have been shown to represent the major Ca^{2+} signal for excitation–contraction coupling in smooth muscle of blood vessels in healthy experimental animals [1–7]. The mechanism underlying these phenomena appears complex and has been subject to numerous investigations in the past two decades. The basic mechanism has been shown to be a propagated wave of calcium induced calcium release (CICR) at the inositol 1,4,5-trisphosphate (IP3) receptors (IP_3R) [8]. While repetitive asynchronous Ca^{2+} wave-like oscillations are responsible for vascular tone and determine the size of the blood vessel lumen [8], synchronized Ca^{2+} oscillations have been associated with vasomotion [5,9]. Moreover, we have shown that refilling of the sarcoplasmic reticulum (SR) via plasma membrane (PM)-SR junctions is required for the maintenance of both the repetitive Ca^{2+} waves and vascular tone [8,10].

Unfortunately, the phenomenon of myoplasmic Ca^{2+} oscillations has thus far not been reported for intact smooth muscle tissue from human blood vessels (although the first report of intracellular Ca^{2+} waves was based on measurements done in smooth muscle cells cultured from humans [11]). In a recent publication, we documented this functional difference between vascular smooth muscles of human and murine origin, while demonstrating a striking difference in the membranous ultrastructure of the smooth muscle derived from the two different sources [12]. The human smooth muscle, which lacked Ca^{2+} oscillations, had very little peripheral SR and practically no PM-SR junctions, while the mice in accordance with previous reports responded to adrenergic activation with asynchronous repetitive Ca^{2+} waves and exhibited abundant peripheral SR and numerous PM-SR junctions. In addition, we noted that while the human blood vessels lacked Ca^{2+} oscillations the Rho-kinase component of their myofilament activation was enhanced [12]. Since the human patients supplying the blood vessels were generally elderly and of compromised health, we hypothesized that in human vascular smooth muscle the degradation of membranous ultrastructure leading to loss of Ca^{2+} wave-like oscillations could be causally linked to the development of vascular dysfunction and disease. Considering the prevalence of human vascular disease and the amount of

* Corresponding author. Fax: +1 604 8226012.
E-mail address: nicola.fameli@ubc.ca (N. Fameli).
[1] Present address: Centre for Integrative Physiology, The University of Edinburgh, Hugh Robson Building, Edinburgh EH8 9XD, United Kingdom.

http://dx.doi.org/10.1016/j.bbrc.2014.01.150
0006-291X/

suffering it inflicts, it is clear that the above putative link between aberrant Ca^{2+} signaling and vascular disease should be further investigated. However, without clear evidence of Ca^{2+} oscillations or waves in human vascular smooth muscle it could be argued that human blood vessels are different from those of experimental animals.

In this communication, we show for the first time that adrenergic stimulation of human mesenteric arteries induces Ca^{2+} oscillations in the medial smooth muscle cells. Since the challenges facing online living human blood vessel research are considerably greater than encountered in the laboratory animal, most of the mechanistic details are usually first established in the latter. The present demonstration that vascular Ca^{2+} signaling in humans involves repetitive Ca^{2+} oscillations provides the clinical relevance for their study in health and disease.

2. Materials and methods

This study was conducted according to the World Medical Association Declaration of Helsinki and informed consent was obtained from all subjects before sampling. Approval was granted by the institutional Ethics Review Board of the Universidad Complutense, Madrid, Spain, for the use of human specimen.

2.1. Tissue collection and preparation

Patients were recruited from those undergoing abdominal surgery at the General Surgery Service (Hospital Clínico Universitario San Carlos, Madrid, Spain). Samples of the mesenteric artery were collected during the surgical procedures, kept in RPMI 1640 (Gibco) 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 saline solution (PSS) and mesenteric arteries 0.3–1.5 mm in diameter were isolated, cleaned from connective tissue and cut into multiple segments that were about 2 mm in length.

2.2. Confocal $[Ca^{2+}]_i$ imaging and myography

The segments of human mesenteric arteries were loaded in PSS with Fluo-4AM (5 μM, Invitrogen, and 5 μM Pluronic F-127, Sigma–Aldrich) for 45 min at 37 °C and then left to equilibrate for 30 min (three 10-min washes) in normal PSS. Depending on the diameter of the vessel, the arterial segments were inverted for easier access to the muscular layer (to avoid rupture smaller segments were left un-inverted) and isometrically mounted on a myograph set on the stage of a confocal microscope for $[Ca^{2+}]_i$ measurements.

For vascular reactivity studies, we employed a modification of the methodology described by Mulvany and Halpern [13], using a 310A Danish Myo Technologies individual isometric myograph. The changes in $[Ca^{2+}]_i$ were measured using an inverted BioRad MRC-1024 laser scanning confocal microscope with a 20× air lens (Nikon Eclipse TE300).

The smooth muscle layer 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 measured changes in Fluo-4AM fluorescence level are proportional to the relative changes in $[Ca^{2+}]_i$. Laser intensity and gain were maintained constant during the experiments, which were all performed at 37 °C. Video capture was set to 900 cycles (approximately 1.25 s/cycle) for each experiment. Phenilephrine (PE) (10 μM, Sigma–Aldrich) was added to the bath 150 s after the start of the video capture and after 300 s the inhibitory drugs (nifedipine, Sigma–Aldrich; cyclopiazonic acid, Sigma–Aldrich; 2-Aminoethoxydiphenyl borate, Tocris) were added. At the same time vascular reactivity was measured with the myograph.

2.2.1. Image and contractility analysis

All data used for the Ca^{2+} traces were analyzed by ImageJ, using the built-in regions-of-interest (ROI) function to select the areas of interest, which were then line-scanned to produce the actual traces. Pseudo-colour visualization was performed by ImageJ, using customized lookup tables to assign colour for each pixel intensity value. Results were graphed using Gnuplot 4.6. Signal amplitude and contractile force attenuation measurements are reported as (mean ± SEM).

2.3. Electron microscopy imaging

Human mesenteric arteries from abdominal surgery were fixed with 4% paraformaldehyde + 2.5% glutaraldehyde in cold (4 °C) 0.1 M Na cacodylate buffer immediately after surgery. Arteries were then dissected, cleaned of the connective and fat tissue, and then cut into 1–2-mm-long segments. Arterial segments were fixed in the same fixative solution for 6 h. The segments were washed five times with cacodylate buffer every 30 min, and left overnight at 4 °C. Post-fixation with 1% osmium tetroxide and potassium ferricyanide in distilled water was done for 1.5 h, after which the segments were washed with distilled water (three 10-min washes) and dehydrated in increasing concentrations of acetone 50, 60, 70, 80, 90, 95 (15 min each) and 100% (three 10-min washes). The segments were then left in a gradual resin infiltration (1:3, 1:1, 3:1 acetone + pure resin) and finally left in pure resin (TAAB 812 mix) at 60 °C overnight. The resin embedded samples were sectioned by diamond knife, and the 80-nm sections were collected onto copper grids and post-stained with 1% uranyl acetate and Reynolds lead citrate for 4 and 3 min, respectively. Smooth muscle cell electron micrographs were obtained using a Jeol JEM-1010 high resolution transmission electronic microscope (JEOL, Tokyo, Japan).

2.3.1. Quantification of the PM-SR reticulum junctions

Electron micrographs of the human mesenteric arteries were converted into digital images, which were analyzed using the freeware inkscape 0.47 (inkscape.org) and an in-house modification of its 'measure.py' script to allow direct output of line measurements into a text file that could be used for quantitative analysis. To measure the extent of linear PM and SR apposition, superficial SR membrane segments and the PM in the individual vascular smooth muscle cell cross-sections were manually outlined and measured in units of pixels in inkscape. The length in pixels of the scale bar in the images provided a calibration gauge. For this analysis, we considered any peripheral SR as part of a PM-SR junction if its membrane was localized at a distance of 30 nm or less from the PM. High magnification whole cell montages were obtained from 11 cells and the entire PM was outlined and measured. Similarly, we measured the segments of junctional SR (Fig. 2A) and calculated the SR/PM ratio to obtain the data in the histogram of Fig. 2B. Measurements are reported as (mean ± SEM).

3. Results

3.1. Phenylephrine (PE)-induced $[Ca^{2+}]_i$ oscillations and contractile response

Calcium oscillations and subsequent contraction were observed in human mesenteric artery smooth muscle after addition of 10 μM PE (Fig. 1). The data for this part of the study were collected from samples from 9 patients and between 5 and 22 cells in each

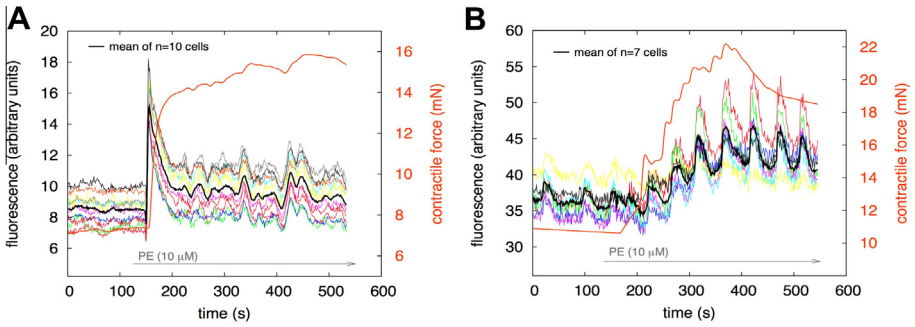

Fig. 1. Ca^{2+} fluorescence and myography traces. Fluo-4AM fluorescence records of PE-induced $[Ca^{2+}]_i$ oscillations in human mesenteric artery smooth muscle (left vertical axis, multicoloured traces) and corresponding myographic measurements of contractile force development (right vertical axis and thick orange trace). The thick black trace is the mean value of the fluorescence traces.

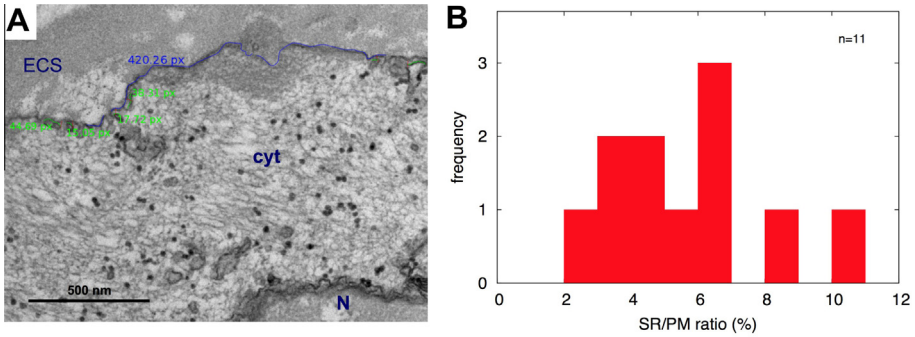

Fig. 2. Ultrastructural study. (A) Representative electron micrograph of human mesenteric artery smooth muscle; coloured tracings as shown were used to measure the extent of the linear SR-to-PM ratio (the PM is traced in blue, the superficial SR in green, the threshold PM-SR separation of 30 nm in red, and the measurements are displayed in pixels (px)—see Section 2.3.1 for details); ECS: extra-cellular space, cyt: cytoplasm, N: nucleus. (B) Histogram of the SR-to-PM ratio observations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sample were imaged (see Table 1). Oscillations were observed in virtually all preparations from 7 of the 9 patients, and presented different amplitude and frequency characteristics from one patient to another. In the majority of cases, all cells in a segment of tissue responded with a large amplitude initial $[Ca^{2+}]_i$ spike shortly after PE stimulation, and then continued to oscillate at much lower (and somewhat variable) amplitudes (Fig. 1A). Occasionally cells displayed no initial large peak, but rather a more gradual and remarkably regular onset of oscillations (Fig. 1B). In all cases, the oscillations appeared to continue to have an elevated average $[Ca^{2+}]_i$ compared to its level prior to stimulation and to maintain their general features as long as the stimulus was present. Moreover, as shown by the mean value of the measured $[Ca^{2+}]$ in both panels of Fig. 1 (thicker black traces), these oscillations appear to be synchronous.

Myographic contractile force measurements were carried out in parallel with fluorescence $[Ca^{2+}]_i$ observations. As illustrated by the thick orange traces in Fig. 1A and B (right side ordinate axis), PE stimulation also provoked contraction in the smooth muscle tissue, which was maintained as long as $[Ca^{2+}]_i$ oscillations were maintained.

3.2. PM-SR apposition measurements

We have previously shown that the presence of PM-SR junctional complexes between the PM and SR is essential for the maintenance of $[Ca^{2+}]_i$ oscillations [14]; a finding that appears to be corroborated by quantitative modeling [10]. We therefore collected mesenteric artery tissue from 5 patients with similar age ranges and health conditions as those whose tissue was used for the confocal microscopy and myography observations reported in Section 3.1 (Table 2). In a series of electron micrographs from those arterial smooth muscle samples (Fig. 2A displays a representative one), we measured the amount of linear PM-SR overlap in 11 cells following the procedure described in Section 2.3. The histogram in Fig. 2B depicts the approximate distribution of our findings in this case, from which we calculate a mean value for the (linear) superficial SR-to-PM ratio of (5 ± 2)%. These numbers likely underestimate the actual PM-SR overlap by a few percent, since, while the superficial SR was almost always clearly visible, the PM frequently had a smeared appearance due to the tangential or oblique orientation at which some sample sections were cut by the microtome knife. In those cases, it is impossible to determine with adequate precision the approximate distance between the superficial SR and the PM and hence determine whether the two membranes actually form junctions or not.

3.3. Possible mechanism underlying the oscillations

In an attempt to elucidate the mechanism that originates the observed oscillations, we studied the effect of the IP_3R inhibitor 2-aminoethoxydiphenyl borate (2-APB), of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) blocker cyclopiazonic acid (CPA), and of the voltage gated Ca^{2+} channel (VGCC) antagonist nifedipine on $[Ca^{2+}]_i$ oscillations and contractile force, after addition of PE. These pharmacological agents were chosen as they inhibit the two main sources of activating Ca^{2+} intake into, and release from, the SR and entry from the extracellular space, as based on previous

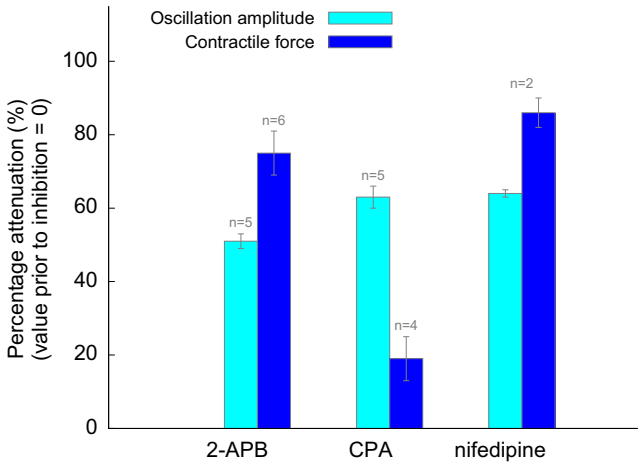

Fig. 3. Effect of inhibitors on oscillation amplitude and contractile force. The "n" numbers shown are the number of arterial segments observed. The number of cells observed in $[Ca^{2+}]_i$ oscillation experiments are: 2-APB, 22, 12, 13, 12, 9, (12); CPA, 9, 11, 8, 11, (16); nifedipine, 6, 16. The numbers in brackets regard the extra "n" of the contractile force bars for 2-APB and CPA experiments.

findings in laboratory animals [8]. We report the results of these experiments by means of the attenuation effect of these drugs on both the oscillatory signal amplitude and on the contractile force attenuation (Fig. 3).

As shown in the bar chart in Fig. 3, a 10 μM dose of 2-APB noticeably reduced the amplitude of Ca^{2+} the signal by more than 50% ($n = 5$); this rendered the oscillatory pattern unobservable in some cases. It moreover had the effect of relaxing the artery by (75 ± 6)% ($n = 6$) of the PE-induced contraction. (In 1 of the 6 experiments the relaxation effect was only about 17% and without this "outlier" the average relaxation was about (87 ± 2)%.)

Addition of 1 μM CPA also reduced or abrogated the amplitude of the Ca^{2+} oscillations, but only provoked minor relaxation of the arterial tissue, except in 1 of the five experiments, in which it almost completely relaxed the tissue (by ≈95%). Due to the skewed distribution of the CPA myographic data, in the bar of Fig. 3 relative to these experiments, we report the (median ± maximum absolute deviation) of the relaxation values, rather than (mean ± SEM).

Finally, we were able to carry out two experiments using nifedipine (1 μM), which had an attenuating effect on both the signal amplitude and contractility of the arteries, as indicated in the rightmost bars in Fig. 3.

4. Patient information

For patient information, see Tables 1 and 2.

5. Discussion

We have observed PE (10 μM)-stimulated $[Ca^{2+}]_i$ oscillations followed by arterial contraction in human mesenteric artery segments collected from patients aged 26 to 71. Samples from 7 of 9 patients showed oscillations. These follow similar patterns, but are nevertheless difficult to characterize quantitatively in terms of their amplitude and frequency due to considerable variability from one patient to the next. We generally observed either one $[Ca^{2+}]_i$ transient followed by several smaller oscillations (Fig. 1A), or a set of larger oscillations (Fig. 1B), both cases inducing an increase in the contractile force (Fig. 1A and B, thicker orange traces), which followed in step with the onset of oscillations. Wave-like $[Ca^{2+}]$ oscillations were first reported in cultured human vascular smooth muscle cells [11], then in native laboratory rat arterial smooth muscle [1], as well as many other mammalian vascular smooth muscle [1–7]. Not only have $[Ca^{2+}]$ oscillations in human vascular smooth muscle intact tissue been difficult to observe, but our own earlier work appeared to suggest that Ca^{2+} signaling in humans may indeed differ from that of other animals [12]. Although the hypothesis was then put forward that the cause for the difference should be sought in the health status of the patients, who volunteered the blood vessels for the study, the question remained open as to whether Ca^{2+} oscillations control vascular smooth muscle contractile activity in humans as well as experimental animals. Our observations reported herein provide evidence to help settle this translationally relevant question. While we did observe oscillations in most patients, they are nonetheless more irregular than those usually observed in healthy laboratory animals. It is possible that, besides the more difficult experimental conditions inherent to work with human tissue, the generally disease state of the patients (see Table 1) be linked to the somewhat deteriorated Ca^{2+} oscillatory signals observed here.

In this respect, the ultrastructural component of our study stemmed from an interest to determine whether there exists a

Table 1
Confocal microscopy experiment tissue donors. HPC: hypercholesterolemia; HPT: hypertension. The symbol × indicates the patient has the condition.

Gender	Age	Diabetes	Smoker	HPC	HPT	Obesity	Sedentary	Neoplasia
F	58	×		×	×	×	×	
F	51		×			×	×	×
F	62		×				×	×
M	26							
M	62	Possible	×		×			×
F	44			×		×		
F	43							
F	71	×			×			×
F	47				×	×	×	

Table 2
Electron microscopy experiment tissue donors. HPC: hypercholesterolemia; HPT: hypertension. The symbol × indicates the patient has the condition.

Gender	Age	Diabetes	Smoker	HPC	HPT	Obesity	Sedentary	Neoplasia
F	42	×	×	×				
M	73		Ex	×	×	×	×	×
M	38							
F	66	×		×		×	×	×
F	18							

correlation between Ca^{2+} signals and intracellular architecture, as we and other researchers have found in previous studies [12,14–16]. Our quantitative image analysis revealed that (5 ± 2)% of the PM in the 11 surveyed cells was apposed to superficial SR membrane (Fig. 2B). Although this may be an underestimate, as we explained in Section 3.2, the level of apposition we find is still significantly less than the value of 15% found in our earlier studies in healthy laboratory animals [4]. This result appears to corroborate the link between loss of PM-SR junctions and diseased state of the tissue [12] and it agrees with the finding that disruption of PM-SR junctions leads to deterioration of Ca^{2+} oscillations [14]. Therefore, it is plausible that the imperfect health of the harvested tissue is reflected in the limited PM-SR apposition observed and in part responsible for the irregularity in the oscillations.

We moreover note that in all cases the $[Ca^{2+}]$ oscillations we observed are synchronous, as is evidenced by the average value traces in Fig. 1A and B (thicker black lines). The appearance of synchronous as opposed to asynchronous oscillations is normally associated with the onset of vasomotion in resistance arteries, which also has a greater dependence on voltage gated Ca^{2+} channels [5,17]. This is consistent with the origin of the vessels available for this study, which were harvested from the peripheral peritoneal region of the patients.

In terms of the mechanism underpinning the observed Ca^{2+} oscillations, within the limitations of the available tissue samples, our results (summarized in Fig. 3) from inhibition of the SERCA pumps (by 1 μM CPA), which prevents SR Ca^{2+} refilling, and of the IP_3R (by 10 μM 2-APB), which blocks SR Ca^{2+} release, point to the involvement of the SR, as we have previously reported [8,10], and suggest that the observed oscillations as well as the corresponding development of contractile force are largely maintained by SR Ca^{2+} mobilization via the IP_3R channels. The finding that 2-APB and CPA have opposing effects on the muscle contractility agrees with the known behaviour of these agents, as we have previously determined [8]. Inhibition of IP_3R by 2-APB tends to impede SR Ca^{2+} release and hence to decrease the efficiency of Ca^{2+} delivery to the myofilaments for contraction. On the other hand, blockade of the SERCA pumps decreases Ca^{2+} sequestration by the SR, thereby increasing $[Ca^{2+}]_i$ and the likelihood of Ca^{2+} interaction with myofilaments, even though the efficiency of the Ca^{2+} delivery mechanism is compromised as indicated by the attenuation of the signal amplitude. Lastly, we find that the VGCC inhibitor nifedipine (1 μM) causes alteration of the oscillatory signal, an observation that corroborates our earlier findings [8], and also relaxes the artery considerably. Although our results make it clear that the Ca^{2+} oscillations in these human blood vessels involve SR Ca^{2+} release via IP_3R, further mechanistic research on human vascular smooth muscle is required to elucidate the precise mechanisms involved.

Nevertheless, this previously unreported observation of α-adrenergic-stimulated $[Ca^{2+}]_i$ oscillations in human native vascular smooth muscle resolves a conundrum that has accompanied this area of research for the past two decades. Our results confirm that, at least from the standpoint of vascular smooth muscle Ca^{2+} signaling, humans behave like animals, and thereby provide the clinical rationale for more detailed mechanistic studies to identify pharmacologic tests.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

Acknowledgments

This work was supported by Fondo de Investigaciones Sanitarias PI080920, Red Temática de Investigación Cardiovascular (RECAVA: RD06/0014/1007, Spanish Ministry of Health) and by Grant No. CIHR MOP-84309 from the Canadian Institute for Health Research. We are grateful to all the nurses and doctors from the Servicio de Cirugía General, Hospital Clínico San Carlos, Madrid, Spain and to the electron and confocal microscopy facility staff of UCM. We also wish to thank Wonjin Kim and Jeremy Hoskins for their help with image analysis, and Fernando Ortego for his technical support.

References

[1] M. Iino, H. Kasai, T. Yamazawa, Visualization of neural control of intracellular Ca^{2+} concentration in single vascular smooth muscle cells in situ, EMBO J. 13 (1994) 5026–5031.
[2] J.H. Jaggar, M.T. Nelson, Differential regulation of Ca(2+) sparks and Ca(2+) waves by UTP in rat cerebral artery smooth muscle cells, Am. J. Physiol. Cell Physiol. 279 (2000) C1528–39.
[3] J.H. Jaggar, Intravascular pressure regulates local and global Ca(2+) signaling in cerebral artery smooth muscle cells, Am. J. Physiol. Cell Physiol. 281 (2001) C439–48.
[4] C.-H. Lee, D. Poburko, K.-H. Kuo, C.Y. Seow, C. van Breemen, Ca(2+) oscillations, gradients, and homeostasis in vascular smooth muscle, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H1571–83.
[5] J.R. Mauban, C. Lamont, C.W. Balke, W.G. Wier, Adrenergic stimulation of rat resistance arteries affects Ca(2+) sparks, Ca(2+) waves, and Ca(2+) oscillations, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H2399–405.
[6] J.F. Perez, M.J. Sanderson, The frequency of calcium oscillations induced by 5-ht, ach, and kcl determine the contraction of smooth muscle cells of intrapulmonary bronchioles, J. Gen. Physiol. 125 (2005) 535–553.
[7] L. Shaw, S. O'Neill, C.J.P. Jones, C. Austin, M.J. Taggart, Comparison of u46619-, endothelin-1- or phenylephrine-induced changes in cellular Ca^{2+} profiles and Ca^{2+} sensitisation of constriction of pressurised rat resistance arteries, Br. J. Pharmacol. 141 (2004) 678–688.
[8] C.H. Lee, D. Poburko, P. Sahota, J. Sandhu, D.O. Ruehlmann, C. van Breemen, The mechanism of phenylephrine-mediated [Ca(2+)](i) oscillations underlying tonic contraction in the rabbit inferior vena cava, J. Physiol. 534 (2001) 641–650.
[9] H. Peng, V. Matchkov, A. Ivarsen, C. Aalkjaer, H. Nilsson, Hypothesis for the initiation of vasomotion, Circ. Res. 88 (2001) 810–815.
[10] N. Fameli, C. van Breemen, K.-H. Kuo, A quantitative model for linking Na+/Ca^{2+} exchanger to SERCA during refilling of the sarcoplasmic reticulum to sustain $[Ca^{2+}]$ oscillations in vascular smooth muscle, Cell Calcium 42 (2007) 565–575.
[11] C.B. Neylon, J. Hoyland, W.T. Mason, R.F. Irvine, Spatial dynamics of intracellular calcium in agonist-stimulated vascular smooth muscle cells, Am. J. Physiol. 259 (1990) C675–686.
[12] J.M. Dai, H. Syyong, J. Navarro-Dorado, S. Redondo, M. Alonso, C. van Breemen, T. Tejerina, A comparative study of alpha-adrenergic receptor mediated Ca^{2+} signals and contraction in intact human and mouse vascular smooth muscle, Eur. J. Pharmacol. 629 (2010) 82–88.
[13] M.J. Mulvany, W. Halpern, Mechanical properties of vascular smooth muscle cells in situ, Nature 260 (1976) 617–619.
[14] C.-H. Lee, K.-H. Kuo, J. Dai, J.M. Leo, C.Y. Seow, C. van Breemen, Calyculin-A disrupts subplasmalemmal junction and recurring Ca^{2+} waves in vascular smooth muscle, Cell Calcium 37 (2005) 9–16.
[15] M. Gherghiceanu, L.M. Popescu, Electron microscope tomography: further demonstration of nanocontacts between caveolae and smooth muscle sarcoplasmic reticulum, J. Cell. Mol. Med. 11 (2007) 1416–1418.
[16] E.D. Moore, T. Voigt, Y.M. Kobayashi, G. Isenberg, F.S. Fay, M.F. Gallitelli, C. Franzini-Armstrong, Organization of Ca^{2+} release units in excitable smooth muscle of the guinea-pig urinary bladder, Biophys. J. 87 (2004) 1836–1847.
[17] C. Aalkjaer, H. Nilsson, Vasomotion: cellular background for the oscillator and for the synchronization of smooth muscle cells, Br. J. Pharmacol. 144 (2005) 605–616.