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Microdomain Ca²⁺ dynamics in mammalian muscle following prolonged high pressure treatments

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Abstract. High pressure (HP) applications are an important thermodynamic tool to influence cellular processes. Especially processes that undergo large volume changes, e.g. opening or closing of ion channels, are in particular susceptible to HP treatments. Such volume changes are extremely difficult to assess for intracellular ion channels, like ryanodine receptors (RyR) residing in the membrane of organelles. In skeletal muscle, RyR act as Ca^{2+} release channels. We previously showed that plasmalemmal Na⁺ and Ca²⁺ ion channels were irreversibly altered after prolonged 20 MPa treatments. Here, changes in microdomain Ca^{2+} levels due to elementary Ca^{2+} release events (ECRE) were monitored using confocal fluorescence microscopy. We studied ECRE in mammalian skeletal muscle following 3 h HP treatments up to 30 MPa to clarify whether RyR induced intracellular microdomain Ca²⁺ dynamics was more susceptible to HP treatment compared to surface membrane ion currents. ECRE frequencies exponentially declined with pressure. ECRE amplitudes and rise times (RT) were quite robust towards HP treatments. In contrast, spatial and temporal ECRE extension showed a tendency towards larger values up to 20 MPa but declined for higher pressures. Activation volumes for pressureinduced persistent ECRE alterations were zero for RT but showed a bimodal behavior for event duration. It seems that although ECRE frequencies are markedly reduced, ECRE morphology is less affected by HP. In particular, RyR opening time is practically unaltered and the observed morphological ECRE changes might reflect alterations in local Ca^{2+} buffers and Ca²⁺ concentration profiles rather than involvement of RyR in mammalian skeletal muscle.

1. Introduction

The vast majority of the earth's biosphere is governed by high hydrostatic pressure environments that can exceed pressures as high as 100 MPa for the deepest ocean trenches [1]. It is still a mystery what precise mechanisms have evolved to enable life exposed to such high pressures and moreover, which compensatory strategies are present in organisms migrating various depth levels, e.g. deep sea fish. High pressure is known to have major effects on biological membranes, e.g. to induce fluid- to gel-like phase transitions [1]. The fluidity of membranes is a prerequisite for their physiological function and depends on factors like structural polymorphism, water content, pH, ionic strength, content of saturated/unsaturated phospholipids, cholesterol, membrane proteins, temperature and pressure itself. Deep-sea animals have been implicated in a regulation of membrane composition in response to environmental challenges termed Joint 21st AIRAPT and 45th EHPRG Int. Conf. on High Pressure Science and TechnologyIOP PublishingJournal of Physics: Conference Series 121 (2008) 112003doi:10.1088/1742-6596/121/1/112003

'homeoviscous adaptation' [2, 3, 4]. Such regulation involves changes in phospholipid or cholesterol fractions, chain length and unsaturation as well as protein content. As a result of this plasticity, simple phase transitions are not common in natural membranes. Another mechanism that has been encountered in deep sea animals is the active production of osmolytes to increased environmental pressures [5, 6]. However, the aforementioned mechanisms are not applicable to diving mammals, e.g. whales or dolphins, or humans in a diving scenario. While diving mammals use sophisticated management of body airspaces or increased oxygen stores in muscle [7], it is predominantly impaired central nervous system function that limits pressure exposure in humans (high pressure nervous syndrome, HPNS) [8].

High pressure (HP) applications to cells and tissues have gained increasing interest in high pressure bioscience in the last years as they allow to elucidate specific interactions that directly or indirectly affect integrative cell functions, e.g. membrane excitability of nerve cells [9] or contractile properties of muscle [10]. Additionally, prolonged HP treatments are suitable to define principle pressure limits for the reversibility of pressure induced specific impairments of cellular and subcellular processes [11, 12].

The activation of skeletal muscle is a complex cascade involving membrane excitation, excitationcontraction (ec) coupling and motor protein activation. Ca^{2+} ions play a crucial role in this finely tuned machinery and perturbations of Ca^{2+} homeostasis are associated with various disease states [13]. Especially the ec-coupling process, involving direct protein-protein interactions of ion channels within the outer cell membrane and within the intracellular membranes of the sarcoplasmic reticulum (SR) are associated with fluctuations in microdomain Ca^{2+} concentrations that take place on a millisecond time scale. Ryanodine receptor Ca^{2+} channels (RyR) within the SR membrane are the site of Ca^{2+} release in muscle and elementary Ca^{2+} release events (ECRE) can be visualized using fluorescent Ca^{2+} dyes in conjunction with confocal microscopy [14, 15, 16].

We have previously shown that there is a rather sharp pressure limit for the reversibility of high pressure induced impairment of contractility [17], membrane function [18] and intracellular global Ca^{2+} homeostasis [19] around 20 MPa to 25 MPa in 3 h long-term pressure exposures. Organelle membranes, such as the SR, have been suggested to be much more susceptible to HP compared to plasma membranes due to their lower cholesterol content [20].

In the present experimental study, we were in particular interested whether prolonged high pressure treatments up to 30 MPa affect elementary Ca^{2+} release events in mammalian skeletal muscle recorded in the post-decompression phase. For this, we evaluated ECRE morphology and frequencies using confocal laser scanning microscopy. Our results show quite unexpected results of relatively stable spatio-temporal morphological parameters of isolated ECRE but lower overall ECRE frequencies following the HP treatment. The results suggest that albeit a larger pressure sensitivity of the SR membrane, channel clusters herein seem to be more pressure resistant compared to ion channels of the plasma membrane.

2. Methods

2.1. Prolonged high pressure treatment of mammalian muscle

For this study, *extensor digitorum muscle* (edl) from adult C57 mice were used. All experiments complied with the guidelines laid down by the Local Animal Care Committee. The preparation of muscles is described in detail in [17]. Excised edl muscles were transferred into small Eppendorff caps completely filled with Ringer solution (see 2.3) and sealed for high pressure treatment. The caps were then inserted into the high pressure vessel that had been pre-cooled to 4 °C. The pressure vessel consisted of an autoclave (PN2500, Wepuko Hydraulics, Metzingen, Germany; effective chamber volume: 4 ml) that was connected to a manual hand spindle (SITEC-Sieber Engineering, Ebmatingen, CH) for pressurization. Water was used as pressurization medium (10 MPa per 0.053% volume change, [17]). A thermoelement to monitor temperature was coupled

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into the vessel and pressure was digitally monitored from a probe within the pressure tubes. To avoid adiabatic heating during pressurization (that additionally was minimized by a very slow pressurization protocol, see below), the autoclave was immersed in a basin filled with a water-glycerol-ethanol mixture kept at a constant temperature of 4 $^{\circ}C$ [17].

Pressurization was carried out using a standardized slow pressurization protocol [17, 18, 19]. Briefly, pressure was increased at a rate of 0.2 MPa per min up to 6 MPa and then at a rate of 0.6 MPa per min until the end pressure of either 10 MPa, 15 MPa, 20 MPa or 30 MPa was reached. This was shown to maximize survival of individual cells [17]. Pressure was kept constant for 3 h and decompression was performed using the reverse procedure.

2.2. Single fibre preparation and post-decompression confocal Ca^{2+} fluorescence microscopy

Single muscle fibres from the the high pressure treated muscle were mechanically dissected while bathing the muscle in relaxing solution (see 2.3). This solution prevents contraction of muscle fibres due to the very low concentration of Ca^{2+} ions and the presence of adenosine-triphosphate (ATP) that relaxes muscle fibres [15]. Control muscles were kept at atmospheric pressure and at 4 °C for the same time period as needed for the complete high pressure protocol and then further processed the same way as high pressure treated muscle. Single fibres were chemically permeabilized using the membrane detergent saponin (Sigma Aldrich, Taufkirchen, Germany, 0.01 % w/v dissolved in relaxing solution) for 2 min. After washout, the solution was exchanged to an internal solution containing high K-glutamate (125 mM, see below). The Ca^{2+} dye Fluo-4 was added to the internal solution to a final concentration of 90 μ M and incubated for ~ 15 min before confocal recording of ECRE. The dye was excited with the 488 nm laser line of an Ar⁺ ion laser and the fluorescence signal was collected between 515 nm and 560 nm using an inverted laser scanning microscope (Leica SP2, Leica Microsystems, Mannheim, Germany). ECRE recordings in single fibres usually started 30 min after the decompression so that the post-decompression phase was considered a new steady-state [18].

2.3. Physiological solutions

Isotonic Ringer solution contained (mM): NaCl 145, KCl 5, CaCl₂ 2.5, MgCl₂ 1, glucose 10, Hepes 10, pH 7.4. Relaxing solution contained: K-glutamate 125, Na₂ATP 5, Na₂CP (CP: creatine phosphate) 10, CaCl₂ 0.13, MgCl₂ 6, EGTA 1, glucose 10, Hepes 10, pH 7.0. Internal K-glutamate solution contained: K-glutamate 124, Na₂ATP 5.3, Na₂CP 10.6, CaCl₂ 0.19, MgCl₂ 7.6, EGTA 0.96, glucose 10, sucrose 10, Hepes 10, pH 7.0. In the latter solutions, small amounts of sucrose were added to adjust osmolarity to 310-320 mOsm in order to avoid shrinking or swelling of muscle fibres between solution exchanges that would otherwise affect triad microstructure and ECRE [21]. The composition of the solution was calculated using the program React II (provided by Dr. G.L. Smith, University of Glasgow). For both relaxing and internal solution, nominal free [Ca²⁺] was 100 nM and [Mg²⁺] was 1 mM.

2.4. ECRE recording protocols and data analysis

ECRE frequency and morphology was determined from image time series (XYT) taken from the fibre middle and repetitive recordings of one spatial dimension along a scan line within the fibre area (XT, 'line-scans', see Fig.1). XYT series consisted of 50 to 200 consecutive images (512x512, 0.8 fps). Although present within the fibre and the external space at equal concentrations, Fluo-4 is known to unspecifically bind to sarcomeric proteins, thus increasing the signal contrast within the fibre [22]. From such images (Fig.1), the cell borders can clearly be visualized. The fibre area scanned in the XYT series was calculated from the voxel size at a given zoom factor. XT images were acquired as 512x512 images with a speed of 2.5 ms per line. ECRE (i.e. Ca^{2+} sparks and embers [15]) were automatically detected in the line-scans using an automated denoising, event detection and analysis algorithm based on the 'à trous'

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implementation of the discrete wavelet transform. The IDL source code (IDL Res. Systems, Boulder, CO, USA) and its application both on synthetic and experimental data in different experimental environments has been published elsewhere [23, 24]. Images were background normalized to obtain a F/F_0 representation. Following wavelet-based denoising, automated event detection was performed using a hard-thresholding technique applied to the wavelet scales, from which the original event was reconstructed [23]. The ECRE spatial morphology and kinetics were quantified by fitting a gaussian to the F/F_0 -X plot from which the ECRE amplitude A and the full-width at half-maximum (FWHM) as a measure for the spatial extent of the ECRE were extracted (Fig.1B). The rise-time (RT) was defined as the time from 10 % over baseline F/F_0 to reach the maximum amplitude and the full-duration at half-maximum (FDHM) was a measure for the ECRE duration [25]. In XYT series, ECRE were detected using a modified version of the denoising and detection algorithm that included background estimation over all images [26]. Due to the fast characteristics of ECRE in mammalian muscle with durations between ten and several tens of ms, isolated ECRE did not span multiple images (0.8 Hz frame rate). ECRE were counted and the frequency was expressed as events per s per mm^2 fibre area. All XYT series were included in the analysis. Note that due to the low overall ECRE frequencies, some XYT series contained no events. This, and the fact that during the scanning of the image ECRE may occur randomly in other parts of the image will be missed (ECRE duration \ll image scan duration), will render our mean values for ECRE frequency statistics a lower limit for true ECRE frequencies. Differences in parameters following each pressure treatment compared to controls were assessed using Student's t-test whereas differences among pressure groups were tested for using either one-way ANOVA or pairwise multiple comparison procedures (Holm-Sidak method). P < 0.05 was considered significant. Outliers were detected in the final bin of data of each pressure using a Grubbs-test at the P = 0.05 level.



Figure 1. Example of ECRE morphology analysis from line-scans. A, example image shows Fluo-4 fluorescence of a single fibre showing an ECRE and the localization of the scan line used for XT imaging. Note that the XYT- and line-scan shown was cut from the 512x512 image for better visualization of the Ca²⁺ spark. B, Fluorescence analysis from a line-scan using the event detection algorithm after wavelet based denoising of the image. Cross within the spark indicates region of peak fluorescence.

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2.5. Activation volumes for irreversible, pressure-induced alteration of ECRE dynamics The ECRE dynamics of activation are described by the rise time RT, the ECRE duration (as an apparent measure for activation and inactivation processes) and is represented by the FDHM. From the pressure dependence of these parameters, activation volumes for the pressure-induced alteration of channel kinetics that persist in the post-decompression phase [12] can be formally calculated according to the equation [27]:

$$\left(\frac{\partial ln\kappa}{\partial p}\right)_T = -\frac{\Delta V^{\ddagger}}{RT} \tag{1}$$

where κ denotes the appropriate rate constants κ_{RT} and κ_{FDHM} related to RT^{-1} and FDHM⁻¹, respectively. R and T have their usual thermodynamic meanings. Null hypothesis was tested in case of very small activation volumes.

3. Results

3.1. ECRE frequency following prolonged high pressure treatments

Fig.2A shows a selection of images from a representative XYT series recorded in a single fibre following a 3 h 10 MPa high pressure treatment. At two time points, ECRE within the fibre can be detected (arrows). Fig.2B shows the frequency distribution in several image series from different single fibres for the pressure treatments indicated. ECRE frequency was (mean \pm SEM) 36.0 \pm 15.2 $\frac{ECRE}{mm^{2}\cdot s}$ under control conditions (atmospheric pressure and 4 °C for 3 h, n= 38), 13.7 \pm 2.3 $\frac{ECRE}{mm^{2}\cdot s}$ following 10 MPa (n= 55 XYT scans), 5.4 \pm 1.6 $\frac{ECRE}{mm^{2}\cdot s}$ following 15 MPa n= 39), 19.0 \pm 4.4 $\frac{ECRE}{mm^{2}\cdot s}$ following 20 MPa (n= 26) and 2.5 \pm 2.7 $\frac{ECRE}{mm^{2}\cdot s}$ following 30 MPa (n= 21) treatments. ECRE frequencies following high pressure treatments showed a tendency for smaller values compared to control conditions that became, however, only significant for 15 MPa treatments (P < 0.03). Among pressure treatments, 10 MPa and 20 MPa treatments showed pairwise significantly larger ECRE frequencies compared to 15 MPa and 30 MPa treatments. The decline in ECRE frequency could be described by an exponential with a pressure constant of 13.6 MPa (dashed line in Fig.2B, r² = 0.76).

3.2. ECRE morphology following prolonged high pressure treatments

Fig.3 shows example distribution histograms for FWHM (A, left panel) and FDHM (B, left panel) in control fibres and following prolonged high pressure treatments (A: 15 MPa, B: 20 MPa). In both cases, a right-shift of the histograms towards larger spatial and temporal morphology values could be observed . This trend was also seen when evaluating all pressures up to 20 MPa (right panels in Fig.3 A & B). Interestingly, for pressures larger than 20 MPa (i.e. 30 MPa) this behavior was reversed and values sharply declined. In contrast, amplitudes and rise-times (RT) were less affected by pressure and were not significantly different from controls with the exception of amplitudes following 15 MPa treatments that were significantly increased. Values among the pressure treated groups were never significantly different as indicated by the bars in Fig.3. For the ECRE morphologies shown in the figure, n= 139 ECRE under control conditions, n= 16 ECRE following 10 MPa, n= 12 following 15 MPa, n= 17 following 20 MPa and n= 3 following 30 MPa treatments were evaluated. Note that for pressures above 20 MPa survival of muscle cells sharply declined during the prolonged 3 h HP treatment in agreement with our previous observations in mammalian muscle [17, 18].

3.3. Activation volumes for pressure induced alteration of ECRE kinetics

Fig.4 shows the rate constant vs. pressure relationships according to eq.1 for the rise-time (RT) and FDHM of ECRE. From this, the activation volumes ΔV^{\ddagger} for the pressure-driven irreversible alteration of release channel function that persisted within the post-decompression phase were



Figure 2. Pressure dependence of ECRE frequency recorded in the post-decompression phase following 3 h high pressure treatments. A, example XY Fluo-4 fluorescence images taken at the indicated time points in a single fibre following a 3 h 10 MPa treatment. Arrows: ECRE. B, ECRE frequency-pressure relation. *: P < 0.05 vs. control.

calculated. ΔV^{\ddagger} was virtually zero for RT indicating no alteration of ECRE activation. On the contrary, FDHM showed ΔV^{\ddagger} values of -18.3Å^3 when considering the whole pressure range. The negative value of ΔV^{\ddagger} would suggest a negative volume change of the underlying process that is accelerated by pressure. However, FDHM tended to increase with pressure up to 20 MPa resulting in temporally more broadened ECRE. Therefore, we assumed a twostep process for pressure-induced alteration in ECRE mechanisms underlying FDHM similar as previously described by us for the activation of tubular Ca²⁺ currents [12]. When applying eq.1 to the pressure range up to 20 MPa, ΔV^{\ddagger} was $\sim +60 \text{Å}^3$ and reversed for larger pressures ($\Delta V^{\ddagger} \sim -225 \text{Å}^3$, Fig.4B).

4. Discussion

In the present study we present, to our knowledge for the first time, a detailed analysis of microdomain Ca^{2+} dynamics in mammalian skeletal muscle following prolonged 3 h HP treatments. Spontaneous elementary Ca^{2+} release events (ECRE) in permeabilized mammalian skeletal muscle fibres have been extensively investigated and characterized in the last couple of years [15, 24, 28, 29].

4.1. Pressure effects on ECRE frequencies

Under control atmospheric conditions, ECRE frequencies were somewhat lower than previously described (~ $40s^{-1}mm^{-2}$ in the present study compared to ~ $150s^{-1}mm^{-2}$ [15]). Due to the stochastic character of ECRE appearance in XYT images, this frequency is affected by various factors like the scanning speed, proportion of fibre area within the image, cytosolic Ca²⁺ and Mg²⁺ concentration [25, 28] or filling of the SR. Following HP treatments, ECRE frequency declined exponentially. The pressure constant of ~ 14 MPa detected in the post-decompression phase is larger than the one found for global decline in resting cytosolic Ca²⁺ fluorescence (~ 5 MPa) but smaller than the decline in mitochondrial Ca²⁺ fluorescence (~ 20 MPa) detected during pressurization using high pressure microscopy [19]. However, these conditions cannot be directly compared. From our previous interpretations, we suggested a pressure-induced Ca²⁺



Figure 3. Pressure dependence of ECRE morphologies recorded in the post-decompression phase following 3 h high pressure treatments. Histograms of data distribution (left) and pressure dependence of mean values (right) for FWHM (A) and FDHM (B). C, D, pressure dependence of mean values for ECRE amplitudes and RT, respectively. *: P < 0.05 vs. control.

leakage from the SR <u>during</u> the HP treatment, either via increased ECRE frequency, reduced SR pump activity or a combination of both [19]. Similar results were also obtained in pressurized SR vesicles from rabbit muscle [30]. It should be noted that in the current study, ECRE were detected in the post-decompression phase. Therefore, a lower overall ECRE frequency would be in agreement with a lower filling of the SR under these conditions.



Figure 4. Apparent activation volumes for pressure driven irreversible alteration of ECRE RT (**A**) and FDHM (**B**) detected in the post-decompression phase after 3 h prolonged HP treatment. See text for details.

4.2. Pressure effects on ECRE morphologies

ECRE parameters of chemically permeabilized fibres under control conditions had amplitudes similar to those given by Zhou et al. [25] but smaller than originally described by us in a previous study [15]. This can be partly explained by a smaller dynamic range for Ca^{2+} fluorescence in the present study due to larger photomultiplier voltages used. This would also result in amplified background fluorescence and reduced signal range. Subsequently, as a portion of low-amplitude ECRE will be left undetected, ECRE frequencies might actually be higher than in the current setting. However, this does not affect our interpretations regarding pressure as settings were kept constant.

Rise times (RT) that reflect the RyR channel open time [28] were similar to previous values [15, 25]. The relative pressure resistance of ECRE amplitudes and RT that is also reflected by the latter's minute activation volume suggest no persistent irreversible alteration of RyR opening and maximum Ca^{2+} -release into the narrow, diffusion restricted triad space [31]. This parallels recent findings from plasma membrane Na⁺ channels that also showed no pressure-induced alteration in activation and even inactivation kinetics despite a reduction in number of functional channels [12]. However, tubular Ca^{2+} channels were much more susceptible to the pressure treatment with markedly slowed activation and inactivation kinetics. The increase in FDHM for ECRE with pressure may also reflect a tendency towards increased inactivation kinetics for RyR, however, this cannot unambiguously be judged at the present state as FDHM is also determined by RT and cytosolic Ca^{2+} buffer properties. Similarly, the spatial and temporal broadening of ECRE found after HP treatments might also reflect altered buffer properties rather than inactivation kinetics of the RyR itself, although further research is needed to address this point.

4.3. Pressure sensitivity of RyR

From the decrease in ECRE frequency a reduction in number of functional RyR by pressure similar to that found for Na⁺ and Ca²⁺ channels can be suggested. However, the pressure sensitivity of RyR seems to be more similar to surface Na⁺ channels but much less pronounced compared to L-type Ca²⁺ channels (DHPR) in our preparation. This is particularly interesting as DHPR and RyR are mechanically linked via a direct protein-protein interaction to confer membrane excitation to SR Ca²⁺ release. Another point to be addressed is the relatively lower

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cholesterol content of endoplasmic reticulum membrane [20]. This would suggest a much higher pressure sensitivity of this organelle and its embedded ion channels. The contrary seems to be true at least for the RyR itself which did not show a major breakdown of function following prolonged high pressure treatments.

Our results are the first to show that ion channels in an intracellular environment may be relatively more protected from high hydrostatic pressure effects compared to surface ion channels in mammalian skeletal muscle.

References

- R Winter and W Dzwolak. Exploring the temperature-pressure configurational landscape of biomolecules: from lipid membranes to proteins. *Philos Transact A Math Phys Eng Sci*, 363(1827):537–562, 2005.
- [2] HMW Okland, IS Stoknes, JF Remme, M Kjerstad, and M Synnes. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comp Biochem Physiol B Biochem Mol Biol, 140(3):437–443, Mar 2005.
- [3] JR Hazel and EE Williams. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog Lipid Res, 29(3):167–227, 1990.
- [4] AG Macdonald and AR Cossins. The theory of homeoviscous adaptation of membranes applied to deep-sea animals. Symp Soc Exp Biol, 39:301–322, 1985.
- [5] AL Samerotte, JC Drazen, GL Brand, BA Seibel, and PH Yancey. Correlation of trimethylamine oxide and habitat depth within and among species of teleost fish: an analysis of causation. *Physiol Biochem Zool*, 80(2):197–208, 2007.
- [6] PH Yancey. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol, 208(Pt 15):2819–2830, 2005.
- [7] PJ Butler and DR Jones. Physiology of diving of birds and mammals. Physiol Rev, 77(3):837–899, 1997.
- [8] AE Talpalar and Y Grossman. CNS manifestations of HPNS: revisited. Undersea Hyperb Med, 33(3):205–210, 2006.
- [9] AA Harper, AG Macdonald, and KT Wann. The action of high hydrostatic pressure on the membrane currents of Helix neurones. J Physiol, 311:325–339, 1981.
- [10] NS Fortune, MA Geeves, and KW Ranatunga. Contractile activation and force generation in skinned rabbit muscle fibres: effects of hydrostatic pressure. J Physiol, 474(2):283–290, 1994.
- [11] L Smeller. Pressure-temperature phase diagrams of biomolecules. Biochim Biophys Acta, 1595(1-2):11-29, 2002.
- [12] O Friedrich, KR Kress, M Hartmann, B Frey, K Sommer, H Ludwig, and RHA Fink. Prolonged high-pressure treatments in mammalian skeletal muscle result in loss of functional sodium channels and altered calcium channel kinetics. *Cell Biochem Biophys*, 45(1):71–83, 2006.
- [13] MW Berchtold, H Brinkmeier, and M Muentener. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol Rev*, 80(3):1215–1265, 2000.
- [14] SQ Wang, C Wei, G Zhao, DXP Brochet, J Shen, LS Song, W Wang, Di Yang, and H Cheng. Imaging microdomain Ca2+ in muscle cells. Circ Res, 94(8):1011–1022, 2004.
- [15] WG Kirsch, D Uttenweiler, and RHA Fink. Spark- and ember-like elementary Ca2+ release events in skinned fibres of adult mammalian skeletal muscle. J Physiol, 537(Pt 2):379–389, 2001.
- [16] H. Cheng, W. J. Lederer, and M. B. Cannell. Calcium sparks: elementary events underlying excitationcontraction coupling in heart muscle. *Science*, 262(5134):740–744, Oct 1993.
- [17] KR Kress, O Friedrich, H Ludwig, and RHA Fink. Reversibility of high pressure effects on the contractility of skeletal muscle. J Muscle Res Cell Motil, 22(4):379–389, 2001.
- [18] O Friedrich, KR Kress, H Ludwig, and RHA Fink. Membrane ion conductances of mammalian skeletal muscle in the post-decompression state after high-pressure treatment. J Membr Biol, 188(1):11–22, 2002.
- [19] O Friedrich, F vWegner, M Hartmann, B Frey, K Sommer, H Ludwig, and RHA Fink. 'In situ' high pressure confocal Ca(2+)-fluorescence microscopy in skeletal muscle: a new method to study pressure limits in mammalian cells. Undersea Hyperb Med, 33(3):181–195, 2006.
- [20] P Mentre, L Hamraoui, G Hui Bon Hoa, and P Debey. Pressure-sensitivity of endoplasmic reticulum membrane and nucleolus as revealed by electron microscopy. *Cell Mol Biol (Noisy-le-grand)*, 45(3):353– 362, 1999.
- [21] CA Martin, N Petousi, S Chawla, AR Hockaday, AJ Burgess, JA Fraser, CLH Huang, and JN Skepper. The effect of extracellular tonicity on the anatomy of triad complexes in amphibian skeletal muscle. J Muscle Res Cell Motil, 24(7):407–415, 2003.

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- [22] S Hollingworth, C Soeller, SM Baylor, and MB Cannell. Sarcomeric Ca2+ gradients during activation of frog skeletal muscle fibres imaged with confocal and two-photon microscopy. J Physiol, 526 Pt 3:551–560, 2000.
- [23] F v Wegner, M Both, and RHA Fink. Automated detection of elementary calcium release events using the a trous wavelet transform. *Biophys J*, 90(6):2151–2163, 2006.
- [24] F v Wegner, M Both, RHA Fink, and O Friedrich. Elementary Ca2+ release events in mammalian skeletal muscle: effects of the anaesthetic drug thiopental. J Muscle Res Cell Motil, 27(5-7):315–326, 2006.
- [25] J Zhou, G Brum, A Gonzalez, BS Launikonis, MD Stern, and E Rios. Ca2+ sparks and embers of mammalian muscle. Properties of the sources. J Gen Physiol, 122(1):95–114, 2003.
- [26] F v Wegner, M Both, RHA Fink, and O Friedrich. Fast xyt imaging of elementary calcium release events in muscle with multifocal multiphoton microscopy and wavelet denoising and detection. *IEEE Trans Med Imaging*, in press, 2007.
- [27] R Meyer and SH Heinemann. Temperature and pressure dependence of Shaker K+ channel N- and C-type inactivation. Eur Biophys J, 26(6):433–445, 1997.
- [28] J Zhou, BS Launikonis, E Rios, and G Brum. Regulation of Ca2+ sparks by Ca2+ and Mg2+ in mammalian and amphibian muscle. An RyR isoform-specific role in excitation-contraction coupling? J Gen Physiol, 124(4):409–428, 2004.
- [29] L Csernoch, J Zhou, MD Stern, G Brum, and E Rios. The elementary events of Ca2+ release elicited by membrane depolarization in mammalian muscle. J Physiol, 557(Pt 1):43–58, 2004.
- [30] A Okamoto, A Suzuki, Y Ikeuchi, and M Saito. Effects of high pressure treatment on Ca2+ release and Ca2+ uptake of sarcoplasmic reticulum. *Biosci Biotechnol Biochem*, 59(2):266–270, 1995.
- [31] YH Jiang, MG Klein, and MF Schneider. Numerical simulation of Ca(2+) "Sparks" in skeletal muscle. Biophys J, 77(5):2333–2357, 1999.