system cells to these transient peak pressures per se do not cause cellular excitation. Instead, it is the concomitant shear forces that reproducibly activate the cells. The resultant signal is amplified and spread by cell-cell propagation arising from a small number of cells that are directly affected by the shear forces (Ravin et al. 2012). Astrocytes have long-range calcium signaling systems that propagate as calcium waves instead of action potentials and modulate neuronal and CNS activity. Blast shock waves simulating those initiating primary blast injury cause a cascade of events in human CNS dissociated cultures in which calcium is elevated mainly in astrocytes, leading to a propagation of calcium waves throughout the network, mediated by ATP signaling. We found that P2 receptor antagonists are able to reduce this response suggesting a potential therapeutic target for treating TBI.

3172-Pos Board B327
Insulin Induces both H2O2 Production and IP3-Dependent Mitochondria Ca2+ Uptake. H2O2 Oxidizes RyR to Elicit Ca2+ Release and GLUT4 Translocation in Skeletal Muscle Cells
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We have described that insulin induces both NADPH oxidase-dependent ROS production and Ca2+ increase in skeletal muscle cells and although insulin-regulated GLUT4 traffic has been widely explored, the role of intracellular Ca2+ handling in this process is poorly understood. We studied insulin-induced GLUT4 traffic in GLUT4myc permanently transfected L6 skeletal muscle cells.
Insulin-induced exofacial exposure of myc epitope, glucose uptake and Akt activation were independent of extracellular Ca2+ levels. Insulin increased H2O2 production measured with the cytosolic molecular sensor HyPer which was inhibited by NAC, a potent antioxidant. p47 substrate of NADPHox labeled only differentiated myotubes. Antioxidant agents, NAC and trolox partly reduced the externalization of myc epitope as did a specific inhibitor of NADPHox and overexpression of catalase. Superoxide dismutase 1 appears to potentiate the insulin effect. H2O2 induced Ca2+ release, which was inhibited by pre-incubation with ryanodine. Cytosol directed parvalbumin-DsRed reduced insulin-dependent exofacial exposure of myc epitope. Moreover, both ryanodine and xestospongin B partly inhibited myc exposure with an additive effect. Insulin induced an increase in the S-glutathionylation state of RyR1, detected by proximity ligation assay. Insulin induced an increase in mitochondrial ROS and Ca2+ levels measured with ratiometric HyPer and PeriCam respectively. The mitochondrial potential and oxygen consumption were also increased by insulin and inhibited by pre-incubation with IP3R blockers. Insulin-dependent GLUT4myc translocation to cell surface was strongly reduced in presence of ruthenium red. These data suggest that insulin induces an increase in cytoplasmic Ca2+ levels by NADPH-dependent RyR1 modifications and IP3R-dependent mitochondrial regulation in skeletal muscle myotubes.
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3173-Pos Board B238
Non-Ligand Mobilization of Intracellular Free Calcium by Nanosecond Pulsed Electric Field (nsPEF)
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nsPEF makes membrane and intracellular structures in living cells permeable to ions and small molecules thus can be used to modulate cell’s signaling pathways and homeostasis. We utilized Fura-2 fast ratiometric recordings to measure changes of intracellular free calcium concentration ([Ca2+]i) in single CHO cells stimulated by 10, 60 and 300 ns PEF. 10 and 300 ns PEF were used for comparison with 60 ns PEF in regard to their potency to make plasma membrane (PM) and/or endoplasmic reticulum (ER) permeable to Ca2+ ions. Single 60 ns PEF evoked transient [Ca2+]i, rise due to Ca2+ influx via PM and Ca2+ efflux from ER with thresholds ~9 and ~19 kV/cm, respectively. The amplitude of Ca2+ rise increased with nsPEF intensity linearly by 8-10 nM per 1 kV/cm (PM) or 5-6 nM per 1 kV/cm when permeabilization of ER was assessed in Ca2+ free solution. This rate of change increased dramatically (to 174 nM per 1 kV/cm) when amplitude of Ca2+ rise reached 100-150 nM. Depleting Ca2+ content of ER showed that such increase was a result of calcium induced calcium release (CICR) activation. Specific blockers 2-APB and dantrolene proved that the activation of IP3 receptors was responsible for physiological mechanism of CICR triggered by nsPEF. 10, 60 and 300 ns PEF had different efficiencies in regard to PM and ER permeabilization for Ca2+ ions. The thresholds of PM and ER permeabilization were indistinguishable for 10 ns pulse while for 60 ns pulse the ER threshold was twofold higher. 300 ns pulse had further reduced potency towards ER permeabilization in comparison to either 10 or 60 ns pulses. These results show that nsPEF can be effectively used to manipulate [Ca2+]i, and activate intracellular signaling pathways bypassing PM receptors.
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Exocytosis & Endocytosis
3174-Pos Board B329
The Regulatory Catalytic Step in Dynamo-driven Membrane Fission
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Pure lipid membranes undergo topological transitions, such as fusion or fission, if external stresses are applied. It follows that proteins conducting topological machinery and modeling in vivo and in vitro to apply Rp-2 and GTPase GRP2 receptor antagonists are able to reduce this response suggesting a potential therapeutic target for treating TBI.

3175-Pos Board B330
Activation of Membrane Fission by Local Elastic Energy Increase at the Edge of Dynamin
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Membrane fission requires the constriction and breakage of a transient neck, splitting one membrane compartment into two. In endocytosis, the GTPase Dynamin forms a helical coat that constrains membrane necks of Clathrin-coated pits to promote their fission. Dynamin constriction is necessary but not sufficient, questioning the minimal requirements for fission. Here we show that fission occurs at the edge of the Dynamin coat, where it is connected to the uncoated membrane. At this location, the specific shape of the membrane increases locally its elastic energy, facilitating fission by reducing its energy barrier. We predict that fission kinetics should depend on tension, bending rigidity and the Dynamin constructory step. To verify that fission times depend on membrane tension in controlled conditions in vitro and in Clathrin-mediated endocytosis in vivo. By numerically estimating the energy barrier from the increased elastic energy, and measuring the Dynamin torque, we show that: 1- Dynamin torque, \( \tau \) in Nm.m, is huge but necessary to achieve constriction, and 2- Dynamin work sufficiently reduces the energy barrier to promote spontaneous fission.

3176-Pos Board B331
The Dynamic pH Domain Variable Loop 1 (VL1) is an Assembly-Independent Sensor of Membrane Curvature
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The role of the membrane-inserting pleckstrin homology (PH) domain variable loop 1 (VL1) in dynamin-catalyzed membrane fission remains unclear. A membrane bending function has been proposed based on the bilayer-couple mechanism and the inability of a membrane insertion-defective VL1 point mutant