used to monitor the formation of such architectures and to study of their ion permeability.

Smooth & Skeletal Muscle Electrophysiology

854-Pos Board B733

ATP Regulates Mammalian Neuromuscular Transmission by Dramatically Decreasing the Resting Muscle Chloride Conductance via P2Y1

Andrew Voss, Julio Vergara.
UCLA Medical School, Los Angeles, CA, USA.

In skeletal muscle, extracellular ATP arises locally from released synaptic vesicles and broadly from active muscle fibers. We examined the direct effect of ATP on developed, innervated mammalian skeletal muscle (*ex vivo* rat *levator auris longus*) using measurements of individual muscle fibers with two intracellular microelectrodes. Near the neuromuscular junction (NMJ), 20μM ATP prolonged the decay of miniature endplate potentials (mEPP tau) by 31 ± 7.5% and extended the membrane potential responses induced by step current pulses ($\Delta E_{m(Ipulse)}$) by 59 ± 3.0%. These responses correlated with an increased input resistance (R_{in}) of 31 ± 2.0%. Analogous increases in non-synaptic regions reveal that ATP acts throughout the muscle fiber. In contrast, 50μM adenosine, a well-characterized metabolite of ATP, induced no apparent increase in mEPP tau, $\Delta E_{m(Ipulse)}$ or R_{in}. Applying established pharmacology showed that the ATP receptor mediating these effects is likely the G-protein-coupled P2Y1, since 20μM ATPγS and ADPβS (slowly hydrolysable analogs of ATP and ADP) and 10μM 2-methylthioadenosine-5′-O-diphosphate (2MeSADP) mirrored the effect of ATP. Furthermore, 20μM MRS2179 blocked activation by 20μM ADPβS. The significant effect of ATP on R_{in} was presumably achieved by closing chloride channels, which maintain the largest conductance of resting muscle. This was confirmed with the chloride channel blocker anthracene-9-carboxylic acid (200-500μM), which mimicked ATP and prevented additional increases by 20μM ATPγS. This ATP response appears unique to mammals, as 50μM ATP induced no analogous increase in the archetypical frog NMJ. Our work outlines a novel mechanism by which physiological levels of ATP regulate synaptic transmission and dramatically alter the resting membrane properties of mammalian skeletal muscle. This has potential implications for the physiology of muscle excitability and fatigue, and the pathophysiology of Thomsen and Becker myotonias.

855-Pos Board B734

Cycloxygenase-2 Inhibitor Celecoxib Is A Potent Activator Of Vascular KCNQ K^+ Channels And An Inhibitor Of L-type Ca^{2+} Channels

Lyubov I. Brueggemann, Kenneth L. Byron.
Loyola University Chicago, Maywood, IL, USA.

Cyclooxygenase-2 (COX-2) inhibitors are important members of the family of non-steroidal anti-inflammatory drugs (NSAIDs). Celebrex® (celecoxib) and Vioxx® (rofecoxib) were introduced in 1999 and rapidly became the most frequently prescribed new drugs in the United States. Vioxx® was voluntarily withdrawn from the market because of a reported increased risk of myocardial infarction and stroke in patients taking the drug for prolonged periods of time. Celecoxib has been reported to inhibit several classes of ion channels, but its effects on vascular smooth muscle ion channels have not been described. Using whole-cell perforated patch clamp techniques we examined effects of celecoxib on K^+ and Ca^{2+} currents in A7r5 rat aortic smooth muscle cells. Application of 10μM celecoxib enhanced K^+ current by 2-3 fold and substantially inhibited Ca^{2+} currents with an apparent positive shift in the voltage-dependence of activation. Both effects were reversible on washout. Nether rofecoxib (10μM), another selective COX-2 inhibitor, nor diclofenac (10μM), a nonselective COX inhibitor, affected Ca^{2+} or K^+ currents in A7r5 cells. We previously reported that KCNQ5 channels are the predominant K^+ channels determining outward potassium current at negative membrane potentials in A7r5 cells. We estimated cumulative dose-response curve of celecoxib on isolated KCNQ5 currents. Celecoxib enhances KCNQ5 current in 3.5 fold with an EC_{50} of 6.9 ± 1.5 μM, without shifting the activation curve. Celecoxib (10μM) was unable to restore KCNQ current inhibited by 100 pM vasopressin (AVP, vasoconstrictor hormone) or 1nM PMA (PKC activator) but inhibition of L-type Ca^{2+} currents (with a positive shift of activation) persisted. The effects of celecoxib, but not rofecoxib, on vascular Ca^{2+} and K^+ channels may explain the differential risks of cardiovascular diseases in patients treated with Celebrex® or Vioxx®.

856-Pos Board B735

Basal Ca^{2+} Entry Controls NFAT Transcriptional Activity, Proliferation And Migration Of Human Vascular Smooth Muscle Cells

Alain Coulombe[1], Yassine Sassi[1], Fabrice Atassi[1], Lahouaria Hadri[2], Stephane N. Hatem[1], Roger Hajjar[2], Anne-Marie Lompre[1], Larissa Lipskaia[2].
[1]UMRS621, INSERM Université Pierre et Marie Curie-Paris 6, Paris, France, [2]Mount Sinai School of Medicine, New York, NY, USA.

We have previously shown that in the rat the sarco/endoplasmic reticulum calcium ATPase, SERCA2a, controls vascular smooth muscle cell (VSMC) proliferation through modulation of the activity of the transcription factor NFAT. Here we tested the hypothesis that SERCA2a, controls human VSMC proliferation by inhibiting voltage-independent Ca^{2+} entry and the NFAT transcription pathway.

Single-channel recording showed that proliferating VSMC possessed high spontaneous voltage-independent basal channel activity (nPo: 0.415 ± 0.050; n=31). SERCA2a gene transfer, using an adenoviral vector, to proliferating VSMCs abolished the activity of these channels (0.091 ± 0.096; n=26) compared to control cells infected with an adenovirus encoding GFP (0.315 ± 0.035; n=42). SERCA 2a gene transfer also down-regulated expression of transient receptor potential channels TRPC4, TRPC5 and stromal interacting molecule 1 (STIM1) suggesting their involvement in the channel activity.

SERCA2a gene transfer also inhibited VSMC proliferation and migration as well as the NFAT activity. Furthermore, NFAT activity was inhibited by depolarization-induced Ca^{2+} influx and by several calcium channel inhibitors such as nifedipine, mibefradil, carboxyamidotriazole and 2-aminoethoxydiphenyl, suggesting involvement of different types of Ca^{2+} channels in the control of NFAT and proliferation. Our data suggest that different types of Ca^{2+} channels are involved in the control of NFAT transcriptional activity and proliferation. By controlling submembrane Ca^{2+} concentration, SERCA2a finely regulated basal Ca^{2+} current *via* regulation of translocation and/or expression of STIM1 and TRPCs.

Acetylcholine Receptors

857-Pos Board B736

Cellular Basis Of Nicotine-induced nAChr Upregulation

Rahul Srinivasan, Rigo Pantoja, Sindhuja Kadambi, Henry A. Lester.
Caltech, Pasadena, CA, USA.

The upregulation and preferential assembly of high sensitivity $(\alpha4)_2(\beta2)_3$ neuronal nicotinic acetylcholine receptors (nAChRs) contribute to aspects of nicotine addiction such as sensitization and tolerance. The cellular mechanisms of these events, however, remain elusive. We employed fluorescently tagged nAChRs to study plasma membrane upregulation by total internal reflection fluorescence microscopy (TIRFM) as well as changes in intracellular receptor stoichiometry using pixel-based Förster's resonance energy transfer (FRET). To delineate the effect of β2 on α4 nAChR trafficking in the absence of nicotine, mouse neuroblastoma (N2a) cells were transiently transfected with either α4-meGFP/wildtype β2 or α4-meGFP/wildtype β4 subunits (m = monomeric; e = enhanced) and imaged at 48 h post-transfection by TIRFM. To set TIRFM parameters, cells were co-transfected with the pCS2-mcherry plasmid, which served as a reference probe. pCS2-mcherry expresses mcherry with a lyn kinase membrane localization signal, allowing visualization of the PM using red emission from mcherry. The α4-meGFP reporter was used to detect receptor expression at the PM. Results showed that the α4-meGFP/wildtype β2 receptors trafficked to the PM in ~10 % of the cells while ~90 % of imaged cells displayed α4-meGFP/wildtype β4 at the PM. In the presence of nicotine (0.1 μM for 48 h), α4-meGFP/wildtype β2 transfected N2a cells displayed a clear increase in receptor trafficking to the PM when visualized using TIRFM. Pixel-based sensitized emission FRET studies on N2a cells transiently transfected with an α4-mcherry and β2-meGFP FRET pair showed that chronic nicotine exposure (1 μM, 24 h) resulted in an increase in assembly of the high sensitivity $(\alpha4)_2(\beta2)_3$ population of receptors, a phenomenon that was blocked by co-incubation with the competitive nAChR antagonist, DhβE (1 μM). These preliminary results point to a modulatory role of β2 subunits as well as a possibly important role for activity-dependent receptor upregulation.

858-Pos Board B737

α-7 Nicotinic Receptor Positive Allosteric Modulators have Varying Kinetic Effects on Desensitization and Current Amplitude

Sarita Yeola, Flora Jow, Yan Tony Lee, Angela Kramer, Tim Lock, John Dunlop, Ravi Peri, Mark R. Bowlby.
Wyeth Research, Princeton, NJ, USA.

The α-7 nicotinic receptor is an important neuronal subtype of ligand gated ion channels. It forms a pentameric homomer that is activated by acetylcholine or nicotine to evoke rapidly activating and desensitizing currents. Activation of α-7 receptors has been implicated as a therapeutic strategy in schizophrenia and Alzheimer disease. Small molecule positive allosteric modulators (PAM's) have been shown to enhance α-7 currents, and are classified as type 1 or type 2 PAMs. The type 1 PAMs enhance the current amplitude but do not alter