

used to guide or parameterize our modeling, and also can be used to compare with our simulation results. Current simulation results show that different adhesion energetic parameters can lead to different multi-cellular patterns, consistent with the experimental data. Future improvements and potential applications are also discussed.

#### 1449-Pos Board B293

##### Relating Electrical Conductance, Connexin 43 Immunostaining, and Cell Shape in Micropatterned Cardiac Cell Pairs

Megan L. McCain<sup>1</sup>, Thomas Desplantez<sup>2</sup>, Nicholas A. Geisse<sup>1</sup>, Helene Oberer<sup>2</sup>, Kevin K. Parker<sup>1</sup>, André G. Kléber<sup>2</sup>.

<sup>1</sup>Harvard University, Cambridge, MA, USA, <sup>2</sup>University of Bern, Bern, Switzerland.

Gap junctions are responsible for cell-cell electrical coupling and maintaining normal cardiac conduction patterns. Connexin 43 (Cx43) is the dominant gap junction protein in ventricular myocytes. Although the relationship between Cx43, conduction, and tissue structure have been extensively studied in engineered monolayers of cardiac tissue, there have been few studies comparing conductance, Cx43, and cell shape on the cell-cell level. We have used micropatterning to control the dimensions of myocyte pairs and study electrophysiological properties at very high resolution. We hypothesized that conductance and Cx43 immunostaining would be directly correlated. We also sought to relate our electrophysiological measurements to cell shape. Using a dual voltage clamp system, we measured the conductance of micropatterned ventricular myocyte pairs, and subsequently fixed and immunostained the same cells for Cx43. Thus, we compared conductance and Cx43 immunostaining serially in the same cell pair. The volume of Cx43 immunostaining was determined using confocal microscopy and quantitative software programs. Using brightfield images, we measured cell dimensions and 2-dimensional cell-cell contact. We studied three types of rectangular cell pairs with varying length to width aspect ratios (3.33:1, 5:1, 6.67:1). The average length of the cell-cell junction ( $R^2=0.99$ ,  $n=23$ ), average conductance ( $R^2=0.92$ ,  $n=22$ ), and average Cx43 immunostaining ( $R^2=0.85$ ,  $n=22$ ) increased linearly relative to the aspect ratio. We found a linear relationship between Cx43 immunostaining and conductance ( $R^2=0.70$ ,  $n=22$ ). A weaker linear relationship was found between conductance and 2-dimensional length of the cell-cell junction ( $R^2=0.51$ ,  $n=23$ ). Our results suggest that cell pairs nearly maximize their contact area, which contributes to increases in both Cx43 and conductance. Cell pairs with higher length-width aspect ratios have more cell-cell contact and therefore higher Cx43 density and conductance. However, Cx43 density is the most important determinant of conductance.

#### 1450-Pos Board B294

##### Role of connexin 32 hemichannels in ATP release from Schwann cells

Carlos Solsona<sup>1,2</sup>, Ezequiel Mas del Molino<sup>1,2</sup>, Xenia Grandes<sup>1</sup>, Laia Bahima<sup>1</sup>, Mireia Martin Satué<sup>1,2</sup>, Rafel Puchal<sup>3</sup>, Luis C. Barrio<sup>4</sup>, Jordi Marsal<sup>1,2</sup>.

<sup>1</sup>University of Barcelona-Idibell, Hospitalet de Llobregat, Spain,

<sup>2</sup>CIBERNED, Barcelona, Spain, <sup>3</sup>Hospital Universitari de Bellvitge (HUB), Hospitalet de Llobregat, Spain, <sup>4</sup>Hospital Ramon y Cajal, Madrid, Spain.

The X-linked Charcot Marie Tooth (CMTX) is an inherited disease due to mutations in connexin 32 (Cx32) gene expressed in Schwann cells (SC) of peripheral nerves. In SC, Cx32 localizes in the paranodes, in the Schmidt-Lanterman incisures and in the cell surface. Cx32 can form "reflexive" gap junction channels as well as functional hemichannels open upon membrane depolarization. We have explored the permeability of Cx32 hemichannels to ATP, in SC and in a heterologous expression system. Murine sciatic nerve trunks release ATP under electrical or mechanical stimulation, as determined by the luciferase reaction. Luminescence imaging revealed that ATP release is especially intense at the SC paranodes, which contain the highest immunofluorescent label for Cx32. Cultured adult SC have a high expression of Cx32 and under mechanical stimulus release ATP being insensitive to exocytosis blockers like brefeldin A. In *Xenopus* oocytes expressing human Cx32, we measured simultaneously the hemichannel currents and the release of ATP elicited by a square depolarizing pulse up to +100 mV. Depolarizing pulses induced characteristic slowly activating outward currents and when the membrane potential returned to the holding voltage tail currents coinciding with the peak of ATP release. The deconvolution of the light signal revealed that the time courses of the tail current and the ATP release were coincident. We established a direct relationship between the amount of ATP released and the amplitude of tail current. Applying positive voltages closer to the ATP reversal potential during the tail current reduced the amount of ATP released. Five different single amino acid mutants of Cx32, described in CMTX, affecting intracellular, extracellular or transmembrane domains, were tested. Those mutations deeply inhibited or abolished the hemichannel currents and the ATP release.

#### 1451-Pos Board B295

##### Regulation Of Neuronal Connexin-36 Channels by pH

Luis C. Barrio<sup>1</sup>, Daniel González-Nieto<sup>1</sup>, Juan Gómez-Hernández<sup>1</sup>, Bélen Larrosa<sup>1</sup>, Cristina Gutiérrez<sup>1</sup>, Ilaria Fasciani<sup>1</sup>, María D. Muñoz<sup>1</sup>, John O'Brien<sup>2</sup>, Agata Zappala<sup>3</sup>, Federico Cicirata<sup>3</sup>.

<sup>1</sup>Hospital Ramn Y Cajal, Madrid, Spain, <sup>2</sup>University of Texas, Houston, TX, USA, <sup>3</sup>University of Catania, Catania, Italy.

Neurotransmission through electrical synapses plays an important role in the spike synchrony among neurons and oscillation of neuronal networks. Indeed, electrical transmission has been implicated in the hypersynchronous electrical activity of epilepsy. We have investigated the influence of intracellular pH (pHi) on the strength of electrical coupling mediated by connexin36 (Cx36), the principal gap junction protein in the electrical synapses of vertebrates. In striking contrast to other connexin isoforms, the activity of Cx36 channels decreases following alkalosis rather than acidosis when it is expressed in *Xenopus* oocytes and N2A cells. This uncoupling of Cx36 channels upon alkalization occurred in the vertebrate orthologues analyzed (human, mouse, chicken, perch and skate). While intracellular acidification caused a mild or moderate increase in the junctional conductance of virtually all these channels, the coupling of the skate Cx35 channel was partially blocked by acidosis. The mutational analysis suggests that the Cx36 channels may contain two gating mechanisms operating with opposing sensitivity to pH. One gate, the dominant mechanism, closes for alkalosis and it probably involves an interaction between the C- and N-terminal domains, while a secondary acid sensing gate only causes minor, albeit saturating, changes in coupling following acidosis and alkalosis. Thus, we conclude that neuronal Cx36 channels undergo unique regulation by pHi since their activity is inhibited by alkalosis rather than acidosis. These data provide a novel basis to define the relevance and consequences of the pH-dependent modulation of Cx36 synapses under physiological and pathological conditions.

#### 1452-Pos Board B296

##### Propagation of Fast and Slow Intercellular Calcium Waves in Primary Cultured Smooth Muscle Cells

Nadia Halidi<sup>1</sup>, Aurélie Formey<sup>1</sup>, François-Xavier Boittin<sup>2</sup>, Jean-Louis Bény<sup>2</sup>, Jean-Jacques Meister<sup>1</sup>.

<sup>1</sup>Laboratory of Cell Biophysics, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, <sup>2</sup>Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland.

Tissue blood flow is controlled by the changes in the diameter of the arteries and arterioles due to the coordinated contraction and relaxation of smooth muscle cells (SMCs) within the vascular wall. The contractile state of SMCs is regulated primarily by the intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ). The increase in  $[Ca^{2+}]_i$  in response to hormonal stimuli propagates from cell to cell along the vessel wall as a wave, and activates the process of contraction. The mechanism underlying this phenomenon, however, is not yet fully revealed.

In this work, we study the onset and propagation of intercellular calcium waves through gap junctions in primary cultured vascular SMCs. For imaging intercellular  $Ca^{2+}$  waves, SMCs seeded along a collagen line and loaded with the fluorescent  $Ca^{2+}$  indicator Fluo-4 were locally stimulated mechanically or chemically. The stimulation evoked two distinct calcium waves: 1) a fast  $Ca^{2+}$  wave (several mm/s), and 2) a much slower  $Ca^{2+}$  wave (few tens of  $\mu$ m/s); both waves propagated to neighboring cells. The fast  $Ca^{2+}$  wave was caused by the propagation of membrane depolarization and subsequent  $Ca^{2+}$  influx through voltage operated channels. This fast wave facilitated the onset and propagation of a slow, but higher amplitude  $Ca^{2+}$  wave that started from the stimulated cell and propagated to neighboring cells. Our results suggest a possible mechanism for intercellular  $Ca^{2+}$  wave propagation through gap junction channels in SMCs.

#### 1453-Pos Board B297

##### Connexin Pore Block By ABG-Sugars

Darren Locke, Andrew L. Harris.

NJMS, UMDNJ - Department of Pharmacology & Physiology, Newark, NJ, USA.

Pore blockers are valuable for structure-function study of membrane channels. Prior work shows novel anthranilamide moieties (ABG) derivatized to maltosaccharides of different lengths (Gn: n-glucose) are size-indexed pore blockers of connexin channels: block occurs with size-match with a segment of the pore lumen, not if the lumen or blocker is too narrow or wide. Permeation studies using the same maltosaccharides derivatized with an uncharged fluorescent group (PA-sugars) show the narrowest part of the pore (size-selective filter) decreases  $Cx32 > Cx26 \approx Cx26/Cx32$ , the last being heteromeric. Efficacy studies of ABG-sugar block from each side of the pore reveal new information about