Gaussian distributions after every time step (*dt=10ps*). Unidirectional particle fluxes were determined by counting particles that crossed the channel (particle gradient *4* to *0*).
Uncharged particle flux was not affected by the pore's mouth shape. When $e=-2e^-$, particle flux depended on pore's shape ($C_{1to2}=1.6\pm0.04x10^6$ *particles/sec/channel [p/s/ch]*; $C_{2to1}=0.75\pm0.03x10^6$ *p/s/ch*). A negative charged ring reduced/blocked particle flux and pore-particle interactions. A positive charged ring doubled particle flux for $C_{2to1}$ only, highlighting the mechanism's complexity.
Particle number at steady-state (SS) during bidirectional fluxes was calculated after placing 8 particles on either side. The average number of particles per cell remained close to 4 at SS and did not vary significantly (*<6%;n=15*) over *6μs;* the time to reach steady state for $C_{1to2}$ was smaller (*1.5μs* and *2.1μs* for $C_{2to1}$). Our mathematical model explains preferential directional fluxes by differences in pore mouth's shape. Identical number of particles at SS indicates thermodynamics compliance.

**503-Pos**

**Propagation Mechanisms of Calcium Waves Arising During Arterial Vasomotion**

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Vasomotion consists in cyclic arterial diameter variations induced by synchronous contractions and relaxations of smooth muscle cells (SMCs) present in the arterial wall. These contractions have been shown to be due to an increase in the intracellular cytosolic calcium concentration. However, the arteries do not contract simultaneously on macroscopic distances and a propagation of the diameter variations can be observed. Our aim was to investigate this propagation. We stimulated endothelium denuded rat mesenteric arterial strips with the vasoconstrictor, phenylephrine (PE) to obtain vasomotion and observed that the contraction waves are linked to intercellular calcium waves through the SMCs. A velocity of about 100 μm/s was measured for the two kinds of waves which could propagate either from the distal to the proximal side of the artery or in the opposite direction. To investigate the calcium wave propagation mechanisms, we used a method allowing a PE stimulation of a small area of the strip. No calcium propagation could be induced by this local stimulation when the strip was in its resting state. However, if a low PE concentration was added to the whole strip, local PE stimulations induced calcium waves spreading over finite distances. The calcium wave velocity induced by local stimulation was identical to the velocity observed during vasomotion. This suggests that the propagation mechanisms are similar in the two cases. Using inhibitors of gap junctions and of voltage operated calcium channels, we showed that the locally induced calcium propagation likely depends on the propagation of the SMCs depolarization. Finally, from the experimental data gathered, it is possible to propose a model of the mechanisms underlying the propagation.

**504-Pos**

**Edema: A Missing Link in the Conduction Velocity-Gap Junction Relationship**

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**Introduction**: Myocardial edema i.e., increased extracellular volume ($V_{ECS}$) occurs in diverse pathologies. Often these states are also linked with sudden cardiac death and conduction slowing, sometimes linked to gap junction (Gj) remodeling. Yet, edema's effect on the conduction velocity (CV)-Gj relationship remains unknown. We hypothesized that edema modulates the CV-Gj relationship.
**Methods**: CV was quantified by optical mapping in Langendorff-perfused guinea pig ventricles while pacing from the anterior epicardium. Tissue water content was estimated by the wet weight (WW) to dry weight (DW; after 24h ≅ 60oC) ratio. $V_{ECS}$ was modulated by perfusion of mannitol (26.1 g/l) or albumin (4 g/l). Gap junctions were uncoupled using carbenoxolone (10, 13 and 50 μM).
**Results**: Mannitol caused edema, increasing WW/DW ratio relative to control ($10.7\pm6$ %, $p<0.05$, n=6). It decreased transverse CV ($CV_T$) by $24\pm4$% and longitudinal CV ($CV_L$) by $9\pm2$% ($p<0.05$, n=4). Consequently, edema increased anisotropy of conduction ($AR_{CV}= CV_L/CV_T$) from $2.30\pm0.16$ to $2.75\pm0.19$ ($p<0.05$). Albumin had the opposite effects. It dehydrated the heart, decreasing WW/DW relative to control ($11.3\pm5$ %, $p<0.05$, n=6). Dehydration increased $CV_T$ by $71\pm10$ % ($p<0.05$, n=4) without significantly affecting $CV_L$. Thus, dehydration reduced $AR_{CV}$ to $1.84\pm0.07$ ($p<0.05$, n=4). Under control conditions, 10 and 13 μM carbenoxolone did not significantly affect CV while 50 μM carbenoxolone slowed $CV_T$ by $25\pm1$ % ($p<0.05$, n=3) but had no effect on $CV_L$. During edema, 13 μM carbenoxolone significantly slowed $CV_T$ by $38\pm9$ % ($p<0.05$, n=4) but had no effect on $CV_L$. Conduction was completely abolished by 50 μM carbenoxolone and mannitol.
**Conclusions**: These data demonstrate that edema slows conduction and unmasks a steeper CV-Gj relationship. Therefore, these data may explain conduction differences between nearly identical models of Cx43 down-regulation. Specifically, $V_{ECS}$ determines whether Gj uncoupling slows conduction.

**505-Pos**

**Increased Intercellular Communication in Activated Cardiac Fibroblasts**

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**Rationale:** A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into myofibroblasts; however the electrophysiological consequences of this phenotypic switch remain largely unknown. **Hypothesis:** Fibroblast activation results in a distinct electrophysiological phenotype that makes the heart more susceptible to arrhythmias. **Methods:** Ventricular fibroblasts were isolated from the ventricles of normal (Fb) and infarcted (MI-Fb) adult rat hearts. Fb and MI-Fb were plated onto myocyte monolayers at densities of 200, 400 and 600 cells/mm$^2$. Cultures were optically mapped with a voltage sensitive dye and conduction velocity (CV) and action potential duration ($APD_{70}$) were obtained. Functional intercellular communication, Cx43 expression levels and distribution were determined. **Results:** At 200 cells/mm$^2$, MI-Fb significantly increased ($22.0\pm0.6$cm/s) CV compared to homocellular myocyte cultures (Myo; $17.8\pm0.4$cm/s). Fb ($145.0\pm3.9$ms) and MI-Fb ($131.1\pm3.7$ms) significantly reduced $APD_{70}$ compared to Myo ($158.6\pm2.5$ms), and $APD_{70}$ was significantly shorter with MI-Fb compared to Fb. At higher fibroblast densities CVs were slower compared to Myo (Fb: $13.8\pm0.4$ and $11.4\pm0.3$cm/s; MI-Fb: $10.6\pm0.3$ and $9.5\pm0.3$cm/s, at 400 and 600 cells/mm$^2$, respectively). Fb and MI-Fb CVs were significantly different at all plating densities. At higher densities $APD_{70}$ was significantly longer for Fb compared to Myo ($171.0\pm2.1$ and $165.7\pm1.8$ms at 400 and 600 cells/mm$^2$, respectively). MI-Fb values were significantly longer at 400 cells/mm$^2$ ($175.8\pm2.7$ms). Cx43 staining was present in contact areas between myocytes and fibroblasts from both sources. Immunoblotting showed a significant increase of Cx43 levels in MI-Fb compared to Fb. Intercellular coupling evaluated with gap-FRAP was significantly increased between myocytes and MI-Fb compared to Fb. **Conclusions:** These data demonstrate fibroblast activation results in important electrophysiological changes that could contribute to the greater incidence of arrhythmias observed in fibrotic hearts. These observations highlight the fibroblast activation process as a potentially new therapeutic target for arrhythmia prevention.

**506-Pos**

**Astrocytes Control Breathing Through pH-Dependent Vesicular Release of Atp**

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Extracellular signalling mediated by ATP has been associated with central chemosensory function. It has been shown that increase in pCO2 in the arterial blood triggers an immediate release of ATP from the chemosensitive regions located on the ventral surface of the medulla oblongata (VMS).
Using in vitro and in vivo preparations and novel genetically encoded Ca2+ indicator based on cyclically permutated GFP - Case 12, we show that astrocytes in the ventral regions of the medulla oblongata are highly sensitive to changes in pH. Decrease in extracellular pH from 7.4 to 7.2 induced transient increases in [Ca2+]i in astrocytes from dissociated neuro-glial cultures prepared from the VMS, in ventral astrocytes of organotypic brainstem slice cultures and in acute horizontal brainstem slices of adult rats, as well as on the VMS in anaesthetized and artificially ventilated rats. ATP receptor antagonists such as MRS2179, PPADS and TNP-ATP effectively blocked [Ca2+]i responses evoked by lowering pH in VMS astrocytes. ATP hydrolyzing enzyme apyrase completely prevented propagation of [Ca2+]i excitation in VMS astrocytes evoked by lowering external pH. These data suggest that Ca2+ responses induced by lowering external pH are mediated by ATP release and subsequent activation of P2 receptors. Inhibitors of vesicular transport brefeldine A and bafilomycin A both effectively abolished [Ca2+]i excitation of VMS astrocytes evoked by lowering external pH. Incubation of astrocytes with FM 1-46 dye (vesicular marker) leads to selective labelling of intracellular vesicles. Acidification of external medium induced decrease in the fluorescence intensity of