activation. Following disruption of caveolae, the time to lysis in 0.02T solution was significantly reduced compared with control cells. In cells fixed for EM, caveolae were defined as invaginations or closed subsarcolemmal vesicles with a diameter of ≈50-100 nm. MBCD and 0.064T hyposmotic swelling significantly reduced the total number of caveolae by 75 and 50% respectively. Both 'open' and 'closed' caveolae were reduced by MBCD, but swelling only affected the 'closed' population. The negative inotropic response observed 6 and 10 min after exposure to 0.064T solution was blocked by the $I_{Cl,swell}$ inhibitor DIDS but enhanced by disruption of caveolae. Our data suggest that swelling causes flattening of 'open' caveolae, in tandem with sarcolemmal incorporation of 'closed' caveolae. We propose that disrupting caveolae removes essential membrane reserves, thereby speeding cell swelling in hyposmotic conditions and promoting activation of mechanosensitive $I_{Cl,swell}$ channels.

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**1315-Pos Board B159**

**Effects of Ion and Water Channels Blockers and Uncouplers on the *Dionaea Muscipula* Ellis Trap Closure**

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The Venus flytrap (*Dionaea muscipula* Ellis) captures insects with one of the most rapid movements in the plant kingdom. Here we present detailed experiments for comparative study of effects of inhibitors of ion channels, aquaporins, and uncouplers on kinetics of the trap closing stimulated by mechanical or electrical triggering of the trap. Two method of inhibitors phytoextraction were used: (1) two 10 μL drops of channels blockers or uncouplers were placed on the midrib of the trap or (2) addition of 50 mL of inhibitors to the soil. Both methods of inhibitors phytoextraction give the same effects on the kinetics of the trap closing. Ion and water channels blockers such as $HgCl_2$, TEACl, $ZnCl_2$, $BaCl_2$, as well as uncouplers CCCP, FCCP, 2,4-dinitrophenol, and pentachlorophenol decrease speed and increase time of the trap closing [1]. We applied for the evaluation of the mechanism of trap closing our new hydroelastic curvature mechanism, which is based on the assumption that the lobes possess curvature elasticity and are composed of outer and inner hydraulic layers with different hydrostatic pressure. The open state of the trap contains high elastic energy accumulated due to the hydrostatic pressure difference between the hydraulic layers of the lobe. Stimuli open pores connecting the two layers, water rushes from one hydraulic layer to another, and the trap relaxes to the equilibrium configuration corresponding to the closed state. The detailed mechanism of the trap closing is discussed.

[1] A.G. Volkov, K.J. Coopwood, V.S. Markin, Plant Science 175 (2008) 642-649.

**1316-Pos Board B160**

**Fluid Pressure-Gated Cation Channel in Atrial Myocytes**

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Regurgitant jets of blood in patients with mitral valve incompetence are known to predispose to atrial fibrillation. To understand cellular basis for the fibrillation induced by the fluid jet, we examined ionic currents induced by a fluid pressure (FP) in rat atrial myocytes. FP was applied by pressurized rapid (~15 dyne/cm$^2$) puffing of bathing solutions onto whole-cell clamped single atrial myocytes. Puffing (1-s long) of normal external solution produced inward current ($I_{FP}$) at a resting membrane potential, which was inactivated independently of FP. The current-voltage relationship of $I_{FP}$ showed inward rectification with a reversal potential of ≈-18 mV. $Ca^{2+}$-free extracellular solution enhanced $I_{FP}$ by ≈7-fold and eliminated the inactivation of $I_{FP}$. $I_{FP}$ was decreased by extracellular divalent cations with the strongest suppression by $Ca^{2+}$ ($Ca^{2+} > Cd^{2+} > Ni^{2+}$). Removal of extracellular $K^+$ or $Na^+$ decreased $I_{FP}$ by ≈46% or ≈35%, respectively. $I_{FP}$ was almost completely suppressed in $K^+$- and $Na^+$-free extracellular solution. Increase of extracellular $Ca^{2+}$ concentration to 75 mM enhanced $I_{FP}$, indicating contribution of $Ca^{2+}$ to $I_{FP}$. $I_{FP}$ was resistant to the blockade of the stretch-activated channel or $Na^+$-$Ca^{2+}$ exchanger. Intracellular $Ca^{2+}$ buffering with 4 mM EGTA did not alter the magnitude and inactivation of $I_{FP}$. $I_{FP}$ was increased to ≈200% immediately after a depletion of $Ca^{2+}$ in the sarcoplasmic reticulum using 10 mM caffeine. Our data provide functional evidence for a novel inwardly rectifying nonselective cation channel in rat atrial myocytes that is gated by fluid pressure. This channel appears to be inactivated by external $Ca^{2+}$-dependent mechanism and accelerated by depletion of the $Ca^{2+}$ store. The FP-dependent cation influx at resting potential may be a possible mechanism for the blood-jet induced atrial fibrillation in mitral valve incompetence.

## Cardiac Electrophysiology I

**1317-Pos Board B161**

**Endothelin-1 Regulates Volume-Sensitive Chloride Current in Rabbit Atrial Myocytes via Reactive Oxygen Species from Mitochondria and NADPH Oxidase**

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Angiotensin II (AngII) signaling and reactive oxygen species (ROS) produced by NADPH oxidase (NOX) are implicated in the activation of volume-sensitive Cl current ($I_{Cl,swell}$) by both beta$_1$-integrin stretch and osmotic swelling. Because endothelin-1 (ET-1) is a potential downstream mediator of AngII and ET-1 blockade abrogates AngII-induced ROS generation, we studied how ET-1 signaling regulates $I_{Cl,swell}$. Under isosmotic conditions, ET-1 (10 nM) elicited an outwardly rectifying Cl current that was fully blocked by the highly selective $I_{Cl,swell}$ inhibitor DCPIB (10 μM) and by osmotic shrinkage. Selective $ET_A$ (BQ-123, 1 μM) but not $ET_B$ blockade (BQ-788, 100 nM) fully suppressed ET-1-induced current. ET-1-induced $I_{Cl,swell}$ also was abolished by inhibitors of EGFR kinase (AG1478, 10 μM) and PI-3K (LY294002, 20 μM; wortmannin, 500 nM), which also suppress stretch- and swelling-induced $I_{Cl,swell}$. ERK inhibitors (PD 98059, 10 μM; U0216, 1 μM) partially and fully blocked ET-1- and EGF- induced currents, respectively, but did not effect $I_{Cl,swell}$ elicited by $H_2O_2$. ET-1 acts downstream from AngII. $ET_A$ blockade (BQ-123) abolished $I_{Cl,swell}$ elicited by both AngII and osmotic swelling, whereas $AT_1$ blockade (losartan, 5 μM) did not effect ET-1-induced $I_{Cl,swell}$. Both NOX and mitochondria are important sources of ROS in cardiomyocytes. Blocking NOX with apocynin (500 μM) or mitochondrial complex I with rotenone (10 μM) both completely suppressed ET-1-induced $I_{Cl,swell}$. In contrast, $I_{Cl,swell}$ elicited by antimycin A (10 μM), which stimulates superoxide production by mitochondrial complex III, was insensitive to apocynin and the NOX fusion peptide inhibitor gp91ds-tat (500 nM). These data suggest that ET-1 and $ET_A$ receptors are required intermediates in AngII-, swelling-, and stretch-induced activation of $I_{Cl,swell}$. Moreover, enhancement of mitochondrial ROS production by ROS from NOX is likely to contribute to activation of $I_{Cl,swell}$ by ET-1.

**1318-Pos Board B162**

**HIV Protease Inhibitors Activate Volume-Sensitive Chloride Current in Ventricular Myocytes by Generating Mitochondrial Reactive Oxygen Species**

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HIV protease inhibitors (HIV PI) have been successfully used to reduce morbidity and mortality of HIV infection. However, their long-term use causes significant side effects including cardiac arrhythmias. Previously we showed that outwardly-rectifying, volume-sensitive Cl current ($I_{Cl,swell}$) is regulated by signaling pathways that elicit production of reactive oxygen species (ROS). Because certain HIV PI recently were reported to augment ROS production, we tested whether HIV PI stimulate $I_{Cl,swell}$ in rabbit ventricular myocytes. Under isosmotic conditions, ritonavir (15 μM, 20 min) and lopinavir (15 μM, 25 min) induced outwardly-rectifying Cl currents (1.5 ± 0.3 pA/pF and 1.9 ± 0.3 pA/pF at +60 mV, respectively) that were fully inhibited by the highly selective $I_{Cl,swell}$-blocker DCPIB (10 μM). In contrast, amprenavir (15 μM, 30 min) and nelfinavir (15 μM, 30 min) did not modulate $I_{Cl,swell}$, and raltegravir (MK-0518, 15 μM, 30 min), an HIV integrase inhibitor, also was ineffective. Two major sources of ROS in cardiomyocytes are sarcolemmal NADPH oxidase and mitochondria. The specific NADPH oxidase inhibitor apocynin (500 μM) failed to inhibit the ritonavir- or lopinavir-induced currents, although we previously found apocynin blocks $I_{Cl,swell}$ activation upon osmotic swelling and stretch. In contrast, rotenone (10 μM, 30 min), a mitochondrial complex I inhibitor that limits electron flux to and ROS production by complex III, blocked 102 ± 4% of ritonavir- and 82 ± 12% of lopinavir-induced $I_{Cl,swell}$. Furthermore, the membrane-permeant, glutathione peroxidase mimetic ebselen (15 μM, 15 min) suppressed $I_{Cl,swell}$ elicited by ritonavir (102 ± 3%) and lopinavir (93 ± 6%). These results suggest that ritonavir and lopinavir activate $I_{Cl,swell}$ via mitochondrial ROS production by complex III. Activation of $I_{Cl,swell}$ by certain HIV PI may contribute to their untoward effects in heart and potentially other tissues.