(Cx43). Their exact molecular effect is still unknown. Previous data show that one analogue prevents phosphorylation on one (serine 297 [S297]) of three new phosphorylation sites on Cx43 (S296, S297, S306) during myocardial ischemia.

Methods and Results: Electrical intercellular coupling were measured at pH 7.2 and during cellular stress (pH_I 6.2 and increased $[Ca^{2+}]_I$) using the double whole-cell patch-clamp method in HeLa cells transiently transfected with wild-type (WT) or mutated Cx43 inserted into a pIRES-eGFP vector. Mutated Cx43 DNA was mimicking a constitutive dephosphorylation on either S297 or S306 on Cx43. Preventing phosphorylation on S297 does not affect gap junction coupling at pH 7.2. Preventing phosphorylation on S306 results in a 40% decrease in coupling at pH 7.2 and in significantly faster and greater uncoupling during cell stress. AAP10, an AAP analogue, 10 nM prevents a decrease in electrical coupling during stress. Also, single channel activity during cell stress can be recorded only in the presence of AAP10, revealing a decrease in unitary conductance from 100 to 60 pS during these conditions. The change in unitary conductance in the presence of AAP10 also occurs during pH 7.2 and when the protein kinase C (PKC) inhibitor BIM (bisindolylmaleimide) is added to the pipette solution. AAP10 also prolongs time to octanol-induced uncoupling during pH 7.2.

Conclusions: AAP10 prevents stress-induced uncoupling and decreases unitary conductance during cell stress and pH 7.2 through a non-PKC-induced pathway. Because electrical coupling increases in the presence of AAP10, we speculate that AAP10 also increases the open probability of the Cx43 gap junction channel.

CHRONIC SINGLE NEPHRECTOMY PROLONGS ACTION POTENTIAL DURATION BY INHIBITING TRANSIENT OUTWARD CURRENTS IN LEFT VENTRICULAR MYOCYTES

Kuan-Cheng Chang, Shih-Sheng Chang, Hsin-Yueh Liang, An-Sheng Lee, Ming-Jai Su. China Medical University Hospital, Taichung, Taiwan, *Institute of Pharmacology, National Taiwan University, Taipei, Taiwan*

Background: Chronic kidney disease (CKD) may contribute to left ventricular hypertrophy, fibrosis, diastolic dysfunction, and impaired coronary flow reserve, which ultimately lead to an increased risk for adverse cardiovascular events. We hypothesized that cardiovascular remodeling is a continuous process starting from the early stage of CKD and sought to explore the cellular electrophysiologic changes in left ventricular myocytes after single nephrectomy (SNx) in rats.

Methods and Results: Adult male Sprague Dawley rats underwent SNx with ventilator support and anesthesia with 2% isoflurane. Left ventricular myocytes were isolated 58 ± 4 days after SNx or sham operation and subjected to patch-clamp studies. Action potential durations (APDs) of SNx myocytes measured at 50% and 90% repolarization (APD_{50} and APD_{90}) were significantly longer than those of sham-operated rats (38.64 ± 2.48 ms vs 25.59 ± 0.79 ms, $P = .002$, and 150.09 ± 4.73 ms vs 95.63 ± 3.06 ms, $P = .0001$, respectively). Resting membrane potential (RMP) of SNx myocytes was less hyperpolarized than that of sham-operated myocytes (-65.16 ± 5.33 mV vs -73.43 ± 3.67 mV, $P = .04$), whereas no significant difference in action potential amplitude was seen between sham and SNx myocytes (102.53 ± 5.10 vs 104.56 ± 2.75, P = NS). APD prolongation was mediated by a reduction in peak I_{to} in SNx myocytes (17.81 ± 1.72 $pApF^{-1}$) compared to sham-operated myocytes (26.74 ± 3.17 $pApF^{-1}$, P = .018), and the partially depolarized RMP was attributed to a 30% reduction of I_{k1} in SNx myocytes. No significant difference was seen in the steady-state I_{to} current (10.93 ± 1.43 $pApF^{-1}$ vs 12.90 ± 1.44 $pApF^{-1}$, P = NS) and the inactivation kinetics ($V_{0.5}$: -29.41 ± 4.90 mV vs -34.89 ± 3.47 mV; slope factor: -8.47 ± 6.91 vs -8.69 ± 4.41, P = NS, respectively) between SNx and sham myocytes. However, the time courses of recovery from inactivation of I_{to} were markedly prolonged in SNx myocytes (τ_f = 0.033 ± 0.003 and τ_s = 0.285 ± 0.028 ms) compared with sham myocytes (τ_f = 0.009 ± 0.004 and τ_s = 0.159 ± 0.006 ms, $P < .05$, respectively). Depolarizing $I_{Ca,L}$ was not different (-9.57 ± 1.11 $pApF^{-1}$ vs -8.88 ± 1.02 $pApF^{-1}$, P = NS) between SNx and sham myocytes.

Conclusions: We demonstrate that APD prolongation and RMP depolarization in SNx myocytes were mediated by inhibition of peak I_{to} and suppression of I_{k1}. Our findings support the hypothesis that cardiac electrophysiologic remodeling might occur during the early stage of CKD.

CENTRAL ROLE FOR MITOCHONDRIA IN REGULATION OF SODIUM CURRENT

Man Liu, Georgia Gaconnet,* Barry London,† Samuel C. Dudley Jr.* *Division in Cardiology, University of Illinois at Chicago and the Jesse Brown VAMC, Chicago, IL, †The Cardiovascular Institute, University of Pittsburgh, Pittsburgh, PA*

Background: A mutant glycerol-3-phosphate dehydrogenase 1-like A280V (A280V GPD1-L) reduces cardiac Na^+ current (I_{Na}) and causes Brugada syndrome. Recent data suggest that this effect is dependent on alterations in nicotinamide adenine dinucleotide (NADH), reactive oxygen species (ROS), and protein kinase C (PKC) activation. Because NADH and PKC can activate ROS production from mitochondria, we investigated the role of this organelle in mediating the effects of mutant GPD1-L and NADH on I_{Na}.

Methods and Results: HEK cells stably expressing the cardiac Na^+ channel were used, and the effects on I_{Na} were assessed by whole-cell patch-clamp recording. A280V GPD1-L caused a 2.48 ± 0.17-fold increase in intracellular NADH level ($P < .001$). NADH application (100 μM) or cotransfection with A280V GPD1-L resulted in a significant decrease in I_{Na} (52% ± 9% and 81 ± 4%, respectively, $P < .01$), which was reversed by 100-500 μM NAD^+, 1 μM forskolin, 5-50 μM chelerythrine, 5 μM superoxide dismutase (SOD), 5-10 μM mitoTEMPO (a specific inhibitor to block mitochondrial superoxide generation), 1-5 μM rotenone (a complex I inhibitor), and 40-80 μM 4′-chlorodiazepam (an inhibitor of mitochondrial benzodiazepine receptor). The decreased I_{Na} induced by 30 nM phorbol myristate acetate (PMA, 60% ± 7%, P_{Na} (51% ± 4%, $P < .01$). L-NAME (an inhibitor for uncoupled NOS) and KN-93 (an inhibitor of CAMKII) had no effect on NADH in reducing Na^+ current.

Conclusions: A280V GPD1-L appears to regulate $Na_v1.5$ by altering the oxidized to reduced NAD(H) balance, which then activates mitochondrial ROS production through a PKC-dependent signaling mechanism. This ROS production leads to reduced I_{Na}. This signaling cascade may help explain the link between altered metabolism, conduction block, and arrhythmic risk.

ST ELEVATION BY SODIUM CHANNEL BLOCKADE IN STRUCTURALLY DISCONTINUOUS MYOCARDIUM: EXCITATION FAILURE BY CURRENT-TO-LOAD MISMATCH

Mark G. Hoogendijk, Mark Potse,*† Bas J. Boukens,* Jan W. Fiolet,* Jacques M.T. de Bakker,*‡ Ruben Coronel.* *Academic Medical Center, Amsterdam, The Netherlands, †Maastricht University, Maastricht, The Netherlands, ‡Interuniversity Cardiology Institute of The Netherlands, Utrecht, The Netherlands*

Background: Right precordial ST elevation after sodium channel blockade has been associated with right ventricular structural discontinuities and sudden cardiac death. The mechanism of ST elevation is debated. We hypothesized that sodium channel blockade causes ST elevation by current-to-load mismatch and excitation failure in structurally discontinuous myocardium.

Methods and Results: In thin (0.5 mm thick) epicardial shavings of porcine ventricles, isthmuses (0.9 mm wide) were created perpendicular to fiber orientation. Activation was mapped electrically or optically (di-4-ANEPPS), and a pseudo-ECG was recorded simultaneously during stimulation (on either side of the isthmus) at increasing pacing frequencies