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independent of cell size, and that calcium cycling protein density is independent of cell area and size. It's well documented that the gap junction protein, connexin-43(Cx43), is highly expressed in peripheral vs. central SAN area. Since it is possible that cell size doesn't completely discriminate between Cx43-negative and positive cells, we measured Cx43-immunolabeling, electrophysiological and Ca^{2+} cycling properties of single isolated SANC.
Freshly isolated adult rabbit Cx43-negative SANC are, on average, smaller ($592.3 \pm 9.2 \mu m^2$, n=579) than Cx43-positive SANC ($747.8 \pm 12.2 \mu m^2$, n=571, $p<0.001$), but there is no difference in the spontaneous AP cycle length based on Cx43 expression (340.2 ± 13.6ms n=30 in Cx43-negative cells, vs. 326.5 ± 9.0ms n=50 in Cx43-positive cells, p=0.39). AP parameters also do not differ between Cx43-negative and positive cells, but the AP of later has a shorter repolarization time (APD90: 136.9 ± 8.6ms vs. 110.2 ± 4.8ms, $p<0.01$), which is not related to cell size. No significant differences are detected in the major characteristics of basal AP-triggered Ca^{2+}-transient or spontaneous Local-Ca^{2+}-Releases during diastolic depolarization between Cx43-negative and positive SANC. Acute β-AR stimulation (1μM isoproterenol) reduces the AP cycle length to the same level in both Cx43-negative and positive SANC (257.3 ± 9.0ms n=9 vs. 271.6 ± 7.7ms n=14, respectively, p=0.25), and no differences of APD90 are detected (p=0.17).
Our results indicate that although different in size, there is no statistical difference between single isolated Cx43-negative and positive SANC of spontaneous AP cycle length or during maximal β-AR stimulation.

3425-Pos Board B286

Suppression of Atrial Fibrillation by Over-Expression of Connexin 43 in a Porcine Model

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Atrial fibrillation (AF) is associated with atrial conduction disturbances caused by electrical and structural remodeling. We hypothesized that expression of the gap junction protein connexin 43 is reduced during AF, and that atrial Cx43 gene transfer would prevent persistent atrial fibrillation. The first aim of this study was to assess whether atrial fibrillation (AF) is associated with connexin remodeling in a porcine model. Second, a strategy to suppress persistent AF by gene therapy was developed and evaluated *in vivo*.
AF was inducedin domestic pigs *via* atrial burst pacing, causing a 62.4% reduction of atrial Cx43 protein. Adenoviruses encoding for connexin 43 (Ad-Cx43) or green fluorescent protein (Ad-GFP) were injected into both atria, followed by epicardial electroporation to enhance transgene expression. Ad-Cx43 treated animals did not exhibit persistent AF during the observation period of 14 days. In contrast, control animals developed persistent AF within 7.4 ± 0.5 days. Rapid ventricular heart rates during AF led to deterioration of cardiac function in control pigs but not in animals treated with Ad-Cx43.
In conclusion, our results highlight the contribution of connexin 43 to atrial fibrillation and demonstrate the viability of electrophysiological gene therapy for prevention of atrial arrhythmias.

3426-Pos Board B287

Altered HCN4 Channel C-Linker Interaction is Associated with Familial Tachycardia-Bradycardia Syndrome and Atrial Fibrillation

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HCN channels underlie the pacemaker current I_f, involved in generation, regulation, and stabilization of sinus rhythm. HCN4 represents the dominant isotype in the sinoatrial node and channel dysfunction is associated with inherited sinus node bradycardia. Here, we report a previously undescribed *HCN4* gene mutation that replaced the positively charged lysine 530 with an asparagine (HCN4-K530N) in a highly conserved region of the C-linker. Six members of a German family carrying the HCN4-K530N mutation developed tachycardia-bradycardia syndrome and persistent atrial fibrillation in an age-dependent fashion. HEK293 cell recordings using whole-cell patch clamp electrophysiology revealed that homomeric HCN4-K530N mutant and wild type channels activate at similar potentials and respond equally upon binding of cAMP. Heteromeric channels, in contrast, showed a significant hyperpolarizing shift in the half-maximal activation voltage. Moreover, the effect of cAMP on channel activation and deactivation properties was significantly increased in heteromeric channels. A comparison of mutant and wild type C-linker domain models suggests that altered subunit interactions between the A' and B' helices and the C' and D' helices of the neighboring subunit may change the intersubunit structural dynamics in heterotetramers of HCN4 wild type and mutant subunits, enforcing inhibition of channel activity by the nucleotide free cyclic nucleotide binding domain in the heterozygous situation. Thus, altered interaction of side chains is tolerated in homomeric mutant channels but interferes with wild type subunits in the heteromeric complex leading to f-channel dysfunction that promotes the development of tachycardia-bradycardia syndrome and persistent atrial fibrillation.

3427-Pos Board B288

Direct cAMP Binding and PKA Phosphorylation Share a Common Gating Mechanism in HCN4 Channels

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Sympathetic regulation of HCN4 channels can occur via two cAMP-dependent pathways: either direct binding of cAMP to a cyclic nucleotide binding domain, or PKA phosphorylation of the distal C terminus. Here, we have investigated the energetic interactions between these two modulatory mechanisms. cDNA encoding wildtype or mutant mouse HCN4 channels was expressed in HEK293 cells, and the voltage-dependence of channel activation was determined in whole cell voltage clamp experiments. Intracellular dialysis of either PKA (20 U/ml) or cAMP (1 mM) shifted the activation midpoint (V1/2) of wild type HCN4 channels by ~10 mV to more depolarized voltages. No additional shift was produced when both cAMP and PKA were introduced together, suggesting that the two modulators share a common final pathway in channel activation. To characterize further the independent effects of each modulator, we used PKA-insensitive (HCN4-Cx4) and cAMP-insensitive (HCN4-R669Q) mutant HCN4 channels. In PKA-insensitive HCN4-Cx4 channels, cAMP significantly shifted the voltage-dependence to more positive potentials, similar to its effect in wildtype HCN4 channels. This result demonstrates that cAMP modulation of HCN4 does not require PKA phosphorylation of the distal C-terminus. In contrast, PKA had no effect on the voltage-dependence of activation in the cAMP-insensitive HCN4-R669Q channels. Taken together, the data are consistent with a model in which direct binding of cAMP and PKA phosphorylation share a final common gating mechanism, however PKA modulation of HCN4 requires an unmodified cAMP binding domain.

3428-Pos Board B289

Electrical Inhomogeneities of Guinea Pig Atrial Tissue in High Resolution Optical Potential Mapping

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Inhomogeneities in the tissue are a determining factor for cardiac function and can be either structural or functional. Using the voltage sensitive fluorescent dye di-4-ANEPPS on incubation stained guinea pig preparations allows to map the tissue's response to externally applied electric shocks. Previous work on ventricular muscle preparations revealed a membrane potential morphology that could be relevant during defibrillation [1]. Using a two pulse stimulus protocol we are able to map the excitation response to a short triggering field pulse and to consequently map the membrane potential morphology resulting from a second field pulse, which is applied in the depolarized state and is attributed to the interaction of the electric field with the tissue. This approach is now carried over to atrial preparations. A custom developed object-positioning and image-stitching method allows for wide view analysis of the atrial tissue structure. Objective-magnifications of 10x, 20x, 40x and 63x enable a multi-scale based analysis with resolutions up to 15 micrometers. This is augmented with information from transmitted light microscopy so that it is possible to correlate the membrane potential patterns with the visible structure of the atrial tissue, providing further insight into atrial excitation and conduction.