the pulse EPR method, and the NASNOX program constitute a tool-kit for un-restricted global structural mapping of nucleic acids and protein/nucleic acid complexes. This tool-kit is being utilized to map the global structure of the packaging RNA (pRNA) in the phi29 bacteriophage DNA packaging motor, which is the strongest known bio-molecular motor. The pRNA forms a ring-shaped complex that is indispensable in motor ATPase activity, yet structural information on pRNA is lacking. We are using the SDSL tool-kit to obtain pRNA inter-helical distances. These constraints are used to determine the spatial packing of the helices and define the global structure of functionally relevant pRNA complexes. This represents the first application of SDSL to study an RNA with unknown structure.

Platform G: Cardiac Electrophysiology

77-Plat KCNE2 Transcript Levels Are Directly Correlated With Heart Estrogen Concentrations

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We have previously shown that the mouse KCNE2 gene is an estrogen-responsive gene and that its transcripts are highly upregulated by estrogen (E2) in ovariectomized (ovx) mice. As male and female have different levels of E2, we investigated whether KCNE2 transcripts are differentially regulated in males and in females at different estral stages: estrus (under the influence of E2 hours after the proestrus E2 surge) and diestrus2 (after a prolonged exposure to low estrogen levels). Real time PCR showed that KCNE2 transcript levels were significantly higher in estrus (10 fold) compared to diestrus as can be expected for KCNE2 being an E2 responsive gene. Interestingly, KCNE2 transcripts were similar in males and in feamles at estrus. Based on these unexpected findings, we speculated that male heart could have high E2 levels. This assumption is conceivable as heart has all the machinery to synthesize locally E2 from testosterone by aromatase CYP450. Plasma and heart E2 levels were measured with the radioimmunoassay technique in males, females at estrus and diestrus, ovx mice and ovx mice treated with E2. Male heart has significantly higher E2 levels than plasma (35±3 pg/ml, n=6 *vs.* 12±0.9 pg/ml, n=5) favoring the hypothesis that male hearts can locally synthesize E2. In fact, male hearts had twice as much E2 levels than females (diestrus 20.2±1.5 n=4; estrus 17.2±0.9 pg/ml, n=4). E2 treatment of ovx mice increased heart E2 concentration from 16±1.4 to 62.7±2.9 pg/ml, n=3. We conclude that:

1. local heart E2 biosynthesis leads to a higher E2 concentration in male hearts;
2. heart KCNE2 transcript levels are directly correlated with the local heart E2 concentrations, and
3. KCNE2 expression is indeed regulated by E2 *in vivo*.

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78-Plat Embryonic Cardiomyocytes Can Functionally Integrate Into Infarcted Myocardium And Reduce The Vulnerability To Post-infarct Arrhythmias

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Transplantation of progenitors or stem cells into the infarcted myocardium is thought to improve the contractile function of the heart, which lacks regenerative capacity. However, proper function of engrafted cells in the infarct requires their electrical coupling with the native myocardium.

To test the coupling of transplanted cells *in vivo* we have used the Ca^{2+}-sensitive circular-permutated GFP (GCaMP2). Embryonic cardiomyocytes (eCM) were harvested from GCaMP2 positive embryos or eCM transduced with a lentivirus encoding GCaMP2 under control of the CMV promoter. GCaMP2 positive eCM showed spontaneous beating and concomitant Ca^{2+} transients *in vitro*. Histological analysis of hearts two weeks after cryoinfarction and injection of GCaMP2 positive eCM revealed stable engraftment and differentiation. Electrical coupling of the transplanted cells was assessed by parallel ECG recording and *in vivo* imaging of cytosolic Ca^{2+} in GCaMP2 positive eCM. For this purpose, the mouse was intubated, ventilated and the chest wall removed. After exposing and stabilizing the heart, GCaMP2 fluorescence was recorded with a macroscope and an EMCCD camera. At physiological heart rates 2:1 or 4:1 coupling of Ca^{2+} transients from engrafted eCM with the native heartbeat could be observed indicating loose electrical coupling. In addition, we could also observe GCaMP2 positive eCM which were not coupled to the native myocardium but displayed slow conduction between grafted eCM within the infarct.

We next wondered whether engrafted eCM affect post-infarct arrhythmia and therefore assessed electrical vulnerability two weeks after infarction by *in vivo* transvenous electrophysiological investigation. VT could be evoked in 96.4% of infarcted mice, however transplantation of eCM reduced VT inducibility to 35.7%, comparable to non-infarcted control mice (38.9%).

Thus, transplanted eCM stably engraft, can electrically couple with the native myocardium and reduce the vulnerability to post-infarct arrhythmia.

79-Plat Characterization of Ion Channels in Human Cardiac Fibroblasts

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Electrophysiology is well established in cardiac contractile myocytes; however, information for ion channels is poorly understood in

human cardiac fibroblasts, though cardiac fibroblasts play a crucial role in maintenance of the extra-cellular matrix in the normal heart, and in myocardial remodeling in the injured and failing heart. The present study was designed to characterize ion channels in cultured human cardiac fibroblast using whole-cell patch clamp and RT-PCR techniques. It was found that multiple ion channels were heterogeneously expressed in human cardiac fibroblasts. They include a big conductance calcium-activated potassium channel current (I_{KCa}) inhibited by 1 μM paxilline and two types of voltage-gated sodium channel currents (I_{Na}) in most human cardiac fibroblasts (70–80% cells). One type of I_{Na} was a slowly inactivated current, sensitive to tetrodotoxin (TTX) inhibition ($I_{Na.TTX}$, IC_{50}=2.2 μM), and another was a fast inactivated current, relatively insensitive to TTX ($I_{Na.TTXR}$, IC_{50}=12 μM). $I_{Na.TTX}$ had a quick recovery time course and positive activation and inactivation potentials relative to $I_{Na.TTXR}$. In addition, a delayed rectifier potassium current (IK_{DR}) and a transient outward potassium current (I_{to}) inhibited by 5 mM 4-aminopyridine, and a chloride current (I_{Cl}) inhibited by the chloride channel blocker DIDS (200 μM) were recorded in a small population of human cardiac fibroblasts (15–30% cells). RT-PCR revealed the molecular identities (mRNAs) of these ion channels in human cardiac fibroblasts, including KCa.1.1 (responsible for big conductance I_{KCa}), $Na_V1.2$, $Na_V1.3$, $Na_V1.6$, $Na_V1.7$ (likely responsible for $I_{Na.TTX}$), $Na_V1.5$ (likely responsible for $I_{Na.TTXR}$), Kv1.5 (likely responsible for I_{KDR}), Kv4.2 (likely responsible for I_{to}), and ClC2 and ClC3 (likely responsible for I_{Cl}). These results demonstrate the first information that multiple ion channels are present in human cardiac fibroblasts, which differ from the well-studied contractile myocytes. Physiological role of these ion channels remains to be studied.

80-Plat A Model Study Of Excitability In The Sinoatrial Node

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It is well established that in the intact sinoatrial node SAN, electrical activity initiates in a central area of small cells, and then propagates via larger peripheral cells to the atria. It is also known that action potential morphology, maximum diastolic potential and upstroke velocity vary between the center and the periphery of the intact SAN. Two primary hypotheses exist to explain heterogeneity in the SAN; the first suggests that differences in electrical activity result from differences in ionic current densities in center versus periphery. The second hypothesis proposes that SAN heterogeneity arises in the intact node primarily from the electrotonic effects of the atria on the cells in the periphery. We have developed a theoretical model to test each hypothesis and to make predictions about determinants of action potential morphology and properties in the SAN. We developed models of single cells and one-dimensional tissue incorporating

1. ionic current heterogeneities that define central and peripheral cells (as reported in some studies) and
2. electrotonic effects alone (reported in others).

Our simulations predict that:

1. Models incorporating intrinsic ion channel heterogeneity to define central and peripheral cells fail to simulate experimentally observed tissue properties.
2. Incorporation of electrotonic effects alone is sufficient to reproduce a range of experimental observations including initiation of the SAN impulse in the center, and importantly, a pacemaker shift to the periphery when the atria tissue is ablated.
3. When we incorporated recent experimental findings into our "electrotonic model" including fibroblasts as a current sink, variable type and density of gap junction proteins and different Na+ and Ca 2+ isoforms, the simulations (17 ms) are in good agreement with experimentally (10–20 ms) measured parameters such as SAN-atrium conduction time.

81-Plat Enhancement of C-type Inactivation of The Cardiac Transient Outward K^+ Channel, Kv1.4, by ω-3 and ω-6 Polyunsaturated Fatty Acids and The Effect of Acidosis, Extracellular K^+ and Mutation of Two-Positively Charged Pore Residues

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Voltage-dependent K^+ channels are inactivated by two distinct processes, N and C-type inactivation. Here we show that C-type inactivation is regulated by -3 and -6 polyunsaturated fatty acids. We have studied the effect of EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid) and AA (arachidonic acid) on Kv1.4, one of the pore-forming subunits responsible for the cardiac transient outward K^+ channel. A mutant Kv1.4 channel that lacks N-type inactivation (fKv1.4 Δ2-146) was expressed in *Xenopus* oocytes, and currents were recorded using the two-electrode voltage clamp technique. EPA, DHA and AA enhanced C-type inactivation in a dose-dependent manner with a K_D of 15, 18 and 43 μM, respectively (n = 5, 7 and 10). We observed an interaction among the effects of DHA, AA, pH and K^+ on Kv1.4: although 30 μM of either DHA or AA markedly enhanced C-type inactivation of Kv1.4 when extracellular pH was 7.4 and extracellular K^+ was 3 mM, it had no effect when the pH was reduced to 5.5 or K^+ was raised to 100 mM (n=5). Replacement of either of two positively-charged residues in the outer pore with a cysteine residue (H508C; K532C) abolished the enhancement caused by EPA, DHA and AA (n = 5). We conclude that EPA, DHA and AA cause a dose-dependent enhancement of C-type inactivation of Kv1.4 via a mechanism it shares in common with extracellular pH and K^+. The effect of polyunsaturated fatty acids on Kv1.4 may involve the formation of a salt bridge between the negatively charged polyunsaturated fatty acids and the positively charged residues (H508 and K532) in the extracellular mouth of the channel.

82-Plat Cysteine Substitution Reveals Novel Inter-subunit Interactions In The Iks Potassium Channel

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The I_{KS} potassium channel, which consists of the alpha subunit (KCNQ1) and beta subunit (KCNE1), is essential for control of action potential duration in the human heart. Loss of function mutations in either subunit can cause long QT syndrome. Gain of function mutations in the S1 helix of KCNQ1 cause atrial fibrillation (AF) and functionally are dependent on association with KCNE1. Previous investigations of inter-subunit KCNE1/KCNQ1 interactions have concentrated primarily on the S6, P-loop, and S4 helices as well as the intracellular termini of KCNQ1 as possible sites of KCNE1 interaction. Here we report novel KCNE1/KCNQ1 interactions revealed by experiments in which we used the spontaneous formation of disulfide bonds by subunit-specific cysteines to report inter-subunit proximity. Cysteines (Cys) were introduced into the extracellular juxtamembrane regions of both KCNQ1 and KCNE1 and crosslinking was determined by DTT-sensitive gel shifts detected under non-reducing conditions on Western blots. Spontaneous crosslinking was tested for 96 permutations of Cys-substituted subunits. We find that Cys substitutions into the extracellular juxtamembrane regions of KCNE1 and S1 and S6 of KCNQ1 cause a gel shift consistent with a KCNE1-KCNQ1 heterodimer whereas substitutions into S2, S3–4, and S5 do not. Whole cell patch clamp analysis of transiently transfected CHO cells expressing selected crosslinking pairs at S1 or S6 revealed a DTT-reversible phenotype of channels that are either constitutively open or once opened, reluctant to close. These data, in concert with the crystal structure of the Kv1.2 K^+ channel, imply that the transmembrane portion of KCNE1 is located between the S1 and S6 helices of two intercalated KCNQ1 subunits and that relative movements between S1/E1 or S6/E1 are important for channel gating. This provides a structural basis to understand the inherited AF mutations of KCNQ1.

83-Plat Doxazosin Induces Apoptosis Of Cells Expressing Herg K^+ Channels

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The antihypertensive drug doxazosin is associated with an increased risk for congestive heart failure and cardiomyocyte apoptosis. Human ether-a-go-go-related gene (hERG) K^+ channels, previously shown to be blocked by doxazosin at therapeutically relevant concentrations, represent plasma membrane receptors for the antihypertensive drug. To elucidate the molecular basis for doxazosin-associated pro-apoptotic effects, cell death was studied in human embryonic kidney cells using three independent apoptosis assays. Doxazosin specifically induced apoptosis in hERG-expressing HEK cells, while untransfected control groups were insensitive to treatment with the antihypertensive agent. An unexpected biophysical mechanism has emerged: binding of doxazosin to its novel membrane receptor, hERG, triggers apoptosis, possibly representing a broader biological strategy in drug-induced heart failure.

84-Plat Characterization Of Action Potential And Inward Currents In Freshly Isolated Ventricular Myocytes From Zebrafish (*Danio rerio*)

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The Zebrafish is a tropical teleost fish that has been the focus of an increasing number of developmental studies. Physiological interest in this species has been spurred on by the ease of mutants which can be induced by chemomutagenesis. However, our understanding of the basic physiology of Zebrafish, in particular cardiac excitation-contraction coupling is limited. Indeed, there is currently no information about the electrical activity of single myocytes freshly isolated from Zebrafish ventricle; this study addressed this point. Viable ventricular myocytes were obtained by enzymatic digestion. The whole cell configuration of the patch clamp was used to record Na current (INa), Ca current (ICa) and action potential (AP). Results are presented as mean±SE and analyzed with paired t-test. Single ventricular myocytes from Zebrafish are long and thin, as described for other fish species. Cell capacitance was 26.0±1.1 pF (n=69). ICa density (test pulse to 0 mV) was −11.4±3.4 pA/pF and INa density (test pulse to −40 mV) was −104±22 pA/pF (n=6). Resting membrane potential was −66±2 mV (n=19). At 0.1 Hz stimulation frequency, AP duration at 25, 50, and 90% repolarization was 50±17, 105±27, 137±35 ms, respectively (n=12), indicating the presence of a plateau phase. Increasing stimulation frequency to 2 Hz significantly decreased AP duration (n=6, $p<0.05$). To conclude, we have developed a method to obtain viable isolated ventricle myocytes from Zebrafish heart. Ionic currents studied present characteristics similar to other fish species. The presence of a plateau during the AP suggests that this species might be appropriate for ion channels related mutation screening of cardiac alteration.

Platform H: Fluorescence Spectroscopy

85-Plat Fluorescence Correlation Spectroscopy in Zebrafish Embryos

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Fluorescence Correlation and Cross-correlation Spectroscopy (FCS and FCCS) have developed into routine tools for the determination