Methods: Therefore, we measured the concentration of norepinephrine, tyrosine hydroxylase (TH) norepinephrine transport carrier (NET) protein, inhibitory G-protein ($G_{i\alpha1+2}$) and cAMP in endomyocardial biopsies from 17 pts with ARVC (age 49 ± 15 SD). Tissue samples from 5 pts without known heart disease were used as controls.
Results: In pts with ARVC, the tissue concentration of norepinephrine (806±82 vs. 1376±96 ng/g tissue; $p < 0.01$) was significantly reduced compared to the control group, thus indicating a depletion of presynaptic (vesicular) tissue norepinephrine storage. Protein expression of TH (5.4 ± 1.8 vs. 2.1 ± 0.4 × 10^6 PI-units; ns) tended to be higher whereas $G_{i\alpha1+2}$ (3.7 ± 0.38 vs. 5.2 ± 0.73 × 10^7 PI-units; $p = 0.06$) tended to be lower in pts with ARVC. Protein expression of NET and cAMP levels were not different between the groups. All values are mean ±SEM.
Conclusion: These data demonstrate for the first time a depletion of presynaptic (vesicular) tissue catecholamine storage and therefore confirm and expand previous findings of autonomic dysfunction in pts with ARVC. Potential mechanisms include an increased release of neurotransmitters to the synaptic cleft and/or reduced function of NET. This hypothesis is in line with previous radionuclide studies demonstrating impaired presynaptic catecholamine recycling and downregulation of postsynaptic β-adrenoceptor density and add important new insights into the pathophysiology and arrhythmogenesis of ARVC with potential future impact on patient management.

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Sustained reentry in co-cultured monolayers of skeletal muscle myoblasts and neonatal rat cardiomyocytes

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Recent clinical trials of skeletal muscle myoblasts (SkMM) for cardiomyoplasty following MI have shown a high incidence of malignant ventricular arrhythmias. We investigated possible cellular mechanisms of SkMM-induced arrhythmia by co-culturing SkMM transduced with a lentivirus expressing GFP with neonatal rat ventricular myocytes (NRVM). SkMMs and NRVMs were grown as a mixed monolayer on 20 mm coverslips in cell ratios of 1:9 or 2:8. Light microscopy demonstrated a relatively uniform distribution of myoblasts and myocytes. Electrical activity was optically mapped using the voltage-sensitive dye di-4-ANEPPS. Cell monolayers were paced with an 8 pulse train at 1 to 5 Hz. In all co-cultured monolayers ($n = 7$), the depolarization wavefront was irregular and wavebreaks occurred even at low pacing rates; conduction block developed between 4.4 and 5Hz, and reentry could be induced. These effects were absent in NRVM-only controls ($n = 7$). Reentrant activity was varied, and consisted of single or figure-of-eight rotors that were drifting, transient, or stable. Average reentry cycle length was 214 ms ($n = 7$) in the co-cultures, compared with cycle lengths of 100ms ($n = 2$) in NRVM-only monolayers. Two co-cultures had rotors that were stable for >60 minutes, while in one co-culture, a stable rotor was present prior to initiating the pacing protocol. Nitrendipine (10 μM) slowed conduction velocity and terminated the reentrant arrhythmias. In summary, we have demonstrated the presence of readily-induced , sustained reentrant activity in co-cultures of SkMM and NRVM.

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Selective activation of εPKC reduces reperfusion arrhythmias and improves recovery from ishemia

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The electrophysiologic consequences of εPKC modulation in ischemic hearts are not known mainly because of the lack of efficient means to *deliver* PKC isozyme modulators into *intact* hearts. We used membrane-permeable peptide εPKC selective *activators* and *inhibitors* and investigated the role of εPKC in ischemia (I) and reperfusion (R) arrhythmias.
Methods: Protein transduction domain from HIV-TAT was used as a carrier for peptide delivery in intact Langendorff perfused guinea pig hearts. Action potentials were mapped using optical techniques during I/R. Hearts were exposed to 30 min stabilization, 15 min of no-flow ischemia, followed by 20 min reperfusion. Peptides (0.5 μM) were infused as follows: a) control (vehicle-TAT peptide; TAT-scrambled agonist peptide); b) εPKC agonist; c) εPKC antagonist.
Results: (see table) The highest incidence of ventricular tachycardia/fibrillation (VT/VF) occurred in hearts treated with εPKC antagonist peptide (100%). More importantly, 100% of hearts treated with εPKC agonist recovered vs 70% in control hearts and 50% of hearts treated with εPKC antagonist. The longest duration of post-reperfusion arrhythmias was 15.6 min in εPKC antagonist group and shortest 4.2 min εPKC agonist group. Minimal APD dispersion was observed 20 min following reperfusion in εPKC agonist maps while dispersion was exacerbated in εPKC antagonist maps.
Conclusion: The results show that εPKC activation by εPKC agonist peptide protects intact hearts from reperfusion arrhythmias and affords full recovery. However, inhibition of εPKC increased incidence of arrhythmias and worsen recovery. This study establishes feasibility of acute delivery of peptides that regulate εPKC activity into *intact* hearts. The results carry significant therapeutic implications for the development of tageted-drugs for acute ischemic heart disease.

INCIDENCE OF REPERFUSION ARRHYTHMIAS

	N	VT/VF	RECOVERY	VT/VF Duration (min)
CONTROL (Vehicle)	10	90%	70%	10.4±3.2
Scrambled ε-PKC agonist	6	83%	83%	8.7±3.3
ε-PKC agonist	7	71%	100%	4.2±1.5 (p<0.05) compared to controls
ε-PKC antagonist	8	100%	50%	15.6±3.0

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A simulation study of atrial fibrillation initiation: Differences in resting membrane potential can produce spontaneous activations at the pulmonary vein-left atrial junction

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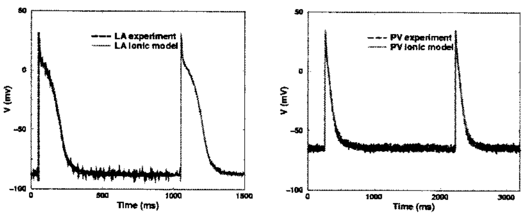
Introduction: Much attention has been given to the role of pulmonary vein (PV) focal activity during atrial fibrillation (AF). However, it still is not clear if PV preparations are auto-oscillatory and can produce ectopic beats when paced at physiological rates. An alternative hypothesis proposes that the resting membrane potentials (RMPs) of left atrial (LA) and PV cardiomyocytes may differ sufficiently to produce, by electrotonic effects, repeated spontaneous activations that propagate through the atria and potentially generate AF.
Methods and Results: Using action potential (AP) recordings obtained experimentally, we developed ionic models of canine PV and LA cardiomyocyte APs that reproduce rate of rise, morphology, RMP (-74 mV in LA versus -65mV in PV), and rate adaptation. To study how different RMPs affected propagation, we created adjoining regions of PV and LA tissue of varying sizes. Electrotonic currents between adjacent regions originated when cells were at rest, due to differences in RMP, with coupling strength governing the spatial voltage profile between the two RMPs. We found that spontaneous activations arose in highly specific circumstances that can be understood by analyzing the interactions among spatial voltage profile, recovery from Na^+ channel inactivation, and excitation threshold (ET). In particular, not only

differences in RMP, but also differences in ET (such as those caused by stretch, memory, aging, and other mechanisms), were necessary for spontaneous activity to arise and propagate into the LA.

Conclusions: PV focal activity leading to AF in some cases may arise not from auto-oscillatory behavior, but rather from experimentally observed RMP differences that result in continuous re-excitation via electrotonic current passage between adjacent regions.



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Cellular mechanisms underlying the development of catecholaminergic ventricular tachycardia

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is characterized by bidirectional VT (BVT), polymorphic VT (PVT) or ventricular fibrillation (VF). Mutations in the genes encoding for the cardiac ryanodine receptor (RyR2) or calsequestrin have been associated with this phenotype. We created an experimental model of CPVT by using low dose caffeine to simulate the leaky RyR.

Methods: A transmural ECG and action potentials (APs) were recorded from epicardial (Epi), M and endocardial(Endo) cells in arterially perfused canine right and left ventricular wedge preparations. After pretreatment with caffeine (100-300 μM), isoproterenol (ISO, 100 nM) was added to the coronary perfusate to induce ventricular arrhythmias. Transmural dispersion of repolarization (TDR) was measured from the simultaneously recorded APs, and from the ECGs (T_{peak}-T_{end} intervals, Tp-e).

Results: Caffeine alone did not significantly alter QT interval or TDR, but facilitated ISO-induced PVCs or VTs. Sustained monomorphic VT originating from epicardium occurred in 9/16 preparations, compared with 3/14 VTs in ISO alone. Delayed afterdepolarization-induced triggered activity was recorded in epicardium. The altered transmural activation sequence of the epicardial ectopic beats resulted in an increase in Tp-e and TDR (35.2 $\pm$ 4.5 to 65.9 $\pm$ 9.8 and 32.1 $\pm$ 3.6 to 52.6 $\pm$ 13.0 ms, respectively, $n = 7$, $p < 0.05$). Single extrastimuli induced PVT in 3/9 preparations. Bidirectional VT occurred in 11/16 wedge preparations due to alternation in the origin of extrasystoles between endocardium and epicardium. Spontaneous PVT developed in 3/16 preparations. Propranolol (1.0 μM) or verapamil (1.0 μM) completely suppressed ectopic activity arising from epicardium, but not endocardium (Purkinje fibers).

Conclusion: Extrasystolic activity arising from epicardium contributes importantly to catecholamine-induced VT/VF under conditions mimicking ryanodine receptor dysfunction. The epicardial origin of the ectopic beats increases TDR, thus providing the substrate for the development of reentrant tachyarrhythmias.

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Synergistic effect of gap junction block and cell density on arrhythmogenesis in cultured cardiac monolayers

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Background: Spontaneously active cultures of embryonic chick heart cells may display periodically beating target patterns, stable spiral waves, or a bursting rhythm driven by the spontaneous onset and offset of spiral waves. The conditions that give rise to these different modes of behavior in culture may also underly arrhythmogenisis in the heart and are not well understood.

Methods: Cell density and cell-cell coupling were simultaneously varied to determine what combination of conditions give rise to different modes of behavior. Cells were plated at high (20×10^3 cells/cm^2), intermediate(10×10^3 cells/cm^2) and low (5×10^3 cells/cm^2) densities and treated with gap junction blocker alpha-glycyrrhetinic acid (aGA) at concentrations of 0, 5, 10, and 35 uM. Monolayers were imaged after 3 days in culture using a macroscopic imaging system that mapped activity over large areas (1 square cm) using calcium sensitive dye (Calcium Green). The results were simulated by varying connectivity and density in a simple excitable media model that incorporates spontaneous activity and a rate dependent fatigue term.

Results: Monolayers preferentially display target patterns at high and intermediate densities and bursting spirals at low densities. Gap junction block converts target patterns to bursting spirals in a manner that depends on cell density: high density monolayers require 10 uM aGA to convert to a bursting rhythm while intermediate density monolayers display bursting spirals with only 5 uM aGA.

Conclusion: Gap junction block and decreased cell density act synergistically to induce a transition from target patterns to bursting spiral waves. This experimental model may explain arrhythmogenisis in disease states such as amyloidosis, fibrosis and ischemia, where both cell coupling and viability are compromised.

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Effects of acute changes in intraventricular pressure on wavebreak and rotor dynamics during ventricular fibrillation in an ovine heart falilure model

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Heart failure (HF) promotes ventricular fibrillation (VF), presumably through a process of electrophysiological remodeling. However, cardiac arrest following VF causes an acute increase in intraventricular pressure, which may also affect VF dynamics via mechano-electrical feedback. We sought to determine the relative contribution of stretch to VF dynamics in isolated failing hearts.

Methods: HF was induced by rapid ventricular pacing for 4-6 weeks in 8 instrumented versus 8 sham-operated sheep. VF was first induced in the opened-chest animal and left ventricular pressure (LVP) was monitored during 3 min of VF. Hearts were isolated and blood-perfused in a Langendorff setup. Optical mapping of the anterior epicardial aspect of both ventricles (area $\approx$16 cm^2) was performed during VF at low (0-5 mmHg) and high (25-30 mmHg) LVP. Maximum dominant frequency (MDF), density of singularity points (SPs) and SP lasting more than one revolution (rotors) were analysed.

Results: At sacrifice, ejection fraction (EF) was 37 $\pm$ 10% in HF and 65 $\pm$ 4% in controls ($p < 0.001$). After 10-20s of VF in situ, LVP reached a similar plateau level in both groups (30 $\pm$ 7 Vs. 28 $\pm$ 7 mmHg, NS). In isolated hearts, at low pressure, VF in HF was slower (8.8 $\pm$ 0.8 Vs 10.1 $\pm$ 1.2 Hz; $p < 0.04$) and more organized; i.e., there were less SPs (14 $\pm$ 5 Vs. 21 $\pm$ 3 SPs/cm^2/sec, $p < 0.01$), less rotors (0.22 $\pm$ 0.1 Vs. 0.84 $\pm$ 0.5 rotors/cm^2/sec, $p < 0.03$) and with longer life spans (167 $\pm$ 10 Vs. 134 $\pm$ 14 ms, $p < 0.001$). We found a positive correlation between EF and SP density ($r = 0.74$, $p < 0.003$). High LVP did not affect frequency, but significantly increased SPs density ($p < 0.004$) to 18 $\pm$ 5 SPs/cm^2/sec in HF and 24 $\pm$ 3 in controls, and rotors ($p < 0.02$) to 0.4 $\pm$ 0.3 rotors/cm^2/sec and 1 $\pm$ 0.5, indicating lower VF organization. There was no interaction between HF and high pressure, i.e. high pressure modified VF parameters in the same manner in HF and controls.

Conclusion: As HF advances, VF organization progressively increases. Acutely increased intraventricular pressure partially reverses these effects by a mechanism that is independent on the remodeling.