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OVEREXPRESSION OF ENDOTHELIAL INSULIN-LIKE GROWTH FACTOR-1 RECEPTORS (IGF-1R): A NOVEL ROLE FOR IGF-1R IN ENDOTHELIAL FUNCTION AND REPAIR

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Introduction Endothelium-derived nitric oxide (NO) is a critical regulator of vascular homeostasis, repair and regeneration. We recently demonstrated that reducing insulin-like growth factor-1 receptor (IGF-1R) numbers in the endothelium, thereby decreasing the proportion of insulin resistant hybrid receptors, enhances NO bioavailability and increases endothelial cell (EC) insulin sensitivity (Abbas*, Imrie*, Viswambharan* *et al. Diabetes* 2011;**60**:2169–78).

Methods To examine the effect of increasing IGF-1R in EC we generated transgenic mice overexpressing the human IGF-1R in EC (human IGF-1R endothelium overexpressing mice (hIGFREO)). Glucose and insulin sensitivity were measured by tolerance testing and plasma insulin and IGF-1 levels were analysed by ELISA. The response of aortic rings to increasing doses of phenylephrine (PE), with and without L-NMMA (a NO inhibitor), were measured ex vivo in an organ bath. NO release, eNOS activity and phosphorylation of eNOS (all in response to insulin) were measured by DAF fluorescence, the conversion of L-arginine to L-citrulline, and western blotting respectively. Endothelial regeneration was investigated by guide-wire injury of the femoral artery with quantification of Evans-blue dyed denuded area and migration assays were performed in response to VEGF by H&E staining.

Results Increased endothelial IGF-1R numbers had no effect on glucose tolerance or insulin sensitivity in hIGFREO mice compared to wild-type (wt) littermates and fasting plasma glucose, insulin and IGF-1 levels were similar. Aortae from hIGFREO mice were hypercontractile to phenylephrine (PE) (E_{max} wt = 0.62 ± 0.045 vs hIGFREO = 0.91 ± 0.045 , $p=0.036$) and had blunted constrictor responses to LNMMA (E_{max} wt = 106.1 ± 30.10 vs hIGFREO = 47.7 ± 9.87 , $p=0.048$) indicating reduced basal NO bioavailability. In response to insulin EC from hIGFREO had: reduced NO release (wt = 4500 ± 1000 vs hIGFREO = 1500 ± 700 , $p<0.05$); reduced eNOS activation (wt = $170\% \pm 25$ vs hIGFREO = $58\% \pm 3$, $p<0.04$); and decreased phosphorylation of eNOS ($p=0.027$). After endothelium denuding arterial injury hIGFREO mice demonstrated accelerated endothelium regeneration (recovered area: wt = $40.27\% \pm 5.7$ vs hIGFREO = $57.25\% \pm 2.3$, $p=0.003$) and enhanced endothelial cell migration under control conditions and in response to VEGF ($p<0.001$).

Conclusions Manipulation of IGF1-receptor numbers may represent a novel strategy for altering insulin sensitivity and vascular NO production. This data demonstrates uncoupling of endothelial NO bioavailability and vascular repair.

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LOCAL β -ADRENERGIC STIMULATION OVERCOMES SOURCE-SINK MISMATCH TO GENERATE FOCAL ARRHYTHMIA

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Background β -Adrenergic receptor (β -AR) stimulation produces sarcoplasmic reticulum (SR) Ca²⁺ overload and delayed after-depolarisations (DADs) in isolated ventricular myocytes. However, in the intact heart, strong electrotonic coupling means that depolarisation from many thousands of cells is required for an action potential to propagate. The mechanisms by which cellular DADs are

synchronised to overcome the source-sink mismatch and produce focal arrhythmia remain unknown. We aimed to determine if localised β -AR stimulation can produce spatio-temporal synchronisation of DADs in the intact heart, and to examine the effects of tissue geometry and cell-cell coupling on the induction of focal arrhythmia.

Methods and Results Simultaneous optical mapping of transmembrane potential (Vm) and Ca²⁺ transients was performed in normal rabbit hearts during subepicardial injections (50 μ l) of norepinephrine (NE, 30–250 μ M) or control (normal Tyrodes). The protocol was performed at baseline and during partial gap junction uncoupling with carbenoxolone (CBX). Local NE produced premature ventricular complexes (PVCs) arising from the application site in all 15 hearts, and a dose-response was evident (low-dose: 0.45 ± 0.62 vs high-dose: 1.33 ± 1.46 PVCs/application, $p<0.0001$). NE-induced PVCs demonstrated areas of abnormal Vm-Ca²⁺ delay at the initiation site, indicating a Ca²⁺-mediated mechanism. PVCs were more inducible with NE at RV vs LV injection sites (1.48 ± 1.50 vs 0.55 ± 0.89 , $p<0.01$) and following CBX (2.18 ± 1.43 vs 1.33 ± 1.46 , $p<0.05$). Analysis of NE tissue exposure and Vm-Ca²⁺ dynamics revealed that differences in focal arrhythmia propensity between RV and LV, and following gap junction uncoupling were due to modulation of source-sink interactions.

Conclusions These data provide the first experimental demonstration that localised β -AR stimulation can produce spatio-temporal synchronisation of SR Ca²⁺ overload and release in the intact heart and highlight the critical nature of the source-sink balance in the initiation of focal arrhythmias.

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ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY-LIKE PHENOTYPE REVEALED BY ENDURANCE TRAINING IN HETEROZYGOUS DESMOGLEIN2 MUTANTS

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Background Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare cardiomyopathy but significantly contributes to sudden cardiac death in young otherwise healthy patients, especially long distance athletes. 5%–10% of patients with ARVC harbour mutations in the extracellular domains of the desmoglein (DSG) 2 gene. To assess the role of DSG2 in the ARVC pathomechanism, mice lacking exons 4–6 of the endogenous DSG2 gene (DSG2mt) were generated. Homozygous DSG2mt/mt mice developed dilatation of ventricles and pronounced fibrosis. Heterozygous DSG2mt/wt mutants, however, did not show such morphological alterations.

Objective To study whether physical exercise provokes a cardiac phenotype in DSG2wt/mt mice they were subjected to endurance training together with wild-type (WT) littermates.

Methods/Results Group swimming training sessions were performed 6 times a week starting with 5 min and gradually incrementing to 90 min/d for 7 weeks. Echocardiography was performed before and after training using a small animal ultrasound unit. Right ventricular (RV) diameters were increased in DSG2wt/mt both compared to pretraining and compared to WT after training. Right ventricular function was also decreased after training compared to pretraining and compared to WT littermates (see Abstract 111 table 1 for values, * $p<0.05$, d = diastolic, s = systolic, lav = long, sav = short axis view, FAC = fractional area shortening, HR = heart rate). Neither left ventricular diameters nor function differed between DSG2wt/

mt and WT littermates. Electrophysiological studies in Isolated Langendorff DSG2wt/mt and WT hearts showed comparable ventricular action potential duration and effective refractory periods. DSG2 mutation correlated with increased arrhythmia inducibility after endurance training. Ventricular arrhythmias were induced in 5 of 8 DSG2wt/mt, but in none of 7 WT hearts during right ventricular stimulation by a single extrastimulus ($p=0.03$). In conclusion, endurance training reveals an ARVC-like phenotype in otherwise healthy and morphologically inconspicuous DSG2wt/mt mice presenting right ventricular dilatation, decreased right ventricular contractility and increased inducibility of ventricular arrhythmias during right ventricular pacing.

Abstract 111 Table 1

Genotype	WT	DSG2 mt/wt
n (females/males)	8 (6/2)	9 (7/2)
HR (bpm)	424 ± 6	435 ± 7
RVlvd (mm ²)	3.67 ± 0.20	$4.29 \pm 0.28^*$
RVsavl (mm ²)	4.76 ± 0.28	$6.26 \pm 0.29^*$
RVsavl (mm ²)	2.10 ± 0.26	$3.50 \pm 0.25^*$
RV FAC (%)	55 ± 4	$44 \pm 3^*$
Age (weeks)	21 ± 0.2	21 ± 0.2
Weight (g)	24.3 ± 0.7	25.5 ± 1.6

112 LOCATION OF SUBSIDIARY ATRIAL PACEMAKERS FOLLOWING ABLATION OF THE SINUS NODE IN THE GOAT

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Introduction The development of sinus node dysfunction in patients may be accompanied by the emergence of subsidiary atrial pacemakers (SAPs) and/or junctional escape rhythms. It has been postulated that biological modification of SAPs may provide a potential alternative to implantation of artificial pacemakers for the treatment of sinus node disease ("biological pacemakers"). We investigated the structural and functional characteristics of SAPs in a chronic goat model.

Methods Adult female goats ($n=7$) underwent epicardial radio-frequency catheter ablation of the sinus node, via right thoracotomy. The endpoint for ablation was defined as a fall of heart rate by 50% accompanied by change in P wave morphology, or the emergence of a junctional rhythm. An epicardial pacing system was implanted for continuous monitoring. Single lead surface ECG, corrected sinus node/subsidiary atrial pacemaker recovery time (CSNRT, CART) and interrogation of the implanted pacemaker were performed at baseline and weekly for 4 weeks following recovery from anaesthetic. After 4 weeks epicardial mapping was performed and location of any SAPs determined.

Results Epicardial mapping preablation confirmed sinus rhythm in all goats and guided attempts at ablation. The predefined acute endpoint of ablation was achieved in six goats. In five goats the ablated surface area was no greater than a 1×2 cm, occupying the cranial half of the intercaval region near the crista terminalis, but was more extensive in two goats (including the animal with failed acute endpoint). Despite profound postablation bradycardia, no animal required pacing for more than the first 24 h post-ablation. Follow-up monitoring revealed an abnormal CART (>500 ms) in three goats, and the P wave morphology remained different from the preablation state in five goats, consistent with a non-sinus rhythm. Repeat epicardial mapping 4 weeks post-procedure revealed stable

SAPs located in the caudal half of the intercaval region (low right atrium) in four goats. In two animals there was evidence of recovery of sinus node function and in a further goat the SAP was located within the interatrial septum or left atrium.

Conclusion Destruction of the sinus node in this experimental model resulted in the generation of chronic SAP activity in all animals. In the majority of cases SAPs were located in the low right atrial free wall (Abstract 112 figure 1) and constitute a promising stable target for electrophysiological modification in a whole animal model of sinus node disease.

Abstract 112 Figure 1 Schematic diagram of location of subsidiary atrial pacemakers. Each number represent the location of SAP of an individual goat (1–7). Animal number 4 not displayed due to inaccessible SAP location.

113 BLOOD THROMBOGENICITY IS INVERSELY RELATED TO CORONARY LESION SEVERITY IN PATIENTS WITH NON ST-ELEVATION ACUTE CORONARY SYNDROME AND TYPE 2 DIABETES MELLITUS

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In patients with type 2 diabetes mellitus (T2DM), non-ST elevation—acute coronary syndrome (NSTEMI-ACS) occurs frequently secondary to non-obstructive coronary lesions than those without T2DM. Pathophysiological events leading to acute myocardial infarction in these individuals remain largely unknown, and subsequently demonstrate a poorer prognosis than those without T2DM.

Methods We evaluated blood thrombogenicity (BT) in 80 patients with and without T2DM 7–10 days after troponin positive NSTEMI-ACS. In accordance to AHA/ESC recommendations all patients received secondary prevention therapy including aspirin and clopidogrel (300 mg loading and 75 mg maintenance doses respectively). Coronary artery disease (CAD) was quantified by coronary