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Free Communications 5: Cardiac muscle

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Chair: Lea Delbridge

Does lignocaine increase the chance of survival from massive heart attack?

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Introduction: Lignocaine (lidocaine) blocks voltage-activated sodium channels and has been used extensively since the 1960s in patients presenting with suspected acute myocardial infarction (AMI). In a review of many clinical trials and publications, Yadav & Zipes (2004) concluded that although prophylactic lignocaine administered after a suspected AMI appeared to reduce the incidence of primary ventricular fibrillation (VF, the fastest and most lethal tachyarrhythmia) by as much as 33%, lignocaine was also associated with an increased incidence of bradycardia (slow heart rate), asystole (no heart beat), and subsequent mortality. Because of this, Yadav & Zipes recommended that on the basis of contemporary information, prophylactic lidocaine should not be used in the management of patients with proved or suspected AMI.

Aim: To determine in an animal model of AMI whether lignocaine reduces the incidence of tachyarrhythmias (VF and/or haemodynamically compromising ventricular tachycardia (VT)) when administered prior to a coronary artery occlusion sufficient to produce an AMI.

Methods: 21 pigs (M+F, 20-35 kg) were sedated with stresnil (1-2 mg/kg im), anaesthetised with thiopentone sodium (10-15 mg/kg iv) and maintained under general anaesthesia with a mixture of isoflurane (0.5 – 2%) in oxygen. Artificial ventilation was maintained at a volume of 15 ml/kg and a rate of 12 breaths per minute. An intravenous saline drip was maintained for intra-operative hydration or lignocaine administration. Blood pressure (BP) and a lead II electrocardiogram (ECG) were monitored, digitised and recorded. Lignocaine (2.5 – 12 mg/kg bolus plus 0.05 – 0.24 mg/kg/min iv continuous infusion) was administered to 11 of the pigs. Following a mid-sternotomy and dissection of the pericardium, the left anterior descending coronary artery (LAD) was ligated 40 min (39 +/- 13 min sd) after the commencement of lignocaine or saline administration mid-way along its length.

Results: The results in Table column 1 refer to the number of animals; the remaining columns refer to all animals. Sustained is defined as lasting longer than 15s and likely to be fatal if not externally reverted.

	animals developing sustained arrhythmia	sustained VTs in 1st 2 h	sustained VFs in 1st 2 h	non-sustained arrhythmias between 1 and 15s in 1st 2 h	total arrhythmias in 3rd hour
Control (n=10)	10	13	43	91	88
Lignocaine (n=11)	6	3	10	70	2

Discussion and Conclusion: The results clearly showed that when lignocaine was administered prior to a coronary artery occlusion it significantly reduced the number of animals which developed a haemodynamically compromising tachyarrhythmia and the number of sustained and non-sustained tachyarrhythmias for all animals.So why then do Yadav & Zipes recommend that lignocaine not be used? Consider the following 2 points: a) After a coronary artery occlusion, the distal tissue becomes ischaemic, hypoxic, and ultimately infarcted. Between the ischaemic region and the surrounding perfused region there is a border zone which receives limited perfusion. Clearly then, iv lignocaine administered after a coronary artery occlusion can not have a pharmaceutical effect on the ischaemic region other than at the border zone. b) Tachyarrhythmias can develop from AMIs in which the occlusion remains intact as well as from AMIs in which the occlusion dissipates and the tissue becomes reperfused. From these 2 points, we can develop 3 scenarios: 1) that an ischaemic region can become reperfused subsequent to an occlusion if the occlusion dissipates, 2) that an occlusion can remain intact but the ischaemic region can be small either because the occlusion is in a small artery or because the border zone is wide as a result of extensive collateral circulation, and 3) that an occlusion can remain intact and produce a large ischaemic region with a narrow border zone. In light of our results and the reduction in incidence in arrhythmias quoted by Yadav & Zipes, we suggest that lignocaine would likely reduce the incidence of arrhythmias in the first 2 scenarios wherein iv lignocaine could perfuse a large portion of the ischaemic region. In contrast, we suggest that in the 3rd scenario, iv lignocaine would never reach the ischaemic region and subsequently it would have no effect on that tissue, irrespective of the dose administered. Finally, we suggest that the lignocaine-related bradycardic and asystolic deaths referred to by Yadav & Zipes may have resulted from overdosing of lignocaine in a setting where it was showing no effect as in scenario 3. In this instance lignocaine, being a sodium channel blocker, would be shutting-down cell conduction. Because of these considerations, we argue with Yadav & Zipes' recommendation and suggest that lignocaine is beneficial in reducing the incidence of tachyarrhythmia in those AMIs where it can be delivered to the ischaemic tissue but the serum levels need.to be kept low so action potentials are not blocked.

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Acute application of n-3 polyunsaturated fatty acids modify calcium sparks in permeabilised rat cardiac myocytes

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Animal studies have demonstrated that acute administration of n-3 polyunsaturated fatty acids (PUFAs) prevent ischemia-induced arrhythmias (Billman, *et al.*, 1997). During and following ischemia, the sarcoplasmic reticulum (SR) becomes overloaded with Ca^{2+} and spontaneous release events occur (Daniels *et al.*, 1991). The subsequent rise in cytosolic [Ca^{2+}] activates sarcolemmal current which can in turn produce after-depolarisations and arrhythmias. PUFAs can reduce the ionic currents responsible for the cardiac action potential and this is believed to be the mechanism for their cardio-protective effects (Xiao *et al.*, 1997).

Studies on the effects of PUFAs on Ca^{2+} handling have shown that 10 μmol/l of eicosapentaenoic acid (EPA) resulted in a 15% reduction in the amplitude of spontaneous Ca^{2+} waves (Negretti *et al.*, 2000). Also 15 μmol/l EPA was found to reduce both the width and duration of Ca^{2+} sparks by ~25% (Honen *et al.*, 2003). When PUFAs were applied directly to the SR Ca^{2+} release channel (Ryanodine receptor, RyR) in artificial bilayers, 30-50 μmol/l caused a 50-80% decrease channel activity. It is not clear if the action of PUFA's on cell Ca^{2+} handing is mediated primarily by the sarcolemma or SR.

This study aimed to determine if PUFAs could directly affect the Ca^{2+} release properties of the intact SR. This was done by measuring the properties of Ca^{2+} sparks in permeabilised cardiac myocytes in which sarcolemmal ion currents did not contribute to Ca^{2+} release within the cell.

Sprague-Dawley rats were anesthetized with sodium pentobarbitone (1ml/kg), the hearts were removed and the cardiac ventricular myocytes were isolated by enzymatic digestion. Following isolation, the myocytes were treated with saponin to permeablise the sarcolemma. Ca^{2+} sparks were viewed using confocal microscopy in line scan mode using the Ca^{2+} indicator fluo-3. Fatty acids tested were oleic acid (OA), arachidonic acid (AA), EPA and docosahexaenoic acid (DHA). Images of Ca^{2+} sparks were collected prior to the addition of fatty acids and at 2 min and 5 min following their addition. Sham experiments were performed to ensure SR Ca^{2+} rundown did not occur.

Spark properties did not vary during experiments in both sham and OA (mono unsaturated fatty acid) treated cells indicating that rundown did not occur. However, PUFA's did affect some spark properties. AA at 50 μmol/l, significantly reduced (10%) spark width within 2 min of exposure. EPA at 50 μmol/l significantly reduced spark intensity (21%) within 5 min. Exposure to 50 μmol/l DHA for 2 min reduced intensity by ~25% and spark mean rate of rise by ~20%. Following exposure for 5 min, spark frequency reduced by ~30% and spark width reduced by ~7%. Even at 30 μmol/l DHA was observed to significantly alter spark properties within 2 min.

The actions of fatty acids on Ca^{2+} sparks in this study were similar to those seen on the open probability of RyRs (Honen *et al.*, 2003). During ischemia, fatty acids are released within the cell by PLA_2. Fatty acid concentrations (all species) up to 0.73 mmol/l have been measured in rat aortic plasma during transient ischemia (Chen *et al.*, 2001). Previously we have shown that 10-20% of membrane fatty acids can release n-3 PUFAs and so it is quite possible for free PUFA levels to reach 70 μmol/l. Therefore under physiological ischemic conditions it is likely that PUFAs could play an important role in protecting myocardium from ischemia by modulating Ca^{2+} handling.

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Abnormal calcium transients and calcium handling protein expression in cardiomyocytes from *mdx* (dystrophic) mice

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Duchenne muscular dystrophy (DMD) is a fatal X-linked genetic disorder caused by deficiency of the cytoskeletal protein dystrophin. DMD patients have extensive skeletal muscle degeneration and a dilated cardiomyopathy (DCM). The *mdx* mouse also lacks dystrophin and in skeletal muscle it exhibits membrane damage and an abnormal influx of Ca^{2+}. The present study was aimed at characterising ventricular performance which may contribute to DCM in *mdx* mice. Ventricular myocytes were isolated from 8-week old wild-type and *mdx* mice and intracellular Ca^{2+} measured with the fluorescent indicator fluo-4 during electrical and caffeine (10 mM) induced stimulation. Protein expression of the ryanodine receptor (RyR), the sarco endoplasmic reticulum calcium ATPase (SERCA) and phospholamban were analysed using immunoblotting techniques. The peak of the electrically stimulated Ca^{2+} transient was significantly greater in *mdx* mice, but the time to peak was significantly shorter. These findings were not the result of increased sarcoplasmic reticulum (SR) Ca^{2+} loading as the caffeine-induced Ca^{2+} transient peak was unchanged in *mdx* mice. The increase in peak calcium transient and the decreased time to peak could be due to the significantly increased levels of RyR protein expression (4-fold), allowing more rapid Ca^{2+} release from the SR during excitation. However, this is not usually found in established DCM (Kubo *et al.*, 2001). It was found that the rate of decline of the electrically stimulated Ca^{2+} transient was significantly slower in *mdx* mice, but the rate of decline of the caffeine-induced Ca^{2+} transient was unchanged, suggesting that the slower removal of Ca^{2+} from the intracellular milieu was a result of decreased SERCA activity, and not decreased sodium-calcium exchanger activity. SERCA protein levels were unchanged, but phospholamban levels were increased significantly (2-fold). The slower rate of decline of the Ca^{2+} transient in *mdx* mice is therefore possibly a result of increased inhibition of SERCA by phospholamban, a finding which is consistent with other studies of DCM (Meyer *et al.*, 1995). This is further supported by the finding that the SERCA/phospholamban ratio was significantly smaller in *mdx* mice. It is concluded that dystrophin deficiency causes impairment in the Ca^{2+} handling properties of *mdx* ventricular myocytes, which may play a role in the development of DCM. Future work will test whether the increase in peak Ca^{2+} transients in the *mdx* mouse is an early compensatory mechanism that reverses as the cardiomyopathy progresses, by investigating myocyte Ca^{2+} handling in older mice.

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Mechanisms underlying the stretch-dependent slow inotropic response in isolated mouse myocardium

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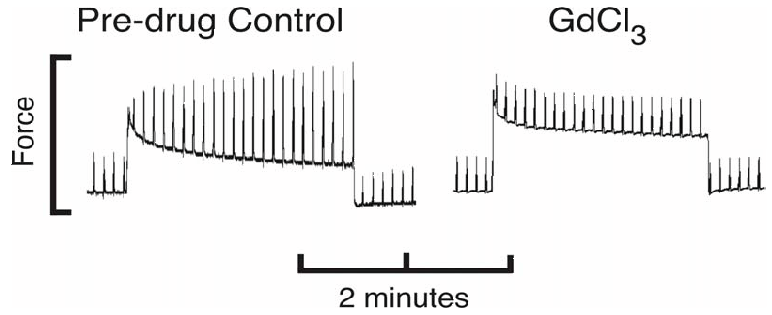
When cardiac muscle is subjected to stretch the force of contraction increases, allowing the intact heart to adjust its output to the body's demand (Allen & Kentish, 1985). This increase in contractility has been shown *in vivo* to occur in two distinct phases. Initially there is an abrupt increase in force that coincides with the stretch, and secondly there is a slower response that develops over a period of a few minutes (the ''slow force response''). The first of these responses is largely due to a change in the sensitivity of the contractile proteins to Ca^{2+}, whereas the slow force response is accompanied by a concomitant increase in the magnitude of the intracellular Ca^{2+} transient (the event that initiates contraction). It has been proposed that stretch-activated channels contribute to Ca^{2+} entry after stretch (Calaghan & White, 2004). The aim of the present study was to reinvestigate the mechanisms underlying the slow force response of cardiac muscle.

Mice were euthanased and cardiac trabeculae or papillary muscles (< 1 mm in length, and 0.1 - 0.3 mm in diameter), dissected from the right ventricle of mouse hearts, were mounted in a muscle chamber between a hook attached to a force transducer and a lever connected to a motor capable of making precise changes in muscle length. Each preparation was then subjected to a step increase in length for 2 minutes whilst isometric force was recorded.



Response of a representative mouse papillary muscle subjected to step increases in length before, and during application of $GdCl_3$.

One minute after the initial length change, active force increased by 77 ± 17% of the force immediately following the stretch (n = 16). Subsequent application of either 400 µM streptomycin, or 20 µM $GdCl_3$ (blockers of stretch-activated channels) reduced the slow force response ($p \leq 0.01$) for identical step increases in length (streptomycin: from 86 ± 25% to 38 ± 14% (n=9), or $GdCl_3$: from 65 ± 21% to 12 ± 7%, n=7), suggesting a possible role for stretch-activated channels in the slow force response.

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Salutary effects of pyruvate are more evident in female than male glut4-deficient mouse hearts

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The mechanisms involved in diabetic cardiac pathology are not well understood. Insulin resistance, defined as a decrease in the ability of insulin to stimulate cellular glucose uptake is often termed the "pre-diabetic" state. Insulin-stimulated glucose uptake in the heart is mediated by the glut4 transporter. It is known that alterations in substrate availability are associated with cardiac hypertrophy, reduced energy production and subsequent cardiac contractile dysfunction (Taegtmeyer *et al.*, 2002). There is some epidemiologic evidence indicating that diabetes has greater negative impact on cardiovascular morbidity and mortality in women than men (Sowers, 1998). The goal of this study was to investigate the metabolic basis for this sex-specific vulnerability in the heart. The inotropic actions of pyruvate, a metabolic product of glycolysis and an oxidizable fuel in the heart, were investigated in a genetic animal model of insulin resistance.

Hearts of age-matched female and male mice (22 week) from three genetic groups were evaluated: wildtype (WT), 'knock-down' (KD, 15% WT glut4) and 'knock-out' (KO, ≤ 5% WT glut4). Mice were anaesthetised with pentobarbitone sodium (70mg/kg, ip), and hearts excised and arrested in iced Krebs-Henseleit buffer. Hearts were perfused (Langendorff-mode) in normoxic conditions with Krebs-Henseleit bicarbonate buffer (37ºC). Left ventricular function was measured using a fluid-filled balloon interfaced to a pressure transducer (MLT884). 5mM glucose with 100uU/ml insulin was provided as the substrate for basal measurements. The perfusate was then supplemented with 5mM pyruvate.

Under basal conditions hearts of female and male glut4-KO mice exhibited significantly reduced developed pressure relative to WT. Hearts of female glut4-KD mice were significantly more functionally impaired than hearts of male glut4-KD mice relative to WT. Pyruvate supplementation significantly improved developed pressure in female and male glut4-KD & glut4-KO hearts. Interestingly, female glut4-KO hearts were most responsive to pyruvate supplementation.

Developed Pressure	**Genotype**	**Basal (mmHg)[#]**	**With 5 mM pyruvate (mmHg)[#]**
Female	WT	137.9 ± 12.9	126.5 ± 11.6
	KD	106.2 ± 13.1 *	139.7 ± 13.3
	KO	108.4 ± 5.7 *	182.7 ± 7.6
Male	WT	151.2 ± 13.3	147.2 ± 6.7
	KD	145.8 ± 6.2 †	175.9 ± 6.3
	KO	91.7 ± 10.4 *	157.4 ± 18.9
[#] $p<0.05$ sex × genotype * $p<0.05$ *vs* WT, †$p<0.05$ *vs* KO			

The acute improvement in isolated function of glut4-deficient hearts after pyruvate supplementation suggests that substrate limitation is a major cause of contractile dysfunction. The responsiveness of glut4-deficient hearts to pyruvate indicates that adaptive metabolic remodelling may have occurred early in development to preserve metabolic 'flexibility'. This adaptation may be accentuated in insulin resistant hearts of females.

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The angiotensin type 2 receptor prevents cell death in neonatal cardiomyocytes of the hypertrophic heart rat

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The Hypertrophic Heart Rat (HHR) displays cardiomyocyte hypertrophy in Association with an apparent reduction in myocyte number in adulthood (Harrap *et al.*). This suggests the possibility of reduced hyperplasia or increased apoptosis during very early cardiac development. The angiotensin AT_1 and AT_2 receptor subtypes have been implicated in both cellular growth and apoptosis, although the precise mechanisms are unclear. Cardiac AT_2 receptor expression is high during early development (Bastien *et al.*), and it has been suggested that AT_2 receptor-mediated actions counterbalance those of the AT_1 receptor. Specifically, it has been proposed that the AT_2 receptor inhibits growth and promotes apoptosis, but data from transgenic and knock-out experiments do not support this hypothesis. The aim of this study was to determine the relationship between cardiac AngII receptor expression levels and neonatal cardiomyocyte growth and apoptotic responses in the HHR compared with their Normal Heart Rat (NHR) control strain.

Cardiac ventricles were freshly harvested from HHR and NHR neonates at post-natal day 2. Tissue AT_{1A} and AT_2 mRNA expression levels were quantified by real-time RT-PCR. Relative to NHR, HHR neonatal hearts exhibited significantly higher AT_2 and lower AT_{1A} receptor expression levels (4.6-fold higher AT_2/AT_1 ratio in HHR compared with NHR).

Neonatal cardiomyocytes were isolated by enzymatic digestion and plated at high density (1250 cells/mm^2). Adenoviruses containing constructs for either the AT_{1A} or AT_2 receptors were created. After 48 hours, myocytes were infected with either AT_{1A} and/or AT_2 receptors to achieve a physiological level of receptor expression (150 fmol receptor protein/mg total cell protein). In addition, to mirror receptor expression in neonatal HHR hearts, cells were infected with AT_{1A} and AT_2 receptors in a 4:1 ratio. Adenoviruses also co-expressed green fluorescent protein (GFP), making possible to identify and morphologically assess infected cells. To assess myocyte apoptosis counts were performed (5 fields from each triplicate well in n = 4 experiments) of infected HHR and NHR cells that displayed vacuolisation. The incidence of apoptosis was studied after 72 hours exposure to 0.1 μM AngII.

When infected with the AT_{1A} receptor alone, HHR myocytes showed significantly higher proportions of apoptotic cells than NHR (22.7%, SE 4.1 vs 1.1%, SE 0.6, $P < 0.001$). With the addition of the AT_{1A} receptor antagonist candesartan (1 μM), the proportion of apoptotic cells in HHR with the AT_{1A} receptor alone fell to levels similar to those seen in NHR (1.8%, SE 0.8). A similar suppression of apoptosis was observed (2.0%, SE 0.9) when the PKC signal transduction pathways that mediate AT_{1A} receptor signalling were inhibited with BIM (1 μM). When cells were infected with both the AT_{1A} and AT_2 receptors, evidence of apoptosis in HHR cells virtually disappeared (0.4%, SE 0.1).

In HHR neonatal cardiomyocytes, intrinsic (presumably genetic) differences seem to predispose to significantly increased AngII-induced apoptosis when the AT_{1A} receptor is expressed in isolation. Co-expression of the AT_{1A} and AT_2 receptors rescues the cells from apoptosis. These findings suggest novel protective physiological mechanisms for the AT_2 receptor in early cardiac growth.

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