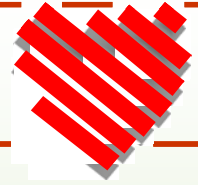
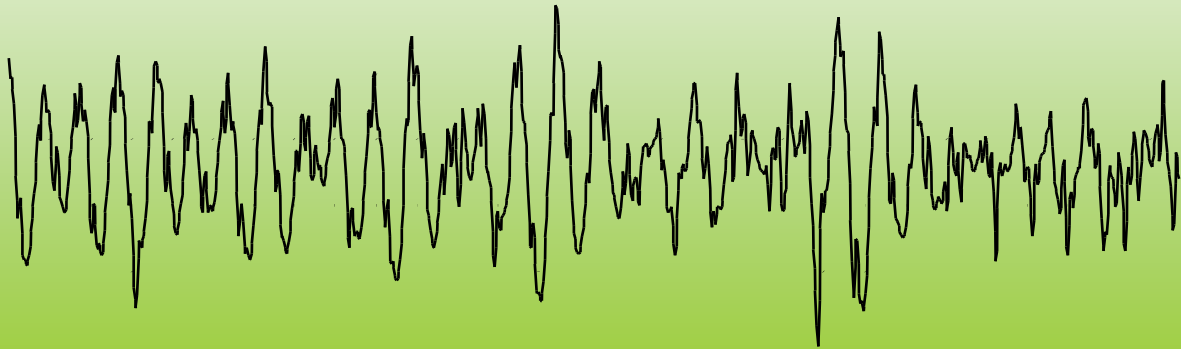


**THE BRITISH SOCIETY FOR  
CARDIOVASCULAR RESEARCH**



*Autumn 2010 meeting*

**The Future of Arrhythmia Research:  
Lambeth Conventions Update**



**Dates: 6<sup>th</sup>-7<sup>th</sup> Sept, 2010**

**Venue: Lambeth Palace, London**

**Abstract Booklet**

**We thank the following meeting sponsors:**



## List of abstracts

LAMBETH CONVENTIONS UPDATE: THE AGENDA. **Curtis MJ, London, UK**

### Poster session on Day 1 in the reception room:

CHARACTERISING CARDIOVASCULAR SAFETY PROFILE OF MUSCARINIC ANTAGONIST, IPRATROPIUM. **Simmons K, Khan J, Hussain A and Maddock HL. Coventry, UK**

CANCER THERAPY DRUG DOXORUBICIN, EXACERBATES MYOCARDIAL ISCHAEMIA REPERFUSION INJURY. **Gharanei AM, Hussain A and Maddock HL. Coventry, UK**

THE BRONCHODILATOR SALBUTAMOL IS ASSOCIATED WITH INCREASED MYOCARDIAL INJURY IN A PRE-CLINICAL HEART ATTACK MODEL. **Nagra A, Maddock H, Hussain A, Coventry, UK**

THE ELECTRO-MECHANICAL WINDOW AS TORSADE DE POINTES RISK-MARKER IN CONSCIOUS AND ANAESTHETISED DOGS AFTER IKS-BLOCKADE.

**van der Linde HJ, Cools F, Vanlommel A, Janssens S, Verrelst J, Van Deuren B, Somers Y, Teisman A, Towart R, and Gallacher DJ. Beerse Belgium**

POSSIBLE ROLE OF THE ULTRA-RAPID DELAYED RECTIFIER POTASSIUM CURRENT ( $I_{K_{UR}}$ ) IN ACTION POTENTIAL REPOLARIZATION IN RABBIT HEART. **Y Cui Y, Wilson C, Turner S, Graham S, McMahon N, Heath B. Ware, UK**

USE OF THE DOG ISOLATED CARDIAC PURKINJE FIBRE ASSAY TO INVESTIGATE A COMPOUND WITH IN VIVO PROARRHYTHMIC ACTIVITY. **Graham S, Wilson C, Cui Y, Heath B, Ware, UK**

INCREASED RISK OF CARDIAC ARRHYTHMIA IN RATS EXPOSED TO DIESEL EXHAUST AIR POLLUTION IS MEDIATED BY THE NOCICEPTIVE TRANSIENT RECEPTOR POTENTIAL A1 (TRPA1). **Hazari MS, Lamb C. Carll AP, Krantz Q, Haykal-Coates N, Winsett DW. Research Triangle Park & Chapel Hill, NC, USA.**

EFFECTS OF DIFFERENT PRECONDITIONING THERAPIES ON ARRHYTHMIAS AND AORTIC BLOOD FLOW IN AN IN VIVO RAT MODEL OF ISCHEMIA/REPERFUSION INJURY. **Ahmed LA, Salem HA, Attia AS& Agha AM. Cairo, Egypt**

VOLTAGE-DEPENDENT AFFINITY OF SOME COMPOUNDS THAT INHIBIT HERG: RAPID DISSOCIATION AND REASSOCIATION? **Milnes JT, McPate M, Dempsey CE, Duncan RS, Leaney JL, Leishman DJ., Hancox JC, Witchel H J. Bristol, Sandwich & Brighton, UK**

IMPAIRMENT OF REPOLARIZATION RESERVE BY COMBINED PHARMACOLOGICAL INHIBITION OF VENTRICULAR POTASSIUM CHANNELS IN RABBITS: IMPLICATIONS FOR ARRHYTHMIA PREDICTION. **Baczko I, Lengyel Cs, Tojbori K, Papp JGy & Varro A. Szeged Hungary**

BOTH THE OPENING AND CLOSING OF GAP JUNCTIONS MAY RESULT IN PROTECTION AGAINST THE ACUTE ISCHAEMIA-INDUCED SEVERE VENTRICULAR ARRHYTHMIAS IN ANAESTHETISED DOGS. **Vegh Á, Papp R, Gönczi M, Kovács M. Szeged, Hungary**

DISTINCT ROLE OF  $Ca^{2+}$ -CALMODULIN DEPENDENT PROTEINKINASE II IN EXCITATION-CONTRACTION COUPLING UNDER CERTAIN CONDITIONS. **Adameova A, Ravingerova T. Odbojarov & Bratislava, Slovak Republik**

DIAZOXIDE-INDUCED ANTIARRHYTHMIC PROTECTION IN THE NON-DIABETIC AND DIABETIC RAT HEART DOES NOT REQUIRE ACTIVATION OF PI3-KINASE/AKT. **Matejikova J, Pancza D, Kolar F & Ravingerova T. Bratislava, Slovak Republik & Prague, Czech Republic**

EXCITEMENT OVER AUTOMATED PATCH CLAMP: ACTION POTENTIALS FROM STEM CELLS AND TEMPERATURE EFFECTS ON HERG INHIBITION. **Haythornthwaite A, Stoelzle S, Haarmann C, Brüggemann A, George M, Fertig N. Nanion Technologies GmbH, Munich, Germany**



## Selected oral presentations on Day 2 in the main auditorium (\*presentation by):

|             |  |
|-------------|--|
| 11.30-11.40 | PREVALENCE OF ARRHYTHMIAS IN NON-IMPLANTED, DRUG-NAÏVE, FREELY MOVING DOGS. <b>Cools F*</b> , <b>Janssens S</b> , <b>Vanlommel A</b> , <b>Teisman A</b> & <b>Gallacher DJ</b> . <b>Beerse, Belgium</b>   |
| 11.45-11.55 | DELAYED CONDUCTION AND ITS IMPLICATIONS IN MURINE Scn5a+/- HEARTS. INDEPENDENT AND INTERACTING EFFECTS OF GENOTYPE, AGE AND SEX. <b>Jeevaratnam K*</b> , <b>Tee SP</b> , <b>Zhang Y</b> , <b>Guzadhur Y</b> , <b>Duehmke R</b> , <b>Grace AA</b> , <b>Lammers W</b> , <b>Lei M</b> & <b>Huang CLH</b> . <b>Cambridge &amp; Manchester UK</b> , <b>Xi'an China</b> , <b>Kuala Lumpur Malaysia</b> & <b>Al Ain, United Arab Emirates</b> |
| 12.00-12.10 | QUANTIFICATION OF SPATIAL DYNAMICS OF CARDIAC ARRHYTHMIAS. <b>Benson AP</b> , <b>Bernus O</b> , <b>Holden AV</b> , and <b>Zhang H*</b> . <b>Leeds &amp; Manchester UK</b>  |
| 12.15-12.25 | MECHANICALLY-INDUCED ARRHYTHMIAS: A NEGLECTED TARGET? <b>Saint DA*</b> , <b>Adelaide, Australia</b>  |

## Abstracts

### LAMBETH CONVENTIONS UPDATE: THE AGENDA

Curtis MJ

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The advances made in pharmacotherapy of cardiac arrhythmias in the last 20 years have been disappointingly meagre. The Lambeth Conventions (Walker et al., 1988) is a guidance for (ventricular) arrhythmia research. It was published more than 20 years ago. It has provided a framework for disciplined experimentation, and offers immutable guidance on experimental practice (use of blinding and randomization, selection of controls, etc.) but it requires an update. It is clear that the community needs to refocus on how new antiarrhythmic drugs will be found. We need to reappraise the available models and approaches. We need to expand the Lambeth Conventions guidance to cover all types of arrhythmias. We need to elaborate guidance for the study of proarrhythmia (e.g., torsades de pointes) and consider guidance for interrogating and targeting channelopathy. On September 6 and 7, 2010, invited and freely-registered participants will consider a series of lecture presentations, and engage in discussions that will inform the preparation of a new Lambeth Conventions guidance.

#### *Reference*

Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM, Cobb SM, Coker SJ, Harness JB, Harron DWG, Higgins AJ, Julian G, Lab MJ, Manning AS, Northover BJ, Parratt JR, Riemersma RA, Riva E, Russell DC, Sheridan DJ, Winslow E, Woodward B. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc Res* 22: 447-455, 1988

## CHARACTERISING CARDIOVASCULAR SAFETY PROFILE OF MUSCARINIC ANTAGONIST, IPRATROPIUM

Simmons K, Khan J, Hussain A & Maddock HL.

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Chronic Obstructive Pulmonary Disease (COPD) is a disease characterised by inflammation of the airways which accounts for the 4th highest cause of global death. The functional role of Muscarinic receptor antagonists such as Ipratropium Bromide (Atrovent) and Tiotropium Bromide (Spiriva) have been shown to successfully improve pulmonary function in COPD patients. Recent studies have associated an increased risk of cardiovascular events in COPD patients currently receiving treatment with Muscarinic receptor antagonists such as Ipratropium (Ogale et al., 2010). The study aimed to investigate the effects of Ipratropium on the myocardium subjected to ischaemia- reperfusion (I/R). Langendorff hearts were subjected to control or I/R in the absence or presence of Ipratropium (1nM, 10nM or 100nM). Hearts underwent triphenyl tetrazolium staining for infarct size assessment. In further studies cardiac myocytes were isolated and exposed to simulated I/R in the absence or presence of Ipratropium (1fM-1mM) and cellular injury was subsequently determined by measurement of live/death ratio and apoptosis using flow cytometry. Administration of Ipratropium (10nM or 100nM) throughout reperfusion significantly increased infarct size to risk ratio (%) compared with non-treated controls (62±2%, 74±4% vs. 52±3%, control P<0.01 respectively). In isolated myocyte experiments, Ipratropium treated groups were observed to significantly increase apoptosis and cell death compared to non-treated controls. This is the first pre-clinical study to show that Ipratropium significantly increases myocardial injury when administered during I/R. Further studies are being investigated to determine the mechanism of action of the cardiovascular events associated with certain bronchodilators.

### *Reference*

Ogale, S. et al., (2010) Chest 137(1):13-19

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## CANCER THERAPY DRUG DOXORUBICIN, EXACERBATES MYOCARDIAL ISCHAEMIA REPERFUSION INJURY

Gharanei AM,\* Hussain A & Maddock HL.

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Drug induced cardiotoxicity is a major concern to the pharmacological industry and it is one of the main reasons for non-approval, re-labelling, warnings and withdrawal of pharmaceutical compounds from the market (1). Doxorubicin is an anthracycline antibiotic used in cancer therapy. Although issues relating to Doxorubicin and cardiac safety have been well established in normal conditions, the effects of this drug on the myocardium during ischaemia-reperfusion have not been investigated in detail to date.

Studies were undertaken in Langendorff hearts and adult / neonatal ventricular myocytes, subjected to ischaemia-reperfusion. Hearts underwent triphenyl tetrazolium staining for infarct size assessment. Treatment groups (n=7-10) were perfused in the presence or absence of Doxorubicin. Following isolation, neonatal or adult cardiomyocytes were subjected to simulated ischaemia- reoxygenation and Doxorubicin was administered at reoxygenation. Cellular injury was subsequently determined by measurement of live/death ratio and apoptosis using flow cytometry. Administration of Doxorubicin in normoxic conditions or during ischaemia-reperfusion significantly increased infarct size to risk ratio (%) compared to respective non-treated controls (30 ± 5% and 81 ± 6% Doxorubicin vs. 10 ± 2% and 65 ± 3% non-treated control, respectively, P<0.01). Doxorubicin also significantly increased apoptosis and decreased cell viability after reoxygenation compared to control. This is the first study to show that the anticancer drug Doxorubicin exacerbates myocardial ischaemia reperfusion injury. Further studies are being undertaken to determine the cellular mechanism via which Doxorubicin mediates increased myocardial injury in conditions of ischaemia- reperfusion.

### *Reference*

(1) Piccini et al., Am Heart Journal 2009; 158: 317-26

## THE BRONCHODILATOR SALBUTAMOL IS ASSOCIATED WITH INCREASED MYOCARDIAL INJURY IN A PRE-CLINICAL HEART ATTACK MODEL.

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Salbutamol is widely used to treat symptoms of chest tightness commonly associated with Asthma. Recent studies have associated increased morbidity and mortality in asthmatic patients with underlying heart disease currently being treated with salbutamol. The study aimed to investigate the effects of salbutamol on the myocardium subjected to ischaemia-reperfusion (I/R). Langendorff hearts were subjected to control or I/R in the absence or presence of Salbutamol (1-100nM) ± CGP-12177 ( $\beta$ 1 adrenoceptor antagonist) or ICI 118551 ( $\beta$ 2 adrenoceptor antagonist). Hearts underwent triphenyl tetrazolium staining for infarct size assessment. Isolated cardiomyocytes were exposed to simulated I/R in the absence or presence of salbutamol (1pM-1microM) ± CGP-12177 or ICI 118551. Cellular injury was determined by measurement of viability, apoptosis and necrosis using flow cytometry. Hypercontracture was also assessed in cardiomyocytes subjected to oxidative stress in the absence or presence of salbutamol. Salbutamol (100nM) significantly increased infarct size to risk ratio (%) compared to controls (76±3% vs. 51±2%, P<0.001, respectively). Administration of Salbutamol in the presence of the CGP-12177 or ICI 118551 completely reversed the cardiotoxic effects of salbutamol (63±4%, P<0.01, 50±2%, P<0.001, respectively). Salbutamol significantly increased apoptosis/necrosis compared to non-treated cardiomyocytes subjected to hypoxia/reoxygenation, the cardiotoxic effect of salbutamol abrogated by CGP-12177 and ICI 118 551. Salbutamol also reduced hypercontracture time in cardiomyocytes subjected to oxidative stress. This study is the first to identify the cardiotoxic effects of salbutamol in a pre-clinical heart attack model. Further studies are being investigated to determine the mechanism of action of the myocardial injury associated with salbutamol.

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## THE ELECTRO-MECHANICAL WINDOW AS TORSADE DE POINTES RISK-MARKER IN CONSCIOUS AND ANAESTHETISED DOGS AFTER IKS-BLOCKADE.

van der Linde HJ, Cools F, Vanlommel A, Janssens S, Verrelst J, Van Deuren B, Somers Y, Teisman A, Towart R, and Gallacher DJ.

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The electro-mechanical window (EMw) is a recently proposed biomarker describing the temporal difference between electrical and mechanical events in beating hearts and is a precursor to identify Torsade de Pointes (TdP) risk in the anaesthetised dog (1). In follow-up studies, conscious dogs (n = 6; telemetered) showed comparable baseline values to anaesthetised dogs (n =20): QT = 250 versus 252 ms, QLVpend = 347 versus 339 ms and EMw = +96 versus +87 ms, respectively.

To compare the EMw in conscious and anaesthetised dogs after Iks blockade (2), 6 conscious dogs were orally treated with JNJ303 (20 mg/kg) and 4 anaesthetised dogs received infusions of JNJ303 (cumulative 2.5 mg/kg). In both conditions JNJ303 induced QT prolongation, a large negative EMw and TdP appeared in 50% of the dogs in each condition (pause-dependent and adrenergic-dependent). In the conscious dogs significant differences in maximum plasma levels (PL), QT and EMw were observed between dogs that induced TdP and those without TdP: PL (4950 versus 1473 ng/ml), QT (427 versus 351 ms) and EMw (-150 versus -68 ms), without differences in RR (642 versus 654 ms) and QLVpend (273 versus 287 ms), respectively. Also in anaesthetised dogs long QT (766 ms) and large negative EMw (-445 ms) at similar PL (5743 ng/ml) were observed. In conclusion: a potent Iks blocker (JNJ303) can cause TdP in conscious and anaesthetised dogs, coupled with a large negative EMw.

### References

1. van der Linde, et al., (2010). *BJP*, DOI:10.1111/j.1476-5381.2010.00934.x.
2. Towart, et al., (2009). *JPTM*, 60, 1-10.

## POSSIBLE ROLE OF THE ULTRA-RAPID DELAYED RECTIFIER POTASSIUM CURRENT (IKUR) IN ACTION POTENTIAL REPOLARIZATION IN RABBIT HEART.

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Safety Pharmacology, GlaxoSmithKline, Park Road, Ware, UK, SG12 0DP

The ultra-rapid delayed rectifier K<sup>+</sup> current (IK<sub>ur</sub>) plays an important role in early cardiac action potential repolarization in a number of species. Although the rabbit heart has proven useful for studying cardiac repolarization, the role of IK<sub>ur</sub> in rabbit heart is less well known, as is the potential contribution of this ion channel to drug-induced changes in repolarization.

The aim of this study was to investigate the effects of 4-aminopyridine (4-AP) and 2-isopropyl-5-methylcyclohexyl diphenylphosphine oxide (DPO-1) on action potential duration (APD) in isolated rabbit atria and ventricular papillary muscles. Intracellular action potentials were recorded at 36-37°C and APD was measured at 20, 50 and 90% repolarization (APD<sub>20</sub>, 50 and 90). Exposure to 4-AP at 50 µM for 30 minutes (min) caused a significant prolongation in APD<sub>20</sub> of 29.0±3.3% and 39.4±9.4% in left atria and right ventricular papillary muscles respectively (stimulated at 1 Hertz (Hz)). In contrast, late repolarization was less affected; increases in APD<sub>90</sub> were 8.6±2.8% and 18.7±5.0% for atria and papillary muscle respectively. The prolongation was reverse frequency- dependent (0.2-1 Hz). Similarly, application of DPO-1 at 0.3 µM for 30 min significantly prolonged APD<sub>20</sub> by 21.9±8.8% and 45.8±23.0% in atria and papillary muscle, respectively. APD<sub>90</sub> was less affected, with increases of 10.8±2.2 and 25.2±13.2% in atria and papillary muscle respectively. In conclusion, it is likely that IK<sub>ur</sub> may play a functional role in repolarization in both rabbit atria and ventricle.

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## USE OF THE DOG ISOLATED CARDIAC PURKINJE FIBRE ASSAY TO INVESTIGATE A COMPOUND WITH IN VIVO PROARRHYTHMIC ACTIVITY.

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The dog isolated cardiac Purkinje fibre assay is commonly used to assess the electrophysiologic effects of drugs known to prolong the QT interval. Drug-induced QT prolongation can be associated with Torsade de Pointes arrhythmias. The aim of this study was to determine if the dog Purkinje fibre assay could distinguish between Compound A, which produced ventricular premature complexes (VPCs) in vivo in the dog via an unknown mechanism, and Compound B, which is from the same chemical and pharmacological class as Compound A but did not produce VPCs. Action potentials (APs) were recorded and the number of VPCs counted. Phase 4 slope, action potential duration at 60 and 90% repolarisation (APD<sub>60</sub> and APD<sub>90</sub>), maximum rate of depolarisation (MRD) and resting membrane potential (RMP) were also measured and compared to time matched vehicle control. Compound A at 100 and 300 µM produced VPCs in 6/8 fibres from 3/3 dogs ranging from 1->50 VPCs during stimulation, and 3-16 VPCs following discontinuation of stimulation (<30 sec). In fibres with VPCs, Compound A significantly increased phase 4 slope at 100 µM. Compound A also significantly increased APD<sub>60</sub> and APD<sub>90</sub> at 30, 100 and 300 µM. In the Compound B group, only occasional VPCs were observed with a similar frequency to control. Compound B had no significant effect on phase 4 slope or the other AP parameters. In conclusion, the dog Purkinje fibre assay was able to distinguish between the proarrhythmic potential of Compound A and Compound B.

## INCREASED RISK OF CARDIAC ARRHYTHMIA IN RATS EXPOSED TO DIESEL EXHAUST AIR POLLUTION IS MEDIATED BY THE NOCICEPTIVE TRANSIENT RECEPTOR POTENTIAL A1 (TRPA1)

Hazari MS,\*(1) Lamb C, (2); Carll AP, (2); Krantz Q, (1) Haykal-Coates N,(1) Winsett DW, (1) Costa DL (1) & Farraj AK (1). (1)National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC, 27711. (2)University of North Carolina, Chapel Hill, NC, 27599.

Epidemiological studies demonstrate a significant association between arrhythmias and air pollution exposure. Sensitivity to aconitine-induced arrhythmia has been employed to examine the factors that increase the risk of such dysfunction. We used aconitine to test whether a single exposure to diesel exhaust (DE) would increase the risk of arrhythmia being triggered in hypertensive rats. We hypothesized that DE exposure increases the risk of arrhythmia due to sensory irritation during and after inhalation. Spontaneously hypertensive rats surgically implanted with radiotelemeters were exposed to 150 ug/m<sup>3</sup> of DE or filtered air for 4 hours. Arrhythmogenesis was assessed 24hrs later in urethane-anesthetized animals by continuous intravenous infusion of aconitine while heart rate (HR) and electrocardiogram (ECG) were monitored. Rats exposed to DE had lower HR when compared to air-exposed animals. Exposure to DE resulted in significantly shorter PR intervals, and significantly prolonged corrected QT (QTc) and corrected JT (JTc) when compared to air exposure. Sensitivity to arrhythmia was measured as the threshold dose of aconitine required to produce ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). Rats exposed to DE successively developed VPB's, VT, and VF at significantly lower doses of aconitine than air-exposed animals. Pre-exposure treatment of rats exposed to DE with a transient receptor potential A1 (TRPA1) antagonist prevented the heightened sensitivity to aconitine-induced arrhythmia. These findings suggest that a single exposure to DE increases arrhythmogenic sensitivity. This heightened sensitivity may be mediated by activation of TRPA1 on airway sensory nerves, which are particularly sensitive to inhaled irritants. (This abstract does not reflect EPA policy.)

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## EFFECTS OF DIFFERENT PRECONDITIONING THERAPIES ON ARRHYTHMIAS AND AORTIC BLOOD FLOW IN AN IN VIVO RAT MODEL OF ISCHEMIA/REPERFUSION INJURY

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**Objective:** The aim of the present study was to compare the cardioprotective effects of different therapies including local, remote, pharmacological and oxidative preconditioning in ischemia/reperfusion injury in rats. **Methodology:** Rats were randomly assigned into the following groups: sham-operated and myocardial ischemia/reperfusion (40min/10min). Other groups were subjected to different preconditioning treatments before the operation. Two groups received oral doses of pioglitazone (10 mg/kg/day) or nicorandil (3 mg/kg/day) for five days. Other two groups were subjected to local or remote preconditioning by three cycles of 5 min of occlusion of either left descending coronary artery or right femoral artery followed by 5 min of reperfusion. The last two groups received either ozone (0.6 mg/kg/day in the first week followed by 1 mg/kg/day in the second week) or oxygen vehicle by rectal insufflations. Heart rate, ventricular arrhythmias and descending aortic blood flow were continuously recorded during the whole operation. **Results:** The arrhythmia score in all groups except ozone was reduced significantly compared to ischemia/reperfusion group. Ventricular premature beats and bigeminy were significantly lowered in local preconditioning and pioglitazone groups. Trigeminy, salvos and ventricular tachycardia were completely abolished in local preconditioning and pioglitazone groups. Torsade de pointe was completely abolished in all treated groups except remote preconditioning. Ventricular tachycardia duration was significantly reduced in all groups compared to ischemia/reperfusion group. Finally, descending aortic blood flow was seriously reduced during the incidence of ventricular arrhythmias which was significant in ischemia/reperfusion group. All different preconditioning therapies show a protective effect against blood flow reduction. This was markedly observed in local preconditioning and ozone groups. **Conclusion:** Different preconditioning therapies exert a protective effect by reducing serious ventricular arrhythmias during myocardial ischemia/reperfusion. This was markedly reflected in part by their blood flow preservative effects.

## VOLTAGE-DEPENDENT AFFINITY OF SOME COMPOUNDS THAT INHIBIT HERG: RAPID DISSOCIATION AND REASSOCIATION?

Milnes JT [1,3], McPate M [1,4], Dempsey CE[1], Duncan RS [1], Leaney JL [2], Leishman DJ [2,5], Hancox JC [1], Witchel HJ [1,6]\*

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Rationale: Voltage-dependent changes in hERG-inhibitor-induced functional inhibition of the hERG potassium (K<sup>+</sup>) channel can potentially be ascribed to state-dependent changes in affinity of the inhibitor for its binding site, but also to allosteric modulation of hERG's gating kinetics and to two-step inhibition processes incorporating an initial non-inhibiting inhibitor-channel encounter complex. In kinetics modulation models and in encounter complex models the inhibitor-channel interaction involves constant residency, while voltage-dependent changes in affinity will manifest rapid equilibrium of the inhibitor-channel complex in some protocols. Methods: Patch clamp of hERG-transfected cells in the 'voltage clamp' mode was performed with a range of protocols involving washing a range of hERG inhibitors on or off during the protocol. Results: The behaviours of both selected inhibitors with positive voltage- and frequency-dependence and of an inverse voltage- dependent inhibitor (BeKm-1) are indicative of apparent changes in channel inhibition concordant with rapid equilibrium. Conclusion: This rapid equilibrium, if fast enough, will affect the predicted net effect on a cardiac hERG channel undergoing voltage-cycling.

### References

[1] Milnes JT, Dempsey CE, Ridley JM, Crociani O, Arcangeli A, Hancox JC, Witchel HJ.

Preferential closed channel blockade of HERG potassium currents by chemically synthesised BeKm-1 scorpion toxin. FEBS Lett. 2003;547:20-26.

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## IMPAIRMENT OF REPOLARIZATION RESERVE BY COMBINED PHARMACOLOGICAL INHIBITION OF VENTRICULAR POTASSIUM CHANNELS IN RABBITS: IMPLICATIONS FOR ARRHYTHMIA PREDICTION

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The reliable assessment of proarrhythmic side effects of drugs in development is essential but remains elusive. Recently, the short-term beat-to-beat variability of the QT interval (QTV) has been suggested as a novel parameter to predict ventricular arrhythmias due to repolarization abnormalities. Here we studied the incidence of Torsades de Pointes (TdP) arrhythmias and its correlation with the frequency corrected QT (QTc) interval and the QTV following combined pharmacological block of IKs and IKr, IK1 and IKr, and IK1 and IKs in anaesthetized rabbits. ECGs were recorded before and after the administration of the IKr blocker dofetilide, the IKs blocker

HMR-1556 and the IK1 blocker BaCl<sub>2</sub> and their combination, intravenously.

Dofetilide significantly increased the QTc interval and induced TdP in 28% of animals, HMR-1556 or BaCl<sub>2</sub> alone had little effect on QTc and did not cause TdP. The combination of dofetilide and HMR-1556, BaCl<sub>2</sub> and dofetilide significantly increased the QTc interval, the QTV and led to a high incidence of TdP (82% and 63%). In conclusion, the QTV and the incidence of TdP increased markedly following the combined pharmacological block of IKs and IKr as well as after combined block of IK1 and IKr. Our results suggest that QTV may be a better predictor of subsequent TdP development than the prolongation of the QTc interval alone. The current model, where repolarization reserve is impaired by pharmacological block of one or more potassium currents, may be utilized for more reliable testing of drugs in development for their possible proarrhythmic side effects.

## BOTH THE OPENING AND CLOSING OF GAP JUNCTIONS MAY RESULT IN PROTECTION AGAINST THE ACUTE ISCHAEMIA-INDUCED SEVERE VENTRICULAR ARRHYTHMIAS IN ANAESTHETISED DOGS

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The objective was to examine whether opening or closing of gap junctions (GJ) modify the acute ischaemia-induced ventricular arrhythmias. In chloralose-urethane anaesthetised dogs myocardial ischaemia was induced by a 60 min occlusion of the left anterior descending coronary artery (LAD). In three groups the GJ opener rotigaptide (RTG; 0.04, 0.2 and 1  $\mu\text{g/kg}\cdot\text{min}^{-1}$ ) was administered in intracoronary infusion, 10 min before and over the occlusion. In other two groups the GJ blocker carbenoxolone (CBX; 50  $\mu\text{M}$ ) was infused also locally, both prior to and during and only during the LAD occlusion. Ventricular arrhythmias were assessed as numbers of single premature beats (VPBs) and tachycardiac episodes, incidences of VT and ventricular fibrillation (VF). Changes in epicardial ST-segment and in total activation time (TAT) were recorded by a mapping electrode. GJ function was assessed by measuring changes in tissue impedance in vivo, as well as GJ permeability and Cx43 phosphorylation in vitro. Compared to the controls all doses of RTG and both administration forms of CBX reduced the number of VPBs (659 $\pm$ 193 vs 149 $\pm$ 49, 163 $\pm$ 50 183 $\pm$ 43, 381 $\pm$ 158 and 317 $\pm$ 104, respectively), the increases in epicardial ST-segment and TAT, and the rapid impedance changes prior to phase Ib. Both drugs preserved GJ permeability but only RTG inhibited the ischaemia-induced dephosphorylation of Cx43. We conclude that there is a critical period of ischaemia during which the uncoupling of GJs becomes particularly important for arrhythmia generation. Shifting uncoupling from this ischaemia interval by either enhancing or delaying the closure of GJs may result in arrhythmia suppression.

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## DISTINCT ROLE OF $\text{Ca}^{2+}$ -CALMODULIN DEPENDENT PROTEINKINASE II IN EXCITATION-CONTRACTION COUPLING UNDER CERTAIN CONDITIONS

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$\text{Ca}^{2+}$ -homeostatic proteins play an important role in  $\text{Ca}^{2+}$  excitation-contraction coupling (ECC); however the involvement of  $\text{Ca}^{2+}$  calmodulin dependent protein kinase II delta (CaMKII) in this process is less known. It has been suggested that CaMKII phosphorylates the key proteins for ECC including L-voltage  $\text{Ca}^{2+}$  channels (LTCC), which subsequently become longer in the open state. Such changes may initiate after depolarizations promoting arrhythmias and contractility disturbances (1). To test this hypothesis, KN-93 (0.5 micromol/l), a CaMKII inhibitor, was administered into the perfusion solution before ischemia and during the first 10 minutes of reperfusion. Incidence of ventricular fibrillation was decreased in the KN-treated rat hearts. Although the duration of arrhythmias did not differ between the groups, arrhythmia score was lower upon CaMKII inhibition. Likewise, the recovery of post-ischemic contractile function was improved in the KN-treated hearts. On the other hand, no additive anti-arrhythmic effects of CaMKII inhibition were observed in the group treated with simvastatin (10 mg/kg, 7 days), which is known to suppress electrical instability (2). In the presence of CaMKII inhibitor in the simvastatin-treated group, the severity of arrhythmias was unchanged as compared with the group without treatment. However, CaMKII inhibitor reversed attenuation of post-ischemic contractile dysfunction in simvastatin-treated rats. CaMKII and LTCC protein levels, which were lower in simvastatin-treated rats, were not influenced by KN treatment. In conclusion, CaMKII is involved in the mechanisms of ECC, however, it seems that under certain conditions, such as statins-mediated cardioprotection, it may have a distinct influence on excitation and contraction. Supported by VEGA 1/0620/10, 2/0173/08.

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## DIAZOXIDE-INDUCED ANTIARRHYTHMIC PROTECTION IN THE NON-DIABETIC AND DIABETIC RAT HEART DOES NOT REQUIRE ACTIVATION OF PI3-KINASE/AKT

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Mitochondrial KATP channels (mitoKATP) activation interacting with phosphatidylinositol 3-kinase (PI3K)/Akt has been suggested as a key element in preconditioning protection against lethal myocardial ischemia/reperfusion injury in healthy heart (1), while its antiarrhythmic effects are less elucidated (2). Experimental studies of diabetes mellitus revealed, besides higher vulnerability to ischemia, paradoxically enhanced ischemic tolerance (3) that has been proposed to share some molecular mechanisms with preconditioning in non-diabetic heart. We explored the involvement of PI3K/Akt in preconditioning-like effect of mitoKATP opener diazoxide on susceptibility to ischemia-induced ventricular tachyarrhythmias in rats made diabetic with streptozotocin (65 mg/kg, 1 week) and in non-diabetic animals. Langendorff-perfused hearts of both groups were subjected to 30-min LAD occlusion with or without prior 15-min perfusion with diazoxide given either alone or combined with PI3K/Akt inhibitor wortmannin administered in concentration that abolished infarct size limitation and Akt activation. Total number of premature ventricular complexes, episodes of ventricular tachycardia and its duration were significantly reduced in the diabetic hearts ( $225 \pm 41$ ,  $5 \pm 2.9$  and  $15 \pm 11$  s vs.  $551 \pm 61$ ,  $11.1 \pm 2$  and  $42 \pm 8$  s in non-diabetic controls, respectively;  $P < 0.05$ ). Pretreatment with diazoxide induced antiarrhythmic effects in both groups, while wortmannin alone did not modify arrhythmogenesis. Bracketing of diazoxide with wortmannin did not reverse antiarrhythmic protection in any of the groups. Conclusions: the results indicate that PI3K/Akt activity is not required for the suppression of ischemia-induced arrhythmias conferred by mitoKATP opening in the normal and diabetic rat heart. Supported by VEGA SR 2/0173/08, APVV SK-CZ-0049-07.

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PREVALENCE OF ECG ARRHYTHMIAS IN NON-IMPLANTED, DRUG-NAIVE, FREELY MOVING BEAGLE DOGS.

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The purpose of this study was to assess the normal prevalence rate of spontaneous arrhythmias in 51 drug-naive, non-implanted, freely-moving Beagle dogs (10 M + 41 F, CEDS, France).

Non-implanted ECG assessment was done in jacketed dogs with 6 external leads and a T47G-encoder (PhysioJacket, ITS). Trained lab personnel visually scrutinized the full 22-hour (day and night) ECG recordings and scored the incidence of different types of arrhythmias.

| Arrhythmia                          | % of the dogs |       | incidence/period |
|-------------------------------------|---------------|-------|------------------|
|                                     | day           | night |                  |
| 1 <sup>st</sup> degree AV-block     | 5.9%          | 15.7% | up to 23         |
| 2 <sup>nd</sup> degree AV-block     | 39.2%         | 39.2% | up to 310        |
| atrial premature complex            | 35.3%         | 45.1% | up to 1006       |
| junctional complex                  | 19.6%         | 33.3% | up to 625        |
| junctional escape complex           | 41.2%         | 58.8% | up to 30         |
| run of junctional complexes         | 17.6%         | 21.6% | up to 165        |
| run of junctional escape complexes  | 13.7%         | 33.3% | up to 23         |
| junctional tachycardia              | 13.7%         | 13.7% | up to 12         |
| ventricular complex                 | 17.6%         | 13.7% | up to 10         |
| ventricular escape complex          | 7.8%          | 13.7% | up to 1007       |
| ventricular premature complex       | 15.7%         | 11.8% | up to 134        |
| run of ventricular complexes        | 3.9%          | 2.0%  | up to 18         |
| run of ventricular escape complexes | 3.9%          | 2.0%  | up to 13         |

Only 1 of these 51 animals showed no arrhythmias during the 22-hour observation period. Based on this assessment of spontaneous ventricular arrhythmias, we judged 12 (23.5%) of these animals to be unsuitable for telemetry implantation for use in safety pharmacology studies.

In conclusion, thorough evaluation of ECG morphology of naïve Beagle dogs before implantation of telemetry instruments will improve conscious telemetered-dog arrhythmia studies.

## DELAYED CONDUCTION AND ITS IMPLICATIONS IN MURINE SCN5A+/- HEARTS: INDEPENDENT AND INTERACTING EFFECTS OF GENOTYPE, AGE AND SEX

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SCN5A haploinsufficiencies are implicated in clinical arrhythmic conditions associated with cardiac conduction disorders. We examined myocardial conduction and its dispersion, and relationships between them, in murine Scn5a+/- hearts modelling such clinical conditions. A 64-channel, multi-electrode array of electrode spacing 0.55-mm compared patterns of right ventricular activation in intrinsically beating Langendorff-perfused, male and female, and young (3 months) and old (>12 month-old), Scn5a+/- and WT hearts, from which monophasic action potentials were also obtained. Mean ventricular activation times (T\*MEAN), spatial dispersions (D\*S) between recording channels within a given cardiac cycle, and maximum activation times, (T\*MAX) representing the slowest possible conduction in any given heart were all higher in old male Scn5a+/- compared to young male and old female Scn5a+/- and old male WT. Temporal dispersions (D\*T) of ventricular activation times at given recording channels were similarly higher in old male Scn5a+/- compared to old male WT. In contrast, T\*MEAN, D\*T, D\*S and were indistinguishable between all WT groups. All groupings of D\*T, D\*S and T\*MAX gave linear correlations with T\*MEAN, each with indistinguishable slopes. In contrast, measures of monophasic action potential duration were indistinguishable between all groups. Genotype, age and sex thus exert significant (P<0.05) independent and/or interacting effects on both myocardial conduction and its dispersion. These variates appeared to influence D\*T, D\*S and T\*MAX through actions on T\*MEAN. Effects on both myocardial conduction and dispersion were greatest in old male Scn5a+/- in direct parallel with features of the corresponding clinical arrhythmogenicity in Brugada Syndrome.

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## QUANTIFICATION OF SPATIAL DYNAMICS OF CARDIAC ARRHYTHMIAS

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The 1987 Lambeth conventions identify an arrhythmia from time series recordings of cardiac electrical activity. All cardiac arrhythmias are spatio-temporal, and involve anisotropic and orthotropic propagation in heterogeneous tissue. Spatial heterogeneities are increased by infarction, or localised ischaemia. Spatio-temporal excitation on the surface of the heart can be reconstructed from body surface maps obtained by multi-channel ECG, and mapped in vivo by multi-electrode recording socks, or visualised optically using voltage sensitive dyes in isolated perfused tissue or whole hearts, at resolutions down to  $\sim 0.2\text{mm}$ , and 0.5 ms. Computational models, based on cell electrophysiology, tissue heterogeneity from molecular mapping by immuno-histochemistry, quantitative PCR and mRNA expression, and tissue anisotropy and orthotropy from diffusion tensor magnetic resonance imaging for cardiac tissues of mouse, rabbit, guinea pig, dog and human are available for the interpretation and simulation in 3-D geometries of these spatiotemporal recordings. Spatial features of the surface activity can readily be mapped e.g. action potential duration (APD) and its restitution, discordant alternans, dominant frequencies and their domains, phase singularities that locate the organisation centres of re-entrant waves at the surface. Such spatial maps allow putative mechanisms for arrhythmias to be tested e.g. the mother rotor hypothesis for fibrillation. We present spatial maps we have obtained of APD, repolarization time, discordant alternans, dominant frequencies and phase singularities during fibrillation obtained from optical imaging experiments (mouse, rat, guinea pig, rabbit and pig) and simulations (mouse, rat, rabbit, dog, human) and quantify the maps and their dynamics.

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## MECHANICALLY INDUCED ARRHYTHMIAS: A NEGLECTED TARGET?

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It is known that stretch of the ventricles is arrhythmogenic [1]. The role that this plays in healthy heart is unclear, but we hypothesised that an exaggeration of this response in hypertrophic hearts may explain the high risk of SCD in patients with hypertrophy and early failure. The hypertrophied heart indeed shows increased sensitivity to stretch induced arrhythmias [2] and increased stretch-activated currents in myocytes from hypertrophied animal and human hearts has been directly demonstrated [3]. We have now extended these observations by examining stretch-induced ectopic beats in isolated hearts from Wistar-Kyoto (control) and SHR [4]. Our results show that hypertrophic hearts are more sensitive to mechanically induced arrhythmias, and that the putative stretch-activated channel blocker streptomycin reduces this sensitivity. Hearts from SHR had a threshold for stretch induced ectopic beats of  $21.2 \pm 3.6$  mmHg ( $n=5$ ) compared to  $49.4 \pm 4.7$  mmHg in Wistar Kyoto ( $n=5$ ),  $p < 0.01$ ). Perfusion of the hearts with  $100 \mu\text{M}$  streptomycin increased the threshold in both (to  $39.7 \pm 9.0$  mmHg in SHR ( $n=5$ );  $p = 0.07$  vs  $69.4 \pm 6.9$  mm.Hg in control ( $n=5$ );  $p = 0.04$ ). These data add to growing evidence that mechanical effects on the myocardium may contribute to arrhythmias in many clinical situations, and suggest that efforts at developing antiarrhythmic agents with this as a target may prove fruitful [5]. As yet there is no guidance for the study of such arrhythmias, and provision for this is required in the Lambeth Conventions update.

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Excitement over automated patch clamp: action potentials from stem cells and temperature effects on hERG inhibition

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The hERG gene encodes a potassium channel responsible for the repolarization of the IKr current in cardiac cells. Given the importance of this channel in the repolarization of the cardiac action potential, and the disturbances of channel function by certain compounds such as anti-arrhythmias and anti-psychotics, this channel has become very important in safety pharmacology testing. Since some hERG-active compounds also exhibit different pharmacology at physiological temperature, experiments performed at this temperature are important in yielding more relevant data in safety screening. In this study, we describe the use of automated patch clamp electrophysiology for recording hERG stably transfected in HEK293 cells. Recordings of the hERG current from up to 8 cells simultaneously could be performed at room temperature (RT) and at physiological temperature. Data will be shown for erythromycin which exhibited a higher potency at 35°C vs. RT. Additionally, using a planar patch clamp workstation, recordings could be made from stem cell-derived cardiomyocytes. Currents mediated by K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> channels could be recorded in the voltage clamp mode. What is more, action potentials in the current clamp mode could also be recorded and pharmacology was performed on action potentials (data will be shown). The use of stem cell-derived cardiomyocytes in safety testing is becoming increasingly important. The ability to test compounds on ion channels in both the voltage and current clamp modes, as well as at different temperatures, may be crucial for future safety testing.