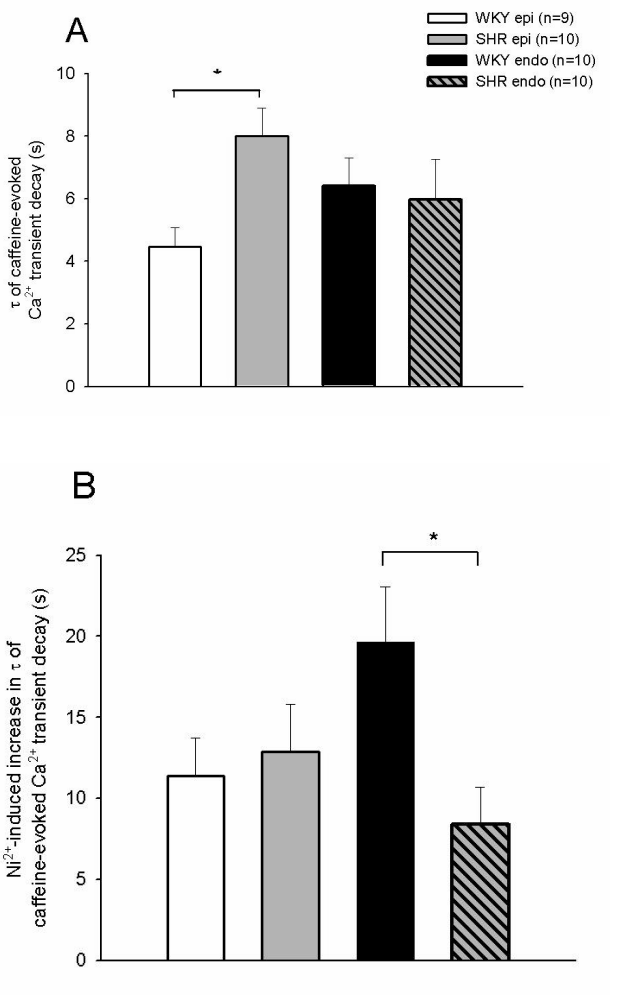
C1

Ca2+ handling in ventricular myocytes isolated from the sub-epicardium and sub-endocardium of the spontaneously hypertensive rat heart.

J. Naz, M.R. Fowler, S.M. Harrison and C.H. Orchard

Biomedical Sciences, University of Leeds, Leeds, West Yorkshire, UK

Pressure overload-induced hypertrophy produces regional changes in cardiac cell structure and function. We have therefore investigated the changes in Ca2+ handing that occur in myocytes from the left ventricular sub-epicardium (EPI) and sub-endocardium (ENDO) of 20 week old spontaneously hypertensive rats (SHR) compared with normotensive age-matched Wistar-Kyoto (WKY) control rats.Rats were killed humanely (Schedule 1 method) and ventricular myocytes isolated by enzymatic dispersion. Cells were superfused with Tyrode solution at 22-24 °C and electrically stimulated via extracellular electrodes. Intracellular Ca2+ was monitored using fura-2.Following a train of 1 Hz conditioning stimuli, rapid application of caffeine (20 mM) was used to release Ca2+ from the sarcoplasmic reticulum (SR). The amplitude of the electrically stimulated and caffeine-induced Ca2+ transients was significantly bigger ($P<0.02$ and $P<0.05$, respectively; unpaired t-tests) in SHR EPI cells than in WKY EPI cells and fractional release remained unchanged. In contrast, there was no significant difference between the amplitude of the electrically stimulated or caffeine-induced Ca2+ transients, or fractional release in ENDO cells from SHR and WKY hearts. The time constant of decay of the caffeine-induced Ca2+ transient (τ; which reflects sarcolemmal Ca2+ extrusion) was significantly greater ($P=0.005$) in SHR EPI than in WKY EPI cells, but was not significantly different in SHR and WKY ENDO cells (Fig. 1A). Simultaneous application of caffeine and 10 mM Ni2+ (to inhibit Ca2+ extrusion via Na/Ca exchange; NCX) increased τ in all cell types (Fig. 1B). In EPI cells, the Ni2+-induced increase in τ was not significantly different between SHR and WKY cells (Fig. 1B). However, the increase in τ was significantly smaller ($P=0.015$) in SHR ENDO than WKY ENDO cells (Fig. 1B). Fig. 1. A. τ of decline of the caffeine-induced Ca2+ transient in WKY and SHR EPI and ENDO cells. B. Ni2+-induced increase in τ in each cell type. * indicates statistical significance.Thus the slower decline of the caffeine-induced Ca2+ transient in SHR EPI cells in the absence of Ni2+ does not appear to be due to lower NCX activity, suggesting that another Ca2+ extrusion pathway may be down-regulated in these cells. In contrast, τ was similar in SHR and WKY ENDO cells in the absence of Ni2+; the smaller effect of Ni2+ in SHR ENDO cells suggests less NCX-mediated Ca2+ extrusion in these cells, and hence that other mechanisms may be up-regulated to compensate for this apparent decrease in NCX activity.



The support of the British Heart Foundation and Wellcome Trust is gratefully acknowledged.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C2

Decreased sorcin expression in a rabbit model of left ventricular dysfunction.

E.B. Elliott, S. Currie and G.L. Smith

Biomedical & Life Sciences, Glasgow University, Glasgow, G12 8QQ, UK

Sorcin is a 22 kDa Ca^{2+} binding protein expressed in cardiac muscle. Recent studies have revealed that sorcin over-expression in cardiomyocytes leads to stimulation of sodium-calcium exchange (NCX) activity (Seidler et al, 2003). NCX expression is altered in many models of heart failure. In a recent study of a rabbit model of left ventricular dysfunction (LVD), increased NCX protein levels but decreased activity were observed (Quinn et al, 2003). The object of this study was therefore to quantitatively examine sorcin expression in this model of LVD. Eight weeks after

ligation of a large branch of the circumflex coronary artery under anaesthetic (100 mg/kg of sodium pentobarbitone which was given by intravenous injection), hearts were removed after an overdose of Euthatal (1 mg/kg). Tissue from the basal region of the LV was removed, homogenised and total protein content was determined. Western blots were performed using the NuPAGE system (Invitrogen) and membranes were probed with a polyclonal antibody against sorcin (Zymed, 1:1000). Sorcin protein bands were normalised against the internal standard GAPDH. Strong signals were obtained from the dimer (44 kDa) and used to quantify sorcin expression. Immunoblot signals due to the monomer were barely detectable under these conditions. This is consistent with reports that sorcin exists as a dimer at physiological concentrations. The average optical density of the sorcin dimer in LVD was significantly lower when compared to that of sham (71.2±11.5% (SEM), $P < 0.05$, $n = 9$). No obvious changes in monomer signals were observed.These results suggest that sorcin expression is significantly reduced in this rabbit model of LVD. Decreased sorcin expression may decrease NCX activity and explain the apparent anomalous changes in expression and activity in LVD.

Quinn et al, J.Physiol, 2003; 553(1): 229-242
Seidler et al, CircRes, 2003; 93: 132-139

This work was financially supported by the British Heart Foundation.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C3

Properties of nuclear Ca^{2+} regulation in isolated rat ventricular myocytes

Z. Yang and D. Steele

School of Biomedical Sciences, University of Leeds, Leeds, UK

Confocal microscopy was used to study nuclear Ca^{2+} regulation in permeabilized cells or in intact myocytes during electrical stimulation. Rats (250-300 g) were killed humanely (Schedule 1) and ventricular myocytes were isolated by enzymatic digestion. Following permeabilization with saponin (10 μg/ml), myocytes were perfused with a mock 'intracellular' solution containing (mM) 100 KCl; 25 HEPES; 0.36 EGTA; 10 phosphocreatine; 5 ATP and fluo-3 acid (5 μM). The free $[Ca^{2+}]$ and $[Mg^{2+}]$ was 200 nM and 1 mM respectively. pH 7.0, 22 ºC. Myocytes typically had two elongated nuclei oriented along the mid axis of the cell. Line scan (x-t) images were obtained by positioning the scan line longitudinally through both nuclei. Under these conditions, prolonged Ca^{2+} release events were consistently detected from the nuclei. The mean duration, amplitude and width of the spontaneous NCR events was 1.78 ± 0.19 s, F/Fo = 2.0 ± 0.04 and 6.1 ± 0.2 μm respectively (n=68). For comparison, the mean half time, amplitude and width of spontaneous Ca^{2+} sparks under the same conditions was 34.3 ± 1.1 ms, F/Fo =2.0 ± 0.1 and 2.6 ± 0.12 μm respectively (n=43). The location of spontaneous nuclear Ca^{2+} release (NCR) events was identified more accurately using syto-11 (250 nM), which fluoresces on binding nucleic acids, allowing the boundary of the nucleoplasm and the NE to be defined. NCR was shown to occur in localised regions at the ends of the elongated nuclei and in-focus events were aligned with the inner surface of the nuclear envelope (NE). Ca^{2+} release events of comparable duration were not detected when the scan-line was positioned transversely across the midpoint of each nucleus or from cytosolic regions, which were clearly outside the nucleus. In further experiments, intact myocytes were perfused with a solution containing mM: 113 NaCl; 5.4 KCl; 1 $MgCl_2$; 1.0 $CaCl_2$; 0.37 Na_2HPO_4; 5.5 glucose; 5 HEPES. 20-22ºC. Cells were then loaded with fluo-3 AM (5 μM for 10 minutes) to allow changes in intracellular $[Ca^{2+}]$ to be detected during field stimulation. Each rise in cytosolic $[Ca^{2+}]$ was followed an increase in nuclear $[Ca^{2+}]$. However, line-scan and x-y images revealed regions at the ends of the nuclei, where $[Ca^{2+}]$ increased rapidly and reached a higher level than the maximum cytosolic $[Ca^{2+}]$, before diffusing inwards towards the centre of the nucleus. The inherently prolonged nature of the spontaneous NCR events detected at the ends of the nucleus in skinned cells suggests the presence of Ca^{2+} release sites with markedly different gating properties to those involved in the generation of cytosolic Ca^{2+} sparks. The experiments on intact cells suggest that synchronised recruitment of these events may serve to amplify and prolong the rise in nuclear $[Ca^{2+}]$, which accompanies each systolic Ca^{2+} transient.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C4

The influence of ageing on action potential duration and intracellular Ca homeostasis in sheep ventricular myocytes

K. Dibb[1], D. Eisner[1], U. Rueckschloss[2], G. Isenberg[2] and A. Trafford[1]

[1]Unit of Cardiac Physiology, University of Manchester, Manchester, UK and [2]Department of Physiology, Martin Luther University, Halle, Germany

Ageing is a significant risk factor associated with the incidence of sudden cardiac death (Priori *et al.* 2001). We have investigated changes in action potential duration and properties of the systolic Ca transient in sheep ventricular myocytes brought about as a result of the ageing process. Young adult sheep (18 months old) and old sheep (>7 years) were euthanased with 200 $mg.kg^{-1}$ intravenous pentobarbitone. Myocytes were isolated from the mid-myocardial layer of the left ventricular free wall by collagenase digestion. Myocytes isolated from young and old animals were stimulated at a frequency of 0.25 Hz in the current clamp mode. Changes in $[Ca^{2+}]_i$ were measured using Fluo-3 AM. Experiments were performed at both 23°C and 37ºC. Data are presented as mean ± SEM from n experiments. Statistical analysis was carried out using a 2 way ANOVA or a Mann-Whitney rank sum test for non normally distributed populations.Action potential duration was increased in the old animals ($p<0.01$, n = 16–17 and $p<0.05$, n = 7 at 23 and 37°C respectively; see figure 1A). The amplitude of the systolic Ca transient was also increased in the old animals compared to the young animals (202±25 & 112±14 nM at 23°C; $p<0.01$, n = 12-17). The increase in the amplitude of the systolic Ca transient in old animals was associated with a decreased rate of rise of the Ca transient in these animals measured as time taken to rise between 10 and 70 % of maximum (35.3±5 & 20.5±2 ms at 23°C; $p<0.001$. n = 15-17). Interestingly 6 from a total of 7 cells isolated from old animals displayed abnormally shaped Ca transients when stimulated at 37°C as shown in figure 1B. This was compared to 1 out of 10 cells isolated from young animals.In

summary there are marked alterations in action potential duration and Ca transient amplitude associated with ageing. Further work is required to determine if these changes are arrhythmogenic and if the abnormally shaped Ca transients recorded from cells isolated from old animals are a direct result of the ageing process or due to the increase in action potential duration.

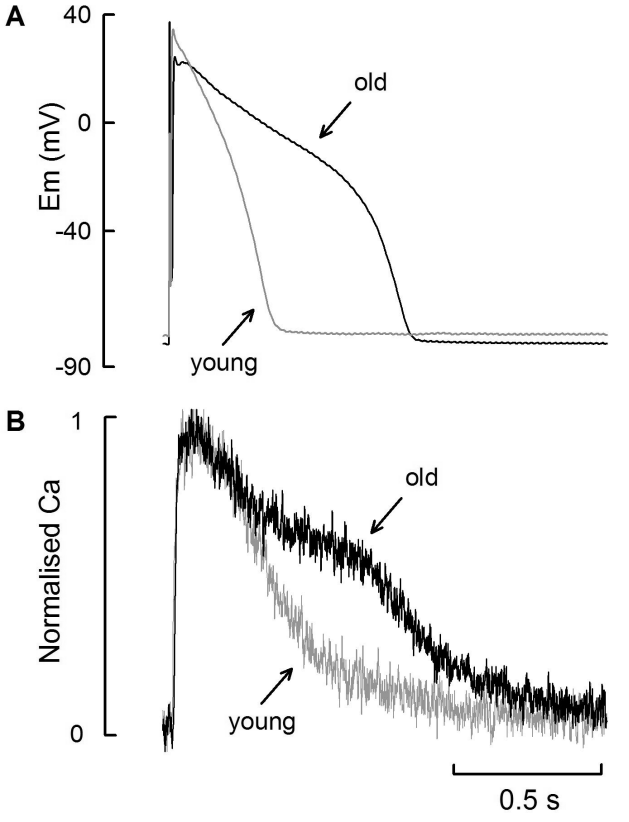


Figure 1. **A**, representative action potentials in myocytes isolated from young (grey) and old (black) animals at 37°C. **B**, normalised Ca transients associated with the action potentials shown in panel A.

Priori *et.al.* (2001) *European Heart Journal*, **22**, 1374-1450

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C5

Substitution of cardiac TnC into rabbit skeletal muscle fibers increases thin filament responsiveness to strong-binding cross-bridges

J.R. Patel, D.P. Fitzsimons and R.L. Moss

Physiology, University of Wisconsin Medical School, Madison, WI, USA

Activation of contraction in striated muscle appears to arise from positive cooperative interactions in the binding of Ca2 and myosin cross-bridges to the thin filament, such that activation of a given segment of thin filament facilitates the activation of neighboring segments. Evidence of cooperative interactions in striated muscle has been inferred from the steepness and biphasic form of the force-pCa relationship, which is steeper at low levels of Ca2 than at high, presumably due to greater intermolecular cooperation at forces less than half-maximal. Furthermore, the mechanisms of cooperative activation appear to differ between skeletal and cardiac muscle, since force-pCa relationships are generally steeper in fast-twitch skeletal muscle fibers. Expression of muscle-specific thin filament proteins (e.g., troponin C) may account for this effect by modulating the responsiveness of the thin filament to strong-binding cross-bridges.To examine possible contributions of troponin C (TnC) isoforms to cooperative activation of the thin filament, rabbit (humanely killed) skinned single psoas fibers were treated to partially extract endogenous skeletal TnC, reconstituted with exogenously added cardiac TnC, and subsequently activated in the presence or absence of a strong-binding, non-force-generating derivative of myosin subfragment 1 (NEM-S1). Relative to control fibers containing endogenous skeletal TnC, substitution of cardiac TnC reduced the steepness of the force-pCa relationship, indicating a decrease in the apparent cooperativity of activation, but had no effect on either the Ca2 sensitivity of force or the rate of force redevelopment during maximal activation. In the presence of NEM-S1, skinned fibers containing cardiac TnC exhibited greater increases in Ca2 -independent force and Ca2 sensitivity of force than fibers containing skeletal TnC. Furthermore, NEM-S1 treatment accelerated the rate of force redevelopment at intermediate levels of activation to a greater degree in fibers containing cardiac TnC, but had no effect on the kinetics of force development in maximally activated preparations.These findings indicate that muscle-specific isoforms of TnC contribute to cooperative activation at submaximal [Ca2] by modulating thin filament responsiveness to strong-binding cross-bridges.

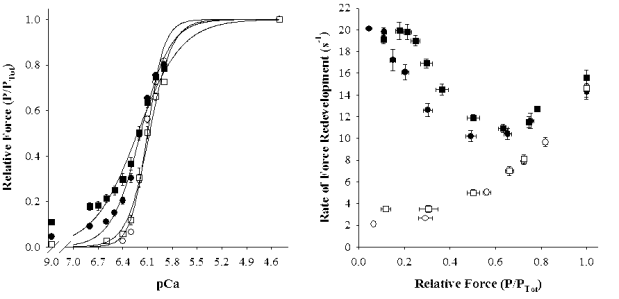

Figure 1. Effects of TnC isoforms and NEM-S1 on the force-pCa relationship (left panel) and the activation dependence of rate of force redevelopment (right panel). All values are means ± SEM, with (○) skTnC (N=11), (●) skTnC/3 mM NEM-S1 (N=5), (■) cTnC (N=6) and (■) cTnC/3 mM NEM-S1 (N=6).

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C6

Cardiomyocyte-specific and global inactivation of the β2 subunit gene of the voltage-dependent Ca^{2+} channel in the mouse

B. Held[1], P. Weissgerber[1], K.R. Chien[2], B. Fleischmann[3], M. Freichel[1] and V. Flockerzi[1]

[1]Experimentelle und Klinische Pharmakologie und Toxikologie, Universitaet des Saarlandes, Homburg, Germany, [2]Institute of Molecular Medicine, UCSD, San Diego, CA, USA and [3]Institut fuer Physiologie 1, Universitaet Bonn, Bonn, Germany

Voltage-dependent Ca^{2+} channels are heteromultimeric protein complexes consisting of a pore-forming subunit α1 and accessory β, γ and α2δd subunits. Of the β subunit, 4 isoforms exist, the β2 is most abundant in heart but also expressed in brain and aorta. In order to test the function of the β2 subunit in vivo, we generated mouse models with either β2 null alleles (β2-/-) or with alleles in which exon 4 of the β2 gene is flanked by loxP sites ($\beta2^{flox/flox}$) for conditional inactivation using the Cre recombinase transgenes. Previously, we have shown that the L-type Ca^{2+} channel current (I_{Ba}) in primary cultured myocytes isolated from β2-/- embryonic mice (E9.5) is reduced, without a change in the action potential duration (Held et al, 2003). Mice were sacrificed according to German guidelines. Similar results were obtained when Ca^{2+} (1.8mM) was used as charge carrier. In cardiac myocytes from E10.5 embryos, the I_{Ca} (1.8mM Ca^{2+}) at 0mV was reduced from -10.5 ± 0.71 pA/pF (n=32) in β2+/+ to -4.2 ± 0.42 pA/pF (n=15) in β2-/- cells ($p<0.05$, ANOVA), no significant differences were observed between β2+/+ and β2+/- cells. To examine whether embryonic lethality is due to cardiac dysfunction, $\beta2^{flox/flox}$ mice were mated with a mouse line expressing the Cre recombinase under a cardiomyocyte-specific promotor. Mice with a cardiomyocyte-specific inactivation of the β2 gene ($\beta2^{-/flox}$; MLC2a-$Cre^{tg/0}$) are not viable. Hearts were isolated at E13.5, separated in ventricle and atrium, and L-type Ca^{2+} current (I_{Ca}, 1.8mM Ca^{2+}) in cardiomyocytes was measured as before. Ventricular cells lacking the β2 gene ($\beta2^{-/flox}$, MLC2a-$Cre^{tg/0}$), had with -4.0 ± 0.3 pA/pF (n=29) a reduced L-type Ca^{2+} current ($\beta2^{+/flox}$; MLC2a-$Cre^{tg/0}$: -6.8 ± 0.6 pA/pF, n=18, $p<0.001$; $\beta2^{+/flox}$; MLC2a-$Cre^{0/0}$: -6.7 ± 1.0 pA/pF, n=6, $p<0.05$; $\beta2^{-/flox}$; MLC2a-$Cre^{0/0}$: -5.9 ± 0.5, n=29; $p<0.01$, ANOVA) as observed with the classical knock-out at E9.5 or E10.5. The data indicate that in cardiomyocytes the β2 subunit and the Ca^{2+} influx through L-type Ca^{2+} channel are essential for development and survival of the mouse embryo.

Held *et.al.*(2003)Pfluegers Arch. 445:S31

We thank for the financial support by the DFG.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C7

Phosphorylation by protein kinase C of HERG potassium channels expressed in a mammalian cell line

S.L. Cockerill, A.B. Tobin, G.B. Willars, N.B. Standen and J.S. Mitcheson

Cell Physiology and Pharmacology, University of Leicester, Leicester, UK

The Human ether a-go-go related gene (HERG) encodes a voltage gated potassium channel expressed in cardiac and neuronal tissues. Attenuation of cardiac HERG current, through either inhibition by drugs or inherited mutations leads to increased risk of arrhythmias. Second messengers such as cyclic AMP and several kinases modify HERG channel activity. We have previously shown that stimulating protein kinase C (PKC) by $G\alpha_{q/11}$ - coupled muscarinic receptor stimulation or elevation of intracellular [Ca^{2+}] results in a sustained decrease of HERG current, a positive shift of activation and acceleration of deactivation kinetics (Cockerill et al., 2003). It has been suggested that PKC modulates HERG by an indirect mechanism, possibly involving phosphorylation of an auxillary subunit in the channel complex (Thomas et al., 2003). The aim of the present study was to determine if PKC activation results in increased phosphorylation of HERG stably expressed in HEK293 cells. Incorporation of ^{32}P into HERG was compared in non-treated cells and cells treated with 1-oleoyl-2-acetylglycerol (OAG) to activate PKC. HERG was immunoprecipitated, proteins were resolved by 10% SDS-PAGE and resulting blots subjected to autoradiography at -80ºC for 20h, as previously described (Budd et al., 1999). Phosphorylation of HERG was quantified using densitometry. Data are expressed as mean±S.E.M from ≥3 experiments. Statistical analysis was by unpaired Student's t-tests with significance accepted at $p<0.05$.Two bands at 155 and 135kDa, corresponding to mature and core glycosylated forms of HERG respectively, were observed in untreated cells, indicatating phosphorylation under basal conditions. A 5min application of 10μM OAG significantly increased the phosphorylation of both bands by 18.1±1.7%. The OAG-dependent increase of phosphorylation could be abolished by preincubating cells with the PKC inhibitor bisindolylmaleimide-1 (3μM) for 15min or by down-regulating PKC by chronic treatment of cells with 1μM phorbol 12-myristate 13-acetate (PMA) for 24h. We have previously shown chronic PMA treatment to down-regulate the α,β,δ and ε isoforms of PKC, demonstrated using western blotting (Cockerill et al., 2003).Both methods of reducing PKC activity had no effect on HERG phosphorylation in non-OAG treated cells suggesting PKC is not responsible for basal phosphorylation of the channel. Our results suggest that HERG is highly phosphorylated under basal conditions and that stimulation of PKC with OAG increases the phosphorylation of the channel, suggesting that modulation of currents by PKC can occur by direct interactions with HERG.

Cockerill SL et. al. (2003) *JPhysiol* **552.P**, 39P
Thomas D et. al. (2003) *Cardiovasc. Res.***59**,14-26
Budd DC et. al. (1999) *Mol. Pharmacol.***56**,813-823

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C8

Expression of ion channel genes in mouse heart

S. Demolombe[1], J. Liu[2], C. Marionneau[1], D. Escande[1] and M. Lei[2]

[1]INSERM U533, Faculte de Medecine, Nantes, France and [2]Physiology, University of Oxford, Oxford, UK

Electrophysiological properties vary in different regions of the heart, which is essential for effective cardiac function. Increasing evidence suggests that cardiac electrical heterogeneity is attributed to regional variability in distribution of ion channel and related genes. Dramatic progress in genomics has led to the discovery of a large number of ion channel genes, but how these genes are expressed in specific regions of the heart and function in relation to its physiological function are largely unknown. We have recently profiled ~500 channel and related genes in sino-atrial node (SAN), atrio-ventricular node (AVN), atrium and Ventricle by using Mouse Known Gene Array. To validate cDNA array results, we have compared relative expression levels of 96 channel genes in these regions with Real-time PCR.Hearts were excised from 20-25 g adult C57BL mice after schedule 1 killing by cervical dislocation. Total RNA was prepared from SAN, AVN, atrium and ventricles as described by Wittwer et al (2002). Real-time PCR arrays, called Micro Fluidic Cards (Applied Biosystems), were used. Ninety-six pre-designed TaqMan probe and primer sets were pre-loaded in the card. Two ng per well of cDNA were thermal cycled and quantitative ion channel expression results were analysed using the 7900HT Sequence Detection System (Applied Biosystems). Results obtained from 2 pools of 10 mice for each tissue, showed that there are regional differences in channel gene expression in murine heart. Channel genes Cacna1d (Cav1.3), Cacna1g (Cav3.1), Cacna1h (Cav3.2), Cacna2d2, Cacnb3, Scn3b, Kcna1, Kcna6, Kcnab1, Kcne3, Gja5 (Cx40) and HCN1 are more highly expressed in nodal tissue (SAN/AVN) than in atrium and ventricle, while channel genes Gja1(Cx43), Cacn2a1c (Cav1.2), Scn5a, Kcnd2, Kcnj11 and Kcnj2 are preferentially expressed in muscle regions. Channel gene expression between SAN and AVN is similar; this could due to the functional and structural similarities between these two nodal regions. However, Pln and Cacn1c are preferentially expressed in ventricle as compared to atria. Inversely, Gja5, Kcna4, Kcnj3 and Kcnq1 are preferentially expressed in atria versus ventricle. The results suggest that regional differential expression of various channel genes underlies electrophysiological heterogeneity of the heart. Regional pattern expression of ion channels genes in heart could provide new targets for designing drugs with high specificity of action.

WIttwer, et al (2002). FASEB J., 01-0792fje.

Supported by ACI, CNRS, INSERM & Wellcome Trust

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C9

Alterations in the properties of the cardiac inward rectifier potassium channel I_{K1} in aged sheep

K. Dibb[1], D. Eisner[1], G. Isenberg[2], U. Rueckschloss[2] and A. Trafford[1]

[1]Unit of Cardiac Physiology, University of Manchester, Manchester, UK and [2]Department of Physiology, Martin Luther University, Halle, Germany

The likelihood of developing cardiac arrhythmias increases with age (Priori et al, 2002). The purpose of the present study was to investigate changes in the cellular electrophysiological properties of the senescent myocardium that may predispose the heart to the development of arrhythmias. Single cardiac myocytes were isolated from the mid-myocardial layer of the left ventricular free wall of aged (>7 years) and young (18 month old) sheep euthanased with 200mg.kg^{-1} intravenous pentobarbitone. Voltage clamp control was achieved using either the whole cell (for I_{K1} measurements) or perforated patch (for action potential measurements) technique with K^+ based solutions. All experiments were performed at 23°C. Statistical analysis was performed using either a Students *t* test or Two Way ANOVA where appropriate.Membrane capacitance increased with age from 157±8 to 211±14pF (n=23-27 cells, P<0.001). Resting membrane potential was unaffected by age (−74.5±0.8mV in young and −74.7±0.9mV in aged sheep, n=17-19 cells, P>0.4). We have also investigated whether ageing is associated with any change in the properties of I_{K1}.In aged sheep, I_{K1} current density increased, e.g. at −130mV from −3.8±0.4pA.pF^{-1} in young sheep to −7.2±0.4pA.pF^{-1} in old sheep (n=10-20 cells, P<0.001). The time to peak current development at −130mV accelerated with age from 19.7±2ms (young) to 5.7±1ms (old) (P<0.0001). Furthermore, the apparent extent of inactivation of the current during a 300ms test pulse was greater in cells from aged sheep (12.8±1 *vs.* 27.5±3%, P<0.0001). Differences in the gating properties of I_{K1} were investigated by examining the voltage dependence of channel conductance (gK/gK$_{max}$). Neither the membrane potential at which conductance is half-maximal (−91.7mV in young and −88.2mV in old) or the voltage required to change conductance *e*-fold (12.3mV in young and 13.2mV in old) altered with age. In summary, ageing is associated with marked alterations in the density and kinetics of the inwardly rectifying potassium channel I_{K1} that helps maintain resting membrane potential. The influence of these changes on the arrhythmogenic potential of the myocardium and the molecular determinants of the changes need to be elucidated.

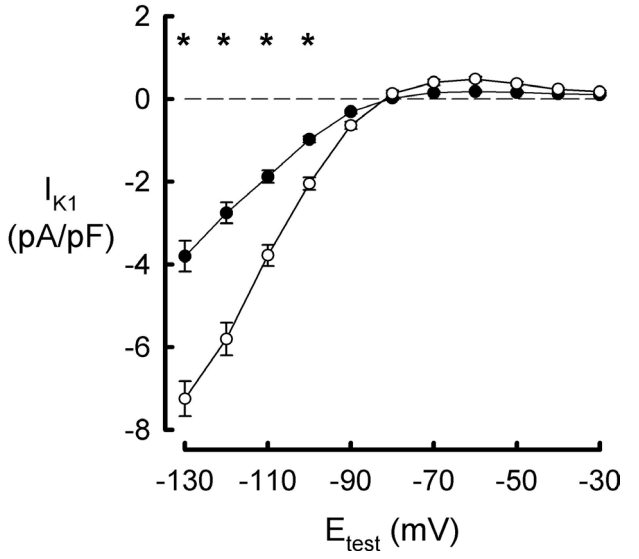

Figure 1. I_{K1} current density in myocytes isolated from young (solid symbols) and aged (open symbols) sheep. * $P<0.05$.

Priori SG *et.al.* (2002). Europace **4**, 3-18.

Supported by The British Heart Foundation and SFB 598

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C10

Cellular electrophysiology of SCN5A-DKPQ long-QT syndrome mutants

M. Lei[1], C. Head[2], C. Goddard[2], C. Huang[2], W. Colledge[2] and A. Grace[2]

[1]Physiology, University of Oxford, Oxford, UK and [2]Departments of Biochemistry and Physiology, University of Cambridge, Cambridge, UK

Long-QT syndrome is characterized by prolongation of the electrocardiographic QT interval and is associated with syncope and sudden cardiac death. One implicated mutation (SCN5A-DKPQ) is predicted to result from delayed inactivation of the cardiac sodium channel although downstream functional consequences are not understood. We have characterized the electrophysiological phenotype of ventricular myocytes from mice harbouring this mutation. Enzymatically dissociated adult ventricular myocytes were prepared from wild-type and SCN5A-DKPQ mice as described by Ashely (2002). Hearts were excised from 20-25 g adult C57BL mice after schedule 1 humane killing by cervical dislocation. SCN5A-DKPQ showed a significant prolongation of action potential (AP) duration (152 ± 18 ms, n=9) compared to wild-type (55 ± 6.6 ms, n=9, $p<0.01$) and early-afterdepolarization like AP. The current density of iNa and late current (see Fig. 1) in SCN5A-DKPQ myocytes are also increased. These findings suggest that the late current could be a major mechanism responsible for the ventricular arrhythmias associated with this mutation.

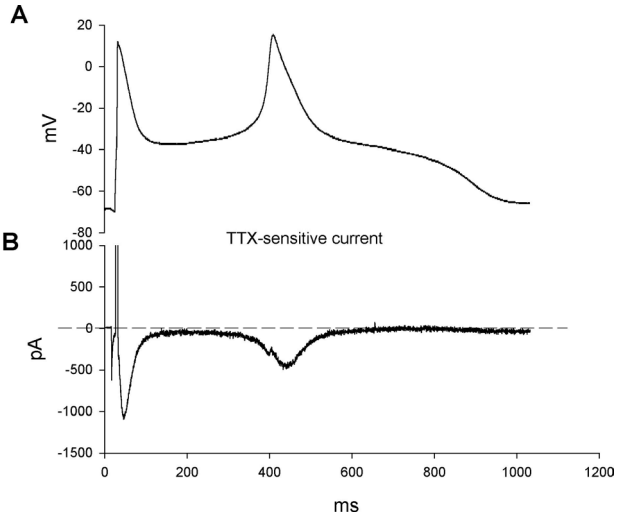

Fig. 1, Representative late INa during action potential in a SCN5a (D/) myocyte.

WITTWER, M., FLUCK, M., HOPPELER, H., MULLER, S., DESPLANCHES, D. & BILLETER, R. (2002). FASEB J., 01-0792fje.

Supported by Wellcome Trust

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C11

The effects of perfusion rate on the dominant frequency of ventricular fibrillation in isolated rabbit heart

J.C. Caldwell[2], F.L. Burton[1], S.M. Cobbe[2] and G.L. Smith[1]

[1]Biological and Life Sciences, Universiry of Glasgow, Glasgow, UK and [2]Section of Medical Cardiology and Exercise Medicine, Glasgow Royal Infirmary NHS University Trust, Glasgow, UK

Clinical studies suggest that as ventricular fibrillation (VF) progresses in humans its frequency decreases and survival likelihood diminishes. Normally myocardial perfusion is minimal during VF. Thus the changes in VF frequency may be associated with progressive ischaemia of the myocardium. Hearts were removed from New Zealand white rabbits under terminal anaesthesia (1mg/kg phenobarbitone) and Langendorff perfused at 40ml/min with Tyrodes solution at 37 degrees centrigrade. Electrocardiogram signals were recorded from electrodes placed in the bath. VF was induced by rapid pacing (50Hz for 8s) and recordings were taken every 30s. Using Fourier transform the dominant frequency (DF) was calculated for each epoch.Figure 1 shows the progressive changes in DF with varying perfusion rates. Perfusion rates lower than control were associated with a decreasing dominant frequency. In general lower perfusion rates produced more rapid declines in DF.These results suggest that dominant frequency changes during VF are caused by reduced myocardial perfusion. Further studies are required to identify the underlying metabolic cause of this effect.

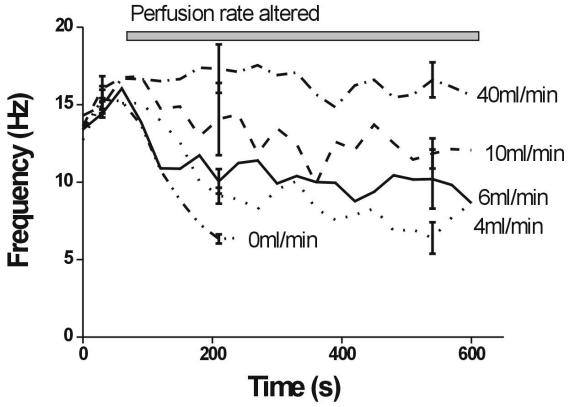

Figure 1 Progression of dominant frequency with time at varying perfusion rates. After 60 seconds the perfusion rates was decreased to levels indicated (n=4 for each criteria).

This study was supported by the British Heart Foundation.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C12

Effect of acidosis on epicardial activation in isolated Langendorff-perfused rabbit hearts

N.L. Walker[2], C.H. Orchard[3], F.L. Burton[1], S.M. Cobbe[2] and G.L. Smith[1]

[1]Biomedical & Life Sciences, Glasgow University, Glasgow, G12 8QQ, UK, [2]Cardiology, Glasgow Royal Infirmary, Glasgow, G32 2ER, UK and [3]School of Biomedical Sciences, Leeds University, Leeds, LS2 9NL, UK

Systemic acidosis and myocardial ischaemia are associated with decreased extracellular and intracellular pH. It has been suggested that this may alter action potential dispersion and conduction, and may thus be arrhythmogenic (Hulme and Orchard 2000). To test this hypothesis, epicardial activation was optically mapped during acidosis, using the voltage sensitive dye RH237. Hearts isolated from adult male New Zealand rabbits under terminal anaesthesia (1 mg/kg pentobarbitone) were Langendorff-perfused with Tyrode's solution containing 3μM cytochalasin D to inhibit movement. Epicardial activation in a 2 x 2cm region of left ventricular free wall was recorded under control conditions (5% CO_2; pH 7.4) and during acidosis (20% CO_2; pH 6.7). The area studied was chosen to include a region of epicardial breakthrough.During pacing of the right atrium at cycle length (CL) of 300, 250, 200 and 150ms, acidosis caused a marked delay in epicardial activation with no obvious change in epicardial conduction (Fig. 1): at CL 250ms, under control conditions the time to earliest activation (Tact) was 99.3 ± 9.0ms (mean±sem, n=3); acidosis increased Tact by 24.3±5.5ms (124±0.8%; $p<0.05$; paired t-test). Decreasing CL to 150ms under control conditions increased Tact to 126.3±6.5ms ($p<0.01$), and acidosis further increased Tact to the extent that 2:1 block was observed in all 3 preparations. Pacing via epicardial-stimulating electrodes demonstrated that epicardial conduction velocity was unchanged (peak in control 70.6±12.9cm/s vs. 64.1±1.7cm/s in 20% CO_2; NS). Endocardial pacing failed to reveal any change in transmural conduction. These data suggest that moderate acidosis has little direct effect on epicardial activation, but prolongs Tact by slowing conduction at site(s) proximal to the ventricular endocardium, possibly at the A-V node, consistent with previous work on the effect of acidosis on the ECG (Aberra et al, 2001).

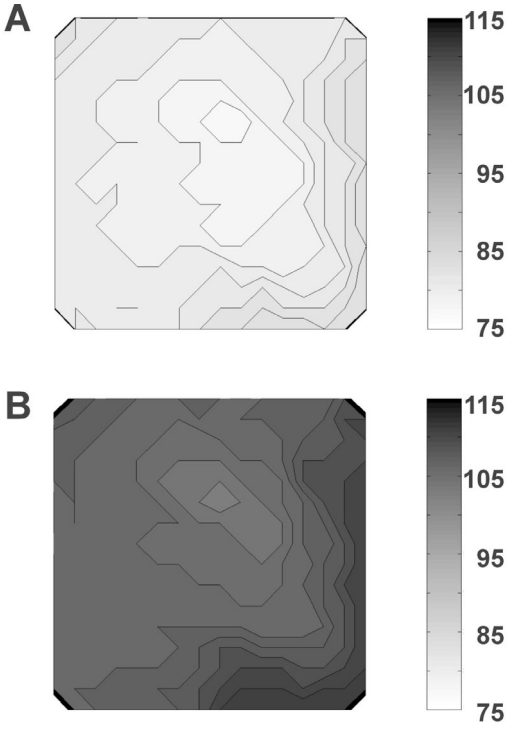

Figure 1. Isochronal maps of epicardial activation from the left ventricular free wall (approximately equidistant from apex and base) at CL 300 ms. Scale on right indicates the activation time, from stimulation of the right atrium to epicardial activation. Panel A: 5%CO_2; B: 20% CO_2.

Aberra, A. et al. (2001) Exp Physiol 86.1, 27-31.

Hulme, J.T. and Orchard, C.H. (2000). Am.J Physiol 278.1, H50-H59.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C13

Progressive short-term effects of streptozotocin-induced diabetes on electrical activity of the heart

F.C. Howarth[1], E. Adeghate[2], M. Jacobson[3] and K. Fatima-Shad[4]

[1]Physiology, United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates, [2]Anatomy, United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates, [3]Engineering, United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates and [4]Science, United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates

Biodata including electrocardiogram (ECG), physical activity and body temperature were monitored using a telemetry system (Data Sciences Int., USA). The system comprised transmitter devices (TA11CTA-F40), receivers (RPC-1), a data exchange matrix

(20CH) and a personal computer for system configuration, control and data storage. Transmitters were surgically implanted in six young adult (200-250g) male Wistar rats under general anaesthesia (sodium pentobarbitone, 45 mg/kg ip). The transmitter devices were inserted in the peritoneal cavity and electrodes from the transmitter were arranged in Einthoven bipolar (Lead II configuration). After full recovery from surgery baseline data was collected for nine days. On day 10, three animals received a single ip injection of streptozotocin (60 mg/kg bodyweight, STZ) dissolved in citrate buffer. Age-matched controls received citrate buffer alone. Biodata were recorded for 5 minutes of every hour, 24 hours per day, during a period of 1 month. From the collected data, secondary physiological measurements were determined including the average 5-minute heart rate (HR), heart rate variability (HRV), and HRV power spectral density (PSD). In order to highlight the effect of STZ-induced diabetes, baseline day 5 and 10 days after STZ treatment were statistically compared using the paired t-test. P-values less than 0.05 were considered significant. Blood glucose was characteristically elevated in STZ-treated (376.3±24.6 mg/dl, n=3) compared to controls (80.0±4.5 mg/dl, n=3). HR during the baseline period was 353±12 beats per minute (BPM, n=6) and was significantly reduced at 10 days after STZ treatment (243±12 BPM) compared to controls (365±13 BPM). HRV during the baseline period was 25±4 BPM (n=6) and was significantly reduced at 10 days after STZ treatment (11±3 BPM) compared to controls (33±3 BPM). Activity during the baseline period was 0.92 counts per minute (CPM, n=6) and was significantly reduced at 10 days after STZ treatment (0.39±0.09 CPM) compared to controls (1.04±0.13 CPM). Body temperature during the baseline period was 37.6±0.1°C (n=6) and was significantly reduced at 10 days after STZ-treatment (36.9±0.1°C) compared to controls (37.6±0.1°C). Defective autonomic regulation of cardiac function might underlie altered HRV in short-term STZ-induced diabetic rats.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

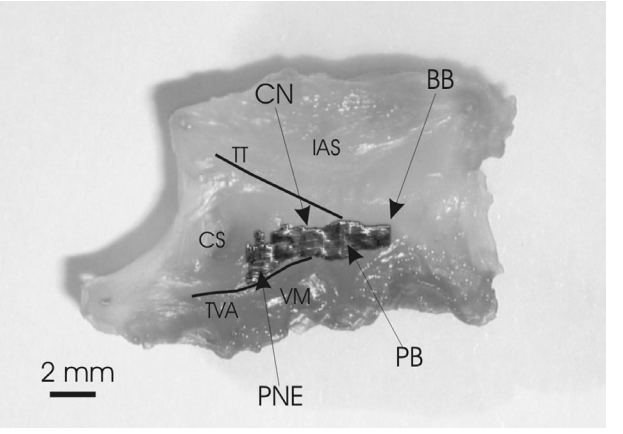
C14

A three-dimensional model of the rabbit atrioventricular node conduction axis

I.D. Greener[1], H. Dobrzynski[1], J. Li[1], V. Nikolski[2], M. Yamamoto[1], R. Billeter[1], I. Efimov[2] and M.R. Boyett[1]

[1]School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK and [2]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA

The atrioventricular (AV) node conduction axis is complex and heterogenous in terms of function and morphology. Functionally, there are dual (fast and slow) conduction pathways leading from the atrial muscle to the compact AV node (Wu, 1982). As well as normal conduction, the dual pathways are also implicated in AV node pacemaking and AV nodal reentrant tachycardia. Despite their functional importance, on an anatomical level, these pathways are poorly understood. To obtain a better understanding of the structure of the AV node, we are constructing a 3-D model of the region of the AV node (the triangle of Koch). Adult New Zealand White rabbits were killed humanely and the AV node region isolated (n = 6; Fig. 1). 10 μm serial sections were cut perpendicular to the tricuspid valve (and the endocardium). 60 sections at 100 μm intervals were stained with Masson's trichrome to show the histology. Adjacent sections were immunolabelled for neurofilament (a marker of the conduction system in the rabbit heart) and Cx43 (gap junction protein known to be present throughout much of the heart, but not in the AV node). Based on the histology and the immunolabelling, the distributions of different cell types (neurofilament-negative ventricular muscle, neurofilament-negative atrial muscle, neurofilament-positive conduction tissue, connective tissue) were identified in each of the 60 sections. Matlab software is being used to develop a 3-D model from these data. As an example, Fig. 1 shows the distribution of neurofilament-positive conduction tissue (dark grey) in the triangle of Koch (from left to right): posterior nodal extension (PNE), compact node (CN), penetrating bundle (PB), bundle branches (BB), interatrial septum (IAS), tendon of Todaro (TT), coronary sinus (CS), tricuspid valve annulus (TVA) and ventricular myocardium (VM). The posterior nodal extension corresponds to the position of the slow pathway. Cx43 immunolabelling was abundant in the working myocardium, but sparse or absent in the conduction tissue. Our data show two different tissue types in the AV node region: neurofilament-negative/Cx43-positive tissue (atrial and ventricular muscle) and neurofilament-positive/Cx43-negative tissue (conduction tissue).



Wu, D. (1982). Pacing Clin Electrophysiol. 5, 72-89.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C15

The surface area: volume relationship of mouse cardiac myocytes does not change following induction of hypertrophy

M.A. Stagg[1], C.M. Terracciano[1] and K.T. MacLeod[2]

[1]Heart Science Centre, NHLI, Imperial College London, London, UB9 6JH, UK and [2]Cardiac Medicine, NHLI, Imperial College London, London, SW3 6LY, UK

Changes in the spatial relationship between the sarcolemma and intracellular compartments may be an underlying reason for

defective excitation-contraction coupling observed in heart failure (Gomez *et.al.*, 1997). These changes may arise from a modified ventricular myocyte surface area: volume ratio (SA:V). The quantification of electrophysiological data are reliant on SA:V. However, this normalization factor is unknown in normal and hypertrophied murine ventricular myocytes and could be of relevance to studies on transgenic mouse models of hypertrophy. In this work, we calculated SA:V in the same myocytes isolated from normal and hypertrophied mouse hearts. Hypertrophy was induced by infusion of angiotensin II for 14 days (Ang II; 2 μg/min/kg) via an osmotic minipump implanted subcutaneously under isoflurane anaesthesia (inhalation 0.5-3.0%). Control mice received saline solution (Veh). Cardiac myocytes were isolated following cervical dislocation and voltage clamped to assess the membrane capacitance, an index of cell surface area (Terracciano *et.al.*, 1998). The same cells were visualised with the fluorescent dye, di-8-ANEPPS, and optically sectioned by confocal microscopy. Cell volume was calculated by 3-D reconstruction of the images. Differences between means were assessed with a one-way ANOVA and Bonferroni post-test. A two-tailed Pearson correlation was used to assess differences between cell membrane capacitance and volume in Veh and Ang II groups.Following angiotensin II infusion, mice showed a significant increase in heart-weight-to-body-weight ratio indicating cardiac hypertrophy (Veh: 7.1±0.4 mg/g (5); Ang II: 9.4±0.7 mg/g (6), $p<0.05$); (means±S.E.M. (*n*)). Ventricular myocyte volume was significantly greater in Ang II, (34.4±1.4 pl (41)) compared with Veh, (27.1±1.5 pl (35, $p<0.001$)). Capacitance was also larger in Ang II, (237±7 pF (41)) compared with Veh, (177±9 pF (35, $p<0.001$)). The surface area: volume ratio was unchanged by hypertrophy: (Ang II: 7.33±0.40 pF/pl (41); Veh: 7.09±0.48 pF/pl (35, p=ns)).We conclude that, following the induction of hypertrophy in the murine heart, the ratio between cell surface area and cell volume is unchanged.

Gomez AM *et al.*(1997) *Science.* **276**, 800-806.
Terracciano CMN *et al.*(1998) *J.Physiol.* **512**, 651-667.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C16

Effect of cardiac hypertrophy upon the expression and activity of the glutamate transporter, GLT1.

N. King[1], J.D. McGivan[2] and M. Suleiman[1]

[1]Bristol Heart Institute, University of Bristol, bristol, UK and [2]Biochemistry, University of Bristol, Bristol, UK

GLT1 and EAAC-1 are two important members of a family of sodium dependent transporters, which are largely responsible for glutamate and aspartate uptake in peripheral tissues (PalacÍn *et al.*1998). In the hypertrophic rat heart there is an increase in EAAC1 protein expression and a faster rate of aspartate transport compared to normal rat hearts (King *et al.* 2002) Nothing however is known about the expression of GLT1 in the hypertrophic heart or about the effect of cardiac hypertrophy on glutamate transport. The aim of this study was to investigate GLT1 expression and its contribution to glutamate transport in sarcolemmal vesicles prepared from control and hypertrophic heart. Hypertrophic hearts were obtained from spontaneously hypertensive rats, whilst normal hearts were obtained from their corresponding normotensive control, Wistar Kyoto rats. All rats were humanely killed by cervical dislocation and the hearts dissected. Cardiac sarcolemmal vesicles were prepared by homogenisation and differential centrifugation (King *et al.* 2001). The initial rate (at 1s) of 0-0.3mM L-[^{14}C]glutamate transport was measured by rapid filtration at room temperature (King *et al.* 2001). These measurements were carried out with/without 0.2mM of the selective GLT1/EAAC-1 inhibitor, L-serine-O-sulphate (SOS) (Palacin *et al.*1998). Western blotting followed by scanning densitometry was used to assess the level of GLT1 expression (King *et al.* 2001).GLT1 was expressed in sarcolemmal vesicles from both normal and hypertrophic hearts. The density of the bands for vesicles from hypertrophic hearts was 11.42±1 1.1 optical density units (OD)/mm^2 which was significantly greater than the 7.28±0.62 OD/mm^2 measured for vesicles from normal hearts (N = 4±S.E., $p>0.02$, Students unpaired *t*test). The maximal velocity of the SOS insensitive sodium dependent component of glutamate uptake was significantly faster at 5.7±1.2 pmol/mg/s in vesicles prepared from hypertrophic hearts compared to the 1.0±0.2 pmol/mg/s (N = 4±S.E., $p < 0.01$, Students unpaired *t*test) measured for vesicles from control hearts. These results suggest that in comparison to the normal heart, the expression and activity of GLT1 is upregulated in the hypertrophic heart.

King N *et al.* (2002).*Pflügers Archiv* **433S**, S439.
King N *et al.* (2001).*Cardiovasc Res* **52**, 84-94.
Palacín M *et al.* (1998). *Physiol Rev* **78**, 969-1054.

Support by the British Heart Foundation

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C17

Increased sarcoplasmic reticulum (SR) calcium content, but not regression of cellular hypertrophy, correlates with clinical recovery from end-stage heart failure using left ventricular assist device (LVAD) and pharmacological therapy

C. Terracciano, J. Hardy, E. Birks, A. Khaghani, N. Banner and M.H. Yacoub

Heart Science Centre, NHLI, Imperial College London, Harefield, UK

LVAD treatment has been used successfully for the management of patients in end-stage heart failure. In a limited number of patients, when associated with pharmacological therapy, this treatment leads to substantial clinical improvement (Yacoub, 2001). Cardiomyocytes isolated from biopsies taken at explantation of the LVAD in patients with clinical recovery showed changes in intracellular Ca2+ homeostasis (Terracciano *et.al.*, 2003). However, whether these specific cellular changes are related to clinical recovery remained unclear. We therefore studied isolated left ventricular myocytes from patients at the time of LVAD implant (LVAD core, n=7 patients), from tissue taken at explantation in patients with clinical recovery (Recovery, n=6) and from patients who did not show clinical recovery, thus requiring transplantation (Transplanted, n=4). Ethical approval was obtained by the Brompton & Harefield NHS Trust Ethics Committee. Informed

consent was obtained from each patient. Data are expressed as mean ±SD. Statistical differences between means were calculated with one-way ANOVA comparisons and Bonferroni post tests.Electrophysiology was performed using switch-clamping and high resistance (20-30 MΩ) microelectrodes. Compared with myocytes taken at implantation, both recovery and non recovery groups showed a similar reduction in cell capacitance (LVAD core:290±61 pF; 7; Recovery:148±28 pF; 5; Transplanted:167±65 pF; 4, $p<0.01$; mean ± SEM; n = patients). Action potentials from patients with recovery shortened significantly compared with myocytes from LVAD core. Such an effect was not observed in the non-recovery group (time -to-90% repolarisation LVAD core: 735±160 ms; 7; Recovery: 454 ±58 ms; 6, Transplanted: 801 ±125 ms; 4, $p<0.01$). L-type Ca current fast inactivation was faster in recovery compared with non-recovery (LVAD core: 16.4±3.3 ms; 7; Recovery:9.3±1.7 ms; 5; Transplanted:18±5.5 ms; 4; $p<0.01$) and SR Ca content was increased in the recovery group compared with both the implant and non-recovery groups (SR Ca content in LVAD core: 30.3±17 μM/l non mitochondrial volume (NMV); 7; in Recovery: 90.4±37 mM/l NMV; 5; in Transplanted: 35±15 mM/l NMV; 4; $p<0.01$).These findings further identify alterations in excitation-contraction coupling, and SR Ca2+ handling in particular, as key functional determinants in patients with heart failure. In contrast regression of cellular hypertrophy did not appear to influence clinical recovery.

Terracciano CMN *et.al.* (2003). *Eur.Heart J.* **24**, 1329-1339.

Yacoub, MH (2001). *Eur.Heart J.* **22**, 534-540.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C18

The role of Ca^{2+} and mitochondrial re-energization in reperfusion injury of adult rat isolated cardiac myocytes following metabolic inhibition or mitochondrial uncoupling

G.C. Rodrigo and N. Standen

Cell Physiology and Pharmacology, University of Leicester, Leicester, UK

Reperfusion injury of cardiac myocytes following metabolic inhibition (MI) is characterised by hypercontracture, loss of Ca^{2+}-homeostasis and contractile function, and is sensitive to the energetic status of the heart (Rodrigo et al. 2002). We investigated the role of mitochondrial re-energization in reperfusion injury by studying cellular events during reperfusion following MI, where NADH and $FADH_2$ concentrations are high, and uncoupling where mitochondria are fully oxidized.Ventricular myocytes were isolated from humanely killed adult rats (Rodrigo et al. 2002) and stimulated at 1 Hz and at 35°C. Contraction was measured with a video system, $[Ca^{2+}]_i$ with fura-2 and $[ATP]_i$ with Mg green. For MI we used substrate-free Tyrode containing 2mM NaCN + 1 mM IAA, and uncoupling used 10 mM CCCP. Both MI and uncoupling caused contractile failure, rigor and an increase in diastolic $[Ca^{2+}]_i$. After 10 min MI $[Ca^{2+}]_i$ was 256 ± 5 nM (mean ± S.E.M. n = 25 experiments) in MI, and 489 ± 18 nM (n = 27) during uncoupling. Reperfusion of MI-treated myocytes with normal Tyrode induced an immediate but transient fall in $[Ca^{2+}]_i$ to 179 ± 6 nM (n = 14) followed by a hypercontracture. However, reperfusion after uncoupling had no immediate effect on cell length or $[Ca^{2+}]_i$. Rather, there was a delay of 2-3 min before a transient fall in $[Ca^{2+}]_i$ accompanied by a hypercontracture. Following hypercontracture $[Ca^{2+}]_i$ climbed steadily in both MI and uncoupled cells, reaching 300-350 nM after 10 min reperfusion. Reperfusion of MI and uncoupled myocytes resulted in a transient increase in ATP, which preceded hypercontracture. However, ATP concentration continued to remain low in MI-treated myocytes but recovered to near control values in uncoupled myocytes. Removal of extracellular Ca^{2+} during MI or uncoupling limited the rise in $[Ca^{2+}]_i$ to 127 ± 12 nM (n = 11), and 100 ± 5 nM (n = 11) respectively, but had no effect on reperfusion-induced hypercontracture. Removing Ca^{2+} during reperfusion prevented the reperfusion-induced increase in $[Ca^{2+}]_i$ in both cases, but only blocked hypercontracture in uncoupled myocytes.Our results support a model for reperfusion injury of MI-treated myocytes where production of ATP secondary to mitochondrial re-energization is primarily responsible for initiating hypercontracture by activating cross-bridge cycling. The reduced severity of reperfusion injury in uncoupled myocytes may be linked to loss of mitochondrial reducing power (NADH, $FADH_2$).

Rodrigo GC *et al.* (2002). *J Mol Cell Cardiol* **34**, 555-569.

Supported by the British Heart Foundation.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C19

Activation of NaH exchange contributes to the slow inotropic response to stretch independently of endothelin 1 in muscle and myocytes from the rat heart

S. Calaghan and E. White

School of Biomedical Sciences, University of Leeds, Leeds, W Yorks, UK

Activation of NaH exchange (NHE) has been shown to contribute to the slow inotropic response to stretch in intact cardiac muscle from several species (see Cingolani *et al.*, 2003). We have addressed 2 questions: Is NHE activated by stretch of the single cardiac myocyte? Is NHE activation secondary to endothelin 1 (ET 1) release? Rats were killed humanely. Papillary muscles were stretched from 88 to 98% of the length at which maximum tension is generated, single ventricular myocytes were stretched by around 7% from resting sarcomere length using carbon fibres. The slow response was calculated as the increase in force at 5 min (myocyte) or 10 min (muscle) after stretch as a % of that recorded immediately after stretch. All experiments were performed in the presence of bicarbonate-based physiological solution at room temperature.The NHE inhibitor HOE 642 (5 μM) significantly reduced the magnitude of the slow response to stretch by around 50% in papillary muscle ($P<0.05$; paired Student's t-test) and by around 80% in the single myocyte ($P<0.01$; Figure 1). However, the non-selective ET 1 receptor antagonist PD145065 (1 μM) had no effect on the magnitude of the slow response in either preparation (Figure 1).

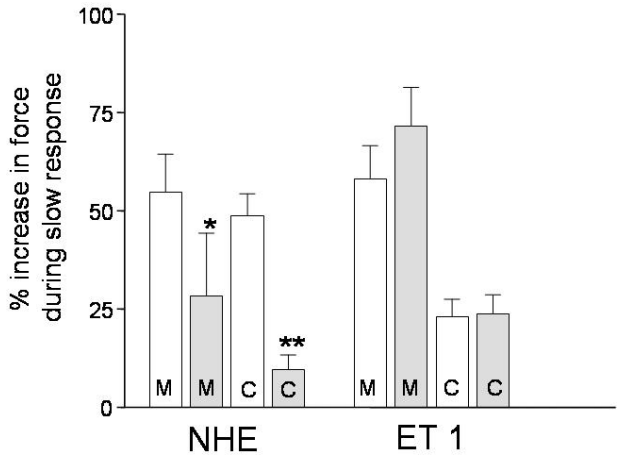

Figure 1. The effect of inhibition of NHE (5 μM HOE 642) and antagonism of ET 1 receptors (1 μM PD145065) on the magnitude of the slow response to stretch in papillary muscle (M) and myocytes (C) from the rat heart. Open bars are under control conditions, shaded bars are in the presence of inhibitor. For data with HOE 642, myocytes were stretched by 7.9 ± 1.5% (mean ± S.E.M; n=6); for data with PD145065, myocytes were stretched by 5.9 ± 0.9% (n=12); for all data with muscle, n=6. * P<0.05 ** P<0.01 compared with respective control conditions (paired Student's t-test).

It has been proposed by several groups that the slow response seen at the level of the myocyte is Na^+-independent. The present data suggests that this is not the case. NHE activation (which will increase intracellular Na^+ which consequently exchanges for Ca^{2+} on the NaCa exchanger) contributes to the slow response in the stretched single myocyte as well as in intact cardiac muscle. Furthermore, we have shown for the first time that NHE activation is not dependent on ET 1 release in either myocytes or muscle from the rat heart. Indeed the lack of effect of PD145065 on the slow response in myocytes or muscle argues against an autocrine or paracrine role for ET 1 in this species.

Cingolani HE *et al.* (2003). *Cardiovasc Res* **57**, 953-960.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.