

1106-185

### Growth Hormone-Releasing Peptide Can Suppress Myocardial Oxidative Stress and Ameliorate Progressive Left Ventricular Remodeling in Cardiomyopathic Hamsters

Mitsunori Iwase, Sahoko Ichihara, Yosuke Kato, Aya Matsushita, Hiroaki Kanazawa, Katsunori Hashimoto, Koji Obata, Kohzo Nagata, Toyooki Murohara, Mitsuhiro Yokota, Takasi Tsunematsu, Yoshihiro Ishikawa, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan, Yokohama City University, Yokohama, Kanagawa, Japan

**Background:** Growth hormone-releasing peptide (GHRP) may act directly on the myocardium and improve left ventricular (LV) function, implicating a potential new approach to the treatment of cardiomyopathic hearts. We hypothesized that cardiac favorable effects of GHRPs might include the attenuation of myocardial oxidative stress.

**Methods and Results:** Dilated cardiomyopathic TO-2 hamsters (6 weeks old) were injected with GHRP-2 (1mg/kg per day, n=8), or saline (n=8) for 6 weeks. F1B hamsters served as controls (n=8). LV functional and structural changes were evaluated by echocardiogram and the extents of myocardial oxidative stress were determined by myocardial 4-hydroxy-2-nonenal (HNE) concentration as well as the tissue-reduced glutathione, oxidized glutathione, and the redox ratio (GSH/GSSG). LV fractional shortening (LVFS) decreased from  $53.0 \pm 1.4$  to  $25.1 \pm 1.0$  % and the LV end-diastolic dimension increased from  $3.7 \pm 0.1$  to  $5.0 \pm 0.1$  mm in untreated TO-2 hamsters between 6 and 12 weeks. Both LVFS and LV dimension were substantially improved by treatment with GHRP-2 ( $41.5 \pm 1.5$  %,  $4.6 \pm 0.1$  mm, respectively). The ratio of LV dimension / LV wall thickness in untreated TO-2 hamsters was significantly greater than that in controls ( $6.42 \pm 0.23$  Versus  $3.73 \pm 0.13$ ,  $p < 0.001$ ), and was substantially normalized by the treatment with GHRP-2 ( $4.78 \pm 0.23$ ). The extent of cardiac fibrosis in TO-2 hamsters with GHRP was significantly less than that in untreated TO-2 hamsters. The level of HNE in hearts of TO-2 hamsters was greater than that in controls ( $1.63 \pm 0.14$  Versus  $0.85 \pm 0.08$  pmol/mg protein), and was normalized by the treatment of GHRP-2 ( $0.71 \pm 0.07$  pmol/mg protein). The redox ratio in hearts of untreated TO-2 hamsters was greater than that in controls ( $2.98 \pm 0.08$  Versus  $1.95 \pm 0.04$ ,  $p < 0.005$ ) and was improved by the treatment of GHRP-2 ( $2.36 \pm 0.08$ ). Myocardial glutathione peroxidase activity in untreated TO-2 hamsters was similar to that in F1B hamsters. **Conclusion:** GHRP can reduce myocardial oxidative stress during the initial development of LV dysfunction, leading to a favorable modification of progressive LV remodeling process in dilated cardiomyopathic hamsters.

1106-186

### Testosterone Inhibits the $\alpha_1C$ Subunit of L-Type $Ca^{2+}$ Channels ( $Ca_v1.2$ ) and $\alpha_{1H}$ T-type $Ca^{2+}$ Channels ( $Ca_v3.2$ ) Stably Expressed in Human Embryonic Kidney 293 Cells

Jason L. Scragg, Richard D. Jones, T Hugh Jones, Kevin S. Channer, Chris Peers, The University of Leeds, Leeds, United Kingdom, The University of Sheffield, Sheffield, United Kingdom

**Background:** Testosterone therapy reduces myocardial ischaemia in men with coronary artery disease (CAD) [1] and improves symptom scores and exercise capacity in men with congestive heart failure (CHF) [2]. These effects may be attributed to a direct vasodilatory efficacy in the coronary [3] and peripheral [4] vasculature. *In vitro* studies suggest this vasodilation is due to a calcium antagonistic action upon voltage-gated  $Ca^{2+}$  channels [5], but this has yet to be investigated directly. **Methods:** We employed patch clamp methodology to study the effect of testosterone (1nM-1 $\mu$ M) upon whole-cell  $Ca^{2+}$  channel currents (using 20mM  $Ba^{2+}$  as the charge carrier) in HEK 293 cells stably expressing either the main pore-forming  $\alpha_1C$  subunit of a human L-type  $Ca^{2+}$  channel ( $Ca_v1.2$ ), or the  $\alpha_{1H}$  T-type  $Ca^{2+}$  channel ( $Ca_v3.2$ ) [6]. **Results:** Testosterone (1nM-1 $\mu$ M) caused a rapid, irreversible concentration-dependent inhibition of L-type  $Ca^{2+}$  currents, with an  $IC_{50}$  value of  $61.0 \pm 0.6$  nM ( $n = 4-9$  cells at each concentration tested). Current-voltage relationships indicated that testosterone caused a similar inhibition at all activating potentials, suggesting that the inhibition was voltage-independent. Testosterone (1nM-1 $\mu$ M) also inhibited currents mediated via T-type  $Ca^{2+}$  channels, but this effect was partially reversible with a lower  $IC_{50}$  value of  $231.9 \pm 0.7$  nM. **Conclusions:** Testosterone acts as an inhibitor of both human L-type and T-type  $Ca^{2+}$  channels, the major voltage-gated calcium channels expressed in vascular smooth muscle. Inhibition of L-type  $Ca^{2+}$  channels occurred at physiological concentrations. This calcium antagonistic action may contribute to the clinical benefits associated with testosterone therapy in men with CAD and CHF.

1. English KM *et al.* *Circ.* 2000; 102, 1906-11
  2. Pugh PJ *et al.*, *Heart* 2003; In Press
  3. Webb CM *et al.* *Circ.* 1999; 100, 1690-96
  4. Pugh PJ *et al.*, *Eur.Heart.J.* 2003; 24, 909-15
  5. Jones RD *et al.* *Br.J.Pharmacol.* 2003; 138, 733-44
  6. Fearon IM *et al.* *Circ.Res.* 2000; 87, 537-539
- This work was supported by the British Heart Foundation.

1106-187

### Both Beta-Blockers and Amiodarone Increase Speed of QT / Heart Rate Adaptation

Peter Smetana, Esther Pueyo, Katerina Hnatkova, Velislav Batchvarov, Marek Malik, St. George's Hospital Medical School, London, United Kingdom

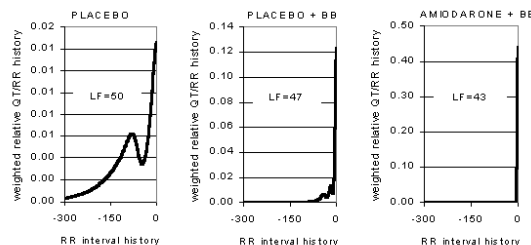
**Background:** Recent studies gave evidence that the combination of amiodarone and beta blocker (BB) treatment is beneficial. This therapeutic option may be relevant in many areas including those of no clear indication for ICD. However, the mode of synergistic action is still to be established. Since both substances were shown to modulate QT/RR relationship we investigated a descriptor of QT rate adaptation speed in Holter recordings of patients from the EMIAT database surviving the 2-year follow-up period.

**Methods:** In 754 (403 amiodarone, 351 placebo) 24-hour Holter recordings obtained 1 month after randomisation into EMIAT RR, and QT intervals were measured automati-

cally on a beat-to-beat basis using a commercial Holter system. The individual QT rate adaptation profile was assessed on a continuous basis by quantifying the weighted impact of RR interval history on each QT interval and expressing this by the so-called lambda factor (LF). The smaller LF, the faster QT interval adaptation to heart rate. Results were compared in patients on and off BB in the amiodarone and the placebo group.

**Results:** Both amiodarone and BB increased the speed of QT adaptation to changes in RR interval (LF: placebo vs placebo+BB,  $50.4 \pm 6.7$  vs  $47.4 \pm 7.7$ ,  $p = 0.00006$ ; amiodarone vs amiodarone+BB:  $47.9 \pm 6.8$  vs  $43.1 \pm 7.5$ ,  $p = 2 \times 10^{-11}$ ). Examples in the figure.

**Conclusions:** Both amiodarone and BB improve adaptation of QT interval to changes in RR interval. This might be one of the reasons for the superior benefit of a combination of the therapies.



1106-188

### Ghrelin Is Synthesized and Secreted by Human Cardiomyocytes Protecting These Cells From Apoptosis

José Ramón González-Juanatey, María J. Iglesias, Roberto Piñeiro, Oreste Gualillo, Montserrat Blanco, Carlos Diéguez, Francisca Lago, Hospital Clínico Universitario Santiago De Compostela, Santiago De Compostela, Spain, Universidad De Santiago De Compostela, Santiago De Compostela, Spain

**Background:** Ghrelin, the endogenous ligand of growth hormone secretagogue receptor (GHS-R), acts on the pituitary and the hypothalamus to stimulate the release of growth hormone (GH) and promote appetite and adiposity. It has also been reported to increase myocardial contractility, induce vasodilation, and protect against myocardial-infarction-induced heart failure. Though principally gastric in origin, it is also produced by other tissues. This work investigated whether cardiomyocytes synthesize and secrete ghrelin, and how its production in these cells responds to stress and exogenous apoptotic agents. **Methods and Results:** RT-PCR showed that cells of the adult mouse cardiomyocyte line HL-1 produce mRNAs for both ghrelin and GHS-R, and competitive binding studies using 125I-labelled ghrelin showed efficient constitutive expression of GHS-R at the surface of HL-1 cells. Immunohistochemistry confirmed the presence of ghrelin in the cytoplasm of HL-1 cells and of isolated human cardiomyocytes in primary culture. Radioimmunoassay showed that ghrelin was secreted by HL-1 cardiomyocytes into the culture medium. Ghrelin did not modify the viability of HL-1 cells subjected to 12 h starvation, but did protect against the apoptosis inducer cytosine arabinoside (AraC). Finally, production of ghrelin mRNA in HL-1 cardiomyocytes was reduced by AraC but increased if exposure to AraC was preceded by GH treatment. **Conclusions:** We demonstrate for the first time that ghrelin is synthesized and secreted by isolated murine and human cardiomyocytes, probably with paracrine/autocrine effects protecting these cells from apoptosis. This new axis may play a beneficial role in myocardial dysfunction and heart failure.

## ORAL CONTRIBUTIONS

825

### Clinical Trials: Cardiovascular Outcomes

Monday, March 08, 2004, 4:00 p.m.-5:30 p.m.  
Morial Convention Center, Room 265

4:00 p.m.

825-1

### Efficacy and Safety of Ximelagatran Compared With Well-Controlled Warfarin in Elderly Patients With Nonvalvular Atrial Fibrillation: Observations From the SPORTIF Trials

S. Bertil Olsson, for the Executive Steering Committee on Behalf of the SPORTIF Investigators, University Hospital, Lund, Sweden, Mount Sinai Medical Center, New York, NY

**Background:** Nonvalvular atrial fibrillation (AF) affects 5-10% of people over 75 years of age, in whom the risk of thromboembolism is higher than in younger individuals. Although adjusted-dose warfarin protects against ischemic stroke, many patients cannot sustain treatment because of bleeding, drug interactions, and coagulation monitoring. The oral direct thrombin inhibitor, ximelagatran (ExantaTM, AstraZeneca), is a potential alternative anticoagulant. Both treatments were compared in patients of at least 75 years.

**Methods:** The SPORTIF III (open-label,  $n = 3410$ ) and V (double-blind,  $n = 3922$ ) trials included 2804 patients at least 75 years of age with AF and at least 1 stroke risk factor randomized to adjusted-dose warfarin (target INR 2.0-3.0) or fixed-dose ximelagatran (36 mg twice daily). The primary endpoint was stroke (ischemic or hemorrhagic) and sys-