SUBCUTANEOUS TESTOSTERONE IMPLANT THERAPY IMPROVES ENDOTHELIUM-DEPENDENT AND INDEPENDENT VASODILATION IN POSTMENOPAUSAL WOMEN ALREADY RECEIVING OESTROGEN Worboys S, Kotsopoulos D, Teede H, McGrath BP, Davis SR. The Jean Hailes Foundation and Monash University Dept of Medicine, Monash Medical Centre, Clayton, Vic, Australia

The gender difference in cardiovascular disease has been partly attributed to higher androgenic hormone levels. Although testosterone in women may not affect lipids, it remains unknown whether it negates favourable oestrogenic effects on endothelial function. We have investigated the effects of testosterone implant therapy on endothelial function [flow mediated vasodilation (FMD)] in women using hormone replacement therapy (HRT). B-mode ultrasound measurements of resting brachial artery diameter, following reactive hyperaemia (endothelium-dependent) and following glyceryl trinitrate (GTN) (endothelium-independent) dilatation were recorded in 33 postmenopausal women stabilised on HRT (> 6 months), at baseline and 6 weeks after a testosterone implant (50mg), with 15 postmenopausal non-users of HRT serving as controls. In the brachial artery baseline resting diameter was similar (0.40 ± 0.01 vs 0.41 ± 0.01 cm, p = 0.5). In the treated group, testosterone levels increased (0.99 ± 0.08 to 4.99 ± 0.3 nmol/L, p<0.001), associated with a mean 42% increase in FMD (6.4% ± 0.7 to 9.1% ± 1.1, p = 0.03). The control group did not change (8.1% ± 1.4 to 5.6% ± 1.0, p = 0.4). There was significantly greater improvement in FMD in the testosterone-treated compared to control group (p = 0.04). GTN induced vasodilatation increased with testosterone treatment (14.9% ± 0.9 to 17.8% ±1.2, p = 0.03).

Conclusion: Exogenous testosterone implants improve both endothelial dependent (flow mediated) and endothelium-independent (GTN mediated) brachial artery vasodilation in postmenopausal women, using long-term oestrogen therapy. The mechanisms underlying these potentially beneficial cardiovascular effects require further investigation.

LIPOCORTIN PROTECTS CARDIAC MYOCYTES FROM ISCHAEMIA IN VITRO. JM Gordon*, RH Ritchie & GJ Dusting. Howard Florey Institute, University of Melbourne, Vic, 3010

Cardiac myocytes (CM) can be protected from a potentially lethal ischaemic insult by preconditioning with transient ischaemia. Although the mechanisms of the protection are still poorly understood, adenosine is a known trigger. We have recently reported that an N-terminal fragment of the annexin peptide lipocortin-1 (LC-$1_{2\text{-}26}$) protects rat myocardium from endotoxin and cytokine-induced depression of inotropic responsiveness[1]. However, the cardioprotective potential of lipocortin against ischaemia/reperfusion injury has not been determined. We tested the hypothesis that lipocortin protects isolated CM against cellular injury in a model of simulated ischaemia. Adult rat CM were incubated in a simulated ischaemia buffer[2] (10 mM 2-deoxy-D-glucose, 20 mM D,L-lactic acid in a HEPES buffer, pH 6.5) for 4h at 37°C. Paired control CM were incubated in normal HEPES buffer (pH 7.4). CM were then allowed to recover in normal medium for 2.5h. The protective effects of adenosine or LC-$1_{2\text{-}26}$ (0.3 μM) during simulated ischaemia were assessed as the reduction in lactate dehydrogenase (LDH) and creatine kinase (CK) activity as a percent of that induced by simulated ischaemia alone (mean±SE). Simulated ischaemia significantly increased LDH activity by 463±111 IU/10^5 cells (n=13, P<0.05 vs control) and CK activity by 5125±1500 IU/10^5 cells (n=13, P<0.05 vs control).

Treatment	↓ LDH activity (% of ischaemia)	↓ CK activity (% of ischaemia)
1 μM adenosine + ischaemia	47 ± 18 % (n=6)*	77 ± 32 % (n=6)
10 μM adenosine + ischaemia	109 ± 25 % (n=6)*	77 ± 46 % (n=6)
0.3 μM lipocortin + ischaemia	78 ± 11 % (n=7) #	81 ± 25 % (n=7) *

* P<0.05, # P < 0.001 (both vs simulated ischaemia alone)

These data indicate that lipocortin protects CM from cellular injury during simulated ischaemia: we now plan to elucidate the intracellular signalling pathways mediating this effect.

1. Ritchie RH et al (1999) Clin Exp Pharmacol Physiol, 26, 522-524
2. Gordon JM et al (1999) Proc Aust Soc Clin Exp Pharmacol Toxicol, 6, 94

AORTIC STENOSIS: EVIDENCE OF PLATELET AND VALVULAR ENDOTHELIAL DYSFUNCTION. Y.Y.Chirkov*, A.S.Holmes, S.R.Willoughby, S.Stewart, S.Chandy and J.D.Horowitz. Cardiology Unit, Queen Elizabeth Hospital, University of Adelaide, South Australia.

Recent investigations have linked the development of aortic valve (AV) calcification with the occurrence of acute coronary syndromes (ACS), suggesting common pathogenetic factors. We have previously demonstrated that (1) platelets from patients with stable angina pectoris and ACS are hyperaggregable and hypo-responsive to nitric oxide (NO) donors such as sodium nitroprusside; (2) the prophylactic anti-anginal agent perhexiline (Pex) relieves symptoms in patients with aortic stenosis (AS) and potentiates platelet responsiveness to NO in patients with angina pectoris. We now evaluated platelet responsiveness to NO in patients with AS, and the possible anti-aggregatory effects of AV endothelium.

Blood samples were obtained from patients (n=12) with symptomatic AS with or without coronary artery disease not treated with Pex. ADP-induced platelet aggregation (impedance aggregometry in whole blood) and its inhibition by SNP were determined, and compared with that of normal subjects (n=32). In 9 patients, aggregation studies were repeated 9±3 (SEM) days after initiation of treatment with Pex; plasma Pex concentration was 0.99±0.28 mg/L.

Parameter	Normals	AS	AS/Pex
ADP Aggregation (Ohms) Men	7.8±0.7	12.9±1.8*	11.4±1.3
Women	9.8±0.6	14.8±2.2*	12.6±2.6
SNP Inhibition (% of control)	54±3	25±5**	46±8#

*p<0.05 and **p<0.01 vs normals; #p<0.05 vs AS

Possible effects of AV endothelium on aggregation were investigated via co-incubation with normal aggregating blood of explanted AV tissue from patients with AS (n=4) or aortic regurgitation (AR, n=2). AR tissue inhibited aggregation by 56 to 84%. With AS tissue, specimens cut from "smooth" areas of AV inhibited aggregation by 28±13%, while specimens cut from calcified areas potentiated aggregation by 21±6%.

Conclusions: (1) AS is associated with platelet hyperaggregability and hypo-responsiveness to NO, which may predispose to development of ACS; hypo-responsiveness to NO is ameliorated by treatment with Pex. (2) AV tissue normally exerts anti-aggregatory effects, presumably via factors released from endothelium; this appears to be diminished in AS.

THE ANTIPSYCHOTIC AGENTS THIORIDAZINE, CHLORPROMAZINE AND CLOZAPINE BLOCK THE HUMAN-ETHER-A-GO-GO-RELATED GENE (HERG) POTASSIUM CHANNEL: CELLULAR MECHANISM FOR PROARRHYTHMIA. H. Tie*, B.D. Walker, C.B. Singleton, J. Bursill, K. Wyse, S. Valenzuela, S.N. Breit, T.J. Campbell. Department of Medicine, Centre for Immunology, and Victor Chang Cardiac Research Institute, St. Vincent's Hospital, Sydney.

Background: Thioridazine and chlorpromazine are commonly used conventional antipsychotic agents which can cause QT prolongation and torsade de pointes. Clozapine is the prototype of the newer atypical agents. Its use has increased markedly due to a superior efficacy to side effect profile. QT prolongation with clozapine has been reported in animal studies. We examined the cellular basis underlying these observations by studying the effects of these agents on HERG potassium channels. These channels are a primary target of drugs causing QT prolongation. **Methods:** We used the whole cell configuration of the patch-clamp technique. Drugs were applied to HERG stably transfected into Chinese Hamster Ovary cells. **Results:** HERG tail currents were elicited at -60mV after a 3.9-sec step from -80mV to +30mV. Thioridazine and chlorpromazine blocked these currents in a concentration-dependent manner, with an IC_{50} of 1.07 ± 0.06 μM and 1.47 ± 0.03 μM respectively. Block by thioridazine 1 μM and chlorpromazine 3 μM displayed no voltage-dependence between -30 and +30 mV, and no use-dependence at stimulation frequencies of 0.1 and 0.5 Hz. Neither drug enhanced the rate of current decay during prolonged depolarizing pulses. Channel inhibition by thioridazine and chlorpromazine was independent of the depolarizing pulse duration. Clozapine also blocked HERG tail currents, with an IC_{50} of 2.63 ± 0.12 μM. Clozapine block also displayed no voltage-dependence. **Conclusion:** Thioridazine, chlorpromazine and clozapine block HERG channels at clinically relevant concentrations *(therapeutic concentration: thioridazine 0.25-6 μM, chlorpromazine 0.6-2.5 μM, clozapine 0.6-2 μM)*. All three drugs appear to preferentially bind to the closed channel state. This blockade likely underlies the QT prolongation associated with thioridazine and chlorpromazine. Serious cardiotoxicity such as myocarditis and cardiomyopathy have been reported with clozapine. This is the first report of HERG channel blockade by clozapine, and suggests that this agent may also proarrhythmic.