

reduction of rate (-6.9%, -10.1%, and -15.9%) and of the EDD (-9.7%, -20.5%, -23.2%, respectively,  $n = 4-7$ ) while no differences were observed in the TOP. MDP and APD values were significantly different from control only at the 0.6 mg/ml dose; in particular APD increased by +15.7% ( $n=7$ ) and MDP became less negative by -6.9% (4.0 mV,  $n=7$ ). The time course of the spontaneous rate decrease proceeded with time constants of 18.4 s, 17.6 s, and 14.2 s for 0.02, 0.2, and 0.6 mg/ml ( $n=4-7$ ), respectively. The effect of the drug was reversible upon wash-out all concentrations.

**Conclusions:** Our preliminary experiments provide the first evidence that TMYX, a drug largely used in the treatment of cardiac arrhythmias in TCM, acts as a dose-dependent spontaneous rate-lowering agent in SAN cells. These preliminary data also indicate that at doses of 0.2 mg/ml or less, the chronotropic effect reflects a selective action on the early diastolic "pacemaker" depolarization, and thus suggests an effect on the If current. Since TMYX is a mixture of several pure compounds, we intend to proceed with the identification of the specific molecule (s) responsible for its chronotropic action.

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#### GW25-e5170

##### Estrogen inhibits cardiac hypertrophy via upregulation of B-type natriuretic peptide expression in primary neonatal cardiomyocytes

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**Objectives:** The main purpose of this study was to determine whether estrogen could regulate cardiac B-type natriuretic peptide (BNP) expression and to examine signal transduction pathway (s) that are involved in this process. The role of estrogen-induced BNP expression in estrogen's anti-hypertrophic effect will be also investigated in this study.

**Methods:** BNP expression and related signal pathway of neonatal rat ventricular cardiomyocytes (NRVCs) under estrogen treatment (E2, 0.1nM~10nM) was analyzed by real-time quantitative PCR, Western-blotting or immunofluorescence. Phenylephrine (PE)-induced cardiac hypertrophy under estrogen with or without BNP neutralizing antibody treatment was indicated by immunostain of cardiac troponin I and real-time quantitative PCR detection of cardiac hypertrophic markers.

**Results:** Our results confirmed that estrogen treatment induced both cardiac BNP mRNA transcription ( $1.89 \pm 0.18$  fold vs control,  $n=4$ ,  $P < 0.05$ ) and protein translation (glycosylated proBNP,  $3.93 \pm 0.38$  fold vs control; non-glycosylated proBNP,  $1.78 \pm 0.29$  fold vs control,  $n=4$ ,  $P < 0.05$ ) in a concentration-dependent manner. Compared with control group, a higher percentage ( $63.3 \pm 12.2\%$  vs  $18.5 \pm 5.8\%$ ,  $n=3$ ,  $P < 0.01$ ) of BNP expression positive NRVCs was also observed in estrogen treatment group, as indicated by BNP immunostaining. Our data also shown that upregulation of BNP expression under estrogen treatment was accompanied by Akt ( $2.10 \pm 0.16$  fold vs control  $n=3$ ,  $P < 0.01$ ) and p38 ( $1.87 \pm 0.39$  fold vs control,  $n=3$ ,  $P < 0.05$ ) phosphorylation but not Erk1/2. Treatment of PI3-kinase/Akt inhibitor LY294002 or p38 MAPK inhibitor SB203580 decreased the estrogen-induced BNP expression in NRVCs ( $P < 0.05$ ). Furthermore, while E2 treatment significantly reduced PE induced cardiomyocytes hypertrophy (cell-surface area, PE  $2.17 \pm 0.36$  fold vs PE+E2; beta-MHC mRNA, PE  $1.76 \pm 0.10$  fold vs PE+E2; alpha-Actin mRNA, PE  $1.70 \pm 0.21$  fold vs PE+E2,  $n=4$ ,  $P < 0.05$ ), BNP specific neutralizing antibody partially reversed the ability of estrogen to block phenylephrine-induced cell size enlargement (cell-surface area, PE+E2+antiBNP vs PE,  $P > 0.05$ ) and hypertrophic marker gene upregulation (beta-MHC mRNA, alpha-Actin mRNA, PE+E2+antiBNP mRNA, PE+E2+antiBNP vs PE,  $P > 0.05$ ) in NRVCs.

**Conclusions:** Our study shows that estrogen enhances intracellular BNP expression in primary cardiomyocytes. Both PI3-kinase/Akt and p38 MAPK signal pathways were involved in estrogen-induced BNP expression. Further, our data suggests that BNP partially mediates estrogen's anti-hypertrophic effect on cardiomyocytes. Our study helps to explain the higher BNP level observed in normal cycling women than men and offers an insight into estrogen-mediated cardioprotection.

#### GW25-e5214

##### Angiotensin II Promotes Atherogenesis by Increasing the Expression of Connexin 43 in Dendritic Cells NIE Wencheng, 5

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**Objectives:** Angiotensin II contributes to atherogenesis and plaque vulnerability, but the mechanisms are not fully understood. Dendritic cells are vital in both inflammation and atherogenesis. To study the relationships between angiotensin II, dendritic cells, and atherosclerosis, we investigated the effects of angiotensin II on the expression of connexin43 in dendritic cells.

**Methods:** Treatment with angiotensin II increased connexin43 expression ( $P < 0.05$ ) and enhanced the induction of connexin43 expression by lipopolysaccharide in mouse bone marrow-derived dendritic cells ( $P < 0.05$ ). The

pro-atherogenesis effect, were attenuated by the angiotensin II type 1 receptor blocker valsartan.

**Results:** In atherosclerotic plaques of mice expressing high levels of endogenous angiotensin II ApoE<sup>-/-</sup>, generated by the 2kidney-1clip method, connexin43 expression in dendritic cells was also increased ( $P < 0.01$ ), accompanied by the upregulation of the dendritic cell maturation marker CD40 ( $P < 0.01$ ) and vulnerable plaque phenotypes. The in vitro and in vivo effects of angiotensin II on connexin43 expression, as well as the proatherogenesis effect, were attenuated by the angiotensin II type 1 receptor blocker valsartan.

**Conclusions:** Angiotensin II may promote atherosclerosis and plaque vulnerability by increasing the expression of connexin43 in dendritic cells and inducing the maturation of dendritic cells through the angiotensin II type 1 receptor, thus altering the inflammatory balance in favor of T helper 1 polarization.

#### GW25-e1648

##### The Ligament of Marshall: a conduit between left stellate ganglion and ventricle as well as a target for treating long QT syndrome induced by cesium chloride

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**Objectives:** Histology and electrophysiology show that the ligament of Marshall (LOM) is richly innervated by sympathetic and parasympathetic nerves in humans and animals. However, the effect of LOM ablation on cardiac autonomic nervous system and ventricular electrophysiology is still unknown. To explore it, we investigate the impact of LOM ablation on left stellate ganglion (LSG) stimulation induced blood pressure and ventricular electrophysiology changes as well as cesium chloride (Cs) induced ventricular arrhythmias.

**Methods:** In 24 anesthetized mongrel dogs, a left thoracotomy exposed the LSG and LOM. Step 1: In 6 dogs, the maximal systolic blood pressure percent change (MSBP%) and ventricular effective refractory period (ERP) change induced by LSG stimulation were measured before and after LOM ablation. Step 2: 18 dogs were assigned into two groups: the control group ( $N=9$ ) and the LOM ablation group ( $N=9$ ). Cs-induced ventricular arrhythmias were recorded and the concentration of Cs required to induce ventricular tachycardia was measured.

**Results:** LOM ablation could markedly attenuate the increase in the MSBP% and the decrease in ventricular ERP induced by LSG stimulation ( $P < 0.05$ ). During 50V LSG stimulation, the MSBP% before and after LOM ablation were 54.32% and 26.96%, respectively ( $P = 0.003$ ). Compared to the control group, the incidence of Cs-induced ventricular arrhythmias could be significantly decreased and the concentration of Cs required to induce ventricular tachycardia was significantly elevated in the LOM ablation group (1.25 vs 0.75mmol/kg,  $P = 0.002$ ).

**Conclusions:** Ventricular electrophysiology changes induced by LSG stimulation could be attenuated by LOM ablation. In Cs-induced LQTS model, LOM ablation could significantly decrease the incidence of ventricular arrhythmias and increase the ventricular tachycardia susceptibility. LOM may act as a conduit between left stellate ganglion and ventricle as well as a target for treating Cs-induced LQTS.

#### GW25-e2116

##### The effects of local cardiac sympathetic ablation on ventricular electrical instability after acute myocardial infarction

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**Objectives:** Increased sympathetic activation after acute myocardial infarction (AMI) is thought to contribute to the potential for fatal arrhythmia. However, whether local cardiac sympathetic ablation can reduce ventricular arrhythmias (VA) is not known. The aim of this study was to investigate the impact of the coronary sinus (CS) and great cardiac vein peripheral nerve ablation on ventricular electrical instability in acute myocardial infarction dogs.

**Methods:** Twenty one anesthetized dogs were randomly divided into sham group ( $n=5$ ) or MI group (MI-placebo group  $n=8$ ; MI-ablation group  $n=8$ ). The dogs were ligated with left anterior descending branch for MI induction. The incidence and duration of VA were monitored for 1h in 1h after ablation. The ventricular effective refractory period (VERP) and spatial distribution of refractory periods were measured before creating MI and in 2 h after ablation. Subsequently the ventricular fibrillation threshold (VFT) was determined in the dogs without spontaneous VF. Finally, the tissues from around CS and great cardiac vein were removed and indicating nerve by immunohistochemical staining of tyrosine hydroxylase (TH). Norepinephrine levels in Coronary Sinus and ventricle tissues Stores as well as infarct size were also detected during the experiment. The other groups were the same as the MI-ablation group.

**Results:** The incidence and duration of VA in MI-ablation group were significantly lower than that MI-placebo group ( $P < 0.05$ ), two dogs in only MI-placebo group died of spontaneous VF. Before creating MI, there were no statistical differences in the VERP and spatial distribution of refractory periods between the three groups, whereas the ventricular ERP of infarct size were prolonged obviously and spatial distribution of refractory periods were decreased when compared with MI-placebo group in 2 h after ablation ( $P < 0.05$ ), VFT also showed an increased trend ( $P > 0.05$ ). During the experiment, NE levels from CS