cardiac remodeling process. Recently we found that there is the signaling pathway of drug-induced long QT interval-regulated kinase 1A (Dyrk1A) modulating the alternative splicing of CaMK II through alternative splicing factor (ASF). Here we aimed to investigate the possible involvement of CaMK II-dependent signaling in renovascular hypertension-induced cardiac hypertrophy, and further explore the complex mechanisms by which valsartan, an angiotensin receptor blocker, inhibited the cardiac hypertrophy.

Methods: Renovascular hypertension was induced by two-kidney one-clip (2KIC). Dyrk1A and ASF protein expression were measured by western blotting, and real-time PCR was used to determine the alternative splicing of CaMK II.

Results: After two-kidney one-clip (2KIC), rats were treated with valsartan (30 mg/kg per day) for 8 weeks; hypertrophic parameter analysis showed that valsartan attenuated cardiac hypertrophy in renovascular hypertensive rats. Western blot analysis showed that valsartan significantly attenuated 2KIC-induced increase in the truncated expression of Dyrk1A, while significantly reversed 2KIC-induced nuclear speckle translocation of ASF in renovascular hypertensive rats. RT-PCR demonstrated that valsartan adjusted 2KIC-induced imbalance in alternative splicing of CaMK II by up-regulating the mRNA expression of CaMK IIIC and down-regulating the mRNA expression of CaMK IIb and CaMK IIa.

Conclusions: The results suggested that valsartan inhibition of cardiac hypertrophy in renovascular hypertensive rats might be at least partly mediated by Dyrk1A/ASF-regulated alternative splicing of CaMK II.

GW25-e2405
Myricetin Inhibits Kv1.5 Channel Expressed in HEK 293 Cells
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Objectives: Myricetin (Myr) is a flavonoid. The previous studies reported that Myr had antiarrhythmic effect. The potential ionic mechanisms are, however, not understood. The present study was designed to investigate the effect of Myr on Kv1.5 channel expressed in HEK 293 cells using a whole-cell patch clamp voltage-clamp technique and western blotting.

Methods: We recorded the current of Kv1.5, expressed in HEK 293 cells with a whole-cell patch voltage-clamp technique and western blotting.

Results: Myr reduced Iqr from 211.0407±48.37848 (pA/pF) to 57.0543±71.75726 (pA/pF) (n=9, P=0.013<0.05). Control condition showed no difference in the current of Myr from 5 min to 20 min. Iqr was 897.20239±67.41077, 906.78865±72.96036, 860.39422±72.42424 and 828.33045±7.5913 at 0.5, 1, 3, 4h, respectively. Moreover, Myr inhibited h Kv1.5 protein in a dose-dependent manner.

Conclusions: We have demonstrated that Myr inhibited Iqr and its protein in hKv1.5, expressed in HEK 293 cell, which was dose-, use- and frequency-dependent. In addition, which may partly explains the mechanism of Myr lowering heart rate.

GW25-e2438
Increased susceptibility of hERG channels to d-sotalol hydrochloride due to a compound mutation L539fs/47-hERG
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Objectives: The purpose of this study was to explore molecular mechanisms underlying the d-sotalol hydrochloride-induced QT prolongation associated with a compound mutation L539fs/47-hERG.

Methods: The L539fs/47-hERG plasmids were transfected into the HEK293 cells stably expressing WT-hERG channels to simulate heterozygous mutant (WT+L539fs/47). We found that treatment with 10-7 to 10-5 mol/L G1 (agonist of the GPER) not only resulted with a significant inhibition of collagen deposition, but also enhanced production of new elastic fibers. Knockdown of GPER using short hairpin RNAs (shRNAs) significantly reduced this effect of G1. Interestingly, we further demonstrated that the pro-elasticogenic effect occurs after the selective activation of GPER subsequent initiates the downstream PKA/CaMKII phosphorylation pathway. PDKA knockdown using shRNAs significantly blocked CREB subunit phosphorylation at Ser-133 and abolished the GPER-mediated expression of elastin. We also demonstrated that G1 induces phosphorylation of CREB contributed to suppression of collagen I synthesis by phosph-CREB-mediated competition for the transcriptional activator CBP1 to Smad transcriptional complexes contributing to collagen I gene transcription.

Results: In summary, our data validate a novel mechanisms in which both anti-collagenogenic and pro-elasticogenic effects occurs after the selective activation of GPER further initiated the PKA/CaMKII pathway, induces a crucial balance between collagenous and elastic fibers that would allow for the best possible resiliency of the post-infarct scars and the optimal cardiac function.

GW25-e2521
Apoe Knockout Mice of Different Weeks of Atherosclerosis Progression of Drug-induced LQTS and TdP-related Symptoms
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Objectives: The Apoe knockout mice progression of atherosclerosis in different time points, to explore different diet of Apoe knockout mice to progression of atherosclerosis.

Methods: 8 weeks of age Apoe gene defects in mice, only 20 is given only to a high-fat diet group, respectively from 8, 12, 16, 20, 24-week-old anesthesia executed 5 points each time 4 lipid and the pathological examination, determination of serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL-c), low density lipoprotein (LDL-c) content, frozen section method line sis icd drug, red oil O staining evaluation atherosclerosis, elastic aorta plaque formation.

Results: 16 weeks of Apoe knockout mice on a high-fat diet group in serum triglyceride levels: 2.20±0.47 mmol/L (P<0.05), total cholesterol: 17.9±1.78 mmol/L (P<0.01) and low density lipoprotein level: 4.32±0.89 mmol/L (P<0.01), blood lipid level three group was obviously higher than that of normal mice, high-density lipoprotein cholesterol (HDL-c) levels in high-fat diet group was obviously lower than normal mice group, the tendency for 0.17±0.65 mmol/L (P<0.01), Apoe knockout mice of atherosclerotic plaque area (29.20±30.16 mm) was significantly higher than the normal mice (3.61±1.41 mm) (P<0.01), along with the age growth and associated with significant difference. With the time length progression of given different diet, Apoe knockout mice gradually aggravate the extent of atherosclerosis, a high-fat diet group than normal diet group of Apoe knockout mice serious pathological changes of atherosclerosis.

GW25-e6356
Lack of non-synonymous mutation in Orai genes of patients with atrial fibrillation
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Objectives: We have demonstrated that Myr inhibit hERG channel protein expression on the cell membrane and resulted in retention in the endoplasmic reticulum. However, the retention and retention was much more serious in the cells expressing heterozygous L539fs/47-hERG compared to the cells expressing WT-hERG. The compound mutation L539fs/47-hERG obviously enhanced the susceptibility of hERG channels to d-sotalol hydrochloride. This may explain the drug-induced LQTS and TdP related symptoms during the administration of sotalol.

GW25-e2511
The activation of the G-protein-coupled estrogen receptor subsequently triggers the PKA/CaMKII phosphorylation pathway causes decline in collagen deposition and parallel stimulation of elastogenesis in cultures of human cardiac fibroblasts
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Objectives: It has been previously reported that the activation of G-protein coupled estrogen receptor (GPER) can alleviate the maladaptive ventricular hypertrophy and arrhythmia that develops in mice after experimental cardiac infarction.

Methods: Our present studies, performed on cultures of human cardiac fibroblasts investigated whether such beneficial effects of this receptor would be exercised through the mechanisms interfering with deposition of major components of extracellular matrix: collagen and elastin.

Results: We found that treatment with 10-7 to 10-5 mOL/L G1 (agonist of the GPER) not only resulted with a significant inhibition of collagen deposition, but also enhanced production of new elastic fibers. Knockdown of GPER using short hairpin RNAs (shRNAs) significantly reduced this effect of G1. Interestingly, we further demonstrated that the pro-elasticogenic effect occurs after the selective activation of GPER subsequent initiates the downstream PKA/CaMKII phosphorylation pathway. PDKA knockdown using shRNAs significantly blocked CREB subunit phosphorylation at Ser-133 and abolished the GPER-mediated expression of elastin. We also demonstrated that G1 induces phosphorylation of CREB contributed to suppression of collagen I synthesis by phosph-CREB-mediated competition for the transcriptional activator CBP1 to Smad transcriptional complexes contributing to collagen I gene transcription.

Conclusions: In summary, our data validate a novel mechanisms in which both anti-collagenogenic and pro-elasticogenic effects occur after the selective activation of GPER further initiated the PKA/CaMKII pathway, induces a critical balance between collagenous and elastic fibers that would allow for the best possible resiliency of the post-infarct scars and the optimal cardiac function.
Objectives: Orai3 is a store-operated Ca2+ channel specific for mammals. Previous studies found that 2-APB, an agonist of Orai3 channel, can either cause or prevent atrial fibrillation in animals. The aim of this study is to determine whether Orai3 mutation is a pathogenetic factor of atrial fibrillation.

Methods: Genomic DNA was extracted from the peripheral blood of 124 patients with atrial fibrillation. The two exons of Orai3 gene were separately amplified from the genomic DNA and sequenced with corresponding primers. The coding sequences were assembled and aligned with the reference sequence from GenBank. Mutation found in the alignment was confirmed by manual check on the original sequencing chromatograms.

Results: Two of the 124 patients were found to carry heterogenic mutation from C to T at position 711 of the nucleic acid sequence. However, this mutation does not lead to any change on translated amino acid sequence.

Conclusions: Due to the lack of non-synonymous mutation in Orai3 gene, we conclude that Orai3 channel is probably not directly involved in the cardiac action potential. The effect of 2-APB on atrial fibrillation is more likely related to other targets of this drug, such as IP3 receptors.

GW25-c3159
Screening of potassium channel mutations in patients with atrial fibrillation
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Objectives: To identify gene mutations of potassium channels that contributed to the pathogenesis of atrial fibrillation.

Methods: Genomic DNA was extracted from the peripheral blood of 124 patients with atrial fibrillation. The coding regions of genes including KCNQ1 (maspin, KCNE1L (KCNE5), KCNQ2 (MIRP1), KCNE4 (MIRP3), KCNJ2 (Kir2.1) and KCNJ4 (Kir2.3) were amplified from the genomic DNA and sequenced with corresponding primers. The obtained sequences were aligned with reference sequences from GenBank. Mutations found in the alignments were confirmed by manual check on the original sequencing chromatograms.

Results: Mutations leading to changes of amino acids and corresponding mutation rates were found: KCN1E1, S37R (0.8%), S38G (92.7%), D85N (0.8%); KCN1E1L, Y131F (0.16%); KCN2E: none; KCNE4, M109V (0.8%); D196E (91.1%); KCNE2, V93E (0.8%); KCNJ4, none.

Conclusions: Genetic mutations on potassium channels are important pathogenic factors of atrial fibrillation. Among the six genes screened KCN1E1 and KCN1E1 showed highest mutation rates. The electrophysiological functionality of these mutants needs to be examined in the future to understand their impacts to the cardiac action potential.

GW25-c3168
Sodium tanshinoneIA sulfonate improves tachycardia-induced electrical remodeling of canine
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Objectives: To determine the effects of DS-201 on electrical remodeling of canine and cell membrane potassium ion channels.

Methods: Mongol canine were used for preparation of animal models with AF through rapid pacing left atrial appendage, and then the effect of DS-201 on AF was determined by frequency and duration of AF. And K,1.5 protein expression in atrial myocytes was detected with western blotting.

Results: It was showed that DS-201 significantly reduced both the frequency and duration of AF (P<0.05, n=5). The frequency of AF was reduced from 7.2+1.31 to 3.12±1.05, and the duration of AF was lowered from 5.2±2.13 s to 0.89±1.23 s. It is interested that DS-201 did not inhibit K,1.5 protein expression but significantly increased its expression.

Conclusions: DS-201 improves tachycardia-induced electrical remodeling of canine by modulating the low-level expression of K,1.5 in AF.

GW25-c3173
Inhibition of TRPC channels by the cardioprotective drug sodium tanshinone IIA sulfonate
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Objectives: Sodium tanshinone IIA sulfonate (STS) is a water-soluble derivative of tanshinone IIA, the major lipophilic component extracted from the root of Danshen (Salvia Miltiorrhiza). STS is clinically used in the treatment of myocardial infarction, coronary artery disease and other cardiovascular disorders. STS can protect the heart against pathological hypertrophy in laboratory animals. However, the direct molecular targets of STS on cardiomyocytes are still unclear. Here we aim to examine the effect of STS on the activity of TRPC channels, which have been suggested to be important mediators of pathological cardiac hypertrophy.

Methods: Intracellular Ca2+ measurement and patch clamp recordings were performed on HEK293 cells stably transfected with human TRPC4 and TRPC5 cDNA. STS was applied to the extracellular solution to test the drug effect.

Results: We found that STS at micromolar concentrations inhibited TRPC4 and TRPC5 channels. The potency of tanshinone IIA on the inhibition of these channels is much lower than that of STS, suggesting the sulfonation of this compound is important for its channel-inhibitory activity.

Conclusions: The inhibition of TRPC channels by STS found in this study is a novel aspect of the cardioprotective pharmacology of this drug. As STS has been used in patients by injection with safety approval, our results suggest that blockade of TRPC channels is a potentially safe strategy for clinical therapy.

GW25-c3207
β1-adrenoceptor Autoantibodies Induce Repolarization Abnormalities and Increase Susceptibility to Ventricular Arrhythmias in Guinea Pigs
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Objectives: The objective of this study was to investigate whether monoclonal autoantibodies against the second extracellular loop of β1-adrenergic receptor (β1-AR mAb) induce directly ventricular arrhythmias and to clarify the electrophysiologic mechanisms.

Methods: To identify the function of β1-AR mAb, the binding of β1-AR mAb with the β1-adrenergic receptor (β1-AR) on the HCC2 by laser scanning confocal microscopy and the effects of β1-AR mAb on the beat frequency in cultured ventricular myocytes of neonatal rats were observed. A langendorff perfused heart model was used in this study to explore the direct roles of β1-AR mAb in arrhythmias.

Results: Results showed that β1-AR mAb may bind with β1-AR and increase the beat frequency of ventricular myocytes of neonatal rats, which was similar to autoantibody-induced the second extracellular loop of β1-adrenergic receptor (β1-AA) isolated from patients, thus β1-AR mAb might be seen as the tool to stay β1-AR. β1-AR mAb induced ventricular premature contractions, and enhanced the excitability of ventricular fibrillation by decreasing the threshold of ventricular fibrillation (β1-AR mAb group: 9.0±1.5 V; Control group: 11.0±2.1 V, P<0.05, n=5/group) and prolonging the duration of ventricular fibrillation (β1-AR mAb group: 1650.8±155.0 min; Control group: 1000.1±127.1 min, P<0.05, n=5/group); β1-AR mAb increased the susceptibility to ventricular arrhythmias as a result of repolarization abnormalities by reducing corrected QT intervals (0 min: 360.0±11.1 ms; 10 min: 333.0±14.0 ms, P<0.05, n=5/group) and prolonging late phase repolarization of monophasic action (MAPD20, 40, 60, 80, 100, 120, 140, 160 ms) (0 min: 360.0±11.1 ms; 10 min: 333.0±14.0 ms, P<0.05, n=5/group) in isolated guinea pig hearts.

Conclusions: It is concluded that β1-AR mAb could induce directly ventricular arrhythmias attributed to the increase of the susceptibility of ventricular arrhythmias by causing repolarization abnormalities.

GW25-c3301
Cardioprotective Effect of Pinacidil on Rats Heart with Transient Hypoxia and Reperfusion Injury
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Objectives: The aim of this study was to evaluate the cardioprotective effect of pinacidil postconditioning on rat hearts with transient hypoxia and reperfusion.

Methods: An acute myocardial anoxia-reperfusion rat model was created by ligating coronary arteries for 10 min and subsequent reperfusion for 60 min. Twenty-four rats in 4 groups received different treatments: normal hearts as control (N), isolation of coronary arteries for 10 min and subsequent reperfusion (A/R) only (N), pinacidil postconditioning on rat hearts with transient hypoxia and reperfusion (A/R) only (N), pinacidil postconditioning to protect A/R-injured hearts.

Results: The left ventricular systolic pressure and maximum -dp/dt in A/R groups are significantly higher than those in the control group (P<0.01). The left ventricular pressure developed, maximum +dp/dt, and heart rate in the A/R group were slightly decreased. The pinacaidl-postconditioning group has better cardiac function recovery after ischemia/reperfusion than the A/R group (P<0.01). In addition, using the patch-clamp technique, significant differences in the mean open time and conductance value were found in the pinacidil group relative to the A/R group. The expression of apoptosis proteins (Bax, Bcl-2) were increased during A/R, while the Bcl-2 protein expression decreased. A significant difference was found in the pinacidil treatment group relative to the A/R group.

Conclusions: Pinacidil postconditioning can exert cardioprotective effects on A/R-injured rat hearts, which may indicate a potential application of pinacidil postconditioning to protect A/R-injured hearts.