ABSTRACTS

Abstracts for Posters:

P1.

A COMPARATIVE STUDY ON THE ISOLATED PANCREATIC b-CELLS OF OBESE (+*DB*/+*DB*) AND LEAN (+*DB*/+*M*) MICE

Alice LS Au,[1] Rebecca KY Lee,[1] SW Seto,[1] Ingrid MF Wong,[1] SK Kong,[2] Aaron HP Ho,[5] George PH Leung,[3] SW Chan,[4] YW Kwan[1]
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Pancreas plays an important role in glucose homeostasis, insulin secretion and the development of diabetes mellitus (DM). So far, most type 2 DM studies were performed on single pancreatic islets and/or β-cells of normal animals incubated in a specially-designed culture medium that "mimicked" the hyperinsulinemic/hyperglycemic conditions. However, a successful isolation of single, viable β-cells from animal models for human DM research (e.g. obese/diabetic (+*db*/+*db*) mice) has not been reported. In addition, a comparison of the pharmacological responses of single pancreatic β–cells of the obese/diabetic and lean mice is unknown. In this study, age-matched (female; ~6 month-old) non-diabetic (+*db*/+*m*) (~30 g) and diabetic (+*db*/+*db*) (~60 g) mice were used. Islets of Langerhans of both species were successfully isolated and the single viable b-cells were harvested. The basal $[Ca^{2+}]_i$ level and the $[Ca^{2+}]_i$ change in response to ionomycin (2.5 μM) challenge were significantly smaller in the β-cells of +*db*/+*db* mice, compared to +*db*/+*m* mice. Immunofluorescent analysis revealed a lesser degree of insulin staining in the β-cells of +*db*/+*db* mice, compared to +*db*/+*m* mice. An enhanced outward K^+ current amplitude in response to isopimaric acid (10 μM, a BK_{Ca} channel opener) was recorded in the β-cells of +*db*/+*m* mice. In contrast, isopimaric acid did not alter/suppress the K^+ current amplitude in the β-cells of +*db*/+*db* mice. Thus, our results demonstrate that the β-cells of +*db*/+*db* mice respond differently which may have significant clinical implications.

Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

P2.

24-HOUR EFFECTS OF BISOPROLOL/HYDROCHLOROTHIAZIDE COMBINATION COMPARED WITH VALSARTAN IN CHINESE HYPERTENSIVE PATIENTS

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Background and aim: Diuretic-based combination represents an alternative option for first-line antihypertensive therapy. We compared the efficacy of β1-blocker bisoprolol with hydrochlorothiazide (Lodoz) with valsartan on 24-hour ambulatory blood pressure in Chinese patients.

Methods: After a placebo run-in, 23 hypertensive patients were randomized to open-label treatment with once daily Lodoz 2.5 (2.5mg bisoprolol/6.25mg hydrochlorothiazide) or valsartan 80 mg. Dosage was up titrated every 4 weeks until patients reached target blood pressure (BP). Twenty-four-hour ambulatory (A) BP monitoring was conducted at weeks 0, 8 and 16. Twenty-four-hour ABP were analysed by time-structured cosinor which involved the least square fit of a 24-hour cosine curve, estimation of a rhythm-adjusted mean (MESOR), and measures of the extent and timing of predictable change within a day (circadian amplitude and acrophase). BP response rate was defined as the percentage of patients with clinic diastolic (D) BP ≤90mmHg or ≥10mmHg decrease after treatment. Control rate was percentage of patients with DBP ≤90mmHg after treatment.

Results: Both Lodoz and valsartan significantly reduced clinic, 24-hour as well as night-time and day-time BP. Both response and control rates in the Lodoz group were 100% compared to 72.7% ($p>0.05$) and 63.6% ($p=0.02$) in the valsartan group. The Lodoz group showed significantly greater reductions in rhythm-adjusted mean of 24-hour DBP (MESOR-DBP) and daytime DBP than the valsartan group ($p<0.05$). The MESOR-BP was decreased by 20.84/12.99mmHg in Lodoz group as compared with 14.33/7.66mmHg in valsartan group. The Lodoz reduced day-time BP by 17.81/10.64mmHg and night-time BP by 22.95/14.65 mmHg, whilst valsartan lowered day-time BP by 11.24/3.93 and night-time BP by 15.47/10.60 mmHg.

Conclusion: We concluded that the low dose combination of bisoprolol and hydrochlorothiazide was more effective than valsartan for 24-hour blood pressure control in terms of MESOR-DBP and day-time DBP.

ABSTRACTS

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P3.

INTERMEDIATE CONDUCTANCE CA^{2+}-ACTIVATED K^+ CHANNELS IN PORCINE CORONARY ENDOTHELIUM UNDER HYPOXIC EXPOSURE

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Objectives: The importance of endothelial intermediate conductance Ca^{2+}-activated K^+ channels (IK_{Ca}) in endothelial function has been revealed by the involvement of these channels in the function of endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO). Endothelial dysfunction is observed in many pathological conditions, including hypoxic / ischemic states. Little is known about the effect of hypoxia on the activity of IK_{Ca} and therefore, this study was designed to assess whether the electrophysiological property of IK_{Ca} is altered under hypoxic exposure.
Methods: Endothelial cells (ECs) were enzymatically isolated from porcine coronary arteries. Primary cultures of ECs were used for patch-clamp study. Hypoxia (PO_2: 25-40 mmHg, 10 min) was elicited in a sealed chamber bubbling with 95%N_2-5%CO_2. Whole-cell IK_{Ca} currents were compared in ECs with or without exposure to 1-hr hypoxia.
Results: Hypoxic exposure markedly reduced whole-cell K^+ current (21.3± 1.1 pA/pF to 9.8±1.5 pA/pF, at 100 mV, $P<0.05$) and the current activated by IK_{Ca} /SK_{Ca} activator 1-EBIO (from 31.8±3.3 pA/pF to 19.2±2.8 pA/pF, $P<0.05$). The inhibitory effect of IK_{Ca} blocker TRAM-34 on the current was more significant in the normoxic group than that in the hypoxic group. IK_{Ca} current was reduced from 12.2±2.0 pA/pF to 1.9±0.4 pA/pF after hypoxic exposure ($P<0.05$).
Conclusions: Hypoxia reduces the activity of endothelial IK_{Ca}. This may be an important mechanism underlying the endothelial dysfunction under hypoxic / ischemic conditions.
Acknowledgments: This study was supported by Hong Kong RGC grant (CUHK4651/07M) and CUHK direct grants 2041388 & 2041384.

P4.

THE EFFECT OF AGING AND HYPERTENSION ON ENDOTHELIAL-DERIVED HYPERPOLARIZING FACTOR (EDHF)-MEDIATED RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Aging is accompanied by endothelial dysfunction due to the reduced bioavailability of nitric oxide (NO) and endothelium-derived hyperpolarizing factors (EDHF). EDHF-mediated vasodilatation is present in aged or hypertensive animals. However, it appears impaired in animals when both conditions are combined. The present study examined the hypothesis that the release of EDHF is augmented to compensate for a reduced bioavailability of NO during the early stages of endothelial dysfunction, but that its release is reduced as endothelial dysfunction progresses. Endothelium-dependent relaxations to acetylcholine (Ach) were obtained in superior mesenteric arteries isolated from spontaneously hypertensive rats (SHR) at week 15, week 36, week 60 and week 72, using age-matched Wistar Kyoto (WKY) rats as controls. The contribution of EDHF to these relaxations was identified as the residual response in the presence of the cyclooxygenase inhibitor, indomethacin, and the NO synthase inhibitor, L-NAME.
Inhibition of the EDHF signaling cascade alone with a combination of TRAM-34 and UCL 1684 did not affect ACh-induced relaxations in both SHR and WKY rats at all ages. The NO-mediated relaxation was comparable in arteries of both SHR and WKY at all ages. However, in the combined presence of indomethacin and L-NAME, the ACh-mediated relaxation was reduced in arteries of 36 weeks old SHR and abolished at 60 weeks. In arteries of 60 weeks old WKY there was a reduction of the relaxation to acetylcholine. This EDHF-mediated response was not affected in the presence of the gap junction inhibitors, carbenoxolone and GAP-27, but, was inhibited in the presence of the Na^+/K^+-ATPase inhibitor, ouabain. These findings suggest that under control conditions NO can fully account for endothelium-dependent relaxations in mesenteric arteries of SHR and WKY rats. The endothelium releases EDHF to compensate for NO in the presence of L-NAME in young SHR and WKY rats. However, the ability of the endothelium to release EDHF is impaired by aging. In addition, the present results suggest that the Na^+/K^+ pump but not gap junctions, are involved in the actions of EDHF in the mesenteric artery of the rat. The expression and/or activity of these proteins may be reduced by aging, thus leading to an impaired compensatory EDHF-mediated relaxation in the absence of NO.

ABSTRACTS

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P5.

TISSUE-TYPE PLASMINOGEN ACTIVATOR: A POSSIBLE CANDIDATE OF ENDOTHELIUM-DERIVED HYPERPOLARIZATION FACTOR?

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It is well established that endothelial cells release endothelium-derived hyperpolarizing factor (EDHF), which dilates blood vessels. However, the identity of EDHF has not yet been clearly understood. Tissue-type plasminogen activator (t-PA) is a serine enzyme secreted by endothelial cells. It plays a critical role in the regulation of hemostasis by converting plasminogen into plasmin which initiates fibrinolysis and restricts propagation of the clot beyond the site of vascular injury. Besides, t-PA is used in the treatment of acute myocardial infarction because of its fibrinolytic property. Interestingly, both t-PA and EDHF are released from endothelial cells upon the stimulation by bradykinin and substance P, and the release occurs in response to the increase in intracellular calcium concentration. The aim of the present study was to investigate the vasodilatory effect of t-PA and whether this effect could account for the EDHF-dependent vasodilation. The results of tissue bath study showed that exogenously addition of t-PA elicited dilation of porcine coronary arteries in a dose-dependent manner, with an EC_{50} value of 0.008 ug/ml. The vasodilatory effect of t-PA was the same when the endothelial cells were removed, suggesting that t-PA-induced vasodilation was endothelium-independent. However, different from EDHF, the vasodilatory effect of t-PA was not affected by the pretreatment with tetraethylammomiun (a non-selective potassium channel blocker) and iberiotoxin (a large conductance calcium-activated potassium (BK_{Ca}) channel blocker). To study the EDHF response, porcine coronary arteries were pretreated with L-NAME (which blocks nitric oxide synthesis) and indomethacin (which blocks prostacyclin synthesis) before dilation by bradykinin. Our results showed that plasminogen activator inhibitor-1 (PAI-1), an inhibitor of t-PA, did not change the EDHF response. In addition, enzyme-linked immunosorbent assay (ELISA) study revealed that the endogenous release of t-PA from porcine coronary arterial endothelial cells, upon the stimulation by bradykinin and substance P, was in a negligible level and was far below the concentration which could cause vasodilation. We therefore conclude that t-PA can dilate porcine coronary arteries. However, our data do not support the hypothesis that t-PA is a candidate of EDHF.

P6.

INHIBITION OF THE RELAXATION TO CNP BY ENDOTHELIUM-DERIVED NITRIC OXIDE IN THE PORCINE CORONARY ARTERY

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C-type natriuretic peptide (CNP) is produced by endothelial cells and has been proposed as an endothelium-derived hyperpolarizing factor (EDHF). The present experiments were designed to define the mechanism underlying relaxations to CNP in coronary arteries and to determine the role of the endothelial cells in the response. Porcine coronary arteries rings were studied in organ chambers for isometric tension recording. Concentration-relaxation curves to CNP were obtained during contractions to prostaglandin $F_{2\alpha}$ or endothelin-1. The experiments were performed in the absence or presence of the inhibitor of nitric oxide synthase ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), the inhibitor of cyclooxygenase indomethacin, the TP-receptor antagonist S18886 the inhibitor of soluble guanylyl cyclase ODQ, or a cell permeable analog of cyclic GMP (cGMP). In rings with, but not those without endothelium, the relaxation to CNP was potentiated by L-NAME. Incubation with ODQ and cGMP did not affect the response to CNP. Likewise indomethacin and S18886 did not affect the relaxation. These experiments suggest that in porcine coronary arteries endothelium-derived NO reduces the relaxation caused by CNP. This effect of NO is not related to inhibition of soluble guanylyl cyclase or the production of vasoconstrictor prostaglandins.

ABSTRACTS

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P7.

PPARγ AGONIST ROSIGLITAZONE AMELIORATES ENDOTHELIAL DYSFUNCTION IN TYPE II DIABETIC (DB/DB) MICE

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Peroxisome-proliferator-activated receptor (PPAR) agonists have been shown to exert beneficial effects against vascular disorders including hypertension and atherosclerosis. PPARγ activation also modulates glucose and lipid metabolism associated with type II diabetes. The present study investigated whether chronic PPARγ activation by rosiglitazone could ameliorate endothelial dysfunction in an animal model of type II diabetes, db/db mice. db/db mice were treated with rosiglitazone or vehicle for 6 weeks. Plasma glucose was monitored during the treatment. Plasma metabolic parameters including insulin, lipid, and adiponectin were determined. Vascular reactivity in isolated blood vessels was studied in myograph. Protein expressions were detected by Western blotting. Rosiglitazone treatment significantly improved endothelium-dependent relaxations in aortas, renal arteries, and resistant mesenteric arteries of db/db mice. Western blotting results demonstrate a down-regulation of angiotensin type I receptor (AT_1R) and nitrotyrosine. Rosiglitazone also increased the phosphorylation of eNOS at Ser^{1177} and phosphorylation of 5'AMP-activated protein kinase (AMPK) at Thr^{172} without affecting total eNOS or AMPK level. Rosiglitazone significantly improved glucose tolerance, reduced plasma insulin, total cholesterol, and triglyceride level. Moreover, rosiglitazone increased the plasma adiponectin level. 12 hrs treatment of adiponectin in organ culture improved endothelial function in db/db aortas, and increased phosphorylation of eNOS and AMPK. Taken together, the present results show that the vasoprotective effect of rosiglitazone in type II diabetes is mediated through reducing oxidative stress and increasing NO bioavailability, which may be related to adiponectin-AMPK signaling cascade. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme)

P8.

LACTIC-ACID-INDUCED ATP RELEASE FROM RAT SKELETAL MUSCULAR L6 CELLS IS MEDIATED BY CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) CHANNELS

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Background and Objective: We had previously shown that lactic-acid-infusion increased the interstitial ATP concentration of perfused rat skeletal muscle in-vivo, which could be inhibited by $CFTR_{inh-172}$, a specific inhibitor of CFTR. A stable rat skeletal muscular L6 cell line was used in this study to confirm that skeletal muscle cells (rather than nerve or vascular cells) were responsible for the acidosis-induced ATP release; the role of CFTR in mediating the ATP efflux during lactic acid incubation was confirmed using RNA interference (RNA_i) technology.
Methods: L6 cells transfected with or without siRNA for the *Cftr* gene were incubated with 10 μM lactic acid in the culture medium for 3 hours at 37°C. At the end of the incubation period, the cells were collected and blotted for CFTR expression by western blotting technique. HPLC was used to measure the ATP and adenosine concentrations of the collected bathing medium.
Results: CFTR expression was significantly increased in L6 cells incubated with 10 μM lactic acid, compared to the cells left untreated. Both CFTR siRNA(s), which target different sequences of the *Cftr* gene, suppressed CFTR expression in L6 cells. In L6 cells without *Cftr* gene silencing, 10 μM lactic acid incubation significantly increased extracellular ATP and adenosine concentrations to a similar extent; however when the CFTR was suppressed by CFTR siRNA, the lactic-acid-incubation-induced increases in ATP and adenosine outputs were prevented, suggesting that the increased ATP was released from L6 cells through CFTR channels during lactic acidosis, and this ATP was extracellularly converted to adenosine to increase the extracellular adenosine concentration.
Conclusions: These data confirmed that skeletal muscle cells were the source for the increased extracellular ATP during a lactic acid challenge; and this ATP efflux was mediated by CFTR channels.

ABSTRACTS

Abstracts for Posters:

P9.

CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 INVOLVES IN ENDOTHELIUM-DEPENDENT CONTRACTIONS IN THE AORTA OF THE SPONTANEOUSLY HUPERTENSIVE RATS

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Phospholipase A2, a regulatory enzyme found in most mammalian cells, catalyzes membrane phospholipids to arachidonic acid. There are two major cytosolic types of the enzyme, calcium-dependent (cPLA2) and calcium-independent (iPLA2) phospholipase A2. Calcium plays a crucial role in endothelium-dependent contractions. The present study investigated whether or not iPLA2 plays a role in such responses in the aorta of the spontaneously hypertensive rat (SHR). In endothelial cells, iPLA2 was densely distributed. At 10 μM, selective iPLA2 inhibitor, bromoenol lactone (BEL), at the concentration of, abrogated endothelium-dependent contractions induced by both acetylcholine and A23187. At 5 μM, only the contractions induced by acetylcholine were inhibited. Incubation with arachidonic acid methyl ester together with BEL restored the contractions. Same results can be obtained from the measurement of the release of the prostacyclin. Store-operated calcium channel (SOC) was also proved to be involved in the contraction process by using a SOC inhibitor, SKF96365. Thus, iPLA2 plays substantial role in generating endothelial-derived contracting factors and both calcium-dependent and -independent pathways areinvolved in the process.

P10.

EFFECTS OF DEXMEDETOMIDINE IN THE MESENTERIC ARTERY AND THE AORTA OF ENDOTOXIN INDUCED RAT

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Introduction: Dexmedetomidine is an anesthetic agent, with known α_2 adrenergic effects, used both in humans and in animals. The present experiments were designed to compare the vascular effects of dexmedetomidine in rat arteries during normal and septic conditions.
Methods: Ten weeks old male normal Sprague Dawley rats were used. The mesenteric arteries and thoracic aortae were dissected and suspended into 5 ml organ chambers for isometric tension recording. In some experiments, endotoxin from E-coli lipopolysaccharide (O55:B5) was injected 10 mg/kg intraperitoneally 2 or 24 hours before the start of experiments.
Results: In the mesenteric arteries, dexmedetomidine caused concentration-dependent relaxations (with a maximal response averaging 50%). It induced smaller relaxations in the rat aorta (maximal response averaging 10%). At concentrations above 100 nM, the relaxation was reverted to a concentration-dependent contraction in the mesenteric arteries. In the presence of L-NAME (a nitric oxide synthase inhibitor) and after the removal of the endothelium, the drug-induced relaxation was abolished in mesenteric arteries. The secondary contraction was reduced when prazosin (α_1 adrenergic antagonist) was added to the bath solution. After endotoxin administration, the relaxations in mesenteric arteries were reduced to around 20% in both 2 and 24 hours treatment durations. Endotoxin did not affect the magnitude of the secondary contraction, which were still abolished in the presence of prazosin.
Conclusions: The vascular effects of dexmedetomidine depend on the vascular bed studied. The vasodilatation caused by dexmedetomidine is nitric oxide and endothelium-dependent in the rat mesenteric artery. This relaxation can be reduced during sepsis, while α_1 adrenoceptors are responsible for the secondary contraction at higher concentrations of dexmedetomidine.

ABSTRACTS

Abstracts for Posters:

P11.

RENIN INHIBITION IMPROVES ENDOTHELIAL FUNCTION IN SPONTANEOUS HYPERTENSIVE RATS

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Aliskiren is the first orally effective renin inhibitor approved for the treatment of hypertension. The present study aims to investigate whether direct renin inhibition by aliskiren could improve the impaired endothelial function and NO bioavailability in spontaneous hypertensive rats (SHRs). SHRs and Wistar Kyoto rats (WKYs) were treated with vehicle (control) and aliskiren for 8 weeks. Blood pressure was monitored biweekly. Changes in vascular reactivity in isolated aortas and renal arteries were studied in organ bath and myograph. Protein expression of endothelial nitric oxide synthase (eNOS), angiotensin II type 1 receptor (AT_1R) and nitrotyrosine were detected by Western blot analysis. Vascular superoxide production was measured by dihydroethidium (DHE) staining. Blood pressure lowering effect of aliskiren was prominent in SHRs but not in WKYs. Aliskiren treatment improved endothelial function in SHRs by restoring the impaired endothelium-dependent relaxations to acetylcholine and decreasing the exaggerated endothelium-dependent contractions to acetylcholine in the presence of L-NAME. DHE staining and western blot on nitrotyrosine revealed the vascular superoxide anions and peroxynitrite levels were significantly lower in arteries of aliskiren-treated SHRs. The present results also demonstrate that treatment with aliskiren can restore the phosphorylation of eNOS at ser^{1177} without affecting the total eNOS level, as well as decrease the protein expression of AT_1R in SHR arteries. By contrast, aliskiren has minimal effects on WKYs. Taken together, the present study provides novel evidence demonstrating that direct renin inhibition can effectively protect endothelial function in hypertensive rats by augmenting NO bioavailability, which supports the therapeutic benefit of aliskiren in patients with hypertension. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme)

P12.

IMPROVED ENDOTHELIAL FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS STUDY OF ENDOTHELIAL NITRIC OXIDE SYNTHASE ENHANCER

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Objectives: Endothelium-derived nitric oxide (NO) plays a pivotal role in maintaining vascular homeostasis. NO-deficiency has been demonstrated in many cardiovascular disorders, including hypertension. We studied whether AVE3085, a newly developed endothelial nitric oxide synthase (eNOS) transcription enhancer, improved endothelial function in spontaneously hypertensive rats (SHR) under both acute and chronic conditions.
Methods: The isometric force study was performed with rat aortas. Protein expression of eNOS and phosphorylated eNOS (p-eNOS) was determined by Western blot. In chronic study, AVE3085 was administrated by oral gavage once daily for 4 weeks before thoracic aortas were removed. Rats receiving vehicle (5% methylcellulose) daily for 4 weeks served as control. Endothelium-dependent relaxation of rat aortas was induced by acetylcholine (ACh) in phenylephrine-precontraction (n=8).
Results: In the acute study, pretreatment with AVE3085 (10 μM) for 2 hr markedly increased the ACh-induced relaxation in the aorta of SHR (50.2±4.5% vs. 26.9±4.4%, P<0.05). eNOS and p-eNOS protein expression were significantly higher in the SHR treated with AVE3085. Four- week oral feeding of AVE3085 dramatically reduced the blood pressure in the SHR rats (170.0±4.0 vs. 151.8±1.8 mm Hg, P<0.001) with response to ACh augmented (50.1±3.4% vs. 19.7±7.0%, P<0.05) in the aorta. Compared with SHR without AVE3085 treatment, removal of endothelium and L-NAME pretreatment both enhanced phenylephrine-induced contraction in AVE-treated animals (P<0.05).
Conclusions: The present study demonstrated that AVE3085 improved the NO-related endothelial dysfunction in SHR and the functional improvement is associated with the upregulation of eNOS gene expression.
Acknowledgements: This study was supported by Hong Kong RGC grant (CUHK 4651/07M) and DUHK direct grants 2041388 &2041384.

ABSTRACTS

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P13.

BONE MORPHOGENIC PROTEIN 4 INDUCES ENDOTHELIAL CELL APOPTOSIS

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Expression of bone morphogenic protein 4 (BMP4) can be induced by disturbed flow and oxidative condition in endothelial cells. BMP4 stimulates the production of reactive oxygen species (ROS) and causes endothelial cell dysfunction, leading to some inflammatory diseases, such as atherosclerosis. However, the molecular mechanism of BMP4-induced endothelial cell apoptosis was not fully understood. In this study, we investigated the signaling pathway of BMP4-induced apoptosis in primary cultured rat aortic endothelial cells (RAECs). Flow cytometry and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were used to detect apoptosis. Protein expressions of caspase-3 and pro-caspase-3 were detected by Western blot. The production of superoxide anions was detected by dihydroethidium (DHE) staining. BMP4 induced a time- and concentration-dependent endothelial cell apoptosis through the release of intracellular superoxide anions and activation of caspase-3. SB 202190 (p38 MAPK inhibitor), SP 600125 (JNK inhibitor) but not PD 98059 (ERK inhibitor) inhibit the increase in expression of caspase-3 which demonstrates the involvement of p38 MAPK and JNK signaling in BMP4-induced apoptosis. In conclusion, the present study demonstrates that release of superoxide induced by BMP4, a novel proinflammatory factor, in RAECs may trigger p38 MAPK and JNK signalings which then activate caspase-3 and cause endothelial cell apoptosis. (Supported by GRF and CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme).

P14.

MULTIPLE ION CHANNEL BLOCK OF CHLOROFORM IS INVOLVED IN ITS ARRHYTHMOGENIC EFFECT

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Objective: Although chloroform is no longer used in clinic as an anesthetic due to its acute intoxication leading to lethal arrhythmias as well as depression of the central nervous system, it is still widely used in industrial production as organic solvent, and involved in the cases of suicide and homicide. The present study was designed to investigate the electrophysiological basis of the arrhythmogenic effect of chloroform.
Methods: Whole-cell patch clamp technique was employed to study effects of chloroform on inward rectifier K^+ channel (Kir2.1), human cardiac ether-a-go-go related (hERG) K^+ gene, Nav1.5, or pacemaker gene (HCN2) stably expressed in HEK 239 cells. Isolated rat heart was also used in this study.
Results: Chloroform showed obvious arrhythmogenic effect in isolated rat heats at a concentration of 10 mM. Although it (10 mM) had no effect on human cardiac Kir2.1 channels, chloroform inhibited the pacemaker HCN2 channel and human cardiac I_{Kr} (i.e. hERG) channels in a concentration-dependent manner, with IC_{50} of 4.57 μM and 4.29 μM respectively. The inhibition of hERG channel was recovered to 78.3% on washout. In addition, chloroform suppressed human cardiac Nav1.5 currents to 75.5%, 52.4%, and 17.2% of control at 5, 10 and 15 mM, respectively.
Conclusion: These results demonstrate that chloroform blocks multiple cardiac ion channels, I_{Kr}, I_{Na}, and the pacemaker HCN2, which likely account at least in part for the chloroform-induced lethal arrhythmias. These findings may be helpful in seeking effective treatment of acute chloroform intoxication.

P15.

PROTECTIVE EFFECT OF APIGENIN ON NEURONS AGAINST CEREBRAL ISCHEMIA/REPERFUSION INJURY VIA REGULATING THE ACTIVITY OF ATPASE

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Objectives: To determine whether apigenin, the main component of Flos Chrysanthemi, protects neurons against cerebral ischemia/reperfusion injury and its underlying mechanism.
Methods: Primary cultured hippocampal neurons were prepared from newborn Sprague-Dawley rats, and the model of oxygen-glucose deprivation/reperfusion (2 h/24 h) was used. Neuronal viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and cell injury was evaluated by lactate dehydrogenase (LDH) leakage rate. The percentage of apoptotic cells was measured by using Hoechst 33258 staining. Adult male Sprague-Dawley rats were subjected to four-vessel-occlusion for 10 min followed by reperfusion for 24 h, and the activities of Na^+, K^+-ATPase and Ca^{2+}, Mg^{2+}-ATPase were measured by spectrophotometry.
Results: Oxygen-glucose deprivation/reperfusion decreased the cell viability and increased LDH leakage rate and percentage of apoptotic cells. Compared with oxygen-glucose deprivation/reperfusion group, apigenin (1-100 μmol/L) treatment significantly increased the cell viability, decreased LDH leakage rate and the percentage of apoptotic cells in a dose-dependent manner. Apigenin (200 $mg \cdot Pkg^{-1}$, *i.p.*) markedly inhibited the decrease of ATPase activities induced by global cerebral ischemia/reperfusion.
Conclusion: Apigenin protects neurons against ischemia/reperfusion-induced cell injury via, at least partly, regulating the activity of ATPase.

ABSTRACTS

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P16.

INVOLVEMENT OF ION CHANNELS IN PROLIFERATION OF HUMAN CARDIAC FIBROBLASTS

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Objective: Cardiac fibroblasts play a central role in the maintenance of extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodeling in the injured and failing heart. Excessive fibroblast proliferation and increase in the extra-cellular matrix will increase myocardial stiffness and cause ventricular dysfunction and subsequent heart failure. Our previous study demonstrated that four types of ionic currents, I_{KDR} (voltage-gated delayed rectifier K^+ current), I_{KCa} (big conductance Ca^{2+} -activated K^+ current), $I_{Cl.vol}$ (volume-activated chloride current), and I_{Na} were present in cultured human cardiac fibroblasts. Little is known about the functional involvement of these ion channels in cardiac fibroblasts, and the present study was therefore designed to examine the possible involvement of I_{KDR}, I_{KCa}, $I_{Cl.vol}$, and I_{Na} in proliferation of human cardiac fibrioblasts.
Methods and results: Using MTT assay, we found that cell proliferation of human cardiac fibroblasts was remarkably suppressed the I_{KDR} blocker 4-aminopyridine (3.0 mM, by 27.2%, $P<0.05$ vs vehicle control), the specific big conductance I_{KCa} blocker paxilline at 1 and 3 μM (by 12.0% and 58.4%, $P<0.05$ vs control), and the volume-regulated chloride channel blocker NPPB (200 μM, by 12.1%, $P<0.01$) or DIDS (200 μM, by 25.9%, $P<0.01$ vs control) with 48 h incubation. However, sodium channel blocker, TTX (1 and 10 μM), had no significant effect on proliferation of human cardiac fibroblasts.
Conclusion: These results demonstrate that IKDR, big conductance I_{KCa} and $I_{Cl.vol}$, but not I_{Na}, regulate the proliferation of human cardiac fibroblasts. The further study will be performed using specific siRNAs targeting to specific ion channel genes to investigate how these channels modulate cell cycle progression and whether proliferation-related kinases are affected by these interventions.

P17.

ROLE OF MONOAMINE OXIDASES IN THE EXAGGERATED 5-HT-INDUCED TENSION DEVELOPMENT IN VITRO OF HUMAN PRE-ECLAMPTIC UMBILICAL ARTERY

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We investigated the role(s) of monoamine oxidases (MAOs) on the altered 5-hydroxytryptamine (5-HT)-induced tension development of the isolated umbilical artery of preeclamptic (PE) pregnancy of Chinese women. An enhanced 5-HT-induced tension development of the umbilical artery of PE pregnancy was observed when compared with that of normal pregnancy. The enhanced component of 5-HT-induced tension development was eradicated by clorgyline (a MAO_A inhibitor). Blockade of eNOS (N^{ω}-nitro-L-arginine methyl ester), 5-HT transporter (citalopram) and 5-HT receptor subtypes ($5HT_{2B}$, SB 204741; $5\text{-}HT_{2C}$, RS 102221; $5\text{-}HT_7$, SB 269970) of the umbilical artery of normal pregnancy mimicked the enhanced 5-HT-induced tension development as observed in the PE tissues. In contrast, no apparent changes in 5-HT-induced tension development of the umbilical artery of PE pregnancy were observed with the same pharmacological manipulations. A decreased protein expression levels of MAO_A and eNOS (no iNOS expression was detected), and an increased expression of PTEN were demonstrated in the umbilical artery (endothelium intact) lysate of PE pregnancy, compared to that of the umbilical artery of normal pregnancy. Thus, in the umbilical artery of PE pregnancy, a decrease of MAO_A and eNOS protein expression levels is probably associated with, or responsible for, the exaggerated 5-HT-induced tension development.
Acknowledgements: This project was financially supported by Direct Grants for Research (The Chinese University of Hong Kong) (Reference no.: 2007. 1.079).

ABSTRACTS

Abstracts for Posters:

P18.

EFFECTS OF HIGH GLUCOSE (25 MM) ON HMG COA REDUCTASE AND CAVEOLIN-1 PROTEIN EXPRESSION IN PORCINE ISOLATED PANCREATIC ISLETS

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Recent studies have demonstrated that inhibition of HMG CoA reductase by statins inhibited the glucose-induced insulin release in various animal models, and uses of statins provide beneficial outcomes in diabetic patients. However, the biochemical existence of HMG CoA reductase in the pancreas, and the effects of hyperglycaemia on HMG CoA reductase are unknown. In this study, we hypothesised that glucose levels modulate the expression of HMG CoA reductase and caveolin-1 in porcine isolated pancreatic islets. Fresh pancreatic islets were harvested and incubated in medium supplemented with normal (5 mM) or high (25 mM) glucose for 24 and 48 hr before subjecting to Western blot analysis. Our results demonstrate, for the first time, the expression of HMG CoA reductase in porcine isolated pancreatic islets. Under high glucose (25 mM) conditions (48 hr), a significant increase and decrease of the expression of HMG CoA reductase and caveolin-1, respectively, were observed. In contrast, there was apparent change in the expression of HMG CoA reductase and caveolin-1 of the pancreatic islets incubated under the normal glucose (5 mM) conditions (24 and 48 hr). Thus, our results suggest that hyperglycaemia has significant modulatory effects on HMG CoA reductase expression/functions of porcine pancreatic islets. The physiological significance of HMG CoA reductase in diabetes mellitus remains to be determined.

Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

P19.

SIMVASTATIN SUPPRESSES CYTOKINES-INDUCE INOS EXPRESSION IN PORCINE ISOLATED PANCREATIC ISLETS

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Inflammation is important in the pathophysiology of diabetes mellitus. Inflammatory cytokines such as IL-1β, TNF-α and INF-γ induced iNOS expression and inhibited insulin secretion from the pancreatic islets of various animal models. Statins (HMG CoA reductase inhibitors) possess anti-inflammatory effects but the effects of statins on the inflammatory cytokines-induced iNOS expression of the pancreatic islets are unknown. In this study, we hypothesised that simvastatin (a HMG CoA reductase inhibitor) modulates the cytokines-induced iNOS expression of freshly harvested porcine isolated pancreatic islets. The harvested pancreatic islets were incubated with or without IL-1β (10 ng/ml) and TNF-α (10 ng/ml), alone or in combination, for 24 hr. In some preparations, the islets were pre-incubated with simvastatin (10 μM; 60 min) before the addition of cytokines. The protein expression of iNOS of the pancreatic islets was evaluated and compared. IL-1β and TNF-α, applied alone, failed to induce iNOS protein expression. In contrast, a combination of IL-1β and TNF-α markedly induced iNOS expression which was significantly suppressed by simvastatin. Thus, we have demonstrated that a combination of IL-1β and TNF-α regulated iNOS expression of porcine isolated pancreatic islets which was sensitive to the presence of simvastatin. Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

ABSTRACTS

Abstracts for Posters:

P20.

PROTECTIVE EFFECTS OF RALOXIFENE AND ESTROGEN ON RAT PROSTATE ENDOTHELIAL CELL LINE YPEN-1 DAMAGED BY HYPOXANTHINE-XANTHINE OXIDASE (HXXO) OXYGEN RADICAL DONOR SYSTEM

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Reactive Oxygen Species (ROS) have been traditionally regarded as toxic by-products of aerobic metabolism. ROS, however, also act as intracellular signaling molecules and can mediate phenotypes in vascular endothelial cells, which may be physiological or pathological in nature. Estrogen has been demonstrated to protect different types of cells from apoptosis induced by various substances. Selective estrogen receptor modulators (SERMs) such as raloxifene are compounds that have both estrogen agonistic and estrogen antagonistic properties. They can function in the same way as estrogen in some tissues (e.g. bone) and more like antiestrogen in some other tissues (e.g. breast) and have important clinical applications. This study is to examine the effects of estrogen and raloxifene on the HX-XO (hypoxanthine-xanthine)-induced death of rat prostate endothelial cell line YPEN-1 and the mechanism of its protective effects. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay showed that in the control group, cell survival rate decreased significantly. After DAPI staining, in the estrogen and raloxifene-pretreated group the number of cells with apoptotic morphology decreased significantly. The mechanism of estrogen and raloxifene protection was studied using two-dimensional gel electrophoresis coupled with mass spectrometry. In comparison with controls cells, the differential proteomic analysis of YPEN-1 treated by HX-XO revealed the variation of some proteins such as rab GDP dissociation inhibitor β-2, histone H2B, alcohol dehydrogenase, peroxiredoxin, adenylate kinase isoenzyme, dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, protein disulfide-isomerase A6 and peptidyl-prolyl cis-trans isomerase A. Raloxifene and estrogen were shown to restore the altered proteins to the control level. These proteins induced by estrogen or raloxifene might play an important role in protecting endothelial cells from oxidative stress-induced cellular damage. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme).

P21.

INHIBITORY EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) ON NUCLEOSIDE TRANSPORTERS IN HUMAN AORTIC SMOOTH MUSCLE CELLS

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Objective: It is known that the equilibrative nucleoside transporters (ENTs) play an important role in adenosine functions because they fine-tune the extracellular concentrations of adenosine. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of pain and inflammation. NSAIDs affect vascular functions due to their inhibitory effect on cyclooxygenase, which attenuates the synthesis of vasoactive prostanoids. In the present study, we sought to investigate whether or not NSAIDs could act on ENTs in vascular smooth muscle cells.

Methods: The ENT activity in human aortic smooth muscle cells (HASMCs) was determined by measuring the initial rate of [^{3}H]adenosine uptake. Since ENTs can be subdivided into ENT-1 and ENT-2, the effects of NSAIDs on these specific isoforms will also be studied using PK15NTD/ENT-1 and PK15NTD/ENT-2 cells.

Results: The effects of different NSAIDs (100 μM) on [^{3}H]adenosine uptake were screened. Aspirin and naproxen had no effect on [^{3}H]adenosine uptake. Etodolic, ibuprofen and ketoprofen inhibited [^{3}H]adenosine update by 15% while indomethacin, mefenamic acid and piroxicam inhibited adenosine uptake by 30%. Sulindac inhibited [^{3}H]adenosine uptake by 20% but its active metabolites sulindac sulfide and sulindac sulfonate inhibited [^{3}H]adenosine uptake by 80% and 30%, respectively. Sulindac sulfide inhibited [^{3}H]adenosine uptake in PK15NTD/ENT-1 cells with IC_{50} values of 22.6 ± 3.35 μM and kinetic study revealed that sulindac sulfide was a competitive inhibitor of ENT-1. In contrast, sulindac sulfide did not affect the [3H]adenosine uptake in PK15NTD/ENT-2 cells.

Conclusions: Among the NSAIDs studied, sulindac sulfide appears to be the most potent in inhibiting the ENT-1-mediated adenosine uptake in HASMCs. It suggests that sulindac sulfide may affect the vascular functions through its potential effect on regulating the availability of adenosine in the vicinity of adenosine receptors.

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P22.

EFFECT OF 2',3'-DIDEOXYADENOSINE ON THE RELAXATION OF RAT BASILAR ARTERIES

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Objective: 2',3'-Dideoxyadenosine (ddA) is a nucleoside analogue, which is used as an antiviral drug for HIV-infected patients. At the same time, ddA is an inhibitor of adenylyl cyclase. It has been suggested that ddA reduces vasodilation in rabbit iliac arteries because ddA decreases cAMP level in endothelial cells, which in turn inhibits gap junction and reduces the endothelium-dependent hyperpolarization factor (EDHF) response (Griffith et al., 2004). However, evidence has demonstrated that EDHF responses in other vascular beds are not necessarily linked to gap junction. Therefore, it is hypothesized that ddA may show different effects on different types of blood vessels.
Methods: The effect of ddA on rat basilar arteries was investigated using wire myograph.
Results: Pre-incubation of ddA did not affect the acetylcholine-induced endothelium-dependent relaxation of basilar arteries. In contrast, ddA itself caused relaxation of basilar arteries in a dose-dependent manner (2 x 10-6-2 x 10-4 M). The vasorelaxing effect of ddA was unaffected even the endothelium was removed. In addition, although ddA is an adenosine analogue, the vasorelaxing effect of ddA was not inhibited by ZM 241385 (1 x 10-6 M, an adenosine receptor blocker).
Conclusion: The present findings have demonstrated that the effect of ddA on rat basilar arteries is distinct from that on rabbit iliac arteries. ddA reduces vasodilation in rabbit iliac arteries but exerts vasorelaxing effect on rat basilar artery through an endothelium-independent and adenosine receptor-independent mechanism.
Reference: Griffith TM, Chaytor AT, Edwards DH, Daverio F, McGuigan C. Enhanced inhibition of the EDHF phenomenon by a phenyl methoxyalaninyl phosphoramidate derivative of dideoxyadenosine. Br J Pharmacol 142: 27-30; 2004.

P23.

MOLECULAR MECHANISM AND CHARACTERIZATION OF MATERNALLY INHERITED ESSENTIAL HYPERTENSION

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Objective: We aimed to observe the relationship between the mitochondrial tRNA mutation and the essential hypertension by examining the mutation of four tRNA ($tRNA^{Lys}$, $tRNA^{Ile}$, $tRNA^{Gln}$ and $tRNA^{Met}$). We also wanted to explore the inherited signs and clinical characters of maternally inherited essential hypertension.
Methods: We collected the data of general information, blood routine test, blood biochemical examination and color Doppler echocardiography examination of the subjects. We extracted DNA from subject's white blood cell, and amplificated the target fragment using the special primers. We then purified the PCR products, and then we directly sequenced them. At last, we analysised the sequencing results and blasted it on net. We also made a comparative analysis of the collected data of the essential hypertension subjects who carried tRNA mutation and those who did not carry mutation using the methods of 1:1 case-control study.
Results: (1) From the mutation analysis of mitochondrial DNA of 2,000 essential hypertensive subjects, we totally found 26 mutation sites in 57 subjects, and 22 mutation sites were new. The most frequently occurrence of the mutation site was A4386G in tRNAGln gene, next to this was G4394A in the same tRNA gene. (2) The onset ages of the individuals carrying the mutation were earlier than those who did not bear them, which was not associated with the change of body mass index. (3) tRNA mutations significantly affected serum lipids, blood electrolyte, blood creatinine, blood urea nitrogen and heart structure and function, and different tRNA mutations produced different effections. (4) Most essential hypertensive patients had maternally inherited history, which fulfilled the feature of mitochondrial hereditary.
Conclusion: 1. Mitochondrial tRNA mutations might result in the change of their structure and function, and then damaged the blood metabolism, the balance of the blood electrolyte, the steady-state of the blood cells and the heart structure and function, which were involved in the progress of the essential hypertension. (2) Part of the essential hypertension patients clinically presented the characters of maternal inheritance, which might be associated with the tRNA mutation.

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P24.

UP-REGULATION OF ENDOGENOUS NITRIC OXIDE PRODUCTION IN RAT ADRENAL GLAND IN CHRONIC HYPOXIA

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Background: Adrenal gland is an effector of the sympathetic nervous system, which plays an important role in the cardiovascular response to hypoxia. We have shown the endogenous nitric oxide (NO) produced by NO synthases (NOS) is increased by hypoxia in oxygen-sensitive tissues. Yet, there is a paucity of information on the adrenal NO production under chronically hypoxic (CH) conditions simulating in subjects sojourning to high altitude or patients with chronic cardiopulmonary diseases.
Hypothesis: CH increases the endogenous NO production and the NOS expression in adrenal gland.
Methods: Normoxic (N) and CH rats were exposed to air and 10% O_2 for 7 days, respectively. The level of endogenous NO was measured by electrochemical microsensor placed on the surface of superfused adrenal gland slices. The expression of NOS in adrenal gland was determined by RT-PCR and Western Blot. RESULTS: L-arginine (Arg, 1 mM) increased the adrenal NO level. The Arg-induced NO elevation was significantly more in the CH group than that of the N group. By contrast, the endogenous NO level was decreased by NOS inhibitor L-NMMA (100 μM). The effect of L-NMMA on the endogenous NO production in CH slices was more significant than that of the N group. Moreover, the mRNA and protein expression of NOS in adrenal gland was also increased in the CH group. Comparable results were observed in PC12 cells.
Summary: Chronic hypoxia upregulates the NOS expression and endogenous NO production in rat adrenal glands. The elevated NO production in the adrenal gland may play patho- or physiological roles in the activation of sympathetic-adrenal axis in responding to chronic hypoxia.

P25.

VOLTAGE-DEPENDENT ANION CHANNEL IS INVOLVED IN THE APOPTOSIS OF CELL LINES CARRYING MITOCHONDRIAL DNA A4263G MUTATION

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In this report, we studied the effect of voltage-dependent anion channel (VDAC) contributed to the apoptosis of the cell lines carrying mitochondrial DNA A4263G mutation. We established lymphoblastoid cell lines derived from 3 symptomatic and 1 asymptomatic hypertension individuals in the family carrying A4263G mutation compared with 3 control cell lines. The mitochondrial potential ($\Delta\Psi m$) was detected by flow cytometry and the co-localization of VDAC and Bax was evaluated by confocal laser scanning microscopy. The results showed that the expression of VDAC and Bax of the lymphoblastoid cell lines in individuals carrying mtDNA A4263G mutation increased compared with control group, while the expression of small conductance calcium dependant potassium (sK_{Ca}) had no change. The confocal imaging showed co-localization of VDAC/Bax on the outer membrane of mitochondrial of the cell lines from individuals carrying mtDNA A4263G mutation, while the interaction was not seen on control group. Flow cytometry showed mitochondrial potential of cell lines from individuals carrying mtDNA A4263G mutation decreased 32% compared with control group ($P<0.05$) and this difference was attenuated by Cyclosporin A (CsA, 2μM), a blocker of VDAC. In conclusion, the change of expression of mitochondrial VDAC and subcellular co-localization of VDAC/Bax leads to the significant increase of mitochondrial permeability and apoptosis of cell lines carrying mtDNA A4263G mutations.

ABSTRACTS

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P26.

RELATIONSHIP BETWEEN QRS TIME AND LONG-TERM EFFECT OF VENTRICULAR RESYNCHRONIZATION IN ELDERLY

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Objective: The purpose of the study is to explore the relationship between QRS time and long-tern effect of ventricular resynchronization in the treatment of congestive heart failure in elderly.
Methods: Consecutive elderly patients with congestive heart failure were selected with the criteria of: (1) Sinus rhythm. (2) NYHA 3-4 class. (3) LVEF≤40%. (4) LVED ≥55mm. (5) delta ventricular ejection time≥30ms. (6) moderate mitral insufficiency. (7) refractory to conservative medication. Patients were divided into wide (>120 ms) and narrow (≤120 ms) QRS group according to the QRS time on surface electrocardiogram. The parameters of procedure, pacing and follow-up between two groups were comparable (P<0.05).
Results: Twenty-seven elderly patients (male 21 cases, mean age 67.3± 5.6years) were enrolled. Sixteen and eleven patients were divided into wide and narrow QRS groups respectively (176.8±13.2ms vs116.5±8.6ms). Twenty-seven tri-chamber pacemakers were successfully implanted without mortality and complications. After procedure, QRS time was shortened by 31.2±5.7ms in wide QRS group and prolonged by 11.4±3.2ms in narrow QRS group. Two groups were followed by 15.4±3.8 months with event free. Compared with pre-procedure, both groups had significant improvement in NYHA, LVEF, LVED and delta ventricular ejection time (P<0.05). There were no significant different between two groups in above parameters during follow-up (P>0.05).

	NYHA(class)		LVEF(%)		LVED(mm)		Delta ejection time (ms)	
	Control	F/U	Control	F/U	Control	F/U	Control	F/U
Wide QRS	3.3±1.6	2.5±1.2	31.9±7.2	47.3±8.6	62.8±5.7	47.1±4.9	46.6±8.9	33.6±5.1
Narrow QRS	3.1±1.5	2.6±1.7	34.2±6.5	45.8±7.7	63.2±5.3	46.3±5.2	45.7±6.1	31.4±3.9
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Conclusions: Ventricular resynchronization has long-term improvement effect in elderly patients with congestive heart failure and selected by delta ventricular ejection time. QRS time in surface electrocardiogram has no influence on this long-term effect.

P27.

ANTIARRHYTHMIC EFFECT OF ETHYL ACETATE EXTRACT FROM FLOS CHRYSANTHEMI ON RATS

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Objective: To investigate the effect of ethyl acetate extract from Flos Chrysanthemi (EFC) on experimental arrhythmia induced by ischemia/ reperfusion or aconitine in rats and its underlying mechanisms.
Methods: Arrhythmia model in intact rat was induced by aconitine (30 μg/kg body weight, *i.v.*). In isolated Langendorff-perfused rat hearts, regional ischemia and reperfusion was induced by ligation (for 30 min) and release (for 15 min) of left anterior descending artery. The effect of EFC on ventricular fibrillation threshold (VFT), effective refractory period (ERP), and diastolic excitation threshold (DET) in rat heart was measured. The action potentials of papillary muscle in rat right ventricle were recorded by conventional glass microelectrode technique and four parameters including resting potential (RP), amplitude of action potential (APA), maximal velocity of phase 0 depolarization (V_{max}), and action potential duration at 90% repolarization (APD_{90}) were measured.
Results: We found that (1) EFC significantly decreased the number and duration of ventricular tachycardia (VT), delayed the occurrence of ventricular premature beats (VPB) and VT induced by aconitine. Arrhythmia score of the EFC group was lower than that in aconitine-treated group. (2) EFC markedly prolonged the ERP and increased the VFT in the isolated perfused rat hearts during ischemia and reperfusion, but did not affect the DET. (3) EFC significantly decreased V_{max}, prolonged APD_{90}, but had no effect on RP and APA in papillary muscle from the right ventricle compared with ischemia/ reperfusion group.
Conclusion: EFC antagonizes the arrhythmia induced by aconitine, and decreases the vulnerability of I/R myocardium, which may be mediated by the inhibition of Na^+ influx and Na^+ channel inactivation kinetics and the decrease of K^+ efflux during repolarization, thus increasing myocardial electrophysiological stability.

ABSTRACTS

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P28.

FUNCTIONAL EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID-RELATED CHANNELS IN CHRONICALLY HYPOXIA HUMAN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

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Transient receptor potential vanilloid (TRPV)-related channels are nonselective cation channels pertinent to diverse physiological functions. Multiple TRPV channel subtypes have been identified in different tissues and cloned. The aim of this study was to investigate the role of TRPV channels in hypoxia-induced proliferation of human pulmonary artery smooth muscle cells (PASMCs) and its possible signal pathway. RT-PCR, real-time PCR and Western blot were used to detect the expression of TRPV in human PASMCs. Cell number was determined with a hemocytometer. Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) was measured using a dynamic digital Ca^{2+} imaging system. The mRNA of TRPV1-4 was detected in human PASMCs and chronic hypoxia up-regulated expression levels of the TRPV1 gene and protein. The ability to proliferate, the resting $[Ca^{2+}]_{cyt}$, and CPA-induced capacitative Ca^{2+} entry in human PASMCs were enhanced significantly by chronic hypoxia compared with the control, and these effects were inhibited in a dose-dependent manner by capsazepine, a TRPV1 channel inhibitor. These results suggested that TRPV1 may be a critical pathway or mediator in chronic hypoxia-induced proliferation of human PASMCs.

P29.

A STUDY ON MATERNALLY INHERITED HYPERTENSION AND MITOCHONDRIAL DNA POINT MUTATION A4263G IN A LARGE CHINESE FAMILY

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Objective: To find a novel mitochondrial DNA point mutation A4263G, we characterized clinically and evaluated hereditarily a large Chinese family with the characteristics of maternally inherited hypertension.
Methods: The mitochondrial DNA point mutation A4263G was detected by sequence analysis of mitochondrial DNA from enrolled patients with essential hypertension. Then we collected and did statistic analyses on the clinical data of this family.
Results: All the members with mitochondrial DNA point mutation A4263G were maternal members, a finding consistent with the maternal inherited characteristics. The morbidity of hypertension in the maternal members is up to 53.8%, while that in the nonmaternal members is only 11.8% ($P<0.01$); the onset age of hypertension is tend to be advanced (from 64.3±5.0 y to 23.3± 2.9 y); the levels of blood glucose, total cholesterol and sodium of maternal members were different with those of nonmaternal members ($P<0.05$), while the results of echocardiogram has no difference between two groups. Finally, the blood pressure of maternal members was relevant with age, smoking, height and high salt diet.
Conclusions: By far all findings, including the same mitochondrial DNA point mutation in all maternal individuals and clear pattern of maternal inheritance, suggested mitochondrial DNA point mutation may be associated with hypertension and play an important role in onset of hypertension.

ABSTRACTS

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P30.

ANTIARRHYTHMIC EFFECT OF ATORVASTATIN ON ISCHEMIA/REPERFUSION RATS

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Objective: To investigate the effect of Atorvastatin (Ator), a traditional blood-lipid lowering drugs, on arrhythmia induced by ischemia/reperfusion (I/R) in SD rats and to explore its underlying mechanisms.

Methods: In isolated Langendorff-perfused rat hearts, arrhythmia was produced by regional ischemia and reperfusion via ligation (for 30 min) and release (for 15 min) of left anterior descending artery. The effect of Ator on ventricular fibrillation threshold (VFT), effective refractory period (ERP), and diastolic excitation threshold (DET) in rat heart were measured.

Results: We found that 10 μmol/L Ator significantly decreased the number and duration of ventricular tachycardia (VT), delayed the occurrence of ventricular premature beats (VPB) and VT induced by ischemia/reperfusion. Ator also prolonged the ERP and increased the VFT (P<0.05, 0.01 vs. I/R group) in the isolated perfused rat hearts during ischemia and reperfusion, but did not affect the DET. However, L-NAME cancelled these antiarrhythmic effects induced by Ator.

Discussion: The results suggest that Ator exerts its antiarrhythmic effects against ischemia/reperfusion. This antiarrhythmic mechanism may be related to that Ator could prolong the ERP and increase VFT to enhance the cardiac electrophysiological stability through NO pathway. However, the precise mechanism of NO involving in the antiarrhythmic effect of Ator against ischemia/reperfusion needs to be further explored.

P31.

W-7, A CALMODULIN ANTAGONIST, DIRECTLY BLOCKS HUMAN ETHER A-GO-GO RELATED GENE POTASSIUM CHANNELS STABLY EXPRESSED IN HEK 293 CELLS

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W-7 is a well-known calmodulin antagonist and believed to be a potential anti-arrhythmic agent. The purpose of the present study was to determine whether W-7 would block human ether a-go-go-related gene (hERG or Kv11.1) potassium channel and human Kv1.5 and Kir2.1 channels. Whole-cell patch voltage-clamp technique was used to record membrane currents in HEK 293 cells expressing hERG, hKv1.5, or hKir2.1 gene. We found that W-7 blocked hERG current (I_{hERG}) in a concentration-dependent manner (IC_{50}: 3.5 μM). Blockade of hERG channels showed a closed channel blocking property. Steady-state activation $V_{0.5}$ of hERG channels was negatively shifted by 9.3 mV (from -5.1±1.3 mV of control to -14.4±1.9 mV, n=11, P<0.01), while inactivation V0.5 was negatively shifted by 9.9 mV (from -57.5±2.4 mV of control to -67.4±2.7 mV, n=7, P<0.01) with application of 3 μM W-7. The S6 mutant Y652A, F656V and the pore helix mutant S631A had a reduced channel block by W-7, and IC_{50} was 5.5 μM, 12.0 μM, and 24.6 μM, respectively. In addition, W-7 inhibited human Kv1.5 channel (IC_{50}: 6.5 μM) and human Kir2.1 channel (IC_{50}: 15.1 μM). The block also showed a closed channel blocking property. These results indicate that W-7 is a multiple-channel blocker by binding to the closed channels of hERG, hKv1.5 and Kir 2.1. The concentrations of ion channel blockage are lower than that of calmodulin inhibition; thus, caution should be taken when it is used as a calmodulin inhibitor or a potential anti-arrhythmic agent.

ABSTRACTS

Abstracts for Posters:

P32.

EGFR KINASE REGULATES HUMAN CARDIAC NA(V)1.5 CURRENTS

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Nav1.5 is the pore-forming a-subunit protein of the cardiac sodium channels which plays a pivotal role in the initiation and propagation of the cardiac action potential. It is generally believed that cardiac sodium current (I_{Na}) is regulated by protein phosphorylation. The present study was designed to determine whether protein tyrosine kinases (PTKs) regulate human cardiac Nav1.5 channels stably expressed in HEK 293 cells using a whole-cell patch clamp technique.

It was found that human cardiac INa was enhanced by epidermal growth factor (EGF, 100 ng/ml) or the protein tyrosine phosphatases (PTPs) inhibitor orthovanadate (1 mM). The selective EGFR kinase inhibitor AG556 (5 μM) remarkably inhibited INa amplitude, shifted the inactivation voltage toward negative potentials, and slowed the recovery of INa from inactivation These effects were countered by orthovanadate. However, insulin and the Src-family tyrosine kinase inhibitor PP2 had no significant effect on I_{Na}.

These results suggest that EGFR kinase (but not Src-family kinase) and PTPs regulate human cardiac Nav1.5 channels stably expressed in HEK-293 cells. EGFR kinase positively, while PTPs negatively modulates the channels. Additional experiments are required to confirm tyrosine phosphorylation level of Nav1.5 using immunoprecipitation and Western blot analysis and to find out the tyrosine phosphorylation site(s) of Nav1.5 using site-directed mutagenesis.

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CARDIOPROTECTIVE OF INTERMITTENT HYPOBARIC HYPOXIA AGAINST ISCHEMIA/REPERFUSION INJURY IN RAT HEART

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Abstract: The aim of this study was to investigate the mechanisms of cardioprotection against ischemia/reperfusion-induced injury in rat hearts adapted under intermittent hypobaric (IHH) hypoxia. Adult male SD rats were put into the hypobaric chamber (simulated 5000m) for 6h daily, lasting 42 days. Isolated hearts were subjected to 30 min global ischemia followed by 30 or 120 min reperfusion. Glibenclamide, 5-hydroxydecunoate or pinacidil were administrated before ischemia. Cardiomyocytes $[Ca^{2+}]_i$ were measured using a digital CCD. The activation and translocation of PKC-α, ε, and δ isozymes, Bax and Bcl-2 were examined by Western Blotting; Incidence of apoptosis in cardiomyocytes was determined by TUNEL.

Results: Post-ischemic functional recovery of LVDP and ±dp/dtmax were better in IHH hearts. [Ca2+]i in cardiomyocytes from normoxic hearts gradually increased during ischemia and kept at higher level during reperfusion. However, in cardiomyocytes isolated from IHH hearts, $[Ca^{2+}]_i$ kept at lower level during ischemia and reperfusion. Glibenclamide and 5-hydroxydecunoate respectively abolished this effect. However, they had no effects on nomoxic myocytes. Pinacidil attenuated calcium overloading during ischemia and reperfusion in normoxic myocytes, but had no effect on IHH myocytes. IHH up-regulated the baseline protein expression of particulate fraction of PKC-α, ε, and δ. Ischemia and reperfusion induced the particulate/cytosolic ratios of PKC-α, ε, in IH hearts was higher than those of normoxic hearts, and the particulate/cytosolic ratio of PKC-δ, in IH hearts was higher than that of normoxic hearts during ischemia period. Ischemia/reperfusion-induced apoptosis was significantly reduced in IH group. After ischemia/reperfusion, the expression of Bax in both cytosolic and membrane fractions were decreased, and the expression of Bcl-2 in membrane fraction was upregulated in IHH hearts.

Conclusions: The results indicated that KATP channels and PKC contributed to the cardiaoprotection afforded by IHH against ischemia/reperfusion injury. The elimination of calcium overload might underlie the mechanism of cardioprotection. IHH attenuated ischemia/reperfusion-induced apoptosis via increasing the ratio of Bcl-2/Bax in membrane fraction. (The study was supported by grants: No.30393130; 2006CB504100)