ABSTRACTS

Abstracts for Posters:

P1.

LUTEOLIN REDUCES CARDIAC DYSFUNCTIONS AND MITOCHRONDRIAL OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Aim: To investigate the effects of luteolin on cardiac functions and mitochondrial oxidative stress in streptozotocin (STZ)-induced diabetic rats.
Methods: Male Sprague-Dawley rats were randomly divided into a normal control group, a luteolin control group, a diabetic group, and diabetic groups orally administered with a low dose (10 mg/kg/d) or a high dose of luteolin (100 mg/kg/d) for eight weeks. The body weight, blood glucose, cardiac functions, left ventricular weight, myocardial collagen, and reactive oxygen species (ROS) levels were assayed. The cardiac mitochondrial ROS level, superoxide dismutase (SOD) activity and the mitochondrial swelling were measured.
Results: Treatment with luteolin had no effect on the blood glucose but reduced the losing of body weight in diabetic rats. High dose of luteolin markedly reduced the ratio of ventricular weight and body weight, increased the left ventricular develop pressure, and decreased the left ventricular end diastolic pressure in diabetic rats. The myocardial levels of ROS and collagen, the cardiac mitochondrial ROS level, and the mitochondrial swelling in diabetic rats were all markedly reduced by high dose of luteolin. Furthermore, high dose of luteolin significantly increased the mitochondrial SOD activity in diabetic rat hearts.
Conclusion: Treatment with luteolin for 8 weeks markedly improves the cardiac function, which may be related to reducing mitochondrial oxidative stress and mitochondrial swelling, in diabetic rats.
(This work was supported by grants from the National Natural Science Foundation of Zhejiang Province (Y206179))

P2.

MATERNALLY INHERITED HYPERTENSION IS ASSOCIATED WITH THE MITOCHONDRIAL $tRNA^{Ile}$ A4295G MUTATION IN A CHINESE FAMILY

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Objective: Mutations in mitochondrial DNA have been associated with cardiovascular disease. We report here the clinical, genetic, and molecular characterization of one three-generation Han Chinese family with maternally transmitted hypertension.
Methods: Members of this Chinese family underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. Genomic DNA was isolated from whole blood and the entire mitochondrial gene was amplified by PCR. PCR fragments were purified and subsequently analyzed by direct sequencing analysis.
Results: Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the known hypertension-associated $tRNA^{Ile}$ A4295G mutation and 33 other variants, belonging to the Asian haplogroup D4j. The A4295G mutation, which is extraordinarily conserved from bacteria to human mitochondria, is located at immediately 30 end to the anticodon, corresponding to conventional position 37 of $tRNA^{Ile}$. The occurrence of the A4295G mutation in several genetically unrelated pedigrees affected by cardiovascular disease but the absence of 242 Chinese controls strongly indicates that this mutation is involved in the pathogenesis of cardiovascular disease. Of other variants, the $tRNA^{Glu}$ A14693G and ND1 G11696A mutations were implicated to be associated with other mitochondrial disorders. The A14693G mutation, which is a highly conserved nucleoside at the TwC-loop of $tRNA^{Glu}$, has been implicated to be important for tRNA structure and function. Furthermore, the ND4 G11696A mutation was associated with Leber's hereditary optic neuropathy.
Conclusion: The combination of the A4295G mutation in the $tRNA^{Ile}$ gene with the ND4 G11696A mutation and $tRNA^{Glu}$ A14693G mutation may contribute to the high penetrance of hypertension in this Chinese family.

ABSTRACTS

Abstracts for Posters:

P3.

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

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The mitochondrial voltage-dependent anion channel (VDAC) is increasingly implicated in the control of apoptosis. We have studied the effects the mitochondrial DNA (mtDNA) A4263G $tRNA^{Ile}$ mutation on VDAC expression, localization, and apoptosis. Lymphoblastoid cell lines were derived from 3 symptomatic and 1 asymptomatic members of a family with hypertension associated with the A4263G $tRNA^{Ile}$ mutation as well as from control subjects. Mitochondrial potential ($\Delta\Psi_m$) and apoptosis were measured by flow cytometry; co-localization of VDAC and Bax was evaluated by confocal microscopy. Expression of VDAC and Bax in mtDNA A4263G cell lines was found to be increased compared to controls, while expression of the small conductance calcium-dependant potassium channel (sK_{Ca}) was unchanged. Confocal imaging revealed co-localization of VDAC/Bax on the outer mitochondrial membrane of A4263G cell lines but not from controls. Flow cytometry indicated that the mitochondrial potential was decreased by 32% in A4263G cells versus controls while rates of apoptosis were increased ($P<0.05$). The difference was attenuated by Cyclosporin A (CsA, 2 μM), a blocker of VDAC. We conclude that increased expression of mitochondrial VDAC and subcellular co-localization of VDAC/Bax increases mitochondrial permeability and apoptosis in cell lines carrying the mtDNA $tRNA^{Ile}$ A4263G mutation.

P4.

THE ROLE OF PPAR-GAMMA IN TELMISARTAN-INDUCED INHIBITION OF CONTRACTIONS IN MOUSE MESENTERIC RESISTANCE ARTERIES

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Upon the activation of renin-angiotensin system (RAS), angiotensin II stimulates the angiotensin II type 1 (AT_1) receptor through triggering NAD (P)H oxidase to produce superoxide anions, also referred as reactive oxygen species (ROS) in vascular cells. The elevated level of ROS dramatically reduces the bioavailability of nitric oxide (NO), leading to endothelial dysfunction. Recently, telmisartan has been suggested to possess a partial peroxisome proliferator-activated receptor-γ (PPAR-γ) activity in addition to being a general angiotensin II-receptor blocker (ARB). Since the studies of the role of PPAR-γ in telmisartan have been vastly lacking in blood vessels, the present study is to examine the hypothesis that telmisartan-induced inhibition of agonist-elicited contractions in mouse mesenteric resistance arteries is due to an increase in basal NO level which is mediated via a PPAR-γ-dependent mechanism. The arteries taken from C57 mouse were suspended in myograph for isometric tension measurement. Incubation of telmisartan (0.1-10 μmol/L) for 24 hours reduces 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U46619)-induced contractions in a concentration-dependent manner. Co-treatment of the rings with 300 nmol/L GW9662 (a PPAR-γ antagonist) antagonized the effect of 10 μmol/L telmisartan. The inhibitory effect of telmisartan (10 μmol/L) on contractions was prevented by actinomycin D (10 μmol/L). Both a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester, and a guanylyl cyclase inhibitor 1H [1,2,4] oxadizolo-[4,3-a]quinoxalin-1-one, abolished the telmisartan (10 μmol/L)-induced inhibition of U46619-elicited contractions. The present results suggest that additional endothelial NO production is probably the mechanism that accounts for the telmisartan-induced inhibition of vasoconstriction and the NO production is likely to be PPAR-γ-dependent. (Supported by GRF grants and CUHK LKS Institute of Health Sciences)

ABSTRACTS

Abstracts for Posters:

P5.

DPP4 INHIBITOR SITAGLIPTIN PROTECTS AGAINST ENDOTHELIAL DYSFUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Sitagliptin, a highly selective DPP-4 inhibitor, acts by inhibiting the inactivation and degradation of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), is an effective anti-diabetic drug for the treatment of type 2 diabetes. Little is known about the beneficial effects of sitagliptin against vascular dysfunction associated with hypertension. The present study aimed to investigate whether sitagliptin can protect endothelial function in spontaneously hypertensive rat (SHR). Changes in vascular tone were studied in myograph and the protein expression was detected by Western blotting. The level of nitric oxide was determined by confocal microscopy using DAF-FM fluorescent dye in primary culture of SHR aortic endothelial cells. 12-hr treatment with 10 μM sitagliptin or 10 nM GLP-1 agonist exendin-4 both augmented the acetylcholine-induced endothelium-dependent relaxations in SHR renal arteries and these effects were abrogated by GLP-1 receptor antagonist exendin 9-39. Three-week treatment with sitagliptin led to a significant improvement in endothelium-dependent relaxations in SHR renal arteries. The protective effect of sitagliptin was abolished by exendin 9-39, while exendin 9-39 had no effect on acetylcholine-induced endothelium-dependent relaxations in SHR and WKY renal arteries. Sitaglitapin treatment also increased phosphorylation of eNOS at Ser^{1177} in cultured endothelial cells. Moreover, sitagliptin and exendin-4 enhanced nitric oxide production in primary culture of SHR aortic endothelial cells, which was blocked by exendin 9-39. In conclusion, the novel results suggest that sitagliptin improves endothelial function in SHR by increasing NO bioavailability, which provides functional implications of the GLP-1 signaling pathway in the cardiovascular system. (Supported by GRF and CUHK LKS Institute of Health Sciences)

P6.

PHARMACOLOGICAL INHIBITION OF ADIPOCYTE-FATTY ACID BINDING PROTEIN (A-FABP) IMPROVES ENDOTHELIAL FUNCTION IN MALE APOLIPOPROTEIN E-KNOCKOUT MICE

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Adipocyte-fatty acid binding protein (A-FABP) modulates inflammatory responses in macrophages and may play a role in formation of foam cells and atherosclerotic plaques. A-FABP is markedly upregulated in regenerated porcine coronary arterial endothelial cells. The project were designed to investigate the presence (or not) of A-FABP as well as endothelial function at early stages of atherosclerosis in the aorta of 8, 12 and 18 weeks old male C57 apolipoprotein E-knockout ($ApoE^{-/-}$) mice. The effect was determined by treatment with a selective A-FABP inhibitor, BMS 309403, in 12-weeks old $ApoE^{-/-}$ mice. A-FABP was detected by immunoflorescent staining in the endothelium of the aorta at 12, but not 8 weeks. In myograph experiments, the endothelium-dependent relaxations to acetylcholine and UK14304 (a selective α_2-adrenoceptor agonist) were reduced significantly in the $ApoE^{-/-}$ mice at 8 and 12 weeks on, respectively, compared to those obtained in wild type mice. Relaxations to the calcium ionophore A23187 were diminished significantly only from 18 weeks. Treatment with the A-FABP inhibitor significantly improved the relaxation to acetylcholine and UK14304 but not that to A23187 without affecting the plasma lipid profile. In conclusion, A-FABP was detected in male atherosclerotic-prone $ApoE^{-/-}$ mice since the age of 12 weeks. Endothelial dysfunction was observed as early as at 8 weeks of age and deteriorated until 18 weeks, as judged from the reduced relaxations to acetylcholine, UK14304 and A23187. Endothelial dysfunction can be alleviated by treatment with an A-FABP inhibitor, suggesting that A-FABP may be a novel target for the treatment of endothelial dysfunction.

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P7.

INVOLVEMENT OF CFTR AND KATP CHANNEL IN ACIDOSIS-INDUCED ATP RELEASE FROM L6 CELLS

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Introduction: In our earlier study in dog skeletal muscle, muscle contractions brought about an increase in interstitial adenine nucleotides and a decrease in muscle pH. Depression of the pH of rat soleus or EDL muscle with lactic acid infusion also stimulated ATP release, which could be inhibited by CFTRinh172, an inhibitor of CFTR, or glibenclamide, an inhibitor of both KATP channels and CFTR.
Objectives: In this study, we used RNA interference to selectively silence expression of the channel proteins, in order to confirm whether both CFTR and KATP channels are involved in pH-depression-induced ATP increase.
Methods: Specific siRNAs for CFTR and the KATP channel were transfected into L6 cells, followed by lactic acid treatment for 3 hours. As a control, other L6 cells were transfected with the siRNA for MAPK, a protein unrelated to ATP release. The protein expression of MAPK, CFTR and the KATP channel was determined by Western Blotting, and the effects of pH depression on the accumulation of ATP and adenosine in the culture medium were determined using HPLC.
Results: The protein expression of MAPK, CFTR and KATP channel was decreased significantly after siRNA knock down. Lactic acid treatment significantly increased the accumulation of ATP and adenosine in the medium surrounding the L6 cells in non-transfected cells. Silencing of MAPK did not affect the lactic-acid-induced ATP release, but silencing of either CFTR or the KATP channel abolished the lactic-acid-induced increases in extracellular ATP and adenosine.
Conclusions: Lactic-acid-induced pH depression stimulated ATP release from L6 cells, which may involve both CFTR and the KATP channel.

P8.

PUERARIN PROTECTS AGAINST HIGH GLUCOSE-INDUCED APOPTOSIS BY INHIBITING CALPAIN ACTIVATION IN HUVECS

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Objectives: The aim of this study is to investigate whether puerarin could protect against high glucose-induced apoptosis by suppressing calpain activation in human umbilical vein endothelial cells (HUVECs).
Methods: HUVECs were exposed to normal glucose (5.5 mmol/L) or high glucose (33 mmol/L) for 48 h. Then cell apoptosis and caspase-3 activity were determined. The expression of heme oxygenase-1 (HO-1) mRNA was evaluated by RT-PCR analysis. The activation of calpain and HO activity were also detected.
Results: Compared with the normal glucose group, exposure of HUVECs with high glucose for 48 h resulted in the significant increases in calpain and caspase-3 activity, and apoptosis, which were prevented by co-incubation with puerarin (10^{-6}, 10^{-5}, or 10^{-4} mol/L) in a concentration-dependent manner. HO-1 mRNA expression and HO activity were decreased in HUVECs treated with high glucose for 48 h. Compared with high glucose group, co-incubation HUVECs with puerarin and high glucose induced the increases in HO-1 mRNA expression and HO activity. HO-1 inhibitor protoporphyrin IX zinc (II) abolished the inhibitive effect of puerarin on high glucose-induced calpain and caspase-3 activation, and apoptosis.
Conclusion: The data show that puerarin protects against high glucose-induced endothelial cells apoptosis by a mechanism involving upregulation of HO-1 expression and inhibition of calpain activity.

ABSTRACTS

Abstracts for Posters:

P9.

HIGH CONCENTRATIONS OF EPIGALLOCATECHIN GALLATE INDUCE CONTRACTIONS OF THE RAT AORTA DUE TO PRODUCTION OF REACTIVE OXYGEN SPECIES, ACTIVATION OF CYCLOOXYGENASE, PRODUCTION OF PROSTANOIDS AND STIMULATION OF TP-RECEPTORS

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Objective: Although regular consumption of green tea is believed to be beneficial for the cardiovascular system, a previous study revealed that high concentrations of the green tea catechin epigallocatechin gallate (EGCG) causes contractions of the aorta of spontaneously hypertensive rats (SHR). The present studies were aimed to investigate the mechanisms underlying these EGCG-induced contractions.

Methods & Results: Isometric tension was measured in isolated aortic rings from 36-week-old male SHR. From 10^{-6} to 10^{-4} M EGCG induced concentration-dependent contractions in preparations both with and without endothelium, which were potentiated by L-NAME (inhibitor of endothelial NO synthase) and abolished by indomethacin (inhibitor of cyclooxygenases) or the thromboxane-prostanoid (TP) receptors antagonist S18886. The extracellular antioxidants SOD and catalase and the intracellular antioxidants apocynin, DETCA and deferoxamine, but not tiron partly reduced the response, while the combined treatment with all intracellular antioxidants abolished the contractions. The release of prostanoids end-products [including prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$ and thromboxane B_2] was significantly increased by EGCG as measured by enzyme immunoassay kits. The intracellular reactive oxygen species (ROS) concentration was measured by confocal microscopy. A burst of ROS was observed upon exposure to a 10^{-4} M of EGCG, which was unaffected by indomethacin or S18886, but was attenuated in the presence of antioxidants.

Discussion & Conclusions: In the rat aorta, high concentrations of EGCG induce contractions through the production of ROS, activation of cyclooxygenase, production of prostanoids and stimulation of TP receptors. The production of ROS is the initial step, and can be attributed to pro-oxidant effects of EGCG. This burst of ROS leads to the activation of cyclooxygenases in both the endothelium and the vascular smooth muscle, followed by the production of prostanoids. These prostanoids in turn stimulated TP receptors and finally caused contraction.

P10.

PROTEIN TYROSINE KINASES REGULATE HUMAN CARDIAC Kv4.3 CHANNEL

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Background. The transient outward K^+ current I_{to} (encoded by Kv4.3) plays an important role in the phase 1 rapid repolarization of cardiac action potentials in the heart. Modulation of I_{to} by intracellular signal transduction is not understood. The present study was designed to determine whether hKv4.3 channel (α-subunit of human cardiac I_{to}) is regulated by protein tyrosine kinases (PTKs) in HEK 293 cells stably expressing human Kv4.3 gene using a whole-cell patch clamp technique.

Results. It was found that human cardiac Kv4.3 current amplitude was remarkably inhibited by the broad-spectrum PTK inhibitor genistein (10 μM), and the inhibition was partially antagonized by the protein tyrosine phosphoatases (PTPs) inhibitor orthovandate (1 mM). It is interesting that the selective EGFR (epidermal growth factor receptor) kinase inhibitor AG556 (10 μM) reversibly reduced Kv4.3 current, and the inhibitory effect was almost fully countered by orthovandate. In addition, the Src-family kinase inhibitor PP2 (10 μM) also decreased hKv4.3 current and the effect was partially antagonized by orthoavanadate. Immunoprecipitation and Western blot analysis revealed that tyrosine phosphorylation level of hKv4.3 channel was reduced by genistein, AG556 or PP2. Their reduction of hKv4.3 channel phosphorylation level was reversed by orthovanadate.

Conclusion: These results demonstrate that hKv4.3 channel is regulated by both EGFR kinase and Src-family kinases. EGFR and Src-family kinases favor tyrosine phosphorlation of the channel, and therefore may affect the cardiac electrophysiology.

ABSTRACTS

Abstracts for Posters:

P11.

LARGE-CONDUCTANCE Ca^{2+}-ACTIVATED POTASSIUM AND ETHER-À-GO-GO POTASSIUM CHANNELS REGULATE PROLIFERATION OF HUMAN MESENCHYMAL STEM CELLS

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Background: Bone marrow-derived mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine; however, cellular physiology is not fully understood in human MSCs. The present study was to explore the potential role of the dominant functional ion channels, large-conductance Ca^{2+}-activated potassium (BKCa) channel, ether-à-go-go potassium (hEAG1) channel, and sodium channel, in regulating proliferation of human MSCs using whole-cell patch clamp and cell proliferation assay approaches.
Results: We found that the BKCa channel blocker paxilline (1 μM) almost fully inhibited BKCa current (from 6.76±0.99 pA/pF of control, to 0.02±0.09 pA/pF at +100 mV, n=5, P<0.05) in human MSCs. The hEAG1 channel blocker astemizole (0.5 μM) significantly reduced hEAG1 current from 4.28±1.86 pA/pF to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 μM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole at 0.3, 0.5, and 1 μM decreased cell proliferation to 96.5, 80.5, and 45.8%, respectively. However, the sodium channel blocker tetrodotoxin (1 μM, fully blocked sodium current) had no effect on proliferation in human MSCs. ^{3}H-thymidine incorporation assay demonstrated that both paxilline and astemizole reduced DNA synthesis rate in a concentration-dependent manner. Flowcytometry analysis displayed that inhibition of BKCa channel with 1 μM paxilline or hEAG1 channel with 0.5 μM astemizole accumulated cells at G0/G1 phase (from control 68.9% to 80.5% for paxilline; to 79.2% for astemizole).
Conclusion: Our results demonstrate that BKCa and hEAG1 channels, but not sodium channel, participate in the regulation of cell proliferation by promoting G0/G1 cells into cell cycling progression.

P12.

AMELIORATION OF HYPERGLYCEMIA-INDUCED MITOCHONDRIAL ROS GENERATION OF SINGLE PANCREATIC ISLET β-CELLS OF OBESE/DIABETIC MICE BY CHRONIC N-ACETYL-L-CYSTEINE

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Background: Mitochondria are the principal source of reactive oxygen species (ROS) in pancreatic islets β-cells and impairment of mitochondrial functions is intrinsically related with diabetes mellitus. Hyperglycemia-induced ROS production by mitochondria is an important aspect in β-cell glucose toxicity. However, most previous studies were performed in either normal islets/single β-cells or insulinoma cells which were bathed in high glucose medium which could not mimic the pathophysiological conditions.
Objectives: To compare and measure hyperglycemia-induced mitochondria ROS generation of primary pancreatic islet β-cells of obese/diabetic (+*db*/+*db*) and lean/control (+*db*/+*m*) mice, and the effects (acute and chronic) of N-acetyl-L-cysteine (NAC) on ROS generation.
Methods: Collagenase-dissociated single pancreatic islet β-cells of C57BL/KsJ obese/diabetic (+*db*/+*db*) mice which exhibit phenotypes of the human T2DM were harvested, and the effects (acute, 10 min; chronic, 24 h) of NAC (20 mM) on high glucose-induced mitochondrial ROS generation were evaluated. Mitochondrial ROS levels were estimated by MitoTracker Red (reduced form) (a selective fluorescence probe for mitochondrial ROS measurement) using con-focal microscope.
Results: A trend of, but a non-significant, higher resting/basal ROS level was observed in single pancreatic β-cells of +*db*/+*db* mice compared to +*db*/+*m* mice. High glucose (15 mM) application gradually caused an increase in ROS levels in single pancreatic β-cells of +*db*/+*db* mice whereas no apparent change was observed in +*db*/+*m* mice. Chronic (24 h), but not acute (10 min), treatments with NAC (20 mM) ameliorated high-glucose induced ROS generation in single pancreatic β-cells of +*db*/+*db* mice.
Conclusions: Hyperglycemia elicited mitochondrial ROS generation only in single pancreatic β-cells of +*db*/+*db* mice. Chronic NAC (a well known anti-oxidant) pre-treatment eradicated high glucose-induced ROS generation. Current study is underway to elucidate the underlying mechanism(s) involved in the differential effects of high glucose on ROS generation as well as the identification of the particular mitochondrial ROS generating system.
Acknowledgements: This project was financially supported by GRF Grant (to YWK) (Reference number: 2410565).

ABSTRACTS

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P13.

APIGENIN AMELIORATES VASORELAXATION IN DIABETIC RATS INDUCED BY STREPTOZOTOCIN

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Objective: To explore the effect of apigenin on endothelium-dependent vasorelaxation in isolated rat aortic rings from streptozotocin (STZ)-induced diabetic rats.
Methods: Diabetes was induced in male Sprague-Dawley rats by STZ treatment (60 mg/kg i.p.) and all rats were randomly divided into a normal control group, an apigenin control group, a diabetic group, and diabetic groups orally administered with a low dose (10 mg/kg/d), a medium dose (50 mg/kg/d) or a high dose (100 mg/kg/d) of apigenin for eight weeks. Then the thoracic aorta was rapidly dissected out and the acetylcholine (ACh)-induced endothelium-dependent vasorelaxation and phorbol 12-myristate 13-acetate (PMA)-induced constriction was measured on the organ bath system. The levels of reactive oxygen species (ROS) and the activity of nitric oxide synthase (NOS) were measured in aortas.
Results: The blood glucose was elevated compared to citrate treated control rats (30.2±4.4 mM *vs.* 4.9±0.9 mM, $P<0.01$) and there was an increased aortic generation of ROS (191.5±21.1% of control, $P<0.01$) and a decreased aortic constitutive NOS activity (4.0±0.5 U/mg protein in control group *vs.* 0.7±0.2 U/mg protein in diabetic group, $P<0.01$) in diabetic rats after eight weeks of STZ treatment. Acetylcholine (ACh)-induced relaxation was impaired (E_{max}: 84.3±3.6% in control group *vs.* 46.4±6.0% in diabetic group, $P<0.01$) whereas PMA (1 μM)-induced constriction was increased (E_{max}: 105.9±9.2% of KCl in control group *vs.* 129.3±12.2% of KCl in diabetic group, $P<0.01$) in aortic rings. Treatment with apigenin dose-dependently enhanced vasorelaxation to ACh ($P<0.01$), markedly decreased aortic ROS production ($P<0.01$) and increased NOS activity ($P<0.01$) in diabetic rats. The increase of constriction to PMA was also markedly inhibited by apigenin in diabetic rats ($P<0.05$).
Conclusion: The results indicate that apigenin dose-dependently reverses the decrease of endothelium-dependent vasorelaxation in diabetic rat aortic rings, which may be mediated by reducing PKC activity and ROS production induced by diabetes and maintaining the activity of constitutive NOS.
Acknowledgement: This work was funded by the grant from Zhejiang Provincial Natural Science Foundation of China (Y206179).

P14.

CAROTID PLAQUES AND LEFT VENTRICULAR HYPERTROPHY IN OLDER PATIENTS WITH HYPERTENSION AND DIABETES: ASSOCIATION WITH BLOOD PRESSURE AND GLYCEMIC CONTROL STATUS

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Background and Objective: Hypertension and type 2 diabetes mellitus, which are frequently co-exist, are leading risk factors of coronary heart disease (CHD) and stroke. Both carotid artery plaques and left ventricular (LV) hypertrophy are well-established predictors for the occurrence of cardiovascular events. Our study aimed to investigate the relationship between prevalence of carotid plaque, LV mass and the control status of blood pressure and glycemia in older patients with hypertension and type 2 diabetes.
Methods: From March 2009 to August 2009, a total of 10,468 people aged 60 years or over participated in "Health Promotion Sojourn for Retired Cadres", a program sponsored by the China National Committee on Aging. Before their traveling, all participants undertook a comprehensive health examination which, in addition to a range of procedures commonly covered by a typical annual check-up in China (measurement of blood pressure, height and weight, fasting lipids and glucose, complete blood cell count, urinalysis, kidney, liver, and thyroid function testing, chest X-ray, electrocardiography), also included ultrasonography of the carotid artery and echocardiograph examination. For subjects with a history of diabetes, HbA1c was also measured. Data of 678 participants (512 males and 156 females, mean age 68.3 years) with self-reported hypertension and diabetes but without history of CHD or stroke were analyzed. A carotid plaque was defined as a localized protrusion of the internal part of the vessel wall into the lumen, and LV mass was determined according to the formula introduced by Devereux et al: $0.80x\{1.04x[(\text{septal thickness} + \text{LV internal diameter} + \text{posterior wall thickness})^3 - (\text{LV internal diameter})^3]\}+0.6$ g. Blood pressure control status was categorized into 4 groups: ideal: ≤120mmHg, adequate: 121-140 mmHg; inadequate: 141-160 mmHg, and poor: ≥161 mmHg, according to patient's systolic blood pressure; and glycemic control status was categorized into 3 groups: ideal: <6.5%, adequate: 6.5%-7.5%, and poor: >7.5%, according to the HbA1c level. Differences between groups were tested with ANCOVA. The independent relationship between plaque and blood pressure, HbA1c level and other risk factors was tested by logistic regression analysis. Multiple linear regression analysis was performed to evaluate the impact of risk factors on LV mass.
Results: Among 678 participants, 392 (57.8%) had carotid plaques. Prevalence of carotid plaque increased with poorer hypertension control (23.3% in the ideal group to 72.8% in the poor group, P=0.003) but not glycemic control (P=0.14). Logistic regression showed that systolic blood pressure but not HbA1c level was significantly associated with carotid plaque after adjustments for age, sex, body mass index, duration of smoke, total serum cholesterol and HDL cholesterol. The mean LV mass was 176 ± 44g. Multiple linear regression analysis showed systolic blood pressure as the only variable significantly associated with LV mass.
Conclusion: In older patients with hypertension and diabetes, blood pressure control rather than glycemic control, is associated with carotid artery plaques and LV hypertrophy.

ABSTRACTS

Abstracts for Posters:

P15.

OXIDATIVE STRESS AND LOCAL INFLAMMATION IN RAT ADRENAL MEDULLA IN CHRONIC HYPOXIA

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Background: Chronic hypoxia (CH) leads to cardiopulmonary changes in subjects sojourning at high altitude, and pathophysiological changes in patients with chronic obstructive pulmonary disease. Sympathetic activation of the adrenal medulla plays an important role in the cardiovascular response to hypoxia. Oxidative stress triggered by a variety of stimuli including hypoxia can mediate cellular damages with increased productions of reactive oxygen species and free radicals in local tissues.
Hypothesis: oxidative stress induced by CH, leads to local inflammation and cellular injury in the rat adrenal medulla.
Methods: Normoxic (N) and CH rats were exposed to air and 10% O_2 for 7 days, respectively. The adrenal medulla was harvested for the measurement of markers for oxidative stress, malondialdhyde (MDA) and nitrotyrosine (NTR), and for the histological analysis of macrophages infiltration and TUNEL staining for apoptosis. Also, the expressions of NADPH oxidase subunits $p22^{phox}$ and NOX-4 were examined by RT-PCR.
Results: The MDA level was significantly increased in the CH group, when compared with the Nx control. Image analysis also showed significantly more % adrenal medulla area with positive immunostaining of NTR than that of the Nx group. In addition, macrophage marker ED1-immunoreactivity was remarkable in the CH group, suggesting a local inflammation. Also, there was an increase in apoptotic cells in the adrenal medulla of CH rats. Moreover, the mRNA expression of $p22^{phox}$ was increased in the CH group, suggesting an involvement of NADPH oxidase in the oxidative stress.
Summary: Results support our hypothesis that CH-induced oxidative stress is involved in the local inflammation and apoptosis in the adrenal medulla. The role of the NADPH oxidase in the CH-induced oxidative stress awaits further investigation.

P16.

CHRONIC INTERMITTENT HYPOXIA INDUCES OXIDATIVE STRESS AND DECREASES NO PRODUCTION IN THE CAROTID ARTERY OF RATS

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Obstructive sleep apnea (OSA) syndrome is a risk factor of hypertension and stroke. Chronic intermittent hypoxia (CIH) leads to oxidative stress and tissue injury. We examined the hypothesis that CIH-induced oxidative stress plays a pathophysiological role in the endothelial dysfunction in rat carotid artery. Adult Sprague-Dawley rats were exposed to IH treatment mimicking a severe OSA condition for 14 days. The carotid arteries were harvested for the malondialdehyde assay, PCR and Western-blotting analysis, and the measurement of nitric oxide (NO) with electrochemistry. Levels of malondialdehyde were significantly elevated in the hypoxic group when compared to the normoxic control. Also, the mRNA expressions of NADPH oxidase ($gp91^{phox}$, $p22^{phox}$) were markedly increased in the hypoxic group, indicating an involvement in the CIH-induced oxidative stress. In addition, the protein level of phosphorylated eNOS (ser1177) and the NO levels were notably lowered in the hypoxic group. These results suggest that oxidative stress induced by the CIH treatment deteriorates the endothelial function of the carotid artery with decreased NO bioavailability. These data may be clinically relevant to the increased risk for cerebrovascular disease in OSA patients.

ABSTRACTS

Abstracts for Posters:

P17.

RESPONSE OF TRPC3 CHANNELS TO ACUTE HYPOXIA

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Background & Objectives: Transient receptor potential channels (TRPs) have been recognized as novel players in the regulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that is essential to cell function. The TRPC3 channel is an important family member of TRPs and sensitivity of these channels to reactive oxygen species has been demonstrated in recent studies. However, little has been known regarding the effect of ischemia/hypoxia on these channels. Therefore, in this study, we investigated the response of the TRPC3 channel to acute hypoxia.
Methods: Human embryonic kidney cells (HEK293 cells) were transiently overexpressed with TRPC3 gene and exposed to either normoxia or acute hypoxia (10 min, PO_2<10 mmHg). Patch-clamp study of ionic currents was performed in whole-cell configuration. Protein expression was determined by western blot.
Results: Application of 1-oleyl-2acetyl-sn-glycerol (OAG, 100 μM), the membrane permeable DAG analogue, evoked significant cation current in TRPC3-overexpressing HEK293 cells (3.1±0.4 *vs.* 1.9±0.3 pA/pF, p<0.01) but not in wild-type cells (2.0±0.4 *vs.* 1.9±0.4, p>0.05). Acute exposure to hypoxia for 10 min enhanced the increase of current induced by OAG (5.7±0.9 *vs.* 3.0±0.4 pA/pF in normoxia cells, p<0.05). OAG failed to induce current change with the presence of specific anti-TRPC3 antibody in both normoxia (1.9±0.2 *vs.* 2.1±0.1 pA/pF, p>0.05) or hypoxia-exposed cells (2.0±0.4 *vs.* 2.4±0.4 pA/pF, p>0.05). Protein expression of TRPC3 was not altered by acute hypoxia.
Conclusions: Acute hypoxia enhances the electrophysiological activity of the TRPC3 channel. This may be a mechanism involved in $[Ca^{2+}]_i$ dysregulation under hypoxic / ischemic states.
Acknowledgments: This study was supported by Hong Kong RGC grant (CUHK4651/07M) and CUHK direct grants 2041388 & 2041384.

P18.

NITRIC OXIDE DOES NOT AFFECT THE RELEASE OF ENDOTHELIUM-DERIVED CONTRACTING FACTOR PGF2α IN THE HAMSTER AORTA

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We have recently observed endothelium-dependent contractions in the young hamster aorta treated with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), an inhibitor of nitric oxide (NO) production and identified a likely endothelium-derived contracting factor (EDCF), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). However, it remains unsolved whether the masking effect of endothelium-derived NO on endothelium-dependent contractions is due to its inhibition to the EDCF release which is mediated by COX-2 or NO plays a dominant role counteracting the EDCF-mediated contractions of vascular smooth muscle cells. The present study investigates the interaction between NO and EDCF. Aortic rings from young golden hamsters (aged ~3 months) were suspended between two stainless steel wires in myograph for the measurement of isometric tension. The release of $PGF_{2\alpha}$ was measured by enzyme immunoassay. Endothelium-dependent contractions were elicited by acetylcholine (ACh) only in the presence of L-NAME. Incubation of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 3 μM) also unmasked ACh-induced contractions. Sodium nitroprusside (SNP, 1 μM) inhibited endothelium-dependent contractions which were unmasked by L-NAME but not by ODQ. Co-incubation of L-arginine (1 mM) with L-NAME abolished ACh-induced endothelium-dependent contractions. $PGF_{2\alpha}$ elicited greater contractions in hamster aortas without endothelium or with endothelium treated with L-NAME. SNP attenuated the $PGF_{2\alpha}$-induced contractions. ACh-stimulated release of $PGF_{2\alpha}$ was similar regardless of the presence of L-NAME and SNP. Our results indicate that the presence of NO dose not affect the $PGF_{2\alpha}$ release, but inhibits ACh- or exogenous $PGF_{2\alpha}$-induced contractions, it is thus likely that NO exerts a downstream effect on vascular smooth muscle cells to inhibit endothelium-dependent contractions, instead of a direct inhibition on the COX-2 activity and subsequent $PGF_{2\alpha}$ production.
(Supported by GRF and CUHK LKS Institute of Health Sciences)

ABSTRACTS

Abstracts for Posters:

P19.

PPARδ ACTIVATION PROTECTS ENDOTHELIAL FUNCTION IN DIABETES

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Recent evidence highlights the therapeutic potential of peroxisome proliferator-activated receptor-δ (PPARδ) agonists to increase insulin sensitivity in diabetes. The implication of PPARδ activation in the regulation of cardiovascular function is unclear. The present study investigates whether PPARδ activation can improve endothelial function under hyperglycemic conditions and in type 2 diabetes. Vascular reactivity of mouse aortas was studied in myograph. Protein expressions were detected by Western blotting. Reactive oxygen species (ROS) production was measured by dihydroethidium fluorescence, and nitric oxide (NO) production was quantified by DAF-FM dye using confocal microscopy. GW0742 and GW501516 (1 μM, PPARδ agonists) improved endothelium-dependent relaxations, inhibited endothelium-dependent contraction induced by acetylcholine, and suppressed the cyclooxygenase-2 (COX-2) up-regulation in the aortas of diabetic db/db mouse. PPARδ agonists prevented hyperglycemia-induced impairment of endothelium-dependent relaxation in aortas of wild type mice, but not in PPARδ$^{-/-}$ mice. PPARδ agonists reduced the ROS production induced by hyperglycemia and in db/db mouse aortas. Moreover, PPARδ activation increases eNOS phosphorylation at Ser^{1177} and Akt phosphorylation at Ser^{473}, without modulating the protein expression of total eNOS and Akt. Importantly, PPARδ activation enhanced the NO production in cultured endothelial cells. Taken together, the present study provides novel evidence for the endothelial protective effects of PPARδ activation in diabetes through triggering Akt/eNOS signaling cascade, reducing oxidative stress, and inhibiting COX-2 upregulation. The novel findings of the present investigation provide useful insights into new therapeutic strategies against the development of vascular dysfunction in diabetes.

P20.

CYCLOOXYGENASE-DERIVED PROSTANOIDS MEDIATE ENDOTHELIAL DYSFUNCTION INDUCED BY ADVANCED GLYCATION END PRODUCTS

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Diabetes increases the formation of advanced glycation end products (AGEs) on the vascular wall and AGEs are closely linked to the development and progression of diabetic atherosclerosis. The present study tests the hypothesis AGEs can have a direct effect on blood vessels to reduce endothelium-dependent relaxations through an increase in the production of cyclooxygenase (COX)-derived prostanoids. Aortas from both non-diabetic db/m^{+} and diabetic db/db mice were studied. Mouse aortas were incubated for 24 hours with AGEs and their impact on vascular reactivity was assessed in myograph. AGEs impaired acetylcholine-induced endothelium-dependent relaxations of non-diabetic mouse aortas without affecting sodium nitroprusside-induced relaxations. Treatment with aminoguanidine (100 μM, AGE inhibitors) (1) prevented AGE-induced endothelial dysfunction in non-diabetic mouse aortas; and (2) improved endothelium-dependent relaxations in db/db mouse aortas. AGE-induced effects were abolished by indomethacin and COX-2 inhibitor (NS398, 3 μM) but not COX-1 inhibitor (sc-560, 0.3 μM), suggesting an involvement of COX-derived prostanoids in mediating AGEs-induced damaging effects. S18886 (100 μM, thromboxane-prostanoid receptor antagonist) also abrogated the AGE-induced attenuation in endothelium-dependent relaxations. EIA assay further revealed that AGEs increased $PGF_{2\alpha}$ production in mouse aortas under the stimulation of acetylcholine. Importantly, AL8810 (1 μM, FP receptor antagonist) could prevent AGE-induced effects. Taken together, the present results demonstrate that AGEs impair endothelial function possibly through an increased production of COX-2 derived $PGF_{2\alpha}$. The novel findings of the present investigation may provide useful insights into new therapeutic strategies against diabetic atherosclerosis by targeting COX-2-derived $PGF_{2\alpha}$ and FP receptor. (Supported by GRF and CUHK LKS Institute of Health Sciences)

ABSTRACTS

Abstracts for Posters:

P21.

DIFFERENTIAL EFFECTS OF DPP4 INHIBITOR SITAGLIPTIN IN MODULATING VASCULAR TONE

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Sitagliptin, a newly developed anti-diabetic drug, inhibits the activity of dipeptidyl peptidase-4 (DPP-4) which improves glucose homeostasis in diabetes. Little is known about the effects of sitagliptin in the modulation of vascular function. The present study examined its impact on the vascular tone in several types of blood vessels isolated from male Sprague-Dawley rats. The artery was suspended in organ bath or in myograph for measurement of isometric force. Sitagliptin causes concentration-dependent relaxations in phenylephrine-contracted aortas, carotid, femoral, and renal arteries and the presence of endothelium plays a role in aortas and femoral arteries but not in other arteries. In contrast, sitagliptin produces a contractile effect in coronary arteries, which can be reversed by nifedipine (voltage-sensitive calcium channel blocker) and pinacidil (potassium channel activator). Sitagliptin-induced contraction in coronary artery is dependent on extracellular calcium ions, but is independent of the presence of endothelium. The results suggest that sitagliptin may exert a direct effect on arteries, probably by elevating intracellular free calcium concentration in rat coronary artery smooth muscle while producing the opposite effects in smooth muscle cells of other systemic arteries. The detailed mechanisms are being investigated. The novel results obtained from the present study may help to define the safety profile of sitagliptin in combating against vascular complications in diabetes. (Supported by GRF and CUHK LKS Institute of Health Sciences)

P22.

RELATIONSHIP OF GENETIC VARIANTS IN GENE ENCODING ADRENOMEDULLIN WITH HYPERTENSION AND DYSGLYCAEMIA IN HONG KONG CHINESE

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Introduction: Adrenomedullin (AM) is a vasodilatory peptide that acts directly via cAMP and indirectly via endothelial nitric oxide. It also facilitates the differentiation of pre-adipocytes and affects lipolysis and glucose uptake. Therefore, we investigated the association of common genetic variants in the gene encoding adrenomedullin (*ADM*) with hypertension and dysglycaemia in the Hong Kong Chinese population.
Methods: We genotyped 4 SNPs of *ADM*, rs3814700, rs11042725, rs34354539 and rs4910118, in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2), which has a median follow-up time of 6.4 years. Dysglycaemia includes impaired fasting glucose (≥6.1 mmol/L), impaired glucose tolerance (2h glucose ≥7.8 mmol/L) and diabetes.
Results: The minor T allele of SNP rs4910118 was significantly associated with lower systolic blood pressure (β=-0.057, P=0.0079) and mean arterial pressure (β=-0.054, P=0.014) at baseline after adjusting for covariates, but not at follow-up. However, none of the SNPs was significantly associated with prevalent or incident hypertension. Although dysglycaemia was not significantly associated with any of the SNPs at baseline, the minor A allele of the SNP rs11042725 was significantly associated with the development of dysglycaemia during follow-up (OR=1.30, P=0.018) and dysglycaemia at follow-up (OR=1.24, P=0.0093), after adjusting for covariates.
Conclusion: Our study provides preliminary evidence for a role of the adrenomedullin gene in influencing blood pressure and the development of diabetes.

ABSTRACTS

Abstracts for Posters:

P23.

PHOSPHODIESTERASE INHIBITION AMELIORATES TP RECEPTOR-MEDIATED IMPAIRMENT OF VASORELAXATION INDUCED BY CYCLIC AMP-ELEVATING DILATORS

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Objects: To examine whether stimulation of TP receptors impairs endothelium-independent relaxations to cyclic AMP-elevating agents via increasing the activity of phosphodiesterases (PDE).

Methods: Rat carotid arteries without endothelium were isolated and suspended in myograph for the measurement of changes in isometric tension; the tissue content of cyclic AMP was assayed by enzyme immunoassay kit; and TP receptor was detected in vascular wall by immunohistochemistry.

Results: In phenylephrine-contracted rings, relaxations induced by isoprenaline (receptor-mediated) and forskolin (receptor-independent) were markedly reduced by the presence of U46619; the attenuated relaxations were prevented by acute treatment with S18886, the selective TP receptor antagonist but not by protein kinase C inhibitors. The reduced relaxations were partially restored by IBMX (non-selective PDE inhibitor), cilostazol (PDE3 inhibitor), rolipram (PDE4 inhibitor) or by Y27632 (RhoA/Rho kinase inhibitor), but not by T0156 (PDE5 inhibitor). U46619 diminished isoprenaline- or forskolin-stimulated rise in cyclic AMP and this effect was inhibited by cilostazol or rolipram.

Conclusions: The present results suggest that activation of TP receptors impairs cyclic AMP-dependent vasorelaxations partly via PDE- and RkoA/Rho kinase-dependent mechanisms.

P24.

RELATIONSHIP BETWEEN ABACAVIR AND RISK FACTORS OF CARDIOVASCULAR DISEASES

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The use of abacavir (a nucleoside reverse transcriptase inhibitor), in the treatment of HIV infection, has been shown to increase the risks of stroke and myocardial infraction.[1] The underlying mechanism is hitherto unclear. It is known that thrombosis and endothelial dysfunction are closely related to the development of these cardiovascular disorders. Therefore, we hypothesized that administration of abacavir may result in the damage on endothelial functions or acceleration of thrombotic process.

Sprague-Dawley rats (330-350 g) were treated with abacavir (16 mg/kg/day) for 28 days by gavage. Isometric tensions of basilar artery and mesenteric artery were measured. Messenger RNA and proteins expressions of factors related to endothelial function and inflammation were measured by QPCR and Western blotting, respectively. In addition, the plasma level of CD40L, a platelet-derived factor which is commonly used as a marker of platelet activation, was measured by ELISA kit.

Our results showed that the maximum relaxations of both basilar artery and mesenteric artery by acetylcholine were not different between the control group and abacavir-treated group, though the value of IC_{50} was larger in the control. The data of QPCR and Western blotting showed that there were no significant change in the mRNA and protein levels of eNOS, COX-1, COX-2 and ICAM-1 in aorta after the treatment with abacavir. However, a higher plasma level of CD40L was detected in the abacavir-treated group.

The results of this study suggested that abacavir upregulates the platelet activity, which may increase the chance of thrombosis and result in a higher risk of cardiovascular events.

ABSTRACTS

Abstracts for Posters:

P25.

MELAMINE AND ITS DERIVATIVE CYANURIC ACID IMPAIR RENOVASCULAR FUNCTION AND REDUCE RENAL BLOOD FLOW IN RATS

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The contamination of milk products with melamine in Mainland China caused a widespread public health concern. The present study aims to examine whether ingestion of melamine and cyanuric acid can impair renovascular function and reduce renal blood flow in rats. Melamine (60, 300 or 600 mg/kg/day) and cyanuric acid (150 mg/kg/day) were administered to 5-week-old rats daily. Vascular function of isolated intralobal renal arteries was assessed in myograph. Renal blood flow was examined by functional magnetic resonance imaging (fMRI). Chronic administration of melamine at 600 mg/kg/day to rats for 3 months significantly reduced acetylcholine-induced endothelium-dependent relaxations (EDR) in renal arteries without altering sodium nitroprusside-induced endothelium-independent relaxations. Chronic exposure to melamine also augmented the endothelium-dependent contractions (EDC) in renal arteries. Acute 30-min incubation of thromboxane-prostanoid (TP)-receptor antagonist (S18886, 100 nM) in melamine-treated arteries rescued the impaired EDR and abolished the augmented EDC. By contrast, S18886 did not affect EDR in vehicle-treated arteries. Combined treatment of melamine and cyanuric acid for 5 days led to a significant reduction of renal blood flow as detected by fMRI, while melamine or cyanuric acid alone had no effect. The results from the present study suggest that chronic exposure to high dose of melamine can damage renovascular function, probably through increases in cyclooxygenase-derived prostanoids which act on the TP-receptor to reduce the EDR and cause EDC in renal arteries. The detailed mechanisms underlying renovascular dysfunction caused by melamine and its derivative cyanuric acid are currently under investigation. (Supported by HKSAR Food and Health Bureau Grant)

P26.

ROS DOES NOT CONTRIBUTE TO THE ACUTE DEVELOPMENT OF NITROGLYCERINE TOLERANCE IN RAT AORTAS

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The development of nitrate tolerance limits the clinical efficacy of nitric oxide (NO) donors. Several cellular mechanisms have been proposed to explain nitrate tolerance and the increased production of ROS is one of the possibilities. The present study examined if ROS participated in nitroglycerine tolerance which occurs acutely in aortas isolated from male Sprague-Dawley rats. The aortas were exposed to 30 μM nitroglycerine (with and without pre-incubation of ROS inhibitors) for 90 min and rinsed out four times before phenylephrine was added to cause a steady contraction. Subsequently, nitroglycerine was added cumulatively to the bathing solution to induce relaxations. Nitroglycerine-induced relaxations were severely reduced after 90 min-nitroglycerine exposure. Treatment with apocynin, tempol and tiron plus DETCA did not rescue the impaired relaxations. Angiotensin II, H_2O_2 and hypoxanthine plus xanthine oxidase (which can generate ROS as determined by electron paramagnetic resonance) did not impair nitroglycerine-induced relaxations. The studies on changes in the activity of NAD(P)H oxidase and ROS levels in the vascular wall are being carried out. The preliminary results indicate that ROS may not be involved in the acute occurrence of nitroglycerine tolerance. However, it is still unclear whether chronic treatment with antioxidants can delay the development of nitroglycerine tolerance (supported by CUHK LKS Institute of Health Sciences).

ABSTRACTS

Abstracts for Posters:

P27.

INVOLVEMENT OF PLASMA MEMBRANE MONOAMINE TRANSPORTER IN SEROTONIN UPTAKE IN VASCULAR SMOOTH MUSCLE CELLS

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Serotonin (5HT) is a potent vasoconstrictor. It has been reported that 5HT can be taken up by the rat aortas through the serotonin transporters (SERT). This 5HT uptake mechanism may play a crucial role in fine-tuning the availability of 5HT at its cognate receptors. However, many studies have demonstrated that a significant part of 5HT uptake in blood vessels is insensitive to the blockade by SERT inhibitor fluvoxamine, suggesting that other transport system(s) are also involved in the 5HT uptake in blood vessels. Plasma membrane monoamine transporter (PMAT) is a novel polyspecific organic cation transporter that can transport organic cations such as 5HT. PMAT is strongly expressed in kidney and brain. However, it is hitherto unclear whether PMAT is present in blood vessels.

The aim of this work was to study the role of PMAT in 5HT uptake in vascular cells. Results of RT-PCR demonstrated the presence of mRNA of PMAT in human brain microvascular smooth muscle cells (HBMSMCs) but not in human brain microvascular endothelial cells (HBMECs). The [^{3}H]5HT uptake in HBVSMCs was increased with time and was saturable with a Michaelis-menten constant of 50.36±10.2 mM. This low affinity of 5HT transport was consistent to the characteristics of PMAT. Moreover, 30% of the [^{3}H]5HT uptake in HBMSMCs was inhibited after the PMAT expression is silenced by siRNA. Interestingly, the result of semi-quantitative RT-PCR showed that mRNA expression of PMAT in basilar arteries of spontaneous hypertensive rats is higher than that of normal Wistar Kyoto rats. Taken together, our study suggests that PMAT is present and is involved in the 5HT uptake in the vascular smooth muscle cells. Upregulation of PMAT may be associated with hypertension and it warrants further investigation.

P28.

ACUTE VASCULAR EFFECT OF ALDOSTERONE ON RESISTANCE ARTERIES FROM NORMAL AND HEART FAILURE RATS

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Heart failure is a complex disease which involves numerous genetic, neuroendocrine and environmental factors. Some evidences have emerged in resent years to support the direct role of aldosterone in heart failure, independent of its regulation on blood volume and fluid, electrolyte metabolism. This study was designed to investigate the effect of aldosterone on resistance arteries from normal and heart failure rats. Acute heart failure of rat was induced by coronary artery ligation. Five weeks after the surgery, echocardiography showed the cardiac dysfunction. The hearts were then excised and Masson trichrome staining of cross sections revealed a visible myocardial infarction and fibrosis. Segments of third-order branches of the mesenteric arteries were isolated for isometric tension recording. We found that in normal rats, aldosterone (10^{-9} M - 10^{-7} M) caused further contraction in mesenteric arteries precontracted by phenylephrine (PE, 10^{-6} mol/L), but aldosterone did not cause vasocontraction in the arteries from heart failure rats. In the mesenteric arteries from normal rats, pre-incubation of aldosterone (3×10^{-8} M) for 10 min decreased the contractile response to low concentration of PE (1×10^{-7} - 1×10^{-6} M), but enhanced the contractile response to high concentration of PE (3×10^{-6} - 3×10^{-5} M). This effect was abolished by eplerenone (2×10^{-6} M), an inhibitor of aldosterone receptor. However, in the arteries from heart failure rats, aldosterone (3×10^{-8} M) decreased the contraction induced by PE (1×10^{-7} - 3×10^{-5} M), which was partly blocked by eplerenone (2×10^{-6} M). These results indicate that aldosterone has biphasic effect on contractile response to PE of normal rat arteries, mediated by aldosterone receptor. The effect of aldosterone on heart failure rat arteries is monophasic, reducing the sensitivity to PE, which is partly mediated by aldosterone receptor.

ABSTRACTS

Abstracts for Posters:

P29.

ION CHANNELS AND THEIR ROLE IN CELL PROLIFERATION OF 3T3-L1 PREADIPOCYTES

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Background: Mouse 3T3-L1 peradipocytes are widely used for metabolic study; however, cellular physiology (e.g. functional ion channel expression) is not fully understood. The present study was to investigate ion channel expression and functional role of them in regulating cell proliferation using whole cell patch voltage clamp technique, RT-PCR, Western blot, and cell proliferation assay in undifferentiated 3T3-L1 preadipocytes.

Results: We found that three types of ionic currents were present in 3T3-L1 preadipocytes, including a Ca^{2+}-activated K^+ current (I_{KCa}) in 39% cells, an inwardly-rectifying K^+ current (I_{Kir}) in 15% cells, and a chloride current (I_{Cl}) only in 8% cells under isotonic conditions. Interestingly, I_{Cl} was observed in all cells with hypotonic (0.8T) insult, suggesting that it is a volume-sensitive I_{Cl} ($I_{Cl.vol}$). I_{Kir} was inhibited by Ba^{2+}, and I_{KCa} was inhibited by the intermediate conductance I_{KCa} channel blocker clotrimazole. I_{Cl} was reduced by the chloride channel blockers DIDS. RT-PCR revealed significant expression of mRNAs: KCa3.1 for I_{KCa}, Kir2.1 for I_{Kir}, and Clcn3 for $I_{Cl.vol.}$ Proteins of these channels were detected using Western blot analysis. Proliferation assay demonstrated that blockade of I_{KCa} with clotrimazole or $I_{Cl.vol}$ with DIDS inhibited cell proliferation in a concentration-dependent manner. Flowcytometry analysis showed that clotrimazole (3 μM) and DIDS (200 μM) accumulated the cells at G0/G1 phase (from control 49.91±2.8% to 57.05±3.6% for clotrimazole, $P<0.05$; to 61.08±4.3% for DIDS, $P<0.05$).

Conclusions: These results demonstrate the first information that three types of functional ion channel currents, including intermediate-conductance I_{KCa}, $I_{Cl.vol}$, and I_{Kir}, are heterogeneously present in 3T3-L1 preadipocytes. I_{KCa} and $I_{Cl.vol}$ participate in the regulation of cell proliferation.

P30.

DISTINCT EFFECTS OF SIMVASTATIN ON CYTOSOLIC Ca^{2+} CHANGES AND Ca^{2+}-SENSING RECEPTOR EXPRESSION OF ISOLATED PANCREATIC ISLETS β CELLS OF OBESE/DIABETIC MICE

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Background: Diabetics often have hyperlipidemia as a co-morbidity. In previous clinical and animal studies, statins (HMG CoA reductase inhibitors) provided cholesterol-independent beneficial effects in diabetic patients/animal models. Activation of Ca^{2+}-sensing receptor (CaR) resulted in insulin release in human isolated pancreatic islets.

Objectives: To compare and measure the effects of simvastatin (SIM, a HMG CoA reductase inhibitor) (10 nM, 24 h incubation) on protein expression of CaR and ionomycin- and caffeine-elicited $[Ca^{2+}]_i$ changes of single pancreatic islet β-cells of obese/diabetic (+*db*/+*db*) and lean/control (+*db*/+*m*) mice.

Methods: Collagenase-dissociated single pancreatic islet β-cells of C57BL/KsJ obese/diabetic (+*db*/+*db*) mice (which exhibit phenotypes of human T2DM) were harvested. The protein expression of CaR was evaluated using Western Blot. Ionomycin- and caffeine-induced $[Ca^{2+}]_i$ changes were measured using Fluo-4 fluorescent imaging techniques. Glucose (5 and 15 mM)-induced insulin release was measured (by ELISA).

Results: Protein expression of CaR, but not HMG CoA reductase, was lowered in pancreatic islets of +*db*/+*db* mice (~60% of +*db*/+*m* mice) compared to +*db*/+*m* mice, and it was partially restored by SIM. A relatively small ionomycin (1 μM)- and caffeine (5 mM)-induced $[Ca^{2+}]_i$ change was observed in single pancreatic β cells of +*db*/+*db* mice compared to +*db*/+*m* mice, and it was restored after SIM treatment. An attenuated glucose (5 and 15 mM)-induced insulin release was consistently observed in the pancreatic islets of +*db*/+*db* mice and the suppressed glucose (15 mM)-induced insulin release in pancreatic islets of +*db*/+*db* mice was partially restored by SIM.

Conclusions: The biochemical existence of HMG CoA reductase and CaR in pancreatic islets of +*db*/+*db* and +*db*/+*m* mice was confirmed. The suppressed ionomycin-induced $[Ca^{2+}]$i and glucose-mediated insulin release of pancreatic islets β-cells +*db*/+*db* mice could be restored by SIM suggesting that SIM could be used in treating T2DM.

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ABSTRACTS

Abstracts for Posters:

P31.

MODULATORY EFFECTS OF SIMVASTATIN ON INSULIN RELEASE OF PIG PANCREATIC ISLETS OF LANGERHANS

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Background: Type 2 diabetes mellitus (T2DM) is a metabolic disease and many T2DM patients have hyperglycemia as a result of deficiencies in insulin secretion (β-cells dysfunction). A locus on chromosome 9 is linked to cholesterol levels and DM, and diabetic patients with dyslipidemia are now receiving HMG CoA reductase inhibitors (statins which are mainly for lowering blood cholesterol). However, there is no consensus on whether statins consumption can modulate insulin release in patients and animals with DM**.**

Objectives: To evaluate the effects of simvastatin (SIM, a HMG CoA reductase inhibitor) on insulin release of pig isolated pancreatic islets bathed in glucose medium (5.6 and 25 mM), and the underlying cellular mechanisms involved.

Methods: Fresh pig pancreases were collected from a local slaughterhouse. Pancreas was finely cut into small pieces and isolated pancreatic islets were handpicked under the dissecting microscope. The collected pancreatic islets were cultured in RPMI solution supplemented with glucose (5.6 and 25 mM) with and without SIM (24 h incubation) before they were subjected to different assays (Western blot, and insulin release using ELISA).

Results: The biochemical existence of HMG CoA reductase was confirmed, and only the protein expression of p-HMG CoA reductase was elevated in high glucose medium. Changing glucose from 5.6 to 25 mM resulted in ~5-fold increase in insulin release. Under normal glucose (5.6 mM) condition, SIM (10 μM) treatment (24 h) markedly enhanced (~ 9-fold) glucose-induced insulin release. In contrast, SIM pre-treatment did not modify insulin release from pancreatic islets bathed in high glucose (25 mM) medium.

Conclusions: The biochemical existence of HMG CoA reductase in pig pancreatic islets was confirmed. The expression of the inactivated form of HMG CoA reductase (p-HMG CoA reductase) was elevated under hyperglycemic conditions. SIM pre-treatment only enhanced insulin release from pancreatic islets bathed in normal but not high glucose medium suggesting that SIM may not provide beneficial effects in patients with T2DM.

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P32.

THE FLAVONOID KAEMPFEROL ENHANCES SODIUM NITROPRUSSIDE-INDUCED RELAXATION IN PORCINE CORONARY ARTERIES VIA ACTIVATION OF POTASSIUM CHANNELS

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Kaempferol is a major flavonoid component of the Chinese medicine, Carthamus tinctorius, which has been used to treat cardiovascular diseases. Previous studies demonstrated that kaempferol potentiated sodium nitroprusside (SNP)-induced relaxation at concentrations that did not directly relax porcine coronary arteries. This study aimed to investigate the mechanisms of this vascular action of kaempferol using organ bath technique. In the presence of indomethacin, SNP-induced relaxation was significantly enhanced by kaempferol in porcine coronary arteries with and without endothelium. These potentiations were partially inhibited by iberiotoxin, a big conductance calcium-activated potassium channel (BK_{Ca}) blocker, but was not affected by TRAM-34 or UCL-1684, selective inhibitors of intermediate and small conductance calcium-activated potassium channels (IK_{ca} and SK_{ca}), respectively. Carbenoxolone, a gap junction inhibitor, and KT5720, a protein kinase A inhibitor, also did not affect the potentiation by kaempferol in arteries with or without endothelium. These findings suggest that kaempferol activates BK_{Ca} in vascular smooth muscle to enhance SNP-induced relaxation in porcine coronary arteries, while IK_{ca}, SK_{ca}, protein kinase A and gap junctional proteins do not play a role.