ABSTRACTS

Abstracts for Oral Communications:

OC1.

CONTRACTILE RESPONSE TO HYPOXIA IN THE PORCINE CORONARY ARTERY

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The contractile response to hypoxia has been studied in the lung, and is termed hypoxic pulmonary vasoconstriction. However this response is also observed in coronary arteries. The present study investigated the mechanism underlying this contractile response in isolated porcine coronary arteries. Isometric tension was measured in rings with or without endothelium. In quiescent preparations, the contractile response to hypoxia was only observed in rings with endothelium and was abolished by indomethacin and terutroban, which shows the involvement of cyclooxygenase products and TP receptor activation, respectively, in this phenomenon. In contracted preparations, the hypoxic response was also endothelium-dependent, but was abolished by L-NAME and ODQ, suggesting the involvement of cyclic GMP. Assay of the cyclic GMP content showed no change upon exposure to hypoxia in preparations with and without endothelium. These experiments suggest that these hypoxic coronary contractions depend on more than one signaling pathway.

OC2.

HEIGHTENED SYSTEMIC OXIDATIVE STRESS CRITICALLY ACCELERATES WORSENING ATHEROSCLEROSIS IN THE LATE CARDIOVASCULAR CONTINUUM

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Background: Both increased oxidative and inflammatory stresses are implicated in atherogenesis. However, little is known about their role in atherosclerotic progression in patients already at the advanced cardiovascular continuum.
Objective: To investigate the impact of oxidative and inflammatory stress on the progression of carotid atherosclerosis in patients with established ischemic stroke.
Methods: A total of 43 consecutive patients (mean age 65.7±8.8 years; male 70%) with primary or recurrent ischemic stroke (>6 months) were recruited from our medical outpatient clinics. High resolution ultrasound (Agilent Sonos 5500, Philips, USA) was used to assess burden of carotid atherosclerosis in terms of maximum intima-media thickness (mIMT). Serum malodialdehyde (MDA) and high-sensitivity C-reactive protein (hsCRP) were respectively measured as markers of systemic oxidative and inflammatory stress.
Results: These patients showed a mean mIMT of 2.25±0.98 mm. Serum MDA (Pearson r=0.32, *P=0.035*) and hsCRP (Pearson r=0.41, *P=0.007*) were both positively associated with mIMT. Adjusting for potential confounders by multivariate model (age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking history, use of aspirin/statins/antihypertensives and body-mass index), each 1 μM increase in serum MDA independently predicted increase in mIMT by 0.79 mm (95%CI [0.23-1.36], *P=0.008*). Furthermore, each 1 mg/L increase of hsCRP was independently predictive of increase in mIMT by 0.06 mm (95%CI [0.01-0.12], *P=0.017*). Hyperlipidemia and diabetes accounted for IMT increase by 0.56 mm (95%CI [0.04-1.08], *P=0.037*) and 0.53 mm (95%CI [0.01-1.05], *P=0.046*) respectively.
Conclusions: This study demonstrated that systemic oxidative stress strongly accelerates secondary progression of carotid atherosclerosis in patients with established ischemic stroke, independent of and above all conventional risk factors including systemic inflammation. This suggests that effective reduction of oxidative stress should be a major therapeutic target in patients at the advanced cardiovascular continuum.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

CONTROL OF ADENOSINE FORMATION BY VASCULAR ENDOTHELIAL CELLS

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Introduction: Adenosine is formed extracellularly by ecto-5'-nucleotidase (5'N) during exercise vasodilation. It has been suggested that vascular endothelial cells act as a source of adenosine during hypoxia. However, it is not known whether the adenosine originating from endothelial cells is formed intracellularly by cytosolic-5'-nucleotidase or extracellularly by ecto-5'-nucleotidase.
Objectives: In this study, we investigated whether vascular endothelial cells were capable of forming adenosine intracellularly in sufficient quantities for it to diffuse out into the extracellular space.
Methods: Vascular endothelial cells from hindlimb muscles of young male SD rats were isolated and purified. In the first series of experiments, cells were homogenised, and the cytosolic- and ecto-5'Ns were separated by differential centrifugation. 5'N in the cell homogenates was assayed at different pH values, as previously reported (Le & Ballard, 2007). Enzyme kinetic parameters, such as *Vmax*, *Km* and *Kcat* were calculated using the Eadie-Hofstee equation. In the second series of experiments, the cultured primary endothelial cells were exposed to normoxia or hypoxia (19% or 2% O_2) or pH 6.0 for 24 hours. The effects of hypoxia or low pH on the accumulation of adenosine in the culture medium were determined using HPLC.
Results: The highest value of *Vmax* occurred at pH 7.5 for ecto-5'N and at pH 7.0 for cytosolic-5'N, but the *Vmax* for the cytosolic-5'N was 2-3 times higher than that for the ecto enzyme across the pH range 6.0-8.0. The *Km* for both enzymes decreased with pH. Thus, *Kcat* for both enzymes was increased at low pH. Accumulation of adenosine in the medium surrounding the cultured endothelial cells was not changed greatly by a reduction in the pH to 6.0, but it was increased by around 50% following 24 hours exposure to hypoxia; preliminary data suggest that AOPCP, an inhibitor for ecto-5'N, did not significantly decrease the formation of adenosine in hypoxia.
Conclusions: Cytosolic-5'N has a higher activity than ecto-5'N, which is the opposite of the situation in muscle cells, suggesting that adenosine may be formed intracellularly in vascular endothelial cells. Furthermore, hypoxia significantly increased the adenosine formation by primary endothelial cells, confirming the capability of vascular endothelial cells to form adenosine during hypoxia.
Reference: GY Le, HJ Ballard. Properties of Adenosine-Metabolising Enzymes Extracted from Vascular Endothelial Cells. J HK Coll Cardiol 2007;15(2):85.

OC4.

ADIPOCYTE-FATTY ACID BINDING PROTEIN (A-FABP) IS PRESENT IN THE ENDOTHELIUM OF MALE APOLIPOPROTEIN E-KNOCKOUT MICE

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Adipocyte-fatty acid binding protein (A-FABP) modulates inflammatory responses in macrophages. A-FABP has been detected in cell cultures derived from porcine regenerated endothelium. These findings suggest a role for A-FABP in the formation of foam cells and atherosclerotic plaques. The present experiments were designed to investigate the presence (or not) of A-FABP and endothelial function in aortae of 8,12 and 16 weeks old male C57 (strain: B6.129P2) apolipoprotein E-knockout ($ApoE^{-/-}$) mice. A-FABP was detected by immunohistochemical staining in the endothelial layer of the aorta at 12, but not 8 weeks. Endothelium-dependent relaxations were measured in a myograph and compared to those obtained in aortae of C57 wild type mice. The relaxations to acetylcholine were reduced significantly in the $ApoE^{-/-}$ mice from 8 weeks on while those to the calcium ionophore A23187 were diminished significantly only from 12 weeks on. The endothelium-independent relaxation in response to the nitric oxide donor sodium nitroprusside was not affected in $ApoE^{-/-}$ mice. In conclusion, A-FABP was detected in male atherosclerotic-prone C57 $ApoE^{-/-}$ mice since the age of 12 weeks. Endothelial dysfunction was observed as early as at 8 weeks of age to judge from the reduced endothelium-dependent relaxations to acetylcholine. The dissociation between the absence of A-FABP and the presence of endothelial dysfunction at 8 weeks suggests that the presence of A-FABP at an older age is a consequence rather than a cause of the insufficient production of nitric oxide.

ABSTRACTS

Abstracts for Oral Communications:

OC5.

GENISTEIN ACUTELY POTENTIATES ACETYLCHOLINE-INDUCED RELAXATION THROUGH A G-PROTEIN COUPLED PATHWAY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives: Genistein, a phytoestrogen rich in soy beans and soy products, was reported to be a vasorelaxant. This study examined the receptor and related signaling pathways in the rapid vascular actions of genistein.
Methods: Isometric tension was measured in isolated aortic rings from 32-weeks-old male spontaneously hypertensive rats (SHR).
Results: Acute exposure to genistein at 10 μM, a concentration with no direct relaxation effect, potentiated acetylcholine (ACh)-induced relaxation and reduced ACh-induced contraction in the presence of L-NAME (100 μM). Both actions were insensitive to 10 μM actinomycin D (transcription inhibitor) and 10 μM cycloheximide (translation inhibitor). The potentiation of ACh-induced relaxation by genistein in the absence or presence of indomethacin was inhibited by 10 μM NF023 and 10 μM GP antagonist-2A, the selective G_i and G_q α-subunit antagonists, respectively, but not by 10 μM NF449, a selective G_s α-subunit antagonist. Interestingly, NF023, NF449 and GP antagonist-2A did not alter the inhibitory effect of genistein on ACh-induced contraction. To further elucidate the mechanism of the vascular response given by genistein, the involvement of G-proteins was inspected in A23187-induced relaxation and contraction. NF023 and GP antagonist-2A, but not NF449 inhibited the potentiating effect of genistein on A23187-induced relaxation in the presence of indomethacin. Reduction of A23187-induced contraction by genistein was unaffected by all three G-protein inhibitors.
Conclusion: These results demonstrate that rapid vascular actions of genistein in modulating ACh-induced relaxation and contraction responses in SHR are mediated by non-genomic pathways. $G\alpha_i$ and $G\alpha_q$, but not $G\alpha_s$, were involved in the potentiating effect of genistein in ACh and A23187-induced relaxations, but none were involved in the inhibitory effect of genistein in ACh and A23187-induced contractions. Involvement of G-proteins in the enhancement of ACh-induced relaxation by genistein suggests that genistein exerts its effect through a putative G-protein coupled phytoestrogen receptor.

OC6.

ESTROGEN SUPPRESSES THE CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II THUS CONFERRING CARDIOPROTECTION

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Estrogen confers cardioprotection by down-regulating β_1-adrenoceptor and suppressing the expression and activity of protein kinase A. We hypothesized that estrogen may also protect the heart by suppressing the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), another signaling messenger activated by β_1-adrenoceptor via the Gs protein that enhances apoptosis. We first determined the expression of CaMKII in the heart of sham operated rats and ovariectomized rats with and without estrogen replacement. Both CaMKIIδ and phosphorylated CaMKII were up-regulated in the heart from ovariectomized rats, which was restored to normal by estrogen replacement. We then determined the injury and contractile responses to ischemic insult with or without β-adrenoceptor stimulation with isoprenaline (10^{-7}M) in isolated perfused hearts and isolated ventricular myocytes. The infarct size and lactate dehydrogenase release from the heart in response to ischemic insult were significantly greater after ovariectomy. Similarly the cardiac contractility, the amplitude of the electrically induced intracellular Ca^{2+} transient, which is directly correlated to the shortening of the myocyte, and TUNEL-positive cells, were also greater in the ovariectomized rats upon ischemia/reperfusion in the presence or absence of isoprenaline. Most importantly, the responses to ischemic insult in ovariectomized rats were reversed not only by estrogen replacement, but also by blockade of CaMKII with a selective inhibitor, KN93 (2.5 μM). The observations indicated that estrogen confers cardioprotection by suppressing the CaMKII. The CaMKII isoform involved may be CaMKIIδ. The effect of estrogen on CaMKII is independent of β-adrenoceptor in addition to its effect of down-regulating the receptor.

ABSTRACTS

Abstracts for Oral Communications:

OC7.

ACUTE SIMVASTATIN INHIBITS THE IKATP CHANNELS OF PORCINE CORONARY ARTERY SMOOTH MUSCLE CELLS

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Statins (3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase inhibitors) have been shown to provide beneficial effects on cardiovascular system. Compared to the cholesterol-lowering properties, the effects of simvastatin (a commonly-used statin) on ion channel gatings of coronary artery smooth muscle cells have not been fully explored. In porcine isolated coronary artery, the cromakalim (10 nM-10 μM)- and pinacidil (10 nM-10 μM)-induced relaxation was inhibited by simvastatin (3 and 10 μM). In single cells of human left internal mammary artery and porcine coronary artery, simvastatin (1, 3 and 10 μM) suppressed the cromakalim (10 μM)- and pinacidil (10 μM)-mediated opening of the whole-cell IK_{ATP} channels, and it was eradicated by okadaic acid (100 nM). Simvastatin (10 μM) and AICAR (1 mM) elicited a compound C (1 μM)-sensitive [^{3}H]-deoxy-glucose uptake and an increase in $[ATP]_i$ of the coronary arterial cells. Simvastatin caused a time- and concentration-dependent increase in phospho-AMPKα-Thr^{172} and phospho-PP2A-Tyr^{307}. Simvastatin-induced phospho-PP2A-Tyr^{307} was eradicated by okadaic acid, ryanodine, KN93, phloridzin (1 mM), ouabain (10 μM), in $[glucose]_o$-free and in $[Na^+]_o$-free conditions. The enhanced phospho-AMPKα-Thr^{172} expression was abolished by compound C, ryanodine (100 μM), KN93 (10 μM) and in $[Ca^{2+}]_o$-free conditions. Acute simvastatin enhances glucose uptake and ATP formation which resulted in an inhibition of PP2A-Tyr^{307}. PP2A inhibition favored the subsequent Ca^{2+}/CaMKK-mediated AMPKα-Thr^{172} phosphorylation and thus inhibition of the vascular IK_{ATP} channels opening.

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OC8.

POLYOL PATHWAY CONTRIBUTES TO THE IMPAIRMENT OF CALCIUM HOMEOSTASIS IN POST-ISCHEMIC REPERFUSED RAT HEARTS

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A number of studies have shown that the polyol pathway contributes to ischemia-reperfusion (I/R)-induced myocardial infarction due to depletion of ATP. However, whether this glucose metabolic shunt also contributes to I/R-induced cardiac contractile dysfunction is not clear. In the present study, we show that post-ischemic contractile functions of the isolated perfused hearts was improved by pharmacological inhibition of aldose reductase (AR) or sorbitol dehydrogenase (SDH), two enzymes in the polyol pathway. I/R-induced contractile dysfunction is most likely due to impairment in Ca^{2+} signaling as indicated by lower amplitude of the Ca^{2+} peak, longer time to reach the peak, and slower return to base level. All these abnormalities were significantly ameliorated by treatment with AR or SDH inhibitors. Furthermore, we show that inhibition of AR or SDH protected the activity of SERCA and RyR, two of the key players in Ca^{2+} signaling mechanism that regulates cardiac contraction, from I/R-induced inactivation. During I/R polyol pathway probably contributes to the inactivation of SERCA and RyR by decreasing the level of GSH and increasing the level of superoxide.

ABSTRACTS

Abstracts for Oral Communications:

OC9.

A CENTRAL ROLE OF PKC_{δ} AND ACTIVATION OF P38 MAPK AND ERK1/2 PATHWAYS IN ANGIOTENSIN II-MEDIATED UPREGULATION OF COX-2 IN ENDOTHELIAL CELLS

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High circulating angiotensin II (Ang II) levels were reported in patients with hypertension and other vascular complications, while cyclooxygenase-2 (COX-2) has been regarded as the culprit of vascular inflammation and found to be localized in atherosclerotic plaque. Although both Ang II and COX-2 are associated with vascular inflammation and remodeling, it remains elusive whether COX-2 plays a direct role as a downstream event in mediating Ang II-induced vascular pathogenesis. The present study aimed at investigating the relations between Ang II stimulation and COX-2 expression and intracellular signaling pathways linking these two pro-inflammatory factors. Primary endothelial cells were freshly cultivated from thoracic aorta of Sprague Dawley rats. The expression level and activation of relevant proteins with and without drug treatment were examined by Western blot analysis. COX-2 expression was augmented with increasing Ang II concentration (3-100 nM), and it reached a maximum (>15-fold increase compared with control) after an 8-hour incubation with 100 nM Ang II. Ang II type 1 receptor (AT1R) blocker (losartan) and RNA synthesis inhibitor (actinomycin-D) inhibited such upregulation. Of the well-known transcriptional pathways tested, only the inhibitors of p38 MAPK and ERK1/2 (SB 202190 and PD 98059, respectively) significantly decreased the COX-2 expression, each producing ~ 50% reduction. Co-treatment with SB 202190 and PD 98059 caused further reduction, suggesting a joint mediation through p38 MAPK and ERK1/2. Although these signaling molecules are known to be redox-sensitive, inhibitors of reactive oxygen species (ROS) failed to alter the COX-2 upregulation. By contrast, PKC inhibitor (GF109203X), and particularly the specific PKC_{δ} inhibitor (rottlerin), but not PKC_{α} inhibitor (Go 6976), prevented both the phosphorylation of ERK1/2 and COX-2 expression. The pivotal role of PKC in Ang II-induced COX-2 expression was further supported by the stimulatory effect of a phorbol ester (phorbol 12-myristate 13-acetate, PKC activator) on COX-2 expression, which was again inhibited by GF109203X and rottlerin. The present results suggest an essential role of PKC_{δ} and subsequent activation of p38 MAPK and ERK1/2 in Ang II-mediated COX-2 upregulation and this response did not involve ROS. Since elevated Ang II and COX-2 induction serve as prerequisites for vascular complications, this study provides an important molecular basis for further elucidation of how altered COX-2- derived products participate in vascular inflammation (Supported by GRF 465308, CUHK Focused Investment Scheme and Li Ka Shing Institute of Health Sciences).

OC10.

CHRONIC INTERMITTENT HYPOXIA ELEVATES OXIDATIVE STRESS AND IMPAIRS CALCIUM HOMEOSTASIS IN RAT CARDIOMYOCYTES

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Objectives: This study examined the hypothesis that chronic intermittent hypoxia aggravates oxidative stress and deteriorates calcium (Ca^{2+})-handling in rat cardiomyocytes.
Methods: Adult male 2-month old Sprague-Dawley rats were daily administered with melatonin (MIH, 10 mg/Kg/day of body weight, i.p.) or vehicle (VIH, 2% ethanol in normal saline) and exposed to intermittent hypoxia (inspired oxygen alternating from 21 to 5±0.5% oxygen per minute for 8 hr/day) for 4 weeks. Levels of malodialdehyde (MDA) and the mRNA expression of anti-oxidant enzymes in the rat hearts were measured respectively by colorimetric study and reverse transcription-PCR. Changes in sarcoplasmic reticulum (SR) Ca^{2+}-handling were measured by spectrofluorometric study with isolated fura-2-loaded cardiomyocytes; also differences in protein levels and activities of SR-Ca^{2+} handling proteins were determined by Western blot and $^{45}Ca^{2+}$ flux study in the ventricular myocytes.
Results: The ratio of heart/body weight and the level of MDA were significantly increased in the VIH group compared with the normoxic control and MIH group. In addition, the mRNA levels of catalase and Mn-superoxide dismutase in the VIH group were much lower than that of the normoxic and MIH groups. Furthermore, spectroflurometric studies indicated that decreases in SR-Ca^{2+} content and the Ca^{2+}-overloading induced by metabolic inhibition/anoxia were remarkable in VIH cardiomyocytes compared to the normoxic control. Also, protein levels and activities of SR Ca^{2+}-ATPase and sarcolemmal Na^{+}-Ca^{2+} exchanger were markedly reduced in VIH cardiomyocytes. Moreover, these Ca^{2+} handling impairments in the cardiomyocyte were significantly less in the MIH group than that of the VIH group.
Conclusions: Our results demonstrate that chronic intermittent hypoxia, simulating severe levels of obstructive sleep apnea in patients, induces oxidative stress that could cause the impairment of Ca^{2+} handling in the cardiomyocyte leading to cardiac injury.