1033-94 **Calcineurin Inhibition Does Not Prevent Left Ventricular Hypertrophy but Does Suppress Its Molecular Markers in a Rat Model of Low-Renin, Mild Pressure Overload**

Aisling C. McMahon, Victoria L. Benson, Scott H. Kesteven, Michael P. Feneley, *St Vincent's Hospital, Sydney, Australia, Victor Chang Cardiac Research Institute, Sydney, Australia.*

Background: The role of the Ca^{2+} dependent calcineurin (Cn) signalling pathway in the pathogenesis of pressure overload left ventricular hypertrophy (LVH) is controversial. The aim of this study was to investigate the relationship between the Cn pathway and the development of LVH in a rat model of low-renin, mild pressure overload.
Methods: Male Wistar rats were randomly assigned to receive either 1 mg/kg FK506 daily IP (FK) or an equivalent volume of saline, IP (S). After 3d, half of each group was then subjected to banding of the aortic arch (B). The remaining animals were sham-operated controls (C). LV / body weight ratios (LV / BWt) and molecular markers of hypertrophy were studied 7 and 21d after surgery. Relative expression (normalised to the saline-control [SC] group) of ANF, α-skeletal actin and BNP in LV tissue samples was assessed by RT-PCR.
Results: At 7d, there was no increase in LV / BWt in SB animals compared with SC. Consistent with this finding, there was no change in the expression of the molecular markers examined. In contrast, after 21d an increase in LV / BWt of 13±8% ($p<0.005$) was found in SB; but there was no difference between SB and FKB in the degree of LVH induced (0.0023±0.0002 vs 0.0023±0.0003; n.s.). Peak aortic pressure gradients were similar in SB and FKB at 21d (35±15, n=10 vs 33±16 mmHg, n=9 respectively) and banding *per se* did not cause an increase in plasma renin activity. The RT-PCR data (n=5-6 per group) at 21d indicated a 4-fold increase in ANF expression ($p<0.03$), a 10-fold increase in α-skeletal actin expression ($p<0.04$) and a 9-fold increase in BNP expression ($p<0.06$; n.s.) in SB compared with SC animals; no change in the expression levels of these markers was observed in FKB animals.
Conclusions: 1. FK506 does not prevent pressure overload hypertrophy in this model, suggesting that the Cn pathway *per se* is not critical for the development of LVH, when renin is not elevated. 2. The supression of molecular markers associated with LVH without supression of LVH in FKB indicates that these proteins are causally related to the Cn cascade but not necessary for the genesis of LVH. 3. Collectively, these data suggest that alternative intracellular signalling pathways are responsible for LVH in this model.

1033-95 **Stimulation of Arachidonic Acid Release From Mas-Transfected COS Cells Is Not Restricted to Angiotensin-(1-7) and Does Not Involve AT1 and AT2 Receptors**

Insa de Buhr, Martin Vahl, Thomas Walther, *Department of Cardiology and Pneumology, Free University of Berlin, Berlin, Germany.*

Background: Besides angiotensin (Ang) II, other Ang peptides, as Ang III (Ang-(2-8)), Ang IV (Ang-(3-8)), and Ang-(1-7) also have biological activities. Especially Ang-(1-7) has become an angiotensin of interest, since its vascular and baroreflex actions counteract those of Ang II. Recently, it could be demonstrated that Ang-(1-7) is a functional ligand of the G protein-coupled receptor Mas. We wanted to examine whether other Ang receptors are involved in the ligand/receptor interaction and if other Ang metabolites also affect the signalling pathway of Mas.
Methods: COS cells were transfected with a pcDNA-Mas construct (Mas-transfected cells). COS cells, transfected only with the plasmid pcDNA, served as control cells. Cells were incubated with (^{3}H)arachidonic acid (AA) for 18h. 15 min after adding the angiotensins and their antagonists, the amount of (^{3}H)AA release was measured by liquid scintigraphy. To evaluate the specifity of Ang-(1-7) binding, we used, beside the specific Ang-(1-7) antagonist A-779, antagonists for the angiotensin receptors AT1 (Irbesartan) and AT2 (PD123319).
Results: Control cells were found to react on incubation with 10-8 M Ang-(1-7), Ang III, or Ang IV with a slight increase in (^{3}H)AA release (35%±9%, 14%±4%, 12%±5%;n=8). The incubation of Mas-transfected COS cells with Ang-(1-7) resulted in 265%±45% (n=8) increased (^{3}H)AA release compared to control cells. Ang III and IV elevated the amount of (^{3}H)AA release by 111%±26% and 112%±23% (n=4), whereas incubation with Ang I and Ang II showed no changes. The (^{3}H)AA release induced by Ang-(1-7) was totally blocked by A-779 but preserved by PD123319 and Irbesartan.
Conclusion: Ang-(1-7) stimulates the AA pathway specifically via the Mas receptor. Ang III and Ang IV also stimulate AA release from Mas-transfected cells. In contrast, Ang I and Ang II have not been effective. The fact that A-779 blocks (^{3}H)AA release suggests A-779 to be a specific antagonist of the Mas receptor. Neither AT1 nor AT2 receptors are involved in the interaction between Ang-(1-7) and Mas. These findings could open new goals in cardiovascular therapy by pharmacological manipulation at the Mas receptor.

1033-96 **Characterization of a Novel Inositol 1,4,5-Trisphosphate Receptor/Calcineurin Signaling Pathway in Cardiac Hypertrophy: Effects of Heparin**

Zhiming Zhu, Shanjun Zhu, Yongjian Yang, *Department of Hypertension and Endocrinology, Chongqing Institute of Hypertension, Daping Hospital, Chongqing, People's Republic of China.*

Background: Cardiac hypertrophy may be regulated primarily by hypertrophy-stimulating factors. However, little is known about the cardiac growth-inhibitory factors that negatively regulate the formation of cardiac hypertrophy. Recently, a calcium-dependent calcineurin (CN) / nuclear factor of activated T-lymphocytes-3 (NFAT3) / GATA4 signaling pathway was found to be involved in cardiac hypertrophy. Heparin has been shown to prevent growth and proliferation of cardiomyocytes besides its well-characterized anticoagulant action. Heparin specifically inhibits the 1,4,5-trisphosphate receptor (InsP3R) which is an intracellular calcium release channel. We postulated that activation of InsP3R mediated CN / NFAT3 / GATA4 pathway have a potential role in regulation of cardiomyocyte hypertrophy and heparin may antagonize this signaling pathway. Methods and Results: primary neonatal rat cardiomyocytes were cultured using established techniques. Fluorescence-spectrophotometric measurements using fura2 showed that InsP3 caused a dose-dependent increase of cytostolic free calcium concentration in cardiomyocytes (IP3 100 nmol: 138±7 vs 223±8 nmol/l, $p<0.01$). InsP3 increased 3H-thymidine (IP3 100 nmol: 35637±175 vs 138106±2494 cpm/well, $P<0.01$) and 3H-leucine (IP3 100 nmol: 838±19 vs 1534±119 cpm/well, $p<0.01$) incorporation in a time- and dose-dependent manner. Immunoblotting showed that InsP3 significantly enhanced the expression of c-fos, c-myc, alpha-actin and beta-MHC in cardiomyocytes. Administration of InsP3 time-dependently increased the expression of CN, NFAT3 and GATA4. The InsP3-induced CN expression was blocked by heparin administration. Conclusions: The study indicates that the InsP3 sensitive calcium pool is related to cardiac hypertrophic gene regulatory pathways. Antagonizing the InsP3R/CN/NFAT3/GATA4 signaling pathway may be a new therapeutic target for cardiac hypertrophy. (Supported by grant from NSFC No. 39725013).

POSTER SESSION

1034 Pathophysiology of Large and Small Arteries

Sunday, March 17, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

1034-69 **Lysophosphatidylcholine Is a Major Contributor to the Synergistic Effect of Mildly Oxidized Low-Density Lipoprotein With Endothelin-1 on Vascular Smooth Muscle Cell Proliferation**

Takuya Watanabe, Takashi Katagiri, Rajbabu Pakala, Claude R. Benedict, *Third Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan, Division of Cardiology, University of Texas-Houston Health Science Center, Houston, Texas.*

Background: Endothelin-1 (ET-1) and oxidatively modified low-density lipoprotein (LDL) are associated with essential hypertension and atherosclerosis. We assessed the effect of mildly oxidized LDL (mox-LDL) and highly oxidized LDL (ox-LDL) and their major oxidative components, i.e., reactive oxygen species (ROS), lysophosphatidylcholine (LPC), and 4-hydroxy-2-nonenal (HNE) and their interaction with ET-1 on VSMC proliferation.
Methods: Growth-arrested VSMCs isolated from the rabbit aorta were incubated with different concentrations of native LDL, mox-LDL, ox-LDL, hydrogen peroxide (a donor of ROS), LPC, or HNE with or without ET-1. DNA synthesis in VSMCs was measured by [3H]thymidine incorporation.
Results: Mox-LDL, ox-LDL, hydrogen peroxide, LPC, HNE, or ET-1 stimulated DNA synthesis in a dose-dependent manner. Maximal effect was observed at 5 microg/ml for mox-LDL (162%) or ox-LDL (154%), 15 microM LPC (156%), 5 microM hydrogen peroxide (177%), 1 microM HNE (144%), and 0.1 microM ET-1 (195%). By contrast, native LDL was without any significant effect. When added together, there was no synergistic effect of native LDL, hydrogen peroxide, or HNE with ET-1 on DNA synthesis. However, the effect of mox-LDL (0.1 microg/ml), ox-LDL (0.5 microg/ml), or LPC (10 microM) was potentiated by ET-1 (114 to 338%, 133 to 425%, 118 to 333%, respectively). The mitogenic effect of mox-LDL, ox-LDL, or LPC and their interaction with ET-1 were inhibited by defatted albumin (10 microg/ml), antioxidant N-acetylcysteine (400 microM), the NADPH oxidase inhibitor diphenylene iodonium (1 microM). The mitogenic effect of ET-1 and its interaction with mox-LDL, ox-LDL, or LPC were inhibited by the ETA/B receptor antagonist TAK044 (1 microM) or the MAPK kinase inhibitor PD098059 (10 microM). The synergistic interaction of mox-LDL, ox-LDL, or LPC with ET-1 was completely reversed by the combined use of N-acetylcysteine and TAK044.
Conclusions: Our results suggest that mox-LDL, ox-LDL, and their major phospholipid component LPC act synergistically with ET-1 in inducing VSMC proliferation via the activation of redox-sensitive and MAPK pathways.

1034-71 **Chronic Reduction of Carotid Blood Flow Causes Salt-Sensitive Hypertension in Rats**

Masato Nishimura, Hiroyuki Kobayashi, Tetsuya Hashimoto, Toyoshi Fukuda, Koji Okino, Noriyuki Yamamoto, Toshikazu Yoshikawa, Toshihiko Ono, *Toujinkai hospital, Kyoto, Japan, Kyoto Prefectural University of Medicine, Kyoto, Japan.*

Background: Obstruction of extracranial carotid artery is reportedly associated with systolic hypertension in adults. We asked in the present study whether chronic reduction of carotid blood flow elicits salt-sensitive hypertension through regulation of the renin-angiotensin system (RAS) in the brain.
Methods: Bilateral internal carotid arteries of 12-week-old male Wistar rats (n = 40) were ligated at one-week interval. Carotid-ligated or sham-operated rats were treated with high salt (8% NaCl diet and 1% NaCl drinking water) or low salt (0.3% NaCl diet and distilled water) for 6 weeks. Systolic arterial pressure (SAP) was measured using tail-cuff method, and diurnal urinary excretion of arginine vasopressin and norepinephrine was measured once a week. At the end of the experiment, expression of the RAS messenger RNAs in the hypothalamus and lower brainstem was measured using competitive RT-PCR method. Effects of a 6-day intracerebroventricular infusion of CV-11974 (10 mg/kg/d, n = 8, 50 mg/kg/d, n = 8), a blocker of angiotensin II type-1 receptor, or vehicle (artificial cerebrospinal fluid, n = 8) on SAP, heart rate, urinary excretion of vasopressin and norepinephrine were investigated in carotid-ligated rats administered with high-salt for 6 weeks.