1027-26 Reactive Oxygen Species Act as Mediators in Endothelial Cell Signalling Pathways

Kaikobad Irani, Steven Sollott, Loren Rosolowski, Toren Finkel, Maitrayee Sundaresan, Pascal J. Goldschmidt-Clermont. *The Johns Hopkins University, Baltimore MD, Gerontology Research Center, NIA, Baltimore MD and Cardiology Branch, N.I.H., Bethesda MD*

Generation of reactive oxygen species(ROS) in response to conditions such as ischemia-reperfusion has been associated with cellular damage. However, recent evidence suggests that ROS may also be produced in response to cytokines and growth factors. We sought to investigate the role of ROS in endothelial cell signalling pathways. Addition of vascular endothelial cell growth factor (VEGF 0.5 nM) to human aortic endothelial cells (HAEC) resulted in a significant though transient increase in ROS as assessed by the H_2O_2 sensitive fluorophore dichlorodihydrofluorescein diacetate (DCF-DA). Furthermore, direct addition of H_2O_2 or H_2O_2 + sodium orthovanadate (NaOV) to HAEC produced a marked and dose-dependent increase in protein tyrosine phosphorylation. Specific proteins involved in tyrosine kinase signalling activated by H_2O_2 and H_2O_2 + NaOV included the VEGF receptor (flt-1) itself, mitogen activated protein kinase (MAPK) and phospholipase C-gamma. In addition, concentrations of H_2O_2 (+/– NaOV) that lead to tyrosine phosphorylation also induced changes in the actin superstructure and membrane ruffling. This suggests that ROS such as H_2O_2 act as intermediaries in endothelial signalling pathways and may mediate essential cellular functions such as migration and mitosis in response to growth factors and changes in O_2 tension.

1027-27 Transcriptional Regulation of Inducible Nitric Oxide Synthase Expression in Cardiac Myocytes

Carmine V. Oddis, Mitchell S. Finkel. *University of Pittsburgh, Pittsburgh, PA*

We have previously reported that interleukin-1β (IL-1) alone induced the transcription of nitric oxide synthase (iNOS) in isolated neonatal rat cardiac myocytes (CM). CM cultured with IL-1 for 48 hrs. stimulated nitrite (NO_2^-) production, stained positively for iNOS protein and revealed mRNA for iNOS by RT-PCR. Cytokines have been shown to stimulate nuclear translocation of the transcription factor, NF-κB, in lymphocytes. The specific NF-κB inhibitor, pyrrolidinedithiocarbamate (PDTC), inhibited IL-1 induced NO_2^- production by CM (6.7 $\pm$ 0.6 vs. 0.9 $\pm$ 0.3 nmol/1.25 $\times$ 10^5 cells/48 hrs.; $p < 0.01$; $n = 12$). Immunohistochemical staining revealed that PDTC blocked IL-1 stimulated NF-κB translocation into the nucleus of CM. Tetrahydrobiopterin (BH_4) is a co-factor for NOS activity. GTP cyclohydrolase I is the rate-limiting enzyme in de novo BH_4 synthesis. The GTP cyclohydrolase I inhibitor, 2, 4-diamino-G-hydroxy-pyrimidine (DAHP), also blocked IL-1 stimulated NO_2^- formation by CM (6.7 $\pm$ 0.6 vs. 0.3 $\pm$ 0.1 nmol/1.25 $\times$ 10^5cells/48 hrs.; $p < 0.01$; $n = 12$). RT-PCR revealed no iNOS mRNA in cells treated with IL-1 + PDTC. Cardiac myocytes treated with IL-1 + DAHP did express iNOS mRNA. Neither PDTC nor DAHP had any effect on GTP-cyclohydrolase I mRNA expression in cardiac myocytes. The membrane permeable analogue of BH_4, methyl-BH_4, (mBH_4), only partially reversed DAHP inhibition of NO_2^- formation. (6.7 $\pm$ 0.6 vs. 2.4 $\pm$ 0.3 nmol/1.25 $\times$ 10^5 cells/48 hrs.; $p < 0.01$; $n = 12$). Thus, IL-1 stimulated iNOS expression in CM requires nuclear translocation by NF-κB, but optimal NO_2^- production additionally requires GTP cyclohydrolase activity.

1027-28 Tyrosine Kinase Regulation of Phospholipase D-Protein C Kinase Pathway in Ischemic Preconditioning

Nilanjana Maulik, Masazumi Watanabe, Arpad Tosaki, Daniel Engelman, Yu-Feng, W. Huang, Richard M. Engelman, John A. Rousou, David Deaton, Joseph Flack, Dipak K. Das. *University of Connecticut School of Medicine, Farmington, CT*

Translocation and activation of protein C kinase (PKC) has been implicated in ischemic preconditioning. In an attempt to identify the intracellular signaling pathway leading to PKC activation, we have examined the role of protein tyrosine kinase (PTK) in ischemic preconditioning. Isolated rat hearts were perfused for 10 min with or without a PTK inhibitor and then preconditioned by four times repeated ischemia (5 min), each followed by 10 min of reperfusion. Hearts were switched to working mode and subjected to 30 min ischemia followed by 30 min of reperfusion. Preconditioning led to the activation of PTK [45% vs. 20% for control] and phospholipase D (PLD) [72 $\pm$ 5.2 vs. 52 $\pm$ 4.6 for control] in concert with the increase in diacylglycerol (DAG) formation [50 $\pm$ 2.1 vs. 32 $\pm$ 2.9 for control] and PKC activation [52% vs. 25% for control]. Activation of TPK and increased second messenger production were associated with the enhanced tolerance of the hearts to ischemic reperfusion injury as evidenced by the significantly increased developed pressure (DP) [45.4 $\pm$ 1.4 vs. 35.4 $\pm$ 1.8 for control], dp/dt_{max} [2055 $\pm$ 89 vs. 1520 $\pm$ 63 for control], arterial flow [24.3 $\pm$ 1.2 vs. 14.4 $\pm$ 0.7 for control] and coronary flow [19.6 $\pm$ 0.6 vs. 17.1 $\pm$ 0.4 for control] in the ischemic-reperfused hearts. Pretreatment of the hearts with the TPK inhibitor completely abolished the preconditioning phenomenon in conjunction with the inhibition of TPK and restoration of PLD, DAG and PKC at the control levels. The results of this study demonstrate that PTK–PLD signaling plays a role in ischemic preconditioning and activation of PKC.

1028 Genetics of Lipid Disorders

Wednesday, March 27, 1996. Noon–2:00 p.m.
Orange County Convention Center, Hall E
Presentation Hour: Noon–1:00 p.m.

1028-74 Abnormal HDL_3 and Apo AI-Mediated Cellular Cholesterol Efflux in Subjects With Familial HDL Deficiency

Michel Marcil, Larbi Krimbou, Betsie Boucher, B.C. Solymoss, Jeffrey S. Cohn, Jacques Genest Jr.. *Clinical Research Institute of Montreal and Montreal Heart Institute, Québec, Canada*

We have identified and characterized 4 probands with severe familial HDL deficiency (FHD) characterized by a lack of mature, α-migrating LpAI HDL particles. Despite thorough clinical, biochemical and molecular characterization, no known cause of HDL deficiency has been identified in these families. We examined the possibility that a cellular defect may cause an impaired HDL-mediated cholesterol efflux in FHD. Fibroblasts were obtained from skin biopsies of normal and FHD subjects. Using cholesterol-loaded cells, selective radiolabeling of cellular cholesterol pools were used for efflux studies. First, confluent growth-arrested cells were labeled with [^{3}H]-cholesterol to specifically enrich plasma membrane as established by sucrose density gradient ultracentrifugation; most of the [^{3}H]-cholesterol (80–95%) was found within the plasma membrane. In these conditions, normal HDL_3 (1 mg/mL of proteins) was able to desorb [^{3}H]-cholesterol from normal and FHD cells in a time dependent (up to 24 hours), linear kinetic fashion, with no difference in rates of efflux between FHD and normal cells. Second, to introduce a high fraction of label into intracellular compartments, cells were labeled with [^{3}H]-cholesterol during growth. Under these conditions, apo AI or HDL_3 (100 μg/mL of proteins) had a markedly reduced effect in promoting radiolabeled cholesterol efflux in FHD cells (25–30%) compared with cells from normal subjects. When using [^{3}H]-mevalonolactone to label cells, significantly less newly synthesized sterols are found in the plasma membrane of FHD cells, compared with normal cells (≈2–3-fold). This data show that intracellular cholesterol efflux is abnormal in FHD fibroblasts and suggest a defect in cellular trafficking of cholesterol to the cell membrane. This would lead to the formation of small, lipid-depleted nascent HDL particles (preβ mobility LpAI particles) that are rapidly cleared from plasma.

1028-75 Abnormal Regulation of the LDL-R and HMG CoA Reductase Genes in Subjects With Familial Hypercholesterolemia With the "French Canadian Mutation"

Lu Yu, Shiqiang Qiu, Jacques Genest Jr.. *Clinical Research Institute of Montreal; Montréal Heart Institute, Montréal, Québec, Canada*

Familial hypercholesterolemia (FH) is seen with high frequency in Québec, Canada. A large deletion (> 10 kb), 5' end of the low density lipoprotein receptor (LDL-R) gene includes exon 1 and the promoter region, and is the major mutation of the LDL-R in FH subjects in Québec (≈60% of cases). No mRNA is produced from the allele bearing the mutation. LDL-R protein expression is under the control of the non-deletion allele. We studied the regulation of the LDL-R and HMG CoA red. genes in response to lovastatin (Lova) in cultured human skin fibroblasts. We determined mRNA levels of the LDL-R and HMG CoA red. genes by RNase protection assay in skin fibroblasts obtained from controls ($n = 3$) and subjects with FH ($n = 6$). We measured ^{125}I-LDL binding on skin fibroblasts grown in the presence of lipoprotein deficient serum with, or without Lova. Control subjects exhibited coordinate regulation of the LDL-R and HMG CoA red. genes in response to Lova, 0.1–25 μM, for 0 to 24 hours. Correlation coefficients between

mRNA levels of both genes were 0.97 in controls, 0.96 in FH. Although the genes were coordinately regulated in FH fibroblasts, there was a marked up-regulation of the HMG CoA red. gene compared with the LDL-R gene in FH subjects. Peak mRNA levels of the LDL-R gene was approximately 5.31 fold over LPDS in controls, 1.73 fold in FH. ^{125}I-LDL binding studies confirm that FH subjects increase the amount of LDL receptors in response to Lova, 5 μM. We conclude that the LDL-R and HMG CoA reductase genes are expressed in coordinate regulation in fibroblasts from subjects with FH due to the > 10 Kb deletion, but with a proportionately greater up-regulation of the HMG CoA reductase gene. Some subjects with FH due to the > 10 Kb deletion of the LDL-R gene who fail to respond to HMG CoA red. inhibitors have abnormal LDL-R gene up-regulation in response to Lova *in-vitro*.

1028-76 Linkage of the Apo CIII Microsatellite With Isolated Low High Density Lipoprotein Cholesterol

Cecilia M. Devlin, Karen I. Zeller, Valerie L. Prenger, Michael Miller. *Baltimore Veterans Affairs Medical Center; University of Maryland Medical Center, Baltimore, MD*

Low levels of high density lipoprotein cholesterol (HDL-C) (< 35 mg/dL) and its primary apolipoprotein, apo AI, are associated with premature coronary artery disease (CAD) even with desirable total cholesterol levels (< 200 mg/dL). To evaluate the familial basis of isolated low HDL-C, a nationwide search of normocholesterolemic subjects with HDL-C < 20 mg/dL was conducted. Seven unrelated probands (mean HDL-C = 9 ± 5 mg/dL) and 96 biological family members were identified. Five probands developed premature CAD (mean age = 45 ± 4). Extracted genomic DNA from each subject was used to determine whether a highly polymorphic region within the apolipoprotein AI-CIII-AIV gene complex segregated with isolated low HDL-C. The size of the alleles of the $(C_aT_b)_c$ microsatellite within intron 3 of the apo CIII gene was assessed following PCR amplification and denaturing polyacrylamide gel electrophoresis. Using quantitative sib-pair analysis there was strong evidence for linkage of this microsatellite with the reduced HDL-C phenotype ($P < 0.005$). These results suggest a potentially important role for the apo CIII microsatellite region in the genetic screening of families with isolated low HDL-C associated with premature CAD.

1029 Myocardial Infarction: Basic II

Wednesday, March 27, 1996, 3:00 p.m.–5:00 p.m.
Orange County Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

1029-37 Can Lactate, Per Se, Induce Cardiac Preconditioning?

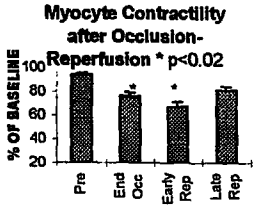
Francesca Aresta, Gary Gerstenblith, Konrad Vandegaer, Robert G. Weiss. *The Johns Hopkins Hospital, Baltimore, MD*

The contractile benefits associated with cardiac "preconditioning" (IPC) induced by exposure to brief periods of ischemia, hypoxia, and to adenosine may be related to activation of K_{ATP} channels. Lactate also activates K_{ATP} channels. To test whether repeated transient lactate exposures, resulting in tissue lactate levels similar to IPC, but in the absence of ischemia, can provide similar "preconditioning" benefits, isolated, retrogradely perfused rat hearts were subjected to either a "lactate-preconditioning" protocol (LP) consisting of two 5-minute exposures to 15 mM lactate and two 5-minute periods of reflow with normal buffer ($n = 6$), to a previously reported IPC protocol composed of two 5-minute ischemia reperfusion cycles (IP, $n = 5$), or control perfusion (C, $n = 5$). Subsequently all hearts underwent 30 minutes of normothermic, total ischemia (I) followed by 30 minutes of reflow. Tissue levels of lactate (10.5 ± 0.3 versus 10.5 ± 0.5 mmol/gWW) and contractile dysfunction (developed pressure (DP) 89.2 ± 4.4% and 89.8 ± 3.9% of initial) were similar in LP and IPC hearts, respectively, before the prolonged ischemia period. The recovery of DP after 30 min of I, however, was higher in IPC hearts than in C and LP reaching, respectively, 56.8 ± 3.4%, 14.2 ± 6.8%, and 9.5 ± 3.6% of the baseline values. EDP was lower during reperfusion in IPC hearts than in LP and C hearts, and there were no significant differences between the latter two groups (36.2 ± 3.5, 82.0 ± 2.9, and 81.2 ± 0.5 mmHg, respectively). Thus, transient lactate exposure resulting in tissue lactate levels similar to IPC does not improve contractile recovery after prolonged ischemia and therefore cannot be a mechanism which explains the protective effects observed in preconditioned myocardium.

1029-38 Release of Soluble Myocardial Depressant Activity by Reperfused Myocardium

Rupinder Brar, Anand Kumar, Gary Schaer, Steven Hollenberg, Tony Hursey, Yifan Sun, Joseph Parrillo. *Section of Cardiology, Rush Medical College, Chicago, IL*

Myocardial ischemia followed by reperfusion is associated with injury and myocardial stunning. Circulating cytokines may play a role in reperfusion injury. *Methods:* We examined effect of serum from dogs undergoing LAD occlusion and reperfusion on contractility of beating myocytes. Anaesthetized dogs ($n = 3$) were subjected to 3 hours of LAD occlusion and 3 hours of reperfusion. Control dogs ($n = 3$) underwent sham occlusion. Coronary sinus serum from each dog was collected preocclusion (PRE), end occlusion (END OCC 170 min), early reperfusion (EARLY REP, 5 min) and late reperfusion (LATE REP, 170 min). Myocardial depressant activity in eac.: sample ($n = 6$) was evaluated using a previously described assay employing incubation of serum with isolated contracting cultured rat cardiac myocytes. Myocyte contractility was assayed by measuring the amplitude of displacement of a latex bead embedded in the cell membrane at baseline and at 30 minutes following incubation with serum. Results are mean ± SEM of the baseline displacement. *Results:* Significant myocardial depressant activity was seen at END OCC ($p = 0.02$), and EARLY REP ($p = 0.001$). The effect was largely reversed at LATE REP (see figure). Control dogs showed no significant depression.



Conclusions: These findings demonstrate circulating myocardial depressant activity in efferent blood after coronary occlusion, with the greatest depression occurring during early reperfusion. Soluble circulating mediators may be important in the pathogenetic mechanisms causing reperfusion associated myocardial depression (stunning).

1029-39 High Extracellular K$^+$ During Hypoxic Preconditioning Episodes Attenuates the Post-Ischemic Contractile and Ionic Benefits of Preconditioning

Keith M. Baumgarten, Gary Gerstenblith, Robert G. Weiss. *The Johns Hopkins Hospital, Baltimore, MD*

Hypoxic preconditioning (PC) improves contractile recovery and decreases calcium loading following ischemia (I) and reperfusion (R). This may be mediated via activation of K_{ATP} channels and a resulting decrease in the trans-sarcolemma K gradient. To test whether changing the trans-sarcolemma K gradient during the PC period changes its benefits, isolated isovolumic rat hearts were subjected to two, 5 min intervals of hypoxia, separated by 5 min of normoxic reflow, in the presence of normal K (5 mM, NmlK-PC) and of high K (10.3 mM, HlK-PC). Developed pressure (DP) and cellular Ca and K, by atomic spectroscopy using KCoEDTA as an extracellular marker, at 45 min of R after 30 min of total I were compared to those of control hearts (C), which did not undergo any PC. (Results at 45 min of R: mean ± SD; Ca_i^{++} before I = 4.1 ± 2.0)

	DP as % of initial	K_i^+ (μmol/g dry)	Ca_i^{++} (μmol/g dry)
C	14.1 ± 8.0 (n = 14)	166 ± 46.5 (n = 9)	19.4 ± 6.8
NmlK-PC	72.2 ± 20.7 (n = 12)	231 ± 17.7 (n = 5)	12.9 ± 3.3
HlK-PC	31.7 ± 19.6 (n = 12)	186 ± 54.6 (n = 4)	22.9 ± 1.6

Thus, the trans-sarcolemmal K gradient during the PC period influences PC effects; decreasing the gradient decreases preconditioning's favorable influences on contractile recovery and calcium loading during reperfusion.