MOLECULAR MECHANISMS OF VASCULAR DISEASES

Organizer: Victor Dzau

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Keynote Address (joint)

G 001 REGULATION OF REGULATORS, David Baltimore, The Rockefeller University, New York, New York 10021 and the Whitehead Institute for Biomedical Research, Cambridge, MA. 02142. The most conceptually challenging issue of differentiation is not how gene expression is regulated. -- Jacob and Monod largely solved that problem -- but how the regulators are regulated. To achieve a differentiated, stable phenotype requires that a cell program numerous genes in quite precise ways, using robust regulatory strategies to assure long-term stability. In no cellular system is there a satisfactory description of regulation, but as more regulators are isolated, and the genes encoding them are cloned, elements of regulatory strategies are emerging. One is that regulators occur in structurally-related families and that often members of a family interact among themselves. The leucine zipper, helix-loop-helix and rel-related proteins (especially NF-κB) are examples. Also regulators may be sequestered by inhibitors (as IκB for NF-κB or Id for helix-loop-helix proteins) and therefore, the regulation of the inhibitor becomes a crucial component of the system. Certain regulators positively activate themselves, contributing to stability. Also, when their products are not required, many, maybe all, genes are maintained in the non-expressed state by active silencing methods and counteracting silencing may be a crucial aspect of allowing gene expression. Individual genes are affected by a medley of regulators, thus the ensemble of gene products that define any one differentiated state is produced by a multitude of regulators, each maintained by its own particular set of interactions.

Paracrine Regulation of Vascular Tone

G 002 NORMALIZATION OF CEREBROVASCULAR REACTIVITY IN HYPERCHOLESTEROLEMIC RABBITS BY THE EDRF PRECURSOR, John P. Cooke, Eben Alexander, Peter McL. Black, E. Rossitch, Jr. Division of Neurosurgery, Harvard Medical School; Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305.

Excessive vasoconstriction and reduced endothelium dependent vasodilation is observed in vessels from hypercholesterolemic animals and man. This seems to be largely due to the reduced release of endothelium-derived relaxing factor (EDRF) from these vessels. Current evidence suggests that EDRF is derived from the metabolism of L-arginine. We hypothesized that the release of EDRF from hypercholesterolemic vessels could be restored by administering the precursor to EDRF, L-arginine. Basilar arteries from hypercholesterolemic or normal rabbits were harvested, cannulated for perfusion at physiologic pressure, and changes in vessel diameter monitored by video microscopy. In comparison to normal vessels, those from hypercholesterolemic animals vasoconstricted more to KCl, endothelin (E), and 5-hydroxytryptomine 5-HT). Vasodilation to acetylcholine (ACH), but not that to verapamil, was impaired in the hypercholesterolemic vessels. In vitro administration of L-arginine (3 mM) for 45 minutes, normalized vasodilation to ACH and vasoconstriction to E, 5 HT, and KCL in the isolated vessels from hypercholesterolemic animals. This effect was stereospecific, and dependent upon the presence of the endothelium. To conclude, these data confirm that hypercholesterolemia attenuates endothelium dependent relaxation, and enhances the sensitivity of those vessels to vasoconstrictors. In vitro administration of L-arginine normalized vascular reactivity in these tissues. Thus, hypercholesterolemia induces a reversible endothelial dysfunction that may be corrected by supplying the precursor of EDRF, L-arginine.

G 003 VASCULAR EFFECTS OF LEUKOCYTES, Donald D. Heistad, J. Antonio G. Lopez, Mark L. Armstrong, and Andreas Mugge, VA Medical Center and University of Iowa College of Medicine, Iowa City, IA 52246

Leukocytes have received attention in relation to development and progression of atherosclerotic lesions. We have proposed an additional role for leukocytes: they may contribute to spasm of atherosclerotic arteries. This hypothesis is based on responses to injection of a peptide, f-met-leu-phe (fMLP), which activates leukocytes and releases their vasoactive products. Injections of fMLP have little effect in the limb of normal monkeys, and produce marked vasoconstriction in atherosclerotic monkeys (1).

Complement has been demonstrated on endothelium over atherosclerotic lesions. We have found that injection of complement C5a, to simulate endogenous activation of leukocytes, produces marked vasoconstriction in atherosclerotic but not normal monkeys.

We have attempted to determine whether blood-borne leukocytes or leukocytes in the arterial wall account for constrictor responses of atherosclerotic arteries in vivo. Addition of fMLP to arterial segments in vitro did not produce contraction of atherosclerotic arteries. Injection of complement C5a in the limb perfused with a blood-free solution in situ did not produce constriction of atherosclerotic arteries. Both studies suggest that circulating leukocytes or leukocytes adherent to the endothelium, not leukocytes in the arterial wall, account for constriction of atherosclerotic arteries.

We have examined mediators of leukocyte-induced vasoconstriction. Studies in vitro indicate that activated human leukocytes produce endothelium-independent contraction in arteries from normal and atherosclerotic monkeys. Contraction is mediated in part by oxygen radicals (probably hydroxyl radicals) and in part by a stable factor.

We have examined effects of regression of atherosclerosis. Regression of lesions was induced by feeding atherosclerotic monkeys a normal diet for 18 months. Regression restores endothelium-dependent vascular responses to normal in vitro and abolishes hyperresponsiveness to serotonin in vivo. Leukocytes in the intima are lost during regression, but they persist in adventitia. Abnormal vasoconstrictor responses to fMLP in vivo return towards, but not to, normal during regression of atherosclerosis.

1. Lopez, JAG, et al. Circ. Res. 65:1078-1086, 1989.

G 004 POSSIBLE ROLE OF ENDOTHELIN IN REGULATION OF VASCULAR TONE Tomoh Masaki, Masashi Yanagisawa, Sadao Kimura and Katsutoshi Goto, Institute of Basic Medical Sciences,University of Tsukuba, Tsukuba, Ibaraki 305 Japan

Endothelin (ET) is a potent vasoconstrictor peptide produced by endothelial cells. Analysis of human genomic gene of endothelin revealed the existence of three different types of endothelins designated as endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). Northern bolt analysis with the cDNA clones for these three types of endothelins and autoradiographic studies of various tissues with iodine-labelled endothelin demonstrated that endothelins and their receptors are wide - distributed, not only in vascular endothelial cells but also in many kinds of cells, suggesting the variety functions of endothelins.

ET is a 21 amino acid peptide with two disulfide bonds. ET induces vasoconstriction by three types of mechanisms, i.e. activation of phospholipase C releasing inositol 1, 4, 5 - trisphosphate, calcium channel activation and increase in the sensitivity of pharmacomechanical coupling mechanism to intracellular calcium ion. In addition, ET stimulates the phospholipase A_2 to produce vasoactive prostanoids.

Exogenously administrated ET induces initial depressor response followed by sustained increase in blood pressure. The pressor response is entirely accountable for direct vasoconstrictor action of ET on peripheral vessel. The relative effect of the vasorelaxation and vasoconstriction induced by ET depends on the vessel employed. Mesenteric artery is very sensitive to ET. Mesenteric arterioles are more sensitive than the venules. Since human plasma level of ET is around 1.5 pg/ml, the circulating ET may not induce vasoconstriction. On the other hand relatively low dose of arginine vasopressin or angiotensin II induces ET-production. Therefore, in physiological state, probably angiotensin II or arginine vasopressin stimulates the endothelial cell to produce ET, and the ET released into the muscular layer may induce and maintain the vascular tone.

Molecular Mechanisms of Vascular Diseases

Molecular Biology of Vascular Smooth Muscle

G 005 STRUCTURE AND EXPRESSION OF SMOOTH MUSCLE MYOSIN HEAVY CHAINS. M. Periasamy, P. Babij, and S. White. Dept. Physiology & Biophysics, Univ. Vermont, Burlington, VT 05405

We have isolated and characterized two distinct cDNA clones encoding smooth muscle myosin heavy chain (SMHC) from rat & rabbit smooth muscle tissues. These two SMHC cDNAs were identical for the most part but diverged at the 3' coding and in a portion of 3' UT regions. One type of cDNA clone (rat-RAMHC21) encoded 43 unique amino acids from the point of divergence of the two cDNAs. The second type of cDNA clone (rat-RAMHC-15) encoded a shorter carboxyl terminus of 9 unique amino acids and was the result of a 39 nucleotide insertion. Genomic cloning and DNA sequence analysis of the SMHC gene from rat indicated that the two types of cDNA clones, were generated from a single gene by the mechanism of alternate RNA splicing of a single exon (39 nt) at the carboxy-terminus. Western blotting analysis using antibodies raised against unique carboxyl terminus peptide of the two SMHC clones established that the two cDNA clones correspond to SM1 and SM2 myosin proteins previously identified.

To further understand the physiological relevance of the two MHC isoforms, we have analyzed their expression during smooth muscle development, and in animals with spontaneous hypertension and thyrotoxic treatment. The expression of the SMHC isoforms are developmentally regulated, and SM1 appears to be the predominant form in fetal smooth muscle (both in rat and rabbit) and the level of SM2 gradually increases through neonatal stages. Both isoforms are coexpressed in most smooth muscle tissues and their ratio is about 60% to 40% and may vary slightly depending on the tissue. Further the expression of SM1 and SM2 isoforms are not drastically altered in the vascular smooth muscle tissus of spontaneously hypertensive rats. Hyperthyroidism in rats induced significant reduction in the MHC mRNA levels in most smooth muscle tissues but did not alter the MHC mRNA level in the aortic smooth muscle. On the other hand, protein analysis indicated no significant change in the MHC protein content (SM1/SM2) in these tissues. The above data indicates that thyroid hormone may preferentially affect the mRNA stability without altering the relative abundance of the MHC protein.

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Babij P & Periasamy M (1989) J. Mol. Biol. 210:673.

Growth Factors and Signal Transduction (joint)

G 006 CHRONIC INFLAMMATION, PDGF, TGFß, AND SMOOTH MUSCLE PROLIFERATION, Russell Ross, Edouard J. Battegay, Elaine W. Raines, Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195

Atherosclerosis is thought to be a specialized form of chronic inflammation. Smooth muscle proliferation is a hallmark of atherosclerosis and is responsible for restenosis following bypass surgery and PTCA. A number of cells present in developing lesions of atherosclerosis, including activated endothelium, smooth muscle, monocyte/macrophages, and platelets, are capable of elaborating a number of growth-regulatory molecules, including the different dimeric forms of PDGF and at least one form of TGFß. Both PDGF-B-chain transcript and protein and TGFß transcripts are increased in lesions of atherosclerosis. In cultured quiescent smooth muscle cells, TGFß modulates smooth muscle proliferation by altering expression of both PDGF A-chain and both PDGF cell-surface receptors. The amount of TGFß per cell determines whether the smooth muscle response to TGFß is proliferation or inhibition of proliferation. Released PDGF is cleared very rapidly from the circulation. However, smooth muscle cells and endothelial cells can selectively store PDGF on extracellular binding sites. This extracellular compartmentalization appears to be mediated through the alternatively spliced exon 6 of the PDGF-A chain and a homologous region in exon 6 of the PDGF-B chain. Thus directional migration and proliferation of smooth muscle in response to PDGF can be modulated by other growth-regulatory molecules and by the form of PDGF secreted. Such regulation may determine the outcome of chronic inflammation that precedes and accompanies fibroproliferative diseases such as atherosclerosis. Supported in part by NIH grants HL-18645 and HL-03174.

G 007 TGF-β IN THE CARDIOVASCULAR SYSTEM. Michael B. Sporn, Anita B. Roberts, Thomas S. Winokur, Kathleen C. Flanders, James K. Burmester, Ursula I. Heine, Lalage M. Wakefield, David Danielpour, Su Wen Qian

The three isoforms of TGF-β (TGF-β1, -β2, and -β3) are omnipresent, multifunctional peptide signalling molecules. Recent studies indicate that they play an important role both in the development and the activities of the cardiovascular system. Their normal and pathologic physiology in both the heart and the peripheral vascular system will be discussed. Special emphasis will be given to the role of TGF-β in response to ischemic or anoxic injury of the heart.

G 008 MOLECULAR MECHANISMS OF TRANSCRIPTIONAL REGULATION IN YEAST, Kevin Struhl, Ph.D., Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

The yeast GCN4 protein binds to upstream promoter sequences of 30-100 genes involved in amino acid biosynthesis and coordinately induces their transcription in response to amino acid starvation. GCN4 binds as a dimer to a 9-bp region, with optimal binding to the palindrome ATGACTCAT. The 60 C-terminal amino acids of GCN4 are sufficient for specific DNA binding and also for dimerization. The GCN4 DNA-binding domain is similar in sequence to the jun oncoprotein, the oncogenic version of the vertebrate AP-1 transcription factor. Moreover, GCN4 and jun bind the same DNA sequences, and jun efficiently activates transcription in yeast cells indicating a basic similarity in the molecular mechanism of eukaryotic transcriptional activation. The GCN4 DNA-binding domain contains a "leucine zipper" region that is sufficient for dimerization; however, the leucine residues are not critical.

In addition to the DNA-binding domain, transcriptional activation by GCN4 requires a short acidic region in the center of the protein. Acidic regions of 35-40 amino acids are sufficient for full activation when fused directly to the DNA-binding domain. The activation region is a repeated structure composed of small units that act additively which presumably interacts with other proteins of the transcriptional machinery.

The *his3* promoter contains two functionally distinct TATA elements, T_R and T_C, but only T_R can activate transcription in combination with GCN4 or GAL4. The sequence TATAAA is sufficient for T_R to activate transcription, but almost all single bp substitutions abolish function. Interestingly, the TATTTA double mutant is functional and 3 mutations activate transcription in combination with GCN4 but not GAL4. Finally, a wide variety of sequences completely unrelated to TATAAA can serve as the T_R element. These observations suggest that multiple proteins can perform the TATA function for transcriptional activation.

G 009 FOS-JUN STORY, Lynn J. Ransone, Jane Visvader, V.J. Dwarki, Kim Morley, Penny Wamsley and Inder M. Verma, Molecular Biology & Virology Laboratory, The Salk Institute, La Jolla, CA 92037.

Gene expression is modulated by the specific interaction of nuclear proteins with unique regulatory sequences in the genome. Nuclear oncoproteins fos and jun (AP-1) cooperate in forming a very stable heterodimeric complex that binds to the AP-1 site with increased affinity. The "leucine zipper" domain of both fos and jun is necessary for the formation of this heterodimer. We have undertaken a mutational analysis of (1) individual leucine residues, (2) neighboring amino acids within the "leucine zipper" domain, and (3) residues in the highly basic domain of both the fos and jun proteins, to examine the contribution of these amino acids to the formation of a stable fos/jun heterodimer, the formation of jun homodimers and the binding potential of the heterodimeric complex to the AP-1 site. Mutations of single residues within the "leucine zipper" domain had no effect on protein complex formation. However, mutagenesis of the first leucine of the heptad repeat in either fos or jun drastically reduced binding of the complex to DNA. Mutations within the fos and jun basic regions and alteration of the spacing between the basic and "leucine zipper" domains indicate that the basic region of fos has a crucial role in determining the DNA binding affinity of the transcriptional complex. This is further substantiated by making site specific mutations in the basic amino acids in fos protein. We have also generated fos-jun chimeras to access the role of "leucine zipper" in the formation of homodimer. Mutation in the leucine zipper domain affects not only the protein-protein association, but also DNA binding. Conversely mutation in the DNA binding domain influences Fos-Jun heterodimer formation. We have generated DNA binding mutants of Jun (JunΔRk) which are functional transdominant negative mutants. F9 cells producing JunΔRk cannot be differentiated by retinoic acid. The role of fos/jun complex in transcriptional transactivation will be discussed.

Angiogenesis and Vasculogenesis in Developmental Biology

G 010 THE bFGF SYSTEM IS INVOLVED IN VASCULAR DEVELOPMENT AND CAN BE MANIPULATED TO INHIBIT SMOOTH MUSCLE PROLIFERATION, Ward Casscells, M.D., NIH, BETHESDA, MD

In bioassays, administered bFGF promotes mesoderm induction, angiogenesis, neuronal survival and neurite extension, specific endocrine functions and proliferation of most cells of mesodermal, ectodermal and even endodermal origin. These properties are shared by other members of the FGF family and non-FGF factors as well. The discovery of VEGF and PD-ECGF (angiogenic factors which are mitogenic only for endothelial cells) together with the inability of anti-bFGF antibodies to inhibit tumor angiogenesis, has raised doubts as to whether endogenous bFGF is involved in natural angiogenic processes. By heparin-affinity chromatography, mitogen assays, Northern and Western blots and immunohistology using multiple specific antibodies for bFGF, we find expression of bFGF in capillaries invading and proliferating in developing organs such as heart and spinal cord, and also after coronary ligation in the adult rat. An increase was also noted when endothelial monolayers were wounded in vitro, and the resulting migration and proliferation were inhibited by anti-bFGF antibodies. Thus, some role for bFGF in angiogenesis is likely.

No data have implicated bFGF in smooth muscle development or atherogenesis; some groups report no mitogenic or chemotactic effect of bFGF on smooth muscle cells (SMC). We find, by Northern analysis and in situ hybridization, Western analysis, immunohistology and radioligand binding, that bFGF and FGF receptors are expressed in embryonic vascular SMC. Adult SMC express these mRNAs and proteins after balloon injury or on culturing. We also find bFGF to be a potent mitogen for SMC in low serum conditions. Yet antibodies that neutralized added bFGF caused little inhibition of SMC proliferation in either optimal or suboptimal FGF-free serum, perhaps due to intracrine bFGF function or use of growth factors other than bFGF. Thus, anti-bFGF antibodies are unlikely to prevent SMC proliferation in vivo. But the ribosome inactivator saporin, once conjugated to bFGF, entered and killed proliferating (but not quiescent) SMC in vitro and in vivo; a single intravenous dose inhibited SMC proliferation after balloon injury.

In summary these data suggest roles for bFGF in angiogenesis and in smooth muscle proliferation during development and after vascular injury. Moreover, the re-expression of FGF receptors after vascular injury makes possible the selection ablation of SMC using a single self-targeting dose of a bFGF-toxin.

G 011 EMERGENCE OF ENDOTHELIAL CELLS DURING EMBRYOGENESIS, Françoise Dieterlen-Lièvre and Luc Pardanaud, Institut d'Embryologie Cellulaire et Moléculaire, 49bis, av. de la Belle Gabrielle, 94736 Nogent s/Marne Cédex, France.

Angiogenesis, actively investigated in adult normal or tumoral tissues, occurs by formation of new endothelial cells from preexisting ones. On the other hand, *de novo* emergence of endothelial cells in the embryo - conveniently termed vasculogenesis- is still an enigmatic process. The avian embryo is a favorable model, because of the various experimental approaches possible at the morphogenetic and organogenetic periods. Cells, tissues, territories or rudiments can be combined or exchanged *in ovo* between two closely related species, chick and quail. The rationale is based on the presence in quail nuclei, of a nucleolus-associated mass of heterochromatin that serves as a marker. Several monoclonal antibodies further make it possible to identify various cell lineages in one of the two species only. In particular we have developed a monoclonal antibody (mAb), QH1, which has affinity for the quail hemangioblastic lineage (endothelial and hemopoietic cells) (1). A central issue is to define an angioblastic anlage, i.e. to identify the mesodermal sublineage that gives rise to endothelial cells, to precise when and where it becomes determined, how organ rudiments acquire their angioblasts and how these collaborate with other precursors in vessel wall construction. By means of the quail nuclear marker or QH1 affinity detected after various experimental patterns (neural anlage exchange, yolk sac chimeras, organ rudiment transplantation), the following points have been established: 1) endothelial precursors arise in the area vasculosa as well as the area pellucida of the early blastodisc; 2) mesectodermal cells, derived from the head neural crest, have no vasculogenic capacity but do give rise to pericytes; 3) during organogenesis, mesoderm behaves differently according to the germ layer it is associated with: emergence of endothelial cells appears restricted to mesoderm which contacts endoderm transitorily or permanently, while mesoderm associated with the ectodermal germ layer is colonized by extrinsic angioblasts (2). Another question mark concerns the existence of a common precursor for endothelial cells and hemopoietic cells, the hemangioblast. The product of two nuclear oncogenes, c-*ets*1 and c-*myb*, respectively expressed during differentiation of these two lineages (3), may serve as markers to discriminate early steps of divergence.

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(3) Vandenbunder B., Pardanaud L., Jaffredo T., Mirabel M.A. and Stéhelin D. Complementary patterns of expression of c-*ets*1, c-*myb* and c-*myc* in the blood-forming system of the chick embryo. Development, 1989, 106, 265-274.

G 012 REGULATION OF VASCULAR SMOOTH MUSCLE CELL DIFFERENTIATION, Gary K. Owens, Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia, 22908

There is considerable interest in identifying factors and mechanisms that regulate the differentiation of vascular smooth muscle cells (SMC) during both normal development of the vascular system, as well as in various pathological states such as atherosclerosis, and pulmonary hypertension - diseases characterized by accelerated proliferation of SMC and alterations in SMC phenotype. This presentation will focus on two areas of investigation in my laboratory in which we have explored control of SMC differentiation.

The first involved examination of the interrelationships between growth state and differentiation in SMC, and in particular, determination of the effects of platelet derived growth factor (PDGF) isoforms and other mitogens on expression of SMC-specific contractile proteins in cultured rat aortic SMC. Results demonstrated that human (platelet-derived) PDGF-, and rPDGF BB-induced mitognesis was associated with marked decreases in expression of a number of SM specific contractile proteins including SM alpha-actin, SM myosin heavy chains, and SM alpha-tropomyosin at both the mRNA and protein levels. In contrast, serum-induced proliferation was not associated with reductions in SM alpha-actin expression, suggesting that suppression of muscle specific contractile protein expression in SMC, unlike in skeletal muscle, may not be obligatory for cell cycle entry. Of particular interest, PDGF AA was not mitogenic for these SMC, but induced an increase in SM alpha-actin, and SM myosin heavy chain expression. Results demonstrate a differential effect of PDGF isoforms in SMC, and suggest a possible role for PDGF isoforms in control of SMC differentiation.

The second involved development and characterization of an inducible SMC lineage system that could be used to attempt to isolate master differentiation control genes or lineage determination genes for SMC (see related Abstract by Owens et al. in this volume). Clonal lines of cells were isolated from retinoic acid treated mouse P19 embryonal carcinoma cells that expressed SM alpha-actin, SM myosin heavy chain, as well as responsiveness to a variety of contractile agonists. Results provide evidence that SMC lineages can be induced by retinoic acid treatment of P19 embryonal carcinoma cells, and establish the utility of this system for attempting to isolate lineage or master differentiation control genes for SMC.

Supported by grant R01 HL-38854 from the National Institutes of Health.

G 013 MANIPULATING VASCULAR DEVELOPMENT IN EMBRYONIC STEM CELLS AND CHIMAERIC MICE.

R. Lindsay Williams, Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Parkville, Victoria 3052, Australia.

Embryonic stem (ES) cells, the totipotent outgrowths of preimplantation embryos can be cultured in vitro and will develop normally forming all somatic and germ cell tissues when introduced into the embryonic environment. Thus ES cells provide a source of early embryonic cells for studying embryonic differentiation and development in culture. Furthermore they provide an efficient method for the introduction of both dominant and recessive mutations into mice with a number of advantages over more conventional techniques for generating transgenic mice.

Studies of the c-src family of tyrosine kinases have been carried out using retrovirus vectors which express src mutants or middle T antigen (which increases the tyrosine kinase activity of the endogenous $pp60^{c\text{-}src}$). ES cells have been derived which stably express either v-src (in collaboration with C. Boulter, University of Cambridge, UK) or middle T antigen. Chimaeric embryos derived from ES cells expressing middle T antigen initially develop normally, however, these embryos fail to develop beyond mid-gestation. This is due to the formation of multiple endothelial tumours, haemangiomas, which disrupt blood vessel formation in the developing foetus and yolk sac (1). Endothelioma cell lines derived from these lesions induce haemangiomas within hours of injection into rodents or chicken and quail embryos. The rapidity of tumour formation appears to be due to the to the recruitment of non-proliferating host endothelial cells into the haemangioma (2). In contrast v-src does not promote endothelial tumours in chimaeric embryos or mice suggesting that the tissue specificity of tumour formation of these two potent oncogenes is different.

To investigate the events leading to haemangioma formation during embryogenesis we are currently attempting to develop an in vitro model for tumour formation. We are using the ability of ES cells to differentiate into embryoid bodies which resemble yolk sacs (3,4) to follow the events which occur during the formation of in vitro yolk sacs by ES cells expressing middle T antigen.

In a separate series of experiments specific growth control genes (including members of the c-src gene family) are being mutated by homoloogus recombination in ES cells so as to introduce null mutations of these genes into mice. Using this methodology we hope to identify genes which play a crucial role during embryonic development. The latest results obtained in these experiemts will be reported.

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Structural Modulation by Hemodynamic & Vasoactive Stimuli

G 014 **FLOW MODULATION OF ENDOTHELIAL STIMULUS-RESPONSE COUPLING AND TRANSDUCTION.**

Peter F. Davies. Department of Pathology, Pritzker School of Medicine, The University of Chicago, Chicago, IL60637.

At the interface between flowing blood and the arterial wall, a confluent monolayer of endothelial cells operates as a signal-transduction system for hemodynamic forces associated with flow. Such forces are involved in the physiological regulation of vessel tone, hypertension, and the localization of focal atherosclerotic lesions. Investigations of the effects of shear stress, pressure and stretch upon endothelial biology have therefore been conducted with a view to linking the precise flow forces to the vessel wall biology.

Hemodynamic forces can be resolved into two principal components; (1) pressure, acting perpendicular to the endothelial surface, and (2) shear stress, the frictional force tangential to the cell surface in the direction of flow. The endothelium is the major recipient of wall shear stress, while underlying smooth muscle cells and the extracellular matrix, together with the endothelium are subjected to pressure. Stretch is associated both with shear stress and pressure. By the use of in vitro techniques, together with correlative in vivo studies, a catalog of endothelial responses to defined flow forces has been established. Some responses occur within milliseconds, others over periods of days. Flow sensing structures in the cell have not been identified. It is unclear whether or not the rapid responses are primary flow sensing mechanisms essential for the later structural adaptations to flow. However using ATP-induced intracellular calcium mobilization as a model for the acute responses to flow noted in the presence of agonists, dual mechanisms of flow sensing by endothelial cells have been identified. In the first, flow influences the effectiveness of agonist-receptor association by modulating the mass transport of agonist (ATP) in the diffusion boundary layer adjacent to the cell surface. In the second, flow forces exert direct effects upon the cell which influence calcium homeostasis. Recent studies of adhesion sites at the basal surface of endothelial cells have revealed rapid changes in their distribution when the cells are subjected to flow. Real time measurements of adhesion in living cells using confocal image analysis suggests that focal adhesion plaques may be involved in the flow responses.

G 015 MECHANICAL DETERMINANTS OF INTIMAL THICKENING, Seymour Glagov, Christopher K. Zarins, Hisham Bassiouny and Don P. Giddens; University of Chicago and Georgia Institute of Technology

Arteries adapt to sustained changes in wall shear stress or tensile stress by alterations in dimension, configuration and composition. Increased flow rate causes arteries to enlarge until lumen radius results in restitution of normal baseline wall shear stress of about 15 dynes/cm^2. Reductions in flow result in narrowing of the lumen to increase flow rate and maintain baseline wall shear stress. Evidence from both human vessels and experimental models indicate that this response may include intimal thickening. At the human carotid bifurcation, intimal thickening occurs in a region of relatively low wall shear stress, flow separation and complex departures from axially aligned, unidirectional flow. About experimental coarctations in the monkey, intimal thickening was inversely related to the level of wall shear stress. Since wall shear rate is inversely proportional to the third power of the radius, a small change in effective radius is usually sufficient to reestablish baseline values. Increases in effective radius result also in increased mural tension. Compensatory increases in mural composition may include intimal widening. Computations of mural tensile stress which take into account only the width of the media may yield values which are abnormally elevated compared to regions without intimal thickening. If, however, media and intima are taken as total wall thickness, mural tensile stress may approach normal values for the particular location. Reactive intimal thickening occurs in two principle morphologic forms: a) intimal fibromuscular hypertrophy (IFH), an orderly, layered, usually self-limiting, widening which includes smooth muscle cells and matrix fibers, echoing the architecture of the artery media, and b) intimal hyperplasia (IH), a fairly uniform accumulation of cells with smooth muscle and/or myofibroblast features, often in a myxoid stroma, with some formed fibers, but without a well-differentiated layered architectural organization. Localization of IH at anastamoses or where vessels are deformed suggests that an adaptive, well differentiated IFH reaction may be prevented by persistent local low shear conditions, such that the reaction proceeds to occlusion or distortion incompatible with adequate flow. Transitions and superimpositions among IFH, IH and atherogenesis (AP) would be expected to occur in response to modifications of flow, wall tension and geometry and in relation to the imposition or modulation of clinical AP risk factors. Such transitions are found in human arteries. The molecular mechanisms which regulate these responses and define their limits remain to be illuminated.

G 016 **TENSION DEPENDENT CHANNEL ACTIVATION.** Frederick Sachs, Department of Biophysical Sciences, SUNY, Buffalo, N.Y. 14214

All cells respond to mechanical inputs, but the mechanisms for generating the responses are generally unknown. One mechanism that we do know about is ion channels sensitive to membrane tension. These channels appear in virtually all cells, and with varying selectivities. The most common selectivites are non-seletive cation and potassium selective. The channel gating may depent posti tively on tension (stretch-activated) or negatively (stretch-inactivated). We have been able to study the stress and strain in patches during channel activation and have been able to implicate a cytoskeletal linkage to the channels. The cytoskeletal linkage is neither actin nor tubulin based upon treatment with appropriate reagents. The physiological role of the channels has been difficult to determine because we have no highly selective blockers. Recently we have been able to record the stress induced calcium influx in intact heart cells using fluorescence microscopy. These results show that mild stress, comparable to the stress procduced by bringing a patch pipette in contact with a cell, can produce enough calcium influx to set off a wave of calcium-induced-calcium release. Such stresses are likely to be encountered inthe normal activation of the heart and perhps insmooth muscle as well. The imaging technique may be beginning to teach us the role of the channels in cells and tissues.

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Lipoproteins and Other Risk Factors on Vascular Pathobiology

G 017 HYPERCHOLESTEROLEMIA, LIPOPROTEINS, AND ENDOTHELIAL CELL DYSFUNCTION. Richard A. Cohen, M.D., Vascular Biology, Boston University School of Medicine, Boston, MA. 02118.

The normal coronary artery endothelium releases vasodilators and vasoconstrictors which regulate vasomotor tone. Previous studies have demonstrated that atherosclerosis in man as well as that induced by hypercholesterolemia in animal models, is associated with abnormalities of endothelium-dependent vasodilation. Relaxations caused by some, but not all receptor-mediated endothelium-dependent agents are impaired, whereas those to the calcium ionophore, A23187, are relatively preserved. This suggests that atherosclerosis causes selective endothelial cell dysfunction which can not be explained by intimal thickening or damage. Endothelial cells exposed to hypercholesterolemia for 10 weeks *in vivo* demonstrate similar abnormalities without atherosclerosis. To determine if endothelial cell dysfunction in hypercholesterolemia is caused by exposure to elevated levels of low density lipoproteins (LDL), the vasoactive effects of LDL have been studied *in vitro* on isolated normal arteries and selectively by inhibiting the release of endothelium-derived relaxing factor (EDRF). In cultured endothelium, LDL increase cell calcium, and inhibit the agonist-induced production of EDRF. The primary vasoactive effects of LDL *in vitro* are due to its trace metal catalyzed oxidation by free radicals and may be prevented by lipid soluble antioxidants and antioxidant enzymes. Hypercholesterolemia is accompanied by increases in peroxidation of lipids, and prevention of peroxidation during hypercholesterolemia *in vivo* may restore endothelium-dependent relaxations. Thus, endothelial cell dysfunction caused by hypercholesterolemia may be explained by actions of LDL on the endothelium.

G 018 LIPOPROTEIN MEDIATED EVENTS IN EARLY ATHEROGENESIS, Alan M. Fogelman, Judith A. Berliner, Mahamad Navab, Ali Andalibi, Feng Liao, Susan Imes, Linda L. Demer, Susan D. Cushing, Jeong Kim, Mary Territo and Aldons J. Lusis, Department of Medicine, University of California Los Angeles School of Medicine, Los Angeles, CA 90024-1679.

Minimally modified low density lipoprotein (MM-LDL), derived by mild oxidation has been shown to induce certain inflammatory responses in vascular cells in tissue culture. These include: induction of monocyte (but not neutrophil) adherence to endothelial cells (EC), induction of EC production of colony stimulating factors (CSF), and induction of EC and smooth muscle cell production of monocyte chemotactic protein (MCP-1) (1-3). In vivo, μg quantities of MM-LDL injected into mice caused CSF activity to increase from approximately 300 CFU/ml to 2,000 CFU/ml 5 h after injection of MM-LDL but remained near control levels after injection of native LDL. Additionally, within 4 h after injection of MM-LDL, mRNA for JE, the mouse homologue of MCP-1, was markedly induced in various tissues, and scanning electron microscopy revealed a significant increase in the number of leukocytes adherent to the aortic endothelium. In an artery wall model where human aortic endothelial and smooth muscle cells are separated by a collagen layer, LDL stimulates migration of monocytes into the collagen layer even in the presence of serum. This stimulation is blocked by inhibitors of oxidation and by HDL suggesting that the co-cultures can modify LDL and that this modified LDL induces migration. In vivo and in vitro cells exhibit differential sensitivity to the effects of MM-LDL. Preliminary work in vitro suggests that a determinant of this sensitivity is the level or the inducibility of manganese dependent superoxide dismutase in the cells.

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G 019 THE ROLE OF APOLIPOPROTEIN(a) IN ATHEROSCLEROSIS

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Numerous studies have shown that elevated levels of the plasma lipoprotein Lp(a) are correlated with an increased risk of atherosclerosis. There is a thousand-fold variation in Lp(a) levels in the population. Most of the variation of plasma concentration is genetically determined and refractory to diatary and drug manipulation. Lp(a) closely resembles low density lipoprotein [LDL], but contains a distinguishing protein, apolipoprotein(a) [apo(a)]. Sequence determination revealed that apo(a) contains unexpected similarity to plasminogen, marked by numerous repeats that resemble the fourth kringle domain of plasminogen. Apo(a) is a large glycoprotein that exhibits great variation in size which is due to inherited differences in the number of repeated domains.

The normal and pathological roles of Lp(a) remain obscure, but its structure has suggested some link between thrombosis and plaque development. Other investigators have shown that Lp(a) binds endothelial cells and components of blood clots and extracellular matrix, including partially degraded fibrin and firbonectin. We have demonstrated high affinity binding of Lp(a) to macrophages. Each of these properties could lead to the development of atherosclerosis by interfering with the normal process of clot lysis or by delivering cholesterol-laden particles to the interior of blood vessels.

Molecular Mechanisms of Cell-Cell Interactions

G 020 MOLECULAR GENETIC STUDIES OF INTERCELLULAR ADHESION MOLECULE -1 IN THE MOUSE,

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Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily and has been shown to bind to LFA-1, which is a member of the integrin superfamily. ICAM-1 is felt to play a critical role in endothellal leukocyte interaction and thus may play an important role in vascular pathophysiology. As a step towards genetic manipulations of ICAM-1 in the mouse we cloned and sequenced the cDNA for ICAM-1. The cDNA has been used to study the time course and tissue distribution of mRNA levels of ICAM-1 in the mouse after stimulation with endotoxin. The cDNA has also been use to map and clone the gene for murine *Icam-1*. Five overlapping lambda clones were obtained by screening several mouse genomic libraries. These clones span the entire gene and include approximately 6 kb of 5' flanking sequence and 12 kb of 3' flanking sequence. With over 95% of the exon material sequenced, the *Icam-1* gene appears to be composed of 7 exons. Exon 1 contains the translation start signal and the entire peptide leader sequence. Exons 2 through 6 contain sequences that encode for immunoglobulin domains 1 through 5 respectively. Each exon encodes for a single immunoglobulin domain. Exon 7 contains sequence encoding the transmembrane domain, cytoplasmic region, and the polyadenylation site. Two genomic clones have been used to develop a construct to disrupt *Icam-1* in the mouse using homologous recombination in embryonic stem cells.

G 021 DEVELOPMENTAL, MICROENVIRONMENTAL, AND ACTIVATION-LINKED REGULATION OF CELL ADHESION MOLECULES INVOLVED IN LEUKOCYTE TRAFFICKING, Eugene C. Butcher, M.D., Louis J. Picker, Mark A. Jutila, T. Kei Kishimoto, Ellen L. Berg; Department of Pathology, Stanford University, Stanford, California 94305-5324 Leukocyte-endothelial cell recognition/adhesion regulates leukocyte trafficking *in vivo*. A number of leukocyte and endothelial cell surface molecules important in leukocyte-endothelial recognition have been identified. These include members of known cell adhesion molecule families (the immunoglobulins, the integrins) as well as two novel classes of cell adhesion molecules. The lymphocyte surface homing receptor for peripheral lymph nodes and related inducible endothelial cell ligands for neutrophils (ELAM-1 and GMP140), define a new lectin-related adhesion molecule family. H-CAM (CD44, the Hermes antigen), a widespread molecule involved in lymphocyte-HEV interactions, is a cell surface adhesion molecule related to cartilage proteoglycan core and link proteins. In addition, tissue-specific vascular adhesion molecules for circulating lymphocytes, the "addressins", have been identified in mucosal lymphoid organs and in peripheral lymph nodes and skin. Functional studies imply the existence of a number of other cell adhesion molecules which remain to be identified.

Leukocyte-endothelial cell interactions demonstrate exquisite tissue-, inflammation-, and cell type-specificity. This specificity is determined 1) by the developmental regulation of these diverse cell adhesion molecules, both during leukocyte and endothelial cell differentiation and activation; 2) by the regulated expression of some of these adhesion molecules in response to local microenvironmental stimuli - - - e.g., induction of neutrophil and monocyte adhesion molecules on endothelial cells by cytokines; and 3) by modulation of the function of cell adhesion molecules at the cell surface---for example, the activation-linked enhancement of Mac-1 function, and simultaneous proteolytic cleavage of the lymph node homing receptor (the MEL-14 antigen) during neutrophil activation at sites of extravasation during acute inflammation. The regulation of these cell adhesion events by developmental, microenvironmental, and activation-linked mechanisms provides an intriguing model for the determination of cell-specific heterotypic interactions, and is clearly of central importance in regulating local inflammatory and immune responses.

G 022 LEUKOCYTE INTEGRIN INTERACTION WITH INTERCELLULAR ADHESION MOLECULES. Michael S. Diamond, Antonin R. de Fougerolles, and Timothy A. Springer. Department of Pathology, Harvard Medical School, and The Center for Blood Research, Boston, MA 02115.

The leukocyte integrin family is comprised of three members, LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150,95 (CD11c/CD18), and is responsible for adhesive events in the immune system. Intitally, we observed that LFA-1 bound to purified ICAM-1 (CD54) and this interaction could explain some of the adhesive events in the immune system including T cell and NK cell killing, and leukocyte adhesion to endothelial cells. This presentation will focus on two interactions between leukocyte integrins and intercellular adhesion molecules: Mac-1-ICAM-1 and LFA-1-ICAM-2. Using a transfectant cell adhesion assay to purified protein coated to plastic, we find that ICAM-1 is a counter-receptor for Mac-1; this interaction may explain one mechanism of how activated neutrohils attach to stimulated endothelial cells. However, this adhesion differs from LFA-1-ICAM-1, in its temperature dependence, avidity, and in the individual antibodies which block the interaction. Previously, using an expression cloning system, we found a second molecule, ICAM-2, that served as a counter receptor for LFA-1. Two monoclonal antibodies have been raised and with these we have characterzed the cell surface expression, tissue distribution, and some of the functions of ICAM-2 in leukocyte-leukocyte and leukocyte-endothelial cell interactions.

Molecular Mechanisms of Vascular Diseases

Pathologic Interactions of Hemostasis/Thrombosis

G 023 PHARMACOLOGIC INHIBITION OF SUBENDOTHELIAL PLATELET DEPOSITION AFTER ARTERIAL INJURY, Tim A. Fischell, Aaron V. Kaplan, Wing-Hung Leung, Gordon W. Grant, Lawrence L-K Leung, Stanford University Medical Center, Stanford, CA, 94305.

Platelet deposition at the site of arterial injury may play an important role in both abrupt vessel closure and restenosis after balloon angioplasty (1). Using a perfused whole vessel *ex-vivo* model (2) we evaluated the ability of thrombin inhibition with D-Phe-Pro-ArgCH2Cl (PPACK) and/or inhibition of the membrane fibrinogen receptor (GPIIb/IIIa), with the monoclonal antibody 7E3 to reduce platelet deposition following balloon angioplasty-induced arterial injury. The effect of local delivery of PPACK into the arterial wall by an infusion balloon catheter was also evaluated. Fresh rabbit aortas were mounted in a perfusion bath. One-half of the mounted arterial segment was dilated with a 5 mm balloon angioplasty catheter, with the other half serving as the "Control" (uninjured) segment. The vessels were then perfused with human blood at physiologic pressure and shear rates for 30 minutes. The blood was treated with heparin (2U/ml, n=7), PPACK (10μM, n=4) or 7E3 (10μg/ml, n=4). In an additional set of experiments PPACK (10 μM, n=9) was locally delivered at the site of arterial injury via an infusion ("Wolinsky") balloon catheter. Platelet deposition in each segment was assayed using ^{111}In-labeled platelets and scanning electron microscopy. With heparin 8.2±2.2 x 10^6 plt/cm^2 were deposited at the site of balloon injury compared to 0.7±0.2 x 10^6 plt/cm^2 in the uninjured segments ($p<0.02$). Platelet deposition at the site of balloon injury was reduced by 47% by PPACK and 70% by 7E3, compared to heparin (p values <0.04 and 0.02 respectively for PPACK and 7E3). The local delivery of PPACK by the infusion balloon catheter also significantly reduced platelet deposition by 46% compared to heparin treated segments injured by conventional balloons ($p<0.01$). Reduction of platelet deposition in the PPACK and 7E3 treated segments was confirmed by scanning electron microscopy.
Conclusions: At shear rates observed in non-stenotic coronary arteries, 1) PPACK and 7E3 reduce platelet deposition at the site of arterial injury significantly more than heparin, 2) Inhibition of platelet deposition by PPACK demonstrates the importance of thrombin in platelet thrombus formation, 3) The 7E3 data suggest that ~70% of platelet deposition after arterial injury is GPIIb/IIIa dependent, with ~30% attributable to platelet-subendothelial adhesion, 4) Local delivery of PPACK by an infusion catheter is feasible and achieves an antiplatelet effect comparable to "systemic" delivery in our model.

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G 024 STRUCTURE AND FUNCTION OF PHOSPHATIDYL INOSITOL METABOLIZING ENZYMES, Philip W. Majerus, Washington University School of Medicine, St. Louis, MO 63110.
New pathways of inositol phosphate metabolism have been discovered and functions for these metabolites have been proposed. In particular phosphatidylinositols appear to serve important functions during cell proliferation and motility. We have recently defined a pathway for biosynthesis of the newly described 3-phosphate containing inositol phospholipids. These lipids have been found in many cell types and tissues and represent a distinct pathway of phosphatidylinositol metabolism. The turnover of 3-phosphate containing inositol lipids is accelerated by thrombin in platelets. We have recently defined the pathway for biosynthesis of these lipids in platelets utilizing a method in which we measured the rate of incorporation of phosphorus into each position of the inositol ring. In this way the pathway is defined as PI➡PI3P➡PI(3,4)P_2➡PI(3,4,5)P_3. In NIH 3T3 cells stimulated with PDGF the same pathway was found. The initial enzyme in this pathway, phosphatidylinositol 3-kinase, was originally discovered in immunoprecipitates from transformed cells. However, we now find that this enzyme forms complexes in platelets in response to thrombin. Thus, formation of these lipids may serve a function distinct from growth. Recent studies suggest that 3-phosphate inositol lipids function as lipids and participate in actin polymerization and cytoskeletal rearrangements. Inositol cyclic phosphates have also been found to participate in control of cell proliferation. The isolation of cyclic hydrolase cDNA has allowed transfection of this enzyme into NIH 3T3 cells to overexpress hydrolase. These studies indicate that cells with increased cyclic hydrolase grow to a lower density at confluence than control cells. This relationship has been found in many cell types with differences in cyclic hydrolase ranging over 100-fold. Those cells with high cyclic hydrolase and correspondingly low levels of cyclic inositol phosphate grow to lower density at confluence than low enzyme expressing cells. These studies suggest that inositol cyclic phosphate levels determine the density to which cells can grow. Thus cyclic hydrolase is anti-proliferative and could be an example of an anti-oncogene.

G 025 Endothelial Cell Fibrinolytic Assembly: Modulation by Lp(a). Ralph L. Nachman, Cornell University Medical College, New York, N.Y. 10021.

Endothelial cells play a critical role in thromboregulation by promoting the assembly of a membrane bound fibrinolytic system. Cultured endothelial cells synthesize and secrete t-PA which can bind to a specific endothelial cell surface receptor. The membrane attachment or binding site preserves the catalytic activity of t-PA and protects it from its major physiological inhibitor (PAI-I). N-terminal glutamic acid plasminogen (Glu-PLG), the main circulating fibrinolytic zymogen, also interacts specifically with the endothelial cell surface. Binding is associated with an approximately 10-fold increase in catalytic efficiency of plasmin generation by t-PA which reflects conversion of Glu-PLG to its plasmin-modified form, N-terminal lysine plasminogen (lys-PLG). Endothelial cells in culture as well as cells in vivo readily convert surface Glu-PLG to Lys-PLG. This modification of circulating plasminogen at the endothelial cell surface further enhances the fibrinolytic potential of the blood vessel wall.

Lipoprotein(a) (Lp(a)), an LDL like particle that contains the plasminogen-like apolipoprotein a (apo (a)) is elevated in patients with atherosclerotic coronary artery disease. Lp(a) competitively inhibits plasminogen binding to the endothelial cell and interferes with surface-associated plasmin generation. Lp(a) accumulates in atherosclerotic lesions and is present immunohistochemically in the microvasculature of inflammatory tissues. In cultured endothelial cells, Lp(a) enhanced PAI-I antigen, activity and steady state 3.4 k and 2.4 kb mRNA levels without altering t-pa antigen or mRNA transcript levels. LDL treatment of endothelial cells selectively raised the level of the 3.4 kb mRNA species of PAI-1 without a concomitant increase in PAI-I activity or antigen. Endothelial cell exposure to Lp(a) did not cause generalized endothelial cell activation since the functional activity and mRNA levels for tissue factor, platelet derived growth factor (PDGF) and interleukin-6 (Il-6) were not elevated following Lp(a) exposure. These data suggest a molecular mechanism whereby Lp(a) may support a specific prothrombotic endothelial cell phenotype, namely by increasing PAI-I expression. Vascular deposition of Lp(a) may provide a link between impaired cell surface fibrinolysis, chronic thrombogenesis and progressive atherosclerosis.

Models and Mechanisms of Vascular Injury and Remodeling

G 026 MECHANICAL FACTORS INFLUENCING ARTERIAL GROWTH CONTROL, B. Lowell Langille, Department of Pathology, University of Toronto and Max Bell Research Centre,The Toronto Hospital, Toronto, Ontario, Canada, M5G 2C4.

Arteries adapt to chronic changes in blood flow through endothelium-dependent adjustments in vasomotor tone, then by remodeling of the vessel media. Early vasodilation in response to increased perfusion is mediated by EDRF, but an unknown vasoconstrictor contributes to responses to decreased blood flow. In rabbits, remodeling of mature arteries entrenches diameter changes with 1-2 weeks, but this entrenchment occurs without net changes in major wall constituents, as assessed by measurements of vessel wall elastin, collagen and DNA levels.

In contrast, experimental blood flow changes during development modulate subsequent arterial accumulation of wall constituents. This finding implies that developmental blood flow changes provide cues that adjust arterial growth rates to levels appropriate for the perfusion requirements of peripheral tissues. Accordingly, we used perinatal sheep preparations to show that the profound changes in blood flow distribution that occur at birth are accompanied by parallel modulation of arterial growth rates. Both cellular and matrix constituents of the vessel wall are affected. The studies also provided evidence for a novel mechanism of arterial adaptation to postnatal arterial pressure changes. Blood pressure rises from fetal levels of 45 mmHg to about 80 mmHg by 3 weeks post partum.

Taken together, these findings imply that diseases that alter vascular perfusion will influence arterial structure, possibly in ways that affect disease progression. Such influences may be particularly important during pre- and postnatal vascular development. The cellular mechanisms that mediate blood flow-related growth modulation are poorly understood.

G 027 TRANSGENIC RATS: NEW MODELS FOR HYPERTENSION

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Primary hypertension is considered a polygenic inherited disorder with up to six genes involved. Much is known about the molecular biology of the renin-angiotensin system, one of the key components of blood pressure homeostasis, but the role it plays in the development of hypertension is still largely unknown. To investigate their individual roles in the physiology of blood pressure control, we are introducing RAS gene constructs into the germline of the mouse and the rat. Though widely used, transgenic technology is almost exclusively limited to the mouse since this species is genetically well characterised and its short generation time and ease of handling facilitate experimentation. To overcome size limitations of the mouse in certain areas of cardiovascular research, and to complement mouse studies already underway, we have established transgenic rats as a new tool for hypertension research.

We have succeeded in generating transgenic rats harboring the mouse Ren-2 gene in their genome. Two independent lines of transgenic rats have been established which exhibit extreme hypertension (systolic pressure 180-260 mm Hg). Since the hypertensive phenotype segregates with presence of the transgene, this suggests that expression of the mouse renin gene is responsible for the hypertension. A survey of tissues indicates that the transgene is expressed in a tissue-specific manner, the highest expression being found in the adrenal gland. Since plasma renin levels are relatively normal and kidney renin levels are lower than normal, these animals may represent a model for normal or low renin hypertension with high tissue renin. We have further characterised the morphology of these animals, and now demonstrate that they exhibit classic symptoms typical of the vascular damage due to extreme hypertension.

G 028 CELLULAR BASIS OF ABNORMAL VASCULAR STRUCTURE IN HYPERTENSION, Michael J. Mulvany, Niels Korsgaard. Department of Pharmacology, Bartholin Building and Department of Pathology, Aarhus County Hospital, Aarhus University, 8000 Aarhus C, Denmark.

This contribution will review work done in our laboratory concerning the effects of hypertension and of long-term antihypertensive treatment on media:lumen ratio and on media smooth muscle cell volume (V_{SMC}) in isolated segments of mesenteric resistance arteries (i.d. ca. 200 μm) from spontaneously hypertensive rats (SHRs). The work has been done using a myograph [1] and an unbiased stereological principle, the "disector" [2]. In untreated 12 wk old SHRs, media:lumen ratio is increased [3], but V_{SMC} was similar to that seen in control normotensive Wistar-Kyoto rats (WKYs). The increased media:lumen ratio seen in the SHR vessels appears in part to be due to "remodelling" (i.e. a redistribution of existing cells [4]). However, treatment of SHRs from age 4 to 24 wk with four different drugs (metoprolol (130 mg/kg/day), isradipine (42 mg/kg/day), captopril (60 mg/kg/day) or perindopril (1.5 mg/kg/day)) caused a small reduction in V_{SMC}, which correlated with the antihypertensive effect of the drugs [5]. Measurements using the myograph showed that, in the vessels examined, there was no effect of treatment either on media volume or - combining these data with the disector data - on the number of smooth muscle cells: the observed decrease in media:lumen ratio was due to remodelling. Induction of hypertension using the 1-kidney,1-clip Goldblatt technique resulted in an increase in both media:lumen ratio and in V_{SMC}, but no change in number of smooth muscle cells [6]. The results suggest that both genetic hypertension and antihypertensive treatment may affect resistance vascular structure primarily by remodelling existing smooth muscle cells. By contrast, some forms of experimental hypertension appear to affect vascular structure by a hypertrophic response.

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G 029 RESPONSE OF ARTERIAL WALL TO INJURY , Michael A. Reidy
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Injuries to the arterial wall are known to initiate smooth muscle cell replication and formation of intimal lesions and yet there is little information regarding factors which control this process. In a recent study we found that platelets were not essential for SMC replication following balloon catheter injury yet their absence did influence cell movement and lesion size. Changes in SMC replication, however, were observed when carotid arteries were totally denuded of endothelium by a procedure which caused little trauma to the underlying arterial wall (SMC Thymidine index 15% vs 1.5%). Platelets adhered to this denuded surface and transcripts for PDGF A-chain, PDGF receptor and TGF-ß changed in an identical manner to that observed in arteries subjected to balloon catheter injury. This result suggested that trauma to the arterial walll accompanied with release of endogenous factors might influence SMC growth. Addition of bFGF to animals was found to cause a dramatic increase in SMC replication of denuded arteries (11.5% vs 54.8%) and lead to an increase in size of intimal lesions. Furthermore administration of an antibody to bFGF immediately prior to balloon catheter injury significantly reduced SMC replication.These studies suggest that arterial injury may initiate SMC replication via the release of endogenous bFGF.(Supported by NIHHL 03174-35 and 41103.)

Clinical Implications and Future Directions

G 030 THE EFFECT OF HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS ON VASCULAR FUNCTION IN HUMANS, Mark A. Creager, M.D., Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

In vitro and in vivo studies in animals have suggested that cholesterol impairs endothelium-dependent vasodilation, even in the absence of atherosclerosis. To determine whether hypercholesterolemia impairs vasodilator function in humans, vascular reactivity was measured in forearm resistance vessels of normal subjects and patients with hypercholesterolemia (1). Each subject received intrabrachial artery infusions of methacholine, which releases endothelium-derived relaxant factor, and nitroprusside, which directly stimulates guanylate cyclase in vascular smooth muscle. Forearm blood flow was measured using venous occlusion plethysmography. Maximal vasodilator potential was determined during reactive hyperemia. The forearm blood flow response to methacholine was attenuated in hypercholesterolemic subjects, suggesting impaired endothelium-dependent vasodilation. In addition, the forearm blood flow response to nitroprusside was less in hypercholesterolemic subjects, indicating some impairment in endothelium-independent vasodilation. Reactive hyperemic blood flow was similar in each group of subjects. To determine whether endothelium-dependent vasodilation could be restored in hypercholesterolemia, subjects were treated with L-arginine, the precursor of nitric oxide. L-arginine augmented the forearm vasodilator response to methacholine, but not nitroprusside, in patients with hypercholesterolemia. It is concluded that 1) Endothelium-dependent vasodilation is impaired in resistance vessels of humans with hypercholesterolemia in the absence of atherosclerosis; and 2) L-arginine improves endothelium-dependent vasodilation in these individuals.

Additional studies were conducted in normal subjects and patients with peripheral atherosclerosis to determine whether endothelial function was impaired in resistance vessels of patients with atherosclerosis. Methacholine and nitroprusside were infused intra-arterially. Calf vascular resistance, via venous occlusion plethysmography, was determined at baseline, and during each drug infusion. The calf vascular resistance response to methacholine, but not nitroprusside was blunted in the patients with atherosclerosis. It is concluded that in humans with peripheral atherosclerosis, endothelium-dependent vasodilation is impaired in resistance vessels.

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G 031 GENE THERAPY OF CARDIOVASCULAR DISEASE. David A. Dichek. Molecular Hematology Branch, National Heart Lung and Blood Institute, Bethesda, MD 20892.

We are using retroviral vector-mediated gene transfer to develop new therapeutic strategies for the treatment of intravascular thrombosis and hypercholesterolemia. A retroviral vector containing a human t-PA cDNA was constructed and used to transduce adult sheep endothelial cells. Analysis of the conditioned medium of t-PA transduced cells using t-PA antigen and activity assays as well as zymography demonstrated a large increase in fibrinolytic activity (1-2 orders of magnitude) which is clearly attributed to the overexpression of human t-PA protein. In order to begin to test the ability of these fibrinolytically enhanced cells to prevent thrombosis in vivo we developed protocols for seeding the cells onto intravascular stents and vascular grafts and for quantitating cell retention after exposure to physiological flow conditions. In separate experiments a retroviral vector containing a human LDL receptor cDNA was constructed and used to transduce primary skin fibroblasts from Watanabe Heritable Hyperlipidemic rabbits (an LDL receptor deficient strain). Correction of the rabbits' genetic defect was confirmed by in vitro assays including immunoprecipitation and LDL binding. Autogenous gene corrected cells reimplanted into donor rabbits survived for at least 4 weeks in vivo, as determined by both immunohistochemistry and recovery of LDL-binding cells from the implants. The potential of these two approaches for the treatment of thrombosis and hypercholesterolemia will be discussed.

G 032 BASIC MECHANISMS CONTRIBUTING TO NEOINTIMAL PROLIFERATION FOLLOWING MECHANICALLY-INDUCED ENDOTHELIAL INJURY IN VIVO, James T. Willerson, The University of Texas Medical School at Houston and the Texas Heart Institute at Houston, TX 77030

We have developed a canine model in which neointimal proliferation occurs within 10-21 days following mechanical injury to the endothelium at sites of experimentally-created coronary artery stenosis. The neointimal proliferation developing in this model has the same morphologic characteristics as the neointimal proliferation found after balloon angioplasty.

We have studied 27 chronically-instrumented dogs following the placement of an external coronary constrictor around the proximal portion of the left anterior descending coronary artery. The application of the constrictor was associated with endothelial injury. A Doppler flow probe was placed proximal to the coronary artery stenosis so that continuous coronary flow velocity could be monitored. In these studies, we have found that there is a significant relationship between the frequency and severity of cyclic coronary flow alterations mediated by recurrent platelet attachment and dislodgement and the subsequent development of neointimal proliferation at the site of the coronary stenosis and endothelial injury ($r=0.87$, $p<0.01$). Animals with frequent and recurrent cyclic flow variations generally developed moderate or severe neointimal proliferation. In contrast, animals with infrequent or no cyclic flow variations generally developed minimal neointimal proliferation. In addition, combined therapy by bolus injection and constant infusion of thromboxane A_2 synthesis inhibitor and receptor antagonist and a serotonin receptor antagonist prevented cyclic flow alterations in the awake, unsedated animals with coronary stenosis and endothelial injury and prevented or markedly attenuated the neointimal proliferation in these same animals. These results suggest a close relationship between the magnitude of platelet deposition at sites of endothelial injury and coronary artery stenosis and the subsequent development of neointimal proliferation in this experimental model.

G 033 PHENOTYPIC CORRECTION OF HEPATIC LDL RECEPTOR DEFICIENCY. *J.M. Wilson, #C.H. Wu, *M. Grossman, +N.R. Chowdhury, #G.Y. Wu, and +J.R. Chowdhury, Departments of Medicine, *University of Michigan Medical School, Ann Arbor, MI, #University of Connecticut, School of Medicine, Farmington, CT, and +Albert Einstein College of Medicine, Bronx, NY.

Gene therapy is being considered in the treatment of several acquired and inherited diseases. Previous studies have focused primarily on bone marrow cells as targets for gene transfer, however, many diseases will require genetic modification of other somatic tissues such as the central nervous system or the liver. We have been developing methods for directing gene replacement therapies to the liver. Familial hypercholesterolemia (FH) is an autosomal dominant disorder in humans caused by a deficiency of the receptor for low density lipoproteins (LDL) that we have used as a model for developing liver-directed gene therapies. Features of this genetic disease that make it a particularly useful model include 1) the biochemical, pathophysiological, and clinical aspects of FH have been well-described and the corresponding normal gene is cloned and available, 2) phenotypic correction of the metabolic abnormalities associated with FH will probably require gene transfer into the hepatocyte because the liver is the primary organ responsible for degradation of LDL and the only organ capable of excreting cholesterol, 3) an authentic animal model for FH exists, the Watanabe heritable hyperlipidemic (WHHL) rabbit, and 4) no effective conventional therapy exists for homozygous deficient FH other than combined liver/heart transplantation.

We have used the WHHL rabbit to develop two different types of gene replacement therapies. The ex vivo approach to gene therapy involves harvesting hepatocytes from an LDL receptor deficient animal, transfecting a functional LDL receptor gene into the hepatocytes using recombinant retroviruses, and transplanting the genetically modified cells back into the affected animals. A potentially more effective and less morbid approach to the genetic treatment of FH is to target the delivery of a functional LDL receptor gene to hepatocytes in vivo. Hepatocyte targeting methods are based on interactions with the hepatocyte specific receptor, the asialoglycoprotein receptor. Progress in the development of ex vivo and in vivo gene replacement therapies for FH will be presented.

Late Abstract

SIGNAL TRANSDUCTION BY PDGF RECEPTORS INVOLVES DIMERIZATION AND ASSOCIATION WITH SIGNALING MOLECULES, L.T. Williams, J.A. Escobedo, V.A. Fried, H. Ueno, and W.M. Kavanaugh Howard Hughes Medical Institute and Cardiovascular Research Institute, University of California San Francisco, CA 94143 and Department of Cell Biology and Anatomy, New York University, Valhalla, New York 10595

To study the role of receptor dimerization in signal transduction we have used mutants of the PDGF β receptor (PDGFR) that bind PDGF but lack intrinsic kinase activity. When co-expressed with wild type receptors, these mutants formed heterodimers with wild type PDGFR in a PDGF-dependent manner. A heterodimer consisting of wild type PDGFR and a PDGFR with a truncated tyrosine kinase domain was defective in autophosphorylation, whereas the ligand-induced homodimer of wild type receptor was autophosphorylated. When co-expressed with wild type receptors in Xenopus oocytes, the mutant PDGFR abolished signal transduction by wild type PDGFR but did not affect signal transduction by wild type FGF receptors (FGFR). A similar mutant of the FGFR blocked signal transduction by wild type FGFR but not by PDGFR. These findings show that receptor dimerization is required for PDGF receptor signal transduction, suggest that autophosphorylation is intermolecular reaction between the components of a receptor dimer and establish a unique approach to inhibit receptor function in vivo.

Autophosphorylated PDGFR associates with several cellular molecules and phosphorylates some of them on tyrosines. We have recently focused on an 85 kDa protein (P85) that associates with ligand-activated PDGFR and is one of the major tyrosine-phosphorylated proteins in PDGF-stimulated cells. We purified P85 using tyrosine-phosphorylated PDGFR as an affinity reagent and cloned the cDNA that encodes P85. P85 contains two SH2 domains but no identifiable kinase motifs. Thus P85, like GTPase activating protein, may associate with PDGF receptor through its SH2 domains. We showed that P85 binds to a 16 amino acid segment of PDGFR the that contains phosphotyrosine at position 719. This segment of PDGFR also binds phosphatidylinositol 3 kinase (PI3 kinase), an enzyme that has been implicated in the regulation of growth of both normal and transformed cells. P85 expressed in cos cells competes with PI3 kinase and 110 kDa protein for binding to the receptor. Thus P85 is either a subunit of PI3 kinase or an antagonist that competes with PI3 kinase for binding to the receptor. Thus signaling seems to involve ligand-induced receptor dimer formation, intermolecular autophosphorylation of receptors, and the binding of SH2 domain-containing proteins to autophosphorylation sites on the receptor. The SH2 domains appear to recognize phosphotyrosine in a specific sequence context.

Molecular Mechanisms of Vascular Diseases

Molecular Biology of Smooth Muscle

G 100 DISPROPORTIONATE EXPRESSION OF NA,K-PUMP SUBUNIT mRNAs IN PROLIFERATING VASCULAR SMOOTH MUSCLE CELLS. J.C. Allen, C.L. Seidel, T.A. Pressley‡ S.S. Navran, and R.M. Medford† Dept. Medicine, Baylor College of Medicine, University of Texas Medical School‡ Houston, TX, and Emory University† Atlanta, GA.

When freshly dispersed smooth muscle cells from canine saphenous veins are seeded in 10% fetal calf serum there is an initial rise in cytoplasmic Na^+ due to a low number of Na pump sites. This is followed by an increase in pump site abundance and a reduction in Na, resulting in cell proliferation (Allen et al 1989. Am. J. Physiol. 256:C793). To assess the regulation of pump number and its role in vascular smooth muscle proliferation we measured pump alpha and beta subunit mRNAs. Freshly dispersed, 2, 4 day and confluent cells showed a beta-1 message on Northern blots with a full length dog kidney cDNA. Although no alpha-1, alpha-2, or alpha-3 message was detectable by Northern analysis using a variety of alpha-sequence probes or RNase protection, an $mRNA_{alpha-1}$ was detected by PCR indicating low abundance. This amplification was performed with RNA after reverse transcription, using two sets of primers made to a full length rat alpha-1 cDNA: one set made to sequences near the 5' end, the other to sequences spanning exon 11 and exon 13. The products of these amplifications were consistent in predicted size and sequence, confirming the presence of $mRNA_{alpha-1}$. Further specificity was confirmed, since primers to alpha-2 cDNA gave no positive products. These data indicate there are large differences in the relative abundance of $mRNA_{alpha-1}$ and $mRNA_{beta-1}$. If pump site subunit content in this tissue is 1:1, translational or post-translational control of subunit mRNAs must be dissimilar, allowing the coordinate expression of functional pump sites necessary for the regulation of proliferation.

G 101 MODULATION OF SMOOTH MUSCLE MYOSIN HEAVY CHAIN (MHC) GENE EXPRESSION BY SERUM AND THYROXINE. Philip Babij, Sachiyo Kawamoto, Sheryl White, Robert Adelstein and Muthu Periasamy. Dept. of Physiology, Univ. of Vermont, Burlington VT 05405 and Laboratory of Molecular Cardiology, NIH. Bethesda, MD 20892.

Smooth muscle MHCs SM1 and SM2 are expressed exclusively in vascular and non-vascular smooth muscle cells and the two isoforms are generated by alternative splicing of a single gene at the carboxyl terminus. To study regulation of MHC expression, primary rat aorta smooth muscle cells were grown in 10% serum and harvested when subconfluent (5 days) or confluent (7-12 days). Compared to intact aorta as control, RNase protection analysis showed that SM1 mRNA was unchanged in both proliferating and quiescent cultured smooth muscle cells. In contrast, the level of SM2 mRNA decreased to 20% of control in cells grown in the presence of serum mitogens. A similar pattern of unchanged SM1 mRNA and decreased SM2 mRNA levels was observed in twice passaged smooth muscle cells harvested at subconfluency. Mitogen withdrawal for 2-4 days after cells reached confluency had no effect on SM1 mRNA level and did not restore decreased SM2 mRNA to control levels. These results demonstrate that primary smooth muscle cells grown in the presence of serum maintain normal levels of SM1 mRNA but show modulation of SM2 expression. Western blotting analysis with SM1 specific antibody showed normal levels of SM1 protein, but SM2 MHC protein was barely detectable with SM2 specific antibody. Modulation of smooth muscle MHC expression was also observed in vivo in adult male rats made thyrotoxic for 20 days. RNase protection showed that both SM1 and SM2 mRNA levels decreased to 20-30% of control in all smooth muscle tissues studied except aorta which maintained normal levels of SM1 and SM2 mRNAs. SDS-PAGE showed that there was no difference in the total amount of SM1 and SM2 MHC proteins between control and thyrotoxic animals. These results suggest that thyroxine may have unique effects on expression of smooth muscle MHC mRNAs.

G 102 ELEMENTS OF THE CHICKEN SM ALPHA ACTIN PROMOTER REQUIRED IN CIS FOR SMOOTH MUSCLE-SPECIFIC TRANSCRIPTIONAL ACTIVATION, Randal S. Blank, Tim C. McQuinn, Kunio Takeyasu, Robert J. Schwartz, and Gary K. Owens, Department of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908 and Departments of Pediatrics and Cell Biology, Baylor College of Medicine, Houston TX 77030.

Previous studies have defined sequences within the 5' flanking region of the chicken SM alpha actin promoter which are required for transcriptional activation in emybronic chicken myoblasts and fibroblasts. To better understand transcriptional regulation of this gene in smooth muscle cells (SMC), we transfected chicken SM alpha actin promoter-CAT fusion plasmids into SMC from rat aorta. In marked contrast to observed effects in chicken skeletal muscle and fibroblasts, both p122CAT (-122 to +19) and p151CAT (-151 to +19), constructs containing conserved CBAR elements, elicit only small increases in CAT reporter gene activity in cultured rat aortic SMC. Interestingly, addition of the sequence between -151 and -257 (p257CAT) resulted in 7 and 12 fold increases in CAT activity over that observed with p151CAT and p122CAT, respectively. Results of these studies demonstrate that regions upstream of the CBAR elements may exert potent regulatory effects on transcription, and that SMC utilize mechanisms for the transcriptional regulation of the SM alpha actin gene which differ dramatically from those utilized in other cell types.

G 103 **CLONING OF A Na^+/H^+ ANTIPORTER cDNA FROM RAT AORTIC SMOOTH MUSCLE CELLS.**

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The Na^+/H^+ antiporter is a transmembrane protein which plays an important role in regulating intracellular pH. Intracellular alkanization is a necessary step during cell division. Several growth factors including: PDGF, EGF, thrombin, and angiotensin II increase Na^+/H^+ antiporter activity in rat aortic smooth muscle cells (RASM).To understand the mechanism for Na^+/H^+ antiporter activation by these factors, we have cloned a portion of the Na^+/H^+ antiporter cDNA from RASM by the polymerase chain reaction.

Single strand cDNA was transcribed from RASM poly A^+ mRNA stimulated by PDGF for 24hr. The upstream oligonucleotide was 5'TCCCACCACTGGAACTGGACCTTCG3' and the downstream oligonucleotide was 5'TCCGGAGCAGCATCTGGTTCCAGGC3'. After the first amplification, a 792 bp product, the exact size expected based on the human cDNA sequence, was obtained. With a third oligonucleotide, 5'CTGTCAGAAGGTGGTCCAGGAACT3', we have reamplified this product and a 384 bp product was obtained and cloned in M13.

The sequence of the Na^+/H^+ rat antiporter in this region is very homologous with the human antiporter (1623-2007) at the nucleotide level (92%) and the amino acid level (100%).

With this probe we have screened a rat aortic cDNA library from Dr Mark Taubman, and have obtained several clones. We plan to use this cDNA sequence to study several RASM mutants which lack Na^+/H^+ antiporter activity but express Na^+/H^+ antiporter mRNA.

G 104 Na^+ PUMP DENSITY/UPREGULATION CORRESPONDS TO VARIATION IN CANINE VASCULAR SMOOTH MUSCLE CELL MITOGENIC RESPONSE, T.F. Feltes, S.S. Navran, C.L. Seidel, J.C. Allen, Depts. Pediatrics and Medicine, Baylor College of Medicine, Houston, TX 77030.

The Na^+ pump (NaP) is relatively sparse in vascular smooth muscle cells (VSMCs) but may regulate mitogenesis via control of intracellular Na^+ (Na_i). We have observed vessel specific and maturational differences in onset of proliferation in primary (1^o) cultures of canine VSMCs. We studied coronary artery (CA), saphenous vein (SV) and pulmonary artery (PA) VSMCs in 1^o culture with 10% fetal bovine serum (FBS) from adult (a) and <21 d.o. newborn (nb) canines and measured cell#, Na_i, NaP# and cell volume. Onset of proliferation in a-SV, a-PA and all nb-VSMCs appeared by Day 3 in culture while onset was delayed in a-CA VSMCs until Day 5. Na_i increased followed by a doubling of NaP# and decrease in Na_i to baseline levels prior to onset of proliferation in all cells. The increase in a-CA VSMC NaP# was delayed compared to all other cell types and was accompanied by a sustained increase in Na_i. Cell volume at Day 1 in culture demonstrated no significant differences in nb-CA,SV or PA VSMCs. However, a-CA VSMCs were significantly larger than a-SV, a-PA and all nb-VSMCs while Day 1 NaP# was similar for all cells tested making NaP density for a-CA VSMCs relatively reduced. These data suggest that differences in NaP density and rate of upregulation may account for the maturational and vessel heterogeneity observed in VSMC mitogenesis.

G 105 INDUCTION OF COLLAGENASE IV IN CULTURED VASCULAR SMOOTH MUSCLE CELLS

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Rearrangement and production of extracellular matrix by vascular smooth muscle cells (SMC) in arteries are main features in the pathogenesis of arteriosclerosis. During the process of SMC migration into the intima breakdown of the matrix surrounding the cells seems to be important. Therefore, expression of proteinases such as collagenase may be relevant. We have investigated the inducibility of collagenase IV (CIV; 95kD) in cultured SMC by different stimuli. Rabbit aortic SMC were cultured in serum free medium (SFM) containing insulin, transferrin, and thyroglobulin. After 48h of incubation in SFM, CIV was detected by immunofluorescence using a polyclonal antibody. Cells positive for CIV were counted and expressed as percentage from total cell number. CIV was not expressed in SMC in SFM. Induction could be achieved only by stimulation with phorbolester and PDGF (Platelet-derived Growth Factor) B-B homodimer (25ng/ml were able to induce immunoreactive CIV material in 16% of the SMC). Stimulation with PDGF A-A homodimer did not induce CIV, although the cells appeared to possess both receptor subtypes. Cells could be induced to proliferate after addition of PDGF A-A and they expressed mRNA for both PDGF receptor types. Other cytokines such as Interleukin 2, Tumor Necrosis Factor-alpha, and Transforming Growth Factor-beta did not induce CIV. This study suggests a specific role for PDGF in expression of CIV in rabbit aortic SMC, which may be mediated through the ß-type PDGF receptor.

G 106 Chimeras of Calmodulin, Troponin C and Parvalbumin: Probes of Structure-Function Relationships in Smooth Muscle Contraction. Sam George, Zenghua Su, and Anthony Means, Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston TX 77030.

The calmodulin-myosin light chain kinase interaction is the key regulatory step in the final common pathway through which calcium initiates vascular smooth muscle contraction. A full understanding of the molecular nature of this interaction could lead to new strategies for pharmacologic intervention in disease states such as angina, hypertension, and congestive heart failure. To establish which regions of calmodulin (CaM) are involved in binding and activation of smooth muscle myosin light chain kinase (MLCK), we constructed a series of chimeric proteins in which CaM's functional domains were exchanged with the corresponding domains of two homologous calcium binding proteins, troponin C (TnC) and parvalbumin (Parv). Using chimeras in which domain I of CaM and TnC were exchanged, we have shown that neither domain I nor domains II-IV of TnC can substitute for the corresponding region of CaM without loss of ability to activate MLCK (*J Biol. Chem.* **265**: 9228). Further mutagenesis studies established that calcium binding in the first domain of CaM plays an important role in binding to MLCK, whereas some residues affecting CaM's surface charge distribution in the first domain significantly affect CaM's ability to activate MLCK. We have now expressed and purified several new chimeras of CaM, TnC, and Parv to evaluate the roles of CaM domains II, III and IV in MLCK activation. We expect that these mutagenesis studies will enhance our understanding of CaM's structure-function relationships, and more precisely define the structural features of CaM that are required to activate MLCK and initiate smooth muscle contraction.

G 107 POLYAMINE TRANSPORT AND ORNITHINE DECARBOXYLASE (ODC) ACTIVITY IN HYPOXIC PULMONARY ARTERY SMOOTH MUSCLE CELLS (PASMCS). Mark N. Gillespie, Cheryl Haven-Doll, and Jack W. Olson. Division of Pharmacology and Experimental Therapeutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082.

Hypoxia causes remodeling of the pulmonary circulation that is dependent on increases in lung polyamine contents. Mechanisms by which polyamines are regulated in hypoxic lung cells are unknown, but ODC activity, the initial enzyme in *de novo* biosynthesis, is depressed and polyamine transport is augmented in lungs from hypoxic rats. To determine if hypoxia directly influences polyamine regulatory mechanisms in pulmonary vascular cells, we examined ^{14}C-spermidine (SPD) transport and ODC activity in bovine PASMCs cultured under normoxic or hypoxic (culture medium Po_2: 18-30 torr) conditions. Uptake of ^{14}C-SPD was concentration-dependent, exhibited saturation kinetics, and prevented by metabolic inhibition. In control cells, ^{14}C-SPD uptake was characterized by Km and Vmax values of 0.8 μM and 4.1 pmoles/min/10^6 cells, respectively. While Km was unaffected by hypoxia, Vmax was increased to 25.7 pmoles/min/10^6 cells. The hypoxia-induced increase in ^{14}C-SPD transport was prevented by cycloheximide and actinomycin D, thus highlighting the importance of protein and RNA synthesis. In contrast to induction of transport, ODC activity in control cells, 48 pmole/mg protein, was reduced by 50% to 24 pmole/mg protein in hypoxic cells. These findings indicate that hypoxia alters polyamine regulatory mechanisms in PASMCs and suggest that increases in lung polyamine contents necessary for hypoxic vascular remodeling may be ascribed to induction of transport in cells of the pulmonary circulation. (supported by HL-38495 and HL-02055).

G 108 FIRST ENTRY INTO M PHASE CAUSES A LARGE ACCUMULATION OF NON-MUSCLE MYOSIN IN RAT VASCULAR SMOOTH MUSCLE CELLS. David J. Grainger, T. Robin Hesketh, James C. Metcalfe and Peter L. Weissberg. Department of Biochemistry, University of Cambridge,Tennis Court Road, Cambridge, CB2 1QW, U.K.

Vascular smooth muscle cells (VSMCs) from rat aortae contained very little non-muscle myosin heavy chain (MHC) immediately after dispersal and the protein did not accumulate if the cells were held in G_0/G_1 phase by witholding serum or were held in first S phase by the addition of bromo-deoxyuridine (BrdU). However, non-muscle MHC accumulated by >20-fold per cell during first M phase when over 80% of the cells divided between 48h and 72h after addition of serum. Delaying the addition of serum caused a delay in the accumulation of the non-muscle MHC until the cells subsequently entered M phase. If the cells were held in M phase at the metaphase/anaphase boundary by nocodazole, the accumulation of non-muscle myosin still occurred although division was blocked. When the cells were pulse-labelled with [^{35}S]-methionine, it was found that non-muscle MHC was one of the major proteins being made and that its synthesis occurred at similar rates throughout the cell cycle. This implied that the rate of degradation of the protein before first M phase was much faster than in M phase when the protein accumulated rapidly. This was confirmed by direct measurements of the rate at which [^{35}S]-methionine labelled non-muscle MHC disappeared from the cells which gave a half-life for the protein of about 8h before M phase but about 5 days in post-mitotic cells, ie an increase of approximately 15 fold. The data are consistent with the hypothesis that there is a mechanism in VSMCs which shortens the half-life of the protein before first M phase and that the accumulation of non-muscle MHC by the increase in half-life at first M phase is necessary for division of these cells.

G 109 PLATELET DERIVED GROWTH FACTOR-BB DECREASES SMOOTH MUSCLE MYOSIN HEAVY CHAIN EXPRESSION IN CULTURED VASCULAR SMOOTH MUSCLE CELLS, Bethany J. Holycross, Randal S. Blank, Maria M. Thompson, Michael J. Peach, Gary K. Owens, Departments of Pharmacology and Physiology, University of Virginia School of Medicine, Charlottesville, VA, 22908

We have previously shown that treatment of postconfluent, quiescent rat vascular smooth muscle cells (SMC) with platelet-derived growth factor (PDGF)-BB dramatically reduced smooth muscle (SM) α-actin synthesis, in part, via a destablization of the SM α-actin mRNA. In the present studies we have focused on the expression of another SM specific protein, SM myosin heavy chain (SM-MHC) to determine whether the actions of PDGF-BB on SM α-actin are protein-specific or may represent a global ability of PDGF-BB to induce a loss of the SMC differentiated phenotype. SM-MHC synthesis was assessed by gel electrophoretic analysis of protein from cells labeled with ^{35}S-methionine. SM-MHC synthesis was markedly decreased in PDGF-BB treated cells compared to cells maintained in SFM. Northern blot analyses showed that SM-MHC mRNA expression was decreased by 5-10 fold in PDGF-BB vs vehicle-treated, post-confluent, explant-derived or enzyme dispersed SMC. Results of nuclear run-on analyses showed no inhibition of SM-MHC transcription 24 hours following PDGF-BB treatment, suggesting that the decrease in SM-MHC mRNA levels was mediated via post-transcriptional destablization of the SM-MHC mRNA. These findings suggest that circulating or locally produced PDGF-BB may play a generalized role in the modulation of the SMC phenotype to a less differentiated state, a characteristic of SMCs in atherosclerotic blood vessels.

G 110 ENDOTHELIN-1 STIMULATION OF PHOSPHOLIPASES C, D AND A_2 IN VASCULAR SMOOTH MUSCLE. Allan W. Jones, Yu Liu, Brinda Geisbuhler and Nancy Cook. Department of Physiology, UMC, Columbia, MO 65212

Endothelin-1 (ET-1) is a potent vasoconstrictor which has been proposed to act via Ca-channels and/or by receptor regulated events. We measured the effects of ET-1 (10-100 nM) on PLC ($[^3H]IP_2$ formation), PLD (incorporation of ethanol into phospholipids, ^{32}PEt), and PLA_2 (6-keto-$PGF_{1\alpha}$ formation) in rat aorta. ET-1 increased $[^3H]IP_2$ 3.5 fold, ^{32}PEt 18 fold and 6-keto-$PGF_{1\alpha}$ by 46 $\pm$ 9 pmol/(mg wet wt. x 30 min.) above basal levels. The changes in PLD and PLA_2 exceeded those to norepinephrine (NE, 30 μM) by 2-4 times, while the changes in PLC were 2-fold smaller than those induced by NE. ET-1 also stimulated ^{42}K efflux (Ca-dependent), but it did not exhibit the phasic response associated with NE. It is concluded that ET-1 is a regulator of phospholipase-2nd messenger signalling, including the novel lipase, PLD. ET-1 has a relatively larger effect on PLD and PLA_2, while NE stimulates PLC to a greater extent. The difference in the phospholipase profile could underlie differences in functional responses. (Supported in part by Grants HL315852 and 30519).

G 111 DECREASED LEVEL OF SARCO(ENDO)PLASMIC RETICULUM Ca^{2+}ATPase mRNA IN AORTAS FROM SPONTANEOUSLY HYPERTENSIVE RATS, Thierry, H. Le Jemtel, Francine Lambert, Michel Clergue, Anne-Marie Lompré. INSERM U275, ENSTA - Ecole Polytechnique, Centre de l'Yvette, Palaiseau, France.

The level of expression of the sarco(endo)plasmic reticulum Ca^{2+}ATPase gene 2 (SERCA 2) was measured in thoracic aorta (TA) and abdominal aorta (AA) from spontaneously hypertensive rats (SHR) and compared to that of normotensive wistar kyoto (WKY) rats. Ca^{2+}ATPase mRNA was quantitated by S1 nuclease mapping analysis using concomitant hybridization with a a- smooth muscle actin probe to normalize Ca^{2+}ATPase mRNA to a smooth muscle specific mRNA. In WKY, the relative amount of Ca^{2+}ATPase mRNA was greater in TA than in AA: 94.8 ± 17.5 vs 50.1 ± 8.44 ($n=10$, $p<0.05$). When compared to WKY, the amount of Ca^{2+}ATPase in SHR was substantially reduced in TA: 35.9 ± 5.3 vs 94.8 ± 17.5, ($n=10$, $p<0.01$) and to a lesser extent in AA ie. 38.1 ± 3.3 vs 50.1 ± 8.4, ($n=10$, NS). Decreased ability to store calcium may impair relaxation and contribute to increased impedance of large vessels in SHR.

G 112 EXPRESSION OF RAT TRANSLATIONAL PRODUCTS OF THE SMOOTH MUSCLE MYOSIN REGULATORY LIGHT CHAIN GENES, Bradley D. Ralsher, and James W. Grant, Department of Pediatrics, Washington University School of Medicine. St. Louis, MO. 63110

The smooth muscle myosin regulatory light chain (RLC) contributes to both the force generation and stability of myosin in smooth muscle and non-muscle cells. In the rat, two conserved myosin RLC genes (RLC-A and RLC-B) encode isoforms with 95% amino acid homology, with both mRNA expressed in a wide variety of rat tissues. (Grant, JW et al, J. Cell. Biol. 111:427-435, 1990). Comparison of RLC-A and B mRNA expression in selected rat smooth muscle cell-like (A7R5 and A10) and non-muscle (C6, NRK, R2) cellular lines reveal message detected in all cell lines, but RLC-A in greatest abundance in the smooth muscle lines and RLC-B primarily in the non-muscle lines. Immunoblots of whole tissue and cellular protein homogenates, after standard SDS/PAG electrophoresis, using monoclonal antibodies to chicken gizzard RLC antigen reveal the RLC protein is detected in all rat tissues, but in greatest abundance in smooth muscle tissues. Examination of the homogenates from the rat cell lines, reveals that the RLC protein is greater than 5 fold more abundant in the smooth muscle-like A7R5 and A10 cell lines. This is in contrast to the non-muscle cell lines which contain the antigen at only minimally detectable levels, using both the protein and DNA content as a basis of comparison. Dot blot analyses of the steady state mRNA levels reveals that unlike the difference in RLC protein content, the mRNA levels are < 2 fold different in all cell lines. This data is consistent with regulation of RLC gene expression at either the translational or post-translational level to achieve the smooth muscle contractile phenotype.

G 113 INSULIN ATTENUATES AVP-INDUCED CALCIUM TRANSIENTS AND VOLTAGE-DEPENDENT CALCIUM CURRENT IN VASCULAR SMOOTH MUSCLE (VSM) CELLS, J.L. Ram, P.R. Standley, F. Zhang, M.B. Zemel and J.R. Sowers, Dept. of Physiology and Division of Endocrinology and Hypertension, Wayne State University, Detroit, MI 48201

Hypertension associated with diabetes may be due to the absence of normal insulin attenuation of VSM contractility. In order to investigate the mechanisms by which insulin attenuates VSM contractility, effects of insulin on responses of cultured VSM cells (a7r5) to arginine vasopressin (AVP) and membrane potential were investigated. In normal cells, the lowest [AVP] to produce a >5% rise in $[Ca^{2+}]_i$ was 10^{-7} M. Treatment with insulin (1 and 100 mU/ml) increased the [AVP] needed to produce the same rise in $[Ca^{2+}]_i$ to 10^{-6} M and 10^{-5} M AVP, respectively. The response to AVP was significantly reduced within 60 min of insulin application. AVP-elicited inward current (whole-cell patch clamp) was significantly reduced by 90 min of insulin treatment (100 mU/ml), from a peak current of -103 $\pm$ 27 pA (normal) to -37 $\pm$ 15 pA (insulin-treated). In the same cells, peak voltage-dependent calcium-dependent inward current was unaffected by insulin. However, the current-voltage curve was shifted to the right by insulin, achieving 20% of the maximal inward current response at -44 $\pm$ 4 mV in control cells, but requiring depolarization to -28 $\pm$ 4 mV in insulin-treated cells. Thus, insulin may reduce VSM contractile responses by attenuating agonist-mediated rises in $[Ca^{2+}]_i$ mediated, in part, by reductions in calcium influx through both receptor-and voltage-operated channels.

G 114 CONTROL AND FUNCTION OF MYOSIN ISOFORMS IN VASCULAR SMOOTH MUSCLE, C.L. Seidel, D.S. Rickman, J.C. Allen, A.M. Kahn*, Dept. Medicine, Baylor Colllege of Medicine and University of Texas Medical School*, Houston, TX 77030

Proliferating vascular smooth muscle cells cultured in serum decrease their content of muscle specific myosin heavy chain (SMHC) while increasing their content of non-muscle myosin heavy chain (nmMHC). The purpose of this work was to determine both the mechanism of control and function of this shift. Using a SMHC specific c-DNA probe (M. Periasamy, U.Va.), Northern analysis indicated that the temporal decrease in SMHC protein was preceded by a decrease in mRNA suggesting transcriptional regulation. Since myosin can function in either migration or contraction, the relationship between the expression of a specific myosin isoform and these cellular functions was determined. Cells containing only SMHC reversibly shortened in response to KCl and serotonin while those containing nmMHC did not. Cells expressing nmMHC migrated in response to serum, while those expressing only SMHC did not. To determine if in vivo vascular proliferation was associated with similar changes in myosin isoform expression and altered function, canine saphenous veins grafted into the femoral artery were examined after one month. Control veins contained only the normal two SMHC's (SMHC1-62%; SMHC2-38%) while grafts contained SMHC 1 & 2 as well as nmMHC (26%). Grafting did not affect the proportion of SMHC 1 & 2 relative to total SMHC content. Grafted veins also exhibited a reduction in maximum force per myosin. These data suggest that in vitro and in vivo, the presence of nmMHC is associated with a reduced contractile ability and that the reduced contractile ability of vein grafts may be due in part to the expression of a myosin isoform that is better suited for migration (ie. nmMHC) than for force development (i.e. SHMC).

G 115 THE DEVELOPMENTAL EXPRESSION OF MYOSIN HEAVY CHAIN ISOFORMS IN RAT AND RABBIT SMOOTH MUSCLE. Sheryl White, Janet Woodcock-Mitchell, John Mitchell, Philip Babij, Robert Low, and Muthu Periasamy. Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405.

Two smooth muscle myosin heavy chain isoforms (SM1 and SM2), as well as cellular myosin isoforms, are expressed during the course of smooth muscle development. The pattern of myosin expression was examined at 14 and 17 through 21 days fetal development in rat and at 23, 25 and 29 days in rabbit smooth muscles using SM1 and SM2 specific antibodies, a polyclonal smooth muscle myosin antibody and a human platelet myosin antibody. These antibodies were used to analyze myosins via Western blotting and immunohistochemistry on whole embryo sections. In the rat, cellular myosin is detected as early as 14 days, primarily around developing airways and blood vessels, while SM1 myosin is barely detectable at 17 days in stomach and intestine smooth muscle. The SM2 isoform is the last to be detected at approximately 20-21 days, just prior to birth. In rabbit, the SM1 isoform can be detected in aorta and visceral smooth muscle at 23 days fetal development, while SM2 was detectable at low levels only in bladder prior to birth (29-31 days). It is interesting to note that the pattern of expression of SM1 and SM2 in fetal visceral tissues (SM1 being the more abundant) is the complete opposite to the pattern of expression in these tissues in the adult rabbit where SM2 is the more abundant form.

G 116 ISOLATION AND HETEROLOGOUS EXPRESSION OF A cDNA ENCODING BOVINE INOSITOL POLYPHOSPHATE 1-PHOSPHATASE, John D. York and Philip W. Majerus, Washington University, Division of Hematology-Oncology, 660 S. Euclid, Box 8125, St. Louis, MO 63110
Inositol polyphosphate 1-phosphatase, an enzyme of the phosphatidylinositol signaling pathway, catalyzes the hydrolysis of the 1 position phosphate from inositol 1,3,4-trisphosphate and inositol 1,4-bisphosphate. The function(s) of the substrates and products of inositol polyphosphate 1-phosphatase is not fully understood. The protein was isolated from calf brain, digested with trypsin or CNBr, and the amino acid sequence of several peptides was determined. Degenerate oligonucleotide primers were designed from amino acid sequence and used to synthesize an 80 bp fragment by the polymerase chain reaction. This product was used to isolate a 1.6 kD cDNA with an open reading frame of 400 amino acids, 185 bp of 5' untranslated region, and 171 bp of 3' untranslated region followed by a putative poly A tail. The coding region of the cDNA was inserted into an expression vector that was used to obtain the recombinant protein from *E. coli* cells. The recombinant enzyme (M_r=44 kD) had a specific activity and other properties similar to native bovine brain inositol polyphosphate 1-phosphatase. It hydrolyzed both inositol phosphate substrates and was inhibited by lithium ions. The enzyme shows minimal sequence similarity to inositol monophosphate phosphatase, the other enzyme inhibited by lithium ions in the signaling pathway. The ability to express active inositol polyphosphate 1-phosphatase in bacteria will facilitate studies aimed at identifying the catalytic and regulatory domains of the enzyme. Expression of this protein in various eukaryotic cells may allow us to understand the function(s) of Ins(1,4)P_2, Ins(1,3,4)P_3, and their metabolites within cells.

Molecular Mechanisms of Vascular Diseases

Endothelium as Interface Between Circulating and Tissue Factors

G 200 CLONING AND SEQENCING OF THE MOUSE ICAM-1 GENE, Christie M. Ballantyne, James E. Sligh, Jr., Xiao-Yuan Dai, William E. OBrien, and Arthur L. Beaudet, Section of Atherosclerosis and Cardiology, Department of Medicine and Institute of Molecular Genetics , Baylor College of Medicine and Howard Hughes Medical Institute, Houston, Tx 77030

Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily and has been shown to bind to LFA-1, which is a member of the integrin superfamily. ICAM-1 is felt to play a critical role in endothelial leukocyte interaction and thus may play an important role in vascular pathophysiology. As a step towards genetic manipulations of ICAM-1 in the mouse we have cloned, characterized and sequenced the gene for murine *Icam-1*. Five overlapping lambda clones were obtained by screening several mouse genomic libraries. These clones span the entire gene and include approximately 6 kb of 5' flanking sequence and 12 kb of 3' flanking sequence. With over 90% of the exon material sequenced, the *Icam-1* gene appears to be composed of 7 exons. Exon 1 contains the translation start signal and the entire peptide leader sequence. Exons 2 through 6 contain sequences that encode for immunoglobulin domains 1 through 5 respectively. Each exon encodes for a single immunoglobulin domain. Exon 7 contains sequence encoding the transmembrane domain, cytoplasmic region, and the polyadenylation site. Two genomic clones have been used to develop a construct to disrupt *Icam-1* in the mouse using homologous recombination in embryonic stem cells.

G 201 CHARACTERIZATION OF CULTURED ENDOTHELIAL CELLS ISOLATED FROM TRANSGENIC MOUSE HEMANGIOMAS, Victoria L. Bautch[1], Linda Kolpack[2], Richard G. Azizkhan[2], and Nathalie DuBois[1], Department of [1]Biology and [2]Surgery, University of North Carolina, Chapel Hill, NC 27599

To determine whether transgenic endothelial tumor cells retain properties of normal endothelial cells and thus can model normal angiogenic responses, we have isolated endothelial cells from transgenic mouse hemangiomas. Endothelial cell tumors arise in transgenic mice carrying the intact polyoma early region. Endothelial cells from tumor material were isolated by cell sorting using a fluorescent endothelial cell marker (Di-I-Ac-LDL, (DiI)). We sorted two times with three passages between sorts and we have obtained cells that are uniformly positive for DiI staining. The cells have a doubling time of 5-10 days in culture and have been carried for about 34 passages from the animal. Two morphological populations are observed: small cobblestone-looking cells and large cells. Both populations are positive for von Willibrand's Factor and polyoma T antigen by immunostaining. The large cells represent a small percent of the population and their number does not vary upon passage, suggesting that they are senescent endothelial cells. Cell morphology varies with different substrata, and the cells form capillary-like structures on Matrigel but not on gelatin. We are currently characterizing this phenomenon and studying the expression of several different angiogenic factors and proteases in the cultured cells. The cultured endothelial cells (passages 10, 17, 22, 26, 29) were injected s.c. into non-transgenic histocompatible hosts. Hemangiomas developed at the site of injection with a latency of 2-3 weeks, and the tumors were fatal to the host at 4-6 weeks after injection. These observations indicate that the cultured cells have retained endothelial cells properties.

G 202 SYNTHETIC CHIMERIC ANTICOAGULANT PEPTIDE, Frank C. Church, Jeanne E. Skibbens and Joan L. Woods, Center for Thrombosis and Hemostasis, University of North Carolina School of Medicine, Campus Box 7015, Chapel Hill, NC 27599

Heparin has been used for almost 50 years for the treatment of thrombotic disorders in hospitalized patients. The clinical use of heparin as an antithrombotic drug is occasionally associated with some significant side-effects including hemorrhage and heparin-associated thrombocytopenia; thus, these complications emphasize the need for new anticoagulants to aid in the treatment of thromboembolism. We investigated the biochemical properties of a synthetic chimeric anticoagulant peptide **(CAP)** that contains an Arg-Gly-Asp **(RGD)** tripeptide, the versatile cell recognition signal of extracellular matrix components, coupled to a carboxyl terminal fragment of the bloodsucking leech protein hirudin (residues 53-64): WGRGDSANGDFEEIPEEYL **(RGD-hirudin)**. Compared to hirudin(53-64), RGD-hirudin has essentially the same properties and acts (i) as an inhibitor of the fibrinogen clotting activity of α-thrombin with an IC_{50} (concentration resulting in 50% inhibition) of 0.6 μM, (ii) as an anticoagulant using human plasma clotting assays *in vitro*, and (iii) as an inhibitor of α-thrombin-stimulated platelet aggregation with an IC_{50} of 7 μM. RGD-hirudin and hirudin (53-64) interact with the same site on α-thrombin, and peptide binding does not adversely influence thrombin inactivation by the plasma proteinase inhibitor antithrombin (with or without added heparin) but binding does protect thrombin during trypsinolysis. Unlike hirudin(53-64), RGD-hirudin mediates integrin-specific cell attachment, and in solution it inhibits cell adhesion (using fibroblasts and endothelial cells). The bifunctional action of CAP suggests that a class of synthetic peptides derived with this structural motif in which a specific cell recognition sequence "targets" the peptide to a thrombus with ensuing thrombin inhibition may have therapeutic value as an anticoagulant.

G 203 LIPOPROTEIN (a) REGULATES PLASMINOGEN ACTIVATOR INHIBITOR-1 INHIBITION OF TISSUE-TYPE PLASMINOGEN ACTIVATOR, Jay M. Edelberg*, Christopher F. Reilly#, and S. V. Pizzo*, *Departments of Pathology and Biochemistry, Duke University, Durham NC 27710, and #Department of Pharmacology, Merck, Sharp & Dohme Research Laboratories, West Point PA 19486

Elevated levels of Lipoprotein (a) [Lp(a)] are associated with an increased risk of atherosclerotic disease and a depression of fibrinolytic activity. Lp(a) may regulate both the activation and inhibition of the fibrinolytic system. Lp(a) inhibits in vitro plasmin generation by competing with plasminogen for the active site of fibrin(ogen)- and heparin-bound tissue-type plasminogen activator (t-PA) (Edelberg et al, *Thrombosis Research 57*, 155-162; Edelberg & Pizzo, *Biochemistry 29*, 5906-5911). In vitro studies of the inhibition of t-PA by its principal plasma inhibitor, plasminogen activator inhibitor-1 (PAI-1) demonstrate that fibrinogen and heparin increase the rate of inhibition by four-fold, and vitronectin increases the rate by six-fold. The dissociation constants of fibrinogen, heparin, and vitronectin for the inhibition reaction are 200 nM, 20 nM, and 600 pM, respectively. Lp(a) protects t-PA from inhibition by PAI-1 in the presence of fibrinogen or heparin. Lp(a) had no effect on the inhibition in the presence of vitronectin or in the absence of fibrinogen or heparin. The dissociation constants of Lp(a) suppression of this inhibition reaction in the presence of fibrinogen and heparin are 11 nM and 10 nM, respectively: these constants correspond to physiologic levels of Lp(a) of 4.2 mg/dL and 3.8 mg/dL, respectively. These experiments suggest that physiologic levels of Lp(a) may depress and prolong fibrinolysis by protecting fibrinogen- and heparin-bound t-PA from PAI-1 inhibition,while inhibiting plasminogen activation by the bound t-PA. Pathophysiologic levels of Lp(a) may further protect PAI-1 from inhibiting t-PA, but may also prevent such a large fraction of available plasminogen from t-PA activation, that fibrinolysis would be greatly inhibited.

G 204 **SMOOTH MUSCLE CELL PROLIFERATION AFTER VASCULAR INJURY IS INHIBITED BY AN ANTIBODY AGAINST bFGF**, Volkhard Lindner, Michael A. Reidy, University of Washington, Department of Pathology, Seattle, WA 98195

Smooth muscle cell (SMC) proliferation with subsequent intimal thickening is a characteristic response of the arterial wall to a denuding injury but it is unclear what factors initiate this response. In this study we examined the role of endogenous bFGF and arterial injury and found that the proliferation of SMCs after balloon catheter denudation of the rat carotid artery was significantly reduced when an antibody raised against human recombinant bFGF was injected prior to injury. Groups of rats received an intravenous injection of either nonimmune IgG or anti-bFGF IgG (Protein-G purified from rabbit serum, 10mg/rat) prior to surgery and 5 minutes later the artery was denuded of endothelium by balloon catheterization. SMC proliferation in these arteries was determined by autoradiography following injection of ^{3}H-thymidine (24, 32, and 40 hours after denudation). The ^{3}H-thymidine index of medial SMCs was significantly reduced after antibody injection as compared to animals receiving the nonimmune IgG (1.5% vs. 7.6%). These data support the hypothesis that SMC proliferation after arterial injury is largely due to release of bFGF from damaged cells in the vessel wall. *(Supported by NIH grant HL 03174-35).*

G 205 IN VITRO ENDOTHELIAL CELL PRODUCTION OF PROSTANOIDS: EVIDENCE FOR THE EXISTENCE OF A HUMORAL FACTOR IN THE ETIOLOGY OF HYPERTENSIVE TOXEMIA OF PREGNANCY AND ITS POSSIBLE PHARMACOLOGIC SUPPRESSION. Charles Lox, Department of Obstetrics/Gynecology, Texas Tech Univ.HSC. Lubbock,Tx. 79430

Alterations in the ratio of the vasoconstricting platelet aggregator Thromboxane (TxA2) and the vasodilatory deaggregator prostacyclin (PgI2) is suspected to be involved in the pathology of pregnancy induced hypertension. These 2 prostanoids were measured in the extracted tissue culture media from endothelial cells obtained from normal term umbilical veins exposed to normal maternal serum or serum from women with severe toxemia of pregnancy (preeclampsia). Production of PgI2 decreased while TxA2 increased in those cells exposed to the toxemic sera. In Vivo, this would tend to exacerbate the clinical manifestations of the disease. When these cells were further exposed to aspirin, indomethacin, or omega-3 fatty acid, the fatty acid reversed this abnormal production of both PgI2 and TxA2, while aspirin reduced TxA2 production. This suggests that omega-3 fatty acid selectively reverses the unfavorable levels of prostanoids induced by the humoral factor. This could indicate a more favorable pharmacologic use of omega-3 fatty acids in the treatment of toxemia of pregnancy, than the presently utilized compound, baby aspirin.

G 206 EARLY ENTEROVIRAL REPLICATION IN THE MURINE MODEL OF MYOCARDITIS: IMPORTANCE IN ACUTE MYOCARDIAL INJURY AND RELATIONSHIP TO PERSISTENCE OF CARDIAC INFECTION. Bruce McManus, Lawrence Chow, Charles Gauntt, Karen Klingel, Reinhard Kandolf, Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE and Max Planck Institute for Biochemistry, Martinsried, FRG.

The importance of enteroviral replication in the direct injury of myocardium during enteroviral infection in the mouse model remains unestablished. Utilizing in situ hybridization with ^{35}S-labeled cDNA probes to conserved regions of the enteroviral genome in ventricular sections of 6 adolescent inbred murine strains: A/J(H-2^a), C57BL/6J(H-2^b), BALB/cJ(H2^d), DBA/2J(H-2^d), B10.D2/nSnJ(H-2^d), C3H/HeJ(H-2^k) infected with 2x10^5 TCID of 4 variants of coxsackievirus B3 (SH,CG,ST,NR), we assessed the relationship of early viral replicative foci (preceding visible myocardial injury or inflammation) to injury and repair in animals studied at days 3,4,7,13 and 21 post inoculation. Infected foci at days 3,4, & 7 were strongly correlated with myocarditic injurious lesions on day 7 and healing lesions on day 21. Widely dispersed viral replication reached a peak between day 3 & 7, and typical autoradiographic signals in contiguous cells reflected direct cell-to-cell spread of virus. By day 7, replication was localized to the viable margins of necrotic inflamed lesions. Animals with the greatest number of viral foci during acute infection had most frequent viral persistence within healing lesions at day 21. The greater the susceptibility of the murine host and the greater the virulence of the viral variant the greater was the likelihood of both early and late viral replication. Viral replication has a prominent role in direct injury and perhaps altered healing of myocarditic hearts.

G 207 RECOMBINANT EXPRESSION AND CHARACTERIZATION OF THE 121 AMINO ACID FORM OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF). RL Mitchell, M Silva, K Wood, S Thompson. California Biotechnology Inc., Mt. View, CA.

VEGF is a newly-identified member of the PDGF family of growth factors. Three mature forms of human VEGF have been predicted based on cDNA cloning and PCR analysis of VEGF mRNA. These forms are 189, 165, and 121 amino acids in length. Isolation of VEGF-121 from native or recombinant sources has not been reported. Therefore, an expression vector containing the cDNA for the 121-amino acid form of human VEGF was introduced into CHO cells. The transfected CHO cells secrete recombinant human VEGF-121 and correctly cleave the signal peptide. Secreted VEGF-121 differs from VEGF-165 in many respects. Due to a loss of basic residues near the C-terminus of the protein, VEGF-121 has an acidic pI and does not bind to heparin Sepharose. Like the 165 amino acid form of VEGF, VEGF-121 is modified by N-linked glycosylation and dimers of VEGF-121 can form between glycosylated or non-glycosylated VEGF monomers. Data on the biological activities of VEGF-121 will be presented.

G 208 ION TRANSPORT AND ENDOTHELIAL CELL VOLUME, W. Charles O'Neill[1,2] and Janet D. Klein[1], Renal Division, Department of Medicine[1], and Department of Physiology[2], Emory University School of Medicine, Atlanta, GA

Endothelial integrity is dependent in part on maintenance of cell volume. The changes in endothelial cell morphology that occur with vasoactive peptides, hemodynamic forces, and pathologic conditions probably involve changes in cell volume as well, and are resposible in part for altered endothelial permeability. Because ion transport, which is responsible for cell volume regulation in other cells, is also altered under these conditions, we have studied the relationship between ion transport and cell volume in cultured bovine aortic endothelial cells. K transport (^{86}Rb flux), cell volume ([^{14}C]urea space), and ion content (atomic absorption) were measured using 0.1 mM ouabain to inhibit the Na-K pump (PUMP) and 50 uM bumetanide to block Na-K-Cl cotransport (COTR). K flux not blocked by either was assigned to K conductance (LEAK). COTR mediated net K influx and its inhibition caused cell shrinkage. Net influx via COTR required the maintainance of a low cellular Na by PUMP. Net K efflux occurred through LEAK. Cell shrinkage activated COTR and inhibited LEAK, with restoration of cell volume through net influx of Na and K. Opposite changes occurred in swollen cells, with restoration of volume via net efflux of K. Bradykinin increased LEAK, leading to cell shrinkage and activation of COTR. Endothelial volume is thus governed by the balance between COTR and LEAK, poising the cells for rapid volume changes and volume regulation through coordinate inhibition and activation of these fluxes. Structural responses in endothelium may involve changes in cell volume related to ion transport. [Supported by NIH DK 01643 and American Heart Association of GA]

G 209 ANGIOBLAST DIFFERENTIATION AND MIGRATION FORMING EMBRYONIC VASCULAR PATTERN ARE INFLUENCED BY GROWTH FACTORS AND ECM, Thomas J. Poole, Department of Anatomy and Cell Biology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210

The angioblasts are the embryonic precursors of endothelial cells which originate within the body of the embryo. We have been using a monoclonal antibody, QH-1, and microsurgery to determine how angioblasts assemble into the rudiments of the major blood vessels. The extent and type of directed angioblast migration define three distinct modes of vessel morphogenesis. Vessel rudiments may organize in place, a process termed **vasculogenesis**, either from angioblasts originating at the rudiment's location or from angioblasts which migrate as individual cells or small groups to that site from distant locations. The dorsal aortae form by the first type of vasculogenesis. The endocardium, ventral aortae and posterior cardinal veins form by the second type. New vessels may also form by sprouting from preexisting vessels, a process called **angiogenesis**. The intersomitic and vertebral arteries are the first vessels to form by angiogenesis, sprouting off the rudiments of the dorsal aortae. The construction of quail/chick chimeras has demonstrated that angioblasts have a tremendous propensity for migration within the embryo, and that the differentiation of endothelial cells from mesoderm may be influenced by the embryonic environment. For example, a quail somite transplanted into the head of a chick host (10 somite stage donor and host) gives rise to many labelled angioblasts which migrate away from the graft site as sprouts, small groups and individual cells. In contrast, a quail somite transplanted in place of a chick somite differentiates into only a few angioblasts. *In vitro* studies indicate that this apparent induction of endothelial cell differentiation from somitic mesoderm may be produced by fibroblast growth factors (acidic and basic). [Supported by grants from the National Science Foundation, American Heart Association and The Brownstein National Foundation for Diabetes and Related Disorders.]

G 210 PHORBOL ESTER INHIBITS ENDOTHELIN AND STIMULATES PROSTACYCLIN SYNTHESIS INDUCED BY HUMAN SERUM IN HUMAN VASCULAR ENDOTHELIAL CELLS

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In clarifying the regulation of the production of endothelin-1 (ET-1) and prostacyclin (PGI2) by cultured human umbilical vein endothelial cells, we found that human serum stimulated ET-1 and PGI2 synthesis concentration- and time-dependently (ET-1 synthesis was stimulated 4.0±0.37 -fold and PGI2 2.0±0.17 -fold by 10 % of the serum during incubation for 24 h, from 9 determinations and 3 separate experiments). Since ET-1 activates protein kinase C and vascular endothelium is one of the targets of ET-1, we investigated the effect of a protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), on human serum induced ET-1 and PGI2 synthesis. PMA inhibited the serum induced ET-1 synthesis, whereas PGI2 synthesis was stimulated. The lowest effective concentration of PMA was 1 nM and the maximal response was obtained with 100 nM (4.6±0.18 times the control for PGI2 and 0.23±0.22 times the control for ET-1, from 9 determinations and 3 separate experiments). The effect of PMA was detectable after 5 h of incubation and lasted at least for 50 h. This is the first demonstration that human serum stimulates the production of ET-1 and that the activation of protein kinase C has a negative feedback effect on the production of ET-1.

G 211 PULSATILE STRETCH OF ENDOTHELIAL CELLS *IN VITRO* STIMULATES PHOSPHOINOSITIDE PATHWAY, Oscar Rosales, Bauer Sumpio, Dept. of Surgery, Yale Univ. Sch. Med., New Haven, CT.

Bovine aortic EC respond to cyclic deformation *in vitro* with an increase in cell proliferation and prostacyclin synthesis. The aim of this study was to determine the effect of mechanical stretching of EC on the production of inositol phosphates (IP), diacylglycerol (DAG) and protein kinase C (PKC). For the IP assay, EC ($2x10^5$/25 mm) were grown on flexible-bottomed plates, labelled with ^{3}H-myoinositol for 48 hours and then stretched with vacuum to either 0, 1, 10, or 100 cycles of 24% elongation-relaxation at 60 cycles/min. Reactions were terminated with 15% trichloroacetic acid, and IP (dpm/10^5 cells ± SD, n = 12) separated by column chromatography. For the DAG assays, unlabelled EC were subjected to similar conditions as above, the lipids extracted with chloroform/methanol and analyzed for DAG (picomoles ± SD, n = 5) by utilizing *E. coli* DAG kinase to phosphorylate DAG to phosphatidic acid in the presence of ^{32}P-ATP. ^{32}P-phosphatidic acid is then resolved on thin layer chromatography. PKC (pmole/μg protein, n = 3) was determined in cytosol (c) and membrane (m) based on the presence and absence of $CaCl_2$ and phosphatidylserine. *p<0.05 compared to 0 cycles.

Cycles	IP_1	IP_2	IP_3	DAG	PKC_c	PKC_m
0	592±32	459±11	386± 7	9± 3	0.3±0.1	0.7±0.1
1	613±22	465±13	486±10*	12± 2	0.3±0.3	1.3±0.2*
10	803±27*	596±22*	385± 8	18± 4*	0.2±0.1	0.4±0.3
100	609±15	482±18	367± 8	9± 2	0.3±0.2	1.7±0.6*

We conclude that these second messengers are available to mediate some of the effects of cyclic stretch on the vascular endothelium.

G 212 LOW DENSITY LIPOPROTEIN RECEPTOR-DEPENDENT EICOSANOID SYNTHESIS IN HUMAN MONOCYTES/MACROPHAGES Peter B. Salbach, Eberhard v. Hodenberg, Philipp Hugger, Julia Kossmann, Uwe Janssen-Timmen, Christine Blattner, Reinhard Ziegler, §John A. Glomset, and Andreas J. R. Habenicht; University of Heidelberg, School of Medicine, Department of Internal Medicine; 69-Heidelberg, Bergheimerstr. 58, F.R.G.; §Howard Hughes Medical Institute Laboratory SL-15, University of Washington, Seattle, WA 98195, USA.

Low density lipoprotein (LDL) effects that are unrelated to cholesterol metabolism have not been studied in great detail. We have shown previously that the LDL receptor pathway delivers arachidonic acid (AA) for prostacylin and prostaglandin E_2 formation in PDGF-stimulated skin fibroblasts and that LDL inhibits the rate limiting enzyme of the PG synthesis pathway, the PGH synthase (1). This study suggested a new role for the LDL receptor. Here we investigated the possibility that the LDL pathway delivers AA for prostaglandin, thromboxane, and leukotriene biosynthesis in cultured human macrophages. This question was of interest to us, because macrophages, together with fibroblasts, accumulate at sites of inflammation, and because each cell type forms a specific pattern of AA metabolites. Incubation of human blood-derived macrophages with LDL that had been reconstituted with (^{14}C)arachidonyl cholesteryl ester (recLDL) led to the formation of labeled prostacyclin, thromboxane, and PGE_2. When the cells were preincubated in the presence of recLDL to allow the LDL derived AA to be incorporated into cellular phospholipids, and then stimulated with the chemotactic peptide fMLP, significant amounts of labeled leukotrienes B_4 and C_4 were formed. The production of eicosanoids appeared to be mediated by the LDL receptor because chloroquin, an inhibitor of lysosomal activity, prevented formation of eicosanoids from rec-LDL but not from unesterified ^{14}C-AA. Our results suggest that macrophages are capable of utilizing AA of LDL to produce several distinct eicosanoids that are likely to play important roles in inflammation and possibly cardiovascular disease.

(1) Habenicht AJR, Salbach PB, Goerig M, Jannsen-Timmen U, Blattner C, King W, Glomset JA; Nature 345, 634-636 (1990)

G 213 CLONING AND SEQUENCING OF THE MOUSE cDNA FOR GMP-140, William E. Sanders, Christie M. Ballantyne, William E. O'Brien and Arthur L. Beaudet, Section of Cardiology, Department of Medicine and Institute of Molecular Genetics, Baylor College of Medicine and Howard Hughes Medical Institute, Houston, TX 77030

Granule membrane protein 140 (GMP-140) is an integral membrane glycoprotein located in the α-granules of platelets and in the Weibel-Palade bodies of endothelial cells. This member of the "selectin" family of adhesion molecules is found on the plasma membrane of activated platelets and endothelium and mediates the binding of these cells to monocytes and neutrophils. GMP-140 may play an important role in endothelium-leukocyte interactions and in the clearance of activated platelets thus providing a link between inflammation and hemostasis. A single clone for GMP-140 was isolated from a mouse lung cDNA library using the human cDNA as a probe. Northern blot analysis of mouse tissues with the murine cDNA probe revealed no detectable baseline expression in the liver, lung, heart, kidney, thymus, spleen, or brain. Treatment of mice with lipopolysaccharide (LPS) endotoxin caused a marked increase in mRNA for GMP-140 in these tissues. The most prominent increase was observed in liver and lung with peak levels of mRNA four hours after LPS treatment. Based on these data, a cDNA library was constructed from liver taken after LPS stimulation, and nine additional cDNA clones were isolated. These clones are currently being characterized. At the present, 30% of the coding region has been sequenced. The overall homology on a nucleotide level between human and mouse cDNA sequence is 80%. A region consisting of tandem consensus repeats related to those in complement-binding proteins is present in the mouse cDNA. The amino acid identity of mouse to human in sequenced areas of this domain is 83%. The complete transmembrane and cytoplasmic domains show 83% and 89% amino acid identity respectively. Also, seven genomic clones have been isolated and characterization is in progress. In order to further elucidate this glycoprotein's function in thrombosis and inflammation, one goal is to generate mouse mutations in GMP-140 using homologous recombination in embryonic stem cells.

G 214 CHANGES IN CYCLIC AMP LEVELS IN ENDOTHELIAL CELLS SUBJECTED TO PULSATILE STRETCH, Bauer Sumpio, Toshiaki Iba, Dept. of Surgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

EC form the inner lining of blood vessels and are subjected to both shear stress and a pressure-induced pulsatile stretch. Most studies, however, are performed with EC under stationary conditions. We have previously demonstrated that subjecting EC in culture to cyclic stretch will modulate their production of various substances including tissue plasminogen activator (tPA). Since other studies indicate an inverse relation between intracellular levels of cAMP and tPA levels, we hypothesize that EC exposed to mechanical stretch will have changes in intracellular cAMP levels which may then account for the enhanced tPA secretion. To study this, EC were obtained from bovine aorta and maintained in DMEM containing 10% fetal bovine serum and other substrates. EC were seeded in culture plates with flexible membrane bottoms ($2x10^5$/ 25 mm well) and grown to confluence. Vacuum was used to deform the membrane bottoms. EC were pretreated with 5 mM IBMX, a phosphodiesterase inhibitor, and then subjected to 24% strain at 60 cycles/min (0.5 s elongation alternating with 0.5 s relaxation) for various time periods. After cyclic stretch, cAMP was extracted with 65% ethanol and measured by radioimmunoassay. The results are fmol/10^3 cells ± SD, n= 4-6, *$p < 0.05$, (unpaired t-test).

	0	3min	5min	10min	30min	60min
cAMP	17.4±1.7	11.3±2.0	6.7±0.8	3.7±0.3	3.0±0.2	6.9±2.3

The results show 10 min after initiation of cyclic stretch, cAMP levels reached the lowest level. This depletion continued for about 30 min before returning slowly to baseline. Thus, pulsatile stretch will decrease intracellular cAMP levels and may account for the increase in tPA production. Cyclic stretching may stimulate the fibrinolytic activity of EC.

G 215 TGFβ1-INDUCED MESENCHYMAL ACCUMULATION AND ANGIOGENESIS IN THE CHICKEN CHORIOALLANTOIC MEMBRANE: POTENTIAL FIBROBLAST AND ENDOTHELIAL CELL INTERACTIONS. Edmund Y. Yang and Harold L. Moses, Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

TGFβ1 is a potent angiogenic factor *in vivo* (Yang, E.Y., and Moses, H.L., *J. Cell Biol.* 111:731-741, 1990). This capacity cannot easily be reconciled with the direct growth inhibitory effects of TGFβ1 on endothelial cells *in vitro*. We hypothesize that during both wound healing and embryogenesis, TGFβ1 may function indirectly, via an intermediate cell type, to induce angiogenesis. When TGFβ1 is applied to the chicken chorioallantoic membrane (CAM) a specific sequence of effects are observed. Fibroblasts migrate rapidly (4-8 hours) towards the point of TGFβ1 delivery. They then become growth inhibited and begin to express increased collagen and sulfated glycosaminoglycans. Extracellular matrix is expressed in graded manner consistent with the diffusion of TGFβ1 into the CAM. In contrast, endothelial cells in the form of capillary sprouts are not observed until later (12-16 hours). Capillary growth occurs predominantly through regions of previous fibroblast accumulation and extracellular matrix deposition. These observations suggest that fibroblasts may interact intimately with endothelial cells during TGFβ1-induced angiogenesis. Fibroblast-derived extracellular matrix or growth factors may act in a paracrine manner to stimulate angiogenesis.

Molecular Mechanisms of Vascular Smooth Muscle Cell Growth

G 300 INDUCTION OF PLATELET-DERIVED GROWTH FACTOR EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS BY LOW DENSITY LIPOPROTEIN, Peter Barath, Meina Ren and James Forrester, Cedars-Sinai Medical Center, Los Angeles, CA 90048 We hypothesized that low density lipoprotein (LDL), a general cell activator may have role in induction of growth factor expression. The aim of this study was to determine the effect of LDL on platelet-derived growth factor (PDGF) A chain transcription in vascular smooth muscle cells (VSMC). Methods: Rat aortic SMC were cultured in DMEM + lipoprotein deficient serum for 48 hours and in DMEM + serum free medium for an additional 48 hours. Cells were then incubated with 250 ug/ml LDL (experimental group, LDL) or without LDL (negative control, C) for 6 hours. A human osteogenic sarcoma cell line served as positive control (U-2OS). Total RNA was extracted and assayed for the presence of PDGF A chain mRNA transcripts using a 1.3 kb human PDGF A chain cDNA probe. Results: LDL increased the signal intensity of all three PDGF A transcripts, especially that of the 2.3 kb. Relative density of PDGF A transcripts on the Northern blot was: 2.9 kb C:6,887, LDL:301,330, U-2OS:386,880; 2.3 kb C:10,669, LDL:621,690, U-2OS:694,480; 1.7 kb C:48,664, LDL:85,203, U-2OS:87,472. Conclusion: LDL is a strong and selective activator PDGF A transcription in cultured VSMC. LDL may have a role in the initiation of SMC proliferation via induction of PDGF A expression.

G 301 **URIC ACID STIMULATION OF VASCULAR SMOOTH MUSCLE CELL PROLIFERATION IS DEPENDENT ON PDGF-A CHAIN EXPRESSION.** Gadiparthi N. Rao and Bradford C. Berk, Cardiovascular Division, Dept. of Medicine, Emory University School of Medicine, Atlanta, GA.

An association between hyperuricemia and atherosclerosis has been established for many years. Recent data suggest that uric acid is generated locally in the vessel wall by the action of xanthine oxidase. This enzyme, activated during ischemia/reperfusion by proteolytic conversion of xanthine dehydrogenase, catalyzes the oxidation of xanthine, thereby generating free radicals and uric acid. Because of the potential role of ischemia/reperfusion in vascular disease, we studied the effects of uric acid on rat aortic smooth muscle cells (RASM) growth. Uric acid stimulated RASM DNA synthesis, as measured by ^{3}H-thymidine incorporation, in a concentration-dependent manner with half maximal activity at 150 μM. Maximal induction of DNA synthesis by uric acid was ~70% of 10% calf serum and equal to 10 ng/ml PDGF-AB or 20 ng/ml FGF. Neither uric acid precursors (xanthine and hypoxanthine) nor antioxidants (ascorbic acid, glutathione and α-tocopherol) were found to be mitogenic for RASM. Uric acid was mitogenic only for vascular cells, stimulating RASM and endothelial cell growth but not fibroblasts or renal epithelial cells. The time course for uric acid stimulation of VSMC growth was slower than serum suggesting induction of an autocrine growth mechanism. Uric acid stimulated the expression of PDGF-A chain mRNA in quiescent RASM cells by 5-fold over control (peak at 8 h). Uric acid-induced ^{3}H-thymidine incorporation was completely abolished by incubation with PDGF-A chain polyclonal antibodies. Thus uric acid stimulates RASM growth via an autocrine mechanism involving PDGF-A chain. These findings suggest that generation of uric acid by such processes as ischemia/reperfusion contribute to atherogenesis and intimal proliferation.

G 302 **THE PHOSPHOLIPASE C-COUPLED SEROTONIN RECEPTOR MEDIATING RAT AORTIC SMOOTH MUSCLE CELL GROWTH IS THE 5-HT$_2$ SUBTYPE.** Marshall A. Corson, R.W. Alexander, and Bradford C. Berk, Cardiovascular Divsion, Dept. of Medicine, Emory University, Atlanta, GA

Proliferation of smooth muscle cells in arteries is associated with contractile hypersensitivity to serotonin (5-HT). We hypothesized that proliferating cells express increased numbers of phospholipase C (PLC)-coupled 5-HT receptors (5-HTR), which mediate contraction via Ca^{2+} mobilization and growth via phosphatidylinositol turnover. To test this we compared 5-HT$_2$R and 5-HT$_{1C}$R mRNA levels in normal rat aorta and phenotypically modulated rat aortic smooth muscle cells in culture. Cultured cells were chosen as a model for proliferative cells *in situ* because of their phenotypic resemblance. We found by Northern blot analysis that cells in culture expressed 50-fold higher levels of 5-HT$_2$R mRNA than aorta. 5-HT$_{1C}$R mRNA was not detected in either case. The 5-HT$_2$R in cultured cells activated PLC, as demonstrated by a 500% peak increase in $[Ca^{2+}]_i$. 5-HT stimulated a 10-fold increase in c-*fos* mRNA accumulation, which was inhibited by the 5-HT$_2$R antagonists ketanserin or LY 53857. 5-HT (1 μM) stimulated cell growth, as shown by a 50% increase in ^{3}[H]-thymidine incorporation. These growth-related signals indicate that the 5-HT$_2$R is expressed in these cells and is functionally coupled. The data suggest that the same receptor responsible for 5-HT contractile hypersensitivity in atherosclerosis and restenosis is partially responsible for the increased smooth muscle proliferation observed in these disease states.

G 303 PDGF ISOFORMS AND THEIR RECEPTORS: THE PDGF β-RECEPTOR SUBUNIT CAN ALSO BIND PDGF A-CHAIN.

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Platelet-derived growth factor (PDGF) is a dimer, where two different PDGF-chains (A, B) can form three possible isoforms (AA, AB, BB). Likewise there are two different receptor subunits (α, β), and high affinity binding of PDGF ligand requires association of two receptor subunits [R.A.Seifert et al., (1989), J. Biol. Chem., 264, 8771-8778]. It was proposed that the α-subunit can bind either the B- or the A-chain but the β-subunit could only bind the B-chain. According to this model PDGF-AA should not bind to the ββ-receptor.

We examined this hypothesis by studying the competition of ^{125}I-PDGF-BB binding by all three isoforms of PDGF on human skin fibroblasts (AG 1523), cells which express predominantly the β receptor subunit. All isoforms competed with ^{125}I-PDGF-BB for the binding to the ββ-receptor. However, PDGF-AA was about 10^4 times less effective at competing than the PDGF-BB homodimer. The PDGF-AB heterodimer gave a medium value, i.e. was about 10^2 times less effective.

From these observations we conclude that the β receptor subunit can also bind the PDGF A-chain, but to a much lesser extent than the B-chain. These results are in accordance with a model which explains the binding of PDGF to its receptor as a cooperative binding of two receptor-subunits to two independent sites on the ligand dimer. In such a way two relatively weak associations between a receptor subunit and a ligand subunit would result in a stronger binding in the dimeric, noncovalent receptor complex.

G 304 EFFECTS OF CYCLIC STRETCH ON GROWTH, ION TRANSPORT, AND GENE EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS.

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In situ, vascular smooth muscle (VSM) cells are subjected to continual cyclic deformation. Most work on growth factors in VSM has been carried out in static culture dishes. To study the effects of cyclic deformation on cell growth, VSM were grown in rubber bottom plates subjected to cyclic stretch (60 cycle/min, 24h). Stretch increased the basal rate of thymidine incorporation 2.7 ±0.3 fold and increased cell number from 1.7 to 2.4 x 10^5 cells/dish. DNA synthesis was maximal from 24-30 h after initiation of stretch. Stretch also increased thrombin (1 U/ml)-induced thymidine incorporation from 15 ± 3 fold over control to 33 ± 7 fold. Stretch did not significantly enhance the growth response to PDGF (5 ng/ml; 28 ± 4 fold, PDGF; 33 ± 5 fold, PDGF + stretch). We then asked if stretch affects the early signaling events induced by growth factors. Stretch did not affect the increase in intracellular Ca^{2+} after either thrombin (8.2 fold, thrombin; 7.7 fold, thrombin + stretch) or PDGF (3.9 fold, PDGF; 3.6 fold, PDGF + stretch). Stretch also did not alter the intracellular pH response to thrombin (0.10 pH unit, thrombin; 0.09 pH unit, thrombin + stretch) or to PDGF (0.09 pH unit, PDGF; 0.08 pH unit, PDGF + stretch). In contrast to its effects on ion transport, stretch did alter the expression of certain genes. Stretch enhanced the response of c-*myc* to thrombin (2.6 densitometry units, thrombin; 3.4 units, thrombin + stretch) and increased expression of the PDGF A-chain both in control cells (7.3 units, control; 19 units, stretch) and in thrombin-treated cells (28 units, thrombin; 45 units, thrombin + stretch). In conclusion, cyclic stretch appears to be an important factor in the growth of VSM. This effect is probably not mediated at the level of early signal transduction, but most likely at the level of gene expression.

G 305 THIOL PROTEASE INHIBITORS AS ANTIPROLIFERATIVE AGENTS IN VASCULAR SMOOTH MUSCLE.

Keith March, Brian Patton, David Hathaway. Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46202.

Smooth muscle proliferation is a prominent feature of the vascular response to mechanical injury. Accordingly, modulation of proliferation has important therapeutic implications for angioplasty restenosis. We have recently identified a subclass of thiol protease inhibitors (TPI) that reversibly inhibit bovine aortic smooth muscle cell (BASMC) proliferation in vitro. In order to further define the nature of this inhibition, two-color flow cytometry (FCM) and Northern analysis of mRNA were employed. FCM was performed using propidium iodide and fluorescein-labeled anti-bromodeoxyuridine demonstrating total and synthesized DNA, respectively. Treatment of BASMC with benzyloxycarbonyl-leu-norleucinal (Calpeptin) at 100 μM and acetyl-leu-leu-norleucinal (TPI-1) at 50 μM was shown to cause a block of PDGF- as well as serum-inducible cell cycle progression at a point prior to the G1-S boundary, reducing the percentage of bromodeoxyuridine-positive cells from 87 to 5 over a 24 hr labeling period. Addition of TPI-1 at various times following serum addition to serum-deprived BASMC showed 80% of the maximal block of DNA synthesis even when added 6 hrs after serum. Block of cell cycle progression was gradually lost as the delay from serum to TPI-1 application was increased from 6 to 12 hrs. By Northern analysis of mRNA following serum addition, TPI-1 caused a 4-fold decrease in the transient elevation of fos proto-oncogene as well as a decrease in the levels of both muscle and non-muscle actin mRNA induced early after serum addition. Combined with FCM data, these results suggest more than one molecular site potentially involved in inhibition by TPI of the progression from G1 to S phase in BASMC.

G 306 MULTIPLE ENHANCER ELEMENTS MEDIATE THE INDUCTION OF c-FOS BY ANGIOTENSIN II IN RAT AORTIC SMOOTH MUSCLE (RASM) CELLS. Allen J. Naftilan, Gretchen K. Gilliland and Carol S. Eldridge. Hypertension Program, U. of Alabama at Birmingham, Birmingham, Al. Angiotensin (Ang) II results in the transcriptional activation of the protooncogenes c-fos, c-jun and c-myc as well as the gene for PDGF A-chain. Becouse of its importance in cell growth we investigated the molecular mechansim by which Ang II induces c-fos. RASM cells were cotransfected with plasmids containing portions of the mouse c-fos gene linked to the chloramphenicol acetyl transferase (CAT) gene and the pMSV-β-galactosidase plasmid. After serum starvation for 48 hours, the cells were stimulated with Ang II ($5x10^{-6}$M), fresh media comtaining 10% fetal bovine serum or vehicle (20 mM acetic acid), harvested in 6 hours and assayed for protein, β-galactosidase activity and CAT activity by thin layer chromatography. After exposure to x-ray film, the spots were counted and corrected for β-galactosidase activity. Four plasmids were used; -356 CAT with base pairs -356 to +109 of the c-fos gene which includes the SRE; -151 CAT with base pairs -151 to +109 which eliminates the SRE; -96 CAT with base pairs -96 to +109 which contains the cAMP response element; and -56 CAT which eliminates all enhancer elements. The results from 6 experiments for each plasmid are given below as corrected % acetylation:

	Ang II	Serum	Vehicle
-356 CAT	21.5 ± 2.1	31.8 ± 1.6	0.83 ± 0.18
-151 CAT	11.2 ± 1.0	17.3 ± 2.0	0.70 ± 0.06
-96 CAT	1.13 ± 0.09	1.67 ± 0.16	0.04 ± 0.02
-56 CAT	0.04 ± 0.02	0.06 ± 0.02	0.01 ± 0.003

These results indicate that Ang II induces c-fos via an interaction of two enhancer elements, one being the SRE and the second is a unuique element located between base pairs -151 to -96.

G 307 REGULATION OF HUMAN VASCULAR ENDOTHELIAL CELL PROLIFERATION AND GENE EXPRESSION BY ANTIPROLIFERATIVE CYTOKINES, M.K. Offermann* and R.M. Medford+, *Winship Cancer Center and the Department of Medicine, +Division of Cardiology, Emory University, Atlanta, GA. 30322

Many cardiovascular disease states are characterized by abnormalities in myogenic or endothelial cell proliferation that may be due to either abnormal expression of, or response to, a variety of regulatory cytokines. Vascular endothelial cells elaborate several important cytokines with antiproliferative properties such as TGFβ, IFNα/β, and IL1. We have tested the hypothesis that these cytokines utilize both independent and interacting molecular and cellular signalling pathways to mediate their antiproliferative effects. As measured by the percent inhibition of ^{3}H-thymidine incorporation into exponentially growing human umbilical vein endothelial (HUVE) cells, each cytokine alone [IFNα (19%), TGFβ (12%) and IL1 (38%)] exhibits significantly weaker antiproliferative effects than in combination [IL1+IFNα (61%), IL1+IFNα+TGFβ (69%)]. At the molecular level, these cytokine interactions induced dramatic changes in proliferation-associated gene expression that could not be predicted by individual cytokines. By Northern analysis, c-myc mRNA accumulation was unchanged by incubation with any cytokine alone. However, the combination of IFNα and TGFβ led to an 80% decrease in c-myc mRNA. 2'5'-Oligoadenylate synthetase (OAS) regulates a latent RNase L activity that is implicated in alteration of cell growth-associated mRNA levels. OAS mRNA was undetectable in control, TGFβ or IL1 treated HUVE cells but exhibited a strong induction in the presence of IFNα. In combination with IFNα, either TGFβ or IL1 alone amplified the IFNα induction of OAS mRNA an additional two-fold, and with both cytokines, over four-fold. Thus, OAS may play a role in the enhanced antiproliferative response to cytokine combinations. These results suggest that antiproliferative cytokines play a major role in inhibiting HUVE cell proliferation even in the presence of supplemental growth factors. We have also identified several candidate genes whose altered expression could be involved in the dramatic antiproliferative responses to combinations of cytokines.

G 308 SELECTIVE COMPARTMENTALIZATION OF PDGF CONTAINING EXON 6 ENCODED SEQUENCES ON EXTRACELLULAR BINDING SITES OF ENDOTHELIUM AND SMOOTH MUSCLE, Elaine W. Raines and Russell Ross, Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195

Sequestration of growth factors by arterial wall cells may be involved in controlling vascular wall proliferation. The platelet-derived growth factors (PDGFs) may be one of the principal growth-regulatory molecules responsible for the migration and proliferation of smooth muscle cells that result in focal thickening of arteries in atherosclerosis. PDGF can be assembled as a disulfide-bonded homo- or heterodimer from two distinct polypeptide chains (PDGF-A and PDGF-B). Alternative usage of exon 6 and 7 of the PDGF A-chain can give rise to two forms of the A-chain, a long and a shorter, truncated form. In the course of attempting to develop antibodies specific to these distinct C-termini of the two forms of the PDGF-A chain, we observed some unique properties associated with the peptide representing the sequence of the alternatively spliced exon 6 of the PDGF-A-chain (peptide A_L). Peptide A_L induces a dose-dependent, temperature-independent release of PDGF from cultured endothelial cells and smooth muscle cells. Endothelial cells treated with peptide A_L release principally PDGF-BB, whereas treated smooth muscle cells release primarily PDGF-AA. The inducible release of PDGF by peptide A_L is shared by the homologous peptide encoded by exon 6 of the PDGF-B chain. Specific extracellular binding sites for exon 6-derived peptides of the A- and B-chains of PDGF are observed. We hypothesize that translation of exon 6 of the A- or B-chain of PDGF results in selective compartmentalization of these forms of PDGF on extracellular binding sites for the exon 6-encoded sequences. However, the alternatively spliced form of PDGF-A chain lacking exon 6 encoded sequences would be secreted. Such differential compartmentalization may provide a means whereby PDGF can act as a growth factor involved in either cell-cell interactions or in a broader context as a paracrine growth factor. Supported in part by NIH grants HL-18645 and HL-03174.

G 309 IDENTIFICATION OF A NOVEL cDNA WHICH IS DIFFERENTIALLY EXPRESSED IN RESPONSE TO PLATELET-DERIVED GROWTH FACTOR AND ANGIOTENSIN II IN VASCULAR SMOOTH MUSCLE CELLS

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Growth of vascular smooth muscle cells (VSMC) is an important feature of atherosclerosis and hypertension. Mitogens, such as platelet-derived growth factor (PDGF), and vasoactive hormones, such as angiotensin II (Ang II), activate many of the same transmembrane signals and early growth-related genes. However, in cultured adult rat aortic VSMC, PDGF is hyperplastic (stimulating both protein synthesis and cell division) whereas Ang II is hypertrophic (stimulating protein synthesis and increasing cell size without cell division). In order to identify molecular events that might distinguish the responses of VSMC to PDGF and Ang II, a cDNA library was constructed from rat aortic VSMC and differentially screened with ^{32}P-labeled cDNA derived from cells treated for 3 hrs with either 10^{-6}M Ang II or 20 ng/ml PDGF (BB). Several clones, hybridizing to PDGF ^{32}P-cDNA with an intensity of at least 5-fold greater than that of Ang II ^{32}P-cDNA, were isolated. Nucleotide sequencing of one clone, pRASM 9, revealed a novel cDNA. By Northern blot analysis, this cDNA corresponded to an mRNA of ≈ 1 kB that was constitutively expressed at very low levels in quiescent (i.e., serum deprived) VSMC. Addition of either PDGF or 10% calf serum to quiescent cultures resulted in a marked induction of pRASM 9 mRNA, beginning at 30 min and peaking at 2 hrs. In contrast, 10^{-6}M Ang II had no effect on pRASM 9 mRNA levels. Induction of pRASM 9 is an early event that may be important in distinguishing the response of cultured VSMC to PDGF and Ang II. pRASM 9 may be one of a group of genes whose induction is necessary for VSMC hyperplasia.

G 310 INHIBITION OF PDGF β-RECEPTOR SIGNAL TRANSDUCTION BY DOMINANT NEGATIVE MUTANTS - FORMATION OF INACTIVE RECEPTOR HETERODIMERS,

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Ligand-induced dimerization of PDGF β–receptor has been postulated to be an important step in receptor activation and signal transduction. However it has been difficult to prove this notion. We have taken a new approach to investigate the role of dimerization in receptor function. We have tested mutants of the murine PDGF β-receptor which lack intrinsic kinase activity made by truncation of most of the cytoplasmic region or by a point mutation at the putative ATP-binding site, for their ability to block signal transduction when co-expressed with wild type receptor. Both mutant receptors formed ligand-induced dimers in vivo, indicating that the cytoplasmic region is not required for receptor dimerization. Cross-linking studies in vivo demonstrated that a ligand-induced heterodimer between truncated receptor and wild type receptor was defective in receptor autophosphorylation. When co-expressed with wild type receptor in Xenopus oocytes, both kinase-negative mutant receptors abolished function of wild type receptor as determined by a $^{45}Ca^{2+}$ efflux assay. The inhibitory effects were neither due to suppression of translation of wild type receptor nor competition of available ligand. Signal transduction by co-expressed fibroblast growth factor receptor was not affected by either mutant PDGF receptors. This approach establishes a unique method to inhibit receptor function in vivo and will help to define the physiological role of PDGF receptors in transgenic animals.

G 311 MITOGENIC EFFECTS OF VASOACTIVE PEPTIDES ON RAT VASCULAR SMOOTH MUSCLE CELLS,

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We have investigated the possible mitogenic effects of the vasoconstrictor angiotensin II (angII) and the three isoforms of the endothelin (ET) peptide ET-1, ET-2 and ET-3 and the structurally similar peptide sarofotoxin S6b on rat vascular smooth muscle cells in culture. DNA synthesis was determined by a double antibody technique to detect bromo-deoxyuridine incorporation into the nucleus. Active nuclei were counted on a Quantimet image analysis system. Measurement of intracellular pH demonstrated that angII, ET-1 and platelet derived growth factor (PDGF) all activated the sodium-hydrogen antiport within minutes of stimulation, confirming a common early intracellular signalling pathway. Whilst PDGF and epidermal growth factor (EGF) both induced DNA synthesis when added alone, neither angII nor any of the ET peptides or sarafotoxin S6b was capable of initiating DNA synthesis in the absence of other mitogens. All the ET peptides potentiated the mitogenic effect of low concentrations of PDGF, ET-1 and ET-2 being considerably more potent than ET-3 and sarafotoxin S6b. By contrast angII showed no such effect. These findings suggest that whilst neither angII nor ET have any significant mitogenic effect on vascular smooth muscle cells in the absence of other mitogens, the release of ET at sites of endothelial injury may well enhance the mitogenic action of locally acting PDGF on vascular smooth muscle cells and potentiate the proliferative response.

In Vivo Gene Expressions in Vascular Disease

G 400 LACK OF ANTIPROLIFERATIVE EFFECT OF NIFEDIPINE IN RE-INJURED AORTA. Raymond C. Bush and Christopher L. Jackson. Dagenham Research Centre, Rhone Poulenc Rorer Inc., Essex, U.K. and Department of Pathology, University of Washington, Seattle, Washington 98195, U.S.A.

Nifedipine inhibits the proliferation of arterial smooth muscle cells (asmc) in injured aorta, but only if administration is started early (1). This suggests that nifedipine inhibits the activation of quiescent asmc and will therefore not prevent restenosis after angioplasty. We have tested this hypothesis in a rat model. Rats were balloon catheterised twice at an interval of 3 weeks. Appropriate sham operated controls were also done. Aortic DNA synthesis was measured by injection of [3H]-thymidine 48h after the second injury. Both the first and second balloon injuries produced a rise in [3H] labeled DNA, but nifedipine administration (10mg/kg bid) only reduced the [3H] incorporation after a single injury (-35%, $p<0.05$). Thus nifedipine will inhibit asmc proliferation in "naive" vessels but not in previously injured ones. The findings of the INTACT group (2) that nifedipine reduced the incidence of new coronary lesions, but did not affect existing lesions is consistent with our hypothesis that nifedipine inhibits the entry of quiescent asmc into the cell cycle, but has no effect on cells that are already proliferating.

1. Jackson, C.L. et al (1989) Atherosclerosis, 80, 17-26.
2. Lichtlen, P.R. et al (1990) Lancet, 335, 1109-1113.

G 401 INCREASED SMOOTH MUSCLE CELL FIBRONECTIN SYNTHESIS IN DONOR CORONARY ARTERIES IN A PIGLET CARDIAC TRANSPLANTATION MODEL MAY BE RELATED TO THE GRAFT OBSTRUCTIVE ARTERIOPATHY, Nadine Clausell, John Coles, Marlene Rabinovitch, Division of Cardiovascular Research, Hospital for Sick Children,Toronto, ON, Canada, M5G 1X8.

Progressive coronary artery obstruction as a result of intimal proliferation is a major complication following cardiac transplantation. This lesion is characterized by disruption of internal elastic lamina (IEL) followed by proliferation of smooth muscle cells (SMC) and migration into the subendothelium. Previous studies in our laboratory have suggested that increased SMC migration and associated intimal proliferation in the fetal lamb ductus arteriosus is related to increased fibronectin (FN) synthesis. We therefore studied a piglet cardiac transplant model at four weeks and assessed extracellular matrix (ECM) components in tissue and SMC cultures comparing the native (host) and the transplanted heart (donor) coronary arteries. Structural abnormalities were evident by both light, electron and immunoelectron microscopy (LM, EM, IEM). On LM there was fragmentation of the IEL in the donor compared to the host coronary arteries. On EM, the SMC had switched to a migratory phenotype and on IEM, there was increased FN, especially in the subendothelium. The SMC from donor and host coronary arteries were used at confluence at passages 2 and 3, labelled with [^{35}S]-methionine for 24 hours and the major ECM glycoproteins, FN, laminin and type IV collagen were determined by using gelatin-sepharose extraction. The FN adherent to the column and the eluant containing laminin and type IV collagen were resolved by 5% SDS-PAGE and autoradiography, and quantified. Identification of each ECM component was confirmed by western immunoblot. We demonstrated that donor SMC produce 4 times more FN than the host SMC in serum free medium and 2 times more in presence of serum. There were no differences in the amounts of laminin and type IV collagen synthesized. Our data in the donor coronary artery both *in vivo* and *in vitro* are similar to those observed in the ductus arteriosus and suggests that increased FN may precede and influence SMC migration in the coronary arteriopathy post-cardiac transplantation.

G 402 HYPERTENSION INDUCED ALTERATION IN VASCULAR SMOOTH MUSCLE CELL PROTEOGLYCAN EXPRESSION, Kevin L. Dreher, Deborah Matzura and Kelley Cowen, Geisinger Clinic, 26-17, Weis Center for Research, Danville, PA 17822.

Proteoglycans (PG) are capable of influencing a number of aspects of vascular smooth muscle (VSM) cell behavior such as growth, migration, extracellular matrix content and assembly and morphology. In addition, these macromolecules are capable of complexing with low density lipoprotein (LDL) and thereby may influence the deposition of LDL within arterial tissue. We have examined the influence which hypertension has on VSM cell PG expression using the Dahl S rat strain. Dahl S rats maintained on a 4% NaCl rat chow diet for 7 weeks displayed a mean arterial bp of 185 ± 17 mmHg, whereas a control set of Dahl S rats maintained on a 1% NaCl rat chow diet displayed a mean arterial bp of 116 ± 27 mmHg. Computer assisted morphometric analysis demonstrated a 34% increase in intimal thickening within thoracic aortas recovered from hypertensive rats. Results obtained from in vitro dual-radiolabeling studies demonstrated a 2-fold increase in protein synthesis and a 2.3-fold increase in proteoglycan biosynthesis within thoracic intimal tissue recovered from hypertensive rats. These findings demonstrate that VSM cell PG expression is effected by hypertension. The levels at which VSM cell PG expression is altered, transcriptional versus post-transcriptional, by hypertension is currently being investigated and will be presented. The increase in VSM cell PG expression observed hypertensive rats may be mechanically mediated since: 1) hypertensive rats contain lower renin levels when compared to their normotensive counterparts [J. Iwai, et al, Circ. Res. 32:678-683, 1973]; 2) vasopressin does not play a role in the maintenance of hypertension in Dahl S rats [H. Matsuguchi, et al, Hypertension 3:174-181, 1981]; 3) there was no effect of enalapril maleate on mean arterial bp or thoracic intimal thicknening in Dahl S rats maintained on an 8% NaCl diet for 4 weeks; 4) in vitro radiolabling studies demonstrated that a 30 hour exposure of 1st pg. VSM cells to angiotensin II (100 nM) resulted in a 47% decrease in the incorporation of $^{35}SO_4^=$ into secreted high molecular weight proteoglycans. Collectively, these findings suggest that hypertensive alteration of VSM cell PG expression may be mediated via a mechanosignal transduction mechanism.

G 403 BALLOON CATHETER DE-ENDOTHELIALIZATION OF THE NUDE RAT CAROTID: RESPONSE TO INJURY IN THE ABSENCE OF FUNCTIONAL T LYMPHOCYTES

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The development of an intimal proliferative lesion after balloon catheter de-endothelialization was studied in congenitally athymic nude rats, lacking T lymphocytes. Significant intimal thickening was observed in both the homozygous (nu/nu) and euthymic heterozygous (nu/+) animals six days after injury, which had increased further after ten days. There was no significant difference in mean intimal: medial cross-sectional area between the nu/nu and nu/+ animals at either time point. Approximately 1% of the cells in the neointima of both groups of animals were leukocytes (OX-1 positive); 0.7% were macrophages (ED-1 positive). In neither nu/nu nor nu/+ animals did T lymphocytes (OX-19 positive cells) constitute more than 0.1% of the neointimal cell population. These data suggest that T lymphocytes do not play a significant role in the accumulation of neointimal cells. The presence of macrophages within the lesions raises the possibility that they may be involved in the recruitment and proliferation of smooth muscle cells. In-vitro characterization of nu/nu carotid medial smooth muscle cells demonstrated approximately 500,000 binding sites for PDGF-BB and few PDGF-AA binding sites (<10,000). The mitogenic and chemotactic responses of these cells to the three dimeric forms of PDGF were in accord with this receptor subunit distribution. PDGF accounted for approximately 50% of the mitogenic activity of a rat platelet releasate. PDGF-BB and PDGF-AB were both potent chemotactic agents for the nude rat carotid smooth muscle cells with a peak response at approximately 10 ng/ml. In contrast, PDGF-AA, TGFß, and basic FGF were only weak chemoattractants for these cells.

G 404 THE ROLE OF PLASMINOGEN ACTIVATORS IN ARTERIAL SMOOTH MUSCLE CELL MIGRATION *IN VIVO*, C.L.Jackson and M.A.Reidy, University of Washington, Seattle, WA 98195.

Smooth muscle cell migration from the arterial media to the intima is an essential component in the development of atherosclerotic lesions, and is also a feature of the response of the rat carotid artery to balloon catheter injury. Since the role of plasminogen activators in the migration of other cell types is well established, we have used the carotid artery balloon injury model in rats to investigate the involvement of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) in arterial smooth muscle cell migration. In animals subjected to a sham procedure, the tissue possessed little net plasmin-generating activity. Two days after injury, plasmin generation was slightly increased. By 4 days, when smooth muscle cells began to migrate out of the media into the intima, plasmin generation in the injured left carotid was significantly greater than in the contralateral control vessel. Approximately 90% of this increase was due to tPA. Immunohistochemical analysis of rat carotid arteries showed an increase in tPA staining commencing 3 days after injury, and Northern analysis revealed that message levels for uPA and tPA also increase at this time. In contrast, PAI-1 message showed only a brief elevation 6 hours after surgery. We hypothesise that the proteolytic activity of plasmin, formed from plasminogen by the action of smooth muscle cell-derived tPA, both directly and indirectly facilitates the movement of cells from the media to the intima.

G 405 EXPRESSION OF THE PDGF-INDUCDIBLE GENE *JE* FOLLOWING VASCULAR INJURY IN-VIVO. Jonathan D. Marmur, Michael Poon, Barrett J. Rollins, Mark B. Taubman, Mount Sinai School of Medicine, New York, NY & Harvard Medical School, Boston, MA.

In experimental models of atherosclerosis secondary to balloon injury and/or high cholesterol diet, monocyte infiltration of the vessel wall has been identified as an early event. It has recently been reported that the platelet-derived growth factor (PDGF) inducible gene *JE* encodes a secretory glycoprotein that is a monocyte chemotactic factor. In culture, treatment of growth arrested vascular smooth muscle cells with PDGF results in the induction of this gene within 2 hours and return to baseline is seen by 12 hours. In order to examine whether *JE* is induced *in vivo*, rabbit aortae were subjected to balloon dilatation, following which mRNA was isolated and analyzed by Northern blot hybridization with a ^{32}P cRNA probe. *JE* was constitutively expressed at low levels in control non-ballooned aortae. Following injury, induction of *JE* peaked between 1 and 4 hours; return to baseline levels was seen by 8 hours. The time course of *JE* gene induction seen *in vivo* closely mimics that seen in cultured VSMC treated with PDGF. We have obtained similar *in vivo* results with *KC*, a PDGF-inducible cytokine that is chemotactic for neutrophils. Thus, induction of PDGF-inducible genes is part of the early response to vessel injury. The cytokines *JE* and *KC* may play important roles in defining that response by recruiting monocytes and neutrophils to sites of injury. Control of the expression of these cytokines *in vivo* may provide a means of preventing the progression of atherosclerosis and/or the development of restenosis following balloon angioplasty.

G 406 SUBSTANCE P AND ALTERED VASCULAR PERMEABILITY IN STREPTOZOTOCIN- DIABETIC RATS, Ronald Mathison and Joseph S. Davison, Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 4N1

Diabetes associated dysfunctions in the neurogenic inflammatory response result from sensory nervous system neuropathy and alterations in receptor-effector mechanisms of vascular tissues. We examined the effects of intravenous substance P (SP; 74 nmoles/kg) and pGlu^5,MePhe^8,Sar^9 SP(5-11) (74 nmoles/kg) on the extravastion of Evans blue (EB) in pentobarbital anaethetized control and 4-5 week diabetic (stretozotocin, STZ; 55 mg/kg) rats. We tested the hypothesis that acute insulin treatment (AI, 10 U/kg 3 h previously) by reducing a cellular energy deficit would correct extravastion defects associated with diabetes. Finally, we determined if cellular glucopenia, induced with 2-deoxyglucose (2-DOG, 200 mg/kg 3h previously) would produce diabetic-like responses in normal rats. The extravasation of EB to both SP and SP(5-11) in the urinary bladder and the pinna of the ear was reduced by more than 50% in diabetic rats when compared to controls. Although AI normalized the extravastion response to SP(5-11), this treatment did not affect the attenuated response to SP. Treatment with 2-DOG actually increased extravasation responses to the two peptides in control animals, in contrast to the anticipated reduction. These results suggest that AI treatment reverses attenuated vascular permeability to an agent (SP(5-11)) which acts on endothelial cells, but is ineffective in modifying the action of SP, which is believed to increase vascular permeability by acting on mast cells. The reduced extravastion in diabetic rats may not be due to an energy deficient state, as 2-DOG increased vascular permeability to the two peptides.

G 407 EVIDENCE THAT TRANSFORMING GROWTH FACTOR ß1 (TGF-ß1) STIMULATION OF PULMONARY ARTERY SMOOTH MUSCLE CELL (SMC) COLLAGEN SYNTHESIS IS POLYAMINE-MEDIATED. Jack W. Olson, Urszula Orlinska and Mark N. Gillespie, University of Kentucky, College of Pharmacy, Lexington, KY 40536

The adverse pulmonary arterial structural changes that underlie monocrotaline (MCT)- and hypoxia-induced hypertensive pulmonary vascular diseases in rats are caused by inappropriate cellular growth, differentiation and extracellular matrix production in each layer of the pulmonary arterial wall. Studies from our laboratories have substantiated that polyamines have essential roles in the evolution of pulmonary hypertension. Our recent observation that lungs from MCT-treated rats have greater amounts of a TGF-ß1 like peptide than control rats suggested that TGFß-1 might be a hypertensive stimuli which enhances lung polyamine synthesis and the ensuing vascular remodeling. Therefore we tested whether collagen synthesis by confluent, quiescent bovine pulmonary artery SMC's could be stimulated by TGF-ß1 in a polyamine-mediated fashion. TGF-ß1 increased ornithine decarboxylase (ODC), the first and generally rate-limiting enzyme in polyamine biosynthesis, in a dose and time dependent fashion with the earliest increase at 3 h and maxium response at 12 h (100 pg/ml). Incubation for 12 h with 100 pg/ml of TGF-ß1 significantly elevated both spermidine and spermine contents as compared to controls (5.4±0.1 vs 7.4±0.17; 19.1±0.3 vs 24.5±0.4 nmoles/mg protein respectively), and the incorporation of ^{3}H-proline into collagen (1106 vs 1653 CPM/10^6 cells). Importantly, co-incubation with 1 mM of difluoromethylornithine (DFMO), a highly specific enzyme-activated irreversible inhibitor of ODC, completely prevented TGF-ß1 stimulation of collagen and polyamine synthesis. Methylaminoisobutyric acid transport studies demonstrated DFMO did not inhibit proline uptake. These data suggest that TGF-ß1 stimulation of SMC collagen synthesis is polyamine dependent. (Supported by HL-44084).

G 408 CULTURED HUMAN ATHEROSCLEROTIC PLAQUE SMOOTH MUSCLE CELLS RETAIN TRANSFORMING POTENTIAL AND DISPLAY ENHANCED EXPRESSION OF THE *MYC* PROTOONCOGENE, J.L. Parkes., R.R. Cardell, F.C. Hubbard, A. Meltzer, and A. Penn, Department of Environmental Medicine, New York University Medical Center, New York, NY 10016.

The proliferation of vascular smooth muscle cells (smc) is critical to atherosclerotic plaque formation. The monoclonal hypothesis proposes that the stimulus for this smc proliferation is a mutational event. Accordingly, plaque smc (p-smc) are thought to derive from a stably transformed, and permanently altered cell population that is distinct from the bulk of arterial smc. We have developed strains of p-smc derived from human aortic plaque using a collagen gel procedure, and have shown that these cells differ from smc cultured from healthy vascular wall (HV-smc) in expression of the protooncogene *c-myc*. One human p-smc strain was extensively characterized; these diploid, karyotypically normal cells have a finite life span in culture. Ultrastructural examination revealed two populations; one exhibited classic contractile smc appearance with longitudinally oriented myofilaments and associated dense bodies. The other cell population appeared modulated to a synthetic state, displaying a well developed dilated RER and Golgi apparatus, and a less developed microfilamentous apparatus. Northern blotting revealed a striking (9-15 fold) enhanced expression of the *c-myc* protooncogene in all p-smc strains tested compared to HV-smc. In contrast, the p-smc and HV-smc expressed similar levels of message for the genes, *N-myc, L-myc, Ha-ras, fos, sis*, LDL receptor, EGF receptor, IGF I receptor, IGF II and HMG CoA reductase. Finally, although p-smc are not tumorigenic, DNA isolated from these cells (like DNA from plaque tissue) is positive in the transfection-nude mouse assay. *c-Myc*, however, is not the transforming gene, since no newly introduced human myc gene was detected in any of the p-smc transformants. Thus, human atherosclerotic p-smc possess both an activated *c-myc* gene and a transforming gene which is retained throughout many cell passages. (Supported by AHA Grant-in-Aid 87-993, and NIEHS R01 02143).

G 409 VASCULAR SMOOTH MUSCLE GROWTH IN EXPERIMENTAL ATHEROGENESIS AND HYPERTENSION. Paolo Pauletto, Institute of Clinical Medicine, University of Padova, 35100 Padova, Italy.

Monoclonal anti-myosin antibodies and immunocytochemical procedures showed the existence of a specific differentiation pattern in the smooth muscle (SM) tissue of rabbit aorta during development. Myosin isoform composition and distribution were studied in two experimental models of SM growth: cholesterol-fed and Goldblatt II (two-kidney, one clip) hypertensive rabbits. SM cells which accumulated in the atherosclerotic plaque showed a myosin isoform content of "immature"-type; this proliferative event was accompanied by a consistent increase of a SM cell population with similar antigenic characteristics in the underlyng media. A comparable change in myosin immunoreactivity was also found in the aortic media from hypertensive rabbits. In aortas from Goldblatt rabbits, SM cells with an "immature"-type of myosin isoform expression were distributed as: (1) a continous layers, at the level of the subendothelial region, or (2) foci of variable size, dispersed within the media. These data indicate that both chemical and mechanical injuries to the arterial wall are accompanied by a selective augmentation of a unique medial SM cell population with antigenic properties in common with developing aortic SM.

G 410 TRANSFORMING ELEMENTS ARE DETECTABLE IN EARLY STAGE ARTERIOSCLEROTIC PLAQUES, A. Penn, F.C. Hubbard Jr. and J.L. Parkes, Department of Environmental Medicine, New York University Medical Center, 550 1st Ave., New York, NY 10016

The carcinogen-treated cockerel is a model for studying early stages of arteriosclerotic plaque (plq) formation. Carcinogen administration accelerates plq development in cockerels. Transforming elements are present in DNA from advanced human plq (PNAS 83: 7951,1986). Here we ask whether transforming elements can also be detected at early stages of plq development in cockerels. NIH3T3 cells were transfected with DNA from: a) plq isolated from carcinogen-treated cockerels; b) healthy artery wall underlying the plq; c)T24-human bladder carcinoma cells [positive control]; and d) NIH3T3 cells [negative control]. Approximately 5 x 10^6 cells from each transfected group were injected into nude mice. Tumors appeared in 5/5 mice in groups 'a' and 'c'; no tumors appeared in mice from groups 'b' and 'd'. All five plq DNA-associated tumors hybridized to a cockerel genomic probe. Eight cockerel-specific bands were identified in Eco R1 digests of 1st round (primary) tumors. DNA from a primary tumor was tested in a 2nd round of transfection. 5/5 mice developed tumors following injection with these 2nd round transformants. A single cockerel-specific band (5.2 kb) was seen in Eco R1 digests of 2nd round tumors. No evidence was found for activation of the oncogenes, *Ha-ras*, *Ki-ras*, *src* or *myc* in the plq-associated tumors. Similarly, DNA from plq-associated tumors did not hybridize to probes for Marek disease virus, Herpes Simplex 1, or reverse transcriptase, suggesting that neither herpes viruses nor retroviruses are involved in the transforming activity of plq DNA. These results indicate that transforming elements are a general property of arteriosclerotic plq, and are detectable in plq of young animals.

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G 411 MECHANISM OF CORTICOSTEROID INHIBITION OF *JE* EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS Michael Poon, Hong Zhang, Claire-Lise Rosenfield, and Mark B. Taubman. Brookdale Center for Molecular Biology and Department of Medicine, Mt. Sinai School of Medicine, New York, NY, 10029.

Corticosteroids inhibit the growth and migration of cultured vascular smooth muscle cells (VSMC). The molecular mechanisms underlying the corticosteroid effect on VSMC are poorly understood. We have examined the expression of *JE*, a secretory glycoprotein that functions in part as a monocyte and VSMC chemoattractant. Induction of *JE* mRNA and protein has been found in cultured VSMC treated with platelet-derived growth factor(PDGF) and in rat aortas shortly after balloon injury. We have previously reported that treatment of rat aortic VSMC with dexamethasone (1uM) completely blocked the induction of *JE* mRNA by PDGF (20 ng/ml BB) or 10% calf serum. Complete inhibition of *JE* mRNA expression occurred within one hour of treatment with corticosteroids. To investigate the mechanisms by which corticosteroids effect growth factor-stimulated expression of *JE* mRNA, VSMC were treated with the protein synthesis inhibitor cycloheximide (10uM). Cycloheximide completely blocked the effect of dexamethasone, demonstrating that protein synthesis was necessary for corticosteroid-mediated inhibition of *JE* expression. Levels of *JE* mRNA transcription were measured by nuclear run-on assays and were found to be only minimally affected by corticosteroids. In contrast, *JE* mRNA half life was markedly reduced in the presence of corticosteroids. These results suggest that the inhibitory effect of corticosteroids on *JE* expression is due in large part to a protein-dependent change in mRNA stability and are in contrast to most other corticosteroid-responsive genes, which are regulated at the level of transcription by a protein synthesis-independent mechanism.

G 412 IMMUNOHISTOLOGIC DEMONSTRATION OF PLATELET-DERIVED GROWTH FACTOR(PDGF) IN SKIN BIOPSIES FROM KAWASAKI DISEASE (MUCOCUTANEOUS LYMPH NODE SYNDROME)

Saji Tsutomu,Matsuo Norio,Naoe Shiro, and Nagamine Kazuo,Department of Pediatrics and pathology,Toho University,Tokyo,JAPAN,143

Although the mechanisms and pathogenesis responsible for vasculitis are not fully investigated, it has been thought that specific growth factors may have an effect on intimal thickening and vascular dysfunction. Kawasaki disease(KD) is a systemic pan-vasculitis which predominantly involves small to medium-sized muscular arteries. The involvement of endothelial cells and vascular smooth muscle cells in the initiation, formation and development of aneurysmal dilatation of coronary arteries in KD have been emphasized. In this regard, we explored the localization of platelet-derived growth factor(PDGF) in situ. [Patients and Methods] Sections of skin biopsies from six KD patients were investigated using the mouse anti-human PDGF monoclonal antibody(MOCHIDA). Biopsied skin samples obtained in the acute phase were fixed with 4.C ethanol and stained with ABC technique. [Results] In KD patients skin, PDGF was detected in the epidermis and partly in the dermis. The prominent staining for PDGF was found in the endothelial lining of capillalies. Positive staining for PDGF was particularly associated with mononuclear infiltrates and macrophages. [Conclusions] In KD,it is postulated that proliferation of medial smooth muscle cells after acute degeneration is a key factor in later intimal thickening and dysfunction of coronary arteries. In this study, PDGF may play an important role in the pathogenesis of vasculitis and skin lesions in KD.

G 413 CHEMICAL-, HORMONAL-, AND SYMPATHETIC-DEPENDENT REGULATION OF SMOOTH MUSCLE CELL HETEROGENEITY IN RABBIT AORTA, Saverio Sartore, Smooth Muscle Group, Institute of General Pathology, University of Padova, 35100 Padova, Italy.

Monoclonal anti-myosin isoform antibodies and immunofluorescence techniques have revealed the existence of two distinct smooth muscle (SM) cell populations in the aortic media of adult rabbit. The large majority of medial SM cells contains a myosin isoform of SM-type whereas a minority shows the presence of both SM and nonmuscle (NM) myosin isoforms. Co-expression of SM and NM myosin isoforms can also be found in developing aortic SM and in cultured vascular SM cells. The size of the SM cell population of "immature"-type increases during exogenous- and endogenous-induced hypercholesterolemia as well as in hyperthyroidism. Conversely, a marked diminution of this unique medial SM cell population can be observed in: (1) nifedipine- or in propylthiouracil-treated animals, and (2) alloxan-induced diabetes or after L-DOPA denervation. These data give support to the hypothesis that a specific ("stem"-like ?) vascular SMC population, whose size is regulated in a chemical-, hormonal-, and innervation-dependent manner, is involved in the proliferative events which occur in different pathological conditions of the arterial wall.

G 414 EXPRESSION OF TRANSFORMING GROWTH FACTORS ß 1, 2 AND 3 FOLLOWING VASCULAR INJURY, Thomas S. Winokur, Ward Cascells, Anita Roberts and Michael B. Sporn

The transforming growth factors ß(TGF-ß) are a small family of highly homologous peptides. Mammals express isoforms 1, 2, and 3. TGF-ß1, the original member of the family, has been shown to affect the growth and differentiation of both endothelial and vascular smooth muscle cells in culture. In addition, the mRNA and protein of TGF-ß1 have been shown to be elevated following vascular injuries. Since the time course of expression is not consistent with TGF-ß1 functioning as a sole regulator of vascular repair we have investigated the expression of TGF-ß 1, 2 and 3 in rat aortas and carotid arteries injured by balloon deendothelialization. Northern analysis and immunohistochemistry along with ^{3}H thymidine autoradiography were used to address the possibility that an alternate TGF-ß isoform has a role in repair following vascular injury.

Late Abstracts

ALTERED PDGF-MEDIATED SIGNAL TRANSDUCTION AND INCREASED VASCULAR SMOOTH MUSCLE PROLIFERATION IN SHR, R.C. Bhalla, R.V. Sharma and L.M. Bendhack, College of Medicine, University of Iowa, Iowa City, IA 52242

Quiescent semiconfluent cultured aortic VSM cells from 8-9 week old WKY and SHR were used. The PDGF-stimulated $[Ca^{2+}]_i$ was significantly ($P < .05$) greater in SHR compared to WKY. Further, the lag period for increase in $[Ca^{2+}]_i$ was shorter in SHR than WKY suggesting some differences between SHR and WKY in PDGF receptor coupling mechanisms. PDGF increased [^{3}H]-thymidine incorporation in a dose-dependent manner, differences between WKY and SHR increased progressively with increasing concentrations of PDGF and proliferation of SHR cells was significantly greater at PDGF concentration of 8 ng/ml and higher. Calcium channel antagonists inhibited PDGF-mediated [^{3}H]-thymidine incorporation and increase in $[Ca^{2+}]_i$ in both WKY and SHR however the extent of inhibition was greater in SHR. In the presence of calcium channel antagonists the differences between WKY and SHR in the PDGF-mediated increase in $[Ca^{2+}]_i$ and [^{3}H]-thymidine incorporation were abolished indicating that increased Ca^{2+} influx through voltage sensitive Ca^{2+} channels plays an important role in PDGF-stimulated greater proliferation of SHR VSM cells.

EFFECTS OF ENDOGENOUS NITRIC OXIDE ON BASAL TONE AND ENDOTHELIUM-DEPENDENT RESPONSES OF EPICARDIAL AND RESISTANCE CORONARY ARTERIES IN AWAKE DOGS, Frederick R. Cobb, David E. Chambers, Chang-Chyi Lin, William D. Kuehl, Richard M.J. Palmer, Salvador Moncada, Alan Chu, Duke & VA MC, Durham, NC, 27705 USA & Wellcome Res Lab, Beckenham, England

Recent in vitro studies indicate endogenous nitric oxide (NO) is an endothelial derived relaxing factor (EDRF) that is synthesized from L-arginine and that N^G-monomethyl-L-arginine (L-NMMA) is a specific inhibitor of NO synthesis. To assess the physiologic role of endogenous NO in coronary arterial vasomotion, epicardial coronary dimensions (ultrasonic crystals), phasic coronary blood flow (CBF), and systemic hemodynamics were measured in awake dogs (n=4) before and after 5, 15, 50 and 120 mg/kg (i.v.) of L-NMMA. At each dose, measurements were made during basal conditions and after endothelium-dependent dilation induced by (i) augmented flow induced by reactive hyperemia after 20 sec coronary occlusion and (ii) acetylcholine (Ach), 4 μg left atrial bolus injection. L-NMMA induced dose-related increases in aortic pressure (55 mm Hg at max dose) and vasoconstriction of epicardial arteries (4.7, 6.8, 7.8, 7.9% decrease in diameter at 5, 15, 50 and 120 mg/kg respectively). At $\geq$ 50 mg/kg, L-NMMA decreased CBF (resistance artery constriction), maximum 18%. In contrast, endothelium-dependent dilation induced by augmented flow and Ach respectively were attenuated only after the highest dose of L-NMMA, from 5.7% and 4.2% before to 2.3% and 2.1% after L-NMMA. All changes returned toward control after L-arginine. Thus endogenous NO, an EDRF, modulates basal vasomotor tone and endothelium-dependent responses in epicardial conductance and resistance coronary arteries in intact animals.

MEMBRANE FREE CHOLESTEROL CONTENT MODULATES TRANSMEMBRANE Ca^{++} FLUX AND CONTENT IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS, Wilson S. Colucci, Thomas N. Tulenko, and Russell A. Bialecki, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

We tested the hypothesis that membrane free cholesterol content (FC) modulates transmembrane Ca^{++} flux and cellular Ca^{++} homeostasis in vascular smooth muscle (VSMC) and endothelial cells (EC). Cells were grown in culture medium enriched with liposomes consisting of cholesterol/phospholipid in a 2:1 molar ratio, and compared to cells grown with cholesterol-free phospholipid liposomes. In VSMC, incubation with cholesterol-rich liposomes for 24 hr increased membrane FC in relation to the concentration of liposomes added (mean increase in FC = 85 $\pm$ 25% with liposome concentration of 1 mg/ml). FC-enriched VSMC exhibited a marked increase in both early Ca^{++} uptake rate (from 0.026 $\pm$ 0.003 to 0.158 $\pm$ 0.022 nmol Ca^{++}/mg protein/sec; $p < 0.001$) and net intracellular Ca^{++} accumulation (+98 $\pm$ 38%; $p < 0.01$). The increase in Ca^{++} uptake by cholesterol-enriched cells was partially inhibited by nifedipine, diltiazem and verapamil. In EC, liposome exposure also increased FC content and resulted in an increase in Ca^{++} uptake. These studies show that; 1) membrane FC can be enriched in VSMC and EC, and 2) that an increase in membrane FC results in a substantial change in transmembrane Ca^{++} handling.

REGIONAL DIFFERENCES IN INTRA-ARTERIAL GROWTH CONTROL.

Jo G.R. De Mey, Martin Uitendaal, and Paul Schiffers. Department of Pharmacology, University of Limburg, Maastricht, The Netherlands

We compared growth responses in isolated smooth muscle cells and segments of various large and small arteries of adult normotensive and spontaneously hypertensive rats. Doubling time of isolated cells did, with the exception of the thoracic aorta, not differ between WKY and SHR or between types of large artery. It was 5 times slower for resistance arterial smooth muscle cells than for large arterial cells. Serum-stimulated DNA synthesis was transient in the media of all isolated arteries. It did not result in media hyperplasia but in proliferation of smooth muscle cells outside the tunica media. In relative terms it decreased from renal artery, over carotid, superior mesenteric, pulmonary artery and aorta to resistance arteries. The endothelium reduced DNA synthesis in large but not small arteries of WKY. In large arteries of SHR, DNA synthesis was smaller than in those of WKY and was not altered by endothelium. These data suggest intra-arterial growth inhibition and regional differences in growth characteristics of arterial smooth muscle cells which interact with structural differences and parakrine phenomena to result in regional heterogeneity of vascular growth responses. Our observations also indicate that chronic hypertension reduces (1) maximal growth responses in arterial smooth muscle and (2) growth inhibitory influences of the endothelium.

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GENETIC RELATEDNESS OF THE SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RAT

Daniel L. Ely, Mark L. Johnson and Monte E. Turner, Department of Biology, The University of Akron, Akron, OH 44325-3908

DNA from SHR and WKY rats was analyzed for restriction fragment linked polymorphisms (RFLP's) with four genes. DNA was digested with eight restriction enzymes (BamHI, EcoRI, HindIII, MspI, PvuII, PstI, TaqI, and XbaI), electrophoresed on 0.8% agarose gels and transferred to Zetabind. Cloned gene probes were hybridized to these filters. Summarized in table below are the hybridization results for those enzymes demonstrating RFLP's.

Probe	Restriction Enzyme	Strain	Fragment Size Missing (Kb)
v-Bas	EcoRI	WKY	28
	XbaI	SHR	5.2
c-Mos	EcoRI	SHR	16
v-Abl	EcoRI	SHR	25
	HindIII	WKY	12, 9.8
	XbaI	SHR	6.8
L-Pyruvate Kinase	EcoRI	WKY	13
	XbaI	SHR	13

Using these four genes and this panel of restriction enzymes we analyzed a total of 438 nucleotides and detected a minimum of 8 base changes. This indicates a DNA sequence variation between the SHR and WKY rat of 1 base change per 55 nucleotides. This level of genetic variation seems surprisingly high considering the origin of the SHR strain. These RFLP's will provide useful genetic markers for the localization of the hypertension genes.

THE USE OF RECOMBINANT ADENOVIRUS VECTORS TO DELIVER GENES INTO VASCULAR ENDOTHELIAL CELLS,

Robert D. Gerard and Robert S. Meidell, Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235-9038

We have made use of replication-defective recombinant human adenovirus vectors to deliver genes of interest into primary endothelial cells. Initially, our experiments employed a virus that expresses SV40 T antigen from the SV40 promoter. Infection of human umbilical vein endothelial cells (HUVEC) with the virus and immunofluorescent staining showed that every cell expressed T antigen. In keeping with the known immortalizing function of T antigen in human cells, the infected HUVECs could be passaged in culture much longer than control cells infected with an irrelevant adenovirus. Some clones of infected HUVECs continued to produce increased quantities of PAI-1 and PAI-1 mRNA in response to both bacterial lipopolysaccharide and tumor necrosis factor although other clones did not. Tissue-type plasminogen activator (t-PA) and urinary plasminogen activator expression in clones also varied. However, long term culture of these clones did not result in the generation of permanent cell lines with the characteristics of primary HUVECs. More recently, we have used the adenovirus vector to deliver a gene which expresses a serpin-resistant t-PA into canine aortic endothelial cells (CAEC). Infection of CAEC with this virus causes the accumulation of large amounts of t-PA activity in the culture medium and t-PA mRNA in the cells. Infection of intact endothelium with such a virus may provide a means of efficient gene transfer for the treatment of thrombotic disease.

INDUCTION OF AUTOCRINE TRANSFORMING GROWTH FACTOR BETA1 (TGFB1) MODULATES VASCULAR MYOCYTE GROWTH INDUCED BY ANGIOTENSIN II (AII) BUT NOT BASIC FIBROBLAST GROWTH FACTOR (bFGF), Gary H. Gibbons, Richard E. Pratt, and Victor J. Dzau. Division of Cardiology, Stanford Univ. School of Medicine, Stanford CA 94305

TGFb1 expression has been described in vascular myocytes (VM) within the vessel wall after injury. It is presumed that TGFb1 contributes to the pathogenesis of vascular disease in these models. However, TGFb1 is normally secreted in an inactive form and the growth modulatory role of autocrine TGFb1 is undefined. We hypothesized that autocrine TGFb1 modulates the growth response to other growth factors. This study compares the role of autocrine TGFb1 induction on the growth effects of AII and bFGF on confluent, quiescent rat aortic VM in a defined serum-free medium. Under these conditions AII induces hypertrophy whereas bFGF is a potent mitogen. Both AII (10^{-6}M) and bFGF(10ng/ml) induced a several-fold increase in TGFb1 mRNA levels detected within 4 hrs and sustained for over 8 hrs. The induction of TGFb1 was abolished by protein synthesis inhibition. Blockade of TGFb1 activity with a TGFb1 antibody (TGFAB) unmasked AII-stimulated mitogenesis whereas it failed to potentiate the stimulatory effect of bFGF. Conclusions:1)both AII and bFGF induce TGFb1 gene expression via a mechanism dependent upon *de novo* protein synthesis, 2)AII appears to promote the activation of latent TGFb1 whereas bFGF does not.

INHIBITION OF PLATELET INDUCED, TUMOR CELL INDUCED, AND EXOGENOUS 12(S)-HETE INDUCED ENDOTHELIAL CELL RETRACTION BY PGI2 AND PGI2 ANALOGS. Irma M. Grossi and Kenneth V. Honn, Dept. Radiation Oncology, Wayne State University, Detroit, MI 48202

Tumor cell adhesion to vessel walls is a prerequisite for hematogenous tumor cell metastasis. Adherent tumor cells induce the reversible and nondestructive retraction of adjacent endothelial cells to gain access to subendothelial matrix. Recently we demonstrated that exogenous 12(S)-HETE induces nondestructive and reversible endothelial cell retraction at a concentration of 10^{-8}M. 12(S)-HETE is the major lipoxygenase metabolite of arachidonic acid produced by platelets, and has also been demonstrated to be produced by a number of tumor cell lines. We therefore investigated the role of tumor cell derived and platelet derived 12(S)-HETE on endothelial cell monolayers. Murine Lewis lung carcinoma (3LL) cells were demonstrated to induce endothelial cell retraction when coincubated with murine pulmonary vein endothelial cell monolayers. This retraction was enhanced in a dose dependent manner by addition of homologous platelets. Prostacyclin (PGI2) is the major cyclooxygenase metabolite of arachidonic acid synthesized by endothelial cells. PGI2 has been demonstrated to inhibit tumor cell-platelet interactions and reduce lung colony formation in experimental and spontaneous metastasis assays. We've previously demonstrated that PGI2 and stable analogs of PGI2 inhibit 12(S)-HETE stimulated adhesion of tumor cells to endothelial cells, subendothelial matrix and fibronectin. PGI2 analogs of PGI2 were examined for their effect on 12(S)-HETE induced endothelial cell retraction. Treatment of 3LL tumor cells with PGI2 or analogs of PGI2 prior to coincubation with endothelial cells inhibited the tumor cells from inducing endothelial cell retraction. Treatment of platelets with PGI2 or PGI2 analogs inhibited platelet enhanced tumor cell induced endothelial cell retraction. Treatment of endothelial cell monolayers with PGI2 or PGI2 analogs similarly demonstrated an inhibition of exogenous 12(S)-HETE induced endothelial cell retraction.

INHIBITION OF ENDOGENOUS PRODUCTION OF PDGF BY ANTISENSE OLIGONUCLEOTIDES LEADS TO ATTENUATION OF ANGIOTENSIN II-INDUCED VASCULAR HYPERTROPHY

Hiroshi Itoh, Richard E. Pratt, Victor J. Dzau, Cardiovascular Research Center, Stanford University, Stanford, CA 94305

Angiotensin II (AII) has been shown to be a growth promotor of vascular smooth muscle cells (VSMC) and to induce the gene expression of platelet-derived growth factor (PDGF). To examine if the PDGF expression mediates AII-induced hypertrophy, we examined the ability of antisense oligonucleotides complementary to PDGF mRNA to suppress the endogenous production of PDGF in VSMC and, if so, what was the effect of this suppression on Ang II induced VSMC hypertrophy. Two different antisense and sense oligonucleotides (15mers) directed towards the translation initiation site of PDGF-A mRNA were introduced into VSMC by cationic liposome-mediated transfection, prior to AII exposure. A mitogenic assay using mouse 3T3 cells was developed to examine the presence of PDGF in VSMC conditioned media. With this assay we demonstrated the antisense oligomers blocked the production of PDGF stimulated by AII (10^{-6}M). AII treatment of VSMC stimulated the incorporation of ^{3}H-uridine (RNA synthesis) and ^{3}H-leucine (protein synthesis), by 130% and 70% respectively. Both antisense oligomers (2-5 uM) significantly attenuated AII-induced increase of ^{3}H-uridine and leucine incorporation by 50% and 60% respectively, compared to their control sense oligomers. This inhibitory effect of the antisense oligomers was greater than that observed with neutralizing anti-PDGF antibodies suggesting that the action of the endogenously produced PDGF in VSMC occurs by both an autocrine and intracrine pathway. These data provide evidence for the role of endogenous PDGF as mediator of AII mediated vascular hypertrophy.

AN IN VITRO MODEL FOR ANGIOGENESIS IN THE CHOROIDOCAPILLARIS SUBSEQUENT TO LASER TREATMENT FOR PROLIFERATIVE DIABETIC RETINOPATHY, Laura E. Lantry, Andrzej W. Fryczkowski, Ralph E. Stephens. Departments of Pathology and Ophthalmology, Ohio State University College of Medicine, Columbus, OH 43210.

Clinical studies suggest that during treatment of diabetic retinopathy, laser-induced release of cytokines, as well as production of local thermal gradients may stimulate angiogenesis in the choroidocapillaris. We have developed an in vitro model system to investigate the cellular and subcellular response to this injury in human and non-human primates. We have isolated and maintained microvascular endothelial cells for several passages from the vascular beds of both retina and choroid, of diabetic and normal human, rhesus monkey, and baboon. The cultures have been identified by uptake of DiI-Ac-LDL, and the lack of muscle-specific a myosin. We are hopeful that this model system will help to elucidate the underlying pathology seen clinically in proliferative diabetic retinopathy.

GROWTH FACTORS INDUCE SMC MITOGENIC RESPONSE IN EXPERIMENTAL INTIMAL HYPERPLASIA. Anna Lokshin, Daniel M.Enerson, Edwin W.Naylor, Robert A.Schwartz and D.Brent Kerns, Departments of Surgery and Genetics,West Penn Hospital,Pittsburgh,PA and Department of Surgery, SUNY, Syracuse, NY.

Arteriovenous grafts in our dogs produce an experimental counterpart of intimal hyperplasia (IH) in humans. Immunohistochemical techniques were developed to study the expression of platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-beta), PDGF receptors and c-myc oncogene by smooth muscle cells (SMCs) of IH lesion and adjacent muscular media.

Mitogenic effect of PDGF was assayed on cultured SMCs of these IH lesions and adjacent vessel wall. Thrombus formation markedly decreased the response to PDGF in cultured SMCs. Expression of c-myc oncogene and internalization of PDGF/PDGF receptors complex observed in relation to platelet-fibrin thrombus provided collaborative evidence of mitogenic activity in these preparations.

These data support the concept that PDGF and other mitogens are elaborated in response to thrombus formation and may play both an initial and sustaining role in the production of IH lesions.

LOCALIZATION OF AN LPS RESPONSIVE ELEMENT WITHIN THE HUMAN TISSUE FACTOR PROMOTER, Nigel Mackman, Korbinian Brand and Thomas S. Edgington, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Tissue factor (TF) is the receptor and cofactor for factor VIIa initiation of the coagulation protease cascades. TF is functional as expressed on the cell surface but the gene is normally silent in intravascular cells. Thus, transcriptional initiation and regulation of the TF gene is critical for control of this protease cascade. Transient induction of TF on human monocytes stimulated with LPS appears to be the result of de novo transcription. In this study we have used the monocytic cell line THP-1. LPS stimulation led to a rapid, transient 20-fold increase in TF mRNA and a 10-fold increase in TF activity which peaked 5 hr post-induction. Functional mapping of the TF promoter in transfected cells using luciferase as a reporter has localized an LPS responsive element (LRE) to a minimal 56-bp region; this is sufficient to confer LPS inducibility to a heterologous promoter. This region contains two AP-1 consensus binding sites and a site which appears to bind the transcription factor NF-kB in gel shift mobility assays. These studies elucidate a mechanism for induced TF expression within the vasculature, which has been implicated in the pathogenesis of several thrombohemorrhagic disorders.

REGULATION OF GENE EXPRESSION IN ENDOTHELIAL CELLS EXPOSED TO LAMINAR SHEAR STRESS, Larry V. McIntire, Scott L. Diamond, John B. Sharefkin and Suzanne G. Eskin. Department of Chemical Engineering, Rice University, Houston, Texas 77251

Fluid shear stress elevates tissue plasminogen activator (tPA) messenger RNA levels and stimulates secretion of tPA by cultured human endothelial cells, while plasminogen activator inhibitor type-1 secretion remains unstimulated. Endothelial cells also secrete a 21 amino acid peptide called endothelin (MW 2492) which causes vasoconstriction and smooth muscle cell proliferation. To determine whether hemodynamic forces can affect production of this peptide, cells from the same primary culture of human umbilical vein endothelial cells (HUVEC) were maintained in stationary culture or exposed to arterial shear stress (25 dynes /cm^2) for 24 hours. Total cellular RNA was isolated from the shear stressed and stationary cultures and the relative levels of endothelin mRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were determined using a coupled reverse transcriptase/polymerase chain reaction method. As indicated by the amount of amplification product, endothelin mRNA levels were almost undetectable in endothelial cells subjected to shear stress for 24 hours compared to the normal endothelin levels in stationary controls. In contrast, the mRNA levels for GAPDH were similar in control and shear stressed cells. HUVEC secretion of endothelin was reduced by over 50% after 4 hours of exposure to shear stress of 25 dynes/cm^2. Low levels of shear stress (4 dynes/cm^2) had little effect on endothelin release. These results indicate that while some functions of endothelial cell are stimulated by fluid mechanical forces, other functions such as endothelin production are suppressed. In blood vessels, wall thickening is not found in high shear stress zones and this absence may be due to negative control of endothelin production by the shear stressed endothelium. Most notably, it is the low shear zones of vessels which tend to suffer from wall thickening. The use of engineered endothelial cells as vectors for gene therapy would likely be compromised if unregulated endothelin production in low shear zones caused vessel wall thickening and subsequent blockage.

REGULATION OF VON WILLEBRAND FACTOR AFFINITY FOR THE PLATELET GP IB-IX RECEPTOR, Zaverio M. Ruggeri, Judith A. Dent, Hiroyuki Azuma, Mitsuhiko Sugimoto, and Jerry Ware. Department of Molecular and Experimental Medicine and Committee for Vascular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The interaction between von Willebrand factor (vWF) and platelet glycoprotein (GP) Ib is physiologically regulated so that it does not occur in the circulation but only at a site of vascular injury. However, abnormal vWF present in Type IIB von Willebrand disease has a characteristically increased affinity for GP Ib, to the extent that it binds to circulating platelets and may cause thrombocytopenia. We have identified a single amino acid change, Trp^{550} to Cys^{550}, located in the GP Ib-binding domain of the molecule comprising residues 449-728. Bacterial expression of recombinant fragments corresponding to this domain demonstrated that molecules lacking the native conformation of vWF bind directly to GP Ib in the absence of modulators and with similar affinity whether containing a normal Trp^{550} or a mutant Cys^{550} residue. In contrast, mammalian cell expression of the same segment of sequence yielded molecules with native conformation that, when containing the normal Trp^{550}, did not bind to GP Ib directly but, like native vWF, bound in the presence of ristocetin. However, molecules containing the point mutation (Cys^{550}) behaved like Type IIB vWF, namely bound to GP Ib even without ristocetin modulation and, in the presence of ristocetin, had ten-fold higher affinity than molecules with normal sequence. These results identify a region of vWF that, although not directly involved in binding to GP Ib, may modulate the interaction apparently through conformational changes.

LOCAL RENIN ANGIOTENSIN SYSTEM IN THE NEOINTIMA AFTER VASCULAR INJURY

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Angiotensin II promotes growth of vascular smooth muscle cells in vitro via the autocrine production of growth factors such as platelet-derived growth factor and transforming growth factor-beta. Consistent with this, angiotensin converting enzyme inhibitors prevent myointimal proliferation after balloon injury in vivo. We have demonstrated the presence of a complete renin angiotensin system in the rat aorta and have speculated that local angiotensin production may play an important role during myointimal proliferation. To provide further evidence toward this hypothesis, we examined the localization of angiotensinogen mRNA and angiotensin converting enzyme in control and injured vessels using in situ hybridization and immunohistochemical staining, respectively. Abdominal aorta of Sprague-Dawley rats were studied before or after injury with a balloon catheter. Neointimal hyperplasia developed as documented by a progressive increase in the ratio of neointimal to media thickness from 0.17 at 1 week to 1.17 at 6 weeks post injury. Angiotensinogen mRNA was clearly detected in the adventitia and media of control and injured aorta. However, at one week after injury, the media to adventitia angiotensinogen mRNA ratio was 3 fold higher in the injured aorta suggesting increased gene expression in the media compared to control. Of potential importance, angiotensinogen mRNA was also detected in the neointima of the injured aorta, and this was also highest at one week after injury. Moreover, ACE was detected both in the media and intima of the injured aorta, at levels which surpassed that of the uninjured aorta. These data are consistent with the hypothesis that balloon injury leads to activation of the vascular renin angiotensin system which may participate in the myointimal proliferation.

ANGIOTENSIN II MODULATES EGF INDUCED MITOGENESIS
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Much attention has focused recently on the potential role of angiotensin II (AII) in smooth muscle cell (SMC) mitogenesis. Angiotensinogen converting enzyme inhibitors have been shown to markedly inhibit myointimal proliferation following experimental balloon injury. However, AII has not been shown to consistently induce proliferation of cultured SMC. In the present study we examined the hypothesis that AII modulates the SMC mitogenic response induced by epidermal growth factor (EGF). Cultured aortic SMC isolated from adult spontaneously hypertensive rats were treated with AII and/or EGF in various combinations. Treatment with AII alone resulted in no increase in DNA synthesis (as measured by 3H-thymidine incorporation) at 24 hours and a 40% increase at 60 hours. Treatment with EGF resulted in a 4 fold increase in DNA synthesis at approximately 24 hours and then diminished to a 50% increase at 60 hours. Co-treatment with AII and EGF caused a prolonged mitogenesis (3 fold increase in DNA synthesis at 60 hours) which persisted for at least 72 hours. These results suggest a role for AII in modulating the SMC mitogenic response to EGF.

COMPUTER-ASSISTED MAPPING OF THE REPAIR OF ENDOTHELIAL CELL MONOLAYERS,
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The repair capacity of the endothelium is postulated to be an important factor in the pathogenesis of a multitude of disease states. In order to clarify this relationship, we have established an in vitro model system to test the ability of a confluent monolayer of human endothelial cells (EC) to regenerate a physically induced wound. Photographic evidence has shown that EC derived from different sources repopulate a denuded area at different rates and demonstrate different patterns of regeneration. Specifically, EC derived from human umbilical vein (HUVE) heal more rapidly and show more individual cell motility than human arterial-derived EC (HAE). The response of EC from HUVE and HAE were compared further using time lapse video microscopy and computer assisted image analysis. Data analysis by Autocad reveals specific shifts in cell density over the monolayer as the cells repopulate the denuded area. The results from this work represent progress toward a quantifiable model system for the in vitro study of the mechanisms of maintainence of the endothelium to correlate with ongoing in vivo studies.

NEW BACKCROSS SUBSTRAINS CONFIRM HYPERTENSIVE EFFECT OF THE SHR Y CHROMOSOME,
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The inheritance of blood pressure (b.p.) in the spontaneously hypertensive rat (SHR) is polygenic. To identify the specific genes involved it is necessary to separate the effect of an individual locus and its relationship to b.p. from the other loci. We have demonstrated that the Y chromosome significantly influences b.p. in crosses between SHR and the Wistar Kyoto strain (WKY). Autosomal loci were also involved in increasing b.p., accounting for 57% of the b.p. increase. Crosses were undertaken to isolate each of these genetic components. Through nine generations of crosses we have created two substrains. SHR/y which contains the SHR Y chromosome in a WKY autosomal background and SHR/a which contains a WKY Y chromosome in a SHR autosomal background. Both substrains maintain male b.p. over 185mm Hg. The genetic makeup of each strain has been assayed using known SHR and WKY RFLP markers and these are consistent with the development of these strains, as are DNA fingerprints. The increased b.p. in the SHR/y strain confirm the hypertensive effect of this chromosome, even in a WKY genetic background. These two substrains will be essential in identifying the genetic loci responsible for SHR hypertension, as each factor can be studied without the confounding effects of other loci increasing pressure.