MOLECULAR BIOLOGY OF THE CARDIOVASCULAR SYSTEM

Organizers: Robert Roberts and Joseph Sambrook

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Poster Sessions

Cardiac Muscle and Myogenesis

P 001 COMMITMENT AND DIVERSIFICATION OF CARDIAC PROGENITORS. Arlene Gonzalez-Sanchez, Joseph Bisaha, Carol Eisenberg, Steven Wylie, and David Bader. Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021. The cellular and molecular mechanisms which control cardiogenic commitment are unknown. We have utilized single cell analysis to determine when splanchnic mesoderm is committed to the cardiogenic lineage. Cardiogenic tissue from stage 4 to stage 8 chick embryos was isolated, trypsin dissociated and grown in vitro at clonal density. At various times after plating, cells were examined for the presence of beating cardiac myocytes and were reacted with monoclonal antibodies directed against the myosin heavy chain (anti-atrial, anti-ventricular anti-conduction system (CS), and anti-general sarcomeric MHC antibodies). After 2 days in vitro, single cells from stage 4 to 8 embryos expressed muscle-specific sarcomeric MHCs although the ratio of differentiated myocyte/total cultured cell increased with age of the donor. Interestingly, only a small percentage (<20%) of autonomously differentiated myocytes expressed atrial, ventricular or CS specific MHCs. When the same progenitors were grown at high density, differential MHC expression was observed suggesting a density-dependent influence to this process. These data demonstrate that splanchnic mesoderm is committed to the cardiogenic lineage much earlier than previously described (just after gastrulation). While these cells are capable of independent differentiation in vitro, the elaboration of cell specific MHC expression appears to be a seperate event from cardiogenic commitment. This work was supported by grants from the NIH (HL35776 and HL36775) and the New York Heart Association. D. Bader is an Established Investigator of the American Heart Association.

P 002 PHYSIOLOGICAL CONTROL OF CARDIAC GROWTH, Peter A. Watson, Takashi Haneda and Howard E. Morgan, Weis Center for Research, Geisinger Clinic, Danville, PA 17822
Stretch of the ventricular wall was identified as a major mechanical stimulus for cardiac growth. Hormones, such as catecholamines and glucagon, also accelerated growth. A common feature of these growth stimuli was found to be increased cyclic AMP content of the heart. In this regard, elevation of aortic pressure from 60 mmHg to 120 mmHg resulted in elevated cAMP content, increased cAMP-dependent protein kinase activity, and increased rates of total protein synthesis and ribosome formation in rat hearts. Faster ribosome formation is of greatest physiological importance because greater capacity for synthesis as manifested in greater RNA content is necessary for continued growth. Elevation of aortic pressure in beating and arrested hearts accelerated rates of ribosome formation in either the presence or absence of insulin (400 μU/ml) in the perfusate. When hearts were arrested with tetrodotoxin and exposed to 0.2 mM methacholine a muscarinic-cholinergic agonist which blocked pressure-induced increased in cAMP content in perfused hearts, elevation of aortic pressure to 120 mmHg failed to increase the rate of ribosome formation during the first hour of perfusion. When aortic pressure was maintained at 60 mmHg, exposure to glucagon increased cAMP content and mimicked the effect of elevated aortic pressure to accelerate rates of ribosome formation in arrested hearts perfused in the presence of insulin. Blockade of adrenergic receptors with propranolol and phentolamine prior to and during perfusion failed to prevent the effects of elevated aortic pressure to increase rates of ribosome formation. These studies indicate that increased aortic pressure preferentially accelerates rates of ribosome formation by a cAMP-dependent mechanism and that this mechanism is a major factor in physiological cardiac growth.

P 003 GENERATION OF CONTRACTILE PROTEIN DIVERSITY BY ALTERNATIVE SPLICING, B. Nadal-Ginard, C.W.J.Smith, Maria Gallego, Athena Andreadis, Mary Mullen, and James G. Patton, Howard Hughes Medical Institute. Dept. of Cardiology, Children's Hospital. Dept. Cellular & Molecular Physiology, Harvard Medical School, Boston, MA 021115. Contractile proteins expressed in sarcomeric tissues are encoded by small multigene families whose members exhibit muscle and developmental stage-specific regulation. A combinatorial use of these multigene families has the capacity to generate several hundred different sarcomeres through selective promoter utilization. Several of the contractile protein genes, however, have the capacity to produce several different isoforms by alternative splicing. The combinatorial use of these isoforms increases the potential for biochemically different sarcomeres to several millions. The mechanisms involved in the regulated production of contractile protein diversity through alternative splicing of TnT, α-TM and MLC 1/3 have been analyzed using a combination of in vivo and in vitro splicing systems. The regulation of mutually exclusive splicing of exons in these genes has received particular attention. In this type of splicing, two exons code for the equivalent region of the protein. Only one of the exons is included in each mature mRNA, but never both or none. The inclusion of one member of the pair represents the common and unregulated pathway, the default pattern. The selective inclusion of the other member in specific cell is the regulated pattern. Three different modes of regulation responsible for the mutual exclusivity have been uncovered. In the case of α-TM exons 2 & 3 cannot be included in the same mRNA under any circumstances because of the peculiar organization of the intron between them. The branch point sequence of this intron is too close to the splice donor site to allow splicing. In the case of MLC 1/3 exons 3 & 4, the mutual exclusivity is not absolute. In the absence of other splicing sites, the two exons can be joined. Their mutually exclusive behavior is enforced kinetically. The removal of the intron is more inefficient than the splicing to the flanking exons. For the TnT exons 16 & 17, yet another mechanism is at work. In this case there are intronic sequences located upstream from exon 16 that inhibit its inclusion in all but the regulated cells. Detailed analyses of some of these models together with the elements involved in the selection of a particular member from each pair uncovered novel features of branch point and acceptor site structure and function that play an important role in both constitutive and alternative splicing.

P 004 **FUNCTIONAL ROLE OF CELLULAR ONCOGENES IN MUSCLE DIFFERENTIATION,** Michael D. Schneider, Molecular Cardiology Unit, Departments of Medicine, Cell Biology, and Physiology & Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030

Our studies have concerned three issues: [1] to establish whether the membrane and nuclear proteins encoded by oncogene expression vectors could serve as surrogates for peptide growth factors which regulate muscle-specific genes; [2] to examine the range of differentiated properties controlled by growth factors and oncogenes, with particular emphasis on development of voltage-gated Ca^{2+} channels; and [3] to analyze the potentially contrasting effects of growth factors and oncogenes during cardiac muscle development and hypertrophy. Previously, we incorporated activated oncogene expression vectors into BC3H1 muscle cells. Both C2 muscle cells and BC3H1 cells express dihydropyridine (DHP)-sensitive "slow" Ca^{2+} channels whose ligand-binding properties and kinetics of activation specifically correspond to those of Ca^{2+} channels in skeletal muscle T-tubules. Furthermore, like C2 cells BC3H1 cells express a 6.5 kb DHPR transcript, using probes specific for the skeletal muscle DHP receptor α_1 subunit (DHPR). Properties associated with myogenic differentiation are delayed or partially inhibited in cells containing activated *myc* or *erb*B genes. These included muscle creatine kinase, myosin light chain-3, and nicotinic acetylcholine receptors (NAChR), as well as "fast" and "slow" Ca^{2+} channels. In contrast, an activated Harvey *ras* allele is sufficient to abolish expression of these muscle differentiation products. Little or no DHPR mRNA is found in cells which lack functional "slow" Ca^{2+} channels. Thus, *ras*, by itself, produces a phenotype indistinguishable from the undifferentiated state provoked by transforming growth factor β (TGFβ). Although most muscle-specific genes are coordinately suppressed, interesting exceptions also are noted. For example, both *erb*B and *myc* stimulate α-cardiac actin gene expression (associated with embryonic skeletal muscle) for 12 or more days. Thus, either a membrane or nuclear oncogene can trigger expression of a "fetal" isoform, at least in skeletal muscle cells. Furthermore, although most muscle-specific genes are suppressed by *ras*, a notable exception is the δ subunit of the NAChR, which is inhibited by serum factors, but not by *ras*. Thus, the actual effect of an oncogene on any potential target is not a foregone conclusion.

In cardiac muscle, the peptides TGFβ and basic FGF each elicit complex changes in contractile protein gene expression, which contrast with their suppressive effects in skeletal muscle and correspond to changes provoked by pressure overload. Reciprocal changes are evoked in myosin heavy chain mRNA expression: stimulation of the embryonic β gene, and inhibition of the adult α gene. Related but distinct changes occur in α-actin gene expression, similar to those occurring after aortic constriction, namely, stimulation of skeletal actin seen in embryonic heart, with little or no change in cardiac actin. In contrast, in skeletal muscle, TGFβ inhibits all sarcomeric myosin heavy chains and α-actins. Acidic FGF produces corresponding changes in myosin heavy chain expression, but differs in its effect on actin expression and is a potent inhibitor of both cardiac and skeletal α-actin in ventricular myocytes. Thus, cardiac myocytes are a target for the effects of peptide growth factors on differentiated gene expression, which selectively stimulate "fetal" contractile protein genes. These findings diverge from the known actions of peptide growth factors in skeletal muscle cells and resemble the responses of cardiac muscle seen during hypertrophic growth.

Hypertrophy and Hyperplasia

P 005 CARDIAC, NEUROLOGICAL AND NEOPLASTIC ABNORMALITIES IN v-fps TRANSGENIC MICE, Siu-Pok Yee[1], David Mock[2], Victor Maltby[1], Malcolm Silver[3], Janet Rossant[1], Alan Bernstein[1], and Tony Pawson[1]. [1]Mt. Sinai Hospital Research Institute, 600 University Avenue, Toronto, Canada, M5G 1X5, [2]Department of Oral Medicine and Pathology, University of Toronto, [3]Department of Pathology, University of Toronto.
Transgenic mice that widely express the v-fps cytoplasmic protein-tyrosine kinase develop a variety of independent pathological conditions, including cardiac enlargement and congestive cardiomyopathy, tremors, and lymphoid and mesenchymal tumors. v-fps protein-tyrosine kinase activity in the heart directly correlates with cardiac enlargement, and with severe myocardial and endocardial damage associated with congestive heart failure. Tremors are associated with v-fps expression in the brain. Malignant tumors induced by the v-fps transgene include lymphomas, thymomas, fibrosarcomas and angiosarcomas. Phenotypes such as cardiomegaly and tremors are evident shortly after birth and show 100% penetrance, as expected of traits solely dependent on the v-fps protein-tyrosine kinase. In contrast tumors develop with varying frequencies after latent periods of 2-12 months. The pleiotropic effects of the v-fps transgene suggest the extensive involvement of cytoplasmic protein-tyrosine kinases in normal physiology and abnormal pathology. In particular, mice expressing v-fps in the heart provide a means to investigate the molecular basis for congestive cardiomyopathy and associated heart failure.

P 006 CHARACTERIZATION OF THE RAT HEART SARCOPLASMIC RETICULUM Ca^{2+} ATPase mRNA : TISSUE AND DEVELOPMENTAL REGULATION, A.M. Lompré, D. de la Bastie and K. Schwartz, INSERM U127 Hôpital Lariboisière, 41 boulevard de la Chapelle 75010 PARIS, FRANCE. In muscles cells, the sarcoplasmic reticulum (SR) is the main intracellular organelle devoted to the regulation of the cytosolic Ca^{2+} concentration, and in non muscle cells, the Ca^{2+} homeostasis is maintained by the rough endoplasmic reticulum (ER), by mitochondria and by calciosomes. Ca^{2+} a influx to the SR and ER is mediated by a (Ca^{2+}-Mg^{2+}) - dependent ATPase. cDNA clones specific for the sarcoplasmic reticulum Ca^{2+} ATPase mRNA were isolated from an adult rat heart library and characterized. Using these cDNA clones and S1 nuclease mapping techniques, we analyzed in rat, the expression of the Ca^{2+} ATPase gene during cardiac development and in several different adult striated, smooth and non-muscle tissues. The same Ca^{2+} ATPase mRNA is present in adult slow skeletal muscle and in the ventricles and atria of the heart. Only one Ca^{2+} ATPase mRNA species is detected in cardiac tissue with development. Two mRNA species, the proportion of which vary depending on the tissue, are observed in smooth and non-muscle tissues. One, which is identical to the cardiac isoform, is expressed at high levels in aorta. The other is the main isoform expressed in uterus, liver and kidney, and is identical to the cardiac mRNA through most of its sequence, only diverging at its 3' end.

P 007 INCREASED EXPRESSION OF THE c-*MYC* PROTOONCOGENE IN THE HEART OF TRANSGENIC ANIMALS DURING DEVELOPMENT INDUCES MYOCYTE HYPERPLASIA. Twila A. Jackson, Michael S. Allard, Catherine M. Sreenan, Lisa K. Doss, Sanford P. Bishop, Judith L. Swain. Duke Medical Center, Durham, N.C. and University of Alabama at Birmingham, Birmingham, AL.

The c-*myc* oncogene is postulated to play a role in the transition from myocyte proliferation to differentiation which occurs during cardiac development. In order to examine the *in vivo* effect of increased expression of c-*myc* in cardiac myocytes during development, we produced a strain of transgenic (TG) mice exhibiting constitutive cardiac c-*myc* expression. The dry heart weight (HWt) and the cardiac DNA content (DNA) of TG mice were compared to non-TG (NTG) littermates: (*p<.01 TG vs NTG)

	1 day old (n=65)			15 days old (n=112)		
	HWt (mg)	DNA (μg)	BWt (g)	HWt (mg)	DNA (μg)	BWt (g)
TG	2.4±.6*	45±18*	1.7±.4	14±5*	171±62*	7.5±2.7
NTG	1.7±.4	32±8	1.8±.4	9±4	107±27	7.9±2.7

The increased cardiac DNA content in the TG mice suggested that the cardiac enlargement observed was secondary to cardiac hyperplasia. To document myocyte hyperplasia, isolated cardiac myocytes were prepared from 4 week old TG (n=9) and NTG (n=20) littermates, cell volume determined with a Coulter Counter system, and myocyte number calculated from the median cell volume and heart weight data. The TG hearts contained more than twice as many myocytes compared to the NTG hearts (11.1 vs 4.9×10^6 myocytes; p<.05). In contrast, the median myocyte volume of the TG hearts was significantly less than in the NTG hearts (13491±1465 vs. 20517±4896 fl; p<.05). These results indicate that increased c-*myc* expression in the heart during development leads to cardiac enlargement, and that the resultant cardiac enlargement is at least partially secondary to myocyte hyperplasia. These data also demonstrate that an increase in the number of myocytes attenuates normal post-mitotic hypertrophic growth. In summary, these results suggest that the c-*myc* protooncogene plays a role in the transition from myocyte proliferation to differentiation that occurs during cardiac development *in vivo*. The results also demonstrate the utility of the transgenic mouse model in studying cardiac development in the intact animal.

Growth Factors and Growth Factor-Inducible Genes

P 008 TRANSFORMING GROWTH FACTOR-BETA IN THE HEART AND IN THE EMBRYO, Kathleen C. Flanders, Nancy L. Thompson, Ursula I. Heine, Patura Kondaiah, Sonia B. Jakowlew, Andrew G. Geiser, Anita B. Roberts, Fernando Bazoberry*, Ward Casscells*, Victor J. Ferrans*, and Michael B. Sporn, National Cancer Institute and *National Heart, Lung and Blood Institute, Bethesda, MD 20892.

Transforming growth factor-β (TGF-β) is a 25 kD multifunctional regulator of cell growth and differentiation. Immunohistochemical studies suggest that TGF-β1 plays a role in embryonic development. It is expressed in a tissue and time dependent manner in mouse embryos with intense extracellular staining in mesenchyme or tissues derived from mesenchyme such as connective tissue, cartilage and bone. It is also especially abundant in areas of critical mesenchymal-epithelial interactions. In addition to TGF-β1, there are four other distinct forms of TGF-β which share approximately 70% homology. Preliminary evidence suggests that some of these forms of TGF-β also play specific roles in embryonic development as demonstrated by their distinct patterns of immunohistochemical localization.

TGF-β1 staining is found in the developing mouse heart. Particularly intense staining is seen in the cushion tissue of the forming heart valves, suggesting that TGF-β plays a role in remodeling of that tissue and its secretion of specialized matrix proteins. Intense staining is also seen in cardiac myocytes of mouse embryos. This myocardial staining persists in the adult mouse although it is less intense. Furthermore, in the adult mouse and rat, atrial staining is more intense than ventricular staining. Ligation of the left coronary artery of rats causing infarction of the left ventricle leads to changes in the myocardial staining pattern. There is a loss of staining in infarcted areas with increased staining in the border zone surrounding the infarct. A 7-fold increase in 2.4 kb TGF-β1 mRNA transcript is observed in the infarcted hearts and a novel 1.9 kb transcript is also increased following cardiac injury. Although the role of TGF-β1 in myocardial infarction is not understood, it is known to play a central role in repair of other soft and hard tissues. Again, preliminary evidence suggests that other forms of TGF-β also play a role in the heart. The mouse embryo heart shows staining for TGF-β3 as well as TGF-β1, and cultured chick myocytes express mRNA for both TGF-β3 and TGF-β4. It will be important to understand the specialized roles of these different forms of TGF-β in the development and function of the heart.

P 009 **REGULATION OF A MUSCLE-SPECIFIC ENHANCER BY GROWTH FACTORS AND ONCOGENES.** E. N. Olson, D. Edmondson, E. Sternberg, and L. Gossett, Dept. of Biochem and Mol Biol., M. D. Anderson Cancer Center, Houston, Tx 77030.

During commitment of stem cells to the myogenic lineage, a battery of muscle-specific genes becomes subject to negative control by mitogens and certain peptide growth factors. To define the regulatory network through which growth factor signals repress muscle-specific genes, we have created stable myoblast cell lines that express a series of oncogene products that are thought to transduce growth factor signals from the cell membrane and the nucleus. These studies have revealed that mutationally activated ras mimics the inhibitory effects of FGF and TGF-beta on myogenesis, and reversibly blocks the induction of muscle-specific genes. To begin to identify nuclear targets for the cascades activated by growth factors and ras, we have analyzed regulatory elements associated with the muscle creatine kinase (mck) gene, which is induced during myogenesis. Tissue-specificity, developmental regulation, and high-level expression of mck are controlled primarily by a muscle-specific enhancer located between -1250 and -1048 bp relative to the transcription initiation site. This enhancer is comprised of multiple regulatory elements that interact with combinations of muscle-specific and ubiquitous trans-acting factors. We have identified a myocyte-specific factor that interacts with an element within the mck enhancer that is found in at least one other muscle-specific enhancer. Expression of this factor is suppressed by ras and by mitogens. Activity of the mck enhancer is abolished in myoblasts harboring activated ras genes, suggesting that ras may suppress the myogenic phenotype by preventing the accumulation of regulatory factors required for transcriptional induction of muscle-specific genes. We have recently isolated cDNA clones for a muscle-specific factor that shows homology to a segment of c-myc and the determination gene, myoD1. This cDNA activates the mck enhancer following transient transfection into ras-transformed myoblasts or nonmyogenic cells. Expression of this cDNA in stably transfected fibroblasts is sufficient to induce fusion and muscle-specific gene expression upon exposure to mitogen-deficient medium. Conversely, stable transfection of C2 myoblasts with this cDNA in the anti-sense orientation prevents differentiation. The potential ability of the protein encoded by this cDNA to bind to the mck 5' enhancer is currently being investigated. DNA sequences responsible for lineage-specific activation of this myogenic regulatory gene are also being examined.

Atherosclerosis and Angiogenesis

P 010 **ONCOGENE EXPRESSION IN HUMAN ATHEROSCLEROTIC PLAQUES,** Earl P. Benditt, Department of Pathology, University of Washington School of Medicine, Seattle, WA, 98195. Atherosclerotic plaques are complex lesions that develop over a long time. A variety of cell signals such as growth factors, cytokines and their receptors as well as other factors involved in maintaining cell and tissue structure and function must be involved. Our early simplified notions of regulation of growth do not now seem to fit the observed cell biology and molecular biology involved in development of vertebrate organisms and pathogenesis of proliferative lesions. Minimally, it is clear that there must be rein-control for each of the factors active in the proliferative process. Which regulatory elements may be involved in the orderly proliferation and maintenance of specific cell populations and the factors that may be altered in lesions will be discussed in the light of our recent studies of human and animal tissues and lesions.

P 011 GROWTH FACTORS AND ATHEROGENESIS, Ross R., Department of Pathology, University of Washington, Seattle, WA 98195.

Several growth factors and cytokines can be formed by the four principal cells involved in the lesions of atherosclerosis: the monocyte/macrophage, T lymphocyte, endothelial cell, and smooth muscle cell. The earliest lesion of atherosclerosis, the fatty streak, consists of mixtures of monocyte-derived macrophages and T cells. The T cells can activate the macrophages by secretion of gamma interferon and other monokines. The monocytes can differentiate into macrophages, can secrete both chains of PDGF, TGFα, TGFβ, TNFα, IL-1, and other factors. Many of the cytokines induce secondary gene expression of growth factors in cells such as endothelium and smooth muscle, both of which can express the genes for both chains of PDGF, and can secrete the different isoforms of PDGF. Northern analysis of RNA extracts of nonhuman primate lesions of atherosclerosis demonstrate the presence of these growth factors in the advanced lesions. Monoclonal antibodies specific for T cells, macrophages, and smooth muscle cells demonstrate that fatty streaks consist of principally macrophages and T lymphocytes, whereas fibrous plaques contain numerous proliferated smooth muscle cells with intermixed macrophages, T cells, and in some cases endothelial cells in the form of vasa vasora. The interactions among these different cells can lead to both growth factor and cytokine gene expression, synthesis, and secretion. Cytokines can induce gene expression for other growth factors such as PDGF in endothelium and smooth muscle. Smooth muscle cells are capable of autocrine stimulation by induction of the gene for PDGF-A. Thus the different possible cellular interactions and cytokines and growth factors that result from these can influence the formation and progression of the lesions of atherosclerosis at different times during their development.

Thrombosis and Thrombolysis

P 012 REGULATION OF THE UROKINASE PATHWAY OF PLASMINOGEN ACTIVATION: PROENZYME, INHIBITORS AND RECEPTOR, K. Danø[1], N. Behrendt[1], E. Rønne[1], V. Ellis[1] and F. Blasi[2], [1]Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, 2100 Copenhagen Ø. and [2]Institute of Microbiology, University of Copenhagen, Øster Farimagsgade 2A, 1353 Copenhagen K., Denmark.

Urokinase catalyzed plasminogen activation is regulated in time and space not only by cellular release of u-PA, but also by activation of its proenzyme (pro-u-PA), by the plasminogen activator inhibitors PAI-1 and PAI-2, and by a cellular receptor specific for u-PA (u-PAR) (1). Urokinase is generally released from cells as single-chain pro-u-PA, which has little or no plasminogen activating capacity (2). Plasmin efficiently ($k_{cat}/Km \approx 3 \times 10^6\ M^{-1}s^{-1}$) converts pro-u-PA to the active two-chain form. u-PA is inhibited by PAI-1 and PAI-2, that do not bind to pro-u-PA. Both pro-u-PA and active u-PA bind specifically to u-PAR (Kd $\approx 10^{-10}$ M), a ≈60 kD glycoprotein that has been found at the surface of a variety of cell types. Pro-u-PA can be converted to two-chain u-PA while receptor-bound, and receptor-bound two-chain u-PA can activate plasminogen and bind PAI-1. In some cell types, receptor-bound u-PA is distinctly located at cell-cell and focal cell-substratum contacts (3). Recent findings have shown that cell-bound plasmin, inaccessible to serum inhibitors, can activate pro-u-PA, that surface-bound u-PA can activate surface-bound plasminogen, and that the former reaction is stimulated approximately 50-fold when both plasmin and pro-u-PA are surface-bound (4,5). These findings suggest that cell surfaces are physiological sites for u-PA catalyzed plasminogen activation, in analogy with the role of fibrin deposits in the t-PA pathway. They also indicate that a directional proteolysis may be obtained by selective activation of pro-u-PA at some sites of cell surfaces. It is not known whether pro-u-PA initially is activated by plasminogen or whether as-yet unknown mechanisms of pro-u-PA activation are involved in the initiation.

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Endothelial Cell Interactions

P 013 THE FIBRINOLYTIC SYSTEM OF CULTURED ENDOTHELIUM: REGULATION BY PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1), EXTRACELLULAR MATRIX (ECM) AND VITRONECTIN (VN), David J. Loskutoff, Research Institute of Scripps Clinic, La Jolla, CA 92037
Plasminogen activation provides an important source of localized proteolytic activity during fibrinolysis, ovulation, cell migration, epithelial cell differentiation, and tumor cell invasion. Precise regulation of plasminogen activator (PA) activity thus constitutes a critical feature of many biological processes. This control is achieved in large part through the action of PAI-1, an inhibitor of both urokinase and tissue-type PA (tPA). The molecular cloning of a cDNA for PAI-1 revealed that the mature human protein is 379 amino acids long, lacks cysteines and is a member of the serine proteinase inhibitor (serpin) gene family. It can represent as much as 12% of the total protein released by endothelial cells (ECs), and is also a major component of their ECM. The production of PAI-1, and its deposition into ECM, is stimulated by endotoxin, interleukin-1, tumor necrosis factor, transforming growth factor β (TGFβ), and dexamethasone. These increases are relatively specific for PAI-1, and result primarily from an increased rate of transcription rather than from increased stability of the PAI-1 mRNA. The PAI-1 gene itself is 12.2 kilo-base pairs in length and is organized into nine exons and eight introns. We have isolated and characterized the promoter of the PAI-1 gene. Transfection experiments indicate that it is expressed in a tissue-specific manner, and that glucocorticoid induction is mediated by two distinct regions. The first is a proximal element (-100 to +75 from the transcription initiation site) which has enhancer like properties, and is induced approximately 10-fold by dexamethasone. The second more distal element (-800 to -549) is stimulated another 4-fold by dexamethasone. We are continuing to characterize the proximal element by linker scan analysis and gel mobility shift experiments. PAI-1 exists in various cellular samples (and in blood) in both an active and an inactive (latent) form. It appears to be produced by ECs in the active form, and is then either released into the medium where it undergoes rapid, spontaneous inactivation (t 1/2 = 3 h) or released into the subendothelium where it binds to ECM and is protected from this inactivation (t 1/2 > 24 h). Thus, the specific binding of PAI-1 to ECM, or to binding proteins in blood, regulates its activity. The binding of PAI-1 to ECM is dose-and time-dependent and occurs with an apparent k_d - 60 nM. The binding protein has been purified using affinity chromatography on PAI-1 Sepharose and shown to be Vn both immunologically and by n-terminal amino acid sequence analysis. The significance of these findings will be discussed.

P 014 INTERACTION OF HUMAN POLYMORPHONUCLEAR LEUCOCYTES WITH MONOLAYERS OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. Samuel C. Silverstein and Ada J. Huang. Department of Physiology and Cellular Biophysics, Columbia University, New York, NY 10032
To study the mechanism(s) by which chemotaxing polymorphonuclear leucocytes (PMN) traverse endothelia, human umbilical vein endothelial cells (HUVECs) were grown to confluence on a substrate of human amnion. Endothelial cells in these monolayers exhibit silver stained intercellular junctions, have 6-12 ohm.cm2 of electrical resistance (a measure of their capacity to retard transendothelial passage of ions), and support oncotic pressure gradients sufficient to cause osmotically driven water flow across the monolayers. PMN do not bind to these monolayers spontaneously, nor do they migrate across them, unless stimulated to do so by a transendothelial gradient of a chemoattractant such as LTB4 or fMLP. In response to chemoattractants, PMN penetrate between HUVECs and migrate across the endothelium at random locations throughout the monolayer. To determine whether proteases promote the opening of inter-endothelial cell junctions by PMN we we tested a variety of protease inhibitors on PMN transendothelial migration. Inhibition of PMN elastase and plasminogen activator, and removal of plasminogen from the medium, did not reduce PMN transendothelial migration. Transendothelial migration of PMN was rapid (virtually complete within 10 min). Although one PMN migrated across the monolayer for each HUVEC in it, PMN migration induced no detectable change in electrical resistance of the monolayer or in its permeability to albumin. This was consistent with the extremely close apposition observed between PMN and HUVECs; morphological studies showed that during PMN migration the HUVEC paracellular pathway increased by less than 0.22%. These studies also showed that pseudopods of migrating PMNs invaginated the surfaces of the HUVECs, suggesting the PMN were signalling a change in the HUVEC cytoskeleton. To determine whether chemotaxing PMNs initiate functional changes in HUVECs we measured [Ca+2]i in the HUVECs. Treatment of HUVEC monolayers with chemoattractant alone, or with PMN in the absence of a chemoattractant, did not cause a significant change in [Ca+2]i in the endothelial cells. In contrast, transendothelial migration of PMN in response to a chemoattractant gradient was accompanied by a transient 5-7 fold increase in HUVEC [Ca+2]i. The increase in HUVEC [Ca+2]i was correlated with the period of PMN migration across the monolayer; HUVEC [Ca+2]i returned to resting levels at about the same time (15min) as PMN migration across the monolayer was complete. These results suggest that contacts between HUVEC and PMN membrane proteins and/or secretory products initiate specific changes in the endothelial cells, and that these changes may promote opening of inter-endothelial cell junctions, thereby facilitating the passage of PMN across endothelia.

Signal Transduction and Receptors

P 015 **THE STRUCTURAL BASIS OF ADRENERGIC RECEPTOR FUNCTION**

Brian Kobilka and Robert Lefkowitz, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina.

The alpha-2 and beta-2 adrenergic receptors are both activated by epinephrine, but can be differentiated by selective agonists and antagonists. Activation of alpha-2 adrenergic receptors inhibits the adenylyl cyclase system, while activation of beta-2 adrenergic receptors stimulates it. The two receptors are homologous with each other, rhodopsin and other receptors coupled to guanine nucleotide regulatory proteins (G-proteins). They contain seven hydrophobic domains which may represent transmembrane spanning segments. The function of specific structural domains of these receptors was determined by constructing a series of chimeric alpha-2, beta-2 adrenergic receptor genes. These genes were expressed in Xenopus laevis oocytes or COS 7 cells and their functional properties were studied. The specificity for coupling to the stimulatory guanine nucleotide regulatory protein lies within a region extending from the amino terminus of the fifth hydrophobic domain to the carboxyl terminus of the sixth hydrophobic domain.

The major determinants of alpha-2 and beta-2 adrenergic receptor agonist and antagonist ligand binding specificity are contained within the seventh hydrophobic domain. The results of these studies also suggest that the seventh hydrophobic domain interacts with the third and/or fourth hydrophobic domains. The site of this interaction may contain the ligand binding site. Support for this hypothesis comes from studies of the beta-2 adrenergic receptor expressed as a heterodimer. In these "Split Receptor" experiments, functional beta-2 adrenergic receptors are made by co-expressing RNA encoding the amino-terminal five hydrophobic domains (SR1-5) with RNA encoding the two carboxyl terminal hydrophobic domains (SR67). Neither SR1-5 nor SR67 when expressed alone yields a functional receptor, but when co-expressed these two peptides combine non-covalently to form a receptor capable of binding ligands and activating the adenylyl cyclase system. Furthermore, both fragments become specifically labeled with the beta adrenergic receptor photo-affinity antagonist Iodocyanopindolol diazirine.

P 016 STRUCTURE AND FUNCTION OF G PROTEIN SUBUNITS, Eva J. Neer, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

The G proteins form a complex family with multiple α, β and Y proteins. Each subunit has closely related isoforms. While the most divergent forms have different functions (for example, α_s, a cholera toxin substrate activates adenylyl cyclase while α_i, a pertussis toxin substrate does not), it is not clear that within a subgroup (for example, α_{i-1}, α_{i-2} and α_{i-3}), the proteins have different functions. To add to the complexity, it appears that both α and βY subunits can act as positive activators of cellular enzymes and ion channels.

In order to evaluate the structural regions involved in the function of α and βY subunits, we have analyzed them in three ways: by making discrete chemical modifications, by functional studies of active proteolytic fragments, and by specific antibodies. These studies have allowed us to define functionally important residues in the α and βY subunits.

Ion Channels

P 017 **GATING OF CARDIAC IONIC CHANNELS BY G PROTEINS**, A.M. Brown[†] and L. Birnbaumer[‡], Departments of Physiology and Molecular Biophysics[†‡] and Cell Biology[‡], Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Ionic channels are subject to a number of acute regulatory processes. A process that has only recently been elucidated is membrane-delimited or direct and uses signal transducing, guanine nucleotide binding (G) proteins as activators. Direct gating of ionic channels by G proteins is widespread and must be distinguished from another form of regulation by G proteins which is indirect and uses cytoplasmic second messengers. The simplest way to make this distinction is to reconstitute G protein effects in cell-free membrane systems containing the ionic channels of interest and possibly the receptor-G protein apparatus too. Direct G protein gating is very important in excitable tissues such as striated muscle and neurons and may be obligatory, i.e. necessary and sufficient for channel opening or modulatory, i.e. neither necessary nor sufficient for channel opening. In heart the G protein G_k is obligatory for the atrial muscarinic K^+ channel (K^+[ACh]) which regulates heart rate. The G protein G_s, the stimulatory regulator of adenylyl cyclase (AC), is modulatory for cardiac Ca^{2+} and Na^+ channels and acts by direct and indirect pathways, the latter being the cAMP regulated protein kinase cascade that phosphorylates these channels. Using recombinant α subunits expressed in bacteria we established that G_s had at least two effectors, some G_is probably have at least two effectors, and that α_is are not specific for the K^+[ACh] channel. An interesting question is the relative importance of the dual direct and indirect modulatory G protein pathways for cardiac Ca^{2+} channels. Theory predicts and experiments confirm that the direct pathway is much faster. Another question of interest is whether effectors alter the rate at which G protein regulation occurs. This appears to be the case for G_s and its effectors: AC on the one hand and Ca^{2+} and Na^+ channels on the other.

P 018 MOLECULAR PROPERTIES OF VOLTAGE-SENSITIVE SODIUM AND CALCIUM CHANNELS. William A. Catterall, Department of Pharmacology, University of Washington, Seattle, WA 98195.

Voltage-sensitive Na^+ and Ca^{++} channels mediate inward movements of Na^+ and Ca^{++} that are responsible for the depolarizing phase of the action potential in excitable cells, and Ca^{++} moving into the cell through Ca^{++} channels acts as a signal for multiple intracellular events. Na^+ channels isolated in functional form from mammalian brain consist of a complex of an α subunit (260 kDa) in association with a β1 subunit (36 kDa) and disulfide-bonded to a β2 subunit (33 kDa). The α subunit is common to Na^+ channels from all tissues that have been studied, and mRNA encoding it is sufficient to direct the synthesis of functional sodium channels in *Xenopus* oocytes, although their rate of inactivation is markedly slowed compared to normal brain Na^+ channels. The functional roles of the β1 and β2 subunits are not understood, but may include modulation of channel assembly and gating properties.

Ca^{++} channels isolated from skeletal muscle transverse tubules consist of an α_1 subunit (175 kDa), which is similar is primary structure to the α subunit of the Na^+ channel, and contains the receptor sites for calcium channel modulators. It is associated with a disulfide-linked glycoprotein complex of α_2 (143 kDa) and δ (27 kDa), β (54 kDa) and γ (30 kDa). The purified Ca^{++} channel complex mediates $^{45}Ca^{++}$ influx after reconstitution into phospholipid vesicles, and the activity of the purified channel is modulated by phosphorylation of the α_1 and β subunit by cAMP-dependent protein kinase, as in Ca^{++} channels in intact cells.

The homologous primary structures of the α subunit of the Na^+ channel and the α_1 subunit of the Ca^{++} channel contain 4 repeated homologous domains containing multiple transmembrane segments. Site-directed, antipeptide antibodies have been used to identify separate sites on the Na^+ channel at which cAMP-dependent phosphorylation, binding of α-scorpion toxins, and interaction of specific antibodies modulate rapid Na^+ channel inactivation. Highly conserved S4 α-helical segments in each domain, which are both hydrophobic and positively charged, are postulated to traverse the membrane and form the voltage-sensing elements of the channels according to a "Sliding Helix" model of voltage-dependent gating. These studies begin the development of a functional and topological map for the principal subunit of the voltage-sensitive ion channels.

P 019 THE Ca^{2+} RELEASE CHANNEL OF CARDIAC MUSCLE SARCOPLASMIC RETICULUM, Gerhard Meissner, Kristin Anderson, F. Anthony Lai, Qi-Yi Liu, Eric Rousseau and Harold P. Erickson*, Departments of Biochemistry and Physiology, University of North Carolina, Chapel Hill, NC 27599 and Department of Cell Biology*, Duke University, Durham, NC 27710 USA.
Excitation-contraction (EC) coupling in muscle is acompanied by a rapid release of Ca^{2+} ions from the intracellular sarcoplasmic reticulum (SR). Although the detailed mechanism of SR Ca^{2+} release has not yet been fully defined, rapid mixing-vesicle ion flux and single channel measurements have indicated the presence of a high-conductance SR Ca^{2+} release channel which is activated by micromolar Ca^{2+} and modulated by ATP, Mg^{2+} and calmodulin (1,2). Use of the channel-specific ligand [^{3}H]ryanodine has led to the purification of the cardiac Ca^{2+} release channel as a 30S complex comprised of four polypeptides of M_r ~400,000 (3). Electron microscopy revealed the four leaf-clover structure previously described for the "feet" that span the transverse tubular (T-) - SR junction. The purified 30S complex, upon reconstitution into planar lipid bilayers, induced large monovalent and divalent cation conductances, the activity of which was regulated by the endogenous ligands Ca^{2+}, Mg^{2+}, ATP, as well as by the Ca^{2+} releasing drugs ryanodine and caffeine. These results indicate that the purified 30S ryanodine receptor complex, the T- SR-spanning feet structures, and the SR Ca^{2+} release channel, all are synonymous. Supported by USPHS, MDA and CHF grants.

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Contractility

P 020 VERTEBRATE NONMUSCLE MYOSIN HEAVY CHAIN: CLONING OF THE cDNA AND THE GENE AND PHOSPHORYLATION BY PROTEIN KINASE C, Robert S. Adelstein, David A. Brill, Mary Anne Conti, Sachiyo Kawamoto, Russell I. Ludowyke, Itzhak Peleg, Yvette Preston, Ralph V. Shohet, Michael Simons and Lawrence Weir, NHLBI, NIH, Bethesda, MD 20892.
To study structure-function relationships in vertebrate nonmuscle myosins we initiated the cloning of the cDNA encoding the myosin heavy chain of chicken intestinal brush border as well as the human gene for this protein. The cloned nonmuscle myosin gene appears to be much larger than other myosin heavy chain genes (>85 kb). The cloned cDNA encodes a sequence of 1,950 amino acids, which is approximately 75% similar to the embryonic chicken smooth muscle myosin heavy chain (1). There is markedly less similarity to skeletal and cardiac muscle myosin. These similarities and differences are consistent with the biochemical properties of vertebrate smooth muscle and nonmuscle myosin which, in contrast to striated muscle myosin, are characterized by an actin-activated MgATPase activity that is regulated by phosphorylation of the 20 kDa myosin light chain.
To gain further insight into the role of myosin phosphorylation in regulating the biological activity of vertebrate nonmuscle cells we studied the phosphorylation of myosin in human platelets and in a cultured rat basophilic leukemia cell line, RBL-2H3. Stoichiometric phosphorylation of the platelet myosin heavy chain and 20 kDa light chain was found to occur in vitro following incubation with protein kinase C and in situ following treatment of intact platelets with phorbol ester. The site phosphorylated on the myosin heavy chain was localized to a single serine-containing tryptic phosphopeptide. This same site, along with the serine-1 or serine-2 residue of the 20 kDa myosin light chain was found to be phosphorylated in RBL-2H3 cells following antigenic stimulation. The time course of myosin phosphorylation by protein kinase C was found to coincide with release of histamine by RBL-2H3 cells.

1. Yanagisawa, M. et al., J. Mol. Biol. (1987) 198:143.

P 021 MOLECULAR GENETIC ANALYSIS OF MAMMALIAN SARCOMERIC MYOSIN HEAVY CHAINS, Leslie A. Leinwand, Rebecca Feghali and Elizabeth McNally, Albert Einstein College of Medicine, Department of Microbiology and Immunology, 1300 Morris Park Avenue, Bronx, New York 10461. Vertebrate myosin heavy chains (MHC) are encoded by multigene families whose members show tissue-specific and developmentally regulated expression. In order to define the program of MHC gene expression in human cardiac and skeletal muscle during development, we have isolated cDNA and genomic clones corresponding to 9 human MHC genes. Chromosome mapping has shown that these genes exist in at least two clusters. Human cardiac MHC genes are tandemly arranged on chromosome 14 and human skeletal MHC genes are clustered on the short arm of chromosome 17. Within an organism, the proteins encoded by the MHC genes are diverse, but when compared to their couterparts in other organisms they show striking homology. Examination of MHC mRNA in tissue samples from patients with cardiovascular disease using these cloned DNAs as probes demonstrates dramatic regional shifts in α and β cardiac MHC gene expression. Quantitative differences in ATPase activity between α and β cardiac MHC correlated with altered contractility of the muscle and may make these myosin shifts physiologically significant. We have begun defining the functional domains of the MHC molecule such as ATPase activity and myosin light chain binding sites using E.coli expression followed by in vitro functional analysis of the bacterially expressed protein. Toward that end, we have generated full-length α and β cardiac MHC cDNA clones and determined their complete sequences. The limited number of amino acid differences between them coupled with their different ATPase activities make it likely that we will be able to identify those residues responsible for force generation. Co-expression in E.coli of α cardiac heavy meromyosin with ventricular myosin light chain 1 has allowed the identification of the amino acids on the heavy chain that mediate myosin light chain binding.

P 022 Na,K-ATPase: STRUCTURE-FUNCTION OF EXPRESSION STUDIES, Jerry B Lingrel, John Orlowski, Elmer M. Price and Marcia M. Shull, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, OH 45267-0524.

Na,K-ATPase is an integral membrane enzyme that is responsible for the transport of Na^+ and K^+ across cell membranes. It provides the energy for the co-transport of ions and solutes as well as maintaining cell volume and electrical potential of cells. Recent cDNA cloning in our laboratory has established the primary sequence of the $\alpha 1$ and $\alpha 2$ subunits of this enzyme as well as a new isoform, $\alpha 3$. The predominant form in the heart is the $\alpha 1$ isoform, however, during early development, significant amounts of the $\alpha 3$ isoform are expressed and this form is replaced by $\alpha 2$ following birth. Structure-function studies have identified the first external domain as being responsible for the differential sensitivity to the cardiac glycoside, ouabain observed between rodent and other mammalian species. The basis for this differential sensitivity resides in the replacement of two charged amino acids to uncharged ones in the sensitive form. These studies help describe the site that cardiac glycosides interacts with this enzyme.

P 023 CALMODULIN REGULATION OF SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE, Anthony R. Means[1], Mark F.A. VanBerkum[1], Indrani Bagchi[1], Samuel E. George[2] and Bruce E. Kemp[3], Department of Cell Biology[1] and Division of Cardiology[2] Baylor College of Medicine, Houston, TX 77030; and St. Vincent's Institute for Medical Research[3], Melbourne, Australia.

Myosin light chain kinase (MLCK) is the rate limiting enzyme mediating the Ca^{++} dependent contraction of smooth muscle. The Ca^{++} dependency requires calmodulin (CaM) as the transducer. We have cloned and sequenced CaM and MLCK from chicken smooth muscle. Both cDNAs have been introduced into bacterial expression vectors. Deletion, site-specific and domain-exchange mutagenesis have been utilized to evaluate functional domains in both proteins. The CaM binding domain of MLCK can be roughly divided into thirds where the NH_2-terminal 2/3 serve as a pseudosubstrate and the COOH-terminal 2/3 bind CaM. In the absence of CaM, the pseudosubstrate prototope inhibits interaction with the myosin light chain substrate presumably by steric hinderance. This inhibition is relieved by Ca^{++}/CaM. Deletion of both the pseudosubstrate and CaM-binding prototopes results in a constitutive enzyme. Synthetic peptide analogs to the pseudosubstrate region serve as potent competitive inhibitors of the constitutive enzyme. Deletion or alteration of a single Arg-containing tripeptide at the COOH-end of the CaM-binding prototope prevents CaM binding to and regulation of MLCK. Amino acids in the first Ca^{++} binding domain of CaM are important for regulation of MLCK activity. Alterations in the length or composition of the central helix that divides the NH_2-terminal and COOH-terminal pair of Ca^{++} binding sites of CaM also affect the ability of the Ca^{++} receptor to activate MLCK. Finally changes in the pseudosubstrate prototope of MLCK alter enzyme activation. Whereas the CaM prototope analog will competitively inhibit a number of CaM-dependent enzymes, the pseudosubstrate prototope appears specific for MLCK. We propose that pharmacologic agents designed to mimic the pseudosubstrate prototope interaction with the substrate binding site of MLCK would be very specific inhibitors of this enzyme.

P 024 ABNORMALITIES OF EXCITATION-CONTRACTION COUPLING IN HYPERTROPHIC CARDIOMYOPATHY, James P. Morgan, Sanford Warren, G. Maurice Briggs, Linda Copelas, Marc D. Feldman, Preston Phillips, Mark Callahan Jr.#, Frederick Schoen, William Grossman and Judith K. Gwathmey. Harvard-Thorndike Laboratory of Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and Mayo Medical School, Rochester, MN#.

We tested the hypothesis that intracellular Ca^{++} ($[Ca^{++}]_i$) overload underlies the diastolic dysfunction of patients with hypertrophic cardiomyopathy. Myocardial tissue was obtained at the time of surgery or transplantation from five patients with hypertrophic cardiomyopathy and was compared to control myocardium obtained from eight patients without heart disease. The isometric contractions and electrophysiologic properties of all myocardial specimens were recorded by standard techniques and $[Ca^{++}]_i$ was measured with the bioluminescent calcium indicator aequorin. In contrast to the controls, action potentials, Ca^{++} transients, and isometric contraction and relaxation were markedly prolonged in the hypertrophic myocardium, and the Ca^{++} transients consisted of two distinct components. At physiologic temperature (i.e., 38°C) and pacing rates (i.e., 1 Hz) a state of relative Ca^{++} overload appeared to develop, which produced a rise in end-diastolic $[Ca^{++}]_i$, incomplete relaxation and fusion of twitches with a resultant decrease in active tension development. We also found that drugs which increase $[Ca^{++}]_i$, such as digitalis, exacerbated these abnormalities, whereas drugs that lower $[Ca^{++}]_i$, such as verapamil, or agents that increase cyclic AMP, such as forskolin, prevented them. We have reported similar findings in patients with end-stage heart failure and compensatory ventricular hypertrophy (1,2). These results may explain why patients with hypertrophic cardiomyopathy tolerate tachycardia poorly, and may have important implications with regard to the pharmacologic treatment of patients with hypertrophic cardiomyopathy. At the cellular level, our data indicate that, in hypertrophic cardiomyopathy, alterations in Ca^{++} handling occur at the level of the voltage-dependent sarcolemmal Ca^{++} channels and the Ca^{++} release/re-uptake sites of the sarcoplasmic reticulum. The nature of these changes and approaches to their prevention/reversal may be further elucidated by molecular biological techniques ((1) Circ Res 1987, 61:70; (2) Circulation 1987, 75:331).

P 025 LOCATION OF Ca^{2+}-BINDING SITES IN THE SARCOPLASMIC RETICULUM Ca^{2+}-ATPase. David M. Clarke*, Tip W. Loo*, Kei Maruyama*, Giuseppe Inesi# and David H. MacLennan*. *Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G1L6 and #Department of Biological Chemistry, University of Maryland, Baltimore, USA 21201.

Central to the mechanism of Ca^{2+} transport by the Ca^{2+} ATPase of sarcoplasmic reticulum is the binding and subsequent translocation of two Ca^{2+} per reaction cycle. Clarification of the translocation mechanism would be furthered by the identification of the amino acids which form the high-affinity Ca^{2+}-binding sites. We have used oligonucleotide-directed site-specific mutagenesis of the cDNA encoding the Ca^{2+} ATPase to alter acidic residues in the stalk and transmembrane domains to evaluate their roles in Ca^{2+} binding. Mutation of most Glx and Asx residues in the stalk sector and in the luminal M1-M2 loop, either singly or in groups, failed to alter Ca^{2+} transport. Exceptions were mutants Asn111 and Asn114 in which Ca^{2+}-dependent phosphorylation at the catalytic site occurred, but overall Ca^{2+} transport was inhibited. By contrast, mutation to neutral counterparts of Glu309, Glu771, Asp800 and Glu908, each of which is predicted to lie near the center of putative transmembrane sequences M4, M5, M6 and M8, respectively, resulted in complete loss of Ca^{2+} transport and of Ca^{2+}-dependent phosphorylation of the enzyme by ATP. Phosphorylation of each of the mutant enzymes with inorganic phosphate was observed, however, even in the presence of Ca^{2+} which inhibits phosphorylation in the wild type enzyme, presumably through binding to a functional Ca^{2+} binding site. Identical results were obtained with alteration of Asn796 and Thr799 which are also predicted to lie near the center of transmembrane sequence M6. These results suggest that oxygen atoms in the side chains of these six residues provide the ligands for one or both of the high -affinity Ca^{2+} binding sites. They also suggest that the Ca^{2+} binding sites lie near the center of the transmembrane domain of the Ca^{2+} ATPase.

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Development

P 026 GENE EXPRESSION DURING CARDIAC DEVELOPMENT. **Paul J.R. Barton***, Serge Alonso, Françoise Catala, Arlette Cohen, Ian Garner, Benoît Robert, David Sassoon and Margaret E. Buckingham. Department of Molecular Biology, Institut Pasteur, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France. **Present address*: National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK.

Mammalian cardiac muscle contains two myosin alkali light chains which are the major isoforms present in atrial (MLC1A) and ventricular (MLC1V) muscle (for review see 1), and which are encoded by separate genes located on different chromosomes in both mouse (2) and man (3). We have recently characterised the gene encoding the mouse MLC1A isoform (4). Although the occurrence of an atrial specific (MLC1A) isoform is only seen in mammals, this isoform shows a striking similarity with that of the isoform L23 found in chick. The recruitment of MLC1A to its atrial specific role raises questions concerning the evolution both of the mechanisms regulating this gene and of cardiac atrial muscle. Comparison of the promoter sequences of the MLC1A and MLC1V genes with those of α-cardiac and α-skeletal actin reveals conserved elements which may be involved in their regulation (5). In particular, the MLC genes contain a common sequence element (CCTTTTATAG) similar to the "CC.Ar.GG box" elements found in α-actin genes (6). We are currently investigating the relationship between these conserved elements and the nuclear factors which bind to them.
Cardiac myogenesis is characterised by the activation of cardiac-specific genes, most of which also show developmental modulation in their expression. We have investigated the expression of actin and myosin genes by *in situ* hybridisation to embryonic and foetal tissue sections. Cardiac actin forms a major transcript from the earliest stages of development of the heart (7) and remains the major cardiac isoform in the adult. However, analysis of a mouse mutant carrying an abnormal cardiac actin gene demonstrates that skeletal actin can replace up to 50% of the cardiac actin in the adult heart without any apparent effect on cardiac function (8). During cardiac development this effect is not seen suggesting that the mechanisms regulating actin gene expression are different in this situation. MLC1A transcripts accumulate in developing atrial and ventricular muscle, but are later down regulated in ventricular muscle where they are replaced by those of MLC1V. In addition to the analysis of actin and myosin genes, we have identified a mouse homeobox-containing gene (Hox-7) which, as well as being expressed in the developing limb bud and mandibular arch, is transiently expressed in the cardiac endocardial cushion tissue (9). This tissue later forms the septum and valves of the developing heart and this Hox-7 may therefore be involved in the morphogenesis of this tissue.

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P 027 THE *v-erbA* ONCOGENE AND ITS INTERFERENCE WITH THYROID HORMONE ACTION. Klaus Damm and Ronald M. Evans, Gene Expression Laboratory, The Salk Institute, PO Box 85800, San Diego, CA 92138-9216. The v-*erbA* oncogene and its cellular homologue, the thyroid hormone receptor, are members of a superfamily of transcriptional regulatory proteins that include receptors for steroid hormones and the morphogen retinoic acid. V-*erbA*, one of the two oncogenes found in the avian erythroblastosis virus (AEV), interferes with the normal differentiation program of erythroid precursor cells, possibly by modulating the transcription of target genes involved in this process. To understand the molecular details of this mechanism, the transcriptional activity of both the thyroid hormone receptor and the oncogene product was assessed by their ability to regulate expression of a thyroid hormone responsive reporter construct. Utilizing a co-transfection procedure, the thyroid hormone receptor was shown to activate transcription in a hormone dependent manner. Co-expression of *v-erbA*, which has lost its ability to bind hormone, blocks this induction, suggesting that the oncogene can function as a thyroid hormone receptor antagonist. The contribution of *v-erbA* to erythroblast transformation is most likely the result of functional interference with the thyroid hormone receptor in erythrocyte progenitor cells infected by AEV.

Extracellular Matrix and Embryogenesis

P 028 MOLECULAR GENETICS OF DROSOPHILA CONTRACTILE PROTEINS, Clifford J. Beall, Christine C. Fyrberg, Eric A. Fyrberg, Department of Biology, The Johns Hopkins University, Charles and 34th St., Baltimore, MD 21218
We are using genetic methods to further refine our understanding of contractile protein assembly and function in the fruit fly, Drosophila melanogaster. We will present data relating to the effects of null mutations within actin, myosin heavy chain, and alpha-actinin genes on sarcomeric assembly. We will also document an interesting actin point mutation, wherein the amino acid replacement (glycine6>alanine) appears to retard sarcomere assembly. Electron microscopic examination of the mutant myofibrils (done in collaboration with Mary Reedy of Duke University) has revealed an unexpected degree of torsional flexibility about the head-tail junction of myosin. Finally, we will present characterization of the genes which encode the troponin subunits of Drosophila thin filaments. This analysis has revealed that the stretch-activated mode of contractility characteristic of many insects is fostered by unusual isoforms of tropomyosin or troponin-I.

P 029 ENDOTHELIAL FORMATION OF MESENCHYME: INDUCTION BY AN IN VIVO ADHERON-LIKE COMPLEX, Roger R. Markwald, Corey H. Mjaatvedt and Edward L. Krug, Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226. The spatio-temporal transformation of AV endothelium into mesenchyme in early heart development establishes the pattern for future valves and septa. The process is elicited by a stimulus secreted by the myocardium into its basement membrane (MBM) that is selectively extracted by EDTA, but, if added to collagen gel cultures, the extracts (containing 0.1-0-2.4 ug/ml protein) induced the complete transformation of endothelium into mesenchyme. By EM analysis, EDTA had primarily extracted electron dense, 30 nm particles from the in situ AV-MBM whose protein composition and size resembled glycoprotein complexes - termed "adherons" - secreted into the growth medium of L6 myoblasts (Schubert and LaCorbiere, J. Cell Biol. 94:108-114). To determine if the inductive stimulus resided in the particle, whole extract was centrifuged at 100,000g to produce a pellet of 30 nm particles. Resuspended pellet fraction but not the supernatant fraction, stimulated the AV endothelium (but not that of the ventricle) to decrease expression of cell adhesion molecules and subsequently form mesenchyme. Similar results were obtained with conditioned medium (MCM) from stage 15 myocardial cell cultures. The pelleted fraction was used to prepare a polyclonal antibody termed ES-1 which, if added to culture medium, blocked endothelial formation of mesenchyme and, when absorbed to affinity supports removed the inductive stimulus from EDTA extracts or MCM. Immunoblots of pelleted fractions indicated ES-1 recognized two major (28/31 and 48 kDa) and four minor antigens (63, 88, 104 and 124 kDa). ES-1 did not recognize fibronectin (FN) (confirmed by ELISA) although a 220/240 band in the pelleted extract did bind to anti-FN antibodies. However, the 48 kDa band did bind to a monospecific polyclonal antibody against basic fibroblast growth factor. ES-1, anti-FN , and anti-FGF antibodies similarly stained 0.5-1.0 um aggregates of the 30 nm particles distributed solely to in situ regions of the MBM where mesenchyme formation occurs. These findings indicate that a soluble, multicomponent (30 nm) complex of the myocardial basement membrane can modify expression of adhesion molecules on endothelial cells and promote their transformation to free cells. (Supported by NIH K37-HL 33756).

P 030 MOLECULAR MECHANISMS OF THYROID HORMONE ACTION ON CARDIAC MYOSIN HEAVY CHAIN GENE EXPRESSION, Seigo Izumo, Gideon Koren, W. Reid Thompson III, Bernardo Nadal-Ginard and Vijak Mahdavi, Department of Cardiology, Children's Hospital, Harvard Medical School, Boston, MA 02115

Thyroid hormone (T_3) affects the physiology of the cardiovascular system by modulating the expression of number of genes. This effect is thought to occur through the interaction of chromatin associated T_3 receptors (TRs) with target gene sequences. We recently demonstrated that expression of all sarcomeric myosin heavy chain (MHC) genes is modulated by T_3 in all striated muscle types. One aspect which makes the MHC genes a particularly suitable experimental system to study the molecular mechanisms of TR function, is that these genes exhibit different response to T_3, either positive, neutral or negative. This phenomenon is not only determined by the MHC gene itself, but depends also on the muscle type in which the gene is expressed. By functional analysis carried out in vivo and in vitro, we observed that T_3-mediated induction of the cardiac α-MHC gene, or inhibition of the β-MHC gene, involves similar 16 bp long T_3 responsive elements, which are, however, situated at different distances relative to the transcription start site. This effect is mediated through a direct interaction of the TR with specific cis-regulatory 5' upstream MHC gene sequences. This observation identifies the TRs as T_3 dependent transcriptional trans-acting factors that control MHC gene expression. In addition the TR isoforms α1 and β, which are encoded by distinct genes, display significant differences in their ability to trans-activate/repress MHC gene expression. Expression of these TR isoforms is regulated in a tissue and developmental stage specific manner and is also under negative feedback control by T_3.

Moreover,the TRα gene encodes several mRNA species, through differential processing of its primary transcripts. The protein generated by one of the most abundant TRα mRNA transcripts, TRα2, is identical to TRα1, except for its carboxyl terminal portion, which is different both in peptide composition and length from that of TRα1. When produced in vivo and in vitro, TRα2 failed to bind T_3 or other hormones, or to trans-activate/repress MHC gene expression. This observation constitutes one of the few documented examples that alternative splicing can produce marked differences in the functional properties of a transcriptional factor.

Late Addition

P 031 **REGULATORY GENES THAT CONTROL DETERMINATION OF VERTEBRATE MYOGENIC CELL LINEAGES,** Sonia H. Pearson-White, Deborah F. Pinney, Fabienne Charles de la Brousse, Brian Lathrop, and Charles P. Emerson, Jr., Department of Biology, University of Virginia, Charlottesville, VA 22901.

DNA transfection approaches have been used to identify two regulatory genes that can direct myogenic conversion of 10T½ cells. The high frequency conversion of the mouse embryonic cell line C3H10T½ to stable, clonal myogenic lineages suggested that one or a few regulatory genes control the establishment of the myogenic cell lineage.[1] This expectation was confirmed by the frequency with which 10T½ cells transfected with a cosmid library of human genomic segments yields clonal primary and secondary myogenic transfectants.[2] Experiments are in progress to recover the transfected gene segment, *myd*, from primary and secondary transfected myogenic lines and to characterize the structure and function of this gene. A second myogenic gene, MyoD1[3], is activated in 5-azacytidine-converted and *myd*-transfected myogenic cell lines. MyoD1 also can induce clonal myogenic conversion and promote the differentiation of 10T½ cells when transfected as a cDNA transcribed by heterologous β-actin or SV40 promoter. MyoD1 is a nuclear-localized protein in differentiated myofibers,[4] and bacterially-expressed MyoD1 protein has sequence-specific DNA binding activity, suggesting MyoD1 regulates muscle-specific genes. *In situ* hybridization experiments localize MyoD1 expressing cells to the early somite and limb mesenchyme of quail embryos. These results establish the developmental importance of MyoD1 in myogenic determination in the developing embryo. The roles of *myd* and MyoD1 in myogenic lineage determination and differentiation will be discussed.

[1]Konieczy and Emerson (1984). Cell **38**, 971.
[2]Pinney et al. (1988). Cell **53**, 781.
[3]Davis et al. (1987). Cell **51**, 987.
[4]Tapscott et al. (1988). Science **242**, 405.

Cardiac Muscle; Hypertrophy and Hyperplasia

P 100 TRANSFECTION EFFICIENCY IN FETAL CARDIAC MYOCYTES AFTER ELECTROPORATION WITH RECOMBINANT PLASMID DNA. Peter G. Anderson, Joseph J. Bahl, Bruce E. Markham, Eugene Morkin. University Heart Center, University of Arizona, Tucson, Arizona 85724

A recombinant plasmid (pSV2MCAT) containing the α-myosin heavy chain promoter region linked to the bacterial chloramphenicol acetyl transferase (CAT) coding sequence was used to evaluate thyroid hormone (T_3) induced gene regulation in cardiac myocytes. Isolated myocytes obtained from 18 day fetal rat hearts were suspended in Hepes buffer containing 40 μg of plasmid DNA and 15 μg of M13mp18 helper DNA and were electroporated with a BioRad Gene Pulser (960 μFD at 240 volts). Myocytes were plated ($4x10^6$ cells/dish) on collagen coated dishes in modified Ham's media with or without 10 nM T_3. Electroporation resulted in a 50% reduction in the number of surviving myocytes. After 3 days in culture, plates were fixed in paraformaldehyde and anti-CAT primary antibody was used to immunolabel myocytes containing CAT. Cardiac myocytes transfected with pSV2MCAT and incubated with T_3 had 18.1±0.73% (mean±SEM) CAT positive cells (10 plates, 1000 cells/plate counted). Light and scanning electron microscopy of myocytes fixed in 2% glutaraldehyde immediately after electroporation and 1 hour later did not demonstrate visible membrane defects. Thus, electroporation of isolated fetal cardiac myocytes with plasmid DNA resulted in successful transfection with T_3 promoted CAT expression in approximately 20% of the surviving myocytes after 3 days in culture.

P 101 Mitochondrial DNA D-Loop Structure and Mitochondrial DNA Polymerase Activity in Mammalian Striated Muscles of Differing Oxidative Capacity, Brian H. Annex and R. Sanders Williams, Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, NC 27705

The mitochondrial (mt) DNA content of mammalian striated muscles is known to correlate with its oxidative capacity, but the mechanisms by which muscle tissues regulate mtDNA content is unknown. We have hypothesized that highly oxidative muscles must replicate their mtDNA more frequently than tissues with a lower oxidative capacity, and that this adaptation would be manifested by differences in the structure of the mtDNA and in the activity of DNA polymerase gamma (mt-Pol). We analyzed the ratio of D-loop to full length mtDNA by probing Southern blots with a synthetic oligonucleotide containing a highly conserved sequence within the D-loop region of the mt DNA light strand. In the same tissues, we measured the specific activity of mt-Pol by a sensitive and specific primer extension assay. In a comparison of skeletal and cardiac muscles from the rabbit, cardiac muscle contained 29% D-loop forms, and expressed mt-Pol activity (fmol/min/mg soluble protein) 20-fold greater than that of the glycolytic tibialis anterior skeletal muscles, in which only 7% of mtDNA assumed the triplex form. In a cross-species comparison, bovine heart mt genomes contained only 20% triplex D-loop DNA while the more oxidative mouse heart expressed a higher mt-Pol activity and 76% of its mt genome assumed the triplex structure. These data indicate that muscle tissues faced with a higher demand for energy production via oxidative metabolism have a greater mitochondrial DNA polymerase activity and may maintain a greater percentage of mtDNA in the D-loop form. These findings focus attention upon mtDNA replication as an important determinant of the highly oxidative muscle phenotype.

P 102 Reciprocal regulation of glycolytic and mitochondrial enzyme mRNA transcript levels by O2 tension in beating heart cell cultures. K.A. Webster, L.H. Kedes and N.H. Bishopric, Department of Biochemistry, University of Southern California and Department of Medicine, University of California at San Francisco, 94143. The ischemic myocardium maintains the synthesis of energy for contraction by a compensatory increase in anaerobic glycolysis. The mechanisms by which oxygen availability may regulate the activity of glycolytic and oxidative enzymes in the myocardium are not understood. We have used a cell culture model to examine the effects of O2 tension on the expression of specific myocardial genes. Neonatal rat heart cells were plated to confluence and grown in media containing 10% FCS at atmospheric O2 tension for 3 days. BrdU was added for the first 2 days to retard proliferation of nonmyocardial cells (<5% of total). On d4 cells were transferred to a hypoxic atmosphere (2% O2) and harvested at specific time points. Beating rates were monitored throughout the experiment. Total RNA was extracted and hybridized with cDNAs for pyruvate kinase (PK) and the ß-subunit of mitochondrial ATPase (mATPase) by Northern analyses. PK transcripts were induced in the hypoxic cells at 24 h and increased to 3-5X over control at 72 h. In contrast, mATPase mRNA decreased by 30-40% over the same interval. These results suggest that O2 tension can mediate myocardial metabolic enzyme gene expression and that the genes for oxidative and glycolytic enzyme pathways are reciprocally affected. The myocardial response to ischemia may thus include adaptations at the level of gene expression.

P 103 HYPERTROPHIC CARDIOMYOPATHY: GENES IN THE HLA REGION EXCLUDED AS CAUSE. Paul A. Brink, Jeffrey A. Towbin, Dennis Fink, Rita Hill, J. Fielding Hejtmancik, Robert Roberts. Baylor College of Medicine, Houston, TX, USA 77030.

Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden death in young persons. The cause of HCM is unknown. Familial cases of HCM afford the opportunity to test in an unambiguous way genetic loci that may be responsible for this syndrome. Pedigrees segregating for HCM were collected and linkage analysis performed with serum and DNA markers. Five families (104 individuals, 35 affected) have been investigated. Because previous studies using HLA typing have suggested linkage of HCM to the HLA locus, probes from this region were selected. For linkage analysis strict clinical diagnostic criteria are essential. Diagnosis requires ventricular hypertrophy as detected echocardiographically in the absence of other causes. Persons above age 65 were excluded from linkage analysis. DNA was prepared from whole blood or lymphoblastoid lines and analyzed for possible co-segregation of DNA restriction fragment length polymorphisms (RFLPs) in the HLA region with the disease phenotype. Analysis with the probe PCH6 (D6S10) located within the HLA region excluded a region of 9 cM with a lod score of less than -2. Thus, genes in the HLA region are excluded as the cause of HCM in the pedigrees investigated. Presently, we are systematically studying loci on additional chromosomes as causative of HCM. Locating the HCM locus will enable development of preclinical tests in these pedigrees, strategies for cloning the gene and unravelling the pathology of this disease. Elucidating mechanisms of hypertrophy in this disease may make contributions in understanding cardiac growth and hypertrophy in general.

P 104 CHARACTERIZATION OF LONG-TERM CULTURES OF YOUNG ADULT DOG CARDIAC MYOCYTES Lawrence B. Bugaisky, Candace G. Snider, Terrell W. James, R. Scott Hall and W. Thomas Woods Jr., Departments of Pathology, Cell Biology and Anatomy, and Physiology. University of Alabama at Birmingham, Birmingham, AL 35294.

To examine the utility of cultured dog cardiac myocytes as an in vitro system for future examination of the direct effects of a stimulus at the cell level, we have undertaken a biochemical characterization of these cells. Cardiac myocytes from young adult beagles were obtained by perfusion of the heart with collagenase. The isolated myocytes were then plated onto laminin coated dishes in Basal Eagle's Medium + 5% calf serum. At least 80-90% of the plated cells attach to the culture dishes. The majority of these myocytes maintain their cylindrical shape and evident cross-striations as well as remain trypan blue negative for at least 48 hours. Unlike cultured rat cells, however, a large percentage of the dog myocytes progress directly towards a more spread out morphology from the original cylindrical shape as opposed to "rounding up". Additionally, these cells maintain the capability to undergo at least one additional round of cytokinesis, with a single binucleated cell becoming two mononucleated myocytes. During the first nine days in culture, we have labelled cells with ^{35}S-methionine, and have observed no dramatic changes in the qualitative pattern of protein synthesis. Changes in the relative incorporation between different proteins, however, have occurred. Furthermore, after 15 days in culture the myocytes were still Periodic-Acid Schiff positive and contained evident cross-striations as indicated by anti-myosin staining. Thus, for at least two-three weeks in culture, dog myocytes continue to express a number of highly differentiated characteristics typical of the myocyte phenotype.

P 105 CARDIOVASCULAR HYPERTROPHY IN GROWTH HORMONE TRANSGENIC MICE, Rodney J. Dilley and Stephen M. Schwartz, Department of Pathology, University of Washington, Seattle, WA 98195

Mice transgenic for the growth hormone gene have significantly greater body weights from about one month after birth than do control mice not expressing the transgene. This study of cardiovascular changes in seven-month-old animals showed increased mass of many organs, including the heart, aorta, kidney and small intestine. In the thoracic aorta, cross sectional area of the media was greater in transgenic mice but the wall/lumen ratio was maintained. The greater medial mass arose from smooth muscle cell hyperplasia and was distributed as thicker layers of medial smooth muscle and elastic connective tissue, rather than more layers. In the mesenteric circulation, the average third branch order vessel supplied a larger mass of intestine in transgenic mice and these vessels had a greater medial cross sectional area and media/lumen area ratio than controls. The number of vessels per intestine and the mesenteric branching patterns (the ratio of third order to first order branches) were maintained at levels similar to controls. These results suggest that the mass of the vessel wall and the pattern of mass distribution are controlled differently. (Supported by AHAW grant 87-WA-119.)

P 106 ATRIAL HYPERPLASIA IN TRANSGENIC MICE. Loren J. Field and Mark E. Steinhelper, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York, 11724. We have generated transgenic mice which carry fusion genes comprised of the human ANF promoter and the SV40 large Tumor antigen (ANF-Tag). Although both left and right atria express SV40 oncoprotein, only the right atrium exhibits a hyperplastic response. The diseased appendage can undergo a several hundred fold increase in mass. Cardiac arrhythmias accompany the hyperplasia, increasing in both frequency and severity with atrial mass (Science 239, 1029-1033, 1988). Transplantation studies were performed to characterize the unilateral nature of the tumor phenotype. Subcutaneous implantation of neonatal transgenic right atria into nude mice results in the proliferation of differentiated myocytes which continue to express cardiocyte markers. Similar transplantation of left atria results in no proliferation, suggesting that unilateral tumorigenesis is due to an intrinsic difference between right and left atrial cells. When material derived from frank right atrial tumors is transplanted into syngeneic mice, ectopic tumors are observed at a very low frequency (>5%) and after a long latent period (9 months). These ectopic tumors can subsequently be passed at a high frequency (> 70%) with an average latency of 8 weeks. RNA and protein analyses indicate that right atrial tumors and the ectopic tumors both continue to express many cardiac-specific markers, and exhibit spontaneous electrical activity. A study was initiated to assess the effect of genetic background on the unilateral atrial hyperplasia. Sublines were generated by back crossing a ANF-Tag mouse to either B6 or D2 inbred animals (C57Bl/6J and DBA/2J, respectively). After four generations of back crossing to D2, right atrial hyperplasia is apparent by 3-4 days of age, and the tumors are lethal by 5-7 weeks; moreover the entire atrium is hyperplastic. Animals derived from B6 back crosses exhibit no pathology until on average 1 year of age, and the hyperplasia is focal in nature. The latency and histological appearance of tumors in the B6 background suggests the requirement of a cooperative genetic event. Experiments are under way to identify the loci responsible for these phenotypes.

P 107 BINDING OF THYROID HORMONE RECEPTORS TO SPECIFIC DNA SEQUENCES IN THE 5' FLANKING REGION OF THE α-MYOSIN HEAVY CHAIN GENE, Irwin L. Flink, Richard W. Tsika, Joseph E. Bahl, and Eugene Morkin, University Heart Center, University of Arizona, Tucson, AZ 85724
Thyroid hormone (T_3) is thought to modulate gene expression through interaction with chromatin-associated receptors which have been shown to be products of the c-erbA protooncogenes. High-affinity binding of liver nuclear T_3 receptors and in vitro translated brain and placental c-erbA cDNAs to specific DNA sequences was analyzed using an avidin-biotin complex DNA-binding assay. Double-stranded oligonucleotides were synthesized and biotinylated corresponding to the rat growth hormone gene T_3 response element (TRE_{GH}) containing the sequence (5'CAGGGACGTGACCGCA 3') and to three homologous sequences in the α-myosin heavy chain 5'-flanking region: -100 to -54 (TRE_1), -163 to -116 (TRE_2), and -247 to -216 (TRE_3) base pairs from the transcriptional start site. The results demonstrate that rat liver T_3 receptors bind efficiently to TRE_2 and TRE_{GH} while TRE_3 gave low but detectable binding. Similarly, brain and placental c-erbA products bound only to TRE 2 and 3. Biotinylated oligonucleotides containing unrelated sequences showed no specific binding. Thus, two of the three homologous elements in the 5'-flanking sequences bind in vitro to T_3 receptor subtypes.

P 109 SKELETAL MUSCLE FIBRES EXPRESS CARDIAC TYPE AND REPRESS FAST TYPE MYOSIN HEAVY CHAIN GENES WHEN SUBJECTED TO STRETCH AND ISOMETRIC OVERLOAD. Andrew Scutt, Claire Gilroy, Gerald-F Gerlach, Vladimir Bicik* and Geoffrey Goldspink Unit of Molecular and Cellular Biology, Department of Veterinary Basic Sciences, The Royal Veterinary College, London University, London NW1 OTU * Faculty of Medicine, Kuwait University

Mammalian skeletal muscles consist of populations of slow contracting oxidative fibres which, like cardiac muscle, are adapted for slow repetitive activity and fast contracting fibres which are required for rapid phasic movements. The slow fibres express the same myosin heavy chain as cardiac muscle which means they use ATP at a slow rate and they work efficiently as their rate of contraction is matched to work rate. Using electrical stimulation to control force generation and limb immobilization to alter the degree of stretch we have shown that fast skeletal muscle can be induced to express the slow type (cardiac beta) myosin heavy chain gene within a period as short as a few days. Stretch and electrical stimulation were found to activate transcription of the cardiac slow type gene and to repress expression of the fast type gene. The higher the pulse frequency of stimulation the greater the expression of the slow type genes which leads us to the conclusion that it is isometric overload rather than stimulation frequency per se that results in slow type, (cardiac beta) myosin heavy chain expression. This offers the possibility of preconditioning skeletal muscle prior to it being use for a cardiac assist.
This work was supported by the Wellcome Trust

P 110 IDENTIFICATION OF MULTIPLE PROTEINS WHICH INTERACT WITH FUNCTIONAL REGIONS OF THE HUMAN CARDIAC ACTIN PROMOTER, Thomas A. Gustafson[1] and Larry Kedes[1,2], [1]Department of Medicine, Stanford University, VAMC, 3801 Miranda 151M, Palo Alto, CA 94304 and [2]Departments of Biochemistry and Medicine, Program in Molecular Biology and Genetics, University of Southern California School of Medicine, Los Angeles, CA 95033. 5' sequences of the human cardiac α-actin gene are involved in the tissue-specific and developmental regulation of the gene. Deletion analyses combined with transient expression experiments in muscle cells have previously demonstrated three primary regions of functional importance. We have previously demonstrated binding of a protein indistinguishable from serum response factor (SRF) to the most proximal region and here we examine protein interaction with the remainder of the promoter. Gel shift and footprinting assays revealed at least six distinct nuclear proteins which interact with regulatory regions of the promoter. The transcription factor Sp1 interacts with at least eight sites as demonstrated by footprinting assays and gel-shift analysis with purified Sp1. CTF/NF-I was shown to interact with a single site which had extensive overlap with an Sp1 binding site. SRF was found to bind to three of the four sites which contain the CArG motif [CC (A/T-rich)6 GG], two of which were closely linked to Sp1 sites. Two unidentified proteins with similar but distinct footprints, interact with the second CArG box and were shown to be distinct from SRF. In addition, CArG box 4 was found to interact with SRF and a second distinct protein whose footprint was contained within the SRF binding site. Sequences surrounding the TATA box were also shown to bind proteins and Sp1 was shown to bind to a site immediately downstream from the TATA box. Thus, each of the three functional upstream regions, as defined by transfection assays, are shown to interact with proteins, including Sp1, CTF/NF-I, SRF and two unidentified proteins. In addition, a number of other protein binding sites are also defined whose functional significance is unknown. These results suggest that expression of the cardiac actin gene in muscle cells is controlled by complex interactions among multiple upstream and intragenic elements.

P 111 HUMAN HEART AND PLACENTAL MITOCHONDRIAL CREATINE KINASE ISOFORMS ARE ENCODED BY DIFFERENT NUCLEAR GENES. Robert C. Haas and Arnold W. Strauss, Departments of Internal Medicine and Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110 Creatine kinase (CK) is a pivotal enzyme involved in energy transduction in tissues with high energy demands. CK isoenzymes from muscle (M), brain (B) and mitochondria (Mt) are encoded by nuclear genes. We have previously isolated and characterized the gene and cDNA encoding human placental MtCK. A probe from the coding region of this gene detected abundant MtCK mRNA in skeletal muscle and ventricle, and less in placenta and small bowel by RNA blot analysis. The same probe also demonstrated a dramatic increase in mRNA from differentiating BC3H1 cells. A 5'-untranslated region probe from the human placental MtCK cDNA hybridized only to mRNA from placenta and small bowel, suggesting the presence of at least two tissue-specific MtCK isoforms. We have subsequently isolated and characterized a human heart MtCK cDNA which is similar to the placental cDNA in size and structure, but has a unique primary nucleotide sequence. Both cDNAs bear similar, but distinct, sequences encoding amino-terminal transit peptides, which are presumably essential in the import of these proteins into mitochondria. The overall nucleotide and predicted amino acid sequence identities of these two isoforms are 70% and 90%, respectively. A 3'-untranslated region probe from the human heart MtCK cDNA detected abundant MtCK mRNA in ventricle, much less in skeletal muscle, and none in placenta or intestine, proving the existence of a third, skeletal muscle-specific MtCK isoform, which may be derived from a third MtCK gene, or generated via alternative splicing. Thus, two or more closely related but distinct genes encode MtCK isoforms, which are tissue-specifically expressed in coordination with cytosolic CKs.

P 112 ENTEROVIRUS-INDUCED CARDIOMYOPATHY. Reinhard Kandolf, Philip Kirschner, Annie Canu, Albert Heim, Peter Hans Hofschneider. Max-Planck-Institute for Biochemistry, D-8033 Martinsried and Dept. of Int. Med. I, Univ. Munich, FRG.

An in situ hybridizaton assay capable of detecting enterovirus RNA in myocardial tissue was developed using full-length reverse-transcribed recombinant coxsackievirus B3 cDNA as an enterovirus group-specific probe. To date, in situ hybridization has already proved to be a powerful tool in assessing the presence of enterovirus RNA in myocardial biopsy samples of patients with a clinical suspicion of myocarditis and dilated cardiomyopathy. In patients with dilated cardiomyopathy of recent onset, the most dramatic manifestation of myocarditis, the incidence of enterovirus infection was found to be approximately 30%. Particularly intriguing is the concept of enterovirus persistence in chronic dilated cardiomyopathies, evolving from acute or subacute infections, which is currently being substantiated by the finding of enterovirus persistence in follow-up biopsies of patients with ongoing disease.

An important finding is the observation that enterovirus RNA was not only detected in myocytes but also in small interstitial cells, which agrees with the previous in vitro findings in cultured human heart cells and persistently infected human myocardial fibroblasts. We expect the in situ hybridization technique to have considerable potential for the study of the natural course of the disease and for the evaluation of antiviral therapy.

P 113 THE EFFECT OF WALL STRESS ON THE PRODUCTION AND ACCUMULATION OF VASCULAR ELASTIN AND COLLAGEN, F. W. Keeley, A. Alatawi and A. Cho, Department of Cardiovascular Research , Hospital for Sick Children, Toronto, Canada, M5G 1X8.

Elastin and collagen are major proteins of the large arterial blood vessels. Together they account almost entirely for the physical properties of the vessel wall and enable these vessels to maintain their integrity and function as flexible, high pressure conduits. Normally, once a mature complement of elastin has been achieved in arteries of growing animals, synthesis and accumulation of elastin essentially ceases. However, elastin production may resume in these vessels in several pathological circumstances including hypertension. Using both *in vivo* and *in vitro* models, we are studying the response of arterial vessels to increased blood pressure and wall stress.

In vivo models of hypertension in the rat have shown that the response of elastin production to increased pressure is early, rapid, and proportional to the elevation in blood pressure. However, even at the earliest stage of the response, there is no change in the relative amount of elastin in the vessel wall, suggesting coordinated synthesis of all wall components. Furthermore, all *in vivo* models show a transient response, with elastin production falling as wall stresses are returned towards normal levels due to wall thickening.

In vitro studies in chick aortic tissue confirm that stress-induced elastin and collagen production is rapid and graded, appearing in less than 6 hours and with a threshold comparable to that seen *in vivo*. The response is diminished at high levels of wall stress, and does not require an intact endothelial cell layer. In both *in vivo* and *in vitro* models, low doses of colchicine appear to block the response to increased wall stress without affecting background levels of synthesis, suggesting that the cytoskeleton may be important in the perception of increased wall stress by the arterial cells and the transduction of this stress into increased synthesis of connective tissue proteins. (Supported by the Heart and Stroke Foundation of Ontario)

P 114 REEXPRESSION OF FETAL TYPE mRNAs IN THE VENTRICLE OF CARDIOMYOPATHIC SYRIAN HAMSTERS. Issei Komuro, Masahiko Kurabayashi, Youichi Kato, Fumimaro Takaku and Yoshio Yazaki, The 3rd Department of Internal medicine, University of Tokyo, Tokyo, Japan

Recently, various biochemical changes have been descrived in the heart of cardiomyopathic hamsters. We examined the changes in the cardiac gene expression of myofibrillar and nonmyofibrillar components in the heart of Syrian hamster, Bio 14.6. Using gene-specific DNA probes, we demonstrated that the mRNAs encoding the fetal isoforms of myosin light chain (atrial light chain) and α-actin (skeletal actin) are expressed in the adult ventricle of 10 to 35 week-old cardiomyopathic hamsters but not in the ventricle of control hamsters. In addition, atrial natriuretic factor mRNA, which was expressed in fetal and neonatal ventricle, was abundantly expressed in the ventricle of cardiomyopathic hamsters. The increase in atrial natriuretic factor mRNA was detected from 10 week-old hamster's heart. Since the cardiac function of this age was not impaired yet, this expression of atrial natriuretic factor might not be an adaptive process. These results indicate that reprogramming of cardiac gene expression both of myofibrillar and nonmyofibrillar components might occur in the heart of cardiomyopathic syrian hamsters.

P 115 ANGIOTENSIN II (AngII) AFFECTS CELLULAR PROTO-ONCOGENES AT THE TRANSCRIPTIONAL LEVEL Massimo Levrero,Clara Balsano,Maria Laura Avantaggiati,Claudio Ferri, Paolo De Marzio, Istituto I Clinica Medica, University of Rome "La Sapienza",Rome,00161, ITALY

In vivo studies have demonstrated that angiotensin converting enzyme inhibitors reduce cardiac and vascular hypertrophy in animal and man.Recently,the development of both hyperplasia and hypertrophy in cardiovascular tissues have been linked to an activation of cellular proto-oncogenes.To further investigate the mechanism of AngII induced cellular hypertrophic/proliferative responses we used the NIH-3T3 murine fibroblasts as a model.AngII,added at concentrations ranging from 10^{-4} to 10^{-8}induced cellular proliferation.A clear cut increase of both c-fos and c-myc steady state mRNAs levels was also observed, although the kinetic of the activation was different.To investigate if AngII acts directly or indirectly on c-fos and c-myc regulatory sequences,NIH-3T3 cells maintained in serum-free defined medium were transfected with two recombinant plasmids containing the bacterial reporter gene CAT under the control of c-fos or c-myc regulatory sequences in the presence or absence of AngII. From these experiments and from the results of nuclear run-on transcription assays it is evident that an increased transcriptional rate is likely to be the primary event in AngII induction of c-fos and c-myc expression.Recombinant plasmids carrying specific deletions in c-fos regulatory sequences are beeing used to determine the target sequences for AngII. The activation of cellular proto-oncogenes by AngII may be important in the development of cardiac hypertrophy.

P 116 RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLP) OF RENIN AND ANGIOTENSINOGEN GENES DISTINGUISH HYPERTENSIVE AND NORMOTENSIVE RAT STRAINS. K. Lindpaintner, S. Takahashi, T. Hellmann, K. Murakami*, J. Mullins, D. Ganten. German Inst for High Blood Pressure Res & Dept of Pharmacology, Univ. of Heidelberg, FRG. *Tsukuba Univ, Ibaraki-Ken, Japan

Primary hypertension in both man and experimental animals is considered to result from an inherited anomaly of one or more genetic factors. The pivotal role that the renin-angiotensin system plays for blood pressure homeostasis via both endocrine and para/autocrine mechanisms emphasizes the potential importance which altered regulation or expression of its components may have. We screened genomic DNA from normotensive Wistar, Wistar-Kyoto, Sprague Dawley, Long Evans, and Brattleboro (DI) as well as from spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and a hypertensive strain cross-bred from SHRSP and DI (SHR-DI) for RFLPs using cDNAs for renin and angiotensinogen as hybridization probes. We identified an RFLP for renin with a number of restriction enzymes. Consistently, a short allele was present in all hypertensive strains where a corresponding longer allele was seen in all normotensive strains. Subsequent multiple digestions and hybridization against specific cDNA and genomic fragments mapped the RFLP to a region of the gene which contains exon I, intron I, and part of the 5' upstream sequence. For angiotensinogen, digestion with *PvuII* disclosed an RFLP distinguishing SHRSP from normotensive controls. This RFLP was mapped to exon II of the gene. Our results demonstrate differences in the structure of the renin and angiotensinogen genes distinguishing normotensive from hypertensive rat strains. The association of hypertension with specific RFLP patterns in genes important for circulatory regulation indicate the potential pathogenetic relevance of our findings.

P 117 PARTIAL PURIFICATION AND CHARACTERIZATION OF A CYTOSOLIC FACTOR STIMULATING TRANSCRIPTION IN VITRO DURING THE DEVELOPMENT OF CARDIAC HYPERTROPHY IN RATS. M. Mariappan, J. Kamala and C. Rajamanickam. Department of Biochemistry, Madurai Kamaraj University, Madurai - 625 021, India.

Studies on transcription in vitro in isolated nuclei have indicated that the 100,000 x g supernatant isolated from hypertrophic hearts of aorta constricted animals can induce transcription in nuclei from normal hearts. Further fractionation and partial purification of the cytosol from hypertrophic hearts through DEAE-cellulose and hydroxylapatite (HAP) column chromatography have shown the enrichment of a protein of molecular weight 45,000 daltons by SDS-PAGE analysis in HAP fraction. The HAP fraction shows a 7 fold stimulation in the synthesis of myosin heavy chain and actin messengers as analysed by hybridization with specific cDNA probes. Immunoprecipitation of this 45kDa protein from the HAP fraction with specific antibody inhibits the stimulatory activity of the HAP fraction. Phosphorylation in vitro using endogenous protein kinase activity has revealed the phosphorylation of a similar molecular weight protein both in the cytosol and in the nucleoplasmic fraction. The extent of phosphorylation was found to be greater in the samples from hypertrophic hearts. The immunoprecipitation of the 45kDa protein in the nucleoplasm with the antibody raised against the 45kDa protein of cytosolic origin suggests the possibility of translocation of this protein from cytosol to nucleoplasm.

P 118 NOREPINEPHRINE STIMULATES PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN NEONATAL RAT CARDIOCYTES. Thomas Marino, Kim D'Ambra, and Ruth Anne Walter, Dept. of Anat. Temple Univ. Sch. of Med. Phila. PA 19140

During the first postnatal week, cardiocytes in vivo cease dividing and increase only in size. In vitro, growth in size is enhanced by exposure to α_1-adrenergic agonists. Since adrenergic stimulation causes increases in 1) cell number in proliferating cell types, and 2) c-myc expression in neonatal cardiocytes, our hypothesis was that neonatal cardiocytes in vitro reenter the G_1 phase of the cell cycle (as evidence: expression of c-myc) and progress through S phase and this process is enhanced by the presence of catecholamines. To test this hypothesis cell volume and the expression of PCNA, an auxiliary protein of DNA polymerase-δ and a marker for cells synthesizing DNA, were measured. Neonatal cardiocytes were placed in serum-free (SF) medium with or without 10% serum (S), norepinephrine (NE), NE plus propranolol (NP), or isoproterenol (I). After 4 days the same cells were fixed and stained for immunofluorescence of both PCNA and myosin, or measured on a Coulter Counter resulting in the following (* or ** = significantly different with $P<0.05$):

	SF	S	NE	NP	I
Volume(μm^3)	1391.0±24.6	-	1822.0±25.0*	1854.3±27.3*	1495.3±10.7**
%PCNA* cells	57.0±1.7	68.5±1.0**	80.0±1.4*	79.0±0.6*	69.0±0.6**

In vitro, α_1-adrenergic stimulation results in increases in cardiocyte volume and in the number of cardiocytes synthesizing DNA. Thus, this catecholamine enhanced cell growth may only be that associated with increased DNA synthesis.

P 119 AFFECT OF BETA BLOCKER TREATMENT OR LOW SODIUM DIET ON CARDIAC MYOSIN HEAVY CHAIN GENE EXPRESSION. Charles F. McTiernan and Suhba Sen, Dept. of Heart and Hypertension, Cleveland Clinic Foundation, Cleveland Ohio 44106. Beta blocker therapy is an effective means of reducing cardiac hypertrophy in both rats and humans. Other evidence has suggested that low Na diets can be effective in preventing the development of hypertrophy, and the progression of hypertension in SHR. Both treatments were examined for their effects on the expression of cardiac myosin heavy chain types, since hypertrophy and impaired cardiac function are associated with the V3 (beta MHC) myosin form. SHR and WKY rats were either untreated, treated with atenolol, or placed on a low sodium diet from 1 to 6 months of age. Blood pressure, left ventricle/body wgt ratio, myosin V1/V3 ratios, and level of alpha and beta MHC transcripts were determined. Low sodium diet prevented the development of hypertension in SHR, reduced the hypertrophy index, and raised the percentage of V1 MHC forms (in both WKY and SHR) from 45% to almost 90%. Analysis of mRNA levels indicates that this occured through reduced expression of beta MHC genes. Atenolol treatment reduced SHR hypertrophy, moderately attenuated hypertension, and marginally raised the percentage of V3 forms; in WKY, atenolol almost doubled the percentage of V3 forms (from 27% to 51%). This change was accompanied by an increased beta MHC mRNA level. It appears that both treatments alter beta MHC gene expression. Low Na diet supresses beta MHC expression in both WKY and SHR. Atenolol increases beta MHC expression, more so in WKY than SHR.

P 120 SPECTRIN IMMUNOLOCALIZATION IN THE MAMMALIAN HEART, Dino A. Messina, Yuji Isobe, Grant Hou, Larry F. Lemanski, Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210
The spectrins are a family of cytoskeletal-membrane proteins that have a wide tissue distribution. In the erythrocyte, spectrin is thought to convey structural integrity to the membrane-skeleton by cross-linking actin oligomers and anchoring them to the cell membrane. The function of spectrin in non-erythrocytic tissue is not clear. We are interested in the contribution that cytoskeletal and membrane associated proteins make during cardiac myofibrillogenesis. Frozen, unfixed sections of hamster cardiac tissue reveal staining for human erythrocyte spectrin at the intercalated disc, Z-bands and sarcolemma. Transverse sections show a delicate internal network of spectrin that appears to surround groups of myofibrils. Detergent treated isolated myofibrils exhibit Z-band staining for spectrin. Western blot analyses prove the specificity of our antibodies and confirm the above light microscopic findings. We are currently involved in determining the precise localization of spectrin in cardiac myocytes at higher resolution. Immunoelectron microscopic techniques including deep-etch and carbon/platinum shadowing of newborn cultured cardiac myocytes stained with anti-spectrin reveal a diffuse, membrane-associated pattern of staining. Presently we are utilizing adult, dissociated cardiac myocytes to view cytoskeletal/membrane/ myofibril relationships. Physical permeabilization followed by antibody incubation and replica technology will allow us to view spectrin at the electron microscopic level in three-dimensions, providing additional information concerning the function of spectrin in heart tissue. (Supported by NIH grants HL 32184, HL37702)

P 121 PROMOTER ELEMENTS REQUIRED FOR HORMONAL AND TISSUE-SPECIFIC EXPRESSION OF THE RAT ANF GENE IN THE HEART, Mona Nemer, Stefania Argentin, Isabelle Lihrmann, Ali Ardati and Jacques Drouin, Institut de Recherches Cliniques de Montréal, Montréal, Québec, Canada H2W 1R7
Atrial natriuretic factor (ANF) is a 28 amino acid peptide hormone which is the major secretory product of the heart. ANF has potent natriuretic, diuretic and vasodilator properties and may be an important modulator of water and electrolyte balance. ANF is derived from a larger precursor which is encoded by a single copy gene. In the adult, the ANF gene is mainly expressed in heart atria and to a much lower extent in ventricles. However, both atria and ventricles express the ANF gene at similar high levels in fetal and newborn hearts. Furthermore, the ANF gene is reactivated in various conditions of cardiac hypertrophy in animal models and in man. The ANF gene is also under multihormonal control including induction by glucocorticoids and thyroid hormones. In order to define the molecular mechanisms involved in hormonal, developmental and cardiac specific expression of the ANF gene, we have used DNA-mediated gene transfer to define the activity of the rat ANF promoter in cardiac and non cardiac cells in culture. Rat ANF sequences extending to 3.9 kb upstream of transcription initiation were fused to the coding sequences for human growth hormone. The ANF promoter was only active in cardiac cells. Deletions analysis indicates that 1.6 kb 5'-flanking sequences are essential for promoter activity and glucocorticoid regulation in primary cardiocyte cultures. These sequences contain several sites which bind nuclear proteins present in atrial and ventricular cells as revealed by DNase I footprinting assays. We are currently using site directed mutagenesis to better define specific promoter sequences which interact with cardiac transcription factors and modulate expression of the ANF gene.

P 122 STUDY OF MECHANISMS FOR AGE-RELATED DIFFERENCE IN MYOCARDIAL RESPONSE TO PRESSURE AND VOLUME OVERLOAD IN THE ADULT RAT.
Gur W. Notani, Warren J. Manning, and Jeanne Y. Wei. Divisions of Gerontology and Cardiology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215.

The P450 enzyme system has well characterized markers for hormonal and developmental gene regulation. To test the hypothesis that the age-related difference in hypertrophic response may be in part hormonally mediated, gene regulation of P450 isozymes are under study in two models of left ventricular hypertrophy. In the volume overload model a tear is produced in the aortic valve. The pressure overload state is induced by supravalvular aortic banding. For both models the animals are allowed to recover and serial changes in left ventricular mass are assessed by gated proton magnetic resonance imaging. P450 forms PCN2 is positively regulated by androgens and negatively regulated by growth hormone infusion and thyroid hormone (T4). The form 2C is positively regulated by androgens and pulsatile growth hormone while negatively regulated by continuous infusion of growth hormone.

P 123 VARIATIONS IN THE HEART LEVELS OF PREPROENKEPHALIN A MESSENGER RNA DURING THE PROGRESSION OF THE DISEASE IN CARDIOMYOPATHIC HAMSTERS.
Michel Ouellette, Kenneth Hastings* and Léa Brakier-Gingras, Département de Biochimie, Université de Montréal, Montréal H3C 3J7 and * Montreal Neurological Institute, McGill University, Montreal H3A 2B4, Québec, Canada.
Enkephalins are opioid peptides which participate in the regulation of myocardial function. We have previously shown (Biochem. Biophys. Res. Comm. 1988, 155, 449) that the preproenkephalin A gene, which codes for the precursor of the enkephalins, is transcribed in the atria and ventricles of the hamster heart. In this study, we have used *in situ* hybridization and Northern blot techniques with a preproenkephalin A cDNA probe to investigate the expression of the gene in the heart of an animal model of cardiac hypertrophy and congestive heart failure, the hamster with hereditary cardiomyopathy. Assays were carried out with the atria and the ventricles of cardiomyopathic hamsters at different phases of the disease (30, 60, 120 and 200 days) and with age-matched control hamsters. The levels of preproenkephalin A mRNA decrease in the atria of cardiomyopathic hamsters during the progression of the disease, the largest decrease (about 50%) being observed in the final phase of the disease. In contrast, in the ventricles of cardiomyopathic hamsters, the levels of preproenkephalin A mRNA increase about two- to three-fold in the necrotic phase (60 days) but are not significantly altered at the other phases of the disease. The possible relationship between these variations and the progression of the disease will be discussed.

P 124 ISOLATION AND CHARACTERIZATION OF cDNA AND GENOMIC CLONES ENCODING MURINE CARDIAC TROPONIN C. Michael S. Parmacek and Jeffrey M. Leiden. Howard Hughes Medical Institute and the Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan.
Cardiac troponin C (cTnC) is the calcium binding subunit of the thin filament which regulates excitation-contraction coupling in the heart. In order to better study the transcriptional regulation of this gene as well as to understand the structural basis of troponin C function, we have isolated and characterized the cTnC cDNA and gene. The murine cardiac troponin C cDNA was isolated directly from total cardiac RNA by the technique of polymerase chain reaction (PCR). The murine cTnC cDNA encodes a protein of 161 amino acids which has been highly conserved during evolution. Primer extension and S_1-nuclease analyses revealed that the start of cTnC transcription is located 35 base pairs 5' of the initiation codon. Southern blot analyses demonstrated that the cTnC gene is a member of a multi-gene family. Northern blot analyses showed that cTnC is expressed in both cardiac tissue and slow skeletal muscle (soleus), but is not expressed in fast skeletal muscle (EDL and anterior tibialis). A full-length cTnC genomic clone was identified and structurally characterized. The cTnC gene is approximately 3.5 kilobases in length and is composed of 6 exons and 5 introns which do not appear to separate the gene into functional domains, but instead interrupt the four previously described calcium binding loops. Analysis of the 5' flanking region of the gene revealed the presence of a consensus TATA box 30 bp 5' of the transcription start site. However, no consensus CCAAT, or muscle-specific CArG sequence elements were identified suggesting that this gene may be regulated in a manner which is distinct from other previously described myofibrillar genes.

P 125 AUTOLOGOUS COUNTERPULSATION USING SKELETAL MUSCLE: THE LINEAR CHARACTERISATION OF THE EARLY ADAPTATION OF MUSCLE PROTEIN GENE EXPRESSION, *C.W. Pattison, *D.V.E. Cumming, *C.A. Lovegrove, **D.G. Clayton-Jones, *M.J. Dunn, **A. Scutt, **G. Goldspink, *M.H. Yacoub. *Department of Cardiac Surgery, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, U.K. and **Royal Veterinary College, Royal College Street, London NW1 OTU, U.K.

The benefit of intravascular counterpulsation to the failing heart is well known and the use of autologous skeletal muscle to power auxilllary ventricles in diastole has been encouraging but beset by problems with haemorrhage, thrombus formation (and emboli). In this programme we have created an entirely autologous extra aortic counterpulsation system using latissimus dorsi in an animal model and so far have not encountered similar problems. The linear characterisation of the transformation from fast to slow fibre types upon chronic electrical stimulation was assessed at the molecular level, and the results are presented in conjunction with muscle flap blood flow data and the systemic haemodynamic effects of the counterpulsation system.

These results have allowed us to minimise the delay between operation and the initiation of electrical stimulation and maximise the early haemodynamic benefits of the system whilst avoiding fatigue which is fundmanetal if autologous counterpulsation is to be of clinical importance.

P 126 DIFFERENTIAL GENE EXPRESSION DURING CARDIAC GROWTH STIMULATED BY HYPOPHYSEAL HORMONES. Stanley A. Rubin. Division of Cardiology, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA.

To test the hypothesis that cardiac growth is regulated by gene expression, I studied the early differential gene expression in the heart of hypophysectomized (hypx) rats after hormone replacement by thyroid ($L\text{-}T_3$) and growth hormones. Two rounds of subtraction hybridization were carried out with poly $(A)^+$ RNA from hypx rat hearts and labeled cDNA from a hormonally stimulated hypx rat heart. The excluded fraction from hydroxylapatite chromatography was used to screen a small portion of a cDNA cardiac library in lambda phage made from a hormonally stimulated hypx rat. From a screening of 40,000 recombinants, positive hybridization signals were obtained from 306, and cored plaques were rescreened. Because $L\text{-}T_3$ is known to cause differential gene expression of myosin heavy chain in the hypx heart, positive plaques were screened to exclude alpha myosin heavy chain gene expression. I conclude that differential gene expression suggests that cardiac growth stimulated by hypophyseal hormones is regulated, and that growth factors and growth genes specific for cardiac growth may be identified by this process.

P 127 EXERCISE INDUCES ATRIAL NATRIURETIC PEPTIDE RELEASE FROM ENDOCARDIAL LAYER OF HYPERTROPHIC LEFT VENTRICLE IN GENETICALLY HYPERTENSIVE RATS. Heikki Ruskoaho, Päivi Kinnunen, Tarja Taskinen, Olli Vuolteenaho and Juhani Leppäluoto, Departments of Pharmacology and Toxicology and Physiology, University of Oulu, SF-90220 Oulu, Finland.

Ventricular hypertrophy is characterized by stimulation of ventricular synthesis of atrial natriuretic peptide (ANP). To examine the role of ventricular ANP levels in the secretion of ANP into the circulation, atrial and ventricular levels of immunoreactive-ANP (IR-ANP) as well as ANP messenger RNA (mRNA) were measured simultaneously in the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats at rest and after swimming. IR-ANP concentration in the left ventricle of one-year-old SHR with severe left ventricular hypertrophy was increased in association with the augmentation of ANP mRNA levels. A 30 min exercise in both strains resulted in marked increases in mean arterial pressure, heart rate, blood lactate and in plasma catecholamines and IR-ANP concentration. The increased ANP secretion was associated with a decrease both in left (34-39 %) and right (24 %) ventricular concentration of IR-ANP, transmurally this depletion of ventricular IR-ANP was greatest (28 %) in the endocardial layer of the left ventricle of SHR rats. When studied in vitro using an isolated perfused heart preparation, the hypertrophic ventricular tissue after atrialectomy secreted more ANP into the perfusate than control hearts; in SHR, ventricles contributed 28 % of the total ANP release to perfusate and 8 % in the normotensive control rats. These studies demonstrate that the ventricular source substantially contributes to the circulating level of ANP. The stimulated release of ANP depletes mainly endocardial left ventricular ANP stores and the amount released depends on the hypertrophic state of ventricular tissue.

P 128 REGIONAL DIFFERENCES IN IN VIVO MYOCARDIAL PROTEIN SYNTHESIS DURING POSTNATAL CARDIAC DEVELOPMENT, Allen M. Samarel and Melanie Alm, Department of Medicine, Loyola University Stritch School of Medicine, Maywood, IL 60153

The study of physiological regulators of contractile protein gene expression requires well-controlled experimental models. Therefore, we have examined in vivo rates of protein synthesis in the left ventricular (LV) and right ventricular (RV) free walls of 5 4-d old rabbits to quantitate differences in contractile protein expression in response to regional differences in hemodynamic load. Total protein fractional synthetic rates (Ks) were calculated from leucine specific radioactivity measurements of protein and cardiac leucyl-tRNA 10 min after tracer adiministration. Fractional rates of protein accumulation (Kg) and degradation (Kd) were determined as previously described (AM Samarel, Circulation Research, in press, 1989) (means±SD; *P < 0.05, paired t-test):

	RV	LV
Ks (%/d)	38.8±5.2	52.4±13.0 *
Kg (%/d)	8.0±1.2	10.9± 1.0 *
Kd (%/d)	30.8±6.1	41.5±13.8

The results indicate markedly accelerated rates of LV protein synthesis and growth during adaptation to postnatal hemodynamics. This regional difference in contractile protein expression can serve as a well-controlled experimental system in which to explore work-dependent alterations in myocardial gene expression.

P 129 STIMULATION OF PROTEIN SYNTHESIS BY CATECHOLAMINES IN CULTURED ADULT RAT HEART MYOCYTES. Jane-Lise Samuel,Isabelle Dubus, Francoise Marotte, Claude Delcayre, Lydie Rappaport,INSERM U127, Hopital Lariboisiere, 75010 Paris, France.

The effect of catecholamines on protein synthesis activity of adult myocardium was studied using the experimental model of isolated adult rat cardiac myocytes maintained in culture for 1 to 6 days. Quantitative and qualitative studies of protein synthesis were carried out by incorporation of radioactive amino acids (14-C-phenylalanine or 35-S-methionine) and by gel electrophoresis and autoradiography. After 3 days of culture, the majority of cells maintained their original rod-shape but the cell density decreased progressively (50% after day 3). The level of total protein synthesis and contractile proteins (myosin and actin) were decrease by 20% per mg total protein ($p<0.05$). The addition of isoproterenol or norepinephrin (10nM) to the medium for 3 days resulted in a global stimulation of protein synthesis (18%, $p<0.01$) that was specificaly inhibited by propanolol (10uM). The catecholamines had no effect on the synthesis of myosin and actin. These findings demonstrate that low doses of β-adrenergic agonists have a growth stimulatory effect on adult cardiac myocytes but the regulation of synthesis of myofibrillar proteins appears to depend mainly on other factors such as contractile activity.

P 130 CIS-ACTING ELEMENTS INVOLVED IN TRANSCRIPTIONAL CONTROL OF THE CARDIAC β/SLOW SKELETAL MYOSIN HEAVY CHAIN GENE, Noriko Shimizu, Courtland E. Yockey, Evelyn Dizon, Gwen Prior, Leanne L. Cribbs, *Patrick K. Umeda and Radovan Zak, University of Chicago, Chicago, IL 60637 and *University of Alabama, Birmingham, AL 35294.

The cardiac myosin heavy chain (HC) β gene is expressed in developing skeletal muscles and mammalian slow-twitch fibers. To study the mechanisms controlling transcription of myosin HC gene during muscle differentiation, the 5'flanking portion of the rabbit myosin HC β gene was fused to the chloramphenicol acetyltransferase gene and transfected into primary chick skeletal muscle cultures. Analysis of 5'deletion mutants indicated that muscle-specific activation is mediated by cis-acting regulatory elements within 300 bps of the cap site. Within this region, linker-scanner mutants identified at least three regions nesessary for transcription. The first lies between -275 and -261 (A), the second is near the CAAT box of the gene (B) and the third lies between CAAT and TATA boxes. Each element had little effect on the heterologous HSV thymidine kinase (TK) promoter activity in muscle cells. However, both A and B elements were essential for strong activation of HSV TK promoter in a tissue- and stage-specific manner.

P 131 DIFFERENTIAL REGULATION OF MYOSIN GENE EXPRESSION IN ATRIA AND VENTRICLES DURING CARDIOGENESIS IN THE RAT, Lauren J. Sweeney and Susan W. Kelley, Department of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153

The tissue-specific expression of different myosin heavy chain (HC) isoforms has been well documented in the neonatal and adult mammalian heart, as has the correlation of this expression with myocardial contractility. However, there is little information on this expression during fetal cardiogenesis in any mammal. We have therefore investigated myosin HC expression in the fetal Sprague-Dawley rat heart, identifying the HCa and HCb isoforms in each cardiac chamber by electrophoresis on 5% SDS-PAGE gels. The expression of HCa was then localized by immunofluorescence with a specific monoclonal antibody (R 37). Results showed that atrial myocytes expressed HCa throughout pre- and post-natal development, while the ventricles showed a striking developmental modulation of HC expression. Ventriculocytes expressed the faster-migrating HCb at all stages, as well as a lesser amount of HCa, which was confined to endocardial-surface trabeculae during septation (13-15 days gestation). By 21 days, this HCa population was dramatically reduced, and a small diffuse population of epicardial HCa-expressing cells was seen. These results indicate that localization of myosin isoform expression is crucial for any assessment of its significance during cardiogenesis. (Supported by American Heart Grant-in-Aid to LJS.)

P 132 IDENTIFICATION AND CHARACTERIZATION OF HUMAN CARDIAC PORIN: A VOLTAGE-DEPENDENT CHANNEL INVOLVED IN ATP MOVEMENT ACROSS THE OUTER MITOCHONDRIAL MEMBRANE, Jeffrey A. Towbin[1], Mikeanne Minter[1], Dieter Brdiczka[2], Volker Adams[2], Ferdinando Palmieri[3], and Edward R.B. McCabe[1], [1]Baylor College of Medicine, Houston, TX, USA 77030, [2]University of Konstanz, Konstanz, FRG, [3]Universita Di Bari, 70125 Bari, Italy.

The porins are voltage-dependent, anion-selective, channel-forming proteins located in the outer mitochondrial membrane which are responsible for passage of ATP, as well as for specific binding of hexokinase and glycerol kinase. This porin-kinase complex has direct access to ATP generated by mitochondrial oxidative phosphorylation and may be important in the regulation of glycolysis. Porin had not been described previously in humans but would be expected in organs requiring a large and constant supply of energy. We therefore postulated that porin would occur in human myocardium. Monospecific, polyclonal antibodies to bovine myocardial and rat liver porins were used for immunoblotting after polyacrylamide gel electrophoresis of human heart homogenates from atria, ventricles, papillary muscles, and interventricular septum. These immunoblots demonstrated selective staining of a 34 kD band in each homogenate, identical to that with purified rat liver porin. Also notable was the finding that the vast majority of this staining was found in the pellet after high speed centrifugation (20,000 g) of the homogenate, as would be expected for a mitochondrial protein. The demonstration of human cardiac porin by immunoblotting with rat liver and bovine myocardial porin antibodies is the first demonstration of cross-species identification of the porins. The success of this approach indicates strong homology between porins from a variety of species.

P 133 CHARACTERIZATION OF AN ENHANCER ELEMENT IN THE 5′ FLANKING DNA OF THE HUMAN M CREATINE KINASE GENE. Robert V. Trask, and Joseph J. Billadello, Cardiovascular Division, Washington University School of Medicine, St. Louis, MO 63110

The muscle creatine kinase (MCK) gene encodes a dimeric protein essential to cellular energy metabolism. Expression of the MCK gene is tissue-specific and developmentally regulated. To determine the mechanisms regulating MCK gene expression we have cloned the human MCK gene and constructed chimeric genes containing 2620 base pairs of MCK 5′-flanking DNA upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene in pSVOCAT. A 17.5-fold induction of CAT activity was seen in transfected C_2C_{12} myoblasts in culture during differentiation. Using promoter deletion analysis we have determined that sequences between -935 and -641 base pairs upstream of the MCK cap site confer developmental expression in transfected C_2C_{12} myoblasts. This essential MCK regulatory element was placed in the sense and antisense orientations in the Bam HI site 3′ of the CAT gene in an inactive MCK-CAT fusion construct containing only basal MCK promoter elements. A 7.7 (sense) and 10.7 (antisense) fold increase in CAT activity was observed in transfected C_2C_{12} myoblasts during differentiation, but not in COS-7 or NIH/3T3 cells. The same regulatory sequences also confered tissue-specific and developmental regulation to a heterologous SV40 promoter-CAT gene construct (pUC19pCAT). In a gel shift assay, nuclear proteins from extracts isolated from C_2C_{12} myotubes bind specifically to this MCK cis-acting regulatory sequence. Thus, we have identified a tissue-specific and developmentally-active enhancer vital to the expression of the human MCK gene which presumably is regulated by a nuclear DNA-binding protein expressed in muscle cells.

P 134 MULTIPLE REGULATORY ELEMENTS IN HUMAN α-MYOSIN HEAVY CHAIN PROMOTER, Richard W. Tsika, Joseph E. Bahl, Irwin L. Flink, Leslie A. Leinwand and Eugene Morkin, Albert Einstein College of Medicine, Bronx, NY 10461 and University of Arizona College of Medicine, Tucson, AZ 85724

The rat α-myosin heavy chain (α-MHC) gene is regulated by 3,5,3'-triiodo-l-thyronine (T_3). Because of conservation of sequences in the 5' flanking region between the rat and human α-MHC genes, T_3 responsiveness and tissue specific expression of the human α-MHC gene was studied in rat fetal heart cells and L_6E_9 using deletion mutants of the human promoter region fused to the CAT gene. In heart cells, four cis-elements were identified: 1) a basal level element (BLE) containing CAAT and TATAA sequences; 2) a proximal strong positive element (SPE) that was expressed constitutively; 3) a proximal T_3 responsive element(s) (TRE); 4) a more distal negative element (NE) that abolished constitutive expression but preserved T_3-inducibility. In L_6E_9 myoblasts and myotubes, BLE, SPE and NE were active, but TRE was inactive. Thus, activation of combinations of these cis-element may be sufficient to explain hormonal and tissue-specific control of the human α-MHC gene.

P 135 **STRETCH-INDUCED MUSCLE PROTEIN SYNTHESIS AND PROSTAGLANDIN EFFLUX IN VITRO.** Herman H. Vandenburgh, Sophia Hatfaludy, and Janet Shansky, Department of Pathology, Brown University and The Miriam Hospital, Providence, RI 02906.

Repetitive 20% stretch/relaxations of tissue cultured avian skeletal muscle cells for 60 s every 30 min stimulates muscle hypertrophy. Protein synthesis rates significantly increase 28 to 42% during the first 5 h of mechanical activity. The efflux of prostaglandins $PGF_{2\alpha}$ and PGE_2 are increased 59% and 97%, respectively, during this time period while the efflux of 6-keto-$PGF_{1\alpha}$ is unaltered. Increasing the frequency of stimulation by decreasing rest period lengths from 30 min to 5 min increases protein synthesis rates by 63% and efflux rates of $PGF_{2\alpha}$ and PGE_2 by 86% and 73%, respectively. Rest periods of less than 5 min progressively decrease the elevated protein synthesis rates and prostaglandin efflux. Long-term mechanical stimulation of the cells for 48 h (30 min rest periods) causes continued elevation of $PGF_{2\alpha}$ efflux (110%) and protein synthesis (97%) but PGE_2 efflux returns to control levels by 24 hr. Stretch-induced protein synthesis rates in cultured skeletal muscle thus correlate with $PGF_{2\alpha}$ efflux. Addition of exogenous $PGF_{2\alpha}$ to control muscle cells significantly stimulates protein synthesis. $PGF_{2\alpha}$ may serve as a second messenger in the transduction of mechanical stimuli into the biochemical alterations associated with muscle hypertrophy.

P 136 A 100 kD NUCLEAR PROTEIN FROM C2 MYOTUBES BINDS SPECIFICALLY TO A MUSCLE-SPECIFIC ENHANCER-LIKE ELEMENT FROM THE HUMAN MYOGLOBIN GENE. Franklin C. Wefald, William E. Kraus, and R. Sanders Williams. Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, NC 27710

We have previously identified an enhancer-like, upstream regulatory element (URE) within the 5' flanking region of the human myoglobin (Mb) gene that is necessary for high-level, muscle-specific transcription of heterologous reporter genes under the control of the myoglobin core promoter. Using cross-competition experiments in gel mobility shift assays, and DNAse I footprinting, we observed that C2 myotubes contain a nuclear protein factor that binds specifically to an 18 bp pyrimidine-rich sequence located within the functional MbURE. Nuclear protein extracts were enriched in this binding factor by heparin-agarose and DEAE-agarose chromotography, and renatured Western blots were probed with a synthetic oligonucleotide containing multiple copies of the 18 bp binding motif. This probe bound to a single polypeptide of 100 kD that we term MbBF1. We used the same probe to screen a cDNA expression library prepared in lamda gt11 from mRNA extracted from C2 myotubes. From 22 positive plaques identified on the initial screen, 3 clones survived two additional screens, and will be characterized further by sequencing and by analysis of the recombinant protein in gel shift and footprinting assays. Although MbBF1 binds to a muscle-specific transcriptional regulatory element, the expression of MbBF1 does not appear to be limited to differentiated myotubes: the protein is present in myoblasts and non-muscle cells as well. In addition to MbBF1, adult striated muscles express an apparently different protein (MbBF2), that has identical binding specificity to MbBF1 (DNAse I footprinting), but exhibits a different mobility when bound to DNA probes containing the MbURE. These findings will be discussed in light of current models of cell-specific gene expression in eukaryotes.

P 137 REGULATION OF CARDIAC SARCOPLASMIC RETICULUM Ca^{2+}-ATPase GENE EXPRESSION. A. Zarain-Herzberg[+], J. Lytton[*], O. Saitoh[+], D.H. MacLennan[*], N.R. Alpert[+], and M. Periasamy[+]. [+]Dept of Physiology and Biophysics, Univ. of Vermont, Burlington, VT 05405, [*]C.H. Best Institute, 112 College St., Univ. of Toronto, Ontario, Canada M5G 1L6.

The cardiac sarcoplasmic reticulum (SR) Ca^{2+} ATPase gene encodes two different isoforms: (1) the cardiac/slow-twitch SR Ca^{2+} ATPase and (2) the smooth/non-muscle sarco(endo)plasmic Ca^{2+} ATPase. We tested the hypothesis that the cardiac SR Ca^{2+} ATPase gene is expressed in a tissue specific manner by performing S1 nuclease mapping using the cloned rabbit slow-twitch/cardiac Ca^{2+} ATPase cDNA and the smooth muscle/non-muscle Ca^{2+} ATPase rabbit cDNA probes. S1 nuclease mapping of total cellular RNA from a variety of tissues demonstrated that cardiac muscle expressed the slow-twitch/cardiac isoform almost exclusively, whereas smooth muscle and non-muscle tissues expressed the alternatively spliced smooth/non-muscle isoform almost exclusively, indicating that the alternative splicing of the cardiac Ca^{2+} ATPase gene is regulated in a tissue specific fashion.

In order to understand the mechanism(s) regulating the tissue specific cardiac SR Ca^{2+} ATPase gene expression, we isolated rabbit Ca^{2+} ATPase genomic clones and analyzed the structure of the gene. S1 nuclease mapping and primer extension analysis of the 5' end of the cardiac SR Ca^{2+} ATPase mRNA indicated that a single transcriptional initiation site is located 524 bp upstream of the translation initiation codon. The promoter and 5' flanking regions were sequenced and found to have a "TATA" box at -28 bp, and a "CCAAT" box at -83 bp, and several GC rich elements. To characterize transcriptional regulatory sequences at the 5' end of the gene we are in the process of making deletion constructs using the CAT reporter gene and introducing them into myogenic and non-myogenic cell lines.

Growth Factors; Atherosclerosis and Angiogenesis

P 200 CHANGES IN NA^+ PUMP EXPRESSION DURING PROLIFERATION OF CANINE VASCULAR SMOOTH MUSCLE CELLS (VSMC) IN PRIMARY CULTURE, Julius C. Allen, Charles L. Seidel, and Stephen S. Navran. Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

VSMC were isolated by enzyme dispersion and grown in fetal bovine serum (FBS). Various manifestations of functional expression of the Na pump were studied at times ranging from freshly dispersed cells to confluence (~6 days). At one day there was a large increase in cytoplasmic Na (Na_i) followed by an increase in Na pump expression (^{3}H-ouabain binding) at 2 days. The increased pump density effected a decrease in Na_i, which was associated with the initiation of cell proliferation. ^{86}Rb uptake studies at comparable times demonstrated that the increased pump sites were fully functional.

When amiloride was present throughout (i.e. day 0 to confluence) cell proliferation was delayed, but only minimal effects were observed on both Na_i and Na pump expression.

If VSMC are plated in FCS with low (~1mM) K compared to normal K (6.6mM) both the increase in pump density and cell proliferation are accelerated.

These data suggest that FCS induces Na influx into VSMC by at least two different pathways, one of which is amiloride sensitive. The amiloride sensitive pathway is related to cell proliferation. The amiloride insensitive pathway induces the increase in Na pump density which is necessary to decrease the large mitogenic-induced increase in Na_i. This change in Na_i, coupled with other factors, may be required to initiate cell proliferation. Proliferation may be enhanced in a low K^+ pump inhibited medium because the pump site number increases faster and the cells are able to reduce the high Na_i more effectively.HL34280

P 201 TUMOR NECROSIS FACTOR IN HUMAN ATHEROMA: DETECTION WITH IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION. Peter Barath, Michael Fishbein, James S. Forrester, Richard Helfant, Aldon Lusis. Cedars-Sinai Medical Center and UCLA, Los Angeles, CA 90048.
Since several effects of tumor necrosis factor (TNF) such as neovascularization, altered procoagulant activity and hemorrhagic necrosis are parts of atheroma evolution, we hypothesized that TNF is present in human atheroma. We developed the first immunohistochemical method for detection of TNF using anti rhTNF alpha monoclonal antibody and peroxidase labelling. There were 6 normal artery sections, 11 artery sections with intimal thickening (int. thick.) and 65 sections with atherosclerotic lesions. In addition, we performed in situ hybridization (ISH) in 10 fresh atherosclerotic vessels. Paraffin embedded sections were hybridized with 32P labelled TNF cDNA probe. Results: 1. By IHC TNF was present not only in monocytes (MO) but also in smooth muscle cells (SMC) and endothelial cells (EC):

TNF (+) cells	Normal		Int. Thick.		Ath.
SMC	0/6	*	3/11 (27%)	*	33/65 (61%)
EC	0/6	*	2/11 (18%)		12/65 (18%)
MO	0/6		0/11 (0%)	*	31/65 (47%)*significant difference

2. By ISH, expression of the TNF gene was found in the intimal SMCs which were positive by IHC. Conclusion: 1. Immunoreactive TNF and TNF mRNA is present in the mesenchymal cells (MO, EC and SMC) of the atherosclerotic human arteries. 2. The frequency of TNF positivity increases as the severity of the artherosclerotic lesion increases. 3. TNF may have a role in atheroma evolution.

P 202 ENHANCED PROLIFERATION OF AORTIC SMOOTH MUSCLE CELLS FROM SHR: ROLE OF PLC ACTIVATION AND cFOS EXPRESSION.Maryvonne Baudouin-Legros Jean-Luc Paquet and Philippe Meyer.INSERM U7,Hopital Necker,75015 PARIS.When stimulated by foetal calf serum (10%), aortic smooth muscle cells isolated from spontaneously hypertensive rats(SHR) proliferate quicker and to a larger extent than control cells obtained from normotensive animals (WKY). Serum-induced PLC activation (determined by measuring inositol phosphates formation) and c-fos and c-myc expression are significantly more important in these SHR cells than in WKY ones: these molecular mechanisms therefore appear to be involved in the enhancement of the cell proliferation. However vaso-active drugs such as angiotensin II also stimulate PLC activation and oncogenes expression in the absence of FCS, when they cannot induce any mitogenic action, and are much more active than serum on these molecular reactions. For both parameters, the responses of SHR cells are significantly higher than the WKY ones.These results therefore suggest that PLC activation and c-fos and c-myc expression are not sufficient to induce vascular smooth muscle cell proliferation and that their enhancement in SHR cells is not the only mechanism involved in the impaired growth of these cultures.

P 203 **MOLECULAR CHARACTERIZATION OF ANIMAL MODELS FOR ARTHEROSCLEROSIS, Virginia Benson-Chanda, Harry R. Davis*, Deborah Mullins*, Satwant Narula, Department of Biotechnology-Molecular Biology and *Department of Pharmacology, Schering Research, 60 Orange Street, Bloomfield, NJ 07003. The diet induced hyperlipidemic and ballon-catheter-induced injury rabbit models are widely used to study various aspects of artherosclerosis. Our studies have been directed at following the molecular events from the early onset stage to the stage where the disease is full-blown resulting in lesions. We have followed the transcriptional expression of PDGF A and B chain to delineate their differential and/or aberrant expression, if any, during the course of this disease. Additionally, we have also probed for other implicated factors such as TNF, IL1, GM-CSF, gamma interferon, smooth muscle specific proteins, etc. The results of these studies will be correlated with the histological data to delineate the course of events in the above two models. The implications of these results will be discussed in the context of the known pathology of human disease.**

P 204 ONCOSTATIN M AS A UNIQUE MODULATOR OF ENDOTHELIAL CELL SURFACE PROPERTIES, T. Brown[*], R. Heimark[+], and P. Gladstone[*], Oncogen[*]/University of Washington[+], Seattle, WA 98121/98195.

Inflammatory cytokines such as IFN-γ, TNF-α, IL-1 and TGF-β have been shown to modulate endothelial cell (EC) surface properties with respect to immunologic function and fibrinolytic activity. A structurally unique cytokine, termed Oncostatin M, originally isolated from activated human T lymphocytes and monocytoid cells has recently been expressed as a recombinant protein (rOnco M) and found to differentially alter EC surface properties. EC demonstrated binding of [^{125}I]-rOnco M in a manner consistent with the presence of high affinity cell surface receptors specific for Onco M (Kd = 8 pM). rOnco M modulated the expression of both class I (HLA-A,B) and class II (HLA-DR) major histocompatibility complex (MHC) molecules on EC. Induction of class I and class II by rIFN-γ and class I induction by rTNF-α was inhibited 40-100% by co-treatment with rOnco M. By contrast, rOnco M was ineffective in modulating class II MHC expression on monocytes indicating a target tissue specificity for Onco M. rOnco M also elicited time- and dose-dependent alterations in the fibrinolytic status of the EC surface as measured by a > 3-fold increase in membrane-bound plasminogen activator (PA) activity. Conversely, equimolar concentrations of rTNF-α, IL-1 and rTGF-β decreased basal PA activity. Current concepts point to an inflammatory/immune component in the pathogenesis of atheroma. These findings suggest that Onco M action at sites of inflammatory/immune reactions may contribute in a distinctive manner to a decreased EC involvement in antigen presentation and increased EC involvement in fibrinolysis.

P 205 IDENTIFICATION OF PDGF B CHAIN mRNA IN PURIFIED HUMAN MEGAKARYOCYTES, Martin J. Carrier*, Ann-Marie Gladwin, Rosalia Lelchuk and John F. Martin, Department of Experimental Immunobiology* and Cardiovascular Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, U.K.
Human megakaryocytes (Mks) are large polyploid cells which fragment, in an as yet unknown mechanism, to produce platelets. These in turn contain large amounts of platelet-derived growth factor (PDGF). This is believed to be involved in the pathology of atherosclerosis. To date it has not been possible to identify PDGF mRNA in human Mks. We will present data which demonstrates the presence of PDGF B chain mRNA in these cells, using a c-sis riboprobe specific for this chain. Human Mks were purified from samples of fresh costal bone marrow using magnetic particles coated with an anti-platelet/Mk antibody (Plt-1). mRNA was then spotted onto nitrocellulose and dot/blot hybridisation was carried out.

Preliminary results indicate that mRNA for PDGF B chain is clearly identifiable, in as few as 50000 cells. We are currently quantitating the level of PDGF mRNA in Mks utilising both dot/blot and in situ hybridisation.

P 206 TGF-BETA 1 AND BASIC FGF IN ACUTE MYOCARDIAL INFARCTION, Ward Casscells, Edith Speir, Fernando Bazoberry, Michio Chiba, Nancy Thompson, Kathy Flanders, Michael Sporn, Victor J. Ferrans, and Stephen E. Epstein, Cardiology and Pathology Branches, NHLBI and Laboratory of Chemoprevention, NCI, NIH, Bethesda, MD 20892.
Little is known of the roles of TGF-beta 1 and bFGF in vivo. In normal adult rat hearts we found specific TGF-beta 1 immunoreactivity in cardiac myocytes, with faint stain in endothelial and smooth muscle cells. Immunoreactive bFGF localized to myocytes and endothelial cells. Endothelial labeling index was 0.2%. From 1-24h after coronary ligation, bFGF and TGF-beta 1 staining disappears from necrotic cardiac myocytes. Loss or masking of the N-terminal epitopes cannot be excluded, but true loss of TGF-beta 1-mediated inhibition may permit the neovascularization (endothelial sprouting and 3HTdR uptake) that began at 24h. Release of TGF-beta 1 may be chemotactic for neutrophils and macrophages, seen by 24h (and stained for TGF-beta 1). From 6-48h, coincident with early angiogenesis, fibroplasia, and compensatory myocyte hypertrophy, TGF-beta 1 mRNA and immunoreactivity increased in viable border zone myocytes, endothelial and smooth muscle cells. In contrast to tumor angiogenesis, mast cells were not numerous. By 24-72h bFGF staining was intense in capillaries entering the wound. TGF-beta 1 and bFGF may be involved in the healing, angiogenesis and compensatory hypertrophy of myocardial infarction.

P 207 INDUCTION OF IGF-I GENE EXPRESSION IN RAT AORTA FOLLOWING BALLOON DENUDATION, Bojan Cercek, James S. Forrester, Richard H. Helfant and James A. Fagin, Divisions of Cardiology and Endocrinology, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, California 90048.
Intimal smooth muscle cell proliferation (SMC) dominates the early phase of healing after arterial endothelial injury. Insulin-like growth factor-I (IGF-I) is believed to be an important paracrine regulator of SMC growth in cell cultures. In this study we investigated whether IGF-I gene expression is modulated after arterial injury in vivo. Balloon denudation of rat aortae was performed with an embolectomy catheter introduced through the left iliac artery. Sham operation consisted of laparatomy only. Animals were sacrificed 1, 3 and 7 days after intervention. IGF-I mRNA content was measured by a solution hybridization/RNase protection assay using a riboprobe which differentially protects the two variant mRNA's, IGF-Ia and IGF-Ib, which arise by alternative splicing of the C-terminal E peptide fragment of the gene.
IGF-I mRNA content was (fold induction over control, mean$\pm$SEM) 2.3$\pm$.55 (Day 1) 5.1$\pm$.85 (Day 3) and 9.2$\pm$3.2 (Day 7). IGF-I mRNA content in the sham operated animals remained unchanged, 1.6$\pm$.3 (Day 1), 1.6$\pm$.25 (Day 3) and 1.1$\pm$.03 (Day 7) after sham operation. IGF-Ia and IGF-Ib mRNA's were regulated coordinately, as IGF-Ia/IGF-Ib ratio was 6.3 in the control animals, 8.0 (Day 1), 9.1 (Day 3) and 7.7 (Day 7) after denudation, respectively. Conclusion: In rats, aortic IGF-I mRNA content progressively increases during the first 7 days after balloon denudation and 3' post-transcriptional alternative mRNA splicing appears not to be a regulated event. These findings suggest that IGF-I may be an important paracrine regulator of in vivo SMC proliferation after arterial wall injury.

P 208 **RELEASE OF SMOOTH MUSCLE CELL MITOGENS FROM ARTERIES AFTER BALLOON ANGIOPLASTY.** P. Macke Consigny and Glenda Bilder. Department of Radiology, Thomas Jefferson University and Rorer Pharmaceutical Co., Philadelphia, PA, 19107.

The purpose of this study was to determine if the smooth muscle cell (SMC) proliferation that causes restenosis after angioplasty is due to the release of SMC mitogens from cells within the arterial wall. Balloon angioplasty was performed on one iliac artery in each of 13 rabbits. In one series of experiments (n=3), the dilated and contralateral control arteries were removed 4 days after angioplasty. Each artery was placed in co-culture (Millicell insert) with quiescent rabbit SMC obtained from aortic explants. After 4 days of co-culture, the change in SMC number was determined. When SMC were cultured in 5% platelet-deficient plasma-derived serum (PDS) alone or with a control artery, SMC number increased 2% ± 10 and decreased 10% ± 3, respectively. In contrast, when SMC in 5% PDS were co-cultured with a dilated artery, SMC number increased 93% ± 18, twice the growth of SMC cultured alone in 10% calf serum. In other experiments, control and dilated iliac arteries were removed from 10 rabbits, the intima was denuded of endothelium, and the RNA was extracted. The mRNA for $PDGF_B$ was identified by Northern hybridization using a v-cis probe. mRNA for $PDGF_B$ was found in every artery dilated 4 days before removal (n=8), but none was found in arteries dilated 4 hours before removal (n=2). The mRNA for $PDGF_B$ was found in only 1 of 10 control arteries. We conclude that angioplasty induces the release of factor(s) from the arterial wall that stimulate SMC proliferation and that one of the factor(s) is most likely $PDGF_B$.

P 209 TRANSFORMING GROWTH FACTOR- BETA (TGF-β_1) AND NEONATAL HEART DEVELOPMENT. G.L. Engelmann[a], K.D. Boehm[b],and C. Tannenbaum[a], Cleveland Clinic Foundation[a], and Case Western Reserve University[b], Cleveland, Ohio 44195-5069

Neonatal ventricular myocyte development is characterized by rapid terminal proliferative events and initiation of cellular maturation that yield a finite number of post-mitotic cardiomyocytes adaptive to hypertrophic growth stimuli. TGF-ß peptides are potent regulatory molecules that have been shown to influence proliferation and differentiation of many cell types. We have found, by Northern/slot blot analysis and localization with *in-situ* hybridization studies to cardiomyocytes, that TGF-β_1 transcripts are abundantly expressed in neonatal ventricular tissue; particularly in pups genetically predisposed to cardiac hypertrophy. The time course of TGF-β_1 gene expression is inversely related to the cessation of myocyte proliferation, between days 7-14, and suggests a possible causal relationship. Tissue immunofluorescent studies have co-localized TGF-ß peptide and gene transcript to the developing myocyte. In vitro studies showed that insulin-like growth factor (IGF) stimulation of protein and DNA synthesis (10-50ng/ml) is blunted by TGF-ß (0.5-1ng/ml). Further studies have shown that total cellular RNA content of cultured myocytes is elevated 2.3-fold by IGF-I and this effect is inhibited nearly 80% by TGF-ß. Preliminary analysis by nuclear run-on experiments of specific gene transcripts affected by TGF-ß treatment concurrent with IGF-I stimulation suggest that selective inhibition of cell cycle gene transcripts (i.e., ODC, KC and JE) occur; while muscle specific (i.e., myosin heavy chain) gene expression was unaffected. Our data suggest that locally produced TGF-ßs may influence, in an autocrine manner, neonatal heart development. Supported by Diabetes Assoc. Greater Cleveland and NIH HL42218-01 (GLE).

P 210 PLATELET-DERIVED GROWTH FACTOR (PDGF)-AA HOMODIMER IS A WEAK VASCULAR SMOOTH MUSCLE CELL (VSMC) MITOGEN, Gary H. Gibbons, Richard E. Pratt, and Victor J. Dzau, Molecular and Cellular Vascular Research Laboratory, Brigham and Women's Hospital, Boston, MA 02115.

VSMCs produce a variety of autocrine growth factors. Increased levels of PDGF A chain mRNA are expressed in VSMCs within atherosclerotic plaques and in vitro. We have reported that angiotensin II induced VSMC hypertrophy is associated with increased levels of PDGF A chain but not B chain mRNA and increased secretion of a PDGF-like peptide. However, the role of autocrine production of PDGF-AA in the regulation of VSMC growth is unknown. We studied the effect of recombinant PDGF-AA on confluent, quiescent rat aortic VSMCs in defined serum-free medium. Although PDGF-BB at 20 ng/ml stimulated DNA synthesis 11 fold, 10 fold higher concentrations of PDGF-AA induced only a 40% increase. PDGF-AA stimulated a dose-dependent increase in RNA synthesis with a threshold dose of 200 ng/ml (control uridine incorporation = 1657 ± 10 cpm/well, 100 ng/ml = 1777 ± 69, 200 ng/ml = 2005 ± 49, 500 ng/ml = 2497 ± 92) ($p < .05$). We conclude that PDGF-AA is a relatively weak mitogen of VSMC but may promote hypertrophy.

P 211 BASIC FGF REGULATION OF A Ca^{2+}-DEPENDENT CELL-CELL ADHESION MOLECULE IN ENDOTHELIUM, Ronald L. Heimark and Stephen M. Schwartz, Department of Pathology, University of Washington, Seattle, WA 98195
Intercellular adhesion is likely to play essential roles in the assembly and integrity of embryonic and adult blood vessels. Confluent cultures of fetal aortic endothelial cells demonstrate both Ca^{2+}-dependent and Ca^{2+}-independent adhesion mechanisms. A hybridoma clone was isolated, producing a monoclonal antibody, Ec6C10, that inhibits Ca^{2+}-dependent adhesion in endothelium. There was no inhibition of Ca^{2+}-independent adhesion of endothelial cells or Ca^{2+}-dependent adhesion of smooth muscle cells. Analysis by Western blotting shows that the MAb Ec6C10 recognizes a protein with an apparent molecular weight of 135,000 in endothelium but was not found in uvomorulin-containing cells. MAb Ec6C10 inhibits reorganization of peripheral microfilaments in confluent endothelial cells in a Ca^{2+}-switch assay. Subconfluent growing endothelial cells show a diffuse immunofluorescent staining pattern. As the cells become confluent, the staining is concentrated at sites of cell-cell contact. This pattern resembles that of monolayers after silver staining of cell borders. The properties of this protein are like that of the cadherins of epithelial cells and we suggest it be called V-cadherin. Addition of basic FGF to confluent endothelial cells induces a time-dependent decrease of V-cadherin as shown by quantitative Western blotting, in addition to loss of the peripheral band of microfilaments. Replication is stimulated 20 hours after basic FGF addition and saturation density is increased 2-3 fold. These observations are consistent with early responses in angiogenesis, showing a loss of junctional contact. (Supported by NIH grant HL-03174.)

P 212 BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN THE HEART : LOCALIZATION BY IMMUNOFLUORESCENCE, Elissavet Kardami, Robert R. Fandrich, Lei Liu and Raymond R. Padua. Division of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, University of Manitoba, Winnipeg, Manitoba, Canada. Extracts from cardiac tissue are highly mitogenic for a variety of cell types due largely to the presence of basic fibroblast growth factor (bFGF); concentration of this factor is highest in extracts from atrial tissue. Examination of frozen cardiac tissue sections (rat, bovine) by immunofluorescence, using affinity purified polyclonal antibodies against the (1-24) N-terminal peptide of bovine bFGF shows that muscle fibers clearly accumulate bFGF in their basal laminae, their nuclear membranes and in the intercalated disk areas. Atrial fibers show heavier pericellular bFGF accumulation. Large blood vessels, capillaries and small groups of non muscle connective tissue cells exhibit very strong reactivity. Finally, small, non-muscle, multicellular structures of gland-like morphology, encased between the muscle fibers, sparcely distributed in the bovine ventricular myocardium, stain the brightest with the anti-bFGF antibodies. The observed bFGF distribution conforms largely with the notion that this growth factor is stored in heparin or heparan sulphate rich areas. No qualitative differences in its pattern of localization were established between atria and ventricles. The higher bFGF content of the atrial extracts could be explained by a heavier contribution from capillaries and blood vessels as well as more extensive endomysium and connective tissue in the atria. bFGF is a powerfull mitogen, thought to be involved in tissue vascularization and regeneration but also in mesoderm formation during embryogenesis. Its cellular distribution within the myofibers is intriguing, suggesting an intimate involvement of bFGF with cardiac myofibrillar function.

P 213 LOCALIZATION OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR LIGAND BINDING DOMAIN. MT Keating and LT Williams, Cardiovascular Research Institute, Howard Hughes Medical Institute, University of California, San Francisco, California The amino acid sequences of the platelet-derived growth factor (PDGF) receptor required for ligand binding are unknown, but presumably lie in the receptor's extracellular domain (residues 1-499). Trypsin-treatment of fibroblasts at $4^{o}C$ reduced the apparent molecular weight of immunoprecipitated PDGF receptors from 180 kDa to two forms of 100 and 90 kDa. These fragments were immunoprecipitated by an antiserum that was directed against intracellular receptor residues 934-951, but not by an antiserum directed against extracellular residues 425-446. Thus, trypsin removed at least 85% of the receptor's extracellular domain. Nevertheless, PDGF still induced tyrosine phosphorylation of the 100 kDa, but not the 90 kDa, receptor fragment in ^{32}P-labeled, trypsin-treated Balb/c 3T3 cells. Receptor fragment phosphorylation was induced by all three forms of PDGF, AA and BB homodimers and AB heterodimers. PDGF also induced transcription of the c-fos protooncogene in trypsin-treated cells. These effects were not due to the activation of full length PDGF receptors since no 180 kDa protein was detected by immunoprecipitation or immunoblot. Thus, PDGF receptors lacking residues 1-446 are functionally intact. These findings suggest that one PDGF binding domain resides within receptor residues 446-499.

P 214 REGULATION OF TYPE-1 PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) IN HUMAN CELL LINES BY HORMONES, GROWTH FACTORS AND CYTOKINES, L.R.Lund(1), A.Riccio(2), S.N.Stacey(1), M.Mayer(1,3), B.Georg(1,3), K.Danø(1), F.Blasi(2), and P.A.Andreasen(3). (1)Finsen Laboratory, Rigshospitalet, Denmark, (2)Institute of Microbiology, University of Copenhagen, Denmark, (3)Institute of Biochemistry C, University of Copenhagen, Denmark.

We have performed a comparative study of the regulation by glucocorticoids, transforming growth factor-beta, tumor necrosis factor-alpha, phorbol 12-myristate 13-acetate (PMA) of the production of type-1 plasminogen activator inhibitor (PAI-1) by a variety of human cell lines. Basal PAI-1 accumulation varied more than 5000 fold between the cell lines. The various cells responded differently to each factor and there was no correlation between the responsiveness and the basal PAI-1 production. PMA-induced differentiation of the monocyte cell line U937 and the promyelocytic cell line HL-60 into macrophage-like cells was found to be accompanied by an up to 100-fold increase in PAI-1 accumulation. The PMA-dependent differentiation of HL-60 cells led to acquisition of glucocorticoid inducibility of PAI-1, which is not present in non-adherent cells.

Cycloheximide was unable to inhibit any of the inductions of PAI-1 mRNA, but in some cell lines cycloheximide on its own caused a strong induction of PAI-1 mRNA. When fused to the heterologous reporter gene chloramphenicol acetyl transferase and transfected into responsive cell lines, the PAI-1 5' flanking region is able to confer phorbol ester, transforming growth factor-beta and glucocorticoid responsiveness to the reporter gene. The analysis of the responses of deletion mutants together with DNAse I footprinting and gel mobility shift experiments is being used to map the cis-acting elements in an attempt to clarify the molecular mechanisms behind the regulation of the PAI-1 gene.

P 215 **ISOLATION AND CHARACTERIZATION OF A HUMAN B CREATINE KINASE-RELATED GENE.** Tony S. Ma, Jonah Ifegwu, Robert Roberts, M. Benjamin Perryman, Molecular Cardiology Unit, Baylor College of Medicine, Houston, TX 77030

Human M creatine kinase (MCK) and B creatine kinase (BCK) are coded in the human genome as single copy genes that reside on chromosome 19 and 14, respectively. Another single copy BCK-like gene sequence is demonstrated to be present which shows EcoRI restriction polymorphism of 7.5 and 5.4 kb, as compared to the 16.5 and 12 kb EcoRI restriction polymorphism demonstrated for BCK. We have prepared an EcoRI-digested genomic library in Lambda-Zap, enriched for the BCK-like gene sequence, and recovered the 5.4 kb genomic fragment. This gene is apparently organized into 2 exons. The deduced coding region and 3' untranslated region share 70% and 85% sequence identity, respectively, with those of the BCK cDNA. A TATAA sequence and two CAAT sequences in tandem are present at 55 bp and 115 bp, respectively, 5' to the ATG initiation codon. There are Alu sequences flanking both the 5' and 3' end of the 1st exon. Whether this gene is functional awaits further study. From the known gene structures of human BCK and the presently reported BCK-like gene, it appears that these two genes are closer related to each other in organization than to the human MCK and mitochondrial CK.

P 216 α_1-ADRENERGIC STIMULATION OF PLATELET-DERIVED GROWTH FACTOR A-CHAIN (PDGF-A) GENE EXPRESSION IN RAT AORTA, Mark W. Majesky, Mat Daemen* and Stephen M. Schwartz, Departments of Pathology, University of Washington, Seattle, WA 98195, and *University of Limburg, 6200 MD, Maastricht, The Netherlands

Sympathetic nerves and catecholamines exert trophic influences on structural adaptations of artery wall to changing circulatory demands. Although mechanisms responsible are unknown, this may occur via regulation of expression of specific genes in vessel wall cells. We report here that phenylephrine produced dose-dependent stimulation of PDGF-A gene expression in rat aorta in vivo via agonist occupancy of α_1-adrenergic receptors. Increases in PDGF-A transcript levels were rapid (maximum 6 hr, 10-fold) and transient. Among several tissues examined, reproducible increases (>2-fold) in PDGF-A mRNA levels were found only in the aorta. Analysis of dissected fractions of periaortic fatty/connective tissues versus remaining artery wall (endothelium removed) showed phenylephrine-mediated increases in PDGF-A mRNA levels only in artery wall (presumably smooth muscle). Stimulated PDGF-A gene expression was accompanied by activation of expression of other growth-related genes including c-fos, c-myc and ornithine decarboxylase, but not DNA synthesis. Other vasoactive agonists (angiotensin II, endothelin) had little or no effect on aortic PDGF-A mRNA levels. These findings suggest a mechanism for previously described trophic effects of sympathetic nerves and catecholamines on arterial smooth muscle, i.e., regulation of growth-related gene expression via α_1-adrenergic receptors. (Supported by HL-03174 and HL-26405.)

P 217 REGULATION OF SMOOTH MUSCLE-SPECIFIC MYOSIN LIGHT CHAIN-2 GENE EXPRESSION BY ONCOGENES, C. Chandra Kumar, Sheela R. Mohan, Cecile Chang*, and James I. Garrels*, Department of Tumor Biology, Schering Research, Bloomfield, NJ 07003 and *Cold Spring Harbor Laboratory, NY 11794. We have characterized the human smooth muscle specific MLC-2 cDNA clone, by screening the cDNA library constructed in the vector lambda gt 10 using mRNA derived from umbilical artery. Our results indicate that this MLC-2 isoform is expressed specifically in only smooth muscle tissue and in some non-muscle cells such as fibroblasts and epithelial cells. Analysis of the MLC-2 gene expression in several non-muscle cells led to the finding that this gene is repressed in several transformed cells that contain active oncogenes. Specifically, transformation of human fibroblast cells such as HOS cells either by infection with Kirsten murine sarcoma virus (containing k-ras oncogene) or by chemical carcinogen MNNG (N-methyl-N-nitro-N-nitrosoguanidine) results in the suppression of the smooth muscle specific MLC-2 mRNA level. Revertants of transformed fibroblasts that lack the k-ras oncogene (K-HOS 312H) exhibit normal levels of MLC-2 mRNA, suggesting that activated k-ras oncogene can block the expression of the smooth muscle specific MLC-2 isoform. Using antibodies for MLC-2 and 2-D gel electrophoresis, we have identified three isoforms of MLC-2 (one smooth muscle and two non-muscle), in fibroblast cells. Quantitative 2-D gel analysis of the radiolabelled proteins of normal and transformed cells indicates that the smooth muscle MLC-2 isoform is specifically repressed when cells undergo transformation. Treatment of HOS cells with tumor promoting phorbol ester such as TPA (12-O-tetradecanoyl-phorbol-13-acetate), represses the transcription of the MLC-2 mRNA. We are currently characterizing the genomic clones for MLC-2 in order to identify the elements that mediate transcriptional response to oncogenes and TPA.

P 218 **PEPTIDE GROWTH FACTORS CAN PROVOKE "FETAL" CONTRACTILE PROTEIN GENE EXPRESSION IN CULTURED CARDIAC MYOCYTES.** Thomas G. Parker, Sharon Packer, and Michael D. Schneider, Molecular Cardiology Unit, Baylor College of Medicine, Houston, TX 77030

Cardiac-specific gene expression is intricately regulated in response to developmental, hormonal, and hemodynamic stimuli. To test whether cardiac muscle might be a target for regulation by peptide growth factors, the effect of three peptide growth factors on the actin and myosin gene families was investigated in cultured neonatal rat cardiac myocytes, after serum withdrawal for 48 hr. Messenger RNA levels were determined by Northern blot analysis and are shown, relative to expression in a vehicle control, in the accompanying table. Transforming growth factor β-1 (TGFβ1, 1 ng·ml^{-1}) and basic fibroblast growth factor (bFGF, 25 ng·ml^{-1}) elicited changes in contractile protein gene expression which correspond to those seen during cardiac hypertrophy induced by a hemodynamic load. The "fetal" (β) myosin heavy chain (MHC) was up-regulated, whereas the "adult" αMHC was inhibited. Concomitantly, expression of α skeletal actin (αSkA) was increased, with little or no change in α cardiac actin (αCaA). These findings differ from the suppressive effects of TGFβ1 reported in skeletal muscle. Acidic FGF (aFGF, 25 ng·ml^{-1}) produced even larger reciprocal changes in MHC expression but, in contrast to either TGFβ1 or bFGF, inhibited the expression of both α-actin genes.

	αMHC	βMHC	αCaA	αSkA
TGFβ1	0.34	4.09	1.17	1.97
bFGF	0.47	4.29	0.97	2.50
aFGF	0.14	8.71	0.10	0.33

Thus, peptide growth factors alter the program of differentiated gene expression in cardiac myocytes, and can be sufficient to provoke "fetal" contractile protein gene expression, characteristic of hypertrophy triggered by pressure overload.

P 219 CORRELATION OF mRNA EXPRESSION OF CELLULAR MITOGENS AND SMOOTH MUSCLE CELL PROLIFERATION IN ARTERIES DENUDED OF ENDOTHELIUM, M. A. Reidy, J. Fingerle, Y. P. Au, and M. W. Majesky, Department of Pathology, University of Washington, Seattle, WA 98195

Smooth muscle cells are capable of synthesizing their own mitogens and in vivo balloon catheter denudation leads to a rapid increase in mRNA for PDGF A-chain and TGF-β. An increase in mRNA for PDGF A-chain and TGF-β was observed in balloon catheter injured thrombocytopenic animals with subsequent smooth muscle cell proliferation similar to that observed in control animals (19.6% vs. 19.9%). Differences, however, were observed in the size of the developing intima, and at 4 and 7 days post balloon injury the thrombocytopenic animals had a significantly smaller intima. In another model of endothelial denudation in rat carotid arteries, total loss of endothelium induced an identical increase in expression of mRNA for PDGF A-chain and TGF-β, yet these animals showed only a modest increase in smooth muscle proliferation as compared to that observed in balloon catheter denuded arteries (1.5% vs. 19.6%) and had a significantly reduced (by approximately 50%) intimal lesion area. These data suggest that an increase in mRNA levels for at least two mitogens, namely PDGF A-chain and TGF-β, is not a good predictor of subsequent smooth muscle cell proliferation and that some other mechanism other than platelet mitogen is responsible for the induction of this growth. (Supported by NIH grants HL-03174 and HL-30203.)

P 220 **DISRUPTION OF VASCULAR DEVELOPMENT AND FORMATION OF HEMANGIOMAS BY ENDOTHELIAL CELLS EXPRESSING POLYOMA MIDDLE T ONCOGENE**, Werner Risau, Lindsay Williams+ and Erwin Wagner*, MPI für Psychiatrie, D-8033 Martinsried, FRG; +EMBL, D-6900 Heidelberg, FRG; *IMP, A-1030 Wien, Austria. Chimeric embryos derived from embryonic stem cells expressing polyoma middle T antigen develop multiple lethal hemangiomas within the embryo and the yolk sac at mid-gestation (*CELL 52, 121-131, 1988*). Endothelioma cell lines established from those hemangiomas rapidly proliferate in vitro and induce hemangiomas when injected into syngeneic mice and also in chick and quail embryos. Histological analysis of hemangiomas induced in mice by Mitomycin C treated and radiolabelled cells, and of quail hemangiomas showed that these hemangiomas are host derived. However, transplantation experiments indicate that they require the continued presence of endothelioma cells and are therefore comparable to regressing human tumors. Endothelioma cells do not produce known endothelial cell growth factors or angiogenic factors in vitro suggesting that novel mechanisms are involved in the process of hemangioma formation. Since middle T is known to modulate the activity of src-like tyrosine kinases these molecules seem to be important for the regulation of endothelial cell proliferation. Our vitro model system for the early development of the vascular system from embryonic stem cells (*DEVELOPMENT 102, 471-478*) might allow us to reveal the mechanisms.

P 221 **EFFECT OF AMILORIDE AND HEPARIN ON MYOSIN HEAVY CHAIN EXPRESSION IN PRIMARY CULTURES OF CANINE VASCULAR SMOOTH MUSCLE (VSMC)**, Charles L. Seidel, Cynthia L. Wallace and Julius C. Allen. Sec. of Cardiovasc. Sci., Dept. of Med., Baylor College of Medicine, Houston, TX 77030.

VSMC maintained in the presence of fetal bovine serum (FBS) proliferate and up regulate the expression of non-muscle myosin heavy chain (nmMHC) and down regulate the expression of muscle myosin heavy chain (mMHC). This does not occur in serum free medium (SFM). The purpose of these experiments was to determine the effect on nmMHC expression of: (1) amiloride, an inhibitor of the Na^+-H^+ exchanger involved in mitogen action and (2) heparin, a known inhibitor of VSMC proliferation. MHC expression was determined by quantitative gel electrophoresis.

Within 2 days in FBS, nmMHC represented 44% of the total MHC in saphenous vein muscle cells and increased to 80% by the 6th day. If heparin (50 U/ml) or amiloride (10^{-4}M) were present, nmMHC expression was not detected until the 4th or 6th day, respectively and at these times represented 50% or more of the total MHC. Proliferation was also delayed. After 2 days in FBS, changing to SFM prevented a further increase in nmMHC expression. However, if amiloride was present during the 2 day exposure to FBS, SFM did not prevent the expression of nmMHC.

These data demonstrate that heparin and amiloride antagonize the effect of FBS on nmMHC expression and suggest that the action of amiloride is to block an effect of FBS that is subsequent to the activation of membrane receptors by FBS. (HL 34,280)

P 222 THE STRUCTURAL RELATIONSHIP OF THE mRNAs ENCODING THE PULMONARY AND THE TESTICULAR ISOZYMES OF RABBIT ANGIOTENSIN CONVERTING ENZYME, Ganes C. Sen and Ravi S. Kumar, Department of Molecular Biology, Cleveland Clinic Foundation, Cleveland, OH 44195

Angiotensin converting enzyme (ACE) is a component of the renin-angiotensin system which plays an important role in the regulation of blood presence. Adult testes produce an isozyme of ACE which is distinct from but related to the one produced by other tissues including lungs. In order to be able to study the structural relationship of the two isozymes we have recently isolated cDNA clones for the testicular ACE. The complete nucleoside sequence of one of these clones has been determined. The encoded polypeptide has a predicted molecular weight of 84kD, a typical signal sequence at the amino terminal and a strongly hydrophobic domain near the carboxy terminal suggesting that the enzyme is an extracellular membrane bound protein. The cDNA sequence contained the previously determined sequences of seven ACE-derived peptides thereby confirming the identity of the clone. Northern analyses demonstrated the presence of an ACE mRNA of 2.6 kb in the testes and one of 5 kb in the lungs. Oligonucleotide probes revealed that although the two mRNAs shared extensive sequence homologies the testes mRNA contained specific sequences which were absent from the lung mRNA. The mechanism of synthesis of the two ACE mRNAs in a tissue specific manner is under investigation. (Supported in part by NIH grant HL 35667).

P 223 STRUCTURE OF A HUMAN NONMUSCLE MYOSIN HEAVY CHAIN GENE, Michael Simons, Robert S. Adelstein, David C. Gdula and Lawrence Weir, NHLBI, NIH, Bethesda, MD 20892 We screened a human genomic Charon 4A library with a human nonmuscle myosin heavy chain (NMMHC) cDNA probe and obtained a single 17 kb clone. Sequence analysis of the clone indicated that it encodes approximately 700 amino acids (out of approximately 1950 for the entire NMMHC) and that it does not contain either end of the gene. Several non-repetitive intronic probes from the 3' and 5' ends of the clone were used to screen other genomic libraries including a cosmid library. Ultimately, we were able to obtain clones containing the 3' end of the gene, but none of the clones extended any further 5' than the original clone. In order to obtain the 5' end of the gene we isolated a 1.4 kb human NMMHC cDNA clone which contains about 200 nucleotides of 5' untranslated region and used this clone to probe a cosmid library and isolate several clones. Almost all of the coding regions including the entire rod portion of the myosin molecule and most of the S1 region are represented in our clones. The human NMMHC gene is much larger than any other MHC gene reported. The difference appears to be mainly attributable to an accumulation of large introns in the part of the gene coding for heavy meromyosin.

P 224 PLATELET DERIVED GROWTH FACTOR MODULATES IL-1 ACTIVITIES (INDUCTION OF PGE_2 AND DNA SYNTHESIS) IN FIBROBLASTS AND VASCULAR SMOOTH MUSCLE CELLS, Jai Pal Singh, William J. Chiou, and Paul D. Bonin, The Upjohn Company, Kalamazoo, MI 49001

Induction of prostaglandin E_2 and DNA synthesis in fibroblasts and vascular smooth muscle cells by IL-1 were studied. Our results showed that in cells incubated in medium containing 5% serum, IL-1 acted as a potent stimulator of PGE_2 and DNA synthesis. However, in cells incubated in low serum or platelet poor plasma (lacking PDGF), IL-1 alone failed to stimulate PGE_2 or DNA synthesis. PGE_2 or DNA synthesis in response to IL-1 was restored when purified platelet derived growth factor (PDGF) was included in the culture medium. Effect of PDGF was dose dependent. The concentration of PDGF required to modulate IL-1 responses was in a range similar to that required for mitogenic activity. Induction of PGE_2 synthesis in response to IL-1 and PDGF was initiated after a lag of 2-3 hours and then continued with a rapid rate (30 ng/hr x 10^6 cells) for 6-8 hours. IL-1 receptor binding studies showed that PDGF treatment of Balb/c3T3 cells produced a 2-3 fold increase in cells surface IL-1 receptors. These studies suggest that PDGF may play an important role in modulation of IL-1 responses in connective tissue cells.

P 225 HEART-DERIVED FIBROBLAST GROWTH FACTORS IN VIVO AND IN VITRO, Edith Speir, Zhou YiFu, Michael Lee, Shashi Shrivastav, Stephen E. Epstein, Ward Casscells, Cardiology, NHLBI, NIH, Bethesda, MD 20892. Acidic and basic fibroblast growth factors (aFGF, bFGF) are structurally related polypeptides which are morphogenic, chemotactic, mitogenic and angiogenic. We extracted aFGF and bFGF from adult rat, bovine, canine and human heart and characterized these FGF's by mitogen assays, by RIA and by Western analysis. Various extraction methods did not increase or decrease the yield of FGF's. Rat heart yielded 200ng/g aFGF and 100ng/g bFGF. Bovine LV contained 239ng/g and 305ng/g bFGF vs 195ng/g aFGF 329ng/g bFGF in RA from the same animal. Canine brain or heart yielded 200ng or 300ng of aFGF and 150ng or 265ng bFGF per g tissue. Vascular and capillary endothelial cells contribute to the bFGF content of the heart and vascular smooth muscle cells and neural tissue contribute to the aFGF content of this organ. FGF peptides were isolated from a variety of tissues and cultured cells, but have not been localized to a particular cell type in vivo. We extracted freshly isolated rat heart myocytes and obtained 30ng of aFGF and 16ng bFGF per 10^6 cells. Freshly isolated bovine aorta endothelial cells (BAEC) gave 20ng of aFGF and 10ng of bFGF and fresh rat aorta smooth muscle cells (RASMC) yielded 3ng aFGF and 1ng bFGF per 10^6 cells. When aliquots of BAEC or RASMC were grown in culture and harvested at confluence, BAEC contained 48ng aFGF and 48ng of bFGF in the lysates and 16ng aFGF vs 9ng bFGF in the ECM. RASMC increased aFGF yield to 25ng, bFGF to 11ng in the cell lysates; the ECM contained 5ng aFGF and 4ng bFGF per 10^6 cells. Thus, in BAEC the increase in both FGF's in cultured vs fresh cells is 6 fold, whereas in RASMC it is 10 fold for aFGF and 15 fold for bFGF.

P 226 DURATION OF ISCHEMIA IS CRUCIAL FOR DEVELOPMENT AND REGRESSION OF CARDIAC COLLATERAL VESSELS. **Hitonobu Tomoike, Mitsuru Noma, Masahiro Mohri, Hideo Yamamoto, Hiroaki Shimokawa, Motoomi Nakamura, Research Institute of Angiocardiology and Cardiovascular Clinic, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan**

Gradual occlusion of the coronary artery over a 20 day period, or repetitive occlusion for 5-10 days elicits neovascularization in animal models. The processes involved in this angiogenesis are not well understood. In the present study, command signal for coronary occlusion and left ventricular pressure, ECG, and regional wall motion in control and ischemic areas of dog hearts were telemetered, non-stop. In spite of the same 90 minute coronary occlusion a day, occlusion every 2-min at 32-min intervals (n=11), but not 15-second at 4-min intervals (n=7) led to development of collateral vessels. The extent of native collaterals, determined according to regional dyskinesia during the first 2 min of occlusion of the coronary artery correlated inversely with the rate of collateral development following repeated 2-min coronary occlusion (n=23). To determine the influence of the duration of non-ischemia in the maintenance or augmentation of collateral functions, occlusion of the coronary was released for 24 hours, a time when the collaterals became functional. Regression of collateral function was evident in all dogs (n=7), however, the retained potential progressed with repetition of this protocol and the control state was reached, along with vascular development. Therefore, the duration of ischemia and the level of native collaterals are crucial for the angiogenetic development of coronary collaterals.

P 227 GROWTH FACTOR EXPRESSION AND GROWTH FACTOR DEPENDENCE OF KAPOSI'S SARCOMA DERIVED CELLS IN CULTURE. S. Werner, W. K. Roth and P. H. Hofschneider, Department of Virus Research, Max-Planck- Institute for Biochemistry, 8033 Martinsried, FRG.

We have established cell cultures from Kaposi's sarcoma (KS) biopsies of patients with AIDS-associated and sporadic KS. Cytochemical staining of these cells revealed the expression of endothelial cell markers, indicating that KS cells might be derived from endothelial cells. In spite of these markers they reveal a fibroblast-like morphology. The malignancy in vitro is low. No rearrangements, amplifications or overexpressions of oncogenes which are often involved in human tumors were found. The expression of several growth factors, e.g. acidic and basic fibroblast growth factor could be detected in these cells. These factors might account for the high vascularization of KS tumors. However they are not sufficient to permit an autocrine growth of KS cells in vitro, as their lifespan in 0.5% fetal bovine serum does not exceed 10 passages. By growth assays in Platelet-Poor-Plasma Serum we found that KS cells are highly dependent on externally supplied Platelet-derived growth factor (PDGF) which- in contrast to fibroblasts- is obvious also at physiological concentrations of Ca^{2+}. Finally we report here data on the expression of PDGF and its receptors which lead to an explanation for the extreme PDGF-dependence of KS cells in vitro.

Thrombosis and Thrombolysis; Endothelial Cells

P 300 CLONING AND SEQUENCING OF THE MOUSE cDNA FOR ICAM-1, Christie M. Ballantyne[1], William E. O'Brien[2], and Arthur L. Beaudet[2], [1]Section of Atherosclerosis and Cardiology, Department of Medicine and [2]Howard Hughes Medical Institute and Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030

Intercellular adhesion molecule 1 (ICAM-1) is the first member of the immunoglobulin superfamily which has been shown to bind to a member of the integrin superfamily, LFA-1. ICAM-1 is felt to play a critical role in the interaction of leukocytes with endothelium and thus may play an important role in vascular pathophysiology. As a step in understanding the biological importance of ICAM-1, we have isolated cDNA clones from a mouse thymus cDNA library. These clones cover 23 bp of 5' untranslated sequence through the entire coding region and 3' untranslated region to the polyA sequence with an approximate length of 2.5 kb as compared to the 3.0 kb length of the human cDNA. This difference is due primarily to the shorter length of the 3' untranslated region in the mouse which is 872 bp as compared to 1.3 kb in the human. Southern blots are consistent with a single gene in the mouse. With 85% of the cDNA sequence completed, the overall identity between the mouse and human sequence at the amino acid level is less than 53%. The high percentage of identity in certain regions suggests functional importance which has led to evolutionary conservation. Two such areas occur in domain 2 (by the immunoglobulin classification) and contain within them an RGE and an RGD amino acid (AA) sequence. The RGE mouse sequence corresponds to the RGE occurring at AA 125 in the human and resides in a region of 75% amino acid identity (89% with conservative changes) over 28 AA. The RGD in the mouse corresponds to an RRD in the human occurring at AA 149 and has 89% identity over 19 AA. These areas may be important in binding to LFA-1 as RGD like sequences have been noted to be critical in binding to other integrins.

P 301 NOVEL FIBRINOLYTIC ENZYMES WITH SLOW PLASMA CLEARING RATES

Michael J Browne,Conrad G Chapman,Ian Dodd,Ashiq F Esmail and Jeff H Robinson,Department of Biotechnology,Beecham Pharmaceutical Research Division,Yew Tree Bottom Road,Epsom,Surrey,KT18 5XQ,U.K.

Tissue-type plasminogen activator (t-PA) is used in the clinic for the treatment of acute myocardial infarction;it is,however,rapidly cleared in vivo with a $t_{\frac{1}{2}}\alpha$ of only a few minutes.We have synthesised two new groups of fibrinolytic proteins and measured their clearance rates.The first group is composed of t-PA muteins lacking one or more of the A-chain domains (i.e.F-K_1K_2B,FG-K_2B,FGK_1-B,F--K_2B,---K_2B and F-K_2K_2B). Muteins lacking the growth-factor or kringle 1 domains were cleared more slowly than t-PA in vivo.The second group was produced by fusing the plasmin A-chain to either the t-PA B-chain or the urokinase B-chain via a disulphide bond.Both of these hybrid proteins were cleared very slowly in a number of model systems. The clearance results suggest that some of these novel proteins,particularly the hybrids,may have therapeutic advantages over native t-PA.

P 302 PURIFICATION AND CHARACTERIZATION OF TWO GLYCOSYLATION FORMS OF A RECOMBINANT PLASMINOGEN ACTIVATOR, LY210825, P. J. Burck, D. H. Berg, M. W. Warrick, S. C. Yan, and C. J. Vlahos, Biochemistry and Molecular Biology Research Departments, Eli Lilly and Company, Indianapolis, IN 46285.

A recombinant plasminogen activator, LY210825, which comprises the second kringle and serine protease domains(K2P) of native plasminogen activator, exists in two forms, as evidenced by SDS PAGE, when the activator is purified from mammalian cell culture fluid. The two forms differ in molecular weight, affinity for lysine Sepharose, and interaction with hydrophobic affinity resins. These differences have been exploited to purify the two forms of LY210825. The primary form(I) contains 3.19% carbohydrate, while the minor form(II) contains 5.37% using the phenol-sulfuric acid assay with glucose as a standard. Tryptic peptide mapping of the two forms of LY210825 indicates that form I is glycosylated at ASN 448(native t-PA amino acid sequence numbering) while form II is glycosylated at both ASN 448 and ASN 184. The carbohydrate composition of form I has been determined following its digestion with sialidase and hydrolysis with 2M trifluoroacetic acid. The ratio of glucosamine and mannose to galactose indicates the N-linked carbohydrate is a mixture of di- and tri-antennary complex sugars. Form I has a specific activity(1.5 million IU/mg) approx. twice that of form II using a fibrin plate assay, and the plasminogen activator activity of form I is more enhanced by CNBr fibrinogen peptides than is form II.

P 303 EXPRESSION OF A RECOMBINANT ANTIBODY-TARGETED UROKINASE-TYPE PLASMINOGEN ACTIVATOR IN MAMMALIAN CELLS. C.-C.Chou, P.Zavodny, J.Anagnost, J.Carter, F.Hamud+, H.Lonial, T.Love*, D. Mullins+, S.Narula, M.Petro, T.Quertermous*, M.Runge* and S.Witherspoon. Departments of Biotechnology-Molecular Biology and +Pharmacology, Schering Corporation, Bloomfield, NJ 07003 and *Cardiac Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA02114. An expression plasmid was constructed consisting of a cloned genomic heavy chain (h.c.) variable region from a fibrin specific monoclonal antibody (59D8), cloned genomic constant region of the mouse gamma 2b hc., and the coding region from a genomic clone of low molecular weight single-chain urokinase plasminogen acivator (LMW ScuPA) beginning at Leucine 144. A second plasmid was constructed which contains the human beta-globin 3' untranslated region (utr) in place of the uPA 3'-utr. The original construct (uPA 3'-utr) produced extremely low levels of RNA and protein upon electroporation and gpt selection into h.c. loss variants of the original hybridoma. In-vitro nuclear run-off experiments indicated that the low steady-state level of mRNA was not due to a lowered transcription rate relative to the endogenous h.c. of the 59D8 parent. In contrast, the second construct (human beta-globin 3'-utr) yielded readily detectable recombinant protein after electroporation into h.c. loss variants as above. Expression was monitored by analysis of conditioned media by Western blot, fibrin binding and plasminogen activation assays. Substitution of the uPA 3'-utr with that of human beta-globin resulted in an increase in expression of several orders of magnitude, which may be due to a stabilizing effect of the globin sequences on the mRNA for the recombinant heavy chain-LMW ScuPA chimera.

P 304 IDENTIFICATION OF THE DOMAINS OF t-PA INVOLVED IN AUGMENTED BINDING TO FIBRIN LIMITEDLY DIGESTED WITH PLASMIN, Carlie de Vries, Harry Veerman and Hans Pannekoek, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Dept. of Molecular Biology, Amsterdam, the Netherlands.
Fibrin acts both as a substrate for plasmin and as an assembling surface for plasminogen and t-PA. In the latter function, fibrin strongly potentiates the activity of t-PA and thus is considered as an obligatory cofactor. It is conceivable that the cofactor function of fibrin may alter during ongoing degradation. In agreement with this view are the reported observations that both plasminogen and t-PA exhibit an increased binding to fibrin upon limited digestion with plasmin. We have studied the increase in fibrin binding of t-PA in more detail and have focussed on the identification of the domain(s) that mediate this increment. To that end, we employed a well-defined fibrin binding assay, allowing a controlled plasmin digestion, and metabolically labelled, purified (recombinant) rt-PA. In addition, purified labelled deletion mutants of t-PA were used that precisely lack one or more domains. Previously, we have shown that binding to intact fibrin is mediated by the "finger" (F)- and the "kringle 2" (K2) domain. An optimal increased binding of rt-PA coincides with the appearance of fibrin fragment X. About half of the increased binding of rt-PA can be abolished upon an additional incubation with carboxypeptidase B (CPB) that liberates carboxy-terminal lysine residues. Here, we demonstrate that increased binding due to carboxy-terminal lysines is mediated by the lysine-binding site contained within the K2 domain of t-PA. Furthermore, our data indicate that increased binding that is independent of the presence of carboxy-terminal lysines within fibrin is mediated by the F domain, provided this domain is properly positioned within the t-PA molecule.

P 305 EFFECT OF FIBRIN STRUCTURE AND CONDITIONED MEDIA ON VASCULAR OUTGROWTH FROM TISSUE EXPLANTS, Irene M. Evans, Kim A. Corbett, Catherine M. Intrieri, Charles W. Francis, Department of Biology, Rochester Institute of Technology, Rochester, New York, 14623 and Department of Hematology, University of Rochester, Rochester, New York, 14642.
When abdominal muscle explants are placed in a fibrin clot, growth of vessel-like structures is stimulated after 3-4 days of culture. We investigated the effect of changing the fibrin concentration and fibrin cross-linking on the migration and tube formation of endothelial cells derived from muscle tissue explants. Endothelial cells were identified by Von Willebrand factor and low density lipoprotein staining. Growth was quantitated by micrometer measurements of the leading edge of migrating cells. Migration was found to be an inverse linear function of fibrinogen concentration and was unaffected when clotting was initiated with reptilase instead of thrombin. Growth was found to be dependent on high serum concentrations (10-20% fetal calf serum) and was stimulated by conditioned media from stimulated macrophages cultured in vitro. Conditioned media from CHO cells was also stimulatory. This culture system provides a useful in vitro model for studying the factors involved in vascularization of a fibrin clot.

P 306 INACTIVATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 BY LEUKOCYTE ELASTASE: KINETICS AND MECHANISM, William P. Fay and Whyte G. Owen, Section of Hematology Research, Mayo Clinic/Foundation, Rochester, MN 55905
Leukocyte elastase inactivates plasminogen activator inhibitor-1 (PAI-1) present in the growth substratum of endothelial cells, which suggests an additional mechanism by which leukocytes may regulate fibrinolysis. We studied the kinetics and mechanism of reaction of purified porcine platelet PAI-1 with leukocyte elastase and compared them to those of proteases known to interact with PAI-1. Leukocyte elastase catalytically converts PAI-1 to an inactive, modified inhibitor of slightly reduced molecular weight. The second-order rate constant for the inactivation of PAI-1 by leukocyte elastase is 1.1×10^5 $M^{-1}s^{-1}$. The peptide generated by the action of leukocyte elastase on PAI-1 was isolated and sequenced. Leukocyte elastase cleaves the P_4-P_3 peptide bond of PAI-1 located near the carboxy terminus of the molecule. In comparison, thrombin also converts PAI-1 to an inactive, modified inhibitor, but with a second-order rate constant of only 10^2 $M^{-1}s^{-1}$, and activated protein C (APC) inactivates PAI-1 with a second-order rate constant of 1.1×10^4 $M^{-1}s^{-1}$. The latter rate is not accelerated by protein S or heparin, and APC is inactivated during the reaction. In conclusion: 1) leukocyte elastase inactivates PAI-1 by hydrolysis of a sensitive peptide bond in close proximity to the proposed reactive center of PAI-1, and 2) the kinetics and mechanism of inactivation of PAI-1 by leukocyte elastase suggest that it may function as a physiological regulator of PAI-1 activity.

P 307 STRUCTURE AND FUNCTION OF THE PLASMINOGEN ACTIVATOR INHIBITOR-1 REACTIVE CENTER

Robert D. Gerard, Huda E. Shubeita and Theresa L. Cottey, Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235

Plasminogen activator inhibitor-1 (PAI-1) is a Mr 52,000 glycoprotein member of the serine protease inhibitor (serpin) family. It inhibits plasminogen activators (PA) by forming a stable 1:1 complex with the enzyme. The best current model for the inactivation process suggests that the inhibitor serves as a suicide substrate by presenting a loop of amino acid residues (the reactive center) to the enzyme which closely resemble the plasminogen substrate. However, proteolytic cleavage of the inhibitor results in the formation of a covalent bond and enzymatic inactivation.

We have used a combined molecular genetic, biochemical, and immunochemical approach to study the structure and function of the PAI-1 molecule. Site-directed mutants of both the reactive center P1 and P1' residues (RM) and a "second site" containing the sequence DLKLV have been constructed and expressed at high level in E. coli. The kinetics of inhibition of a panel of serine proteases, including uPA and tPA, have been assessed for the wild type and mutant PAI-1 molecules. The results suggest that PAI-1 is extremely specific for the inhibition of PA, and that its specificity can be modulated by altering the reactive center pair. Alteration of charged residues in the "second site" DLKLV sequence had little effect on the rate or specificity of the inhibition. These results have been complemented by neutralization studies on PAI-1 using anti-peptide antibodies directed against the same two regions of the molecule. Antibodies directed against the wild type reactive center neutralized PAI-1 activity of the wild type, but not the mutant proteins, whereas antibodies against the "second site" had no effect.

P 308 IDENTIFICATION OF FETOMODULIN, A SURFACE MARKER OF DEVELOPMENT, AS THROMBOMODULIN BY GENE CLONING AND FUNCTIONAL ASSAY, Masaru Imada, Sumi Imada, Masahiko Nagumo, Harumi Yamaguchi and Shigeru Katayanagi, Division of Cell Biology, Meiji Institute of Health Science, Meiji Milk Products Co., Inc., Odawara, Japan 250

Fetomodulin (FM) is a cell surface membrane protein that is upregulated in murine fibroblast by cyclic AMP. In early mouse embryos, FM is specifically expressed by parietal endoderm cells, which lead us to hypothesize its role in embryogenesis (Dev. Biol. 122:483, 1987). To prove this, the protein structure was determined by cDNA base sequencing. Double stranded cDNA was prepared using poly-A^+ RNA of fibroblast as an RNA source. Alu I restriction fragments of the cDNA were ligated into a λgt11 expression vector and the library was screened with polyclonal anti-FM antibody. An 80 bp cDNA insert thus obtained was used as a probe to screen a λgt10 library constructed with unfragmented cDNA. Base sequencing of three positive clones has elucidated a partial FM cDNA sequence of 3375 bp. It contained an open reading frame for 535 amino acids followed by a termination codon, TGA, but apparently lacked a part of the amino terminal sequence. Most of the lysylendopeptidase peptides were identified in the deduced sequence, confirming authenticity of the cDNA clones. The study has disclosed an unexpected identity of FM as mouse thrombomodulin (TM). We were also able to demonstrate that FM isolated from fibroblast was capable of binding thrombin and activating protein C upon thrombin binding, supporting functional identity of the two. Stage and tissue specific expression of FM (i.e. TM) in embryonic tissues suggests its role as an embryogenetic protein.

P 309 DEVELOPMENTALLY REGULATED EXPRESSION OF FETOMODULIN: A PROTEIN THAT WAS RECENTLY IDENTIFIED AS THROMBOMODULIN BY GENE CLONING AND FUNCTIONAL ASSAYS, Shigeru Katayanagi, Hideko Iwasaki, Sumi Imada, Masaru Imada, Division of Cell Biology, Meiji Institute of Health Science, Meiji Milk Products Co., Inc., Odawara, Japan 250

Fetomodulin (FM), a surface marker protein of parietal endoderm differentiation (Dev. Biol. 122:483, 1987), was recently identified as thrombomodulin (TM) by gene cloning and functional assays; hereafter referred to as FM/TM. FM/TM localization was examined by immunohistochemical staining of embryos with monoclonal and polyclonal antibodies. The antigen is expressed in embryonic vascular tissues such as ventricular endocardium of 8.5 day of gestation (E8.5), dorsal aorta endothelium (E10.5) and capillary endothelium (E13.5). FM/TM may function as an anticoagulant already in embryonic stages. We were, however, intrigued by the fact that it existed in tissues and membrane compartments that were not previously known to contain the protein. Sinoatrial node cells of E13.5 were positive for FM/TM. Staining of epithelial cells in neuroectoderm, lung bud and hindgut on E10.5 was seen at the site of close cell-cell junction. Also worthy to note is an observation that the expression was frequently transient; e.g. a prominent expression in atrial myocardium on E10.5 became much less distinct on E15.5. Based of these unique spatio-temporal regulation of FM/TM expression, we hypothesize that the protein is endowed with a yet unrecognized function(s). Invertebrate proteins, lin-12 and Notch, which contain repeats of an epidermal growth factor (EGF) motif are well known embryogenetic proteins of nematode and Drosophila, respectively. A six-fold repeat of an EGF motif in FM/TM may be relevant to its function in embryos.

P 310 SPECIFIC ANTIBODY TO HUMAN PLATELET THROMBOSPONDIN AFFECTS THE RECOVERY OF ENDOTHELIAL CELLS IN CULTURE FROM HYPERTHERMIA,
Nika V. Ketis and Jack Lawler*, Department of Anatomy, Queen's University, Kingston, Ontario K7L 3N6; *Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.
In addition to the increased synthesis of the classical heat-shock proteins (28,000, 71,000, 73,000, 90,000, and 100,000 D polypeptides), there is also an increase of an 180,000 D polypeptide in the growth medium of endothelial cells exposed to hyperthermia. Immunoprecipitation with specific antiserum demonstrated that the 180,000 D polypeptide is thrombospondin. During recovery from heat shock, the amount of thrombospondin in the growth medium increases as does the transcription of the gene (J. Cell. Biol. 106: 893 (1988)). In the presence of a monoclonal antibody to human platelet thrombospondin (ascites), the actin, tubulin and vimentin cytoskeletal networks of endothelial cells at 37°C are notably altered. Following heat-shock not only does the vimentin cytoskeleton collapse in the cells but there is also a decrease in the actin filaments. The tubulin network remains basically unaltered. Endothelial cells exposed to hyperthermia followed by the thromobospondin antibody maintain the integrity of the cytoskeletal networks better than cells at 37°C exposed to the antibody. Nonetheless, the recovery of cells from heat shock is prevented by the presence of the thrombospondin antibody. Our data suggests that the recovery of cells from heat shock requires the integrity of thrombospondin and its interaction. We speculate that thrombospondin may be involved in the recovery of endothelial cells from a stressed or injured state. Supported by MRC and NIH grants.

P 311 **HUMAN CORONARY ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) INHIBITS THROMBIN-INDUCED PLATELET AGGREGATION IN VITRO.** David D. Ku, Department of Pharmacology, University of Alabama at Birmingham, AL 35294
Recently, EDRF has been shown to produce a potent inhibitory effect on platelet aggregation. To determine further the role of endothelial cell (EC) production of EDRF and prostacyclin (PGI_2) in the regulation of platelet aggregation and thrombosis, responses of washed human platelets to thrombin in the presence of human coronary arteries (HCA) were studied. In freshly isolated HCA, thrombin (.003-.1 unit/ml) produced an EDRF-dependent, PGI_2-independent relaxation, while in washed platelets, a dose-dependent aggregation was observed. Addition of small HCA segments with intact EC (10-15 mm^2 surface area), but not with disrupted EC, completely inhibited (-100 ± 0.0%, M±SEM of 8 HCA) thrombin-induced platelet aggregation. Pretreatment of HCA with indomethacin (1μM) markedly attenuated the inhibitory effect to -44.8 ± 8.1%, suggesting the importance of endothelial PGI_2 production in the observed antiplatelet aggregatory effect. Activation of human coronary EDRF with the addition of 1μM histamine in these latter indomethacin-treated HCA was also effective in producing a near complete inhibition (-96.9 ± 2.6%) of thrombin-induced platelet aggregation. Presence of atherosclerosis, mechanical disruption of EC or pretreatment with EDRF inhibitor (oxyhemoglobin) selectively attenuated the observed EDRF-induced antiplatelet aggregatory effect. These results suggest that HCA endothelial production of EDRF, in addition to its vasoregulatory action, may also play an important role in the regulation of human platelet aggregation and thrombosis.

P 312 TISSUE PLASMINOGEN ACTIVATOR VARIANTS WITH DUPLICATED KRINGLE DOMAINS. David J. Livingston, William Markland and Daniel Pollock. Integrated Genetics, Inc. Framingham, MA 01701
Tissue plasminogen activator (tPA) is a serine protease which cleaves plasminogen to plasmin in the presence of fibrin. The fibrin affinity of tPA has been reported to reside principally in two domains, the N-terminal "finger" domain and in the kringle 2 domain. We examined how the orientation and interdomain interactions of the kringle 1 and 2 domains in the holoprotein might influence their fibrin affinity. We placed unique restriction enzyme sites in the uterine tPA cDNA and used these sites to duplicate and rearrange the order of the kringle domains. Three variants with duplicated kringle 2 domains showed very low fibrinolytic activity in a fibrin well assay. Two variants with duplicated kringle 1 domains showed fibrinolytic activity greater than the parent protein. Fibrin affinity of the variants in a clot-binding assay was nearly equivalent to the parent protein. Lysine analogs could compete for binding of these variants to fibrin. One variant with a duplicated kringle 1 domain and no kringle 2 domain showed 2/3 of the fibrinolytic activity of the parent protein. We conclude that tPA variants lacking kringle 2 can bind fibrin with high affinity and that these binding interactions involve kringle 1.

P 313 DNAse PROTECTION AND BAND SHIFT ANALYSIS OF HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR GENE PROMOTER REVEALS TWO DISTINCT PROTEIN BINDING DOMAINS, Robert L. Medcalf, Rüegg and §Wolf-Dieter Schleuning, Central Hematology Department, CHUV, CH-1011 Lausanne and §Schering A.G., Biochemistry Institute, 650311, D-1000 Berlin, F.R.G. HeLa cells treated for 24 hours with phorbol-myristate-13-acetate (PMA) produce tissue-type plasminogen activator (t-PA) mRNA. This effect is due to a 15-fold induction of t-PA gene template activity. To identify PMA responsive DNA element(s) within the t-PA gene, we constructed 9 promoter deletion mutants from -1452 to -58 base pairs upstream from the cap-site and fused them to the chloramphenicol acetyl transferase (CAT) reporter gene. PMA inducible CAT activity was lost in transfected cells upon deletion of sequences downstream of position -196. Extracts prepared from non treated and PMA treated HeLa cell nuclei were used for DNase 1 protection experiments. Two specific protein binding sites were localized with both extracts: one containing a cAMP (CRE)-like binding consensus sequence (position-102 to -115), and another one in the first exon (position +60 to +75) containing the conserved AP-2 binding motif. Extracts prepared from nuclei of several cell lines produced similar results. Band shiftanalysis employing double stranded oligomers identical to the two recognition sequences and nuclear extracts prepared from HeLa, Hep G2, HT-1080, U-937, and Bowes melanoma cell lines, demonstrated that the DNA binding activity was sequence specific, and present only in the nuclear fraction. All extracts produced at least two band shifts with oligomer corresponding to the CRE-like consensus. Only a single band shift was observed with the AP-2-like oligomer in all extracts with the exception of extracts from Hep G2 cells, which did not react at all. Hence, at least two distinct nuclear trans-acting factors are involved in the transcriptional regulation the human t-PA gene.

P 314 THE SPECIFIC ACTIVITY OF t-PA DELETION MUTANTS IS STRONGLY DEPENDENT ON THE TYPE OF ASSAY USED; PROBLEMS WHEN INTERPRETING IN VITRO DATA. Gunnar Pohl, Christer Mattsson, Kristina Wikström, Catharina Sterky, Lennart Hansson and Björn Löwenadler, KabiGen AB, and Kabi AB, S-112 87, Stockholm, Sweden.

Six t-PA deletion mutants were constructed, all which lacked the growth-factor (G) domain and the first kringle domain (K1) and had the potential glycosylation site at Asn184 changed to Gln. The mutants varied in presence or absence of finger domain (F) and in the following site mutations: Lys277->Val (V277) and Asn448->Gln (Q448). The molecules were produced in mammalian cells and the specific activity was determined with three different assays using single chain melanoma t-PA as reference.

Fibrin plate assay: All mutants were more active than melanoma t-PA in this assay. The three fingerless mutants had the highest values (3.5-5 fold)

Coupled chromogenic substrate assay. Mutants K2P and K2P-V277 showed low relative values (30%), whereas K2P-Q448 and the three finger-containing mutants had a specific activity which was 90 - 100% of the melanoma t-PA.

Lysis of human plasma clots in vitro: All mutants were less active than melanoma t-PA. The mutants K2P-V277 and FK2P-V277 displayed about 80%, whereas K2P and FK2P had about 60% of the specific activity of the melanoma t-PA. The non-glycosylated variant K2P-Q448 showed an activity of about 50% while FK2P-Q448 was essentially inactive in this assay.

The variable performance of the mutants in vitro complicates predictions of their activity in vivo.

P 315 THE FUNCTIONAL ROLE OF VITRONECTIN (COMPLEMENT S-PROTEIN) AS REGULATORY COMPONENT OF THE HAEMOSTATIC SYSTEM AT THE VESSEL WALL, Klaus T. Preissner, Jürgen Grulich-Henn and Gert Müller-Berghaus, Clinical Research Unit for Blood Coagulation and Thrombosis, Max-Planck-Gesellschaft, D-6300 Giessen (West-Germany).

Vitronectin (VN) (complement inhibitor S-protein) promotes the attachment and spreading of endothelial cells and has been described as potent heparin-binding and -neutralizing plasma protein. Different types of heparinoids including vessel wall-derived heparan sulfate have been shown to be counteracted in their anticoagulant activity by VN. Furthermore, VN is found associated with thrombin-inhibitor complexes in macromolecular ternary products of high molecular weight which still retain the ability to promote attachment of endothelial cells. The association is accompanied by a marked conformational change in the VN-molecule, documented by expression of neo-epitopes or by an increase in binding of VN to other ligands. In addition to plasma, VN has been identified in platelets (Haemostasis 18 (S2), 149; 1988) as well as in the subendothelial cell matrix and appears to be co-distributed with plasminogen activator inhibitor-1 (PAI-1). The procoagulant-like function of VN as major PAI-1-binding protein (JBC 263, 15454; 1988) is not limited to the circulation, since platelet-VN co-elutes with PAI-1 from Zn-chelate-, Con-A-Sepharose and during gel filtration, suggesting a possible complex formation between both platelet components as well. Likewise, both components were detected by a sensitive solid-phase immuno-assay on the intact extracellular matrix of cultured endothelial cells. VN may thereby provide important regulatory functions with respect to the fibrinolytic capacity of the endothelium and is thus unique among the other adhesive proteins, since at sites of inflammation or vessel wall injury the multifunctional repertoire of VN may become locally expressed.

P 316 A SECRETED Ca^{+2}-BINDING GLYCOPROTEIN INHIBITS ENDOTHELIAL CELL SPREADING AND IS EXPRESSED DURING TUBE FORMATION *IN VITRO*, Helene Sage, Luisa Iruela-Arispe, Robert Vernon and Sarah Funk, Department of Biological Structure, University of Washington, Seattle, WA 98195. SPARC (Secreted Protein, Acidic and Rich in Cysteine) is a Ca^{+2}-binding extracellular protein associated with the growth, migration, stress response, and/or differentiation of specific cellular populations. Bovine aortic endothelial cells (BAEC) assumed a rounded morphology in the presence of exogenous SPARC; however, neither attachment nor growth was inhibited. The anti-spreading effect was dose-dependent, non-toxic, cell specific, required Ca^{+2}, and was inhibited by anti-SPARC IgG. The mechanism was RGD-independent and was mediated through interaction with proteins of the extracellular matrix (e.g., type III collagen). BAEC contributing to capillary tube-like structures, which formed spontaneously *in vitro* and were accelerated in the presence of bFGF, stained specifically with anti-SPARC IgG, especially at the tips of the growing tubes. Isolated tubes contained less SPARC mRNA (as percent of total cellular RNA) than subconfluent, actively dividing cells. We propose that SPARC facilitates endothelial cell organization into capillary structures by interaction with components of the vascular extracellular matrix, and that this association is, for certain cells, permissive for cellular migration and/or proliferation.

P 317 A MOLECULAR MECHANISM OF PLATELET ADHESION TO COLLAGEN, Samuel A. Santoro and William D. Staatz, Division of Laboratory Medicine, Washington University School of Medicine, St. Louis, MO, 63110
We have recently defined a Mg^{++}-dependent mechanism of platelet adhesion to collagen which supports a far greater rate and extent of adhesion than does the more intensively studied divalent cation-independent mechanism. Mg^{++}-dependent adhesion of platelets is inhibited by Ca^{++}, is supported by both monomeric and fibrillar collagens, although only the latter supports platelet activation, is supported by types I, II, III, and IV collagen, but not by type V collagen or gelatin, and is not inhibited by RGDS- or RGDT-containing peptides. A monoclonal antibody directed against the Ia component of the platelet membrane Ia-IIa complex specifically inhibits Mg^{++}-dependent adhesion of platelets to collagen. The Ia-IIa complex was purified by affinity chromatography on Concanavalin A-Sepharose and collagen-Sepharose and incorporated into phosphatidyl choline liposomes. The liposomes adhered in a Mg^{++}-dependent manner to collagen, but not to other adhesive proteins. This adhesion duplicated the properties of the Mg^{++}-dependent adhesive mechanism of intact platelets and was also inhibited by the anti-Ia monoclonal antibody. Ca^{++} inhibited adhesion of the liposomes via a simple linear noncompetitive mechanism associated with an alteration of divalent cation-dependent structure within the receptor. We have shown that the platelet Ia-IIa complex is identical to the VLA-2 complex on cells such as lymphocytes and fibroblasts and that the complex likely functions as a Mg^{++}-dependent collagen binding protein on these other cells.

P 318 ADHESION OF BLOOD CELLS TO ENDOTHELIUM IS INHIBITED BY TRANSFORMING GROWTH FACTOR β (TGF-β), Mathew A. Vadas and Jennifer R. Gamble, Division of Human Immunology, Institute of Medical and Veterinary Science, Box 14, Rundle Mall P.O., Adelaide, South Australia, 5000.
The attachment to the endothelium is an essential step in the movement of cells from the circulation into areas of inflammation. Cytokines, such as tumour necrosis factor (TNF) and interleukin-1 increase the adhesive character of endothelial cells resulting in enhanced attachment of neutrophils, lymphocytes and monocytes. We have recently shown (Science 242:97, 1988) that another cytokine, TGF-β inhibits the basal level of adherence of neutrophils to endothelium and is also able to alter the adhesive response of endothelium to TNF. The decrease in adhesive phenotype of endothelial cells following exposure to TGF-β is lost on continued passage of these cells in culture being evidence on 3-6 day old ('young') but not 9-14 day old ('old') endothelial cells. These findings are now extended to resting and stimulated lymphocytes, in which the basal and TNF-α or IL-1 stimulated adhesion is decreased by TGF-β. These phenomena are again only observed on 'young' endothelium suggesting a maturational change in the cells. Thus, TGF-β is the first cytokine described which renders the endothelium non-adhesive to inflammatory cells. This situation may be highly desirable at sites of vascular injury or trauma undergoing extensive endothelial regeneration.

P 319 ISOLATION AND CHARACTERIZATION OF THE HUMAN UROKINASE INHIBITOR GENE (PAI-2) LOCATED ON CHROMOSOME 18 AT 18q21.2-22.

Andrew C. Webb[1], Julie A. Samia[1], Sharon E. Perez[1], Kristin W. Horton[1], Philip E. Auron[2], Thomas B. Shows[3] and Mary G. Byers[3]. [1]Dept. of Biol. Sci., Wellesley College, Wellesley MA 02181; [2]The Arthritis Unit, Massachusetts General Hosp. East Campus, Charlestown MA 02129; [3]Dept. of Human Genetics, Roswell Park Memorial Inst., Buffalo NY 14263.

The molecular cloning of human urokinase-type plasminogen activator inhibitor (PAI-2) by isolation of cDNAs from both monocyte/macrophages and placental sources has been reported previously. Our 1.9kb cDNA encoding the 415 amino acid precursor to PAI-2 was isolated from a lipopolysaccharide (LPS)-stimulated peripheral blood monocyte library (Webb *et al.*, J. Exp. Med. 166: 77, 1987). Restriction fragment probes from this cDNA insert were used to localize the PAI-2 gene to its chromosomal locus and isolate genomic clones. *In situ* hybridization to metaphase chromosomes assigned the PAI-2 gene to the long arm of chromosome 18 at bands 18q21.2-q22. Southern blot analysis and DNA sequencing of genomic clones revealed an organization interrupted by at least six introns and spanning about 9kb of chromosomal DNA. The structure and function of putative PAI-2 promoter elements are being assessed by fusion of genomic sequences to the CAT (chloramphenicol acetyltransferase) reporter gene. Regulation and transcriptional activation of the PAI-2 gene is compared to the interleukin-1β gene, another prominent monocyte/macrophage gene responsive to the same stimulants (e.g. LPS, PMA).

P 320 TRANSCRIPTIONAL REGULATION OF TYPE I PLASMINOGEN ACTIVATOR INHIBITOR IN HEP G2 CELLS BY EPIDERMAL GROWTH FACTOR.

Donald R. Westerhausen, Jr. William E. Hopkins, Burton E. Sobel, and Joseph J. Billadello, Cardiovascular Division, Washington University School of Medicine, St. Louis, MO 63110

Type I plasminogen activator inhibitor (PAI-1) is the primary physiologic inhibitor of tissue-type plasminogen activator (t-PA) and may play an important role in limiting thrombolysis after acute myocardial infarction by attenuating the activity of t-PA after infusion. We have shown that Epidermal Growth Factor (EGF) regulates the expression of steady state levels of PAI-1 mRNA in Hep G2 cells. To determine whether this effect of EGF is mediated at the level of transcription we performed nuclear run-on assays. Hep G2 cells were grown to confluence and subsequently stimulated with EGF (5 ng/ml) or maintained under control conditions. Nuclei were harvested and nuclear run-on assays were performed with 1.0-2.5 x 10^7 unstimulated and stimulated nuclei in parallel. Nascent transcripts labeled with [α^{32}P]UTP were isolated after centrifugation through a CsCl gradient and hybridized to cDNA clones encoding PAI-1, glyceraldehyde-3-phosphate dehydrogenase (GAP), and Factor X immobilized on nitrocellulose membranes. EGF stimulation resulted in a 10-fold increase in PAI-1 gene transcription rate within 3 hr (n = 3 experiments). This result is concordant with the 10-fold increase in steady state concentrations of PAI-1 mRNA determined by dot blotting. The relative transcriptional rate of GAP and Factor X decreased 50%. After incubation of nuclei with α-amanitin (2 μg/ml) transcripts of PAI-1, GAP, and Factor X genes were undetectable. Thus, the expression of the PAI-1 gene in Hep G2 cells is mediated transcriptionally by EGF.

P 321 PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN PROTEIN C FROM 3 MAMMALIAN CELL LINES.

S. Betty Yan, P. Razzano, B. Chao, D.B. McClure and B.W. Grinnell, Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285

Recombinant human Protein C (rHPC), a vitamin K-dependent serine protease, possessing antithrombotic activity was expressed in 3 adenovirus-transformed cell lines; 293 cells (human kidney), AV12 cells (hamster) and BHK cells (hamster). The anticoagulant activity of rHPC in the culture media from 293 cells was fully active while the rHPC from AV12 and BHK cells were partially active. 9 residues of γ-carboxyglutamates (Gla) were found in purified rHPC from 293 and BHK cells. rHPC from AV12 cells were separated into 2 main fractions by pseudo-affinity column chromatography on anion exchange resin ($CaCl_2$ gradient). The rHPC fraction containing 9 Gla has full anticoagulant activity while rHPC fraction containing 6-7 Gla has only 20% anticoagulant activity. The rHPC from all 3 cell lines has the correct N-terminal protein sequence, and 0.5-0.9 residues of erythro-β-hydroxyaspartate. The sugar contents of rHPC, a glycoprotein, from all 3 cell lines were quite different from that of plasma derived HPC. The fucose content was 3-5 fold higher in rHPC. There was also the presence of N-acetylgalactosamine in rHPC. The role of carbohydrate in rHPC is unclear. The reason for the low anticoagulant activity of rHPC from BHK cells is also not known.

Adrenergic and Muscarinic Receptors; Signal Transduction, Ion Channels and Contractility

P 400 ANGIOTENSIN II RECEPTOR MEDIATED STIMULATION OF INOSITOL PHOSPHATES AND CYTOSOLIC FREE CALCIUM IN CULTURED CHICK MYOCYTES, Kenneth M. Baker, Harold A. Singer, and Joseph F. Aceto, Weis Center for Research, Geisinger Clinic, Danville, PA 17822.

We have previously described positive inotropy and increased levels of inositol-1-phosphate as in vitro responses to angiotensin II (AII). AII receptor-mediated stimulation of inositol phosphates was quantified following separation, by high performance liquid chromatography, in cultured chick heart cells prelabeled with L-myo-[1,2-^{3}H(N)]-inositol. A time course indicated that peak responses of AII (10^{-8} M) stimulated increases in inositol-1,4,5-trisphosphate and inositol-1,3,4-trisphosphate were at 15 and 30 seconds, respectively. AII (10^{-8} M) significantly stimulated inositol-1,4-diphosphate (48 ± 26%; basal 6183, stimulated 8972 dpm/mg protein) and inositol-1,4,5-trisphosphate (81 ± 8%; basal 4124, stimulated 7327 dpm/mg protein) above basal levels. There were also observed increases in inositol-1,3,4-trisphosphate and putatively inositol-1,3,4,5,6-pentakisphosphate. AII-stimulated increases in myocyte free cytosolic calcium were monitored with the fluorescent calcium indicator dye Fura-2. There was a biphasic increase in cytosolic free calcium in response to AII (10^{-9} - 10^{-6} M). The peak transient response in cytosolic free calcium after addition of AII (10^{-7} M) occurred at 24 ± 4 sec and was 2.25-fold (396 ± 45 nM) above basal (176 ± 17 nM) levels. The calcium response was blocked or reversed by addition of verapamil (10^{-7} M), lanthanum (0.2 mM) and zero calcium buffer. Bordetella pertussis toxin treatment of myocyte cultures in doses (500 ng/ml) shown to fully ADP-ribosylate 39 and 41 KD α subunits, completely blocked the AII-stimulated increases in inositol-1,4-diphosphate, inositol-1,4,5-trisphosphate, and inositol-1,3,4,5-tetrakisphosphate. The rise in cytosolic free calcium in response to AII was not blocked by Bordetella pertussis toxin pretreatment. Toxin treatment also resulted in the conversion of a two-site ^{125}I-AII binding model (K_{d1} = 0.4 nM, N_1 = 17 fmole/mg protein; K_{d2} = 9.1 nM, N_2 = 58.2 fmole/mg protein) to a single low affinity site K_{d1} = 18 nM; N_1 = 37 fmole/mg protein). These data are not consistent with inositol-1,4,5-trisphosphate regulation of cytosolic free calcium in response to AII in cultured chick myocytes. AII stimulation of cytosolic free calcium appears dependent on external calcium sources.

P 401 THE EFFECTS OF ANGIOTENSIN II AND ADENOSINE ON THE PHOSPHORYLATION OF MYOSIN LIGHT CHAIN IN CULTURED VASCULAR SMOOTH MUSCLE CELLS FROM NORMAL AND HYPERTENSIVE RATS. Hanna Benze, Hsing-Yi Yang, F. Patrick Ross* and Clive Rosendorff, MRC Units for Cardiovascular Research and Mineral Metabolism, University of the Witwatersrand, Medical School, Johannesburg, South Africa.

The regulation of smooth muscle contraction and relaxation is associated with the phosphorylation-dephosphorylation of the 20 kD myosin light chain (MLC). We investigated the effects of angiotensin II and adenosine on the phosphorylation of MLC in primary cultures of smooth muscle cells (SMC) derived from aorta and mesenteric artery of control (WKY) and hypertensive rats (SHR). Extracts of cells incubated with ^{32}P were subjected to 4M urea-SDS electrophoresis, followed by autoradiography and laser densitometry. SHR mesenteric SMC showed significantly higher basal levels of phosphorylated MLC (MLC-P) than SMC from WKY mesenteric artery and SHR aorta. Angiotensin II (1 or 10nM) caused the same increase (30%-50% above basal values) and adenosine (10^{-5}M) the same degree of dephosphorylation (30-40%) of MLC-P in SHR and WKY. The peak time for both effects was 5 min after addition of the drug. These results suggest that the ability of cultured SMC to contract in response to AII or to relax in the presence of adenosine is unaltered in hypertension. However, the basal contractile states of SMC from SHR and WKY show differences which might be related to the pathogenesis of genetic hypertension.

P 402 DIFFERENTIAL REGULATION OF RIGHT AND LEFT VENTRICULAR ß-ADRENERGIC RECEPTORS DURING CHRONIC HYPOXEMIA. Daniel Bernstein, Ellen Voss, Sheila Huang. Department of Pediatrics, Stanford University, Stanford, CA 94305. To determine whether chronic hypoxemia secondary to an intracardiac right-to-left shunt alters regulation of the myocardial ß-adrenergic receptor/adenylate cyclase system, we produced chronic hypoxemia in 9 newborn lambs by creating right ventricular outflow obstruction and an atrial septal defect. Oxygen saturation was reduced to 65-74% for 2 weeks. Eight lambs served as normoxemic controls. ß-receptor density (B_{max}) was determined with the radioligand [^{125}I]iodocyanopindolol in both membrane and light vesicle preparations and adenylate cyclase activity determined during stimulation with isoproterenol, sodium fluoride (NaF), and forskolin. During chronic hypoxemia, B_{max} decreased 45% (hypoxemic, 180.6 ± 31.5 vs. control, 330.5 ± 60.1 fmol/mg) in membranes from the left ventricle (exposed to hypoxemia alone) but was unchanged in the right ventricle (exposed to hypoxemia and pressure overload). The percent of total receptors isolated from the light vesicle fraction was increased in the hypoxemic left ventricles (22 ± 9 %) vs. controls (11 ± 4 %). Ligand affinity (K_D) was not different from control in either ventricle. Left ventricular isoproterenol-stimulated adenylate cyclase activity was decreased by 39% (30.0 ± 4.3 % increase vs. 44.1 ± 9.5 % increase) whereas right ventricular adenylate cyclase activity was unchanged. Stimulation of adenylate cyclase with NaF or forskolin was also not different from control. Circulating epinephrine was increased four-fold in the hypoxemic lambs whereas norepinephrine, T3 and Free T4 were not different from control. These data demonstrate a down-regulation of the left ventricular ß-receptor/adenylate cyclase system associated with an increase in the fraction of internalized receptors during chronic hypoxemia secondary to an intracardiac right-to-left shunt. Furthermore, the expected up-regulation of the right ventricular ß-receptor in the face of an afterload stress failed to occur. We speculate that these abnormalities of cardiac autonomic control may contribute to the decreased ventricular performance seen in patients with cyanotic congenital heart disease.

P 403 VARIABLE RECEPTOR AFFINITY HYPOTHESIS, John A. Bevan, Rosemary D. Bevan and S. Martin Shreeve, Dept. of Pharmacology, Univ. of Vermont, Burlington, VT 05405
Measurements of the contraction of a variety of arteries from three mammalian species to norepinephrine under a variety of circumstances provide evidence that tissue sensitivity and affinity of the α_1-adrenoceptor for norepinephrine co-vary over a range of several orders of magnitude. Variation occurs between species and strains, between different arteries of the same species and with development and disease. The quantitative relationship between sensitivity and affinity suggests that differences in the former can, to a great extent, account for differences in the latter. Furthermore, the variation in affinity appears to be continuous and thus does not provide a basis for functional subdivision of the receptor. There is also evidence that adrenergic antagonist affinity can vary significantly in different blood vessels. The factors that might account for this variation include differences in receptor chemical structure, in the local membrane microenvironment and in a number of intracellular processes. The hypothesis of variable receptor affinity proposes that what appears to be the same receptor on the basis of standard pharmacological criteria may at different times and in different cells exhibit different affinities and that this feature is a major determinant of tissue sensitivity. If this is correct then variation in receptor affinity is an important functionally relevant variable which could account for selectivity of tissue responses to circulating hormones and may represent a mechanism of change in the intact organizm and in disease.

P 404 MOLECULAR CLONING OF SMOOTH MUSCLE CALDESMON, Joseph Bryan, Michiyo Imai, Robyn Lee, James Paul Moore, Wen-Gin Lin and Richard Cook, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030
We have sequenced overlapping clones from plasmid and λ phage smooth muscle cDNA libraries that code for the large form of caldesmon from chickens. The entire sequence is 4107 bp; ~235 bp of 5′ untranslated region, 2127 bp of coding sequence and about 1750 bp of 3′-untranslated sequence. The coding sequence specifies a protein of 709 amino acids with a molecular weight of about 82,000, substantially less than the previous SDS gel estimates of 120-150,000. The anomalous molecular weight estimate in SDS can be correlated with the high glu content (23%). The actin, tropomyosin and calmodulin binding 'head' region, the C-terminal $\frac{1}{3}$rd of the molecule, has a 58 residue sequence with a strong similarity to the Ca^{2+} insensitive tropomyosin binding site of troponin T, but no obvious similarities to known calmodulin binding proteins. The N-terminal tail region contains a repetitive sequence which begins near residue 250 and consists of a 13 amino acid sequence repeated eight times. The general repeat motif is *-(glu)$_3$-(lys/arg)$_2$-(ala)$_2$-(glu)$_2$-(lys/arg)$_1$-(X)-(lys/arg)$_1$-(ala)$_1$-*; where X is glu, gln or ala. The 'head' region has been expressed in a bacterial system and has been shown to bind to calmodulin-Sepharose in a Ca^{2+} dependent manner. Expression of the entire coding sequence yields a protein with an apparent molecular weight of 120-130,000 in SDS gels. We are using the expressed peptides to do additional domain mapping studies. This work was supported by GM26091, HL26973 and funds from MDA.

P 405 ALPHA$_1$- AND ALPHA$_2$-ADRENOCEPTOR-SPECIFIC EFFECTS OF COOLING ON CONTRACTIONS IN THE ISOLATED RAT TAIL ARTERY. Christian T. Harker, Department of Surgery, Oregon Health Sciences Univ., Portland, OR, 97201.
Blood-flow through a rat's tail provides a means of controlled heat loss for thermoregulation. Experiments were designed to assess the effects of acute moderate cooling on postjunctional alpha$_1$- and alpha$_2$-adrenoceptors in isolated segments of arteries from rat tails. Rings of tail arteries from Sprague-Dawley and spontaneously hypertensive rats were suspended in physiological salt solution for the recording of isometric force at 37 or 24 C. Bath medium was gassed with 95% O_2/5% CO_2. Rings were contracted with norepinephrine (NE; 10^{-9}- 10^{-4}M) alone or in the presence of prazosin (Pz; $3x10^{-7}$M) and/or rauwolscine (Rw; 10^{-7}M). Both of these selective antagonists caused rightward shifts of the NE concentration-response curves although alpha$_1$-blockade (Pz) was more effective than was alpha$_2$-blockade (Rw). Cooling caused a significant inhibition of contractile force under control conditions as measured by that concentration of NE required to evoke a contraction equal to 30% of maximal (EC_{30}). This was also true in rings treated with Pz and those treated with both Pz and Rw. In those rings treated with Rw alone, the inhibition caused by cooling was not significant. The results suggest that this vessel possesses both alpha$_1$- and alpha$_2$-adrenoceptors postjunctionally and that neither subtype is potentiated by cooling.

P 406 CLONING AND EXPRESSION OF THE RAT BRAIN TYPE III SODIUM CHANNEL GENE, R.H. Joho, J.R. Moorman, A.M.J. VanDongen, G.E. Kirsch, H. Silberberg, G. Schuster, and A.M. Brown, Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.
Voltage-gated Na channels are membrane-bound proteins and when they are open Na^+ ions diffuse through them at a rapid rate. These channels, which are responsible for generation and propagation of the action potential are the products of a multigene family. At least three cDNA clones for Na channel subtypes from rat brain have been isolated and sequenced, and distinct isoforms are believed to exist in the heart and skeletal muscles based on immunological and RNA hybridization studies. We have assembled, in a transcription-competent vector, a full-length cDNA clone encoding the rat brain type III Na channel. After in vitro synthesis of mRNA, microinjected Xenopus oocytes expressed functional rat brain Na channels from such "cloned" RNA transcripts. We have determined by two-electrode voltage-clamp studies the approximate macroscopic behavior of the type III Na channel. The current threshold is about -30 mV, and the peak of the current-voltage relationship (I-V curve) is about -5 mV. The current peaks at more depolarized test potentials and decays more slowly than that induced by total brain RNA. The K_d for block by tetrodotoxin is 2 nM. An α-scorpion toxin induced an increase in peak current and a new, non-inactivating current component. A β-scorpion toxin shifted activation gating by -25 mV. We are now characterizing the single-channel gating properties of the cloned type III channel in microinjected oocytes.

P 407 GUANINE NUCLEOTIDE SENSITIVITY OF G-PROTEINS COUPLED TO ADENYLATE CYCLASE INHIBITION AND PHOSPHOINOSITIDE HYDROLYSIS IN CHICK HEART CELLS, Linda G. Jones, JoAnn Trejo and Joan Heller Brown, Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093. We have shown that stimulation of muscarinic receptors in dissociated embryonic chick heart cells (CHC) promotes the hydrolysis of phosphoinositides (PI) and inhibits the production of cyclic AMP. The inhibition of adenylate cyclase (AC) is mediated through a guanine nucleotide-binding protein, G_i , and is pertussis toxin (PT)-sensitive. The hydrolysis of the phosphoinositides is PT-insensitive and is presumably mediated through a different G-protein which remains unidentified. We have previously used a permeabilized cell preparation to demonstrate the stimulation of PI hydrolysis by guanine nucleotides providing evidence for G-protein mediation of this response (Jones et al. Circ. Res. 62:299, 1988). We are currently using the permeabilized cell preparation to compare the effects of guanine nucleotides on PI hydrolysis and inhibition of adenylate cyclase in order to define differences in the G-proteins involved. We observe that overnight pretreatment with PT (100 ng/ml) reverses the inhibition of adenylate cyclase (stimulated with isoproterenol or forskolin) by GTP (100 μM) and/or carbachol (1 mM) in permeabilized CHC but does not inhibit guanine nucleotide-stimulated PI hydrolysis. Preliminary results indicate that maximal inhibition of forskolin-stimulated AC by GTPγS occurs at a 10 nM concentration. However, stimulation of PI hydrolysis by GTPγS is half-maximal at 1 μM and is not maximal until 100 μM. In contrast, GTP has the same potency (half-maximal at 100 μM) for both the inhibition of forskolin-stimulated AC and the stimulation of PI hydrolysis. The data suggest a difference in sensitivity for guanine nucleotides of the G-proteins which mediate these two responses.

P 408 MOLECULAR CLONING AND CHARACTERIZATION OF THE HUMAN VENTRICULAR MYOSIN ALKALI LIGHT CHAIN GENE. Masahiko Kurabayashi, Yoshikazu Shibasaki, Issei Komuro, Hidetsugu Tsuchimochi, and Yoshio Yazaki. The Third Department of Internal Medicine, University of Tokyo, Tokyo, Japan.
We have previously shown that the expressions of the β-myosin heavy chain (β-MHC) and ventricular myosin alkali light chain (VLC1) genes are upregulated by pressure overload in human atria. To identify the *cis*-regulatory elements responsible for the isomyosin gene switching, we have cloned and sequenced the gene encoding the human VLC1 gene. Primer extension and S1 nuclease analyses indicate that the major transcription initiation site is located 54 base paires (bp) upstream of the translation initiation codon in both ventricle and slow skeletal muscle. This finding suggests that both tissues use the same transcriptional promoter in different regulatory programs. Within the 686-bp 5'-flanking region, there are 28-bp alternating purine/pyrimidine sequences and two upstream segments exhibiting homology to consensus sequence proposed for viral and several cellular enhancer elements. A comparison of the VLC1 upstream gene sequence with those available for several muscle-specific genes reveals three CCArGG elements and CATTCCT sequence are present in the VLC1 gene promoter region.

P 409 POTASSIUM CHANNEL OPENERS PREFERENTIALLY ATTENUATE NEURALLY-MEDIATED PRESSOR RESPONSES IN PITHED RATS. Rodney W. Lappe, Joseph L. Dinish and George Oshiro. Division Experimental Therapeutics, Wyeth-Ayerst Research, Princeton, N.J. 08540.

The postossium channel openers, chromakalim (C) and pinacidil (P), reduce arterial pressure and vascular resistance by increasing the eflux of potassium from vascular smooth muscle. Previous studies have demonstrated that C is three-fold more potent than P (ED_{30}=80 and 250 ug/Kg, respectively; after i.v. injection in conscious SHR). In the present study, the effects of C and P on pressor responses to endogenous vasoconstrictor stimuli were examined in pithed rats. Changes in arterial pressure and heart rate to increasing intravenous doses of norepinephrine (NE) and angiotensin II (AII) and increasing frequencies of electrical stimulation of the sympathetic nerves (spiral cord stimulation) were monitored before and after intravenous administration of vehicle (V; 20% ethanol in saline), C(1-250 ug/Kg) or P(250-750ug/Kg). V had no effect on the pressor responses in the pithed rats. Injection of C at low doses (1ug/Kg) significaly attenuated the pressor responses to nerve stimulation (Max Decrease=-19$\pm$ 3mmHg) without altering responses to NE or AII. At higher doses of C (80 and 250ug/Kg), the selective effects on neurally-mediated pressor responses were lost, as C markedly suppressed the pressor actions of all three vasoconstrictor stimuli. P failed to alter any of the pressor responses at low doses (250-500ug/kg), but the highest dose of P selectively reduced neurally-mediated pressor responses (Max decrease=-31$\pm$4mmHg). These data indicate that potassium channel openers appear to preferentially antagonize neurally-mediated pressor responses in pithed rats. This interaction may contribute, in part, to the hypotensive actions of the compounds.

P 410 EXPRESSION AND PURIFICATION OF RECOMBINANT MURINE CARDIAC TROPONIN C. Jeffrey M. Leiden, I-Cheng Ho, David Ginsburg, and Michael S. Parmacek. Howard Hughes Medical Institute, and Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI.

Recent studies have suggested that cardiac troponin C (cTnC), the calcium binding subunit of the thin filament, is directly involved in mediating the increases in myocardial contractility observed with increasing sarcomere length (ie. the Frank Starling law). To better understand the structural basis of cTnC function, a full-length murine cTnC cDNA has been isolated. In order to express large amounts of recombinant cTnC in bacteria, the cTnC cDNA was cloned into the pRIT2T prokaryotic expression vector which promotes the expresssion of a Staphylococcal Protein A-cTnC fusion protein from the lambda P_R promoter. In addition a variant of this plasmid was constructed in which a synthetic oligonucleotide encoding a collagenase-cleavable sequence was introduced between the protein A and cTnC coding sequences in order to allow subsequent separation of the two proteins. These two plasmids were introduced into the Y1090 lon^- strain of E. Coli which had been previously cured of the pMC9 plasmid. SDS-PAGE analysis of crude lysates from these bacteria showed that up to 30% of the total protein produced by each strain consisted of the protein A-cTnC fusion protein. The fusion proteins were purified to homogeneity by immunoglobulin-Sepharose chromatography. Purified cTnC was produced by digestion of the immunoglobulin-Sepharose-immobilized fusion protein (containing the collagenase-cleavable sequence) with collagenase. This method of large scale production of purified recombinant cTnC should facilitate structure-function studies of this physiologically important molecule.

P 411 CLONING OF THE CALCIUM RELEASE CHANNEL cDNA FROM RABBIT SKELETAL MUSCLE. Andrew R. Marks, Paul Tempst, Kwang Hwang, Makoto Inui, Christopher C. Chadwick, Sidney Fleischer and Bernardo Nadal-Ginard, Harvard Medical School, Boston, MA 02114. The calcium release channel (CRC), or ryanodine receptor, has been purified from rabbit skeletal muscle sarcoplasmic reticulum (SR). This purified protein (M_r 600,000) is equivalent to the foot structures in the junction of the transverse tubules with the terminal cisternae of SR, and has Ca^{2+} release channel characteristics when incorporated into lipid bilayers. We have obtained amino acid sequence of peptide fragments generated by digestion of the intact CRC and synthesized oligonucleotide primers to construct specific primed cDNA libraries. Several cDNA clones have been isolated which encode the sequences of one or more of the previously sequenced peptides. Northern blot hybridization reveals a 16kb message in rabbit skeletal, and smooth muscle. Elucidation of the molecular structure and function of the CRC should provide further understanding of the mechanisms of excitation-contraction coupling.

P 412 **Functional Coupling and Differential Regulation of Two Na^+,K^+-ATPase Isoforms in Rat Neonatal Cardiocytes**, R.M. Medford, U.Ikeda, T.W. Smith and M. O'Neill, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

At least three physiochemically distinct isoforms of the Na^+,K^+-ATPase (NAKA) α subunit are expressed in a variety of rat tissues and cell lines. The differential regulation of these isoforms by developmental, tissue-specific, ionic and hormonal factors implies a distinct functional role, and molecular regulatory mechanism, for each NAKA isoform. Although several tissues, including the heart, express multiple NAKA isoforms, it is not known whether this represents subpopulations of cells expressing only one isoform or whether different isoforms are expressed simultaneously in the same cell. In vitro rat neonatal cardiocytes (RNC) represents an attractive model in which differential regulation of multiple NAKA isoforms may be studied at both the molecular and functional level. Radiolabelled isoform specific cDNA probes were used to identify and quantitate the expression of α-1, α-2, α-3 and β subunit mRNAs in RNC by Northern filter hybridization. Relative mRNA levels for α-1: α-2: α-3: β were in an approximate ratio of 1.0: 0.0: 0.2: 0.2, consistent with in vivo neonatal hearts. Initial studies demonstrate that the α-1 and α-3 isoforms are also expressed at the sarcolemma and both are functionally coupled to cardiocyte contractility. Changes in amplitudes of contraction of electrically paced single neonatal cardiocytes to increasing concentrations of ouabain, was determined using a phase-contrast optical-video system previously described (J Physiol 325:243,1982). A biphasic, positive inotropic effect was evident in single cells, with EC50's of approximately 0.05 and 10.0 μM ouabain, consistent with the expression of both a ouabain-sensitive (α-3) and ouabain-resistant (α-1) isoform, respectively. This strongly suggests that multiple, functional NAKA isoforms may be expressed in the same cardiac cell and that α-3 is a ouabain-sensitive isoform. In RNC grown for 24 hours in serum-free media, thyroid hormone induces both α-1 and α-3 genes, 3-5 fold, at the level of mRNA accumulation. However, preliminary studies demonstrate that basal α-3, but not α-1, gene expression is dependent on the presence of thyroid hormone in the media. These results suggest that both the α-1 and α-3 genes are differentially regulated within the same cardiac cell in response to hormones and/or serum-growth factors.

P 413 INHIBITORY EFFECTS OF PHOSPHATE AND ARSENATE ON CONTRACTION OF SKINNED SLOW- AND FAST-TWITCH SKELETAL AND CARDIAC MUSCLE: INTERACTION WITH pH. Thomas M. Nosek and Robert E. Godt. Dept. of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912-3000

The effect of phosphate (Pi) on the contractile apparatus of muscle is important because: i) it is a key product of the ATPase reaction; ii) the intracellular level of Pi increases markedly with fatigue or hypoxia; iii) the depression of maximum calcium-activated force (Fmax) by Pi is thought to be due to reversal of the force-producing step of the cross-bridge cycle which leads to a decrease in the number of attached bridges. However, whether a particular ionic species of Pi (i.e $H_2PO_4^{-1}$) is responsible for these effects is controversial and critical to our understanding of the cross-bridge cycle. To clarify this issue, we examined the influence of Pi and its structural analog arsenate (Asi) on contraction of skinned rabbit psoas (fast-twitch), soleus (slow-twitch), and cardiac papillary muscle. Asi decreased Fmax of all three muscle types to a greater extent than Pi. Both Pi and Asi decreased calcium sensitivity of psoas and cardiac muscles, with Asi having the greater effect. The effect of the protonated forms of Pi and Asi on Fmax were evaluated by measuring the response to 30 mM total Pi or Asi at pH 7.4, 7.0, 6.6, and 6.2. In psoas fibers we found that both Pi and Asi were more effective in decreasing Fmax as the pH was lowered (i.e. as the concentration of the diprotonated form increased). On the contrary, soleus and cardiac fibers did not exhibit this behavior. These differences in the effects of Pi and Asi on Fmax in psoas vs. cardiac and soleus muscles, may be related to known differences in their myosin heavy chains. (Support: NIH grants HL/AR 37022 (TMN)) and AR 31636 (REG).

P 414 SEQUENCE ANALYSIS AND CHARACTERIZATION OF A 13.8 kDa PROTEIN INHIBITOR OF PROTEIN KINASE C, J.D. Pearson, D.B. DeWald, N.M. Mozier, H.A. Zurcher-Neely, R.L. Heinrikson, E.D. Fraser*, J.R. McDonald*, M.P. Walsh*, and W.R. Mathews, Biopolymer Chemistry Department, The Upjohn Company, Kalamazoo MI 49001 USA, and *Department of Medical Biochemistry, University of Calgary, Alberta T2N 4N1 Canada

Protein kinase C (PKC) is a widely studied calcium- and phospholipid-dependent enzyme that is involved in a number of cellular activities including transmembrane signaling, growth, and differentiation. Inhibitors of PKC are important tools for decoupling PKC-mediated events. In addition to establishing cellular roles for PKC, PKC-specific inhibitors may be of therapeutic importance in the treatment of cancer and hypertension and as muscle relaxants. An inhibitor isolated from bovine brain and found in many other tissues has been recently described (1), and we have now determined the complete primary structure of this novel protein.

The inhibitor has a pI of 6 and is blocked at the N-terminus. Chemical and enzymatic cleavage of the purified inhibitor followed by gas-phase sequence and FAB-MS analysis of derivative peptides has established that the protein consists of 125 amino acid residues, and has a molecular mass of 13,765. The inhibitor does not show significant sequence homology with any other known protein in the data base.

The protein was found to be an uncompetitive inhibitor of PKC with a K_m (ATP) of 10.6μM and a K_i of 0.46μM. Dot blot analysis showed that the inhibitor was a zinc-binding protein.

(1) J.R. McDonald *et al.* Biochem. J. **242** (1987) 695-705.

P 415 PROTEIN KINASE C ACTIVATION REDUCES THE INTRACELLULAR CALCIUM THRESHOLD FOR AGONIST-STIMULATED PROSTACYCLIN RELEASE. Jeremy D. Pearson, Thomas D. Carter & Trevor J. Hallam[1], MRC Clinical Research Centre, Harrow, Middx, U.K. & [1]Smith Kline & French Research, Welwyn, Herts, U.K.

Agonist-stimulated prostacyclin (PGI_2) release from human umbilical vein endothelial cells (HUVEC) is normally driven by elevations of intracellular calcium, $[Ca^{++}]_i$, above a threshold of 0.8-1μM (Carter et al, Br.J.Pharmacol. 94, 1181, 1988; Hallam et al, Biochem.J. 251, 243, 1988). Protein kinase C activation by phorbol 12-myristate 13-acetate (PMA), however, potentiates ionophore-induced PGI_2 release (Demoll & Boeynaems, Prostaglandins, 35, 243, 1988) but inhibits agonist-stimulated inositol lipid hydrolysis and $[Ca^{++}]_i$ elevations in endothelial cells (Brock & Capasso, J.Cell.Physiol. 136, 54, 1988). We have now studied the effect of PMA on agonist-induced PGI_2 release and determined how it is related to changes in $[Ca^{++}]_i$. Under conditions in which PMA alone did not induce PGI_2 release, pretreatment with PMA caused a dose dependent (EC_{50}=5nM) potentiation of the transient PGI_2 release induced by submaximal doses of agonists (0.02U/ml thrombin, 10nM bradykinin, 0.5μM histamine, 10μM ATP). Maximum potentiation (up to 3.6-fold) was achieved within 5-10 min of pretreatment. PMA did not potentiate PGI_2 release in response to exogenous arachidonate (4μM). PMA alone had no effect on resting $[Ca^{++}]_i$. Pretreatment for 5 min with PMA (1-100nM) dose-dependently reduced peak $[Ca^{++}]_i$ in response to all tested concentrations of ATP, while PGI_2 release in response to submaximal doses of ATP (10-100μM) was potentiated and in response to a threshold dose (1μM) was unaffected. Similarly divergent effects of PMA pretreatment on $[Ca^{++}]_i$ and PGI_2 release were found with the other agonists tested. Concomitant measurement of $[Ca^{++}]_i$ and PGI_2 release from cells exposed to graded doses of ionomycin in the absence of extracellular Ca^{++} demonstrated that PMA pretreatment (5 min; 10nM) shifted the Ca^{++} activation curve to the left and decreased the threshold $[Ca^{++}]_i$ from 0.8-1μM to 0.2-0.3μM. Thus activation of protein kinase C has two consequences. First, it reduces the peak $[Ca^{++}]_i$ achieved in response to agonists. Second, it enhances the sensitivity of PGI_2 synthesis to $[Ca^{++}]_i$. The interaction of these two processes is sufficient to account quantitatively for the observed effects of PMA on PGI_2 synthesis.

P 416 RAT CARDIAC SARCOPLASMIC RETICULUM Ca^{2+} + Mg^{2+} ATPase: CHARACTERIZATION OF cDNA CLONES, AND THYROID HORMONE RESPONSIVENESS OF Ca^{2+} ATPase mRNA IN CULTURED NEONATAL MYOCYTES, Daniel K. Rohrer, Wolfgang H. Dillmann, Department of Medicine, UC San Diego, San Diego, Ca 92103

The sarcoplasmic reticulum (SR) Ca^{2+} + Mg^{2+} ATPase of the rat heart contributes greatly to Ca^{2+} sequestration during diastolic relaxation. As the speed of diastolic relaxation is closely coupled to the rate at which Ca^{2+} is sequestered from the cytosol, this enzyme and all other cardiac Ca^{2+} transporting systems assume important roles as regulators of contractility and performance. The Ca^{2+} pumping ability of the SR is augmented under thyroid hormone excess, and coincident with this is an elevated level of the SR Ca^{2+} ATPase mRNA in vivo. To more strictly define the requirements for thyroid hormone regulation of Ca^{2+} ATPase mRNA, rat neonatal myocytes were cultured in serum-free media with and without T_3. Under these conditions, Ca^{2+} ATPase mRNA levels remained unchanged, while another marker for thyroid hormone action, myosin heavy chain α mRNA, was induced. However, when neonatal myocytes were cultured in the presence of hypothyroid calf or rat serum, addition of T_3 to the media resulted in Ca^{2+} ATPase mRNA accumulation, consistent with the degree of regulation seen in vivo. A rat specific cDNA coding for the Ca^{2+} ATPase mRNA was sought after for two reasons: 1) to avoid species differences between rat and rabbit, and 2) to assist in the future isolation and characterization of this gene and possible DNA regulatory elements. λZAP rat cardiac cDNA libraries were screened using a rabbit 3' cDNA clone to the slow SR Ca^{2+} ATPase (gift, D. MacLennan). The longest positive clone, designated pCC1, is approximately 4000 bp in length, contains all coding and 3' non-coding information, and approximately 250 bp of 5' non-coding information. In summary, a full length cDNA was isolated and used to document the T_3 induced alterations in Ca^{2+} ATPase mRNA. This mRNA is uniquely regulated by T_3, requiring serum specific factor(s) for full regulation.

P 417 cDNA CLONING OF HUMAN SARCOPLASMIC RETICULUM CA-ATPASE.

Terrence L. Scott and Ghazala Ali, Dept. of Muscle Research, Boston Biomedical Research Institute and Dept. of Biol. Chem. and Mol. Pharm., Harvard Medical School, Boston, MA 02114.

The complete amino acid sequence of the human sarcoplasmic reticulum Ca^{2+}- ATPase fast-twitch isoform has been deduced from clones obtained from a fetal muscle cDNA library. The human cDNA has significant homology with that of the rabbit ATPase, but with several important differences, among which are large discrepancies in both the 5' and 3' untranslated regions. The deduced amino acid sequence has extensive homology to the rabbit enzyme, but significant differences at the amino terminus and in other regions. In addition, a major portion of the cDNA corresponding to the human slow/cardiac isoform of the ATPase has been sequenced. Comparisons of the human slow vs. fast twitch isoform sequences and between the human and rabbit ATPases will be examined. Supported by NIH RO132247 and an Established Investigatorship of the American Heart Association to TLS.

P 418 PROTEIN KINASE C REGULATION OF SARCOLEMMAL Ca^{2+} PUMP IN VASCULAR SMOOTH MUSCLE CELLS. Munekazu Shigekawa, Ken-Ichi Furukawa and Yuko Tawada. Department of Molecular Physiology, National Cardiovascular Center Research Institute, Osaka 565, Japan.
We examined the effect of phorbol myristate acetate (PMA), a potent activator of protein kinase C (PK-C), on Ca^{2+} extrusion from cultured vascular smooth muscle cells (VSMCs) incubated in the absence of added extracellular Na (Na_o). Previously, we presented strong experimental evidence that the Na_o-independent Ca^{2+} extrusion from VSMCs is effected by the plasma membrane Ca^{2+} pump (J.B.C. 263, 8058-8065, 1988). Two-min pretreatment of VSMCs with 30 to 300 nM PMA suppressed the intracellular Ca^{2+} transient induced with 1 M ionomycin to about 60 % of the control, whereas it accelerated the concomitant Na_o-independent ^{45}Ca extrusion by up to 20 %. When the Ca^{2+} transient was induced with 0.1 M angiotensin II, the PMA pretreatment markedly suppressed it and also slightly reduced the rate of ^{45}Ca efflux from the cells. These effects of PMA were mimicked by 1-oleoyl-2-acetylglycerol, another PK-C activator, but were abolished by prior treatment of the cells with staurosporine, an inhibitor of PK-C, or prior long incubation of cells with PMA. Analysis of the effect of PMA on $[Ca^{2+}]_i$-dependence of the rate of Na_o-independent ^{45}Ca efflux revealed that PMA increased the maximum efflux rate without a significant change in the apparent affinity for Ca^{2+}. These results strongly suggest that the plasma membrane Ca^{2+} pump in VSMCs can be stimulated by PMA and that PK-C is involved in the regulation of $[Ca^{2+}]_i$ in intact VSMCs.

P 419 **DIHYDROPYRIDINE RECEPTOR GENE EXPRESSION: INDUCTION AND REPRESSION OF FUNCTIONAL "SLOW" CALCIUM CHANNELS INVOLVE A PRETRANSLATIONAL MECHANISM.** Hue-Teh Shih, Helene Bigo, John M. Caffrey, and Michael D. Schneider. Molecular Cardiology Unit, Baylor College of Medicine, Houston, TX 77030

To establish whether formation of the dihydropyridine (DHP)-sensitive "slow" calcium channel in skeletal muscle is regulated by pretranslational mechanisms, DHP receptor (DHPR) mRNA abundance was analyzed by Northern hybridization, using synthetic oligonucleotides derived from the rabbit skeletal muscle DHPR cDNA. A 6.5 kb putative DHPR transcript was identified in differentiated mouse C2 myocytes and post-natal C3H mouse skeletal muscle. Little or no DHPR mRNA was detected in proliferating C2 cells, in agreement with our previous measurements of functional "slow" channels and DHP binding sites. DHPR mRNA was induced at least 10-fold after mitogen withdrawal, and was reversibly suppressed by 0.5 nM transforming growth factor β, which blocks the appearance of "slow" channels and DHPR. DHPR mRNA also was induced by mitogen withdrawal in BC3H1 myocytes, but not in BC3H1 cells stably transfected with a mutant *ras* allele that prevents muscle-specific gene expression. In contrast, DHPR gene expression was not prevented by incorporation of activated oncogenes (v-*erb*B, SV40:c-*myc*) which were permissive for myogenesis and Ca^{2+} channel expression. Similar results were seen with α-skeletal actin and myosin light chain-3. However, expression of the nicotinic acetylcholine receptor (AChR) δ subunit was not suppressed by *ras*. DHPR mRNA was detected in 2-day post-natal C3H mouse skeletal muscle and increased 14-fold by 2 months of age. DHPR mRNA abundance failed to change in soleus muscle 10 and 15 days after tibial nerve transection, despite marked up-regulation of the δ AChR transcript. Thus, developmental regulation of "slow" calcium channels, and the effects of growth factors and cellular oncogenes on calcium channel density measured by biophysical techniques and ligand binding, involve control at a pretranslational level. Furthermore, denervation and *ras* exert discordant effects on the genes encoding DHPR and an AChR sub-unit.

P 420 **ISOFORMS OF CARDIAC TNT DIFFERENTIALLY AFFECT CA-SIGNALLING IN CARDIAC TROPONIN,** **R. John Solaro and Saleh C. El-Saleh. University of Illinois, College of Medicine,Chicago, IL 60680 and University of Cincinnati, College of Medicine, Cincinnati, OH 45267.**
Troponin T (TNT), the tropomyosin (TM) binding unit of the troponin (TN) complex is known to exist in striated muscle as a population of isoforms that change with maturation and possibly load of the heart. We investigated the functional significance of these isoforms in preparations from beef heart, in which two variants (TNT-1 and TNT-2) have been characterized. We probed Ca^{2+}-dependent conformational changes by measuring changes in fluorescence of IAANS, a sulfhydryl group directed reagent. Our results indicate that the state of TNT is sensitive not only to Ca-binding to both classes of sites in cardiac TNC, but also to interactions among thin filament proteins. In studies of the Ca-dependent states of the purified isoforms in reconstituted TN, conformational changes of TNT-1 were more closely associated with Ca-binding to the Ca-Mg sites, whereas conformational changes in TNT-2 were more closely associated with Ca-binding to the regulatory site. TN containing pure TNT-2 showed increased affinity for Ca^{2+}, when compared to TN containing either a mixture of TNT isoforms (3 fold) or pure TnT-2 (2 fold). Our results indicate that the relative abundance of TNT-1 may affect structural aspects of the TN complex, whereas the relative abundance of TNT-2 may serve to alter the Ca-activation process.

P 421 EFFECTS OF RYANODINE ON CONTRACTILE PERFORMANCE OF INTACT LENGTH-CLAMPED PAPILLARY MUSCLE, Ferdinand Urthaler, Alfred A. Walker, Russell C. Reeves and Lloyd L. Hefner, Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, Alabama 35294.
Extent, time-course and mechanisms of the negative inotropic effect of ryanodine (R) were examined in 22 length-clamped ferret papillary muscles paced 12/min at 25°C. After 60 min of exposure to R, 5 μM, a new steady state was attained with forces of 15% of maximum twitch force. R does not pharmacologically excise the sarcoplasmic reticulum (SR) in this preparation. R does not appreciably inhibit the ability of the SR to take up Ca^{2+} as evidenced by the potentiated beats obtained after a short pause that are nearly as large after R as before. On comparing equipotent beats before and after R, we found that R increases the rate at which Ca^{2+} is released during the twitch, if the SR Ca^{2+} stores are equal or similar. Evidence: a larger maximum rate of tension rise and briefer time to peak tension after R. Since R both increases the time SR Ca^{2+} release channels are open and also decreases their conductivity, it must follow that the former effect predominates over the latter in our experiments. R increases leakiness of SR during diastole probably by inhibiting closure of SR Ca^{2+} release channels. Evidence: early peak of restitution curves after R, brevity of time required for a rested state contraction after R, small amplitude of steady state contraction at 12/min. The SR leaks even in the absence of R, but if external Ca^{2+} is so high that Ca^{2+} loss from cells is slowed, or a Ca^{2+} leak into cells via sarcolemma cancels SR leak, then the effects of SR leak are minimized. Evidence: the time required for rested state contraction to occur, or slope of descending limb of restitution curve; however, in presence of R even high external Ca^{2+} cannot prevent rapid depletion of SR Ca^{2+} stores. Even though we have presented evidence for a mechanism whereby R increases the number of open SR Ca^{2+} release channels in both systole and diastole, we do not mean to imply that most of them stay open in diastole otherwise the SR would leak too fast to accumulate any Ca^{2+} for the potentiated beat. Thus, probably most channels close after being open a certain length of time, even in the presence of R.

P 422 OPTIMIZED STROKE WORK IN A DYNAMIC IMPEDANCE, ISOLATED NEONATAL NZW RABBIT WORKING (EJECTING) HEART MODEL USING AN AGE-APPROPRIATE, PHYSIOLOGIC, DEVELOPMENTAL PERFUSATE. Philip J. Vogelsang, M.D., William Marchese, B.S., and Gerald J. Kost M.D.,Ph.D. Department of Pathology, School of Medicine, Davis, CA. 95616.
We developed a unique, neonatal isolated (ejecting) working heart model featuring an input impedance afterload system and physiologic blood perfusate for the study of developmental cardiac pathophysiology. The afterload system incorporates a characteristic resistor (Rc), capacitance chamber, and peripheral resistor (Rp) in series designed using published age-appropriate in vivo hemodynamic data. Our initial experience (n=6 experiments) with the model using washed sheep erythrocytes combined with physiologic buffer (mean hematocrit - 26.5) demonstrates function which approximates reported in vivo results. Mean animal age, weight and wet heart weight were 10 days, 147 gm. and 0.71 gm.,respectively. We report a previously undescribed reference range for the animal model (n=32, age: 6-12 days, weight:64-270 gm.) for total calcium, 8.1-14.1 mg/dL, mean-11.6 +/- 0.23 mg/dL; phosphorous, 5.7-14.7 mg/dL, mean-8.23 +/- 0.30 mg/dL ; albumin (n=13), 2.2-3.5 gm/dL, mean-2.8 +/- 0.11 gm/dL. We studied stroke work (dyne x cm x 1000) with our neonatal (10-12 days) working heart prep using 7.5 (n=2), 9.9 (n=2) and 12 (n=3) mg/dL calcium in a 2-2.5 gm/dL bovine serum albumin/sheep erythrocyte perfusate and found mean sw/gm wet ht. wt. +/- SEM to be 0.82 +/- 0.95, 2.13 +/- 2.08 and 3.10 +/- 0.86; 0.51 +/- 0.71, 1.46 +/- 0.88 and 3.88 +/- 1.18; and (terminated), 0.68 +/- 0.96 and 1.89 +/- 0.65 at 0, 0.75 and 3 hours, respectively. Using the reference range to produce a physiological age-appropriate blood perfusate, optimized stroke work has been achieved with calcium concentrations previously unreported in the literature for this age group.

P 423 CAMP REGULATION OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE BY COOPERATION OF CIS ELEMENTS BOUND BY DISTINCT PROTEINS, Dietmar von der Ahe[1], David Pearson[2] and Yoshikuni Nagamine[2], [1]Max-Planck-Gesellschaft, Clinical Research Unit for Blood Coagulation and Thrombosis, D-6300 Gießen, FRG, [2]Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland. In cultured porcine kidney epithelial cells, LLC-PK_1, urokinase-type plasminogen activator (uPA) mRNA is induced by a cAMP elevating peptide hormone calcitonin. To delineate the regulatory sequences we stably transformed LLC-PK_1 cells with fusion genes containing the wild type or 5'-flanking deletion mutants of the uPA gene linked to the coding sequence for chlor-amphenicol acetyltransferase. We detected a cAMP inducible enhancer around 3.4 Kb upstream of the uPA gene. This enhancer is composed of three domains referred to A, B and C. As shown by DNAase I "footprinting" experiments each domain is bound by a nuclear factor. Using synthetic oligonucleotides corresponding to A, B and C sequence and their combinations, inserted into an enhancerless SV 40 Vector pSV2-CAT we delineated this multisite enhancer complex. The domains A and B were able to confer cAMP resoponsiveness whereas domain C does not show such effect at all. Nevertheless, domains A, B and C together have a much stronger enhancer effect on transcription after hormonal stimulation. In vitro competition studies show that binding to this multisite enhancer is completely disrupted if using the domain C sequence but not the cAMP responsive elements (CRE) A and B as competitors. This suggests that the domain C binding factor may stabilize DNA-protein interactions in the adjacent complexes. Currently we are identifying and isolating these enhancer binding factors.

P 424 MECHANISM OF ACTION OF VASODILATORS : PURIFICATION AND CHARACTERIZATION OF A VASODILATOR-REGULATED PHOSPHOPROTEIN FROM HUMAN PLATELETS , Ulrich Walter and Maria Nieberding, Department of Internal Medicine, Laboratory of Clinical Biochemistry, University of Würzburg,8700 Würzburg,FRG

Cyclic nucleotide-elevating vasodilators such as PG-I_2 and EDRF inhibit both the contraction of vascular smooth muscle cells and the aggregation of platelets at an early step in the activation cascade, and in human platelets, stimulate a pattern of protein phosphorylation which is mediated by cAMP- and cGMP-dependent protein kinases (1-3). Of particular interest is a membrane-associated 50 kDa protein whose phosphorylation is increased both by PG-E_1 (or PG-I_2) and sodium nitroprusside (or EDRF) in intact platelets, or by cAMP- or cGMP-dependent protein kinase in platelet membranes (1).
We have now purified this 50 kDa protein from human platelet membranes to apparent homogeneity by salt extraction, anion-, cation-, and dye ligand chromatography and have carried out initial biochemical and immunological characterizations of this protein. The results suggest that cyclic nucleotide regulated phosphorylation of this 50 kDa protein is an important component of the mechanism of vasodilator action and may represent the molecular basis for the well known synergism of cAMP- and cGMP-elevating vasodilators.
(Supported by the Fritz Thyssen Stiftung and by the SFB 176 of the DFG).
1) Waldmann et al.(1987)Eur.J.Biochem.167,441-448 ; 2) Walter et al.(1988)Eur.Heart Journal (Suppl.H)9, 1-6 ; 3) Waldmann and Walter (1988) Eur.J.Pharmacol., In Press

P 425 CLONING AND EXPRESSION OF MULTIPLE ISOFORMS OF MYOSIN REGULATORY LIGHT CHAIN IN CHICKEN SMOOTH MUSCLE TISSUES. P.Zavodny, M.Petro, H.Lonial, S.Dailey, C.Kumar*, P.Leibowitz*, and S.Narula. Dept. of Biotechnology-Molecular Biology and *Tumor Biology, Schering Corporation, Bloomfield, NJ 07003.
We have isolated cDNA clones from a chicken gizzard cDNA library which encode isoforms of myosin regulatory light chains (RLCs). We have previously reported the sequence of one isoform, here designated type I, which predicts a protein identical to that obtained from amino acid sequencing of isolated, purified myosin light chain two (MLC2). Another cDNA (designated type II) encodes a protein which is 92% homologous to type I. The phosphorylatable threonine and serine residues at positions 18 and 19 of the MLC2 sequence are conserved. The 1379 base pair type II cDNA clone contains a 91 nucleotide (nt) 5' untranslated region (UTR), a 513 nt coding region and a 775 nt 3'UTR (including the polyA tail). The DNA sequence of the coding region of the type II clone is 82% homologous to type I. Most of the DNA non-homology does not affect the protein sequence (the 5' and 3' UTRs are divergent) indicating strong evolutionary conservation pressure to maintain the MLC2 primary sequence. Northern blot analysis reveals the high level expression of type I in aorta and gizzard tissues, with markedly reduced or absent expression in other tissues. In contrast, type II MLC2 is uniformly expressed at a low level in aorta as well as other tissues examined. Southern blot analysis of genomic DNA suggests that the type I and II MLC2 isoforms are present as single copy genes. Based on the sequence analysis of the cDNAs as well as the Southern and Northern blot data we suggest that type I MLC2 corresponds to the smooth muscle RLC while type II represents the non-muscle RLC isoform.

Late Addition

P 426 ***DOWN-REGULATION OF FAST-TWITCH SKINNED FIBER: STRUCTURE/FUNCTION CORRELATION.*** ***J. Gulati, A. Babu and J.A. Putkey, Albert Einstein College of Medicine, Bronx, NY 10461, & University of Texas Medical School, Houston, TX 77225***

Cardiac troponin C has an inactive trigger site and binds one less Ca^{2+} than skeletal troponin C. It depresses the Ca^{2+}-activated tension in fast-twitch fiber (Babu et al, JBC, v.263, 15485, 1988). The present study further characterizes this down-regulation, and tests the structure/function correlations of chicken cardiac troponin C.

Fast-twitch fiber with cardiac troponin C gave reduced force response in 190mM salt (down-regulation). In a control experiment, similarly treated right ventricular trabeculae gave near full force. In 100mM salt the regulation in fast fiber was close to normal.

For structure/function studies using site-specific mutagenesis of cardiac troponin C, bacterially expressed proteins were tested in the fibre. CBN1 and CBN2A were designed by incorporating chicken cDNA of cardiac troponin C into plasmid (Campbell and Putkey,JCB, 107,467a,1988). For CBN1 four inactive amino acids were mutated to repair Ca^{2+}-coordination in the inactive loop. Down regulation of fiber tension was found to persist with CBN1, despite binding nearly 4 Ca^{2+}/mole like skeletal troponin C. Inactivating the second loop as well, in CBN2A, eliminated the tension, but recombination was normal in the fibre.

The results show that (1) salt milieu modifies the regulation of fiber tension by cardiac troponin C, and (2) by mutagenesis, down-regulation is independent of blocked Ca^{2+}-binding in the first loop of cardiac troponin C. (3) Presence of only two active loops in the C-terminus half of CBN2A (and the presumed binding of 2 Ca^{2+}-Mg^{2+}/mole) is sufficient for recombination in fast-twitch fiber.

Development; Extracellular Matrix

P 500 TRANSFORMING GROWTH FACTOR BETA-1 GENE EXPRESSION DURING CARDIAC DEVELOPMENT. R.J. Akhurst, S.A. Lehnert, D. Gatherer and E. Duffie, Duncan Guthrie Institute of Medical Genetics, University of Glasgow, G3 8SJ, UK.
Transforming growth factor beta-1 is a multifunctional regulator of cellular behaviour. It can be growth stimulatory or inhibitory to endothelial cells depending on the environmental milieu of the cell. It can also stimulate the deposition of extracellular matrix material by mesenchymal cells, suggesting that it could play a major role in tissue remodelling during embryogenesis.

We have examined the temporal and spatial expression patterns of the gene encoding TGF beta-1 during murine embryogenesis. Osteoblasts and haematopoietic cells exhibit pronounced levels of expression, as do certain epithelia undergoing tissue remodelling (Lehnert and Akhurst (1988) Development 104 263-273). TGF beta-1 RNA is also observed throughout development of the heart. It is observed as early as 7.5 days post-coitum in endocardial cells and capillaries. At later stages gene expression is relatively elevated in the endocardium of the developing heart valves compared to other areas of the cardiac endothelium. This localised expression persists at least until birth. We postulate that endocardial TGF beta-1 is involved in embryonic remodelling of the heart valves.

P 501 CYCLICAL MECHANICAL STRETCH OF CARDIAC FIBROBLASTS IN VITRO, Wayne Carver, Madan Nagpal, Maurice Nachtigal, Thomas Borg and Louis Terracio, Departments of Pathology and Anatomy, Univ. of South Carolina, Columbia, S.C. 29208.
The mechanisms by which cells percieve and respond to chemical and physical stimuli are being pursued in a variety of experimental systems. Due to the persistant changes in pressure and volume within the heart, this organ provides an excellent model system to examine the response of cells to mechanical forces. An in vitro model system has been developed to examine the effects of cyclic stretch on isolated cardiac cells. Initial observations have demonstrated that heart cells undergoing cyclic stretch are reoriented relative to the direction of stretch and that cytoskeleton reorganization takes place as well. Cyclic stretched cells also increase in overall size, protein synthesis and RNA synthesis. Currently, cDNA probes are being used to analyze changes in specific mRNA species, including collagens, in stretched cells. Preliminary data suggests that changes in collagen mRNA levels may occur in stretched cells which reflect similar changes that have been detected in collagen protein levels in hypertrophied hearts in vivo. These initial observations suggest that this will provide an excellent model system to analyze the mechanisms by which cells respond to mechanical stimulation. Supported in part by NIH Grants HL 37669, HL 24934, HL 42249 and HL 40424.

P 502 VASCULAR SMOOTH MUSCLE CELL PHENOTYPIC MODULATION: ISOLATION OF cDNA SEQUENCES CODING FOR ELEMENTS WHICH MEDIATE THIS PHENOMENON, Gillian W.Cockerill, Julie H. Campbell, Gordon R.Campbell. Department of Anatomy, University of Melbourne, Parkville 3052 Australia. The pathological proliferation of vascular smooth muscle cells is of major imp--ortance in the development of the atherosclerotic plaque. This proliferation can be mimiced when normal aortic smooth muscle cells are seeded in culture at sub-confluent cell density. These predominantly contractile,non-proliferating cells are induced to divide in culture. Prior to logarithmic proliferation of the culture the cells modulate their phenotype, loose contractile filaments and accumulate large numbers of synthetic organelles. To establish the kinetics of phenotypic modulation we have measured the levels of α-actin mRNA in these cells over time. We have shown that the α-actin mRNA in these cells drops rapidly when they are initially seeded in culture, reaching the lowest level by day four, prior to proliferation. Levels of α-actin mRNA steadily accumulate during this proliferative phase and attain the original levels by day nine when the cells are confluent. We have used this behaviour in culture to isolate exclusive sequences important in mediating smooth muscle cell phenotypic modulation. We have made representative cDNA libraries of modulating cells, just prior to proliferation, and selected 22 clones by screening with a subtracted probe. The probe was made by subtracting cDNA from modulating cells against the mRNA from fully contractile non-dividing cells and from proliferating cells. Work is now in progress to analyse these selected clones and establish their functional significance.

P 503 **A UBIQUITOUS FACTOR IS REQUIRED FOR FAST-TWITCH SKELETAL MUSCLE TROPONIN C GENE EXPRESSION**

R. Gahlmann and L. Kedes, Stanford University School of Medicine, Palo Alto, CA 94304

Troponin C is the calcium-binding domain of the troponin-complex regulating muscle contraction in striated muscle. The fast-twitch skeletal muscle troponin C (TnCF) gene is exclusively expressed in fast-twitch muscle fibers while the slow-twitch muscle isoform is expressed in skeletal muscle, cardiac muscle and in some non-muscle cells. We have cloned the human TnCF gene in order to study the mechanism controlling the fiber-type specific expression of this gene. A construct comprising 6 kbp of 5'-flanking sequences of the gene and a reporter gene (chloramphenicol acetyltransferase) was tested for expression in the human fibroblast line HuT12 and in the myogenic cell lines C2, L8 and H9c2(2-1). In transient assays significant expression was only detectable in C2 cells. The differential expression of the TnCF test gene in the three myogenic cell lines may reflect the fiber type specific expression of TnCF *in vivo*. By 5'-deletion analysis we have located a promoter element that increases the promoter activity of the TnCF gene in C2 cells approximately 50 fold. In gel mobility shift assays three nuclear factors were found to bind to this region. All three factors are present in nuclear extracts from muscle and non-muscle cells (C2, L8 and HeLa). The deletion of the binding sites for two of these factors from test-constructs had no negative effect on the expression in C2 cells . One of these two factors has the characteristic binding site of nuclear factor 1 (NF1). Deletion of the binding site for the third factor caused a 50 fold reduction of transcriptional activity in transient assays. This factor binds to the palindromic sequence CTATATATAG. Competition experiments confirmed that this recognition sequence is not related to CarG-boxes ($C[A/T]_6G$) present in promoter elements of actin genes and of the gene c-fos.

P 504 AN ANALYSIS OF THE DISTRIBUTION OF DESMIN DURING CARDIAC MYOFIBRILLOGENESIS IN VITRO IN NORMAL AND CARDIOMYOPATHIC HAMSTERS USING DOUBLE-LABEL IMMUNOFLUORESCENT MICROSCOPY, Guan R. Hou, Yuji Isobe and Larry F. Lemanski, Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

The morphological pattern of redistributions of intermediate filament proteins in correlation with the cardiac myofibril formation was studied in cultured normal and cardiomyopathic hamster heart cells by double-immunofluorescent microscopy. Primary cardiomyocyte cultures from normal and cardiomyopathic hamster neonates were double-immunostained with monoclonal or polyclonal anti-desmin along with a monoclonal anti-myosin heavy chain antibody using a biotin-streptavidin system. At 5 days in culture, normal and cardiomyopathic myocytes exhibited filamentous patterns of desmin localization. The desmin staining did not show an obvious correlation with myofibril alignment at this early stage. At 9 days in culture, most normal myocytes still exhibited filamentous staining with the polyclonal anti-desmin, while they showed very fine spotty staining patterns using monoclonal anti-desmin. In contrast, most cardiomyopathic myocytes, even after 9 days in culture, still retained a staining pattern similar to those seen in 5 day old cultures, although the numbers of the stained filaments increased. These results suggest that the antigenic properties of desmin in cardiac myocytes change with development. In cardiomyopathic cells this conversion of intermediate filament immunoreactivity may be delayed. (This work was supported by NIH grants HL32184 and HL37702 and an AHA grant to LFL).

P 505 THREE-DIMENSIONAL ORGANIZATION OF INTERMEDIATE AND ACTIN FILAMENTS DURING CARDIAC MYOFIBRILLOGENESIS IN VITRO REVEALED BY IMMUNOGOLD REPLICA ELECTRON MICROSCOPY, Yuji Isobe, Guan R. Hou and Larry F. Lemanski, Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

Myofibrillar assembly occurs in the complex filamentous network of the cytoskeleton in cardiac and skeletal myogenic cells. In order to investigate the spatial relationship between the cytoskeleton and myofibrillogenesis, we identified intermediate filaments unequivocally from actin filaments by using immunogold localization of desmin and/or vimentin as well as the myosin subfragment 1 (S1) decoration technique for actin detection in combination with replica electron microscopy. Cultured neonatal hamster cardiac muscle cells grown on coverslips were physically permeabilized and incubated with S1 and/or specific antibodies. Immunogold labeling was subsequently performed using a biotin-streptavidin system. Samples were then freeze-dried and replicated with platinum and carbon. In cultured cardiac myocytes obtained from neonatal hamsters, many individual filaments formed a complex network between myofibrils. These filaments were intimately associated with nascent myofibrils at the various regions along the sarcomeres. By the double staining of S1 and anti-desmin antibody followed by gold probes, most of the inter-myofibrillar filaments were identified as actin filaments by S1 decoration while a few were intermediate filaments stained by antibody/gold complex. Moreover, some finer filaments remained undecorated either by S1 or antibody-gold complex and appeared to interlink the other cytoskeletal elements. (This work was supported by NIH grants HL32184 and HL37702 and an AHA grant to LFL.)

P 506 TWO DIFFERENT NONMUSCLE MYOSIN HEAVY CHAIN mRNAs ARE EXPRESSED IN CHICKEN TISSUES, Sachiyo Kawamoto*, Y. Katsuragawa, Ralph V. Shohet*, M. Yanagisawa, Mary Anne Conti*, A. Inoue, T. Masaki and Robert S. Adelstein*, University of Tsukuba, Japan and *NHLBI, NIH, Bethesda, MD 20892.
Evidence for the presence of two vertebrate nonmuscle myosin heavy chain (NMMHC) genes has been obtained by the laboratory of T. Masaki (Y.K., M.Y., A.I. and T.M.) by isolating two different cDNA clones (2.8 kb and 0.9 kb) from a chicken fibroblast library. These clones encoded the same region of the NMMHC but showed differences throughout the sequence with 73% nucleotide identity. Southern analysis of chicken genomic DNA showed hybridization to different DNA fragments. We (S.K., R.V.S., M.A.C. and R.S.A.) isolated clones encoding the entire coding sequence from a chicken intestinal brush border library which encompassed the 0.9 kb clone. We synthesized two oligonucleotides complementary to the two different mRNAs in an area of marked sequence dissimilarity and confirmed that each probe hybridized to different bands on a genomic Southern blot. We studied the expression of the two nonmuscle MHCs in a number of tissues at different stages of development using Northern blots. Both NMMHC mRNAs were present in all tissues examined (spleen, liver, kidney, brain, fibroblast and muscle) with the exception of intestinal brush border cells. Brush border cells contained mRNA encoding a single NMMHC and this mRNA was predominant in spleen and fibroblasts. Conversely, the second mRNA species was predominant in brain.

P 507 **HISTOLOGICAL LOCALIZATION OF COMPONENTS FROM THE PLASMINOGEN ACTIVATION SYSTEM AND THE CORRESPONDING mRNA IN NORMAL AND TUMOR TISSUE.** Peter Kristensen, Jens Eriksen, Charles Pyke, and Keld Danø. Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark. We have found by in situ hybridization using anti-sense RNA probes that mouse urokinase-type plasminogen activator(u-PA) is synthesized in: epithelium of the kidney distal and collecting tubules, epithelium of ductus deferens, bladder epithelium and a fibroblast-like cell found in gastrointestinal tract. We have previously found that several rat endocrine cells contain tissue-type plasminogen activator (t-PA)(growth hormone, somatostatin and noradrenaline producing cells) and now show that the t-PA containing cells of the adrenal medulla also contain plasminogen activator inhibitor (PAI-1)immunoreactivity. By use of In situ hybridizations we have found that u-PA mRNA is heterogeneously distributed in the invasively growing Lewis Lung Carcinoma and that the cellular content of u-PA mRNA is highest in tumor cells surrounding muscle tissue being degraded. In addition, areas with tissue degradation often contained high u-PA immunoreactivity, but little or no PAI-1 immunoreactivity, allthough the distribution of u-PA and PAI-1 immunoreactivity was simillar in central parts of the tumors. This points to a possible role for PAI-1 in regulating the net extracellular proteolytic actvity in these tumors. A few single scattered cells containing t-PA immunoreactivity were found in central parts of these primary tumors.

P 508 UBIQUITINATED HISTONE H2A (uH2A) IN DIFFERENTIATING MYOTUBE CELLS IS ASSOCIATED WITH SALT-SOLUBLE NUCLEOSOMES WHICH ARE NOT ENRICHED IN ACTIVE OR INACTIVE GENES. John W. Lough, Mary H. Parlow and Arthur L. Haas*, Departments of Anatomy and Cellular Biology and *Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226.
We have shown that uH2A declines 2.5-fold during terminal myogenesis, coincident with reductions in transcriptional activity (Dev. Biol. 119:85; '87). To assess whether this indicates an association of uH2A with active genes, we have used micrococcal nuclease (MNase) digestion and salt extraction to fractionate myotube nuclei. An oligonucleosomal fraction obtained by MNase digestion and extraction in low salt (100 mM NaCl) which comprised only 25% of the nuclear DNA contained 90% of the total uH2A, as revealed by Western blotting. A second ubiquitinated species of 57-kDa (u57) localized to the same fraction. In contrast, a u18 band, possibly uH4, was associated with fractions resistant to MNase digestion and salt extraction. Although MNase recognized a unique structural feature of active myotube chromatin, as evidenced by the appearance of skeletal alpha-actin sequences as a smear rather than the nucleosomal ladder exhibited by inactive and bulk sequences, Southern hybridization revealed that neither the active skeletal alpha-actin gene nor the inactive alpha^{D}-globin gene were exclusively localized in the low salt fraction. These observations are consistent with the following alternative interpretations: (a) that myotube nuclei are heterogeneous in their transcriptional activity and (b) that myotube uH2A is localized in decondensed, soluble euchromatin containing both active or inactive genes. (Supported NIH grants HL-39829 and GM-34009).

P 509 ATRIAL NATRIURETIC FACTOR MESSENGER RIBONUCLEIC ACID AND PEPTIDE IN THE HUMAN HEART DURING ONTOGENIC DEVELOPMENT. Jean-Jacques Mercadier, Marie-Alphonse Zongazo*, Anne-Marie Lompré, Gill Buttler-Brown*, Daniel Gros**, Alain Carayon*, Ketty Schwartz. INSERM Unité 127, Hôpital Lariboisière, 75010 Paris, *CHU Pitié-Salpétrière, 75013 Paris, *INSERM Unité 262, Hôpital Baudelocque, 75014 Paris, **Université d'Aix-Marseille II, 13228 Marseille, France.

Developmental regulation of ANF production by atria and ventricles has been particularly well studied in rats, but little is known about the ontogenesis of myocardial ANF production in man, except that in utero, the ventricular concentrations of IR ANF are high. We have investigated the level of expression of the ANF gene in the human heart during ontogenic development by determining the concentrations of ANF messenger ribonucleic acid (mRNA), of immunoreactive ANF (IR ANF) and of receptor reactive ANF (RR ANF), in myocardial samples of the various heart chambers. We found the level was high and almost identical in the left and right ventricles in utero. It gradually decreased during ontogenic development to reach the low adult levels, with a more rapid decrease in the right than in the left ventricle after birth. In the atria, ANF gene expression was high as early as the 13th week of gestation, was higher in the right than in the left atrium, and appeared little affected by ontogenic development. In conclusion, in man as in rat, the expression of the ANF gene is developmentally regulated. The post-translational processing steps of the peptide in each cardiac chamber at the various stages of development remain important points to be solved in man.

P 510 **Regulation of human aorta smooth-muscle α-actin gene expression.** Takeshi Miwa, Yoshiro Nakano, Shinji Kamada and Takeo Kakunaga; Department of Oncogene Research, Research Institute for Microbial Disease, Osaka University, Osaka, Japan

By using a monoclonal antibody against smooth-muscle α-actin, we detected the expression of this α-actin isoform in the mouse C2 myogenic cells during differentiation into multinucleate myotubes. The transient expression of a CAT gene in C2 myotubes showed that a human aorta smooth-muscle α-actin gene has at least two positive transcription regulatory regions; one is an upstream region from the transcription initiation (-122 bp to -33 bp) and the other is the first intron region (+972 bp to +1152 bp). The positive regions in the upstream and the intron, respectively, contain two CArG boxes (#1 at -62 bp and #2 at -112 bp) and one CArG box (#0 at +1102 bp). CArG box is a ten-nucleotide $CC(A/Trich)_6GG$ element and has previously been implicated in the muscle-specific transcriptional regulation of the human cardiac and skeletal muscle α-actin genes. Gel mobility-shift and methylation interference assays demonstrated specific interaction of C2 cell nuclear factors with CArG #0 and #2 sequences of the smooth-muscle α-actin whereas very weak interaction with CArG #1. Not only guanine residues in the both sides of CArG sequences but also inner and outer DNA sequences seem to take part in this factor binding. The cardiac and skeletal α-actin genes are also co-expressed in myogenesis of C2 cells and in vitro competition experiment showed that CArG boxes in these promoters can bind the same nuclear factors, but they have apparently different binding affinities. Therefore, muscle-specific expression of these α-actin isoform genes might be achieved with ubiquitous transcriptional factors through the CArG box element.

P 511 ANALYSIS OF TRANSGENIC MICE CARRYING THE DBA/2J REN-2 GENE, Mullins J.J.[1] Sigmund C.[2] and Gross K.W.[2], [1]Department of Pharmacology, Univ. of Heidelberg, INF 366, D-6900 Heidelberg, F.R.G., [2] Dept. Mol. Cell. Biol. RPMI, Buffalo, N.Y., U.S.A.

Classically renin is synthesised by the juxtaglomerular cells of the kidney. Recently much attention has been given to extra-renal sites of renin synthesis, and in the ovary the renin-angiotensin system has been shown to be involved in a physiological process distinct from that of blood pressure control, namely ovulation. The mouse provides unique possibilities to study renin molecular biology since certain strains carry an additional renin structural gene, Ren-2. Although Ren-1 and Ren-2 are expressed equally in the kidney, tissue-specific differences in expression are seen at extra-renal sites. We have extended our analysis of the mouse renin genes by introducing the DBA/2 Ren-2 gene into mice homozygous for the Ren-1 gene. Using primer-extention analysis and in-situ hybridisation we have shown that the transgene is expressed in a tissue and cell-specific manner. Additionally, studies with these animals have shown that oestrus-dependant cycling of Ren-2 expression is dependant on a DBA-specific trans-acting factor. We are presently extending such analysis to the rat and a summary of our progress will be presented.

P 512 ANALYSIS OF THE HUMAN ELASTIN GENE STRUCTURE AND 5' REGULATORY SEQUENCES. Joel Rosenbloom, Michael Fazio, Muhammad Bashir, Zena Indik, Helena S. Yeh, Norma Ornstein-Goldstein, Joan C. Rosenbloom, and Jouni Uitto. University of Pennsylvania School of Dental Medicine and Jefferson Medical College, Philadelphia, Pa.19104.
The entire human elastin gene, including the 5' flanking region, was analyzed by extensive DNA sequencing of clones isolated from cosmid and phage libraries. The 45 kb gene contains 34 exons which separately encode functionally distinct hydrophobic and cross-link domains of elastin. The intron-exon ratio is unusually large (15:1), and the introns contain large amounts of repetitive sequences which may predispose to genetic instability. Exon-intron borders are consistently split in a characteristic way permitting alternative splicing in a casette-like manner. Comparison of the genomic and cDNA sequences conclusively demonstrated that there is extensive alternative splicing of the primary transcript which probably accounts for multiple tropoelastins found in different tissues. The 5' flanking region is G+C rich and contains several SP1 binding sites but no consensus TATA box, features previously largely associated with "housekeeping" genes. Primer extension and S-1 mapping of the mRNA indicated that transcription is initiated at multiple sites. These data suggest that the overall regulation of elastin gene expression is relatively complex and potentially takes place at transcriptional and post-transcriptional levels. Definition of human elastin gene structure provides a basis for elucidation of structural mutations and altered regulatory features in diseases affecting elastic fibers. Supported by NIH grants AR 20553, AR 28450 and March of Dimes Grant 1-989.

Late Addition

P 513 ACTIVATION OF PROTEIN KINASES BY CALMODULIN-TROPONIN C HYBRID PROTEINS. Samuel George, Mark Van Berkum, John Putkey, Anthony R. Means, Depts. of Medicine (Cardiology) and Cell Biology, Baylor College of Medicine, Houston TX 77030.
Myosin light chain kinase (MLCK) and calmodulin dependent protein kinase II (KII) are activated by calmodulin (CaM). Cardiac troponin C (cTnC) is structurally remarkably similar to CaM but does not activate these enzymes. Significant structural features of cTnC not present in CaM include an 8 amino acid extension of the NH_2-terminus and an inactive 1st Ca^{2+} binding domain. To investigate whether these differences account for the inability of cTnC to activate MLCK and KII, we made bacterial expression vectors for CaM, cTnC, and hybrids: (1) 1st domain of cTnC, last 3 domains of CaM (TaM), (2) 1st domain of CaM, last 3 domains of cTnC (CaT). We studied the ability of these hybrid proteins to activate MLCK and KII. Both TaM and CaT required high concentrations (> 1 μM) to partially activate MLCK. In contrast, CaT activated KII to >90% of the maximum obtained with CaM, with similar kinetics. TaM did not activate KII. We conclude that differences present in the 1st domain of cTnC (1) contribute to, but are not fully responsible for, the inability of cTnC to activate MLCK; (2) play a substantial role in the inability of cTnC to activate KII. To further investigate these findings, we have constructed, expressed and purified TaM-BM1, a TaM in which Ca^{2+} binding in the 1st domain has been restored by mutagenesis. Enzyme assay data with TaM-BM1 are pending.