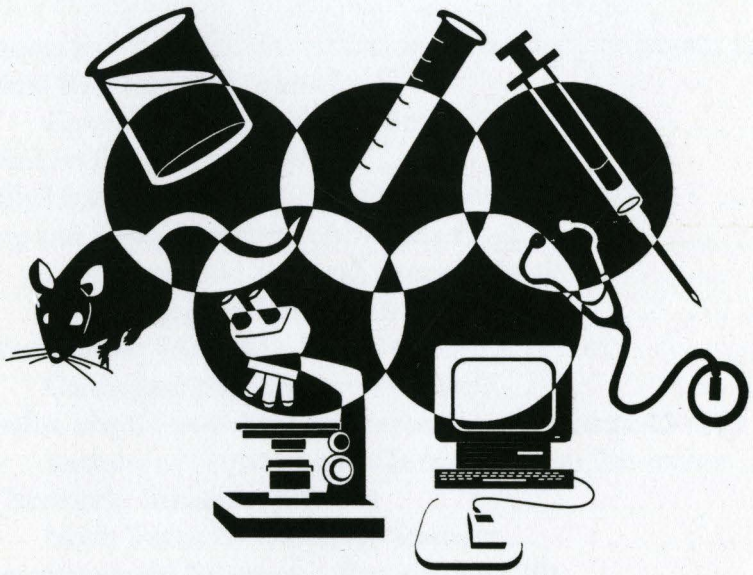
University of North Texas
Health Science Center at Fort Worth

Sixth Annual

Research Appreciation Day

March 25, 1998



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University of North Texas Health Science Center at Fort Worth

Research Appreciation Day

March 25, 1998

TABLE OF CONTENTS

AGENDA

8:00 am - 8:30 am	Assemble Posters	Interdisciplinary Laboratory
8:30 am - 11:30 am	Student Oral Presentation Competition	KIVA Classroom
11:30 am - 1:30 pm	Lunch and Keynote Speaker	Main Auditorium

Welcome
Robert W. Gracy, Ph.D.
Dean
Research and Biotechnology

Overview of RAD '98 Activities
Thomas Yorio, Ph.D.
Dean
Graduate School of Biomedical Sciences

Introduction of Keynote Speaker
Benjamin Cohen, D.O.
Vice President and Executive Dean
Department of Health Affairs

"Extending the Benefit
Primary Prevention of Acute Major Coronary Events
Results of AFCAPS/TexCAPS"
Michael B. Clearfield, D.O.
Professor and Chairman
Department of Internal Medicine

1:30 pm - 2:30 pm	Faculty/Non-Student Poster Session	Interdisciplinary Laboratory
2:30 pm - 5:00 pm	Student/Postdoctoral Poster Competition	Interdisciplinary Laboratory
5:00 pm	Award Ceremony	KIVA Classroom
ALL DAY	Vendor Fair	KIVA Lounge/Hallway

KEYNOTE SPEAKER

Michael Clearfield, D.O., FACOI
Professor and Chairman
Department of Medicine
University of North Texas Health Science Center

AFCAPS/TexCaps is the largest study ever conducted using a cholesterol lowering agent to prevent coronary heart disease. Under the direction of Dr. Clearfield, 2868 participants were randomized at the University of North Texas Health Science Center and followed for five years. The results of this study will have a profound effect on the prevention of coronary heart disease for a large segment of the adult population in this country.

Dr. Clearfield received his D.O. at the Chicago College of Osteopathic Medicine after receiving his B.S. at Albright College in Reading, Pennsylvania. Since 1985, Dr. Clearfield has been Chairman of the Department of Medicine at the University of North Texas Health Science Center at Fort Worth. Dr. Clearfield has also been the Co-Director of the Heart Disease Prevention Clinic since 1990.

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Abbott is committed to the long term success of its product lines within a competitive marketplace. This commitment is demonstrated through aggressive funding of scientific research and development, capital expenditures for manufacturing and distribution, and human resource development resources including substantial training programs.

The Abbott Laboratories Research Achievement Awards are given to the top three student oral presentations, the top three student poster presentations and the top post-doctoral fellowship presentation as determined by a panel of judges.

JUDGES

The 1998 Research Appreciation Day student poster presentation judges are: **David G. Bernard, Ph.D.,** Assistant Professor, Department of Biology, University of Texas at Arlington; **Paul Chippindale, Ph.D.,** Department of Biology, University of Texas at Arlington; **Julie Crider, Ph.D.**, Senior Scientist, Molecular Pharmacology, Alcon Laboratories; **Norman Miner, Ph.D.,** Research Director, MicroChem Laboratory; **Ricardo E. Rodriquez, Ph.D.,** Associate Professor, Department of Chemistry, Texas Wesleyan University; **John Segars, B.S., M.B.A.**, President, Electronic Monitors International, Inc.; **John W. Sheets, Jr., Ph.D.,** Senior Director of Development, Surgical IOL, R & D, Alcon Laboratories, Inc.; **Reginald Stilwell,** Scientific Manager, Johnson & Johnson Medical; **Jill Van Wart Hood, Ph.D.,** Allied Health Coordinator, Department of Biology, University of Texas at Arlington.

The 1998 Research Appreciation Day student oral presentation judges are: **Julia A. Nelson, M.S.**, Vice President for Scientific Affairs, Summa Laboratories, Inc.; **Gerard O'Donovan, Ph.D.**, Chair, Department of Biological Sciences, University of North Texas; **Ron Yasbin, Ph.D.**, Department of Molecular and Cell Biology, University of Texas-Dallas.

VENDOR FAIR

The following companies have provided support for Research Appreciation Day 1998 by participating in our Vendor Fair. For your reference, a list of these vendors appears below. Please join us in thanking these companies for their support of our activities.

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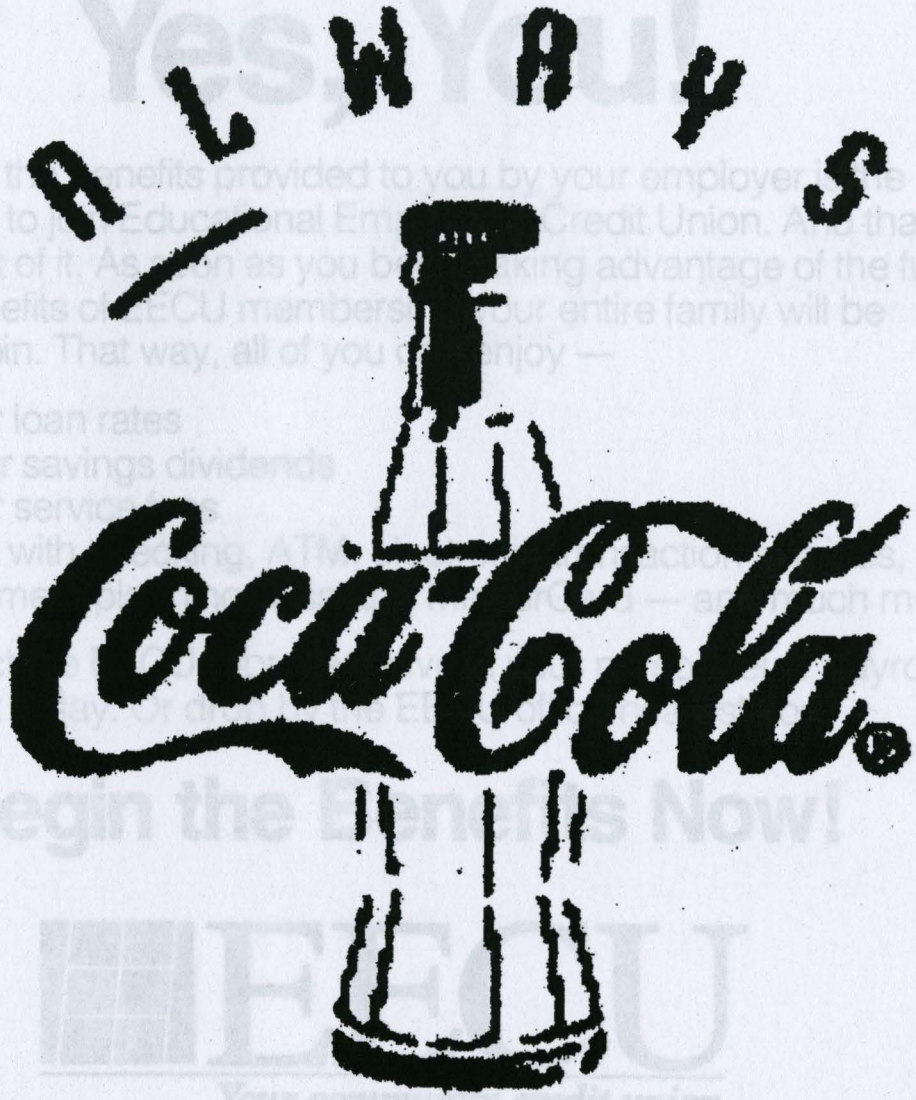
COCA- COLA BOTTLING COMPANY OF NORTH TEXAS

Coca-Cola Bottling Company of North Texas has generously provided the soft drinks for our break this afternoon. Please join us in thanking Coca-Cola Bottling Company of North Texas for its support of our activities.



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TSE/Ridglea Village Travel is a long-standing supporter of the Graduate School of Biomedical Sciences and UNT Health Science Center. Their support of Research Appreciation Day 1998 includes the donation of one round-trip ticket for the first place winner of the student oral presentation competition to travel to a national scientific meeting. Please join us in thanking TSE/Ridglea Village Travel for its continued support of our activities.

TSE

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STUDENT ORAL PRESENTATION COMPETITION

(8:30)	Matthew Crawford	OVER-EXPRESSION OF Bcl-2 IN CULTURED PHOTO-RECEPTOR CELLS PROTECTS FROM PHOTO-OXIDATIVE DAMAGE INDUCES APOPTOSIS BY SUSTAINING NF-κB LEVELS
(8:45)	Kent S. Boles	MOLECULAR CHARACTERISTICS OF 2B4, A HUMAN NATURAL KILLER CELL RECEPTOR
(9:00)	Jennifer A. Jenkins	CORTICOTROPIN-RELEASING FACTOR PRODUCES SENSITIZATION OF THE ANXIOGENIC DISCRIMINATIVE STIMULUS OF mCPP
(9:15)	Srinath Setty	ENDOGENOUS NITRIC OXIDE (NO) MODULATES RIGHT VENTRICULAR MYOCARDIAL OXYGEN CONSUMPTION (MVO_2)
(9:30)	Maria Isabel Taldo	PYRUVATE RESTORES β-ADRENERGIC RESPONSIVENESS OF STUNNED GUINEA PIG MYOCARDIUM
(9:45)	***BREAK***	
(10:00)	J. Storm Shirley	REDUCED CANINE β-ADRENERGIC RECEPTOR (βAR) DENSITY FOLLOWING CHRONIC ENDURANCE EXERCISE
(10:15)	Paramjit Kaur Gill	ENZYME MECHANISM OF CYCLIC ADP-RIBOSE SYNTHESIS AND ITS INTRACELLULAR CONCENTRATION
(10:30)	Harlan P. Jones	TH1 AND TH2 RESPONSE AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN
(10:45)	Lisa Hodge	THE UPPER RESPIRATORY TRACT IS A SEPARATE COMPARTMENT OF THE IMMUNE SYSTEM THAT CAN BE STIMULATED BY MUCOSAL IMMUNIZATION
(11:00)	Bangdong Wei	MUTATIONS ARISING FROM ENDOGENOUS METABOLIC STRESS: ACETATE INDUCES GLYCOGEN MUTATIONS IN A *csrA::KanR* STRAIN OF *Escherichia coli*
(11:15)	Lori Johnson	DETERMINING DIFFERENCES IN GENE EXPRESSION BETWEEN STRAINS OF *STAPHYLOCOCCUS AUREUS* BY SUBTRACTIVE HYBRIDIZATION

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Matthew Crawford

Department: Anatomy and Cell Biology

Graduate Student_x_ Medical Student_x_ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

OVER-EXPRESSION OF Bcl-2 IN CULTURED PHOTO-RECEPTOR CELLS PROTECTS FROM PHOTO-OXIDATIVE DAMAGE INDUCED APOPTOSIS BY SUSTAINING NF-κB LEVELS. ((M.J. Crawford, R. Krishnamoorthy, V. L. Rudick, M. Al-Ubaidi*, N. Agarwal)) UNT Health Science Center, Department of Anatomy and Cell Biology, Fort Worth, TX, *University of Illinois, Dept. of Ophthalmology, Chicago..

Purpose. To determine if over-expression of the bcl-2 proto-oncogene in 661W photoreceptor cells would result in protection from photo-oxidative stress induced changes in NF-κB binding and cell viability. Previous work in our lab has shown that visible light exposure results in oxidative stress to 661W cells as well as down regulation of NF-κB RelA activity and cell death via apoptosis. **Methods.** 661W photoreceptor culture cells were permanently transfected with the pSFFV-neo-bcl-2 plasmid containing the cDNA coding for the full length human bcl-2 gene and neomycin resistance. Following antibiotic screening and clonal selection of the new cell line (B4), SDS-PAGE immunobloting was performed on 661W and B4 cellular extracts using Bcl-2 specific antibodies. Electrophoretic mobility shift assays (EMSA) using NF-κB specific consensus oligonucleotides were performed on nuclear and cytoplasmic proteins from dark and light exposed 661W and B4 cells. The viability of dark exposed 661W and B4 cells were compared with their light exposed counterparts using the Formazan cell proliferation assay. Additionally, the terminal d-UTP end labeling (TUNEL) assay for apoptosis was performed on dark and light exposed 661W and B4 cells. **Results.** Immunoblot results confirmed that the expression of Bcl-2 in the B4 cells was several fold more than that of the 661W cells. EMSA on the dark and light exposed 661W and B4 cells showed that the Bcl-2 over-expression resulted in partial protection from the light induced down-regulation of NF-κB. The light exposed Bcl-2 transfected cells showed a mean increase in viability of 64% over the 661W cells as well as decreased apoptosis, as demonstrated by TUNEL assays. **Conclusions.** These results suggest that Bcl-2 protects 661W cells against photo-oxidative damage induced apoptosis by sustaining NF-κB RelA subunit levels.

Supported by Southern Medical Assoc. and American Osteopathic Assoc.

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Kent S. Boles

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

MOLECULAR CHARACTERIZATION OF 2B4, A HUMAN NATURAL KILLER CELL RECEPTOR

Kent S. Boles and Porunelloor A. Mathew, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107

Natural killer (NK) cells detect and kill cancerous and virally infected cells through receptor recognition that is not dependent on antigen. Target cell lysis is regulated by the binding of NK cell surface receptors that transduce either activating or inhibiting signals depending on the ligation of the target cell's surface molecules. The majority of the know NK receptors are involved in inhibitory signaling and appear to dominate the regulation of killing. A lack of inhibitory signals combined with activating signals leads to NK target cell killing. An activating receptor expressed on murine natural killer and some T cells, termed 2B4 has been previously characterized. Anti-2B4 monoclonal antibodies directed against cultured NK cells greatly enhances their destruction of tumor cells. The molecular characterization and identification of the human homologue of 2B4 could contribute to the immunotherapy of cancer. In the present study, we have isolated several 2B4 cDNA clones from a human NK cell library. A Northern blot of human NK RNA did not hybridize to the mouse cDNA probe, but a Southern blot of human genomic DNA was positive. Therefore, a human genomic library in lambda phage was screened with the full length, murine 2B4 cDNA. A 26 kb clone was isolated that contained a 1.6 kb fragment that maintained hybridization to the cDNA probe. Sequence analysis revealed a 192 bp region with 70% identity to murine 2B4. This region was used as a probe in a Northern against human RNA and hybridized to 3.3 and 2.2 kb bands from a donor sample. The human probe was then used to screen a NK cell cDNA library and 40 positive clones were selected. Four different clones of 700 bp, 1.2 kb, 2.2 kb, and 3.3 kb have been isolated. Preliminary sequence analysis reveals 40% homology between either the 700 bp clone or murine 2B4 compared to the 3' end of the 3.3 kb clone. The 700 bp clone is 63% homologous to murine 2B4. (Supported by NIH grant AI38938)

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Jennifer A. Jenkins

Department: Pharmacology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CORTICOTROPIN-RELEASING FACTOR PRODUCES SENSITIZATION OF THE ANXIOGENIC DISCRIMINATIVE STIMULUS OF mCPP. J. Jenkins, H. Lal and C. Wallis. Dept. Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107

m-Chlorophenylpiperazine (mCPP, a serotonin partial agonist) increases plasma levels of corticotropin-releasing factor (CRF) and corticosterone (CORT). This study was done to determine the effects of 1) exogenously administered CRF and 2) inhibition of CORT synthesis on the anxiogenic discriminative stimulus produced by mCPP. Long Evans rats were trained to discriminate mCPP from saline using a two-lever, food reinforced choice procedure (FR10). Pretreatment (30 min) with the corticosterone synthesis inhibitors, aminoglutethimide (AMG, 0, 5, 10, 20, 40 mg/kg, sc) and ketoconazole (KET, 0, 4.38, 8.75, 17.5 mg/kg, ip) and CRF (0.02, 0.1 and 0.5 ug/ul, i.c.v) produced a significant shift to the left of the dose response curve for mCPP. These data support the hypothesis that CRF produces sensitization of the discriminative stimulus of action of mCPP by increasing its anxiety-like properties. Supported by R01 AA9378 and AA10545.

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Srinath Setty

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ENDOGENOUS NITRIC OXIDE (NO) MODULATES RIGHT VENTRICULAR MYOCARDIAL OXYGEN CONSUMPTION (MVO_2).

Srinath Setty, Xiaoming Bian, H. Fred. Downey. Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, TX 76107.

The role of endogenous NO in modulating MVO_2 is controversial. Results of previous studies are equivocal due to hemodynamic effects of blocking NO release. The present study was designed to evaluate the effect of NO inhibition under controlled hemodynamic conditions. NO release was inhibited by NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) in 14 anesthetized (pentobarbital, 30 mg/kg), open chest dogs. MVO_2 of right ventricle was measured with normal vessel tone and also with maximal vasodilation. MVO_2 was calculated from right coronary blood flow (RCBF) and arteriovenous oxygen extraction. Myocardial segment lengths at end-diastole and end-systole were measured with piezoelectric crystals, and developed force was measured with a miniature force transducer. The slope of the force-length curve during ejection ($\Delta F/\Delta SL$) reflected systolic myocardial stiffness. In group I (n=8), data were obtained as RCP was elevated from 100 to 140 and 180 mmHg, before and 15 min after L-NAME (200 μg/min, i.c.). As RCP was increased to 180 mmHg, RCBF increased by 157 %, and MVO_2 and $\Delta F/\Delta SL$ increased significantly ($p<0.01$). After L-NAME, RCBF decreased at baseline (RCP 100 mmHg, $p<0.01$) and was elevated less as RCP was increased to 180 mmHg (104%; $p<0.01$ vs. pre L-NAME). Although RCBF increased less after L-NAME, MVO_2 and $\Delta F/\Delta SL$ still increased as RCP was elevated ($p<0.05$). These increases were significantly less ($p<0.01$) than those observed at each RCP pre-L-NAME. In group II (n=6), L-NAME induced changes in RCBF were avoided by maximally dilating the Right Coronary (RC) vasculature with Adenosine (3 mg/min, i.c.). Adenosine increased RCBF 609 % at RCP 100 mmHg. As RCP was increased from 100 to 180 mmHg, RCBF increased linearly and similarly pre and post L-NAME with $r^2=0.79$ and $^2r=0.75$, respectively. Under these conditions MVO_2 and $\Delta F/\Delta SL$ increased significantly with RCP. However, L-NAME significantly increased MVO_2 and $\Delta F/\Delta SL$ at each RCP. Our results indicate: 1. Right ventricular MVO2 and function vary with RCP also in the maximally vasodilated RC bed. 2. Endogenous NO decreases MVO_2 at baseline and elevated RCP by maintaining the lower myocardial systolic stiffness. 3. The increase in MVO_2 produced by blocking NO synthase can be masked by the flow mediated decrease in myocardial systolic stiffness.

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: MARIA ISABEL TALDO

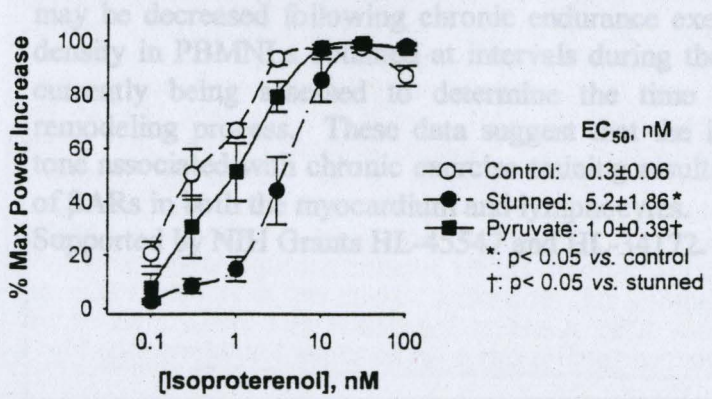
Department: INTEGRATIVE PHYSIOLOGY

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PYRUVATE RESTORES ß-ADRENERGIC RESPONSIVENESS OF STUNNED GUINEA PIG MYOCARDIUM. M. Isabel Tejero-Taldo, Jie Sun, James L. Caffrey, and Robert T. Mallet. Dept. Integrative Physiology, Univ. North Texas Health Science Center, Fort Worth, Texas 76107-2699.

We tested the hypothesis that the response of post-ischemic dysfunctional "stunned" myocardium to ß-adrenergic stimulation was diminished, and that metabolic intervention with pyruvate could restore ß-adrenergic responsiveness to pre-ischemic levels. **Protocol:** Isolated working guinea pig hearts were stunned by 45 min of low flow ischemia, and treatments started 15 min after reperfusion. Dose :response curves (Figure) were generated by measuring the increase in cardiac power from baseline at each dose of isoproterenol and expressing it as a percentage of the maximal increase in power. **Results:** The dose response curve to the ß-agonist isoproterenol (0.1 to 100 nM) was significantly shifted to the right in stunned *vs.* control hearts (Figure). 5 mM pyruvate restored responsiveness of stunned myocardium to near the control level. In separate experiments, 2 nM isoproterenol enhanced function moderately (4.3 fold) in stunned myocardium. 5 mM pyruvate alone increased power 2.4 fold, but the combination of pyruvate and 2 nM isoproterenol increased function 20.8 fold, to near-maximum levels. **Conclusion:** ß-adrenergic responses of stunned myocardium are attenuated, although maximal response was unchanged. Pyruvate restores sensitivity to ß-adrenergic stimulation to pre-ischemic levels, and potentiates cardiac inotropic responses to sub-maximal doses of isoproterenol. Thus, co-administration of pyruvate may allow a reduction in the dose of ß-adrenergic agent necessary to restore optimal cardiac function of stunned myocardium, ameliorating the deleterious effects of those agents on cardiac energetics.



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ABSTRACT FORM

First Author: J. Storm Shirley

Department: Biomedical Sciences

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ABSTRACT

REDUCED CANINE β-ADRENERGIC RECEPTOR (βAR) DENSITY FOLLOWING CHRONIC ENDURANCE EXERCISE.

J.S. Shirley, M.W. Martin, P.A. Gwirtz, and P.B. Raven.

***Cardiovascular Research Institute*, University of North Texas Health Science Center, Fort Worth, TX 76107-2699**

The goal of this project was to test the hypothesis that chronic, repetitive, endurance training results in a decreased density of βARs expressed in the myocardium. Mixed-breed dogs were either cage-rested or performed a nine week progressive treadmill exercise program. Half of the animals in each group received oral doses of the short-acting βAR antagonist, timolol, daily or prior to each bout of exercise. Myocardial microsomal membranes were obtained at the end of the training period for determination of βAR density using ^{125}Iodo-cyanopindolol (^{125}I-CYP) radioligand binding. Specific ^{125}I-CYP binding was decreased ($p<0.05$) by ~ 40% (149 ± 34 fmol/mg protein) in myocardial membranes obtained from exercised animals when compared to untrained animals (250 ± 40 fmol/mg protein). βAR density measured in membranes from animals that received timolol prior to exercise was not significantly different from sedentary controls. Preliminary data indicate that β_2ARs in both canine and human peripheral blood mononuclear lymphocytes (PBMNLs) also may be decreased following chronic endurance exercise training. β_2AR density in PBMNLs obtained at intervals during the exercise program is currently being assessed to determine the time course of this βAR remodeling process. These data suggest that the increased sympathetic tone associated with chronic exercise training results in a downregulation of βARs in both the myocardium and lymphocytes.

Supported by NIH Grants HL-45547 and HL-34172.

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ABSTRACT FORM

First Author: Paramjit Kaur Gill

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ENZYME MECHANISM OF CYCLIC ADP-RIBOSE SYNTHESIS AND ITS INTRACELLULAR CONCENTRATION. P.Gill and R. Alvarez-Gonzalez. Dept. of Mol. Biol. & Immuno., UNTHSC at FW, TX 76107.

Cyclic adenosine diphosphoribose (cADPR) is a recently discovered metabolite of nicotinamide adenine dinucleotide (NAD) that is a potent Ca^{++} releasing agent from intracellular stores. In order to understand the biological significance of cADPR, we must understand the enzyme mechanism by which it is produced and how this is regulated. Incubations of β-NAD^+ with NADase derived from *Neurospora crassa* were carried out and enzyme products were analyzed by HPLC on a Partisil 10-SAX in tandem with a C18 reverse-phase column. A linear production of ADPR and depletion of NAD was observed with time. Addition of 1mM ADPR to the enzyme incubation mixture was made and products were also analyzed. There was no effect of ADPR upon the production of cADPR by NADase. In order to further understand the enzymology of cADPR production by NADases, *Aplysia californica* derived ADPR cyclase, which lacks hydrolase activity, was used to look at the cyclase properties. A linear formation of cADPR was observed with time by HPLC using an AG-MP1 column. Initial substrate saturation kinetics indicated a Km of 602.71 uM and a Vmax of 4.692 umol/min/ug. pH dependent studies were also initiated in order to determine the chemical mechanism of ADPR cyclase. Kinetic parameters at pH 7.0 and 9.0 were determined. The efficiency (Kcat/Km) of the enzyme did not significantly change from 3.7×10^{-6} at pH 7.0 to 3.1×10^{-6} at 9.0. However, the affinity of the enzyme for the substrate (Km) decreased from 602.71uM at pH 7.0 to 409.74uM at pH 9.0. Also, the Vmax decreased from 4.692 to 2.644 umol/min/ug, respectively. The Kcat (rate constant) decreased slightly from 2.26×10^{-3}/sec at pH 7.0 to 1.278×10^{-3}/sec at pH 9.0. A fluorometric assay in which the cADPR intracellular concentration was quantified as either ε-ADPR or ε-AMP has also been developed. First, cells were harvested using a 20%(w/v) perchloric acid treatment to isolate the nucleotide pool. The cell extract was taken through a dihydroxyboronyl Bio-Rex (DHB) resin for affinity chromatography purification of nucleotides containing two or more riboses. Purified material was then treated with snake venom phosphodiesterase to hydrolyze all phoshoanhydride bonds. However under these conditions, cADPR remains intact. The reaction mixture was then purified again using the DHB resin described above. This left cADPR solely in the eluent. Next, cADPR was converted to ADPR, using NAD glycohydrolase isolated from *Bungarius fasciatus* and the products generated were isolated using a third step of affinity chromatography with the DHB resin. Following derivitization of ADPR to ε-ADPR via chloroacetylaldehyde at 60° C and boronate purification on a phenyl boronate agarose column, HPLC- fluorescence detection allowed for the quantification of the cyclic nucleotide. An intracellular concentration of 261.5 nM cADPR was measured in HeLa cells which compares well to a1uM concentration measured via a radioimmunoassay in HL-60 cells.

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Harlan P. Jones

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

TH1 and TH2 RESPONSE AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN Harlan Jones, Haifa Al-Khatib, Lisa M. Hodge and Jerry W. Simecka, Dept. of Microbiology and Immunology, University of North Texas Health Science Center, Forth Worth, Texas 76107

Although intranasal immunization may induce a mucosal immune response, there may be immunopathologic reactions occurring in the lung. In this study, we investigated the T helper cell subsets (Th1 and Th2) responses after intranasal immunization (i.n.) of influenza vaccine plus the mucosal adjuvant, cholera toxin (CT). BALB/c mice were i.n. immunized with influenza vaccine alone or in combination with CT (0 and 7 days). Total RNA was isolated from lung tissue 3 days later. Cytokine mRNA expression of the T helper cytokines (IL-2, IFN-γ, IL-4, and IL-5) were examined by relative RT-PCR using the β2-microglobulin (ß2MGL) housekeeping gene as a standard, to evaluate Th1 and Th2 activation within the lung at day 10. IL-4 and IL-5 mRNA was increased with antigen alone and in combination with CT. However there was no increased expression of IL-2 or IFN-γ mRNA after i.n. immunization with antigen alone. The presence of these Th1 cytokines were found in lungs of mice, given antigen with CT. In comparison, RNAse protection assays demonstrated Th1, and Th2 responses with an increase in IFN-γ and IL-2 after the inclusion of CT. Th1 induction of a delayed type hypersensitivity (DTH) response was shown by increase ear thickness of mice after ear challenge with influenza antigen or CT-B of mice i.n. immunized with influenza antigen and CT. These data indicate that i.n. immunization results in Th2 cell activation which promotes mucosal IgA responses. However, the mucosal adjuvant, CT, appears to promote Th1 cell activation. This work is supported by the American Lung Association of Texas.

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Lisa Hodge

Department: Molecular Biology and Immunology

Graduate Student_X_ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE UPPER RESPIRATORY TRACT IS A SEPARATE COMPARTMENT OF THE IMMUNE SYSTEM THAT CAN BE STIMULATED BY MUCOSAL IMMUNIZATION Lisa M. Hodge and Jerry W. Simecka. Dept. of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107

Mucosal immunity, particularly IgA antibody, is the first line of defense in the upper respiratory tract. In addition, IgG and IgM, are important in preventing viral pneumonia. Vaccination of the upper respiratory tract may induce production of these antibodies, therefore, lowering the risks for respiratory infection. To determine if intranasal immunization is more effective than systemic immunization in priming immune responses in the upper respiratory tract, mice were immunized with whole Philippines (H3N2) influenza vaccine in combination with cholera toxin (CT) either intranasally (IN) or intraperitoneally (IP), followed by a subsequent IN challenge after 7 days. Fourteen days following primary immunization, serum antibody responses were measured by ELISA. Mice that received both primary and secondary IN immunizations produced the highest level of serum, nasal wash and fecal IgA. Serum IgG and IgM levels were high in both IN and IP immunized mice. To further assess antibody responses, anti-influenza antibody-forming cells within lungs, spleens, nasal passages, upper respiratory nodes and lower respiratory nodes were isolated 14 days following primary immunization. In concurrence with serum antibody production, IgA antibody forming cells in these tissues were highest in mice that had received two IN immunizations, while IgG and IgM antibody forming cells were similar between IN:IN and IP:IN immunized groups. In conclusion, we have determined the upper respiratory tract is a separate compartment of the immune system from that stimulated by systemic immunization. (This work is supported by the American Lung Association of Texas).

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Bangdong Wei

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

MUTATIONS ARISING FROM ENDOGENOUS METABOLIC STRESS: ACETATE INDUCES GLYCOGEN MUTATIONS IN A *csrA::KanR* STRAIN OF *Escherichia coli.* B. Wei and T. Romeo. Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

The two carbon compound acetate can serve as a sole carbon source for the growth of *Escherichia coli*, with the induction of the glyoxylate shunt, an anaplerotic bypass through the Krebs cycle. Our laboratory previously identified a global regulatory gene *csrA* (carbon storage regulator) which potently represses glycogen synthesis and gluconeogenesis, activates glycolysis, but does not affect the Krebs cycle. In studies designed to extend our information on the regulatory role of *csrA* in other carbon metabolism pathways, we observed that a *csrA::kanR* mutant is defective for growth on acetate minimal medium. Addition of acetate to a rich growth medium also caused a growth defect in the *csrA* mutant, without affecting the isogenic parent strain. Surprisingly, cultures of the *csrA::kanR* strain grown in the presence of acetate were found to be composed of glycogen biosynthesis mutants. Iodine vapor staining revealed yellow (no glycogen), medium brown, dark brown, and even blue (putatively unbranched glycogen) colonies. The mutations which caused yellow- or blue-staining phenotypes all mapped to the 75 min region of the *E. coli* chromosome, the location of the essential glycogen biosynthesis genes. Pyruvate and Krebs cycle intermediates, including succinate, fumarate, malate, citrate, and 2-ketoglutarate, suppressed the formation of mutants, while glucose, glycerol, lactate, or an *rpoS* mutation did not. We hypothesize that the dual metabolic stress of introducing a *csrA* mutation and adding acetate diverts enough carbon away from the energy-generating steps of the Krebs cycle to inhibit growth, which may be restored either by blocking carbon flux into glycogen or by adding the Krebs cycle intermediates.
(Supported by National Science Foundation)

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Lori Johnson

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DETERMINING DIFFERENCES IN GENE EXPRESSION BETWEEN STRAINS OF *STAPHYLOCOCCUS AUREUS* BY SUBTRACTIVE HYBRIDIZATION. Lori A. Johnson, and Mark E. Hart, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107-2699.

Staphylococcus aureus is a gram positive bacterium causing a wide variety of diseases ranging from abscesses and toxic food poisoning, to more life-threatening complications, such as osteomyelitis and endocarditis. The pathogenesis of *S. aureus* is due in part, to its capacity to produce over thirty cell wall-associated and extracellular proteins; most of which have been identified as the result of extensive *in vitro* studies and determined to be involved in virulence through the use of various animal models. Despite all that is currently known about *S. aureus* , it is still uncertain which genes may be essential for causing staphylococcal disease. One approach in determining which genes might be essential would be to look for differences in gene expression among strains of *S. aureus*. Subtractive hybridization was used to assess differences in gene expression between *S. aureus* strains S6C and 8325-4. Strain S6C is a known producer of staphylococcal enterotoxin B (SEB), while 8325-4 lacks the gene for SEB. Total RNA isolated from post-exponential phase cultures of *S. aureus* strain 8325-4 was photobiotinylated and used for subtraction against cDNA generated from total RNA isolated from S6C grown under similar conditions. Hybrids formed between complementary 8325-4 RNA and S6C cDNA were preferentially removed with streptavidin-coated magnetic particles and the remaining cDNA was analyzed by Southern analysis. Data from these studies indicate that when 8325-4 cDNA was hybridized with 8325-4 RNA, no detectable signal was seen. Signal was observed when S6C cDNA was hybridized with 8325-4 RNA. In addition, subtracted cDNA from S6C was found to hybridize to several regions of *Eco*RI-digested chromosomal DNA from S6C. In contrast, *Eco*RI-digested chromosomal DNA from 8325-4 probed with subtractive S6C cDNA resulted in no detectable hybridizing bands. Experiments currently in progress involve probing subtracted S6C cDNA with *seb* to verify that this gene is represented. It is anticipated that these results will demonstrate that subtractive hybridization can be used as a tool in identifying differences in gene expression between strains of *S. aureus*. (NIH grant AI36934 awarded to M.E.H.)

ETHNOBOTANICAL PRODUCT INVESTIGATION CONSORTIUM

1.	EPIC	EXPLORING THE HEALING POWER OF PLANTS

CLINICAL RESEARCH

2.	David Gibson, Ph.D.	CENTRAL OFFICE OF CLINICAL RESEARCH: THE FIRST YEAR
3.	Cathy Scott	ENTITY INTEGRITY IN REMOTE DATA ENTRY/ACCESS
4.	Patricia Cappelletti	DEFINING EFFECTIVE APPROACHES OF BIOTECH COMPANIES TO COMMERCIALIZE TECHNOLOGY
5.	Harold W. Keller, Ph.D.	INTRODUCTION TO INDUSTRY PRACTICE: A MODEL FOR COLLABORATION

LEWIS LIBRARY

6.	Regina Lee	BIOMEDICAL INFORMATION RESOURCES AT THE LEWIS LIBRARY AND FROM THE NATIONAL NETWORK OF LIBRARIES OF MEDICINE

FAMILY MEDICINE AND PUBLIC HEALTH

7.	John R. Bowling, D.O.	RURAL FAMILY MEDICINE TRACK: STUDENT AND FACULTY PERSPECTIVES ON A RURAL CLERKSHIP EXPERIENCE EXPLORING THE HEALING POWER OF PLANTS
8.	Larry Johnson, LMSW	INFORMATION ACCESS FOR RURAL PRECEPTORS, MEDICAL STUDENTS, AND FAMILY PRACTICE RESIDENTS
9.	Kathryn M. Herron	ACCESS PATTERNS OF A CLINIC-BASED PATIENT EDUCATION WEBSITE FOR INTERNATIONAL TRAVELERS
10.	Claudia S. Coggin, M.S.	RAISING ASTHMA AWARENESS AMONG TEENS

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: **Ethnobotanical Product Investigation Consortium**

Department: **Office of Research and Biotechnology**

Graduate Student____ Medical Student____ Postdoctoral Fellow____ Faculty__X__ Staff____

Read instructions and fit abstract inside the space given below:

EXPLORING THE HEALING POWER OF PLANTS.

Ethnobotanical Product Investigation Consortium. 509 Pecan Street, Fort Worth, TX 76102-4060.

Throughout the world, indigenous peoples have learned which plants kill, which plants heal, and which plants ought to be further tested for their effect on the human body. For example, alkaloids derived from the periwinkle are used in the treatment of leukemia. Plants long used as folk medicines in the mountains of Bolivia show promise of treating HIV. Antibiotics that can help prevent blood clots come from garlic. Because research in this field is interdisciplinary, three local institutions have formed the Ethnobotanical Product Investigation Consortium (EPIC) to identify, characterize and develop natural products from plants. EPIC combines the expertise of botanists at the Botanical Research Institute of Texas (BRIT), natural product chemists at Texas Christian University (TCU) and biomedical researchers and clinical physicians at the University of North Texas Health Science Center (UNTHSC). The collaboration focuses on the development of bioactive products from nature and activities related to such research. EPIC thus provides a unique opportunity to identify potentially important plant species with medicinal value, to isolate and characterize their active components, and to test them for clinical safety and efficacy. EPIC has pooled the unique resources of the three institutions and has developed several research proposals. EPIC also interfaces closely with the pharmaceutical and biotechnology industries in new product research and development.

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: **March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: _____

Department: Central Office of Clinical Research

Graduate Student____ Medical Student____ Postdoctoral Fellow____ Faculty____ Staff____

Read instructions and fit abstract inside the space given below:

CENTRAL OFFICE OF CLINICAL RESEARCH: THE FIRST YEAR

David Gibson, Ph.D., Madelon Petesen, R.N., and Robert Gracy, Ph.D.
University of North Texas Health Science Center, Fort Worth, Texas.

Last year the University of North Texas Health Science Center established a Centralized Office of Clinical Research (OCR), under which all Sponsored clinical research programs have since been managed. During the first year of operation the OCR has been an effective tool for recruiting, administrating and managing clinical research. More importantly, through the efforts of the OCR almost a dozen physician investigators were able to undertake their first clinical trials. Through the Office of Clinical Research these new investigators have been educated and trained in the clinical trial process and have distinguished themselves with the Sponsors. The OCR has established and maintains Standard Operating Procedures (SOPs) and an active Quality Control management system to proactively insure compliance. These SOPs enable investigators and support staff to "come up to speed" quickly and provide guidance for all aspects of the clinical trial process under GCP and FDA regulations. The OCR has been an effective tool for recruiting studies from sponsors and, equally importantly, for recruiting patients for studies at the institution. The effects of having a Centralized Office of Clinical Research on the research and education programs of the Institution and the community will be quantified.

Abstract #2

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: CATHY SCOTT

Department: INSTITUTE FOR CLINICAL RESEARCH

~~Graduate~~ Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ENTITY INTEGRITY IN REMOTE DATA ENTRY/ACCESS.

Cathy Scott and Timothy J. Schreck, Ph.D.
UNTHSC-ICR, Ft. Worth, TX 76107

Clinical research is being challenged to incorporate increasing amounts of data through traditional and developing dataflow channels. These changes require the effective use of emerging technologies and management theory. Implementation of technological changes without symbiotic management paradigm shifts are often disruptive and counterproductive. This has historically resulted in under-utilization of the processes and persistent lag time in maximizing information assets. Clinical research is facing its next evolutionary challenge in remote access data entry and the attending management requirements of phase II, III, IV, and V clinical trials. Those facilities that can implement change ahead of the curve will produce a demonstrable advantage to those who trail the leading edge. The key to success is utilizing intra and internet based dataflow protocols, internal buffers, and strict database administration techniques. The Institute of Clinical Research (ICR) has developed a multipurpose, secure and efficient data collection format that supports both internal and external requirements. The dataflow system incorporates the virtual nature of the net with the consistency of a structured database program. The results of the changes at the ICR indicate noticeable improvements in all phases of the clinical research process: clinical practices, patient recruitment, inventory control, revenues, and a greater overall contribution to the local research community. The result is a data management system that eases the flow of information input as well as output. The flow of information is the critical function of clinical research.

Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Patricia Cappelletti

Department: Biomedical Sciences

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DEFINING EFFECTIVE APPROACHES OF BIOTECH COMPANIES TO COMMERCIALIZE TECHNOLOGY, P. CAPPELLETTI AND H. LAL, DEPARTMENT OF BIOMEDICAL SCIENCES, UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER, FORT WORTH, TEXAS 76107-2699

The objective of the study was to define approaches to commercialize technology by biotech companies affiliated with academic institutions and to determine the most effective ones. The hypothesis tested was that approaches used by biotech companies to commercialize technology significantly influence the success of these companies. A specific combination of approaches was expected to be found most profitable. Availability of marketed products was a measure of success. Five categories of approaches were identified. A survey of biotech companies using a questionnaire provided data on funding, patenting, licensing, new product origins and focus from 85 participating companies. Data were statistically analyzed using multiple regression analyses to test relationships between multiple variables and determine probabilities of success. Results using aggregated data for each category were significant ($p=0.0495$) for success and the first category, but not for the other categories, nor could a combination of these approaches be shown to indicate success. However, analyses of sub-category variables, when tested independently, produced significant results. With one or two academic-sponsored technologies, younger companies ($\leq$10yrs) have a greater probability of success than older companies (>10yrs). For example, a young company with one academic-sponsored technology has a 74% chance of having a marketed product, but an older company has only a 57% chance. The data also suggested that there is a better chance to have a product marketed for companies who have technology transfer offices and who patent prior to publishing than those companies who don't. It was further observed from the data that the chances for a company to have a product on the market increase with age and revenues. In conclusion, overall, these data suggest that approaches used by biotech companies do influence their success. These findings are further confirmed by a case study conducted through a direct interview with the CEO of a biotech company.

Abstract #4

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Harold W. Keller

Department: Office of Research and Biotechnology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

INTRODUCTION TO INDUSTRY PRACTICE: A MODEL FOR COLLABORATION. H. W. Keller, R. W. GRACY, T. YORIO, University of North Texas Health Science Center, Fort Worth, TX 76107-2699

This graduate course serves as a model of collaboration between area industry, small businesses, the City of Fort Worth and the University of North Texas Health Science Center. The idea for this course developed at the Health Science Center and was made possible through the cooperation and networking activities with the City of Fort Worth's Strategy 2000. This group was charged with developing a strategic plan for the city that focused on the health care and biotechnology industries. Networking breakfasts were held monthly where leaders from area businesses, industry and educational organizations met to plan and discuss the economic future of Fort Worth. As an outcome of these meetings, a number of industry representative were invited to discuss the creation of a graduate course that introduced students to the organization and operation of industry practice. This course was offered for the first time in the fall of 1996. It was designed to help graduate students prepare for industry positions and broaden their career path horizons. Sixteen evening class sessions were held with graduate students, postdoctorates, faculty, course instructors, and interested industry leaders in attendance. Twenty-five course instructors came from Abbott Laboratories, Alcon Laboratories, Electronic Monitors, MicroChem Laboratory, TALEM, INC, and the UNT Health Science Center. Instructors volunteered their time to present practical topics to students, including industry organization, regulatory and environmental affairs, research and development (pharmaceuticals and medical devices - instrumentation), manufacturing, quality assurance and quality control, marketing and sales, budgeting, new product development process, clinical studies, intellectual property, entrepreneurial - small business environment and career planning. Students received two credit hours and either a pass or fail grade. Overall course evaluations from the students gave highly favorable ratings for instructional presentation and content, including the importance of the biomedical sciences to industry. Plans call for offering this course again in the fall of 1997. This successful community/health science center collaboration will serve as a model for other courses and joint programs in the future.

Abstract #5

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Regina Lee

Department: Lewis Library

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

BIOMEDICAL INFORMATION RESOURCES AT THE LEWIS LIBRARY AND FROM THE NATIONAL NETWORK OF LIBRARIES OF MEDICINE

Regina H. Lee University of North Texas Health Science Center at Fort Worth Gibson D. Lewis Health Science Library, Fort Worth, Texas 76107.

The Gibson D. Lewis Health Science Library is a Resource Library in the National Network of Libraries of Medicine (NN/LM). The faculty, staff and students of the Health Science Center have access to the biomedical literature of the world through the electronic databases and document delivery partnerships established through the National Library of Medicine's national outreach program, NN/LM. The state's cooperative resource sharing network, TEXSHARE, enables graduate students and faculty from area universities to access the facilities and collections of the Lewis Library. The Library also serves as a community information resource for Fort Worth through contracts and affiliations with hospitals and other agencies. Individual cardholder may use the campus facilities for research and patient care information needs. Through the INTERNET and traditional bibliographic databases, print and nonprint materials, and document delivery services, the Lewis Library provides quality information services quickly and at reasonable cost.

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: John R. Bowling, DO

Department: Family Medicine

Graduate Student ___ Medical Student ___ Postdoctoral Fellow ___ Faculty X Staff ___

Read instructions and fit abstract inside the space given below:

RURAL FAMILY MEDICINE TRACK: STUDENT AND FACULTY PERSPECTIVES ON A RURAL CLERKSHIP EXPERIENCE

Principle Investigator: John R. Bowling, DO
Authors: John R. Bowling, DO; Barbara D. Adams, MSA; Shirley King

University of North Texas Health Science Center at Fort Worth
Fort Worth, Texas 76107

Recognizing the need to increase the supply of primary care physicians practicing in rural and medically underserved areas of Texas, the Department of Family Medicine created a longitudinal program that focuses on training in the skills and knowledge essential for rural medicine. The Rural Family Medicine Track, a series of educational experiences, places emphasis on community-based education that provides student doctors with early and sustained training in the "real world" of rural medicine practice. During Academic Year 1996-97 the third-year, 12-week ambulatory family medicine clerkship was implemented for the first time at eleven rural clinical sites. Students who participated in these rural rotations, and rural physician faculty, completed questionnaires. These questionnaires included items related to attitudes and perceptions regarding the rural experience, value of the educational experience, and track development and implementation. Student and faculty perceptions relating to both cognitive and cultural aspects of the experience showed positive acceptance.

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Larry Johnson, LMSW

Department: Family Medicine

Graduate Student ___ Medical Student ___ Postdoctoral Fellow ___ Faculty X Staff ___

Read instructions and fit abstract inside the space given below:

INFORMATION ACCESS FOR RURAL PRECEPTORS, MEDICAL STUDENTS, AND FAMILY PRACTICE RESIDENTS

Principle Investigators: John Bowling, DO; Samuel T. Coleridge, DO
Authors: Larry Johnson, MSW; Barbara Adams, MSA

University of North Texas Health Science Center at Fort Worth
Fort Worth, Texas 76107

Physicians isolated by distance from medical library resources have difficulty accessing information needed to facilitate student and resident learning and to enhance quality of patient education and care. This project addresses the challenge of linking remote sites with information resources by supplying the needed technology, training, and support. Funds were awarded to purchase computers, communications technology, and training expenses to establish this linkage. The specific aims of this project are twofold: to provide the technology for access to information for teaching sites using an Internet gateway to the medical school library and other health-care databases; and, to provide information access training to physician educators, medical student, and residents. Training in information access will be accomplished by the installation of computers and communications technology to link a site, the Department of Family Medicine, the Gibson D. Lewis Library, and the Internet. Physician educators, students, and residents are being trained to use computer and communication technology to link training sites to electronic databases, Internet resources for patient care, education, and research.

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Kathryn M. Herron

Department: Public Health/ Preventive Medicine

Graduate Student XX Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

ACCESS PATTERNS OF A CLINIC-BASED PATIENT EDUCATION WEBSITE FOR INTERNATIONAL TRAVELERS.

Kathryn Herron, John Licciardone, Doug Squires, Tony Clark.
University of North Texas Health Science Center, Ft Worth, TX 76107

The purpose of this study was to characterize the use of a clinic-based patient education website and to discuss implications for the use of the Internet as a source of health information for consumers. The International Travel Medicine Clinic website was created in 1996 to provide supplemental pre-travel information for clinic patients and prospective international travelers in its north Texas catchment area. Data for 1997 were collected on the 21760 accesses according to domain and type of file accessed (i.e., pre-travel advice, global destination, or immunobiologicals). In the context of pre-travel advice, users most frequently accessed information regarding protection against mosquitoes and other arthropods (40.5% of total accesses). Middle South Asia (8.3%) was the most popular destination queried, followed closely by Mainland Middle America (8.2%), Southern Europe (8.2%), and Eastern South Asia (8.1%). Hepatitis B (20.3%) was the most common immunobiological queried, followed by typhoid fever (16.4%) and yellow fever (13.3%). The most common domain of users was com (32.2%), followed by net (20.5%) and edu (13.0%). The website was accessed by users from nearly every region of the globe on a regular basis and the foreign countries most often represented were Australia (16.5%), Canada (15.5%), and the United Kingdom (14.2%). These results indicate that the website was being utilized regularly as a source of pre-travel health information by consumers from all over the world. With the advent of multiple information systems available online, patients now have the ability to play a more active role in their health management. Certainly, use of the Internet as an educational tool for consumers is a rousing prospect. As the electronic communication revolution continues to unfold, further research is needed to understand the full extent and utility of the Internet as a patient education tool.

Abstract #9

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Claudia S. Coggin, MS, CHES

Department: Department of Public Health and Preventive Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_x__ Staff___

Read instructions and fit abstract inside the space given below:

RAISING ASTHMA AWARENESS AMONG TEENS

Claudia S. Coggin, MS, CHES, Univ. of North Tex Health Science Center; Kristie Aylett, BS, City of Fort Worth Health Department; Diane Peck, RD, LD, MPH, Alaska Department of Health; Susan Ball, MS, Tarrant County Public Health Department; Deborah Jenkins, MA, Texas Department of Public Health

In 1994, more than 2,500 teens visited the emergency rooms at two local hospitals for the emergency treatment of asthma. The 1992 Fort Worth Adolescent Health Survey showed the need to increase the effectiveness of asthma interventions for teens, particulary African-American males. Research showed that members of the target audience often denied having asthma because of the stigma of weakness it carries in their peer group. In 1996, the Tarrant County Asthma Awareness Coalition implemented a collaborative public health promotion initiative incorporating both social marketing tools and health education strategies to address factors that can lead to denial of asthma and its seriousness. The objectives of the health promotion efforts were to develop a asthma education module for local schools, increase physician visits for primary and secondary medical care, reduce visits to the emergency room for asthma conditions, and dispel the belief held by teens that asthmatics are weak. A pilot asthma education module was introduced in four target schools reaching a total of 1,100 students. Local college athletes with asthma were featured on posters, fliers, billboards, and bus placards. The formation of the community coalition lead by the health departments was the first attempt to bring diverse community representatives together in a common campaign targeted at teens. The initial partnering of the health promotion and marketing divisions in the health departments was paramount to the success of the campaign. Including the use of social marketing strategies can enhance the health education campaigns.

Abstract #10

AGING

11.	Thomas J. Fairchild, Ph.D.	GERIATRICS EDUCATION AND RESEARCH INSTITUTE
12.	Janice Knebl, D.O.	RISK FACTORS AFFECTING INDEPENDENT LIVING FOR THE HOMEBOUND ELDERLY
13.	Janice Knebl, D.O.	UNDIAGNOSED DEPRESSION IN THE NURSING FACILITY: RECOGNITION AND TREATMENT
14.	S/D Alayne Kulvicki	THE SEVERITY OF DETRUSOR OVERACTIVITY AMONG INCONTINENT COMMUNITY DWELLING AND AMBULATORY ELDERS
15.	Donald Noll, D.O.	ARE SPECIAL LOW FAT, LOW CHOLESTEROL DIETS FOR THE HOSPITALIZED ELDERLY BEING USED APPROPRIATELY?
16.	Walter J. McConathy, Ph.D.	SENILE DEMENTIA AND SERUM LIPOPROTEINS
17.	Walter J. McConathy, Ph.D.	APOLIPOPROTEIN D AND FLUX OF CELLULAR CHOLESTEROL ACROSS BLOOD BRAIN BARRIER MODEL
18.	Paul Morris, Ph.D.	ADVANCES IN MEASUREMENT OF BIOLOGICAL AGING
19.	John Talent	A DOUBLE STAIN FOR TOTAL AND OXIDIZED PROTEINS FROM TWO-DIMENSIONAL FINGERPRINTS
20.	Yongli Kong	HYPERBARIC OXYGEN-INDUCED MODIFICATIONS OF PROTEINS IN AGING HUMAN FIBROBLASTS
21.	Shelley McDonald	FUNCTIONAL PERFORMANCE CORRELATES WITH LIPID PEROXIDATION IN DIFFERENT BRAIN REGIONS OF THE AGING MOUSE

WOUND HEALING

22.	Dan Dimitrijevich, Ph.D.	WOUND HEALING RESEARCH INSTITUTE
23.	Jami R. Kern	CYTOSKELETAL PROTEIN EXPRESSION IN THE MYOFIBROBLAST PHENOTYPE

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Thomas J. Fairchild, Ph.D.

Department: Special Projects on Aging

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

GERIATRICS EDUCATION AND RESEARCH INSTITUTE
Thomas J. Fairchild, Ph.D., Director. University of North Texas Health Science Center; Fort Worth, Texas 76107

Purpose: To create an "introduction" to the Geriatrics Education and Research Institute for viewers and to create a "lead" poster presentation preceding other posters/abstracts on aging supported or endorsed by GERI.
Methodology: The mission of GERI, a general focus on aging demonstrating a national focus and GERI's focus, and a "food for thought" section constitute the totality of the poster display.
Conclusions: GERI is an inter-disciplinary and multi-disciplinary Institute that focuses on the phenomenon of aging. Its Executive Council reflects most general areas surrounding the study of aging.

Abstract #11

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Janice Knebl, DO

Department: Geriatrics - Department of Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty XX Staff___

Read instructions and fit abstract inside the space given below:

RISK FACTORS AFFECTING INDEPENDENT LIVING FOR THE HOMEBOUND ELDERLY J. Knebl, DO; K. Godwin, Ph.D.; H. Barrett, F. Dark, R. Richwine, H. Truong; University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107.

Purpose: To determine risk factors most likely to jeopardize independence for the home bound elderly, three years of in-home health assessment data (N=149) were analyzed.

Methods: Data for three years were aggregated. Using Statistical Package for the Social Sciences 7.5, analyses included significance and predictive ability of demographic characteristics. Alpha was set at .05. The risk variables included depression, nutrition, cognitive status, and Activities of Daily Living (ADLs) and Instrumental Activities of Daily Living (IADLs), and whether or not the resident received preventive services during the last year (flu shot, pneumovax, PPD, hearing exams, dental exams, eye exams, PAP exams, proctoscopy, and sigmoidoscopy).

Summary: As those between 75-84 years of age increase, it is clear that more elect to live alone; for over 60%, most perform ADLs without assistance. While there appears to be no increases in levels of cognitive impairment, between 28%-42% are moderately or severely impaired. 60% are at moderate or high risk relative to IADLs and nutrition. Finally, between 45%-67% do not receive a flu shot and greater than 80% do not take their pneumovax or PPDs in a timely manner.

Conclusions: Because transportation is problematic for this population and because more are living alone, health promotion, community-based efforts should consider providing services for the home-bound.

(Funded by United Way of Tarrant County through the Area Agency on Aging and supported by the Geriatrics Education and Research Institute of the University of North Texas Health Science Center.)

Abstract #12

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Janice Knebl, DO

Department: Geriatrics - Department of Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty XX Staff___

Read instructions and fit abstract inside the space given below:

UNDIAGNOSED DEPRESSION IN THE NURSING FACILITY: RECOGNITION AND TREATMENT. Janice Knebl, DO, Janelle House, DO, Karen Godwin, Ph.D., Ken Godwin, Ph.D. University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: To determine the degree to which depression is diagnosed in a long term care environment, to determine the degree to which nurses can recognize signs of depression; and to determine the degree to which treatment for depression can be obtained. ***Methods:*** A 175-bed Living Centers of America Nursing Facility in Fort Worth was identified as the research site. Sixty five resident met inclusion/exclusion criteria: the resident must have lived at the facility at least 6 weeks and could not be taking an antidepressant medication. All nursing staff, including Certified Nursing Assistants (CNAs), were trained in the administration of the Geriatric Depression Scale-Short Form and the Behavior Scale for Nonverbal Residents to identify "depression." Both instruments were also used by the Co-Principal Investigator to verify depression status. Data analyses included descriptive analysis to determine the frequency of undiagnosed depression, the frequency of recognition of depression by the nursing staff, and the frequency of treatment for depression following letters of findings to primary care physicians. ***Summary:*** Twenty residents (31% of the study population) were determined to be "depressed" with most over 85 years of age who resided at the facility between one to five years. Nine of these 20 received pharmacologic treatment and 2 of the 9 were referred for psychiatric assessment. ***Conclusion:*** Undiagnosed depression exists in nursing facility residents and can be successfully identified by the nursing personnel. However, most physicians elect not to treat the signs and symptoms of depression in this population.

(Supported by the Living Centers of America and the Geriatric Fellowship Program at the University of North Texas Health Science Center.)

Abstract #13

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: S/D Alayne Kulvicki

Department: Geriatrics/Department of Medicine

Graduate Student___ Medical Student XX Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE SEVERITY OF DETRUSOR OVERACTIVITY AMONG INCONTINENT COMMUNITY DWELLING AND AMBULATORY ELDERS Alayne D. Kulvicki, B.S., Margaret A. Bergmann, M.S., R.N.P., Ruth Kirschner, M.D., Catherine E. DuBeau, M.D., and Neil M. Resnick, M.D. Brigham and Women's Hospital, Boston, MA 02115

Purpose: To determine what urodynamic affect the severity of detrusor overactivity (DO) and to identify a gold standard measuring incontinence frequency in both institutionalized and community-dwelling elders.

Methods: Subjects with detrusor overactivity encoded as the primary or secondary diagnosis after detailed urodynamic testing were selected from an existing database (N=206). 124 met inclusion criteria and were stratified into three functional categories based on mentation and mobility: no impairments (70 subjects), moderate (26), and severe (28) impairment groups. Further stratification considered those taking drugs with an effect on the lower urinary tract (LUT) and those without such medications. After adjusting for mentation, mobility, and medications, we associated more severe DO with increased incontinent episodes. Voiding diaries measuring incontinence frequency averaged over a 24-hour period served as our gold standard. ***Summary:*** Nursing home (64%) and community elders (36%) had an average age of 83 and 82% were female. Out of 20 urodynamic variables measuring bladder proprioception, five showed significant relationships by a student's t test (correlation coefficient ≥ 2.0 and a P value ≤ .05) with the frequency of urinary incontinence. The group without any impairments in mobility and mentation showed significant relationships with the rate of urinary incontinence. Voiding diaries in both ambulatory and institutionalized elderly provide an objective measurement of urinary incontinence frequency and allows for statistical analysis to evaluate the severity of DO. ***Conclusion:*** Urodynamically measured proprioception and UC characteristics can predict the severity of DO in incontinent elders without any impairment in mentation or mobility.

(Funded by the American Geriatrics Society and the Boston University School of Medicine.)

Abstract #14

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Donald Noll, DO

Department: Dept. of Medicine/Division of Geriatrics

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty XX Staff___

Read instructions and fit abstract inside the space given below:

ARE SPECIAL LOW FAT, LOW CHOLESTEROL DIETS FOR THE HOSPITALIZED ELDERLY BEING USED APPROPRIATELY? D. Noll, DO; University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107.

Purpose: To determine if special low fat, low cholesterol diets are being used correctly for elderly persons admitted to the hospital. Low fat, low cholesterol diets are indicated for those with hyperlipidemia. They are relatively contraindicated in those with protein calorie malnutrition, as evidenced by a low serum total cholesterol, low triglyceride, low serum albumen or low total lymphocyte count. **Methods:** Admissions to an acute care hospital between 10/22/97 and 2/22/97 were screened. Those age ≥60 years of age who were placed on some type of low fat, low cholesterol diet were included in the study. **Results**: Forty one men and fifty nine women (N=100) met criteria. 75% of those placed on a low fat, low cholesterol diets did not have hyperlipidemia as defined by a serum total cholesterol greater than 200 mg/dl. Only 19% had "borderline-high" total serum cholesterol between 200- 239 mg/dl and 9% had a "high" serum cholesterol above 240 mg/dl. Low serum cholesterol as defined by a total serum cholesterol ≤160 mg/dl, was present in 36%. Hypoalbuminemia, as defined by a serum albumen ≤3.4 g/dl was present in 44% of the patients. Nineteen percent had a serum albumen level ≤2.9 g/dl. Fifty percent had a low total lymphocyte count below 1500 mm^3. **Conclusions**: The majority of hospitalized elderly persons placed on a low fat, low cholesterol diet do not have hyperlipidemia. A significant percentage show signs of malnutrition as reflected by low serum cholesterol, triglyceride, albumen and low lymphocyte counts.

Abstract #15

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Walter J. McConathy, Ph.D.

Department: Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

SENILE DEMENTIA AND SERUM LIPOPROTEINS. W. McConathy, A.Lacko, J.Kalman, and N.Zachariah University of North Texas Health Science Center, Fort Worth, TX and Albert Szent-Gyorgyi Medical School, Szeged, Hungary.

Even though there are no definitive diagnostic tests except at autopsy, the current view is that dementia of the Alzheimer type (SDAT) represents approximately 70% while multiple infarct dementia (MID) accounts for only about 20% of all cases of senile dementia (SD). To develop tools to differentiate SD, a Hungarian cohort with dementia (SDAT and MID) was studied for abnormalities of the plasma lipid transport system. MID patients ($n = 7$) had an apoE4 allele frequency of 0.43, higher than that of the SDAT group: 0.30 ($n = 26$) indicating apoE4 is not useful to differentiate SD. Like apoE4, sex hormone binding globulin (SHBG) has been associated with atherosclerosis. The SHBG in the MID group was lower than both SDAT and a control group ($p < 0.01$). These observations suggest both SHBG and sex steroid metabolism are deranged in MID. LCAT activity levels were lower in SDAT than in MID suggesting an alteration of reverse cholesterol transport (RCT) in SDAT. To test RCT, serum was used to examine the flux of ^{3}H-cholesterol across a model blood brain barrier (BBB) system. Recovery of ^{3}H-cholesterol for SDAT serum on the basolateral side (cerebral spinal fluid) was significantly lower than the controls ($p < 0.05$) while the MID value was intermediate demonstrating that RCT is most defective in SDAT. Speculation: Alterations in reverse cholesterol transport involving the blood cholesterol esterifying activity, LCAT, could impact membrane composition and structure of endothelial cells at the blood brain barrier. This could contribute to the pathophysiology of SDAT by altering movement of constituents (cholesterol) into and out of the CNS. These findings may be important in understanding the role of arteriosclerosis at the BBB as a potential contributor to the pathophysiology of both MID and SDAT.

Abstract #16

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Walter J. McConathy, Ph.D.

Department: Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

APOLIPOPROTEIN D AND FLUX OF CELLULAR CHOLESTEROL ACROSS BLOOD BRAIN BARRIER MODEL. W. McConathy, Sha Jin, and Pam Brett Department of Medicine, University of North Texas Health Science Center, Ft. Worth, TX 76107.

ApoD, a member of the lipocalin family of proteins, is an apolipoprotein of unknown function and is synthesized by a number of tissues including brain astrocytes. Apolipoproteins D and E are the two principal apolipoproteins of cerebral spinal fluid. The objective was to investigate the potential role of apoD in the movement of cholesterol across a model blood brain barrier (BBB) system. In all experiments, the model BBB consisted of endothelial cells (EC) placed on an insert with 3 u pores, luminal compartment (L), basolateral compartment (BL), and ^{3}H-cholesterol ($1x10^{-6}$ cpm) coated well when examining flux of cholesterol from BL to L. Testing bovine cornea EC and a human EC cell line (huEC, ECV304) demonstrated that the huEC cell line was less permeable by trans endothelial electrical resistance (TEER) and detection of apolipoprotein flux by immunoblotting. ApoD was clearly detected within huEC when added to media by both immunoblotting and fluorescence microscopy while apoAI was not. Use of HDL (HDL+) and apoD free HDL (HDL-) at equivalent cholesterol levels indicated that HDL without apoD is more effective in fluxing cholesterol out of EC. This observation was supported by examining the cholesterol flux of HDL- as impacted by varying apoD levels. Addition of apoD inhibited movement of ^{3}H-cholesterol from huEC. Studies on HDL loading of cholesterol indicated that ApoD inhibits uptake of cholesterol by HDL- when present on the well surface. In conclusion, the human endothelial cell line represents an excellent choice as a relatively impermeable endothelial cell monolayer for studying flux of blood or CSF constituents as perturbed by various pathophysiological conditions; apoD readily enters cells; apoD may play a role in modulating unesterified cholesterol loading of HDL and thus could be an important modulator of reverse cholesterol transport (RCT).

Abstract #17

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Paul Morris, Ph.D.

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ADVANCES IN MEASUREMENT OF BIOLOGICAL AGING.

Paul Morris, Cassandra Beeny, Moukdavanh Stoffel, Reyburn Reynolds, and Michael J. Forster. Geriatrics Education and Research Institute and Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

Various interventions have been purported to slow aging processes, including nutritional supplementation, drug treatment, or lifestyle alterations. Unfortunately, an established methodology for assessing the beneficial or detrimental effects of such interventions on aging does not currently exist. Studies in progress have addressed the potential for assessment of biological aging using non-invasive tests of brain function in mice. If the functional tests were valid indicators of biological age, then it was expected that they should be sensitive to: (i) an experimental intervention resulting in increased longevity, (ii) genetic differences in longevity and, (iii) individual differences in longevity. The functional tests involved assessment of age-related declines in coordinated running, bridge walking, wire-grasping, startle response, and reaction time. These tests were applied at 6-10 month intervals across the life span in longitudinal and cross sectional studies of mice maintained in the UNTHSC pathogen free facility. Sensitivity to experimental intervention was assessed by maintaining separate groups under conditions of either *ad libitum* feeding or 40% restriction of caloric intake. Genetic sensitivity was assessed by comparing declines of brain function performance in C57BL/6, DBA/2, and their F_1 hybrids ($B6D2F_1$). Sensitivity to individual differences in longevity will be assessed by determining the correlation between individual longevity and scores on the functional tests. Caloric restriction resulted in 20-30% increases in longevity and produced parallel shifts in scores of the functional tests. Moreover, the overall rates of decline in test scores with age paralleled differences in longevity of the three genotypes. The relationship between test scores and individual longevity has not yet been considered. Thus far, results indicate that measures of sensorimotor and cognitive performance are valid indicators of biological age, and will provide an essential component of established methods for assessment of potential anti-aging interventions [Supported by NIA grant AG07695]

Abstract #18

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: John Talent

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

A DOUBLE STAIN FOR TOTAL AND OXIDIZED PROTEINS FROM TWO-DIMENSIONAL FINGERPRINTS.

John. M. Talent, M.Sc.; Yongli Kong, Ph.D.; and Robert.W. Gracy, Ph.D.; *Molecular Aging Unit, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107*

Oxidative modification of proteins plays a major role in the etiology of aging and age-related diseases. For example, in Alzheimer's Disease, although evidence points to oxidation of proteins as a causative factor in loss of cognitive abilities, it is not known which specific proteins of the brain are most susceptible to these modifications. Thus, it is of interest to identify the specific proteins which are susceptible to oxidations in vivo. Two dimensional protein fingerprint methods offer the analytical potential for resolution of thousands of individual proteins from tissues, and the oxidized proteins can be visualized with immunological probes. Sensitive methods permit recovery and sufficient amino acid sequencing to identify these proteins. However, for such analyses it is essential to simultaneously analyze both protein content and level of oxidation. We have evaluated several approaches, identified the sources of artefacts and interferences, and developed a double staining procedure that allows visualization and quantitation of total protein patterns as well as the specific oxidized proteins from two dimensional protein fingerprints. The method has been applied to human cells grown in culture and tissue extracts from young and old animals.

Abstract #19

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: YONGLI KONG

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

HYPERBARIC OXYGEN-INDUCED MODIFICATIONS OF PROTEINS IN AGING HUMAN FIBROBLASTS

Yongli KONG, John M. TALENT and Robert W. GRACY
Department of Molecular Biology and Immunology,
UNT Health Science Center, Fort Worth, TX 76107

The accumulation of oxidized proteins in aging is believed to contribute to a variety of age-related diseases including Alzheimer's. Several studies have documented the increased carbonyl content of proteins in aged-tissues. The current study was designed to examine the effects of age on the susceptibility of proteins to the simple oxidative stress of a relatively brief exposure to hyperbaric oxygen. Human fibroblasts obtained from normal human donors were exposed to 100% oxygen for 6 hours with pressures up to 3 atmospheres. The cells were disrupted and proteins resoved in two-dimensional fingerprints. The fingerprints were analyzed by a double stain procedure allowing simultaneous visualization and quantitation of both total proteins and those specifically oxidized and bearing immunoreactive carbonyl groups. The results show that exposure to hyperbaric oxygen causes oxidation of a number of proteins. The number of proteins modified is increased with increasing hyperbaric stress. Cells from old donors were much more susceptible to oxidation than the cells from young donors. These data provide evidence related to the relative number of proteins which may be readily oxidized under mild hyperbaric conditions. They also indicate that with age there is an increased susceptibility of the proteins to oxidative stress.

Abstract #20

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Shelley McDonald

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

FUNCTIONAL PERFORMANCE CORRELATES WITH LIPID PEROXIDATION IN DIFFERENT BRAIN REGIONS OF THE AGING MOUSE. S.R. McDonald, H. Lal, and M.J. Forster. Department of Pharmacology, Geriatrics Education and Research Institute, UNTHSC, Fort Worth, TX 76107.

Oxidative stress is believed to be a causal factor in scenescence. Using the mouse as a model, the current experiment tested the hypothesis that lipid peroxidation in specific regions of the brain causes age-associated impairments of cognitive function and reaction time. Thirty-four C57BL6/JNIA mice, aged 2, 9, 18, or 27 months were tested in a behavioral paradigm that measures learning for escape, avoidance, and simple spatial discrimination (Forster and Lal,1992, *Behav. Pharm. 3:337*). The mice were also tested for reaction time and the concurrent maximum amplitude of response. Following behavioral testing, each mouse was euthanized and the cerebellum, cortex, hippocampus, striatum, hindbrain, and midbrain were removed for biochemical analysis. Lipid peroxidation was determined using the thiobarbituric acid reactive substances assay (TBARS) (Ohkawa et al.,1979, *Analy. Biochem. 95:351*) that measures the colorimetric reaction produced when thiobarbituric acid reacts with malondialdehyde, a secondary product of lipid peroxidation. Group performance declined significantly from 2 to 9 months of age for measures of escape and avoidance, but did not decline further from 9 to 27 months of age. However, lipid peroxidation as measured by TBARS increased as a function of group up to 18 months of age. Correlational analysis revealed that the individual differences in impaired cognitive function are linked to lipid peroxidation at 9 months of age, the earliest age in which performance deficits were evident. These correlations involved only the cerebellum and cortex. The startle reaction demonstrated a general increase in reaction time with increased age and a decreased maximum amplitude of response. The decreased maximum amplitude of response was correlated with individual levels of lipid peroxidation in the midbrain of 27 month-olds. These findings suggest that increases in lipid peroxidation may be a causal factor in age-related impairments of cognitive function and reaction time.

Abstract #21

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: _____

Department: Wound Healing Research Institute

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

A key role of the Wound Healing Research Institute, established in 1992, is to translate research results into viable treatments that minimize the pain and suffering caused by debilitating consequences of problem wounds. Its five-fold mission includes: expanding knowledge of the process of injury and wound healing using novel *in vitro* models and molecular biology techniques; application and innovative approaches such as the use of hyperbaric medicine, growth factors, tissue replacement therapies to problem wounds to prevent amputation and permanent disability; training graduate and medical students, interns and residents in new and interdisciplinary approaches to problem wounds; disseminating knowledge and experience through courses, seminars, conferences and symposia as a part of continuing medical education; and evaluating new pharmaceuticals and devices through all phases of the FDA approval process. Funding from federal, state and private agencies and organizations supports various projects conducted within the institute. Faculty from basic science departments and the departments of general and family practice, internal medicine, pathology, surgery and hyperbaric medicine make up the research staff of the institute.

Abstract #22

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Jami R. Kern

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CYTOSKELETAL PROTEIN EXPRESSION IN THE MYOFIBROBLAST PHENOTYPE

Jami R. Kern*, S.D. Dimitrijevich*¥; Department of *Molecular Biology and Immunology, ¥Wound Healing Research Institute; UNTHSC, FT. WORTH, TX 76107

Wound healing is a process that occurs in stages and involves cellular and matrix components. In the final stages of repair, contraction of the wound occurs. This process is often excessive and causes scarring. For this reason, many studies have been done to look at the role of the major cell type involved in the contraction process, the myofibroblast. This fibroblast phenotype responds to various factors involved in the wound healing process and migrates to the wound site. Once there, it is believed that the wound is closed via contraction by the myofibroblasts. However, the myofibroblasts themselves have not yet been fully examined and understood, partially due to the difficulty in isolating this phenotype. One theory states that fibroblasts adopt the myofibroblast phenotype when they are separated from other cells and must migrate. It is also believed that in order to migrate and function in wound closure, the fibroblasts must alter their cytoskeletal proteins and express more motor proteins, such as α-smooth muscle actin. Therefore, we examined expression of the cytoskeletal proteins; desmin, vimentin, α-smooth muscle actin and myosin light chain. The proteins were extracted from human fibroblasts cultured at either high or low density and with one of three media types. The protein extracted from human umbilical smooth muscle cells was used as a positive control for the motor proteins. The protein analysis revealed that the fibroblasts grown at low density contained an altered expression of cytoskeletal proteins. Results from these and similar experiments will be useful in understanding the molecular mechanism of the wound healing process, especially the contraction of the wound, and will be helpful in treating individuals who suffer from abnormal healing and the painful excessive contraction associated with it.

Abstract #23

CARDIOVASCULAR

24.	Peter Raven, Ph.D.	CARDIOVASCULAR RESEARCH INSTITUTE
25.	Nicolette K. Muenter	THE EFFECT OF SLEEP RESTRICTION ON ORTHOSTATIC CONTROL IN HUMANS
26.	Stephen L. Wasmund	INCREASED ECTOPIC FREQUENCY RESULTS IN INCREASED SYMPATHETIC NERVE ACTIVITY
27.	Robert Carter, III	ROLE OF CENTRAL COMMAND DURING RECOVERY FROM EXERCISE
28.	Walter J. McConathy, Ph.D.	LEFT VENTRICULAR HYPERTROPHY, STRENGTH AND RESISTANCE TRAINING
29.	Paul Fadel	THE EFFECTS OF CHRONIC ENDURANCE EXERCISE ON THE MYOCARDIUM WITH AND WITHOUT β-ADRENERGIC RECEPTOR BLOCKADE
30.	Kevin Gallagher	CHANGES IN VENOUS RETURN ALTER THE PLATEAU OF STROKE VOLUME DURING DYNAMIC EXERCISE
31.	Scott Alan Smith	MODEL OF AORTIC BAROREFLEX FUNCTION IN HUMANS
32.	B. H. Foresman, D.O.	CORONARY ISCHEMIA RESULTS IN SOMATIC DYSFUNCTION IN THE T1-T5 MYOTOMES
33.	Ross Querry	LATENCY OF PEAK TACHYCARDIA DURING GRADED HYPOTENSION
34.	Geoffrey P. Kline	EFFECT OF RENOVASCULAR HYPERTENSION ON CORONARY VASCULAR RESPONSIVITY IN CONSCIOUS DOGS
35.	Martin Farias	LOCATION OF OPIATE RECEPTORS RESPONSIBLE FOR INHIBITION OF VAGAL BRADYCARDIA
36.	Keith Jackson	THE ROLE OF WHITE CELL DERIVED ENKEPHALINS DURING HEMORRHAGIC HYPOTENSION

37.	Steven R. Stuewe	EXERCISE TRAINING INCREASES CREATINE KINASE CATALYTIC CAPACITY IN CANINE MYOCARDIUM
38.	Jie Sun, B.S.	PYRUVATE ENHANCEMENT OF CARDIAC FUNCTION AND ENERGETICS REQUIRES ITS MITOCHONDRIAL METABOLISM
39.	Lara Keyser	ATP AND INOSITOL TETRAKISPHOSPHATE REGULATE Ca^{2+} FLUXES ACROSS CARDIAC SARCOPLASMIC RETICULAR MEMBRANES
40.	Yun Bai	TRANSCRIPTIONAL REGULATION OF CARDIAC HYPERTROPHY SENSITIVE GENES BY AN INDUCIBLE CALCINEURIN EXPRESSION SYSTEM
41.	Michael D. Wilburn	TREATMENT OF SICKLE CELL ANEMIA BY M-15 IN A TRANSGENIC MOUSE MODEL
42.	Adnan Dibas, Ph.D.	MECHANISM OF WORTMANNIN AND ML9 INHIBITION OF VASOPRESSIN-ACTIVATED WATER TRANSPORT IN TOAD URINARY BLADDERS
43.	A. J. Mia, Ph.D.	EFFECT OF VASOPRESSIN ON ENDOSOMES AND CAVEOLAE IN TOAD URINARY BLADDER GRANULAR CELLS
44.	Lawrence X. Oakford, Ph.D.	PRESENCE OF CAVEOLAE IN THE GRANULAR EPITHELIAL CELLS OF RABBIT URINARY BLADDER

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Cardiovascular Research Institute

Department:

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

The Cardiovascular Research Institute (CRI), established in 1995, was developed from the multidisciplinary research efforts of molecular biologists, pharmacologists, physiologists, and physicians in the internal medicine subspecialties of cardiology and pulmonology. A key role of the institute is to integrate basic research findings with the clinical therapeutic problems associated with over 50 million Americans who suffer from cardiovascular diseases.

Institute studies focus on heart disease, with special emphasis on understanding the role of exercise in the prevention of and rehabilitation from heart disease. Research is conducted into the fundamental molecular biologic and cellular mechanisms associated with the improved cardiovascular function, cardio-protection from heart attacks and longer life of those people who have moderate-to-high-activity lifestyles. Both the basic science and clinical divisions of the institute collaborate with pharmaceutical and biotechnology corporations in order to validate new diagnostic, preventive, therapeutic and corrective procedures. Institute activities involve local, national and international partnerships.

The CRI has developed a Center of Sleep Research to investigate the cardiovascular sequalae (Hypertension and heart attack) of sleep apnea, lack of sleep and disturbed sleep. Problems from which more than 20 million people suffer. Additionally, the CRI has established a Center of Physical Medicine to investigate manual methods of treating somatic dysfunction and the use of electrodiagnostic methods in individuals with radiculopathies and low back and neck pain.

This presentation is a collage of projects in progress.

Abstract #24

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Nicolette K. Muenter

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE EFFECT OF SLEEP RESTRICTION ON ORTHOSTATIC CONTROL IN HUMANS

N. K. Muenter, D. E. Watenpaugh, S. L. Wasmund, W. L. Wasmund, and M. L. Smith. University of North Texas Health Science Center, Fort Worth, TX 76107

The purpose of this study was to test the hypothesis that sleep restriction can lead to attenuated cardiovascular responses to orthostasis, and thereby contribute to orthostatic intolerance experienced by many astronauts after space flight. We studied 10 healthy subjects' (ages=22-46) cardiovascular responses to graded lower body negative pressure (LBNP) before and after 4 days of sleep restriction (4 hours/night). Heart rate (electrocardiogram), arterial blood pressure (Finapres), forearm blood flow and vascular resistance (ultrasound) were measured. LBNP tolerance (pressure tolerated and duration of LBNP tolerated) was unaffected by sleep restriction. Arterial pressure tended to be greater post-sleep restriction; however, this increase was significant only for systolic pressure at 60 mmHg LBNP (P=0.038). Heart rate tended to be lower post-sleep restriction; however, this decrease was also significant only at 60 mmHg LBNP (P=0.028). Sleep restriction had no significant effect on forearm vascular resistance or blood flow. Our data suggest that sleep restriction does not attenuate cardiovascular responses to simulated orthostasis as hypothesized, but may slightly augment the ability to maintain blood pressure in the face of an LBNP challenge, though the mechanism for this may not be increased resistance. The lower post-sleep restriction heart rates may simply be a baroreflex-mediated response to the higher post-sleep restriction blood pressures. (Supported by NASA NAGW-5038 and NGT5-50179)

Abstract #25

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Stephen L. Wasmund

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

Increased ectopic frequency results in increased sympathetic nerve activity. S.L. Wasmund, †R.L. Page M.L. Smith, Dept of Integrative Physiology, UNTHSC, †Division of Cardiology, UT-Southwestern Medical Center, Dallas TX.

Single ventricular premature beats (VPB) evoke transient increases in muscle sympathetic nerve activity (MSNA) in humans *(JACC, 13:69, 1989)* and parallel increases in renal and cardiac sympathetic activity in dogs *(JACC, 9:147, 1987)*. In a previous study, it was shown that VPBs occurring one in every ten sinus beats does not alter baseline MSNA *(JACC, 13:69, 1989)*. In this study we sought to test the hypothesis that high rates of ectopy result in chronically elevated sympathetic neural activity. We studied nine patients referred for diagnostic electrophysiologic testing. Arterial pressure (femoral) and MSNA (microneurography) were measured continuously during induced VPBs with a coupling interval of 350 ms. The sinus to VPB ratio was increased progressively from 4:1 to 1:1 And each VPB ratio was sustained for one min. *Results:* Total MSNA (per min) during VPBs at 4:1 and 3:1 were not different from normal sinus rhythm (NSR), but MSNA became progressively and significantly greater at ratios of 2:1 and 1:1 (Table). All data are mean±SE; * P< 0.05.

	NSR	4:1	3:1	2:1	1:1
MSNA (units)	423±77	449±89	544±67	736±104*	817±61*

Conclusions: These data demonstrate that frequent ectopy produce significant sustained increases in MSNA. Since parallel changes in renal and cardiac sympathetic activity occur during singlets and couplets, the changes observed in this study imply that the autonomic milieu for blood pressure regulation and cardiac rhythm control is altered during high frequency ectopy. If cardiac sympathetic activity is augmented as the previous study would suggest, we hypothesize that this effect of ectopy contributes to the relation between high frequency ventricular ectopy and susceptibility to ventricular tachycardia.

Abstract #26

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Robert Carter III

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ROLE OF CENTRAL COMMAND DURING RECOVERY FROM EXERCISE. R Carter, III, DE Watenpaugh, WL Wasmund, SL Wasmund, ML Smith, FACSM. Department of Integrative Physiology, UNTHSC, Ft. Worth, TX 76107-2699.

Previous studies in our laboratory concluded that the initial rapid decrease in blood pressure during inactive recovery from exercise is due, in part, to lack of the skeletal muscle pump, which is an important mechanism for venous return during exercise. In this study, we hypothesized that the role of central command was minimal in arterial blood pressure regulation during recovery exercise recovery. Ten healthy volunteers underwent 3 exercise sessions each consisting of a warm-up followed by 3 min of cycling at 60% of maximal heart rate (HR), followed by 5 min of recovery: 1) seated (inactive); 2) loadless pedaling (active) or 3) passive pedaling, in random order. Thirty min separated the 3 protocols. Mean arterial pressure (MAP, photoplethysography), thoracic impedance (TI), and HR were measured continuously during each bout of exercise. ***Results***: There were no significant differences in the decreases in HR between the inactive and passive pedaling recoveries ($p<0.05$). HR decreased more during passive than during active recovery ($p<0.05$). The decreases in MAP were not different between the passive and active recovery, but significantly less than during inactive recovery. TI was used as an inverse measure of relative changes in central blood volume. TI increased significantly from peak exercise levels during inactive recovery ($p<0.05$). ***Conclusion:*** These data suggest that the contribution of central command in regulation of arterial blood pressure is minimal during recovery from exercise. (Supported, in part, by NIH grant HL-49266)

Abstract #27

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Walter J. McConathy, Ph.D.

Department: Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

LEFT VENTRICULAR HYPERTROPHY, STRENGTH AND RESISTANCE TRAINING W.J. McConathy, R.D. Dickerman, F. Schaller and N.Y. Zachariah. Departments of Biomedical Sciences and Medicine, UNT Health Science Center, Fort Worth, TX 76107.

Idiopathic cardiomyopathy in athletes who use anabolic steroids point to the possible role of steroids in dysfunction. However, the relationship of anabolic steroid abuse to pathological cardiomyopathy remains unknown. Extreme increases in arterial blood pressure occur in the human when performing maximal weight lifting; left ventricular posterior wall (LVPW) thickness is increased in resistance trained athletes (RTA). Our working hypothesis is: Peak blood pressure during resistance training/ strength correlates with degree of left ventricular hypertrophy (LVH). Secondarily, anabolic steroid abuse when coupled with RT would enhance this relationship. Subjects: Male elite, competitive, resistance trained athletes, both users (DU) and nonusers (DF) of anabolic steroids. Methods: Resting transthoracic echocardiography was used for left ventricular measurements. Little difference between cardiac size and function was seen between the 2 groups except for ventricular septal thickness (VST) and LVPW ($p<0.05$). Strength was defined as weight lifted at squat corrected for body mass index (BMI). Using Spearman correlational analyses, strength correlated with both LVPW ($r=0.84$, $p=0.008$) and VST ($r=0.71$, $p=0.05$) in DF but not in DU. In DU, a relationship between strength and both left ventricular end systolic dimension (LVEDs; $r=0.74$, $p<=0.035$) and early diastolic filling velocity (E; $r=0.76$, $p<0.028$) was absent in DF. Pathologic concentric LVH occurring as a compensatory response to blood pressures generated in elite lifters using anabolic steroids remains speculation. This study points to a relationship of strength to LVPW thickness which in our view is related to the intensity of the intermittent hypertensive episodes during weight-lifting. In the DU group, the absence of a relationship of strength with VST or LVPW suggest androgens may accelerate and modify the response to pressure overload as generated by strength. Conclusion: Intermittent hypertensive episodes during weight-lifting can lead to physiological adaptations in the form of concentric LVH and when coupled with anabolic steroids, possible pathologic remodeling of the myocardium.

Abstract #28

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Paul Fadel

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE EFFECTS OF CHRONIC ENDURANCE EXERCISE ON THE MYOCARDIUM WITH AND WITHOUT β-ADRENERGIC RECEPTOR BLOCKADE

P.J. Fadel, R. Welch-O'Connor, K.M. Gallagher, S.A. Smith, R.G. Querry, and P.B. Raven, FACSM. Departments of Integrative Physiology & Cardiovascular Research Institute, University of North Texas Health Science Center at Fort Worth, TX 76107.

The purpose of the present study was to examine myocardial adaptations to chronic endurance exercise training. While it has been well established that chronic endurance training results in decreased resting and submaximal heart rates (HR) and increased cardiac mass, the exact mechanism(s) responsible for these changes remain unclear. Two pairs of untrained males, one heterozygous and one homozygous (identical twins), performed a 12 week run-jog exercise training program. Within each pair, one subject served as a control and trained 4 days/week between 65% - 80% of maximum heart rate reserve (Karvonen formula). The other, experimental subject trained at the same workloads as the control and ingested 10mg of a nonselective β-receptor antagonist two hours prior to each exercise bout. The heterozygous control and experimental subject increased their VO_{2max} values 11.4% and 15%, respectively, indicating a similar training effect. However, while the control subject exhibited a decrease in resting and submaximal HR, the experimental subject's response to training for these same variables remained unchanged. In addition, left ventricular (LV) mass normalized to body weight, determined by magnetic resonance imaging, increased 8.3% and 4% for the control and experimental subject, respectively. The homozygous group showed similar responses with no changes in HR response for the experimental subject and a difference in LV mass of 13.8% and 6.9% for the control and experimental subject, respectively. These results suggest that β-receptor stimulation was a necessary component of the training induced bradycardia and increased cardiac mass. We conclude that the intermittent increases in sympathetic neural signaling produced by exercise training sessions provides an important stimulus for the cardiac changes associated with chronic endurance exercise training.

Abstract #29

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Kevin Gallagher

Department: Integrative Physiology

Graduate Student X Medical Student X Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CHANGES IN VENOUS RETURN ALTER THE PLATEAU OF STROKE VOLUME DURING DYNAMIC EXERCISE

K.M. Gallagher, S.A. Smith, R.G. Querry, K.H. Bryant, R.M. Welch-O'Connor, and P.B. Raven. Department of Integrative Physiology and Cardiovascular Research Institute, UNT Health Science, Fort Worth, TX.

Six average fit (26 ± 2 yrs; $\dot{V}O_{2peak}$ = 38 ± 1 ml•kg^{-1}•min^{-1}) subjects performed dynamic cycling to determine if increases or decreases in venous return affect the plateau that occurs in stroke volume during dynamic exercise. Each subject performed three bouts of exercise to volitional fatigue: control; exercise with Lower Body Negative Pressure (LBNP) of -40 mmHg [to reduce venous return (VR)]; and exercise with volume expansion (VE) to 10% of total blood volume with 6% dextran in saline [to increase VR]. Heart rate (HR, beats•min^{-1}), cardiac output (Q_c, L•min^{-1}) and oxygen uptake ($\dot{V}O_2$) were measured and stroke volume (SV; ml•beat^{-1}) was calculated (mean ± SEM). In the average fit subjects, the mean plateau of SV began at 43 ± 5% of $\dot{V}O_{2peak}$. However, the plateau of SV during LBNP (reduced VR) occurred at a greater percentage of $\dot{V}O_{2peak}$ (53 ± 4), while the plateau of SV during VE 10% (increased VR) occurred at a much earlier percent of $\dot{V}O_{2peak}$ (7 ± 5), which was equivalent to rest. We conclude that the plateau of stroke volume that occurs during dynamic exercise to maximum was primarily determined by venous return and cardiac filling time.

(Supported in part by ACSM Student Research Development Grant & UNTHSC GREAT Grant and NIH # HL45547)

Abstract #30

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Scott Alan Smith

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

MODEL OF AORTIC BAROREFLEX FUNCTION IN HUMANS

S.A. Smith, K.M. Gallagher, R.M. Welch-O'Connor, A.H. O-Yurvati, I.P. Reese, X. Shi, and P.B. Raven. Department of Integrative Physiology & Cardiovascular Research Institute, UNTHSC, Ft. Worth, TX 76107.

The purpose of this investigation was to model isolated aortic cardiac baroreflex function (ABR) in humans. Pharmacologically-induced increases (phenylephrine) and decreases (nitroprusside) in aortic distending pressure were implemented in five healthy young normotensive subjects in order to determine the maximal gain (G_{max}) of the reflex heart rate (HR) response in i) an intact ABR and carotid baroreflex (CBR) and ii) an isolated ABR. Mean arterial blood pressure (MAP) was monitored by an intra-radial arterial catheter. The threshold (THR), saturation point (SAT), response range (RES), centering point (CP) and minimum HR response (HR_{min}) were calculated using the Kent Logistical Model. The ABR was functionally isolated from the CBR by application of neck pressure during the hypertensive response and neck suction during the hypotensive response. Mean ± SE calculations presented in table below:

Condition	G_{max} (bpm/mmHg)	THR (mmHg)	SAT (mmHg)	RES (bpm)	CP (mmHg)	HR_{min} (bpm)
ABR+CBR	2.5±0.8	67.0±7.1	94.5±1.8	51.2±2.6	80.7±4.1	44.3±4.1
ABR	1.4±0.2	66.7±6.5	98.4±3.2	41.5±4.9	82.6±4.8	45.0±3.9

The G_{max} of the ABR was 56% and the response range of the ABR was 81% of the arterial baroreflex (ABR + CBR). We conclude the ABR is the predominant reflex regulator of MAP. (Supported by NIH HL45547)

Abstract #31

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: B. H. Foresman, D.O.

Department: Department of Integrative Physiology

Graduate Student__X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CORONARY ISCHEMIA RESULTS IN SOMATIC DYSFUNCTION IN THE T1-T5 MYOTOMES. B. H. Foresman, D.O., D. A. Vick, D.O., J. L. Dickey, D.O., and P. A. Gwirtz, Ph.D. UNT Health Science Center, Depts. of Biomechanics and Physiology, Fort Worth, TX 76107.

Previous investigations have shown a correlation between somatic dysfunction and cardiovascular disease. These findings could represent a direct somatovisceral reflex or the accumulated effects of indirect mechanisms. This investigation examined if acute coronary artery ischemia would elicit reflex somatic changes in the T1-T5 myotomes. For this investigation, we chronically instrumented eight healthy mongrel dogs to allow measurement of left circumflex artery blood flow (CBF), coronary artery blood pressure (CBP), left ventricular systolic (LVSP) and end diastolic pressures (LVEDP), regional left ventricular contractile function, cardiac output (CO), aortic blood pressure (AoBP), and heart rate (HR). Palpatory assessments were performed by specialists in Osteopathic Principles and Practice who were "blinded" to the intervention. Assessments were recorded under ischemic and sham conditions performed on separate days using a random order and repeated on three separate occasions. To create myocardial ischemia, CBF was reduced by inflating a hydraulic occluder around the circumflex artery; this reduced mean CBF by 40-50%. Five minutes into each intervention cardiovascular measurements and palpatory assessments were collected. As expected, ischemia caused significant decreases in the mean CBF (31.83±2.12 to 20.15±4.24 cm/sec, $p<0.05$), posterior segment shortening (14.4±1.8 to 10.4±2.7%, $p<0.05$) and mean CBP (87.0±3.0 to 50.0±9.0 mmHg, $p<0.05$), while HR increased from 111.0±10.0 to 133.0±11.0. No significant change occured in CO, LVSP, LVEDP, or AoBP. The global assessment of somatic dysfunction in the T1-T5 region increased (1.0±0.2 to 4.0±0.5, $p<0.05$), while no significant difference was noted in other thoracic regions.

Conclusion: This study provides preliminary evidence for a segmental viscerosomatic reflex that is initiated by acute myocardial ischemia and selectively affects the T1-T5 paraspinal muscle groups.

This project was sponsored by the American Osteopathic Association, Grant #96-11-392.

Abstract #32

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Ross Querry

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

LATENCY OF PEAK TACHYCARDIA DURING GRADED HYPOTENSION

R.G.Querry, S.A.Smith, R.Welch-O'Connor, A.H.O-Yurvati, I.P.Reese, P.B. Raven, and X.Shi. Departments of Integrative Physiology & Cardiovascular Research Institute, UNT Health Science Center at Fort Worth, TX

The aim of this study was to test the hypothesis that the latency of the reflex change in heart rate (HR) was related to the magnitude of the change in arterial blood pressure (ABP). Change in mean ABP (ΔMAP, monitored by intra-radial arterial catheter) was elicited by bolus injection of nitroprusside (NT) and phenylephrine (PE) in 6 normotensive healthy young subjects. The rate of ΔMAP appeared to be greater ($P<0.01$) during hypotension than during hypertension, whereas the ratio of ΔHR/ΔMAP was not statistically different between the drugs (see table).

Drug	Dose *μg/kg*	ΔMAP *mmHg*	dMAP/dt *mmHg/sec*	Slope *bpm/mmHg*	Latency *beats*	Latency *sec*	Delta *bpm*
NT	3±0.98	-24±3	-1.49±0.2	-0.71±0.1	12±2.2*	7.6±1.2*	8.4±1.8*
	1±0	-14±2	-1.14±0.2	-0.97±0.2	8±0.8*	6.1±0.6*	6.1±1.6*
PE	1±0	10±2	0.80±0.2	-1.07±0.3	1±0.4*	1.3±0.5*	-1±0.5
	3±0	17±2	0.74±0.2	-0.78±0.1	0.6±0.3	1.1±0.5	-1±0.2

* indicates a significant difference from zero.

During PE induced hypertensive stimuli the bradycardiac latency was minimum. However, the tachycardiac latency, i.e., the time lag between the peak decrease in MAP and the peak tachycardia during NT induced hypotensive stimulation, was significantly ($P<0.01$) associated with ΔMAP, indicating the contribution of cardiac sympathetic activation. Furthermore, the delta tachycardia (the difference between tachycardia corresponding to the peak decrease in MAP and the peak tachycardia) was positively related ($P<0.04$) to the latency. We concluded that cardiac sympathetic mediated tachycardiac latency during graded hypotensive stimuli was dose-response related.

Supported in part by NIH HL45547 & NASA-NSCORT #NAGW-3582

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Geoffrey P. Kline

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF RENOVASCULAR HYPERTENSION ON CORONARY VASCULAR RESPONSIVITY IN CONSCIOUS DOGS

G. P. Kline and P. A. Gwirtz University of North Texas Health Science Center, Dept. of Integrative Physiology, Fort Worth, TX 76107.

Hypertension is associated with a decreased ability of the coronary vasculature to dilate. Whether this is due to increased vasoconstrictor mechanisms or decreased vasodilatory mechanisms is not known. To examine the hypothesis that a dysfunctional endothelium may contribute to this, we compared the effects of blocking the endothelial-dependent vasodilator, nitric oxide (NO) in normotensive dogs to dogs with renovascular hypertension (RVH). Six dogs were chronically instrumented to measure left ventricular systolic and diastolic pressures, maximal rate of rise of LVP, heart rate, mean aortic pressure, and circumflex flow velocity (CFV). After normotensive studies were concluded, RVH was induced in 3 dogs by reducing left renal artery blood flow by 60% causing MAP to rise from 89±6 to 114±7 mmHg. Endothelial NO production was blocked in the normotensive state with 75 mg nitro-L-arginine (L-NA), i.c. Responses to intracoronary (i.c.) norepinephrine (NE), terbutaline (TRB), isoproterenol (ISO), and acetylcholine (ACh) were examined. The following data was collected and are expressed as % change in CFV from basal levels.

	Normotensive	Normotensive + L-NA	RVH
NE (0.3 _g)	79 4	32 7*	39 17
ISO (0.1 _g)	141 44	67 20*	35 2
TRB (10 _g)	142 25	79 16*	74 19
ACh (0.5 _g)	174 30	96 15*	60 2
ADO (20 _g)	179_34	168_46	112_12

*p<0.05 Normotensive + L-NA vs. Normotensive (no statistics performed using RVH group due to small sample size, n=3)

The similar responses observed in the Normotensive+L-NA and RVH groups suggest that the altered coronary vascular responsiveness observed in hypertension involves coronary vascular endothelial damage.

Supported by NIH HL-34172.

Abstract #34

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Martin Farias

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

LOCATION OF OPIATE RECEPTORS RESPONSIBLE FOR INHIBITION OF VAGAL BRADYCARDIA. Martin Farias, K. Jackson, A. Stanfill, and J.L. Caffrey. Dept. of Integrative Physiology, University of North TX Health Science Center, Fort Worth, TX 76107.

The purpose of this study was to locate the opiate receptors responsible for Met-enkephalin-arg-phe (MEAP) inhibition of vagal bradycardia. The location of these receptors has been narrowed to the intracardiac parasympathetic ganglia and the prejunctional nerve terminals innervating the sinoatrial node. In this study three dogs were instrumented with a microdialysis probe inserted into the substance of the sinoatrial node. Nanomolar concentrations of MEAP were infused directly into the sinoatrial node via the microdialysis probe. The functional position of the probe was tested by perfusing norepinephrine which produced an increase in heart rate of more than 30 beats per minute. Vagal stimulations were conducted at .5, 1,2, and 4 Hz during vehicle infusion (saline ascorbate). Diprenorphine, and diprenorphine with MEAP were also infused via the dialysis probe while stimulating the vagus nerve. Cardiovascular responses during vagal stimulation were recorded on-line. Systemic injections of MEAP were given while the opiate receptors in the sinoatrial node were blocked with diprenorphine (opiate antagonist). MEAP introduced into the sinoatrial node via the microdialysis probe reduced vagal bradycardia by more than half. Local blockade of these receptors with the opiate antagonist, diprenorphine, eliminated the effect of MEAP and demonstrated the participation by opiate receptors. When local blockade with diprenorphine was combined with systemic MEAP, the effect of MEAP was similarly blocked suggesting that there are no ganglionic receptors involved in this process. These data are preliminary and more experiments will be needed to validate these conclusions. The findings suggest that the opiate receptors responsible for the inhibition of vagal bradycardia are located in the prejunctional nerve terminals innervating the sinoatrial node. Enkephalin plays a role in cardiac function. This study will broaden our knowledge base regarding opioid role in cardiovascular function.

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Keith Jackson

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE ROLE OF WHITE CELL DERIVED ENKEPHALINS DURING HEMORRHAGIC HYPOTENSION. Keith Jackson, A. Stanfill, M. Farias, D. Yoshishige, B. Barron, and J.L. Caffrey. Department of Integrative Physiology, UNTHSC, Fort Worth, Texas 76107-2699.

The purpose of this research study was to investigate the role of endogenous opiates in cardiovascular function. We reported increases of thirty fold or more in selected enkephalins, met-enkephalin-arg-phe (MEAP), and Peptide B, during sustained hypotension. Initially these increases were assumed to be adrenal medullary in origin. However, similar increases in met-enkephalin were still observed during stress in adrenalectomized animals suggesting another major source of enkephalin. Investigators have recently reported that human white cells synthesize proenkephalin and then process and release a variety of proenkephalin derived products. In order to pursue this investigation, we needed to develop methods for isolating white blood cells and for measuring proenkephalin products within the isolated white cells. Using methods modified from, A. Boyum, (Scand. J. Immunol. 5, Suppl 5: 2-8, 1976) we have validated procedures for reliably isolating white blood cells from small samples of canine blood. Cells were resuspended in RPMI medium, after isolation and adjusted to a concentration of $1x\ 10^6$ cells/ml. The cells obtained from dogs before, after one hour, and after two hours of hemorrhagic hypotension were incubated at 37^0C for periods of one and two hours. After which time, the cell suspensions were centrifuged, the supernatant was saved for analysis and 1ml of hot water was added to the remaining cells to stop all enzymatic reactions. A radioimmunoassay was developed, so that the supernates and white cell extracts could be analyzed. The assay easily measures MEAP and its N-terminally extended precursors peptide-B and proenkephalin with very little cross reactivity. Cells isolated during hypotension have progressively more intracellular enkephalin. When incubated, their content increases dramatically. The content increases more in cells collected later in the hemorrhage. Despite the large increases in content little enkephalin is detectable in the supernate medium. These data suggest that hemorrhagic hypotension stimulates the intracellular accumulation of proenkephalin products. The nature of the stimulus remains to be determined. (Supported by NIDA & local funds)

Abstract #36

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Steven R. Stuewe

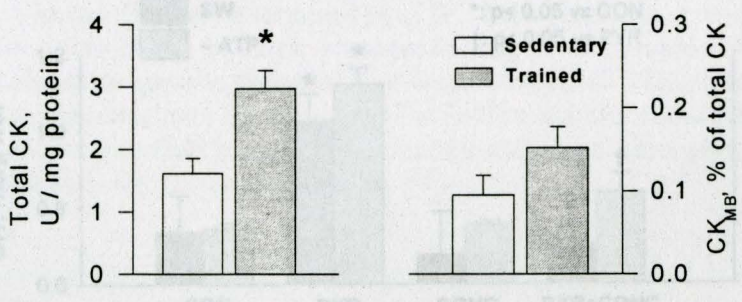
Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EXERCISE TRAINING INCREASES CREATINE KINASE CATALYTIC CAPACITY IN CANINE MYOCARDIUM. Steven R. Stuewe, Patricia A. Gwirtz, Robert T. Mallet. Dept. Integrative Physiology, Univ. North Texas Health Science Center, Fort Worth, TX 76107-2699.

The creatine kinase (CK) energy shuttle efficiently couples energy demand to energy supply in tissues with high and rapidly changing energy demands, including myocardium. This system channels high energy phosphate bonds, generated by oxidative phosphorylation in the mitochondria, to contracting myofibrils and sarcolemmal and sarcoplasmic reticular ion pump ATPases. We recently demonstrated that exercise training increases the activities of several glycolytic and oxidative enzymes in canine left ventricular myocardium. The goal of this study was to determine whether a similar increase in CK capacity occurs in response to training. Mongrel dogs were subjected to a 9 wk treadmill training regimen and sedentary dogs were cage-rested for 4 wk. Stop-frozen biopsies of left ventricular myocardium were obtained from pentobarbital-anesthetized trained and sedentary dogs. CK was extracted in phosphate buffer and assayed at 38°C. CK isoforms (CK_{mito}, CK_{MM}, CK_{MB}) were separated by anion exchange chromatography and assayed. The figure shows total CK activity (U · mg protein^{-1}) and CK_{MB} isoform as percentage of total CK. Exercise training increased total CK activity 84%, from 1.6±0.2 to 3.0±0.4 U · mg protein^{-1} ($P < 0.01$). CK_{MB} as a fraction of total CK activity was unaltered by training. These findings indicate that the capacity of the CK system is increased in response to training due to a proportional increase in all of the CK isoenzymes.



Abstract #37

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Jie Sun, B.S.

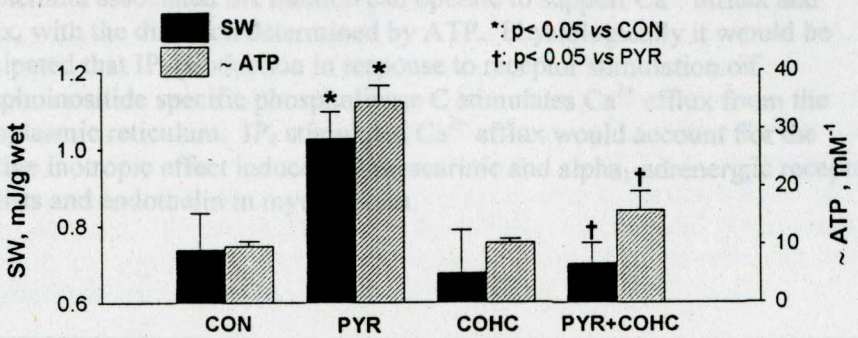
Department: Integrative Physiology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

PYRUVATE ENHANCEMENT OF CARDIAC FUNCTION AND ENERGETICS REQUIRES ITS MITOCHONDRIAL METABOLISM. Jie Sun, Robert T. Mallet, Dept. Integrative Physiology, University of North Texas Health Science Center, Fort Worth, Texas 76107-2699.

Myocardial metabolism of pyruvate augments contractile function and cytosolic free energy of ATP hydrolysis. These favorable inotropic and energetic effects could result from pyruvate catabolism in the mitochondria or from increased ratio of the cytosolic $NAD^+/NADH$ redox couple via the lactate dehydrogenase equilibrium. To differentiate between these two mechanisms, 0.6 mM α-*cyano*-3-hydroxycinnamate (COHC) was administered to isolated working guinea-pig hearts to selectively inhibit mitochondrial pyruvate uptake without altering pyruvate's cytosolic redox effects. In hearts metabolizing 0.2 mM octanoate, 2.5 mM pyruvate (PYR) increased left ventricular stroke work (SW) and power 40%, mechanical efficiency 29%, and cytosolic ATP phosphorylation potential (~ATP) nearly fourfold (Figure), relative to PYR-free controls (CON). $^{14}CO_2$ formation from [1-^{14}C]pyruvate was inhibited 65% by COHC, and octanoate oxidation, *i.e.* $^{14}CO_2$ formation from [1-^{14}C]octanoate, concomitantly increased threefold. COHC prevented pyruvate enhancement of left ventricular function, mechanical efficiency and cytosolic phosphorylation potential, but did not alter respective levels in pyruvate-free control hearts. These results indicate that pyruvate oxidation of cytosolic redox state is not sufficient to increase cardiac function and cytosolic phosphorylation potential when mitochondrial pyruvate transport is disabled, and that mitochondrial metabolism of pyruvate is essential for its favorable cardiac effects.



Abstract #38

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Lara Keyser

Department: Pharmacology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ATP and INOSITOL TETRAKISPHOSPHATE REGULATE Ca^{2+} FLUXES ACROSS CARDIAC SARCOPLASMIC RETICULAR MEMBRANES.

Lara Keyser, Ranga Vasan, Carolyn Quist and Eugene E. Quist. University of North Texas Health Science Center at Fort Worth. Fort Worth, TX, 76107.

Inositol tetrakisphosphate (IP_4) was reported to increase Ca^{2+} uptake into cardiac membranes tentatively identified as junctional sarcoplasmic reticulum (SR). The objectives of this investigation were to identify the membrane site regulated by IP_4 and to further characterize the properties of the IP_4 stimulated Ca^{2+} transporter. In cardiac microsomal membranes fractionated on sucrose gradients, IP_4 stimulated Ca^{2+} uptake was greatest in membranes separating on a 22.5% sucrose cushion. These membranes were enriched in sarcolemma and an ATP dependent Ca^{2+} transport mechanism, susceptible to inhibition by thapsigargin (a SERCA inhibitor). Membranes separating on 30 and 40% sucrose cushions supported greater ATP dependent Ca^{2+} transport, but IP_4 stimulated Ca^{2+} uptake was much less than in the lower density membranes. Analysis of the L-type channel distribution, a transverse tubular/ sarcolemmal marker, indicated that the IP_4 stimulated Ca^{2+} transporter does not co-purify with transverse tubular membranes from rat heart. The IP_4 stimulated Ca^{2+} transporter co-purifies with SR associated with sarcolemma. IP_4 stimulated Ca^{2+} influx was also facilitated by oxalate further characterizing the IP_4 stimulated Ca^{2+} uptake site as SR vesicles associated with the sarcolemmal fraction. In SR vesicles preloaded with $^{45}Ca^{2+}$ and ATP, IP_4 also stimulated Ca^{2+} efflux. Efflux was dependent on the presence of greater than 0.2 mM ATP. These observations indicate that the IP_4 stimulated Ca^{2+} transporter in a sarcolemma associated SR fraction can operate to support Ca^{2+} influx and efflux, with the direction determined by ATP. Physiologically it would be anticipated that IP_4 production in response to receptor stimulation of phosphoinositide specific phospholipase C stimulates Ca^{2+} efflux from the sarcoplasmic reticulum. IP_4 stimulated Ca^{2+} efflux would account for the positive inotropic effect induced by muscarinic and alpha$_1$-adrenergic receptor agonists and endothelin in myocardium.

Abstract #39

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Yun Bai

Department: Molecular Biology & Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

TRANSCRIPTIONAL REGULATION OF CARDIAC HYPERTROPHY SENSITIVE GENES BY AN INDUCIBLE CALCINEURIN EXPRESSION SYSTEM. Y. Bai, H. Zeng, S.R. Grant, Dept. of Molecular Biology UNTHSC@Ft.Worth,TX 76107

Chronic stimulation of cardiac hypertrophy can lead to heart failure in humans. Molecular mechanism(s) driving this adaptive growth process are currently not well understood. It has been shown that adult cardiomyocytes revert to an embryonic program of cardiac gene expression during hypertrophy signaling, and continous up-regulation of cardiac hypertrophy-sensitive genes results in a progression toward heart failure. Recent studies have documented some functional roles for calcium signaling in initiating the hypertrophy response. But it is unclear how these signals are coupled to alterations in cardiac gene expression of the sarcomeric contractile proteins. Preliminary studies from our laboratory suggest that calcium/calmodulin-dependent enzymes play an important role in the cardiomyocyte control of sarcomeric contractile proteins expression. We hypothesize that calcium/calmodulin-dependent kinases and phosphatase, calcineurin (CaN), are principal regulators of transcriptional up-regulation in hypertrophy. We have generated a tetracycline transactivator (tTA) inducible system for CaN expression to regulate the level of CaN in primary cultured cardiomyocytes. We have also investigated this control process in the Pulmonary Arterial smooth muscle cell line, Pac-1. Hypertrophy-sensitive gene expression initiated by exogenous CaN was detected by a promoter-reporter assay. We found that the calcium-regulated protein phosphatase, calcineurin, dramatically activates the transcription of several sarcomeric or embryonic cardiac hypertrophy-sensitive genes. Similar results were observed in Pac-1 cultures. The transcriptional induction of cardiac embryonic genes by CaN is both gene dose- and time-dependent. Transient transcription of reporters reached a maximum 48 hours following transfection. The CaN-specific inhibitor, cyclosporin A, blocked CaN induction of hypertrophy-sensitive gene expression. These results suggest that CaN up-regulates the transcription of hypertrophy-sensitive genes in the cultured neonatal cardiomyocyte and in the smooth muscle cell line, PAC-1. Ongoing studies in this laboratory agree with results from studies on CaN over-expressing transgenic founder mice which demonstrate dramatic cardiac hypertrophy. This work is a collaborative effort with the laboratory of Dr. Eric Olson. The molecular mechanism of CaN transcriptional induction and CaM kinase control of cardiovascular hypertrophy are actively being pursued. These studies will be helpful to develop pharmacologic inhibitors of cardiac hypertrophy and hold to promise of developing second generation drugs targeted to prevent heart failure.

Abstract #40

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Michael D. Wilburn

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Visiting Scientist X

Read instructions and fit abstract inside the space given below:

Treatment of Sickle Cell Anemia by M-15 in a Transgenic Mouse Model. Michael D.Wilburn [1], Fu-mei Wu [2] and Ming-chi Wu [2] PSI Resaerch Center, Inc.[1], Longview, Tx 75607-2643 and Department of Molecular Biology and Immunology [2], University of North Texas Health Science Center, Fort Worth, Tx 76107

Sickle cell anemia is a hereditary disease which afflicts 1 in 400 African-American. Although the molecular mechanism of this disease is well understood, effective treatments are still lacking. Recent progresses in sickle cell research have revealed several breakthrough in therapy, and in tools for drug screening and gene therapy. Hydroxyurea has been approved by FDA as a therapeutic drug for this disease while bone marrow transplantation has also provided as an effective treatment for selected patients. Most important of all is the development of two transgenic sickle cell anemia animal models which mimic human disease. We have synthesized a chemical, α-(S-adenosyl methionine)-O-tocopherol (M-15) which is believed to be an effective anti-sickling agent. Although the mechanism of action of this compound in not known, it is possible that M-15 , like hydroxyurea, induces the expression of Hb-F thus preventing sickling of the abnormal sickle cells. Using the animal model in this project, we will attempt to test the hypothesis that M-15 stimulates Hb-F production in the transgenic sickle cell anemic mice thus prevents the sickle crisis. The same animal model will also be used for testing and screening of potential anti-sickling drugs as well as gene therapy by introducing a constituitively expressed γ-globin gene thus increasing the Hb-F production in the tested animals.

Abstract #41

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Adnan Dibas, Ph.D.

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

Mechanism of Wortmannin and ML9 inhibition of vasopressin-activated water transport in Toad Urinary Bladders

Adnan I. Dibas[1], Abdul J. Mia[2] and Thomas Yorio[1]

[1]Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX. 76107, [2]Jarvis Christian College, Hawkins, TX. 75765

The involvement of myosin light chain kinase (MLCK) in water transport in toad urinary bladder, a popular model of mammalian collecting duct, was investigated. Wortmannin, a potent inhibitor of MLCK, dose-despondently inhibited vasopressin-induced water transport (55 ± 6 % (n=5) at 6 μM), and by 89 ± 2 % (n=4) at 60 μM). A similar inhibition was observed with ML9, another MLCK inhibitor (57 ± 14 % (n=4)). In addition, both inhibitors significantly reduced MLCK-induced phosphorylation of K-MLC_{11-23} peptide (a selective substrate for MLCK, wortmannin inhibited phosphorylation by 89 ± 7 % and ML9 inhibited phosphorylation by 77 ± 4 % (n=4)). Wortmannin and ML9-induced inhibition of water transport was also accompanied by inhibition of endocytosis of fluorescein-isothiocyanate dextran (FITC), an endocytotic marker (wortmannin inhibited endocytosis by 66 % whereas ML9 inhibited endocytosis by 25 %). It is suggested that wortmannin and ML9, inhibitors of water transport, mediate their action at least in part, by inhibition of MLCK and the endocytotic pathway in toad urinary bladder.

This research is supported in part by a grant from the U.S. Department of the Army # DAMD17-95-C-5086

Abstract #42

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Dr. A. J. Mia

Department: Jarvis Christian College/Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF VASOPRESSIN ON ENDOSOMES AND CAVEOLAE IN TOAD URINARY BLADDER GRANULAR CELLS. A.J. Mia*, L.X. Oakford**, A. Dibas** and T.Yorio**. *Jarvis Christian College, Hawkins, Texas 75765 and **UNTHSC at Fort Worth, Fort Worth, Texas 76107

Stimulation of toad urinary bladders by vasopressin increases transepithelial osmotic water flow with insertion of aggrephores (water channels, aquaporins) into the apical plasma membrane. Following cessation of vasopressin, aggrephores are then retrieved into the cytosol as endosomes of various shapes and sizes. These cytosolic endosomes may fuse to form lysosomes and multivesicular bodies or may enter into golgi compartments. We identified a large number of caveolae (microdomain structures) that are derived from the basal plasma membrane that migrate to the apical plasma membrane. The function of these caviolae are uncertain, however, they may be related to vasopressin mediated water transport. Urinary hemibladders, removed surgically from doubly-pithed toads, *Bufo marinus*, were suspended as sacs and used either as control (no hormone) or experimental tissue (100mU/ml vasopressin) under an imposed osmotic gradient. To determine the nature of endosomes and their formation, we used horseradish peroxidase (HRP) at the mucosal or at the serosal surfacee for visualizing endocytosis. Tissues were fixed in cold 2% glutaraldehyde, processed for peroxidase localization and embedded in epon for transmission electron microscopy (TEM). Ultrathin sections, taken on bare nickel grids, were exposed to either uranyl acetate or left unstained. Some caveolae detached from the basal plasma membrane by a process of endocytosis and traversed through the cytosol to the apical plasma membrane. HRP was internalized through the apical plasma membrane as HRP-containing endosomes and not through caveolae. These endosomes appear at various locations of the cytosol of the granular cells, where they fuse into large endosomes. Some endosomes form multivesicular bodies and others end up in lysosomes or in golgi compartments. Internalization of HRP-containing caveolae occurred through the basal plasma membrane and migrated toward the apical surface. What roles the cytosolic endosomes and caveolae play in cycling water channels in this renal membrane model remains to be determined. (Supported by grant: DAMD17-95-C-5086).

Abstract #43

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Lawrence X. Oakford, Ph. D.

Department: Department of Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

PRESENCE OF CAVEOLAE IN THE GRANULAR EPITHELIAL CELLS OF RABBIT URINARY BLADDER

A. J. Mia*, L. X. Oakford** and T. Yorio***

*Jarvis Christian College, Highway 80, Hawkins, TX 75765
**Department of Anatomy and Cell Biology and ***Department of Pharmacology, UNT Health Science Center, Ft. Worth, TX 76107

Previously, we have reported the presence of caveolae, clathrin-coated pits and vesicles in toad urinary bladder (TUB) granular cells, smooth muscle and endothelial cells[1,2]. Here we report for the first time the presence of caveolae and clatharin-coated pits and vesicles in the granular epithelial, smooth muscle and endothelial cells of rabbit urinary bladder (RUB). Comparative transmission and scanning electron microscopy were carried out on TUB and RUB. We found the above structures in both species along the basal and plasma mebranes. Comparative analysis of caveolae indicated little difference in their size and morphology between the amphibian and vertebrate species. However, the RUB granular epithelial cells contained denser cytoplasm and fewer caveolae and secretory granules compared to the TUB epithelial cells. Like in TUB cells, caveolae and vesicles were also found in smooth muscle and endothelial cells of the RUB. Presence of caveolae in TUB and RUB granular epithelia indicate their likely occurrence in the urinary bladders of other vertebrates including human. Since caveolae are known to participate in a variety of cellular processes including transcytosis, endocytosis, pinocytosis and internalization of Simian Virus 40 (SV40) in mammalian cells[3], their functional roles in toad and rabbit urinary bladder cells await further investigation.

1. A. J. Mia *et al.*, FASEB J. **10**(1996)161.
2. A. J. Mia *et al.*, Proc. MSA (1996)934-935.
3. E. Stang *et al.*, Mol. Biol. Cell, **8**(1997) 47.
4. supported in part by Grants DAMD 17095-5086 and NSF BIR-9413907

Abstract #44

NUTRITION AND CHRONIC DISEASE PREVENTION

45.	Walter J. McConathy, Ph.D.	INSTITUTE OF NUTRITION AND CHRONIC DISEASE PREVENTION
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55.	Kimberly Ann Krueger	SITE-SPECIFIC PHOSPHORYLATION OF SYNAPSIN I BY CaM KINASE II IN PANCREATIC βTC3 CELLS: SYNAPSIN I IS NOT ASSOCIATED WITH INSULIN SECRETORY GRANULES
56.	Vijian Dhevan	APO-A1 IS LOCATED WITHIN THE SECRETORY PATHWAY OF TRANSFECTED MDCK CELLS AND ITS EXPRESSION RESULTS IN AN INCREASED GLYCOGEN ACCUMULATION

57. Woo-Jin Chang

INTRACELLULAR GLYCOGEN CONFERS RESISTANCE TO STRESSES SUCH AS HEAT, HYPEROSMOLARITY, AND ACID IN *ESCHERICHIA COLI*

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: _____

Department: Institute of Nutrition and Chronic Disease Prevention

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

INSTITUTE OF NUTRITION AND CHRONIC DISEASE PREVENTION

Michael Clearfield, D.O, Director, and Walter J. McConathy, Ph.D., Deputy-Director University of North Texas Health Sciences Center, Fort Worth, Texas.

Purpose: The long-term mission of this Institute is to promote good health by emphasis on sound nutritional practices. The Institute will have three broad areas of focus: **Basic and Applied Research; Higher Education; and Public Education; and Community Service.**

Activities: The Institute will address the role of nutrition in preventing cardiovascular disease, diabetes, and the improvement of the quality of life during aging. The initial focus will involve cardiovascular risk factors, lipoprotein metabolism, and lipid lowering drugs. In the long term, research activities will focus on the nutritional components and molecular mechanisms of disease processes at the cell, organ, and whole organism level while clinical activities will include the development of programs in the area of diabetes, preventive cardiology, rehabilitation for those with vascular disease, osteoporosis, and dietary intervention in the aging process and cancer.

Summary: Based on available experts at UNTHSC, this Institute will enhance collaborative research between physicians and basic scientists of a number of Departments. More importantly, it will promote interactions with the local and national professional community in the area of sound nutritional practices leading to the prevention of chronic disease.

Abstract #45

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Maya P. Nair

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

EXPRESSION, PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN LECITHIN: CHOLESTEROL ACYLTRANSFERACE

Maya P. Nair, Balachandra J. Kudchodkar, AmirAyyobi, Hydn Pritchard and Andras G. Lacko

We have established a hepatoma cell line, Mc Ardle 7777 (Mc 7777) cells that constitutively express significant quanties of human recombinant lecithin : cholesterol acyltransferace (rLCAT). The Mc 7777 cells were double transfected with LCAT cDNA cloned into a mammalian expression vector. The recombinant Mc 7777 cells were grown to 80% confluency in Dubleccos modified eagle medium with 20% serum. Subsequently the cells were washed and supplied with serum free midium for 24 h periods. The conditioned medium containing rLCAT was harvested at 24 and 48 hrs and subjected to chromatography on a series of three columns (phenyl sepharose, affigel-blue and heparin sepharose). An rLCAT preparation was obtained that was found to be homogeneous by arylamide gel. Previously, we have shown that the physical and chemical properties of Baby Hamster Kidney (BHK) rLCAT were essentially indistinguishable from those of the enzyme species isolated from human plasma. A comparative study of the enzymatic properties of rLCAT has revealed no discernible differences between the rLCAT isolated from the conditioned media of Mc 7777 and BHK expression systems. However, the carbohydrate component of rLCAT from Mc7777 more closely resembles plasma LCAT than the BHK expressed rLCAT. The amount of enzyme per milliliter of the medium was nearly double in the case of Mc7777 compared to BHK expression system. We conclude that for these and other reasons the Mc 7777 cells are superior to the BHK cell line in producing LCAT with the characteristics appropriate for detailed structural atudies.

Abstract #46

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Karen R. Murray

Department: Molecular Biology and Immunology

Graduate Student XX Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

STRUCTURAL DOMAINS OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE

Karen R. Murray and Andras G. Lacko

University of North Texas Health Science Center, Department of Molecular Biology and Immunology, Fort Worth, Texas

Lecithin:cholesterol acyltransferase (LCAT) catalyses the esterification of plasma lipoprotein cholesterol in mammals as part of the reverse cholesterol transport pathway. Studies of the natural mutations of LCAT revealed a putative substrate binding region of the enzyme (residues #121-136) that is totally conserved in six mammalian species. In an effort to develop an immunoassay for LCAT and to obtain additional functional information about this putative substrate binding region, LCAT was probed by immunoassays. Three enzyme linked immunoassay models and three antibodies were utilized in these studies. Two polyclonal antibodies, one against human plasma LCAT and the other against purified recombinant LCAT, and one site directed antibody specifically prepared against a peptide representing the 121-136 region of LCAT, were employed. All antibodies reacted with a recombinant form of purified LCAT secreted by baby hamster kidney (BHK) cells and McArdle 7777 hepatoma cells by both Western Blot and using an antibody sandwich ELISA for LCAT. Only the polyclonal antibodies were able to recognize the enzyme when it was first adsorbed to HDL in a sink immunoassay, or to a hydrophobic surface in a direct coat immunoassay. Direct coat immunoassays demonstrated the needed sensitivity to measure LCAT from cell culture media. These studies suggest that the 121-136 region of LCAT indeed represents a lipoprotein substrate binding domain. Consequently, future studies, probing this region by mutagenesis and confirmational studies should yield valuable information regarding the mechanism of action of this key enzyme in cholesterol transport.

Abstract #47

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Bhalchandra J. Kudchodkar

Department: Molecular Biology & Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

ISOLATION OF LIPID PEROXIDE RICH SUBFRACTION(S) OF PLASMA HIGH DENSITY LIPOPROTEINS.

Bhalchandra J. Kudchodkar, Ladislslav Dory and Andras G.Lacko
Department of Molecular Biology & Immunology. UNTHSC,Fort Worth Texas,76107.

The risk of coronary heart disease increases with elevated levels of plasma low density lipoproteins (LDL) and decreases with the elevated levels of plasma high density lipoproteins (HDL). Therefore, the atherogenic LDL is referred as the ***bad*** and the anti-atherogenic HDL as the ***good*** cholesterol. Recent studies however suggest that when lipid peroxides (LPO), accumulate in HDL, the usually anti-atherogenic HDL is transformed into a pro-atherogenic lipoprotein particle. We have developed a column chromatographic method which, separates the *in vitro* oxidized HDL (OX-HDL) into three fractions containing low (LOX-HDL), moderate (MOX-HDL) and high levels of LPO (HOX-HDL). Retrospective analysis of plasma (stored at -20^0 C for a year) showed the presence of HOX-HDL in the plasma of rats fed palmitate, but not those fed chow, oleate or fish oil. HOX-HDL was also detected in the plasma of rats injected with lead nitrate a known pro-oxidant. HOX-HDL was present in the frozen plasma of rabbits fed cholesterol, but not those fed chow. Suprisingly, HOX -HDL was detected in the frozen human plasma when HDL isolated from large amount of plasma (250ml) was used for fractionation. Among the HDL subfractions, HOX-HDL was found associated predominantly with smaller HDL_3 fraction. Since small HDL_3 particles cross intact endothelium and are found in tissue fluids, we suggest that when high levels of LPO accumulate in the HDL_3, modifications of the particle, including protein modification occur in vivo. These modified particles lose their antiatherogenic properties and become atherogenic i.e. promote the deposition of of lipids into tissues.This method, for the first time, allows us to identify the conditions and the mechanism(s) underwhich anti-atherogenic HDL is converted to pro-atherogenic HDL. This in turn may allow to devise nutritional and/or pharmacological stratagies to prevent and/or decrease their formation in plasma and thus prevent and/or delay atheroscerosis. The method may also help improve the diagnosis of coronary heart disease. (***supported by Faculty research grant***).

Abstract #48

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Samuel S. Chuang

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

A DOWNSTREAM REPRESSOR CONTROLS HEPATIC CELL-SPECIFIC EXPRESSION OF THE HUMAN APOLIPOPROTEIN B GENE.

By Samuel S. Chuang and Dr. Hriday K. Das
University of North Texas- Health Science Center
Fort Worth, Texas, 76116

Atherosclerotic coronary artery disease (CAD) is one of the leading causes of mortality in the United States. Plasma levels of low density lipoprotein (LDL) and its associated apolipoprotein B-100 (apoB) correlate directly with increased risk for development of CAD. Therapeutic modalities presently exist that enable appropriate modulation of LDL metabolism, but therapy has not been specifically directed toward transcriptional regulation of the apoB gene. Reduction of apoB biosynthesis could impact on the predisposition for development of premature CAD. For this reason, we have studied transcriptional regulation of the human apoB gene. Our studies suggest that hepatic cell-specific expression of the human apoB gene is controlled by at least four *cis*-acting elements located between positions -128 and +122. A negative *cis*-acting element (+20 to +40) is located in the first non-translated exon of the human apoB gene. ApoB gene regulatory factor-3 (BRF-3) interacts with the *cis*-acting element (+20 to +40) to mediate apoB expression in the liver. To elucidate the role of the repressor in apoB transcription, BRF-3 has been purified to apparent homogeneity by DEAE-cellulose, heparin-agarose, and DNA-specific affinity chromatography. Purified BRF-3 produced two polypeptide bands with apparent molecular mass of 70 kDa and 67 kDa in sodium dodecyl sulfate polyacrylamide gel electrophoresis detected by a silver stain. Both 70 kDa and 67 kDa proteins have been found to interact specifically with labeled double-stranded oligonucleotide containing BRF-3 binding site in a south western blot and in gel mobility shift assay suggesting that these two polypeptides bind to the sequence (+20 to +40) independently. Purification of BRF-3 will eventually lead to the cloning of genes encoding BRF-3. Availability of these repressor genes may provide novel avenues for developing specific strategies to reduce the biosynthesis of apoB and the risk for developing CAD.

Abstract #49

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

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ABSTRACT FORM

First Author: Eve S. Ettinger

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

IN VITRO EXPANSION OF PANCREATIC ISLETS

Eve S. Ettinger, R.A. Easom, S.D. Dimitrijevich; Department of Molecular Biology and Immunology; UNTHSC, FT. WORTH, TX 76107

Diabetes Mellitus is a leading cause of death in the United States, afflicting over 16 million people. Some consequences of diabetes include blindness, end-stage renal failure and cardiovascular disease. Type I, insulin-dependent diabetes mellitus (IDDM) patients, as well as some non-insulin dependent diabetes mellitus (NIDDM) patients require constant treatment with exogenous insulin to regulate their blood glucose level. A therapeutic approach being studied clinically is implantation of pancreatic β cells of human or porcine origin. The *in vitro* expansion of pancreatic islets is the focus of this study because the islet is an optimal glycemic control unit. Preliminary studies have shown that intact islets can be sustained in a three-dimensional collagen gel matrix for 4-6 weeks. We have also shown that fetal porcine islets sustained *in vitro* can be a source of β cells which appeared to spontaneously aggregate into islets. Immunohistochemical studies demonstrated the sheet of cells growing from the islets contained insulin producing cells, glucagon producing cells, and cells producing both insulin and glucagon. These results suggest the feasibility of propagation and differentiation of functional islet cells *in vitro* and assembly of these into islet like aggregates. The purpose of this study is to examine cell cytodifferentiation into functional islets, as well as to sustain viable islets. This would provide and implantable insulin source, thereby eliminating the need for exogenously administered insulin to diabetic patients.

Abstract #50

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Sarah Boyle

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DEPOLARIZATION- SECRETION COUPLING IN PORCINE ISLET β-CELLS.

Sarah Boyle, Jun Liu*, Richard Easom and Stanley Misler *. Department of Biochemistry and Molecular Biology, University of North Texas health Science Center, Fort Worth, Texas, 76107-2699 and *Department of Internal Medicine and Cell Biology/Physiology and Program in Neuroscience, Washington University, St. Louis, Missouri, 63110

Research on physiology and disease of insulin secreting β-cells would benefit from a source of large numbers of primary β-cells, with response patterns similar to those of human β-cells, for work on transplantation, genetic manipulation (transfection) and hypertrophy/regeneration under stress. Porcine islets, which can be harvested in large numbers may provide such a source. Using patch clamp electrophysiology and membrane capacitance (Cm) tracking, our initial results show that single porcine β-cells display several key features of stimulus-secretion coupling in common with human β-cells. 1) Their voltage dependent Na^+ and Ca^{2+} currents are largest when the cell is depolarized from near rest (-70 mV) to +10mV. 2) Slowly inactivating currents flowing for >100ms trigger "step-wise" increase in membrane capacitance (reflecting exocytosis) that are greatest at +10mV. 3) Cm increases are enhanced by addition of forskolin, a stimmulator of adenylate cyclase. 4) With trains of 10 repeated depolarizations to+10mV, Cm tends to show a progressive "creep" that continues for several seconds after cessation of the train. 5) Endocytosis returns membrane capacitance to baseline values over ~30s. The "slow-to-start, slow–to-stop" exocytosis suggests that average cytosolic Ca^{2+} must bulid up prior to the onset of exocytosis and the decay prior to the cessation of exocytosis. This is supported by simultaneous observation of the time course of voltage-independent Ca^{2+}-activated K^+ currents in these cells..

Abstract #51

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998 .**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Gary Frank Scott

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CALCIUM SENSITIVTY OF β–CELL TRANSCRIPTION FACTOR BINDING TO INSULIN PROMOTER **Gary Frank Scott and R.A. Easom**
Department of Biochemistry and Molecular Biology UNYHSC, Fort Worth, Texas 76107

In mammals, insulin is a hormone essential for the control of glucose homeostasis which is produced exclusively in β-cells of the endocrine pancreas. This study explores the calcium sensitivity of β-cell transcription factor-promoter binding mechanisms which regulate the biosynthesis of insulin in response to glucose. Normal β-cell glucose metabolism raises the ATP/ADP ratio that depolarizes the plasma membrane causing inward Ca^{2+} flux while diabetic β-cells show reduced intracellular Ca^{2+}. Prior research has established a Ca^{2+} role in insulin secretion while the part it plays in biosynthesis ia controversial. Better understanding of the calcium role in glucose-induced insulin production can lead to insight into its impairment in Diabetes Mellitus disease and thus possibly to future improved biomedical treatment.

Insulin biosynthesis by transcription of the insulin gene in the pancreatic β-cell depends on interaction between *trans*-acting transcription factor proteins bound to*cis*-acting DNA sequences in the 5′ upstream promoter. Glucose-induced insulin transcription has been mapped to specific recognition motifs from -197 to -247 in the rat I (rINS) through deletion/mutation studies of these sequences linked in reporter constructs transfected into cultured β-cells. Using oligonucleotide probes representing these glucose-response-elements (GRE) in the electrophoretic mobility shift assay (EMSA), we examined Ca^{2+}-sensitive binding to nuclear extracts from INS-1 cultured insulinoma cells. Three strategies were designed: 1) Ca^{2+} stimulation of permeablized cells, 2) *in vitro* Ca^{2+}-stimulated phosphorylation of nuclear extracts, and 3) *in situ* glucose and K^{+} stimulation of culture medium. These experiments showed Ca^{2+}-mediation of glucose-induced binding inhibited by Ca^{2+}-chelator (EGTA), Ca^{2+}-channel-blocker (verapamil), and Ca^{2+}/calmodulin kinase II-specific inhibitor (KN-93). This evidence suggests that activation by phosphorylation of transcription factors may regulate Ca^{2+}-sensitive binding and thus insulin gene expression in the pancreatic β-cell.

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Harshika Bhatt

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

INHIBITION OF INSULIN SECRETION FROM PANCREATIC β-CELLS BY WORTMANNIN, A MYOSIN LIGHT CHAIN KINASE (MLCK) INHIBITOR. Harshika Bhatt, Michael Lawrence, Barry Conner and Richard A. Easom. UNTHSC at Fort Worth, Fort Worth, TX 76107

It is widely accepted that an increased intracellular Ca^{2+} is critical to glucose-induced insulin secretion. Since insulin secretion is accompanied by protein phosphorylation, it has been proposed that the distal steps of insulin secretion are mediated by cytoskeleton-associated Ca^{2+} /calmodulin-dependent protein kinases. One candidate for this function is myosin light chain kinase (MLCK) which through the phosphorylation of its substrate myosin light chain (MLC) and the subsequent activation of the actin ATPase of actin-myosin microfilaments could trigger a contraction event sufficient to provide the motile force for the shunting of secretory granules to the exocytotic site. There is accumulating evidence in a number of nonmuscle cell types that these mechanisms regulate granule secretion processes. Our hypothesis is that myosin light chain kinase might similarly regulate insulin secretion. The ability of insulin secratogogues, glucose and K^+, to activate MLCK by the phosphorylation of endogenous MLC has been studied using urea glycerol gel electrophoresis or immunoprecipitation of myosin using antiplatelet myosin. Incubation of β-cells and islets with wortmannin, inhibted both glucose-induced and high K^+-evoked MLC phosphorylation in cell monolayers as well as in the cell suspensions. Further, these data was supported by experiments with immunoprecipitation of myosin from ^{32}P-labeled cells and separation of MLC from myosin heavy chain by SDS-PAGE. Wortmannin in concentration ranges required to prevent MLCK activation completely inhibited insulin secretion. These results suggest that MLCK plays a central role in the Ca^{2+} -sensitive insulin secretion from the pancreatic β-cells. (Supported by NIH grant DK-47925).

Abstract #53

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Michael C. Lawrence

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CALCINEURIN ASSOCIATES WITH A NUCLEAR FACTOR OF ACTIVATED T-CELLS (NFAT) DNA-BINDING COMPLEX AND ENHANCES INSULIN GENE TRANSCRIPTION IN PANCREATIC INS-1 CELLS. M. Lawrence, S. Boyle, H. Bhatt and R.A. Easom, UNTHSC, Fort Worth, TX 76107

It has been well documented that nuclear factors of activated T-cells (NFATs) play a critical role in the regulation of cytokine gene expression in immune-system cells, and it has been demonstrated that NFAT can be activated and translocated from the cytoplasm to the nucleus upon activation of the Ca^{+2}/calmodulin-dependent phosphatase, calcineurin. Although transcription factors of the NFAT family have been detected in other tissues outside the immune system, little evidence exists to support the involvement of NFAT in the regulation of gene expression other than that of cytokines. This study identifies four NFAT consensus sequences within the first 620 base pairs of the rat insulin gene promoter and explores the NFAT binding capabilities of two of the sequences in which their presence and location are conserved among other mammalian species, including mice, dogs, and humans. Electrophoretic mobility shift assays showed specific NFAT DNA-binding activity that could be competed with unlabelled probe when incubated with pancreatic INS-1 cell extracts and shifted with extracts preincubated in the presence of anti-calcineurin antibody. Co-transfection experiments with a constitutively active form of calcineurin and a construct containing the rat I insulin promoter showed increased reporter activity over controls. Reporter activity which could be further enhanced by serum and glucose stimulation indicated a synergistic effect between NFAT/calcineurin and other promoter elements to regulate insulin gene transcription. These results suggest that calcineurin can enhance insulin gene transcription by activating NFAT in insulin-secreting cells. (Supported by the Advanced Research Program of the Texas Higher Education Co-ordinating Board)

Abstract #54

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Kimberly Ann Krueger

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

SITE-SPECIFIC PHOSPHORYLATION OF SYNAPSIN I BY CaM KINASE II IN PANCREATIC βTC3 CELLS: SYNAPSIN I IS NOT ASSOCIATED WITH INSULIN SECRETORY GRANULES. K.A. Krueger, E.I. Ings, A.-M. Brun, M. Landt and R.A. Easom, UNTHSC at Fort Worth, Fort Worth, TX 76107 and Washington University School of Medicine, St. Louis, MO 63110.

Increasing evidence supports a physiological role of CaM kinase II in the secretion of insulin from the pancreatic β-cell but the precise sites of action are not known. In the neuron, this enzyme plays an essential role in neurotransmitter release through the phosphorylation of a vesicle associated protein, synapsin I. Because of emerging similarities to the neuron with respect to exocytotic mechanisms, the expression and phosphorylation of synapsin I in the β-cell has been studied. Characteristic of neuronal synapsin I, doublet isoforms of Ia/b, were demonstrated in clonal mouse β-cells (βTC3) and primary rat islet β-cells. By immunoprecipitation, *in situ* phosphorylation of synapsin I was induced in permeabilized βTC3 cells within a Ca^{2+} concentration range shown to activate endogenous CaM kinase II under identical conditions. Proteolytic digests of these immunoprecipitates revealed that calcium primarily induced the increased phosphorylation of sites identified as CaM kinase II-specific and distinct from PKA-specific sites. Immunofluorescence and immunogold electron microscopy verified synapsin I expression in βTC3 cells and pancreatic slices but demonstrated little if any co-localization of synapsin I with insulin containing dense core granules. Thus while this study establishes that synapsin I is a substrate for CaM kinase II in the pancreatic β-cell, it is unlikely that such a phosphorylation event is important for insulin release. (Supported by NIH grant DK-47925)

Abstract #55

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: VIJIAN DHEVAN

Department: Anatomy and Cell Biology

Graduate Student__x__ Medical Student____ Postdoctoral Fellow____ Faculty____ Staff____

Read instructions and fit abstract inside the space given below:

APO-A1 IS LOCATED WITHIN THE SECRETORY PATHWAY OF TRANSFECTED MDCK CELLS AND ITS EXPRESSION RESULTS IN AN INCREASED GLYCOGEN ACCUMULATION. **V. Dhevan and V.L. Rudick, Dept. of Anatomy & Cell Biology, UNT Health Science Ctr., Ft. Worth, TX 76107.**

MDCK cells were transfected with the gene for human apolipoprotein A1 (Apo A1) and one clone (designated N2A) was chosen for further study. To localize Apo A1, paraformaldehyde fixed cells were incubated with antibodies for Apo A1 and/or the following organelle specific markers ß Cop (Golgi), calreticulin (ER) and cathepsin D (lysosomes), followed by incubation with the appropriate fluorescent labeled secondary antibodies. Cells were viewed with a Nikon Microphot fluorescence microscope and photographed using Fuji 1600 ASA color film. Immunofluorescence of double labeled cells revealed Apo A1 to be present in the secretory pathway, particularly within the Golgi. TEM demonstrated that transfected cells had amorphous pools within them that resembled similar structures found in intestinal enterocytes (Caco-2 cells), which endogenously produce Apo A1. Untransfected MDCK cells or those transfected with an empty promoter did not produce such structures. It was postulated that these pools might be glycogen, and cytochemical analyses using periodic acid-Schiff reagent at the light level and bismuth subnitrate at the TEM level indicated that this was so. N2A cells were immunostained for glycogen synthase (GS), and fluorescence microscopy revealed that the GS was associated with the glycogen pools. In addition co-localization of GS with ER (calreticulin) and GS with Apo A1 was also examined. In summary, the presence of Apo A1 within the secretory pathway leads to the accumulation of glycogen within kidney cells. We are currently investigating the mechanism to explain this phenomenon.

Abstract #56

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998.

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Woo-Jin Chang

Department: Molecular Biology & Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

INTRACELLULAR GLYCOGEN CONFERS RESISTANCE TO STRESSES SUCH AS HEAT, HYPEROSMOLARITY, AND ACID IN *ESCHERICHIA COLI*. **Woo-Jin Chang and Tony Romeo, Ph.D. Department of Molecular Biology & Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107**

α−1,4 glucans such as glycogen, starch, and amylose are found in most living organisms including prokaryotes, yeasts, plants, and mammals. Glycogen in *Escherichia coli* is synthesized and accumulated in stationary phase of growth, where bacteria are believed to spend most of their time in the environment. Although studies of the biochemistry, enzymology and genetic regulation of bacterial glycogen have been pursued for the last 40 years, the physiological role of this polysaccharide *in vivo* has been obscure. To elucidate the function of glycogen in *E. coli*, we decided to isolate glycogen-deficient *glg* mutants. Kanamycin-resistant (KanR) transposon mutants were characterized as follows: First, the KanR marker in each mutant was mapped to the region of *glg* gene cluster, 75.5 min. on the chromosome, by P1 bacteriophage transduction. Second, each strain was tested by complementation with plasmid-encoded *glgC* and *glgA* genes, to phenotypically validate the genetic defects. Third, the marker was physically mapped by Southern hybridization. As a result, a *glgA* mutant (TR3-1) and a *glgCA* polar mutant (EG153) were isolated. Those two markers were transferred to a prototrophic *E. coli* strain, MG1655, to create a set of isogenic strains. Stress response assays using a glucose-containing rich medium (Kornberg) revealed that glycogen in *E. coli* confers resistance against heat (57°C, >10,000-fold maximal viability difference was noted), hyperosmolarity (2.5M NaCl, >10,000,000-fold maximal viability difference), and acid (pH 1.32, >1,000 maximal viability difference). Moreover, mutants were sensitive relative to wild type strain only in late-stationary phase cultures, in a good agreement with the physical accumulation of glycogen. Interestingly, the glycogen-deficient TR3-1 mutant was more resistant to hydrogen peroxide (200mM for 30min., about 10,000-fold viability difference was noted with respect to the glycogen-positive parent). However, the resistance profile was reversed when cells were grown in Luria-Bertani broth, where cells do not accumulate glycogen. To our knowledge, this is the first demonstration that *E. coli* glycogen is associated with resistance against various physicochemical insults.

Abstract #57

EYE RESEARCH

58.	James E. Turner, Ph.D.	NORTH TEXAS EYE RESEARCH INSTITUTE
59.	Kristi D. Henderson	EFFECTS OF SUBSTANCE P ON CORNEAL EPITHELIAL CELLS IN VITRO
60.	Robert J. Wordinger, Ph.D.	HUMAN TRABECULAR MESHWORK CELLS EXPRESS NEUROTROPHINS AND NEUROTROPHIN RECEPTORS
61.	Rajnee Agarwal	FAS ACTIVATED APOPTOSIS IN CULTURED HUMAN TRABECULAR MESHWORK CELLS
62.	Alvin E. Finkley	CHARACTERIZATION AND ACTIVITY OF ENDOTHELIN CONVERTING ENZYME-1 IN HUMAN NON-PIGMENTED CILIARY EPITHELIAL CELLS
63.	Grant D. Schafer	TAURINE UPTAKE IN CULTURED HUMAN LENS EPITHELIAL CELLS
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65.	Wendi Lambert	EXPRESSION OF NEUROTROPHINS AND THEIR HIGH AFFINITY RECEPTORS (TRKs) BY CELLS OF THE HUMAN OPTIC NERVE HEAD
66.	Raghu Krishnamoorthy	EXPRESSION OF CD44 mRNA IN THE RETINA DURING DEVELOPMENT AND DEGENERATION
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68.	Raghu Krishnamoorthy	TRANSCRIPTION FACTOR NF-κB AND APOPTOSIS OF MOUSE PHOTORECEPTOR CELLS
69.	Harold J. Sheedlo, Ph.D.	*IN VITRO* RESPONSE OF A TRANSFORMED PHOTORECEPTOR CELL LINE TO GROWTH FACTORS AND AN RPE-SECRETED FACTOR

70. J. E. Turner — PHOTORECEPTOR CELL SURVIVAL PROMOTED BY AN RPE SECRETED FACTOR, A MEMBER OF TRYPSIN PROTEASE INHIBITOR FAMILY

71. Stephen J. Moorman, Ph.D. — COUNTER-ROTATION OF THE ZEBRAFISH *(DANIO RERIO)* EYE IN RESPONSE TO CHANGES IN LIGHT POSITION

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: James E. Turner, Ph.D.

Department: North Texas Eye Research Institute

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

NORTH TEXAS EYE RESEARCH INSTITUTE
James E. Turner, Ph.D., Director. University of North Texas Health Science Center.

Background The North Texas Eye Research Institute (NTERI) was established in 1992 as one of the Centers of Excellence at the University of North Texas Health Science Center at Fort Worth. The purpose of the NTERI is to serve as an academic and research focus for basic and clinical science activities within the vision community of Fort Worth and the surrounding areas. Consequently, over 20 faculty are involved in visual science research and have appointments to the NTERI. These faculty are located in three basic science departments (Anatomy and Cell Biology, Pharmacology, and Biochemistry and Molecular Biology) at the UNT Health Science Center, at the Alcon Research Laboratories and within the ophthalmology community of Fort Worth.

Within NTERI there are a number of groups of faculty interested in retina research, ocular diabetes, autoimmune diseases of the eye, optic nerve regeneration, glaucoma, corneal wound healing and aging. Specific areas of research interests within the various groups include: neovascularization, trophic factors, wound healing, aging, cell death, retina transplantation, glaucoma and diabetic complications. Broad technical areas of expertise are found though faculty interests within the NTERI to include: morphology, cell biology, biochemistry, molecular biology, pharmacology, electrophysiology and clinical expertise. All of the areas of expertise are supported by modern sophisticated equipment and a skilled technical staff.

Abstract #58

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Kristi D. Henderson

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECTS OF SUBSTANCE P ON CORNEAL EPITHELIAL CELLS IN VITRO

Kristi Henderson and S.D. Dimitrijevich

The cornea, a major component of the anterior ocular barrier, is exposed to numerous environmental insults. Corneal epithelium possesses the highest density of nerve endings in the body, and because of this rich innervation the sensory response is the earliest reaction to external stimuli. This response involves the release of neuropeptides such as substance P (SP) and calcitonin gene related peptide (CGRP), which have been suggested to mediate the neurogenic inflammatory response. The effects of SP on the human corneal epithelium have not been extensively studied and are poorly understood. Our recent immunohistochemical examination of human corneal epithelial cells show a strong cytoplasmic presences of SP suggesting a non-neuronal source of this neuropeptide. Also, our studies of human corneal epithelial cell attachment show that SP greatly stimulates this wound healing parameter. In our experiments, we will use cultured corneal cells to study the role of SP in the corneal neurogenic inflammatory cascade. Initially, we will determine if normal human corneal epithelial cells have the NK_1 receptor specific for SP. Then, we will determine the cellular effects of SP on corneal cell attachment and proliferation. We will also determine if SP induces the expression of proinflammatory mediators , cytokines IL1, IL6 and IL8. The results from these studies will also be applicable to the understanding of the neurogenic cascade in other peripheral tissues, such as the consequences of dermal irritation.

Abstract #59

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998 .

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Robert J. Wordinger, Ph.D.

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

HUMAN TRABECULAR MESHWORK CELLS EXPRESS NEUROTROPHINS AND NEUROTROPHIN RECEPTORS. R.J. Wordinger[1], W. Lambert[1], R. Agarwal[1], E. Craig[1], V. Dhevan[1], and A. Clark[1,2] North Texas Eye Research Institute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX.[2]

Purpose: Neurotrophins (NT) are a family of polypeptide growth factors which are involved in the development, maintenance, and regeneration of neurons. In mammals, NT family members include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4/5. Neurotrophins initiate signal transduction by ligand-induced dimerization and activation of 3 specific tyrosine kinase (Trk) receptors (Trk A, Trk B, and Trk C). The preferred receptor for each NT is as follows: NGF / Trk A, BDNF and NT-4 / Trk B, and NT-3 / Trk C. There is growing evidence that NT acting through Trk receptors can stimulate a variety of responses in *non-neural cells* via either paracrine and/or autocrine mechanisms. The purpose of this study was to determine if human trabecular meshwork cells (HTM) express NT and Trk receptors. **Methods:** Neurotrophin and neurotrophin receptor (Trk) mRNA expression was examined by total cellular RNA isolation, RT-PCR, and agarose gel electrophoresis using well characterized, early passage HTM cell lines from 6 day, 6 month, 2 year, 54 year, and 80 year old donors. PCR primers for specific neurotrophins and Trk receptors were designed using Oligo 4.0 (National Biosciences Inc., Plymouth, MN.). **Results**: Using RT-PCR we detected mRNA's for the following NT: BDNF and NT-3. Message for NT-4 was variably expressed being present in day 6, 6 month, and 2 year old cell lines. No mRNA expression was detected for NGF in any cell line. Message for full-length Trk B and C was variably expressed while message for the truncated forms of Trk B and C was expressed in all cell lines. No mRNA expression was detected for Trk A in any cell line. **Conclusions**: To our knowledge, this is the first report that HTM are capable of expressing NT and Trk receptors. Our results demonstrate that cultured HTM cells express mRNA for BDNF and NT-3 as well as mRNA for Trk B, Trk C and truncated forms of both receptors. **Support**: National Glaucoma Program of the American Health Assistance Foundation, Glaucoma Research Foundation, and Alcon Laboratories, Inc.

Abstract #60

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Ragnu Agarwal

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff ✓

Read instructions and fit abstract inside the space given below:

FAS ACTIVATED APOPTOSIS IN CULTURED HUMAN TRABECULAR MESHWORK CELLS. R. Agarwal[1], M. Talati[1], W. Lambert[1], N. Agarwal [1], A. Clark[1,2], and R.J. Wordinger[1] North Texas Eye Research Institute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX.[2]

Purpose: During normal aging cell numbers within the human trabecular meshwork (HTM) decrease. The loss of cells within the HTM is more severe in glaucoma. The mechanism by which the cell populations are reduced is not known. The molecular mechanisms involved in apoptosis have not been studied in the HTM. Fas (CD 95/APO-1) is a widely occurring cell membrane protein which upon binding its natural ligand (Fas L) activates cell death via apoptosis. The purpose of this study was to determine if *ex vivo* HTM tissues and cultured HTM express Fas, Fas L, Bcl-2, Bcl-xl, Bax, and ICE mRNAs and if apoptosis can be activated in cultured HTM cells exposed to a Fas monoclonal antibody (Fas Mab) which mimics the action of Fas L. **Methods**: mRNA expression was examined by total cellular RNA isolation, RT-PCR, and agarose gel electrophoresis from *ex vivo* HTM tissues from donors of 75, 79, and 80 years of age and well chracterized, early passage HTM cell lines from 6 day, 6 month, 2 year, 54 year, and 80 year old donors. Fas activated apoptosis was stimulated by exposing 70% confluent HTM cells to Fas MAb at a concentration of 100-400 ng/ml for 24-48 hours. Control cells were treated with equal concentrations of mouse IgM. Evidence for apoptosis was determined using phase contrast microscopy, TUNEL staining, transmission electron microscopy (TEM), and DNA laddering. **Results**: Using RT-PCR we detected mRNA expression for Fas. Bcl-2, Bcl-xl, and ICE in both *ex vivo* HTM tissues and cultured HTM cells. Message for Fas L and Bax was not detected. Exposure to Fas MAb resulted in significant apoptosis of HTM cell cultures as evidenced by positive TUNEL staining, apoptotic nuclei visualized with TEM, and DNA laddering. Limited apoptotic changes were observed in the IgM treated control cells. **Conclusions**: These results demonstrate that HTM tissues and HTM cells expresses Fas mRNA and other apoptosis mediators and upon activation via Fas Mab, undergo cell death via apoptosis.
Support: National Glaucoma Program of the American Health Assistance Foundation; Alcon Laboratories, Inc.

Abstract #61

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Alvin E. Finkley

Department: Biomedical Sciences

Graduate StudentX Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

Characterization and Activity of Endothelin Converting Enzyme-1 in Human Non-Pigmented Ciliary Epithelial Cells

A. Finkley, G. Prasanna, A. Dibas and T. Yorio. University of North Texas Health Science Center. Fort Worth, TX. 76107.

Endothelins (ETs) are potent vasoactive peptides, present in many ocular tissues including the ciliary epithelium. In the eye, ETs are suggested to lower the intraocular pressure by enhancing the aqueous humor outflow pathway via the trabecular meshwork and Canal of Schlemm. Biologically active ET-1 (21 amino acids) is produced from the precursor big ET-1 (39 a.a.) by a membrane-bound metalloprotease, endothelin-converting enzyme (ECE). One of the important factors that regulate ET levels, is the activity of ECE. In this study, identification of ECE and its activity were done using SV-40 transformed human non-pigmented ciliary epithelial (HNPE) cells. Endothelin and its precursor Big Endothelin (Big ET-1) were detected in HNPE cells by radioimmunoassay and immunofluorescence. Further characterization of the enzymatic activity of ECE (conversion of Big ET-1 to ET-1) was performed using a novel assay involving ^{125}I-Big ET-1. Polyclonal antibodies specific for Big ET-1 but not its product ET-1, were added at the end of the reaction and ^{125}I-Big ET was immunoprecipitated. Counts in the supernatant corresponded to ^{125}I-ET-1 while those in the pellet represented unconverted ^{125}I-Big ET-1. Western blotting using polyclonal antibodies detected ECE-1 in the plasma membrane fraction of HNPE cells but not in the cytosol. Furthermore, crosslinking experiments of ^{125}I-Big ET-1 to ECE-1 enzyme using a combination of crosslinkers of carbodiimide and ethylene glycol-bis(succinic acid n-hydroxysuccinimide ester) (EGS), detected two proteins of 90 and 200 KDa. ECE-1 activity (expressed as the ratio of ^{125}I-ET-1 produced to the total ^{125}I-Big ET-1 incubated) was measured and corresponded to: 20-25% (1hr), 45-63% (3hr) 77-81% (24hr) respectively. Thiorphan (2mM), a potent inhibitor of ECE, abolished ECE-1 activity (12-15%, (3h)). These results suggest that ECE-1 is localized in HNPE cells and is essential for the production of ET-1. The physiological importance of the unusual proteolytic processing by ECE-1 in ocular tissue may reflect on how ET regulates intraocular pressure.

Supported by: Texas Advanced Research Program grant from the Texas Higher Education Coordinating Board.

Abstract #62

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Grant D. Schafer

Department: Anatomy & Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

TAURINE UPTAKE IN CULTURED HUMAN LENS EPITHELIAL CELLS ((G. Schafer[1], S.-W. Chen[1], M. Martin[2], P.R. Cammarata[1]))
University of North Texas Health Science Center, Depts. of Anatomy and Cell Biology[1] and Pharmacology[2], Fort Worth, TX.

Purpose. The association between high-ambient galactose (Gal), the polyol pathway, and aldose reductase (AR) inhibition on *in vitro* [^{3}H]-taurine uptake was examined in cultured human lens epithelial cells (HLE-B3). ***Methods***. The kinetic characteristics of taurine accumulation based on the measurement of *in vitro* [^{3}H]-taurine uptake was determined with cultured HLE-B3 cells incubated in either high ambient galactose or galactose-free, physiologic medium (Phys) under experimental treatments which included aldose reductase inhibition. Competitive reverse transcription-polymerase chain reaction (RT-PCR) was utilized to precisely measure the temporal expression pattern of the human taurine cotransporter gene (hTAUT) under the various culture conditions. ***Results***. [^{3}H]-Taurine accumulation in the presence of 5.5 mmol/l D-glucose (physiologic medium) was rapid and linear for 8 hr. [^{3}H]-Taurine accumulation was monitored during a 3 hr uptake period performed over a concentration range of 1.5 to 400 µmol/l and the data plotted to equations for a two-site fit. The taurine transporter (high-affinity site) revealed a Km of 7.2 ± 1.8 µmol/l and V_{max} of 1891 ± 204 pmol/mg protein/hr, respectively. Chronic exposure (20 hr) of HLE-B3 to 40 mmol/l Gal showed little effect on the high-affinity taurine transport site. There was no apparent change in Km (7.7 ± 3.1 µmol/l) accompanied with a *marginal* decrease in V_{max} (1510 ± 297 pmol/mg protein/hr), suggestive of noncompetitive inhibition. [^{3}H]-Taurine uptake was apparently normalized when sorbinil (0.1 mmol/l) was concomitantly administered with the Gal medium (Km 7.1 ± 2.0 µmol/l, V_{max} 1926 ± 174 pmol/mg protein/hr). The relative expression of hTAUT mRNA was analyzed in Phys and Gal ± sorbinil treatments after 0, 4, 12 and 24 hr of cell culture exposure. hTAUT expression was identical under all treatment conditions. ***Conclusions***. It should be noted that *human* lens epithelial cells, unlike *bovine* lens epithelial cells, display relatively low aldose reductase activity. This point notwithstanding, taurine transport and TAUT gene expression appear little, if at all, affected by intracellular polyol accumulation in cultured *human* lens epithelial cells. Supported by National Health Public Service Award EY05570 (PRC). None

Abstract #63

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: YAMING XUE

Department: ANATOMY AND CELL BIOLOGY

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

HYPOXIA MODULATES GROWTH FACTOR EXPRESSION IN CULTURED HUMAN MÜLLER CELLS. **Y. Xue[1], L. Jingjing[1], A.A. Davis[2], and R.S. Roque[1].** [1]Department of Anatomy and Cell Biology, University of North Texas Health Science Center, Fort Worth, TX 76107; and [2]Department of Molecular Cardiology, University of Texas Southwestern Medical Center, Dallas, TX 75235.

Hypoxia-inducible growth factors are believed to be responsible for the proliferation of blood vessels in retinal neovascularization, while studies in our laboratory indicate that Müller cells release diffusible factors that promote the proliferation of endothelial cells in culture. To determine the role of Müller cells in retinal neovascularization, we have begun to investigate the expression of known angiogenic/permeability molecules in Müller cells cultured under normal or hypoxic conditions. Thus, in the following study, cultured human Müller cells were exposed to hypoxic conditions for variable periods of time and assayed using reverse transcription polymerase chain reaction (RT-PCR), semiquantitative PCR, and Ribonuclease protection assay. Our study shows that cultured human Müller cells express several angiogenic/permeability factors mRNA including vascular endothelial growth factor (VEGF), VEGF-C, basic fibroblast growth factor (bFGF), and angiopoietin-1(ANG-1). Following exposure to hypoxic conditions, VEGF and bFGF mRNA levels in Müller cells were increased but not VEGF-C mRNA. Our study shows that Müller cells express the messages for several angiogenic/permeability factors and that hypoxia modulates the expression of these growth factors in cultured human Müller cells. The hypoxic modulation of growth factor expression in cultured Müller cells support a role for Müller cells in the mechanisms of retinal neovascularization.

(Supported by an American Heart Association, National Affiliate GIA and by NIH EY10766.)

Abstract #64

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Wendi Lambert

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EXPRESSION OF NEUROTROPHINS AND THEIR HIGH AFFINITY RECEPTORS (TRKs) BY CELLS OF THE HUMAN OPTIC NERVE HEAD.

W.Lambert[1], R.Agarwal[1], R.Betton[1], E.Craig[1], A.F.Clark[1,2], and R.J.Wordinger[1]

North Texas Eye Research Institute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, TX[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX[2].

Purpose: Neurotrophins (NTs) are growth factors that promote the development, survival and differentiation of neurons. NT family members include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5). High affinity receptors for NTs are known as trk receptors (tyrosine kinase receptors). NT expression has been detected in many cell lines that also express the corresponding trk receptor suggesting that paracrine and/or autocrine neurotrophin signaling pathways may exist. The lamina cribrosa (LC) region in the optic nerve head consists of two main cell types: lamina cribrosa cells, and ONH astrocytes. Cells from this region come in close contact with retinal ganglion cell axons and may be a paracrine source of NTs for these neurons. Alternatively, these cells may signal each other through NTs. The role of NTs and trk receptors in the normal lamina cribrosa has not been extensively studied. The purpose of this study was to determine which NTs and trk receptors are expressed in cultured human ONH cells. **Methods:** Total cellular RNA isolation, cDNA synthesis, reverse-transcriptase polymerase chain reaction (RT-PCR) and agarose gel electrophoresis were performed using 3 well characterized human LC cell lines, 2 human ONH astrocyte cell lines as well as 3 normal human brain astrocyte cell lines. Total human brain cDNA was used as a positive control. Immunostaining for NTs and trk receptors was done using primary antibodies from Santa Cruz Biotech., Inc. (Santa Cruz, CA) and FITC-labeled secondary antibodies from Sigma Chemical (St. Louis, MO). **Results:** All cell lines studied expressed mRNA for NGF, BDNF, and NT-3. NT-4/5 mRNA was variably expressed. Message for full length trk A, trk B and trk C were also variably expressed. However, mRNA for the truncated forms of trk B and trk C were expressed in each cell line. Immunostaining for the various NTs were positive in all cell lines. Trk A, trk B and truncated trk B immunostaining was positive in all cell lines. Trk C staining showed variable results depending on the cell type. **Conclusions:** Cells of the human optic nerve head express message and protein for neurotrophins and two truncated high affinity receptors. Message and protein for the full length high affinity receptors is variably expressed suggesting that some cells in the optic nerve head may be able to respond to neurotrophins from paracrine or autocrine sources.

Support: None.

Abstract #65

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

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ABSTRACT FORM

First Author: DR RAGHU KRISHNAMOORTHY

Department: ANATOMY & CELL BIOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EXPRESSION OF CD44 mRNA IN THE RETINA DURING DEVELOPMENT AND DEGENERATION. Raghu Krishnamoorthy, Neeraj Agarwal and Michael Chaitin. Department. of Anatomy and Cell Biology and North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107.

In adult mouse neural retinas the cell surface adhesion/receptor molecule CD44 is localized on Müller glial cell apical microvilli which project into the interphotoreceptor matrix. In retinal degeneration slow (rds) mouse retinas, however, CD44 immunolabel increases dramatically throughout the retina during the period of photoreceptor degeneration. For this report, we have studied the expression of CD44 mRNA in Balb/c and rds mouse retinas. Northern blot analysis with a mouse CD44 cDNA probe was used to determine the expression of retinal CD44 transcripts. RT-PCR was used to study the expression of standard and variant forms of CD44 mRNA. The primers for PCR flanked the insertion site for variant exon splicing. Southern blot analysis was used to confirm the identity of the PCR products. The results of the Northern blot analysis demonstrated that in rds degenerative retinas the amount of CD44 mRNA increases at least two times, relative to 18S RNA levels. With PCR analysis, the standard form of CD44 was shown to be expressed at all ages studied for both Balb/c and rds retinas. No preferential expression of CD44 variant forms was noted during development or degeneration.

Abstract #66

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ABSTRACT FORM

First Author: Neeraj Agarwal

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

CHARACTERIZATION OF A SV-40 T-ANTIGEN TRANSFORMED 661W MOUSE PHOTORECEPTOR CELL LINE ((N. Agarwal, M.J. Crawford, R. Krishnamoorthy, H.J. Sheedlo, D.T. Organisciak#, M. Al-Ubaidi*)) Department of Anatomy and Cell Biology, University of North Texas Health Science Center, #Wright State University, Dayton, OH, and *University of Illinois, Chicago, IL.

Purpose. An immortalized mouse cell line (661W) was investigated for characteristic markers for photoreceptor cells. **Methods.** These 661W cells were cloned from retinal tumors of a transgenic mouse line expressing the SV-40 T-antigen under the control of the inter-photoreceptor retinol binding protein (IRBP). These cells were analyzed by immunocytochemistry, RT-PCR and light and electron microscopy. Total RNA from mouse retina and baboon lungs was used as positive and negative controls, respectively, for RT-PCR analysis. Scanning electron microscopy (SEM) was used to study differentiation of outer segments by 661W cells. Rhodopsin generation was measured spectrophotometrically by binding of 9-cis retinal to 661W membranes. **Results.** 661W cells grew in a monolayer exhibiting dendritic-like processes. The doubling time was ~24 hours in media containing 9% fetal bovine serum. Expression of message for the photoreceptor-specific proteins opsin, arrestin, phosphodiesterase (PDE), β-transducin and rds/peripherin were demonstrated by RT-PCR. Immunocytochemistry showed cultured 661W cells expressed photoreceptor proteins arrestin, IRBP, opsin, rds/peripherin, phosducin, α-rod transducin and recoverin. Co-culture and SEM studies suggested 661W cells formed outer segment-like structures. A characteristic rhodopsin peak was observed at 500nm on incubation of 661W membranes with 9-cis retinal upon light bleaching. **Conclusion.** These 661W cells exhibited biochemical characteristics consistent with retinal photoreceptor cells. These cells also developed outer segment-like structures as seen *in vivo*. These cells may provide a valuable tool to study photoreceptor cell biology and disease processes.

None

Abstract #67

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

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ABSTRACT FORM

First Author: Raghu Krishnamoorthy

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

TRANSCRIPTION FACTOR NF-κB AND APOPTOSIS OF MOUSE PHOTORECEPTOR CELLS. ((Raghu Krishnamoorthy, Matthew J. Crawford, Muayyad Al-Ubaidi*, Neeraj Agarwal)) Department of Anatomy and Cell Biology, UNT Health Science Center at Fort Worth, TX, and *University of Illinois, Chicago, IL.

Purpose. The objective of this investigation was to determine the mechanism of photo-oxidative stress induced apoptosis of 661W photoreceptor cells. **Methods.** Cultured 661W photoreceptor cells were exposed to fluorescent visible light (3-4 mW/cm^2) for different time intervals up to 4 hrs with control cells in dark. After light exposure, cytoplasmic and nuclear extracts were subjected to electrophoretic mobility shift assays (EMSA) using end-labeled consensus and mutant NF-κB oligos; immunocytochemistry and immunoblot analysis using specific antibodies and TUNEL for apoptosis. **Results.** Photo-oxidative stress resulted in significant lowering of nuclear as well as cytoplasmic NF-κB activity as shown by EMSA, in light exposed 661W cells. The specificity of the binding of NF-κB was shown by competition with cold NF-κB oligo as well as by mutant NF-κB oligo. These results were further confirmed by immunocytochemistry and immunoblot analysis. Further, inclusion of anti-oxidants and caspase-I inhibitor resulted in protection of NF-κB levels and prevention of apoptosis of light exposed 661W cells. **Conclusions.** NF-κB levels are constitutively expressed in 661W cells. Exposure of 661W cells to photo-oxidative stress leads to down-modulation of NF-κB RelA subunit and increase in caspase-I activity leading to apoptosis of these cells. Inclusion of anti-oxidants and caspase-I inhibitor in the growth medium before light exposure, protects the 661W cells from photo-oxidative stress induced apoptosis

Abstract #68

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ABSTRACT FORM

First Author: Dr. Harold J. Sheedlo

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

IN VITRO RESPONSE OF A TRANSFORMED PHOTORECEPTOR CELL LINE TO GROWTH FACTORS AND AN RPE-SECRETED FACTOR H.J. Sheedlo, J. Malouf, A.M. Brun-Zinkernagel, N. Agarwal and J.E. Turner Dept Anatomy and Cell Biology, North Texas Eye Research Institute, Univ North Texas Health Science Center, Fort Worth, TX

A transformed photoreceptor cell line (661W) isolated from a transgenic mouse line controlled by the promoter construct HIT1 for interstitial retinol binding protein was investigated *in vitro*. Morphological development and proliferation rate of 661W cells at 30-49th passage were studied in response to proteins in conditioned media (CM) of RPE cells, serum, defined media and growth factors, including epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2) and nerve growth factor (NGF). 661W cells were cultured for 1-2 weeks in RPE-CM and growth factors and examined by immunocytochemistry and light and electron microscopy. Cells were also grown in RPE-CM, NGF, EGF and FGF-2 for 3 days and cell numbers were measured by a proliferation bioassay. In this study, we determined that 661W cells expressed opsin by immunofluorescence. Cell growth in defined media conditions, EGF and FGF-2 exhibited multiple processes, while cells cultured in NGF and RPE-CM primarily remained round. 661W cells cultured in serum for 4 days showed process formation. After 3 days, the numbers of 661W cells grown in defined media increased over 2 fold above the plating density. However, cells cultured in defined media with NGF or RPE-CM were 30% and 50%, respectively, of defined media conditions. In addition, 661W cells grown in FGF-2 and EGF were 19% above and slightly below, respectively, control levels. When NGF and EGF were added together at the same concentrations for the conditions described above, the numbers of 661W cells increased slightly above NGF levels to 40% of controls. In conclusion, these 661W cells exhibited process formation *in vitro* suggesting their utility for transplantation in retinas showing photoreceptor cell degeneration. We hypothesize that a protein in RPE-CM, most likely NGF or other neurotrophin, may alter the proliferation of 661W cells by affecting their differentiation or promoting apoptosis.

Abstract #69

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ABSTRACT FORM

First Author: J.E. Turner

Department: Anatomy & Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

PHOTORECEPTOR CELL SURVIVAL PROMOTED BY AN RPE SECRETED FACTOR, A MEMBER OF A TRYPSIN PROTEASE INHIBITOR FAMILY. J.E. Turner, T.H. Nelson, H.J. Sheedlo. Dept. of Anatomy and Cell Biology, North Texas Eye Research Institute, University of North Texas Health Science Center, Ft. Worth, TX.

Purpose. An RPE secreted protein was characterized by amino acid sequencing, tested for its effects on retinal development *in vivo* and *in vitro* and localized by immunocytochemistry. **Methods.** A protein was isolated from media conditioned by neonatal rat RPE cells (RPE-CM) and amino acid sequencing was performed using Edman degradation. The purified RPE protein was injected into the vitreous of postnatal day 7 (P7) Long Evans rats and retinas were examined 7-21 days later by light microscopy and opsin immunocytochemisty. Retinal cells from P2 rats were also cultured with this protein and cell numbers were measured using a cell proliferation bioassay. Proteins of RPE-CM were separated in polyacrylamide gels, transferred to nitrocellulose and probed with a polyclonal antibody to this RPE protein. The protein was immunolocalized in cultured rat RPE cells using this antibody. **Results.** The isolated RPE protein had an amino acid sequence homologous with a family of trypsin protease inhibitors. Significantly increased levels of opsin-immunolabeled ectopic photoreceptor cells were detected in the inner nuclear layer (INL) of retinas of P14 rat eyes injected with the RPE factor when compared to retinas of sham-injected or non-injected control eyes. These cells extended opsin-immunoreactive processes from the inner plexiform layer to the outer plexiform layer, which was never observed in retinas of sham-injected and age-matched control rats. An antibody against this protein recognized an RPE-CM protein that migrated to approximately 65kDa and was shown by immunofluorescence to rim nuclei of cultured neonatal rat RPE cells. In addition, this protein at nanogram levels stimulated a significant increase in P2 retinal cells after 3 days in culture. **Conclusions**. The RPE protein described in this study may potentially be used to arrest photoreceptor cell degeneration in dystrophic animal retinas and human eye diseases such a macular degeneration or retinitis pigmentosa.

Abstract #70

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

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ABSTRACT FORM

First Author: Stephen J. Moorman, Ph.D.

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

COUNTER-ROTATION OF THE ZEBRAFISH (*DANIO RERIO*) EYE IN RESPONSE TO CHANGES IN LIGHT POSITION. S.J. Moorman and J.E. Anwah, UNT Health Science Center at Ft. Worth, [1]Viginia Military Institute.

When a normal zebrafish hatchling is tilted around the transverse body axis (tail-up and tail-down, i.e. pitch) the eye maintains a fairly constant orientation with respect to gravity. This reflex counter-rotation of the eye is thought to be initiated by activity in the gravity sensing portion of the equilibrium receptor system. In addition to gravity, zebrafish use the position of the light for purposes of equilibrium orientation. Here we provide evidence that zebrafish also use the position of the light to initiate reflex counter-rotation of the eye. Zebrafish eggs were collected once a week for 5 weeks within 1 hour after they were laid and fertilized. About 50% of those eggs were transferred into a beaker with aquarium water and about 50% were placed in a NASA designed bioreactor. The bioreactor was designed to simulate a microgravity environment for cells in culture. We have previously shown that zebrafish that hatch from eggs that have been incubated in the bioreactor for 96 hours have irreversible vestibular deficits. The zebrafish eggs/hatchlings were maintained in the bioreactor for 96 hours. (hatching occurs between 48 and 72 hours after fertilization). Reflex counter-rotation of the eye was observed with the hatchling in a capillary tube mounted on an ophthalmic microscope. The orientation of the eye with respect to gravity was noted while each hatchling was illuminated from above and tilted around a transverse axis and then again while illuminated from the side and tilted around a transverse axis. When illuminated from the side, the eye did not maintain a consistent orientation with respect to gravity. However, when illuminated from above, the eye maintained a consistent orientation with respect to the position of the light. Unlike the other reflex eye movements in zebrafish that are functionally mature by 80 hours post-fertilization, this light-induced counter-rotation of the eye did not reach functional maturity until between 96 and 120 hours post-fertilization. Similar to the other reflex eye movements in zebrafish, the development of this light-induced counter-rotation of the eye did not depend on the animal being exposed to light during development. Light induced counter-rotation of the eye has not been reported in any other animal. Supported by NIDCD.

Abstract #71

NEUROSCIENCE AND BEHAVIOR

72.	Harbans Lal, Ph.D.	SUBSTANCE ABUSE INSTITUTE OF NORTH TEXAS (SAINT)
73.	Michael B. Gatch, Ph.D.	BENZODIAZEPINE MODULATION OF ACUTE AND CHRONIC ETHANOL-INDUCED CHANGES IN NOCICEPTION
74.	Michelle Bidaut-Russell	GENDER DIFFERENCES IN ALCOHOL DEPENDENCE, CHILDHOOD CONDUCT DISORDER, AND MAJOR DEPRESSION BETWEEN OFFSPRING OF ALCOHOLIC FATHERS, ALCOHOLIC MOTHERS, AND NON-ALCOHOLIC PARENTS
75.	Beatriz A. Rocha	INTRAVENOUS COCAINE SELF-ADMINISTRATION IN MICE LACKING THE DOPAMINE TRANSPORTER (DAT)
76.	Robert R. Luedtke	CHARACTERIZATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR THE RAT D1a DOPAMINE RECEPTOR
77.	Mingjun Fang	PROTEIN TYROSINE RESIDUE PHOSPHORYLATION OF THE $\gamma 2$ SUBUNIT MAINTAINS THE FUNCTION OF RECOMBINANT $GABA_A$ RECEPTORS
78.	Brandon Lewis	CAPSAICIN STIMULATES HISTAMINE RELEASE FROM MOUSE DURAL MAST CELLS IN VITRO
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ABSTRACT FORM

First Author: Harbans Lal, Ph.D.

Department: Pharmacology and SAINT

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

SUBSTANCE ABUSE INSTITUTE OF NORTH TEXAS (SAINT)

Harbans Lal, Executive Director
University of North Texas Health Science Center, Fort Worth, Texas

The Substance Abuse Institute of North Texas (SAINT) is housed in the Department of Pharmacology and the Department of Psychiatry and Human Behavior. The Institute is a consortium of professionals actively involved in research and education in areas related to the problem of substance abuse.

The SAINT promotes strong interactions between members to develop and extend research programs. Members of SAINT conduct research into the physiological basis of addiction and substance abuse as well as in research aimed at developing new drug therapies which will aid in the withdrawal and abstinence from substances of abuse. Research grants from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) include studies focused on the treatment of alcohol withdrawal as well as studies focused on interactions between ethanol and nicotine. Contracts from the National Institute on Drug Abuse (NIDA) concentrate on developing an antagonist to block the reinforcing effects of cocaine. Other current projects include investigations on the contribution of genetic factors on the consumption of cocaine and use of genetically-modified (knock-out) animals to determine the underlying neurochemical processes involved in cocaine self-administration.

Educational activities of members of SAINT include graduate and post-graduate training of research professional as well as the training of physicians and other health care professionals. The Institute hosts research conferences and cosponsors seminars with area groups. International speakers and visiting scientists are attracted to the University of North Texas Health Science Center campus to interact and perform research with members of SAINT.

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ABSTRACT FORM

First Author: Michael B. Gatch, Ph.D.

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

BENZODIAZEPINE MODULATION OF ACUTE AND CHRONIC ETHANOL-INDUCED CHANGES IN NOCICEPTION

Michael B. Gatch and Harbans Lal

Department of Pharmacology and Substance Abuse Institute of North Texas, University of North Texas Health Science Center, Fort Worth, TX

Ethanol produces antinociception when administered acutely or chronically in rats, and acute withdrawal from ethanol induces hyperalgesia. This study examines the effects of ligands for the benzodiazepine site on the GABA receptor on ethanol-induced changes in nociception. A radiant heat tail-flick assay was used to assess changes in nociception in rats. Acute activity of cumulative doses of ethanol (0.5 – 2.0 g/kg) and diazepam (0.1 – 10 mg/kg), a benzodiazepine site agonist were tested alone and after pretreatment with flumazenil (10 mg/kg). Chronic effects of ethanol were tested in three groups of rats which received 10 days of exposure to a liquid diet. One group received ethanol alone, one group received ethanol and twice daily injections of flumazenil (10 mg/kg), a benzodiazepine site antagonist, and one received a dextrin control diet. Acute withdrawal was tested at 12 hr after removal of the liquid diet. Effects of cumulative doses of diazepam (1.0 – 10 mg/kg) were tested during withdrawal in the ethanol alone group. Acute doses of ethanol produced a small but significant degree of antinociception which was fully suppressed by flumazenil. Acute doses of diazepam did not produce antinociception. Chronic exposure to ethanol produced antinociception on days 2-8. Tolerance developed by day 10 and hyperalgesia was seen 12 hr after removal of ethanol. Administration of diazepam during withdrawal reversed the hyperalgesia induced by ethanol withdrawal. However, flumazenil (10 mg/kg) failed to reverse this effect of diazepam. No antinociception was seen in either the ethanol/ flumazenil or dextrin control groups. These results suggest that the antinociceptive effects of both acute and chronic ethanol are at least partially mediated by GABA receptors, and that diazepam's anti-hyperalgesic effects may not be mediated by the GABA receptor.
(This research was supported in part by NIH grant AA09567-05.)

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ABSTRACT FORM

First Author: Michelle Bidaut-Russell

Department: PHPM

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty XX Staff___

Read instructions and fit abstract inside the space given below:

GENDER DIFFERENCES IN ALCOHOL DEPENDENCE, CHILDHOOD CONDUCT DISORDER, AND MAJOR DEPRESSION BETWEEN OFFSPRING OF ALCOHOLIC FATHERS, ALCOHOLIC MOTHERS, AND NON-ALCOHOLIC PARENTS.

M. Bidaut-Russell; K.K. Bucholz; A.C. Heath; N.G. Martin. Dept. of Public Health/Preventive Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107-2699; Washington University School of Medicine, St. Louis, MO; and Queensland Institute of Medical Research, Brisbane, Australia.

We tested the hypothesis that although a parental history of problems with alcohol would raise the absolute rates of alcohol dependence, childhood conduct disorder, and major depression in both male and female offspring, it would reduce the gender difference in the rates for these disorders compared to that of offspring with non-alcoholic parents. Lifetime prevalence rate of these disorders and of perceived histories of parental problems with alcohol were assessed in 522 complete pairs of unlike-sex Australian twins (paternal alcoholism N=111; maternal alcoholism N=19; no-parental alcoholism N= 392) using the Semi-Structured Assessment for the Genetics of Alcoholism (MINI-SSAGA-OZ). A positive parental history of problems with alcohol was inferred if either one or both twins reported that a parent had alcohol-related problems with health, family, job, police, etc. All twins selected reported having been raised by both biological parents until the age of 16. Male to female odds ratio of alcohol dependence were comparable in offspring of alcoholic fathers and in offspring of alcoholic mothers (OR = 20), while that for offspring of non-alcoholic parents was 6.6. Male to female ratio of childhood conduct disorder was 6.0 in offspring of alcoholic fathers, 1.5 in offspring of alcoholic mothers, and 52 in offspring of non-alcoholic parents. Female to male ratio of major depression was 2.2 among offspring of alcoholic fathers, 1.0 among offspring of alcoholic mothers, and 1.22 among offspring of non-alcoholic parents. These results suggest that the gender of the alcoholic parent may play an important role in gender differences in mental health outcome among adult offspring. (Data collection and data analysis were supported by NIAAA grant AA10974 to M.B.R. and NIH grant AA07535 to A.C.H.)

Abstract #74

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ABSTRACT FORM

First Author: Beatriz A. Rocha

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

INTRAVENOUS COCAINE SELF-ADMINISTRATION IN MICE LACKING THE DOPAMINE TRANSPORTER (DAT).

B. A. Rocha, R. Ator, *B. Giros and *M. G. Caron.

Department of Pharmacology UNTHSC/FW, Fort Worth, TX; *Department of Cell Biology and Medicine, Duke University Medical Center, Durham, NC.

The addictive properties of cocaine have been correlated to the ability of this drug to interact with the dopamine transporter (DAT). The present experiment tested the hypothesis that the binding of cocaine to the DAT is mandatory for its reinforcing effects. For this purpose mice lacking the DAT (KO; n=9) and their wild-type littermates (WT; n=13) were tested for intravenous (IV) cocaine self-administration. Subjects were initially trained to press a lever for food as a reinforcer, and subsequently implanted with an IV catheter. Two days after surgery, mice started cocaine (2.0 mg/kg/inj) self-administration acquisition under a fixed ratio 1 (FR1) schedule of reinforcement. Once acquisition criteria (75% of active lever pressings and at least 10/20 reinforcements within 3-h for three consecutive days) was obtained, mice were switched to FR2 schedule. When a stable baseline of responding for cocaine was obtained, each subject was tested under different doses of cocaine (0.25-4.0 mg/kg/inj) with the number of reinforcements per hour used as the dependent variable. Following dose-response tests, saline substituted for cocaine until extinction behavior was observed. Mice from both genotypes successfully acquired food-shaping in approximately 5 sessions (4.67 ± 1.2 and 5.67 ± 0.9 for KO and WT mice, respectively). However, for meeting cocaine self-administration acquisition criteria KO mice required on average 12.8 ± 6.3 sessions, while WT 5.11 ± 1.9. The latency for acquisition of cocaine self-administration was significantly increased in KO mice ($T= -2.66$; $p=0.05$), but they consistently self-administered cocaine (significant effect of dose of cocaine within subjects ($F(4,32)=7.48$; $p<0.0005$)). Taken together these results showed that cocaine functions as a positive reinforcer for DAT KO mice, suggesting that the absence of the DAT might impair, but it is not critical, for initiation or maintenance of cocaine taking behavior.

Abstract #75

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ABSTRACT FORM

First Author: Robert R. Luedtke

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty ✓ Staff ✓

Read instructions and fit abstract inside the space given below:

CHARACTERIZATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR THE RAT D1a DOPAMINE RECEPTOR.

R.R. Luedtke*, S.A. Griffin Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

A variety of neurological and neuropsychiatric disorders, including Parkinson's Disease, schizophrenia, schizoaffective disorders and tardive dyskinesia, appear to be due to disturbances in the dopaminergic system. Consequently, understanding the regulation and the precise localization of each component of the dopamine receptor system has broad clinical applications. D1-like dopamine receptors, D1a and D1b receptors, share 75% amino acid homology within the transmembrane spanning regions, which construct the neurtransmitter binding site. Therefore, it has been difficult to develop selective pharmacologic reagents for experimental or clinical studies on the role of the two D1-like dopamine receptor subtypes. Our laboratory has developed immunologic reagents that are selective for these two structurally related dopamine receptor subtypes.

A segment of DNA that codes for the carboxy terminus of the rat D1a dopamine receptor was amplified using polymerase chain reaction (PCR). This receptor gene segment was subcloned into a pET-14b expression plasmid which contains an amino terminus "histidine tag". The plasmid was used to transform BL21plysS DE3 *E. coli* cells. Several colonies were evaluated for the ability to express a recombinant peptide (rD1a-COOH). Following induction with 1 mM isopropyl-β-D-thiogalactopyranoside, the recombinant peptide rD1a-COOH was purified by nickel adsorption chromatography and used to immunized BALB/c mice. Hybridomas were prepared and screened using rD1a-COOH with an ELISA assay. Using an immunoblot and immunohistochemical protocols, one monoclonal antibody was found to be immunoreactive with rat D1a dopamine receptors expressed in Sf9 cells, but not immunoreactive with cells expressing D1b, D2 or D3 dopamine receptors or uninfected Sf9 cells. The antibody was found to be immunoreactive with rat brain tissue derived from the caudate and cortical areas. Neuroanatomical immunohistochemical studies indicate that immunoreactivity was observed in the rat caudate and in the cortical areas of the rat brain. *(Supported by NINDS 30507, the Scottish Rite Schizophrenia Research Program and the National Alliance for Research on Schizophrenia and Depression).*

Abstract #76

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Mingjun Fang

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PROTEIN TYROSINE RESIDUE PHOSPHORYLATION OF THE γ2 SUBUNIT MAINTAINS THE FUNCTION OF RECOMBINANT $GABA_A$ RECEPTORS. Mingjun Fang, Renqi Huang and Glenn H. Dillon. Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107

$GABA_A$ receptors are ligand-gated ion channels, composed of varying isoforms of subunits (α, β, γ, δ, and ε). We have previously assessed the functional maintenance of α3β2γ2 $GABA_A$ receptors, and suggested that protein tyrosine phosphorylation may maintain $GABA_A$ receptor function. Here we tested the hypothesis that tyrosine phosphorylation modulates other $GABA_A$ receptor subtypes e.g., α1β2γ2 and α6β2γ2, and subsequently attempted to determine which subunit(s) may be phosphorylated. We used the whole-cell patch clamp technique on recombinant $GABA_A$ receptors stably expressed in human embryonic kidney cells. In receptors composed of α1β2γ2 subunits, we found that current amplitude in response to high and low [GABA] increased over time (run-up), with adequate ATP and Ca^{2+} buffering. In the presence of lavendustin A, a specific inhibitor of protein tyrosine kinase (PTK), current amplitude decreased with time (run-down). Similar results were found in α6β2γ2 receptors. In receptors composed of only α1β2 subunits, addition of lavendustin did not induce any appreciable run-down. Our results support the hypothesis that PTK phosphorylation may maintain $GABA_A$ receptor function. In addition, we suggest this tyrosine phosphorylation occurs at the γ2 subunit of the receptor. (Support: NIH R29 ES07904 and TX ARP 009768-027).

Abstract #77

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Brandon Lewis

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CAPSAICIN STIMULATES HISTAMINE RELEASE FROM MOUSE DURAL MAST CELLS IN VITRO . Brandon Lewis and Edward L. Orr, UNTHSC, Fort Worth, TX, 76107.

The goal of this study was to determine if capsaicin could release histamine from mast cells in the calvarial dura mater *in vitro*. Subject mice were anesthetized, perfused with saline, and then sacrificed. The cranium was exposed and all adherent tissues, including periosteum, were removed. The calvarium and attached dura mater was isolated, then split mid-saggitally and the pieces placed into incubation media (MEM). The calvarial pieces with attached dura were incubated at 37°C for 20 min to allow the tissue to stabilize to a basal state. Each piece was then moved sequentially through 5 vials containing MEM at 5 min. intervals. The final 3 vials for each experimental piece contained 1 μM capsaicin. Samples of incubation media from each vial were assayed for histamine as a measure of mast cell activation. The control and experimental pieces initially showed similar rates of histamine release; however, upon exposure to capsaicin, the rate of histamine release in the experimental sections increased, while the release in the control sections remained fairly constant . After fifteen minutes of treatment, the experimentals had released approximately 20% more histamine. These results show that afferent nerve endings and mast cells remain intact and functional in dural explants and that stimulation of afferent neurons with capsaicin causes release of mast cell histamine, presumably via substance P. This model offers a simplified environment in which to further elucidate the relationship between mast cells and other dural components such as sympathetic efferent neurons.

Abstract #78

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Ming-chi Wu, Ph.D.

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

Prevention of Parkinson's Disease by Glial Cell Derived Neurotrophic Factor in Animal Models.

Ming-Chi Wu[1], Fu-Chou Cheng[2], Lie-Gan Chia[3], and Jon-Son Kuo[2], Department of Molecular Biology and Immunology[1], University of North Texas Health Science Center, Fort Worth, TX, U.S.A., and Department of Education & Medical Research[2], Section of Neurology [3], Taichung Veterans General Hospital, Taichung, Taiwan.

The objective of this study was to test the hypothesis that a glial cell line derived neurotrophic factor (GDNF) can prevent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson-like symptoms in a mouse model, by maintaining the dopamine (DA), 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) levels. Experiments were conducted in two ways. First, animals were injected with GDNF into the striatum followed by multiple subcutaneous injections of MPTP in a successive manner, to mimic chronic exposure to neurotoxins. Second, animals were injected with MPTP successively for seven days followed by a single injection of GDNF. Seven days after the last injection, animals were subjected to locomotive activity assay and sacrificed for dopamine and its metabolite determinations. Tissues of striatum and substaintia nigra were extracted and assayed for dopamine and its metabolites by HPLC. Results from the experiments clearly indicated that administration of MPTP significantly decreased the levels of DA, DOPAC and HVA in the striatum, as well as substantia nigra areas. Pretreatment of animals with GDNF can partially protect against the damage caused by MPTP, by significantly increasing the metabolite concentration in the striatum. Pretreatment with GDNF provides complete protection against MPTP-induced damage in substaintia nigra. Injection of GDNF into the MPTP-treated animals also showed strong restorative effects on the concentrations of these metabolites. In the behavior measurement, increased locomotor activities were seen in all groups treated with MPTP or GDNF or the combination of both. In conclusion, administration of GDNF into striatum exerts protective and reversive effects on the dopaminergic damage caused by MPTP in a chronic experimental situation. Our results further support the potential application of GDNF in prevention and treatment of Parkinson's disease.

Abstract #79

INFECTIOUS DISEASE

80.	Debra L. White	THE GLOBAL REGULATOR CsrA CONTROLS STATIONARY PHASE PROPERTIES THAT MAY AFFECT BACTERIAL PATHOGENESIS
81.	Michelle Wright	ISOLATION AND CLONING OF THE CATALASE GENE(S) FROM *STAPHYLOCOCCUS AUREUS*
82.	Christina A. Malakowsky	CHARACTERIZATION OF CATALASE DEFICIENT VARIANTS OF *STAPHYLOCOCCUS AUREUS* S6C
83.	Lori Johnson	THE EFFECT OF THE ACCESSORY GENE REGULATOR *(AGR)* ON THE IN VIVO GROWTH OF *STAPHYLOCOCCUS AUREUS*

CANCER

84.	Ronald H. Goldfarb, Ph.D.	INSTITUTE FOR CANCER RESEARCH
85.	Kavita Niranjan	IMPROVED DELIVERY FOR CANCER CHEMOTHRAPEUTIC AGENTS

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Debra L. White

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE GLOBAL REGULATOR CsrA CONTROLS STATIONARY PHASE PROPERTIES THAT MAY AFFECT BACTERIAL PATHOGENESIS

Debra L. White, Mark E. Hart, and Tony Romeo. Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

Many of the cellular components which promote the virulence of bacterial pathogens (virulence factors) are expressed when bacterial cultures enter the stationary phase of growth. Studies in *Escherichia coli* have identified a gene, *csrA* (carbon storage regulator), which encodes a global regulator of carbon metabolism and of various genes expressed in early stationary phase. Genome sequencing studies have revealed *csrA* homologs in diverse bacterial species, including numerous human pathogens. Furthermore, a homolog of *csr*A has been recently shown to regulate virulence properties of the plant pathogen *Erwinia carotovora*. A variety of surface properties are also modulated via *csrA* in *E. coli*, suggesting that this gene may likewise affect mammalian pathogenesis. For example, a *csrA* mutant is highly adherent to itself and to glass culture tubes. We have also found that the *csrA* mutant strain, but not its *csrA*$^+$ parent, binds to the dye Congo Red, with the formation of red colonies on Colony Forming Antigen (CFA) medium. Both of these properties suggest that the surface organelles known as curli fimbriae are repressed via *csrA*. Curli are coiled surface structures composed of a single subunit, curlin, which differs from all other known pilin proteins. They are produced under low temperature, low salt, and in late stationary phase and confer the ability to specifically interact with a series of human proteins, including fibronectin, laminin, and MHC type I molecules. Thus, *csrA* may affect the ability of pathogens such as some strains of *E. coli* and *Salmonella* to bind to host tissue and cause infection. In order to directly test this hypothesis, we have cloned and sequenced the *csrA* gene of the model disease-causing organism *Salmonella typhimurium*. The deduced amino acid sequence was found to be identical to that of *E. coli* K-12, although 8 silent mutations were present in the coding region. We are currently manipulating this cloned gene in order to prepare a *csrA* null mutation in the *S. typhimurium* chromosome. The resulting *csrA* deletion strain will be used to characterize the molecular and regulatory properties of the *Salmonella csrA* gene, including its potential role in mammalian infections (National Science Foundation, Robert D. Watkins Minority Fellowships, ASM, GREAT Grant, Department of Research and Biotechnology.).

Abstract #80

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Michelle Wright

Department: Department of Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ISOLATION AND CLONING OF THE CATALASE GENE(S) FROM *STAPHYLOCOCCUS AUREUS* .

Michelle Wright and Mark Hart, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Tx, 76107

Staphylococcus aureus is a gram positive bacterium that is a leading cause of post-operative nosocomial infections. Forty percent of post-operative infections occur at the surgical site, and thirty percent of those infections are attributed to *S. aureus*. Most strains isolated from the hospital environment are resistant to multiple antibiotics, and those also resistant to methicillin require treatment with vancomycin. Vancomycin has been the mainstay for physicians treating patients with multi-drug resistant *S. aureus* infections, but in recent months reports of vancomycin resistant strains have surfaced both abroad and in the United States. Given the seriousness of resistant staphylococci in the hospital environment, it is imperative that new antimicrobial agents be developed.Catalase activity is a key diagnostic feature used to differentiate staphylococci from streptococci. It functions to reduce the toxic effects of hydrogen peroxide generated during aerobic metabolism and has been implicated in the ability of *S. aureus* to avoid phagocytosis by the host organism. In an effort to assess the role catalase plays in staphylococcal disease, it is our purpose to isolate and clone the gene(s) responsible for catalase function in *S. aureus*. Currently we are attempting to complement the catalase mutation in *Escherichia coli* UM255 (*kat G2, kat* E12::*Tn10*) using *S. aureus* chromosomal DNA cloned into the *E. coli* plasmid vector pBluescript. *E. coli* UM255 will be transformed with plasmid DNA by electroporation. Transformants growing in the presence of ampicillin and tetracycline will be screened for complementation by administering hydrogen peroxide and observing colonies for effervescence. Those colonies exhibiting effervescence will be procured for further analysis. Once the identity of the *S. aureus* catalase gene is confirmed, a mutant strain deficient in catalase activity will be generated using allele replacement. Mutant and wildtype strains will be assessed for virulence using the mouse model of cutaneous infection. We believe that the results of these experiments will demonstrate that catalase activity is an important factor for survival and/or virulence of *S. aureus* , and could potentially be a target for antimicrobial drug development.

Abstract #81

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Christina A. Malakowsky

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CHARACTERIZATION OF CATALASE DEFICIENT VARIANTS OF *STAPHYLOCOCCUS AUREUS* S6C. Christina A. Malakowsky[1], Rusty M. Crum[2], and Mark E. Hart[2], [1]Department of Biology, Texas Wesleyan University, Fort Worth, TX. 76105 and [2]Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107.

Staphylococcus aureus is an etiological agent for a number of infections in humans and animals. Diseases caused by *S. aureus* in humans include superficial and deep abscesses, wound infections, osteomyelitis, pneumonia, meningitis, purulent arthritis, septicemia, and endocarditis. *S. aureus* is a gram-positive, non-motile, nonsporeforming bacterium that ferments mannitol and grows in the presence of high salt. In addition, the staphylococci also produce catalase that is routinely used to differentiate the staphylococci from the streptococci. Recently, our laboratory has isolated variants of *S. aureus* S6C which were deficient in catalase production. In addition, some of the catalase variants were also deficient in hemolyic activity on sheep blood agar. In all, four variant types were isolated and found to be stable with respect to their phenotype. In an effort to verify the identity of the variants, they were characterized with respect to their growth in the presence of high salt, their ability to ferment mannitol, and produce coagulase. All four variants grew in the presence of high salt, fermented mannitol, and produced coagulase; presumptively identifying the variants as *S. aureus*. Chromosomal DNA isolated from all four variants and digested with various restriction endonucleases is currently undergoing Southern analysis to determine whether the variants contain the structural genes for lipase, alpha-toxin, delta-toxin, and staphylococcal enterotoxin B; genes characteristically found in *S. aureus* S6C. In addition, a *Sma*I genomic map of all four variants is being generated using clamped homogenous electric field (CHEF) electrophoretic analysis. It is anticipated that results from this study will indicate that all four variants exhibit a *S. aureus* S6C genotype.

Abstract #82

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

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ABSTRACT FORM

First Author: Lori Johnson

Department: Molecular Biology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE EFFECT OF THE ACCESSORY GENE REGULATOR (*AGR*) ON THE IN VIVO GROWTH OF *STAPHYLOCOCCUS AUREUS*. Lori A. Johnson, and Mark E. Hart, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107-2699.

In vitro studies have shown expression of extracellular and cell wall-associated proteins in *S. aureus* is regulated by at least two genetic loci, the accessory gene regulator *(agr)* and the staphylococcal accessory regulator *(sar)*. Mutations in either or both of these regulators result in a pleiotropic effect on exoprotein expression which includes reduced levels of several secreted proteins known to be virulence factors. In addition, a variety of animal models have been used to demonstrate a concomitant reduction in virulence with a mutation in either or both of these regulators. In some of these studies reduced virulence was linked to a decrease in cell number. In an effort to examine the role these regulators play when cells are grown in an in vivo environment, titanium diffusion chambers fitted with 0.22 μm filters were surgically-implanted in the peritoneal cavities of Sprague-Dawley rats. Three days post surgery, chambers were inoculated with 10^3 colony-forming units (cfu) of either *S. aureus* RN6390 or its isogenic mutant RN6911 (Δ*agr::tetA*[M]). Growth was monitored by harvesting chamber contents every six hours and plating on tryptic soy agar (TSA) and mannitol salt agar. In addition, RN6911 was plated on TSA containing tetracycline. Both RN6390 and RN6911 reached a maximum number of cfu by twelve hours from ca. 10^3 cfu to ca. 10^7 cfu (10^4-fold increase). Interesting, both RN6390 and RN6911 decreased in cell number over the next 18 hours before regaining and maintaining their maximum number of cfu over the next 30 hours. No differences in cell numbers were observed between the strains. These data indicate that there are no apparent differences in the ability of the *agr* mutant strain to grow as compared to the *agr* wildtype using the described in vivo environment.

Abstract #83

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 2, 1998**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Ronald H. Goldfarb, Ph.D.

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

Plans are underway to soon establish a new University of North Texas Health Science Center Institute for Cancer Research. The institute will fill the need for establishing leadership in this area of research which has the potential to become an area of growth and excellence for the Health Science Center. It is also envisioned that the Institute for Cancer Research will be a focal point for interactions with private sector biotechnology and pharmaceutical companies. The scope of the institute will include, but will not be limited to, various aspects of basic and translational cancer research. It is also envisioned that the institute will also include strong components of cancer prevention and control, molecular diagnostics, clinical investigations in cancer diagnosis and therapy. Areas under consideration include cancer: cell biology, biochemistry, molecular biology, gene therapy, progression, invasion, angiogenesis/vasculature, metastasis, immunology and experimental therapeutics. A public plenary meeting for the institute has already been held. Future plans will extend the interactions of the institute with other universities, hospitals and administrators with responsibilities for Oncology programs both within the region and nationally. The institute will also provide educational and research training opportunities for graduate and medical students and post-doctoral and clinical fellows.

Abstract #84

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT W

Research Appreciation Day 1998

ABSTRACT FORM

First Author: KAVITA NIRANJAN

Department: MOLECULAR BIOLOGY & IMMUNOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff ✓

Read instructions and fit abstract inside the space given below:

IMPROVED DELIVERY FOR CANCER CHEMOTHRAPEUTIC AGENTS

Kavita Niranjan, Andras G. Lacko, McConathy, Walter and Maya Nair

University of North Texas Health Science Center , Department of Molecular Biology and Immunology, Fortworth, Texas

The purpose of this project is to develop an efficient delivery system of chemotherapeutic agents to tumor cells.Currently, most drugs that are used against malignant tumors attack not only the tumor cells but also normal healthy cells leading to serious side effects. We hope to overcome this problem by using recombinant human high-density lipoproteins (HDL) to deliver the drugs directly into the tumor cells, especially to those that express hdl receptors.HDL has been chosen to be the drug delivery agent since it is much smaller than other delivery agents such as liposomes. The small size of the particle facilitates the penetration into poorly vascularized tumors more efficiently. We have incorporated the chemotherapeutic agent, Doxorubicin into the HDL by sonication in the presence of sodium cholate. We have found from gel filteration experiments that the molecular weight of the HDL/drug complex is ther same as HDL. Subsequently we will screen cultured metastatic tumor cell lines for the incorporation of Doxorubicin and the relative cytotoxicity of the HDL/Drug complex. Cytotoxicity will be assesed by the effect of the drug on the proliferation and the survival of the tumor cell lines. Subsequent to the in vitro assay, screening will be extended to tumor bearing 'whole animals' and eventually to human trials to establish the efficacy of HDL in targeting tumor cells in vivo. Ultimately, this project aims at substantially improving the delivery of anti-cancer drugs to specific target tissues and thus decreasing the drugs toxicity on normal healthy tissues.

Abstract #85

BIOCHEMISTRY AND MOLECULAR BIOLOGY

86.	Sunitha Kumari	POLY(ADP-RIBOSYL)ATION OF P53 IN APOPTOTIC HELA CELLS FOLLOWING DNA DAMAGE
87.	Hilda Mendoza-Alvarez	QUALITATIVE ANALYSIS OF THE CHAIN LENGTH(S) OF ADP-RIBOSE POLYMERS SYNTHESIZED BY POLY(ADP-RIBOSE) POLIMERASE WITH INCREASING SUBSTRATE CONCENTRATIONS
88.	Hilda Mendoza-Alvarez	MECHANISTIC REGULATION OF PARP BY NICKED dsDNA
89.	Rafael Alvarez-Gonzalez, Ph.D.	12TH INTERNATIONAL SYMPOSIUM ON ADP-RIBOSYLATION REACTIONS: FROM BACTERIAL PATHOGENESIS TO CANCER
90.	Pisate John Kamthong	DIFFERENTIAL ACTIVATION OF TRANSCRIPTION FACTORS IN MIA-PACA2 CELLS
91.	Dmitriy S. Ushakov	BIREFRINGENCE OF A-BANDS OF STRIATED MUSCLE FIBERS MEASURED WITH A NOVEL INTERFERENCE MICROSCOPE
92.	Dmitriy S. Ushakov	EFFECT OF ADP ON THE CONFORMATION OF ACTO-S1 COMPLEXES
93.	Yukfung Lee	CLONING AND ANALYSIS OF THE PROMOTER REGION OF 2B4, A MURINE NK CELL RECEPTOR
94.	P. R. Kumaresan	CLONING AND CHARACTERIZATION OF RAT 2B4 GENE

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

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Research Appreciation Day 1998

ABSTRACT FORM

First Author: Sunitha Kumari

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

POLY(ADP-RIBOSYL)ATION OF P53 IN APOPTOTIC HELA CELLS FOLLOWING DNA DAMAGE

Sunitha Kumari, Hilda Mendoza-Alvarez and R. Alvarez -Gonzalez Department of Molecular Biology and Immuno logy, University of North Texas Health Science Center at FortWorth, TX 76107-2699.

HeLa cells in tissue culture were exposed to 50 μM N-Methyl-N′-Nitro-N-nitrosoguanidine (MNNG) for 0.5, 1.0, 1.5 and 2.0 hours to induce programmed cell death (apoptosis). Microscopic examination of HeLa cells showed cell blebbing, cell aggregation and cell degranulation, characteristic features of apoptosis. Apoptotic cells also showed loss of phospholipid symmetry, performed with fluorescently labeled AnnexinV. Similar results were observed when checking for membrane permeability changes utilizing ethidium bromide to label condensed chromatin. Agarose gel electrophoresis of DNA following MNNG treatment revealed the endonucleolytic degradation of chromatin. These results corroborated those obtained with fluorescent microscopy using ethidium bromide staining. The levels of p53 expression (tumor supressor gene) increased concomitantly with the changes in cell morphology, as judged by immunoblotting experiments. Using a western blotting technique, we observed, that p53 co-immunoprecipitated with an antibody specific for the DNA-binding domain of poly (ADP-ribose) polymerase (PARP) and that PARP was co-immunoprecipitated by a p53 specific antibody. Therefore, we went on to examine the possibility that DNA damage-inducible p53 could be poly(ADP-ribosyl)ated. The result of this experiment was negative and was consistent with the conclusion that poly(ADP-ribose) polymerase is degraded proteolytically during the execution phase of apoptosis. By contrast, p53, became a covalent target for poly(ADP-ribosyl)ation, when a crude extract of HeLa cells was incubated with calf thymus PARP in the presence of [^{32}P] radiolabeled NAD$^+$ following MNNG treatment.

The project was supported by grant GM 45451 from the NIH

Abstract #86

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Hilda Mendoza-Alvarez

Department: Molecular Biology & Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

QUALITATIVE ANALYSIS OF THE CHAIN LENGTH(S) OF ADP-RIBOSE POLYMERS SYNTHESIZED BY POLY(ADP-RIBOSE) POLIMERASE WITH INCREASING SUBSTRATE CONCENTRATIONS

Hilda Mendoza-Alvarez, Susana Chavez-Bueno, and Rafael Alvarez-Gonzalez. Department of Molecular Biology and Immunology, University of North Texas Health Science Center. Fort Worth, Texas 76107-2699.

The Auto-poly(ADP-ribosyl)ation of Poly(ADP-ribose)polymerase (PARP) [EC 2.4.2.30] was monitored by measuring the incorporation of [^{32}P] radiolabelled β-NAD$^+$ into acid-precipitable material following incubation of pure enzyme with increasing concentrations of either one of two substrates, β-NAD$^+$, or PARP itself. The [^{32}P]-radiolabelled ADP-ribose polymers synthesized were chemically detached from PARP by alkaline hydrolysis of the mono ester bond between the carboxylate moiety of Glu residues and the ADP-ribose chain. Autoradiographic analysis of the size distribution of free ADP-ribose polymers following High Resolution Polyacrylamide Gel Electrophoresis (HR-PAGE) showed a NAD$^+$-dependent increase in the average size of the ADP-ribose chains as the concentration of this substrate was gradually increased from 0.2 μM to 2.0 mM in the enzyme assay. On the other hand, the average size of the polymer synthesized was unaffected by increasing the concentration of PARP (the enzyme and covalent acceptor protein) from 4.5 to 18 nM. Therefore, we conclude that the size and complexity of the ADP-ribose polymers synthesized during enzyme automodification is determined by the concentration of β-NAD$^+$ and not by the concentration of the acceptor. Interestingly, higher concentrations of PARP (e.g., 27 nM and 36 nM) resulted in the formation of long and highly complex ADP-ribose polymers only. Thus, higher than optimal concentrations of PARP inhibit the automodification reaction at the level of the chain initiation step (substrate inhibition).

Abstract #87

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Hilda Mendoza-Alvarez

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

MECHANISTIC REGULATION OF PARP BY NICKED dsDNA

H. Mendoza-Alvarez and R. Alvarez-Gonzalez. Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107-2699.

We have measured and compared the enzymatic activity of a 40 kDa carboxy-terminal catalytic fragment of poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30] with full length (113 kDa) PARP. We utilized either [^{32}P]3'-dNAD or [^{32}P]NAD as the ADP-ribosylation substrate in the absence or presence of nicked calf thymus dsDNA. The auto-mono(3'-dADP-ribosyl)ation (initiation) reaction catalyzed by PARP with 3'-dNAD showed saturation kinetics as a function of the concentration of nicked dsDNA at constant [PARP]. The initial rates of the initiation reaction also increased with the square of enzyme concentration at constant [DNA]. Therefore, nicked dsDNA is an allosteric activator of PARP that stimulates enzymatic activity by increasing the number of initiation sites and therefore determines the total number of ADP-ribose chains synthesized. By contrast, the enzyme showed a highly complex kinetic behavior as a function of the concentration of nicked dsDNA with NAD. The ADP-ribose polymerization activity initially increased in a bimodal fashion and later decreased at high DNA concentrations. Highly branched polymers of ADP-ribose were synthesized at all nicked dsDNA concentrations tested with high mM NAD. Highly complex polymers were also generated with the 40 kDa catalytic domain with a 500-fold lower enzymatic efficiency in the absence of DNA . The level of activity displayed by truncated PARP agrees with the notion that the DNA-binding domain of this enzyme is localized in the 74 kDa amino-terminus. Thus, while nicked dsDNA allosterically up-regulates the initiation step of polymer synthesis and determines the total number of ADP-ribose chains produced by facilitating PARP dimerization, it is not required for ADP-ribose polymerization.

This project was supported by grant GM45451 from the NIH.

Abstract #88

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Rafael Alvarez-Gonzalez, Ph.D.

Department: Molecular Biology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty__X__ Staff___

Read instructions and fit abstract inside the space given below:

12th INTERNATIONAL SYMPOSIUM ON ADP-RIBOSYLATION REACTIONS: FROM BACTERIAL PATHOGENESIS TO CANCER

ORGANIZER
Rafael Alvarez-Gonzalez, Ph.D.
Associate Professor

Department of Molecular Biology and Immunology, University of North Texas Health Science Center. Fort Worth, Texas 76107.

The 12th International Symposium on ADP-ribosylation Reactions was organized in Cancun, Mexico from May 10 through **May 14, 1997**. The symposium was **sponsored by the University of North Texas Health Science Center at Fort Worth** and the Mexican Society for Biochemistry which celebrated its 40th Anniversary. Financial support for this international event was also provided by the Sweedish company of **Oxigene, Inc.** and California-based **Augoron Pharmaceuticals Inc.** The symposium was organized into the following scientific sessions: **i)** *Functional Analysis of ADP-ribosylation Using Molecular Genetics* (23 presentations); **ii)** *Signalling Processes Involving Eucaryotic and Bacterial Toxin ADP-ribosyltransferases* (22 presentations); **iii)** *Cyclic ADP-ribose and Calcium-Dependent Signalling Pathways* (9 presentations); **iv)** & **v)** *Convergence of Apoptosis, p53 (a Tumor Suppresor Protein), and ADP-ribosylation* (13 presentations); **vi)** *Niacin in Cancer Prevention and Treatment* (16 presentations); and **vii)** *Modulation of Chromatin Function(s) by Poly(ADP-ribosyl)ation* (25 presentations). A total of **44 oral presentations** and **64 posters** were made by **120 scientists** from **16 countries**. Nationalities represented were: Argentina, Austria, Canada, Chile, China, France, Germany, Italy, India, Japan, Mexico, Sweeden, Switzerland, Poland, United Kingdom, and the United States of America.

Abstract #89

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Pisate John Kamthong

Department: Molecular Biology & Immunology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DIFFERENTIAL ACTIVATION OF TRANSCRIPTION FACTORS IN MIA-PACA2 CELLS P. John Kamthong, Fu-Mei Wu, Ming-Chi Wu
Department of Molecular Biology & Immunology, UNT-HSC, Fort Worth, Texas 76107

Macrophage colony-stimulating factor (M-CSF) is a crucial growth and differentiation factor for myeloid cell lines, like monocytes and macrophages. These cells are main effectors in human non-specific immunity. Previous biological activity studies in our lab demonstrated that interleukin-1 (IL-1), phorbal ester (TPA) and calcium/calcium ionophore induce production of M-CSF in cultured human pancreatic cancer cells MIA-PaCa2 and lung tissue fibroblasts CCL-202. Analysis of 5'-enhancer/promoter region of the M-CSF gene reveals several binding sites and putative binding sites for transcription factors, such as NF-kB, AP-1, SP-1, NFAT, NF-IL-6 and EGR-1. Electromobility Shift Assay of the nuclear protein extracts from MIA-PaCa2 cells with the radiolabelled oligonucleotides containing the consensus binding sites for the transcription factors shows differential transcription factor binding when the cells were treated with various stimuli. IL-1--an acute phase cytokine--strongly stimulates NF-kB binding, while it induces AP-1 binding to a lesser extent. TPA mainly stimulates AP-1 binding , while it has lesser effect on NF-kB binding. Calcium and calcium ionophore also stimulates AP-1 binding, but have no effect on NF-kB binding. These results suggest the redundancy in transcription activation by the stimuli, which may be the mechanism to provide multiple level of response to different stimulli. We hypothesize that some or all of the aforementioned transcription factors involve in the expression of M-CSF. Further investigation to detect the changes in mRNA messages, as well as promoter/reporter gene transfection experiments are underway to determine the roles of these transcription factors in M-CSF gene transcription. The increased AP-1 binding in cells treated by calcium/calcium ionophore may be due to higher activities of calcium-sensitive enzymes, such as PKC, PLC, calcium/calmodlin dependent protein kinases/phosphatases in signal transduction pathways leading to increased AP-1 activation. This hypothesis may be tested by experiments with specific inhibitors to the potentially involved protein kinases/phosphatases.

Abstract #90

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Dmitry S. Ushakov

Department: Biochem. and Mol. Biology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

BIREFRINGENCE OF A-BANDS OF STRIATED MUSCLE FIBERS MEASURED WITH A NOVEL INTERFERENCE MICROSCOPE

D.S. Ushakov[1], G.N. Vishnyakov[2], G.G. Levin[2], L.K. Srebnitskaya[3], O.A. Andreev[1,3] & J. Borejdo[1]

[1]Dept. of Biochem. & Mol. Biol., The University of North Texas Health Science Center, Fort Worth, TX 76107, [2]VNIIOFI, Moscow, Russia & [3]ITEB, Pushchino, Russia

Muscle contraction involves cyclical interactions between myosin heads and actin filaments. Myosin heads are thought to generate contractile force by changing the orientation with respect to actin. These changes occur in a part of an A-band where thin and thick filaments overlap. The birefringence of a myosin head is a convenient measure of this orientation. Birefringence of the A-bands of sarcomeres located in the center of a fiber was compared in rigor and relaxation. Birefringence of the I-bands does not change upon transition from rigor to relaxation and it therefore served as a reference. To be able to measure birefringence from small volumes, a novel transmission microscope based on Mach-Zender interferometric principle was constructed. The source of illumination (He-Ne laser) is split into the reference and object channels. The reference channel consists of a quarter wave plate and a piezzo mirror to change the optical path length of the reference beam. The object channel consists of the high numerical aperture (NA) objective and a sample. The reference and object beams are recombined and projected into a CCD camera. Muscle fiber is illuminated with circularly polarized light and 2 images, corresponding to fiber being illuminated parallel and perpendicular to the axis, are obtained by four-frame-phase-shifting algorithm. Birefringence is proportional to the arithmetical difference between these 2 images. The high NA objective makes it possible to visualize individual sarcomeres in a whole muscle fiber. The difference in birefringence between I- and A-bands decreased upon transition from rigor to relaxation. These results support the view that birefringence changes originate in the subfragment-1 portion of myosin and are consistent with randomization of the heads in relaxed muscle. The absolute value of birefringence of a whole muscle fiber increased upon transition from rigor to relaxation. The absolute value of birefringence of a central portion of muscle fiber (excluding fiber edges) decreased upon transition from rigor to relaxation suggesting that Brewster scattering from fiber edges contributes to total birefringence. *Supported by NIAMS.*

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 2, 1998

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: Dmitriy S Ushakov

Department: Biochem and Mol Biology

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Read instructions and fit abstract inside the space given below:

EFFECT OF ADP ON THE CONFORMATION OF ACTO-S1 COMPLEXES.

D.S. Ushakov, O.A. Andreev and J. Borejdo

Department of Biochemistry & Molecular Biology, University of North Texas, 3500 Camp Bowie Blvd, Fort Worth, TX 76107

The conformation of skeletal and smooth muscle myosin subfragment-1 (S1) bound to F-actin was compared in the presence and in the absence of ADP. The conformation was assessed by the ability of carbodiimide to form a zero-length cross-link between the heavy and light chain 1 of skeletal muscle S1 and F-actin and between the heavy chain of smooth muscle S1 and smooth muscle light chains. In agreement with earlier reports, at high or low S1:actin ADP made no difference to cross-linking. However, at the intermediate molar ratios ADP made a significant difference. In contrast, strong dissociating agents -- AMP-PNP and PP_i -- caused complete dissociation of acto-S1 and inhibited cross-linking at any molar ratio. In agreement with earlier reports, ADP had an effect on cross-linking of smooth muscle S1 at high but not at low S1:actin. The effect of ADP is accurately predicted by the two-state model of binding of skeletal muscle S1 (Andreev & Borejdo, 1992) where S1 binds to one actin protomer (state 1) when actin filaments are saturated with S1, and to two protomers (state 2) when filaments are not saturated. At high or low S1:actin, a weak dissociation caused by ADP is unable to change saturation of actin filaments with bound S1, but at intermediate ratios it can, and causes significantly more S1's to bind in state 2 causing changes in cross-linking pattern.

Abstract #92

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ABSTRACT FORM

First Author: Yukfung Lee

Department: Molecular Biology and Immunology

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Read instructions and fit abstract inside the space given below:

CLONING AND ANALYSIS OF THE PROMOTER REGION OF 2B4, A MURINE NK CELL RECEPTOR. **Y. LEE, S.E. STEPP*, P.R. KUMARESAN AND P.A. MATHEW. DEPARTMENT OF MOLECULAR BIOLOGY AND IMMUNOLOGY, UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER, FORT WORTH, TX 76107 AND *DEPARTMENT OF PATHOLOGY, UT SOUTHWESTERN MEDICAL CENTER, DALLAS, TX 75235**

Natural killer (NK) cells are large granular lymphocytes that mediate the killing of various tumor cells and virally infected cells. The molecular basis of NK cell recognition and activation by target cells is poorly understood. We have previously identified, cloned and characterized a receptor, 2B4, expressed on murine NK cells. It is found that 2B4 is not only expressed on all NK cells, but also expressed on macrophages and those T cells which display NK-like killing. Comparison of 2B4 with sequences in the GeneBank database showed that 2B4 is a novel member of immunoglobulin supergene family. In order to further understand the transcriptional regulation of the 2B4 gene, we obtained a genomic 2B4 clone in the P1 system including the sequence of the 2B4 gene promoter region and determined the sequence 5' to the first exon by cycle sequencing. The promoter region was PCR amplified and subcloned into pCR2.1 vector. Using nuclear protein extract from a T cell line, CTLL-2, the interaction of the promoter with nuclear proteins was studied by electrophoretic mobility shift assay (EMSA).The result showed that a 100bp 5' fragment (-89 to +11) of the promoter region interacted with nuclear protein(s) expressed only in CTLL-2 cells, which express 2B4, whereas, such specific binding was not seen in Jurkat, Peer (both T cell line) or Sp2/o (B cell line) cells which do not express 2B4. Competition EMSA showed that the binding was sequence specific. Nested deletion of the 2B4 promoter region and analysis by the reporter gene chloramphenicol acetyltransferase yielded varying levels of CAT expression suggesting the presence of two cis-acting elements in the 2B4 promoter.

Abstract #93

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1998

ABSTRACT FORM

First Author: P. R. Kumaresan

Department: Molecular Biology and Immunology

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Read instructions and fit abstract inside the space given below:

CLONING AND CHARACTERIZATION OF RAT 2B4 GENE.

P.R.Kumaresan, S.Stepp Y.Lee And P.A.Mathew. Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Texas 76107.

Cytotoxic function of NK cell is regulated by cell surface receptors that deliver transmembrane signals. Interactions of these NK receptors with ligands on target cells may deliver inhibitory or activating signals to NK cells. we have previously identified and characterized two receptors expressed on NK cells. One molecule, 2B4 is expressed on all NK cells and appears to transduce a positive signal for killing and other molecule 5E6 is expressed on a subset of NK cells and transduce a negative signal. 2B4 is a 66 Kda monomer protein and belongs to the immunoglobulin gene superfamily. In the present study we have isolated and characterized a rat homologue of the murine 2B4 genes from RNK-16 cDNA library. Genomic Southern blot identified several bands and Northen blot showed three mRNA transcripts at 4kb, 2.5kb and 1.5kb when hybridized with full length 2B4 cDNA. Two of the genes isolated showed more than 65% homology at nuclear level and 68% homology at protein level with murine 2B4. Rabbit antisera was generated against a peptide antigen designed from the extracellular region of the predicted sequence. Immunoprecipitation of surface labeled RNK-16 cells with rat 2B4 antisera identifies an approximately 60Kda protein, which when deglycosolated is approximately 40kDa. The predicted aminoacid sequences of the two clones suggest that the encoded protein may correspond to a secretary form or a GPI linked form of rat 2B4 molecule.

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