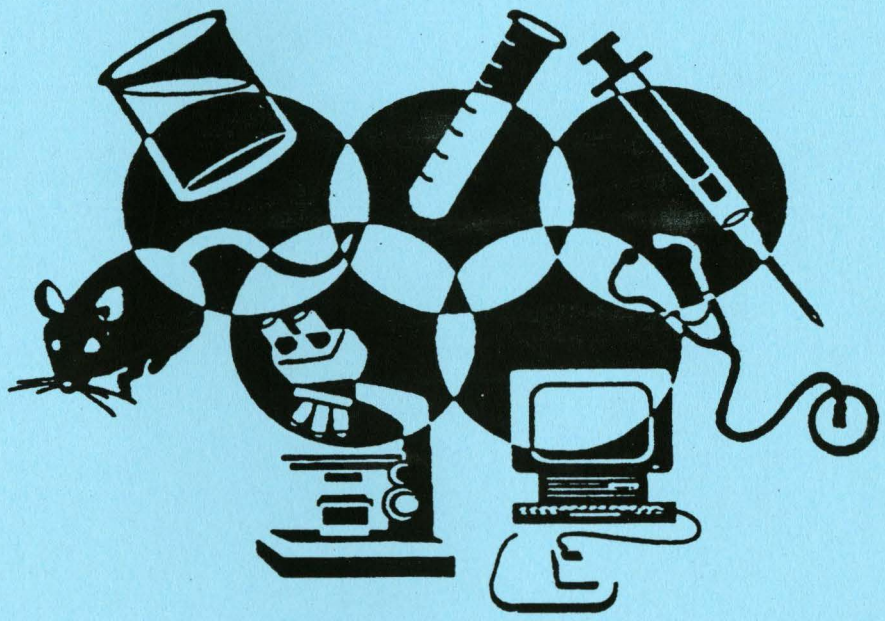
University of North Texas
Health Science Center at Fort Worth

Seventh Annual

Research Appreciation Day

March 24, 1999



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University of North Texas Health Science Center at Fort Worth
Research Appreciation Day
March 24, 1999

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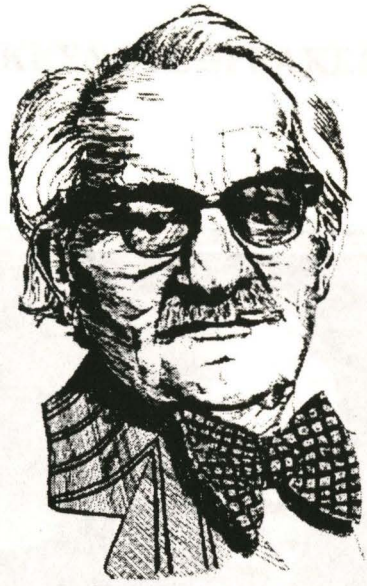
AGENDA

7:00 - 8:00 AM	Assemble Posters	Interdisciplinary Laboratory
8:00 - 9:30 AM	Faculty/Non-Student Posters Session	Interdisciplinary Laboratory
9:30 - 11:30 AM	Student/Postdoctoral Poster Competition	Interdisciplinary Laboratory
11:30 AM - 1:30 PM	Lunch and Keynote Speaker	Luibel Auditorium
	Welcome	Robert W. Gracy, Ph.D. Dean Office of Research and Biotechnology
	Overview of RAD '99 Activities	Thomas Yorio, Ph.D. Dean Graduate School of Biomedical Sciences
	Introduction of Mr. E. Bruce Street, Sr.	Robert W. Gracy, Ph.D.
	Introduction of Scott Grundy, M.D., Ph.D.	Walter McConathy, Ph.D. Department of Internal Medicine
	Introduction of Keynote Speaker	Scott Grundy, M.D., Ph.D. UT Southwestern
	Award Presentation	Drs. Gracy, McConathy and Mr. Street
	"The Dietary Reference Intakes: Progress and Confusion"	George H. Beaton, Ph.D. 1999 Roger J. Williams Award in Preventive Nutrition Recipient
1:30 PM	Open Reception with Dr. Beaton	ME1-806
1:30 - 4:30 PM	Student Oral Presentation Competition	Everett Hall
5:00 PM	Award Ceremony	Everett Hall
ALL DAY	Vendor Fair	Everett Hall Lounge/Hallway



The Roger J. Williams AWARD

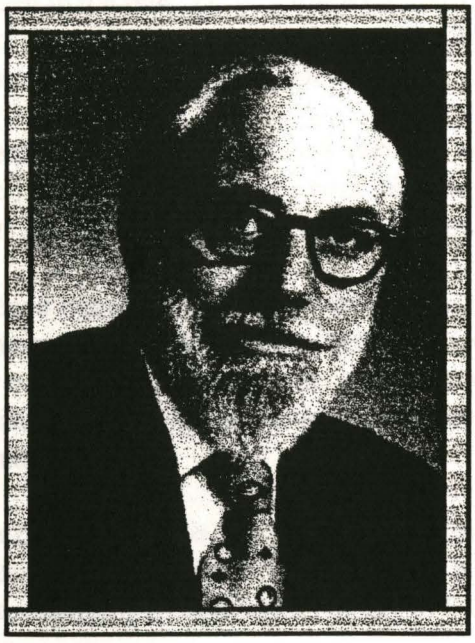
IN PREVENTIVE NUTRITION

1 9 9 9

The Roger J. Williams Award in Preventive Nutrition is made possible by an endowment established by Mr. E. Bruce Street, Sr. and his wife, Mrs. Virginia Street. It is Mr. Street's belief that human nutrition is an area of unrealized individuals who have made important contributions to this area.

Mr. Street was a Regent of the University of North Texas and the University of North Texas Health Science Center at Fort Worth (formerly known as TCOM).

KEYNOTE SPEAKER



George H. Beaton, Ph.D.
Professor Emeritus, Nutritional Sciences
University of Toronto, Canada

George H. Beaton, Ph.D., is a foremost authority on the theoretical basis of formulation and application of dietary guidelines. He has been a leading figure in the evolution of current concepts of estimating nutritional requirements. This evolution of thought has grown out of his own research and by participating in many committees responsible for setting dietary guidelines and nutrition policy. The concepts emerging from Dr Beaton's work are the foundation for the current paradigm for dietary reference intakes (DRIs) for North America. Dr. Beaton's research and theoretical studies have called into question many longly held positions about the relations between intakes and nutrient needs of different populations. The readjustment of these positions resulting from Dr. Beaton's critique has led to a fundamental modification of approaches to the development of nutrition guidelines. Not only has he made fundamental contributions at the experimental level, but his theoretical analysis of requirement estimates and application constitutes a significant body of influential work in the nutrition field.

ABBOTT LABORATORIES
RESEARCH ACHIEVEMENT AWARDS

Abbott Laboratories has been in the business of improving lives for more than a century. Our 56,000 employees around the world are dedicated to discovering, developing and marketing innovation health care solutions across the spectrum of health care.

With a shared commitment to the advancement of medical science, Abbott's 56,000 employees worldwide have a devoted their careers to achieving our ambitious mission. Around the world, people demand the highest quality in every product the company manufactures.

This dedication to excellence has led to an outstanding record of long-term financial performance and continuous growth. Abbott consistently ranks high in the performance measures that determine the Fortune 500 and other comparisons of the world's leading corporations. For example, Abbott's worldwide sales of $11 billion ranks 124th in revenues in Fortune magazine's 1997 ranking of the largest U.S. based corporations.

Headquartered in the northern suburbs of Chicago, Abbott has manufacturing, distribution operations, and joint ventures in 44 countries. The company's products are marketed in more than 130 countries. Abbott Diagnostics Division has a facility in Irving, Texas where they manufacture diagnostic instrumentation.

Abbott maintains its standing among the world's most respected health care companies by developing innovative products at the leading edge of medical technology. This year we will reinvest more than $1 billion in research and development. In addition, Abbott is on schedule in implementing company-wide strategies to ensure our Year 2000 readiness. These initiatives not only benefit our shareholders and customers, they provide long-term stability and commitment to the communities where we are located, to our suppliers and to our employees.

Every day, Abbott scientists are making news by discovering innovative medical technologies to improve your health. Their efforts support our overriding objective: to improve lives.

See Abbott Laboratories online at http://www.abbott.com

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

ALCON LABORATORIES
RESEARCH ACHIEVEMENT AWARDS

THE ALCON GROUP

The Alcon group is the global leader in the research, development, manufacture and marketing of ophthalmic products, including surgical instruments and accessory products, intraocular lenses, prescription drugs and contact lens care solutions. The Alcon group is wholly-owned by Nestle S.A.

Founded in Fort Worth, Texas in 1947, the Alcon group now employs 10,000 individuals around the world. Total sales for 1998 exceeded $2.1 billion, with activity in more than 170 markets. One of the cornerstones of Alcon's success is the company's commitment to Research and Development. Housed at the company's headquarters in Fort Worth is the 400,000 square-foot William C. Conner Research Center, the largest and most sophisticated eye research center in the world. Over the next five years, Alcon plans to spend nearly $1 billion on eye-related research, more than any entity outside of the National Eye Institute.

The Alcon Laboratories Research Achievement Awards are given to the top two postdoctoral poster presentations as determined by a panel of judges.

FORT WORTH MEDTECH CENTER
INNOVATION AWARD

The Fort Worth MedTech Center, Inc. , a private non-profit business incubator founded in February 1998, provides specialized and emerging medical and technology companies in Fort Worth.

The mission of the Center is to attract, grow and graduate successful medical and technology companies that are financially viable and freestanding and to encourage job creation in the Fort Worth medical and technology community.

The Fort Worth MedTech Center exists to foster and assist new business ventures through the critical first years of existence. These ventures must support medical and/or technology-oriented products or services. These ventures will ultimately provide economic gains, employment opportunities, and tax-base expansion in Fort Worth.

The Incubator selectively invests time, money and expertise in emerging young companies and entrepreneurs that demonstrate the potential to generate significant sales and job creation in Fort Worth. Medical and technology companies such as these also diversify the Fort Worth economy and make it less reliant on a single industry, while creating high-wage and high-quality jobs.

The Fort Worth MedTech Center provides a wide range of specialized business services that, in a pro active approach, complements the needs of the start-up companies. In addition, the Center offers introductions and connections to a network of corporate investors, such as venture capitalists, investment and merchant bankers and private investors, also known as angel investors. These services are available to the start-up companies to increase their chances of success, to ensure a high graduation rate and sound decision making by the entrepreneurs participating in the Incubator.

See Fort Worth MedTech Center online at http://www.medtech.org

JUDGES

The 1999 Research Appreciation Day student poster presentation judges are:

David G. Bernard, Ph.D., Assistant Professor
Department of Biology, University of Texas at Arlington

Ken Boyce, Environmental Specialist
United States Environmental Protection Agency

Paul Chippindale, Ph.D., Assistant Professor
Department of Biology, University of Texas at Arlington

Guy Dixon, Ph.D., Laboratory Manager
Tarrant County Public Health Department

Mike Dixon, Ph.D., Chair and Associate Professor
Department of Biology, Texas Wesleyan University

Julia A. Nelson, M.S., Vice President for Scientific Affairs
Summa Laboratories, Inc.

Iok Hou Pang, Ph.D., Principal Scientist and Adjunct Associate Professor (UNTHSC)
Alcon Laboratories, Inc.

Ricardo E. Rodriguez, Ph.D., Associate Professor
Department of Chemistry, Texas Wesleyan University

Reginald Stilwell, Scientific Manager
Johnson & Johnson Medical

The 1999 Research Appreciation Day student oral presentation judges are:

Julie Crider, Ph.D., Senior Scientist
Molecular Pharmacology, Alcon Laboratories, Inc.

Art Goven, Ph.D., Faculty Executive Assistant to the Chancellor and Professor
Biological Sciences, University of North Texas - Denton

Jill Van Wart Hood, Ph.D., Allied Health Coordinator
Department of Biology, University of Texas at Arlington

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Madison, WI 53711
(972) 790-8642

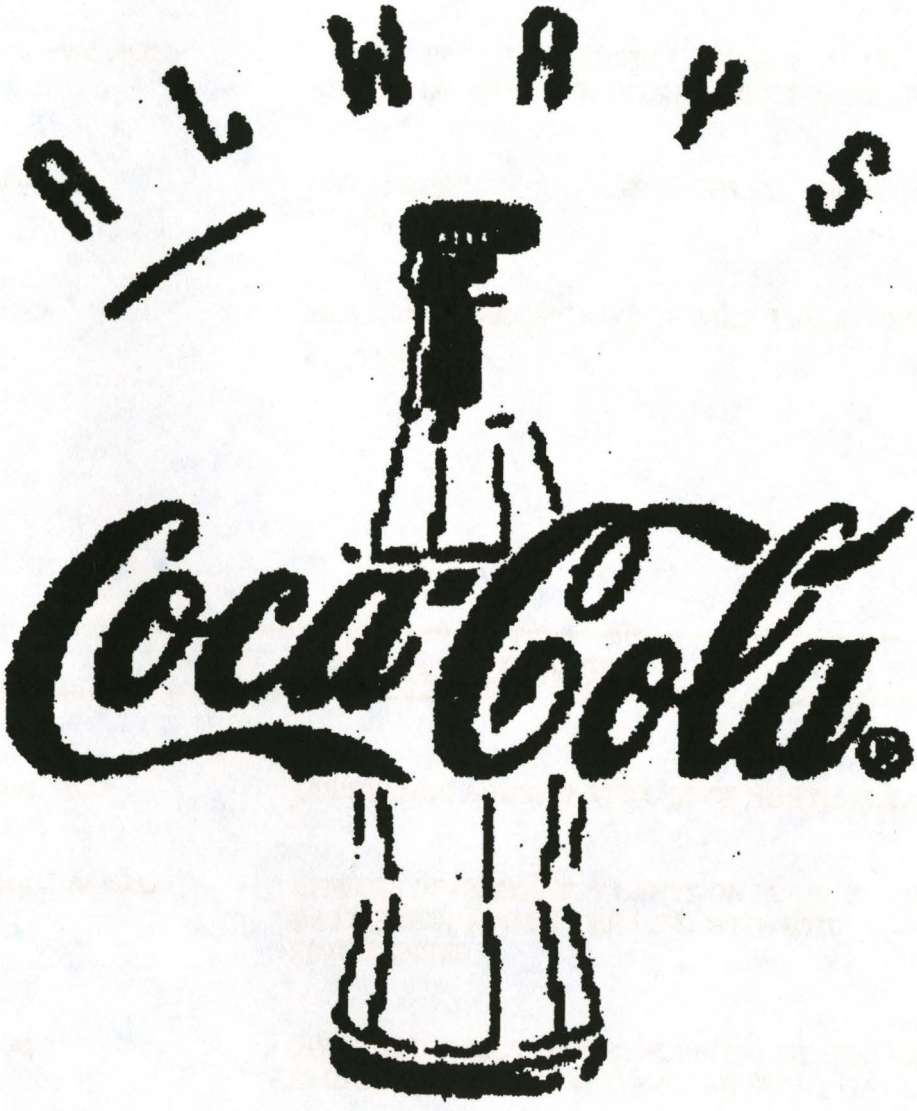
Representative: Natalie Luke, Ph.D.

TRAVEL SERVICE EVERYWHERE
RIDGLEA VILLAGE TRAVEL

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 1999 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.

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.



MUSIC AND MEDICINE

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2.	Miriam Henoch, Ph.D.	EAR CANAL RESONANCE AND SPECTRAL CHARACTERISTICS OF MUSICIANS' INSTRUMENTS AS RISK FACTORS IN PERFORMANCE INDUCED HEARING
3.	Miriam Henoch, Ph.D.	SOUND PRESSURE LEVELS WITHIN A UNIVERSITY JAZZ ENSEMBLE: ARE STUDENTS AT RISK FOR HEARING LOSS?
4.	Kris Chesky	UNT MUSICIAN HEALTH SURVEY: DATA ON MUSICIAN HEARING LOSS
5.	Kris Chesky	MUSICIANS' PERCEPTIONS OF WIDESPREAD DRUG USE AMONG MUSICIANS

NEUROSCIENCE

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8.	Monica Yagle	[^{3}H] ETHYNYLBICYCLOORTHOBENZOATE ([^{3}H] EBOB) BINDING IN RAT CEREBELLUM AND RECOMBINANT $GABA_A$ RECEPTORS

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Bernard Rubin, D.O.**

Department: **Music and Medicine**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

TEXAS CENTER FOR MUSIC AND MEDICINE. Bernard Rubin, D.O., & Kris Chesky, Ph.D. Department of Medicine, and UNT College of Music, University of North Texas Health Science Center, Fort Worth, Texas 76206.

The talent, energy, and hard work of musicians and other performers give us the pleasures and deeper experiences, and the diversions we need to enrich our lives. Like athletes, musicians require health, strength and stamina to perform in today's competitive music world and to meet their own high artistic expectations. Training for a career in music is difficult and even the most successful performers may be subject to severe and, at times, crippling stresses and strains.

In many cases the ailments and disabilities of musicians arise from the specific uses of the body required to perform, such as the overuse disorder of an instrumentalist's hand, or they may be related to performing itself, such as the common but nonetheless debilitating anxiety of stage fright. Even though the disciplines of medicine and music have long recognized that musicians were susceptible to particular performance-related diseases, very little knowledge exists about how, when, and why these diseases develop or how to prevent them. Furthermore, a great many musicians still have difficulty in finding the specialized care they need.

For these reasons, a group of professionals at the University of North Texas and the University of North Texas Health Science Center have developed the Texas Center for Music & Medicine. Considered as the pioneering arts medicine group in the Southwest, it is dedicated to the study, prevention, diagnosis, and treatment of illness that may arise in the course of a musician's career.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Miriam A. Henoch, Ph.D.**

Department: **Music and Medicine**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

Ear Canal Resonance and Spectral Characteristics of Musicians' Instruments as Risk Factors in Performance Induced Hearing Loss.

Miriam A. Henoch, Ph.D. and Kris Chesky, Ph.D, Department of Speech and Hearing Sciences, College of Music, University of North Texas, Denton, Texas 76203.

The purpose of the present study was to examine the effects of ear canal resonance on the spectral characteristics of sounds produced by musicians' instruments in relation to risk factors associated with noise induced hearing loss. Nine musicians, each playing a different instrument, were chosen as subjects for the investigation. The spectrum levels of sounds produced by musical instruments were measured both inside and outside of the ear canal during performances of sustained Gs, paired at constant intensity levels and for three different octaves. With direct reference to the measured peak resonance of the musician's ear canal, comparisons were made between sound pressure levels inside the ear canal to sound pressure levels outside the ear canal. It was found that ear canal resonance has the potential to generate an increase in the sound pressure levels inside the ear canal of the performing musician by as much as 22 dB SPL. The implications for increased risk for hearing loss as a function of the interaction between ear canal resonance and the frequency spectrum of specific instruments is discussed.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Miriam Henoch, Ph.D.**

Department: **Music and Medicine**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

SOUND PRESSURE LEVELS WITHIN A UNIVERSITY JAZZ ENSEMBLE: ARE STUDENTS AT RISK FOR HEARING LOSS?
Miriam Henoch, Ph.D., Kris Chesky, Ph.D., & Bernard Rubin, D.O.
Department of Speech and Hearing Sciences, College of Music, and Department of Medicine, University of North Texas, Denton, Texas 76206.

University music students are exposed to a wide range of sound pressure levels (SPLs) in a variety of non-orchestral ensembles including concert band, marching band, and lab jazz band. The sound pressure levels produced may vary depending on the size and type of the ensemble, selection of performance literature, performance practices, and the acoustical characteristics of the performance venue. A survey of literature indicates a lack of studies that quantify sound pressure levels generated in these non-orchestral settings. Most studies have focused on classical musicians in an orchestral environment. Furthermore, the extent of intersection variability within non-classical ensembles has not been studied. This information is important for the development of preventative strategies for the protection of hearing loss in musician populations. The purpose of this investigation was to determine SPLs produced within a typical university jazz band setting in order to determine if students were exposed to levels that exceeded risk criteria established by OSHA (1983) for industrial workers.

Subjects for this investigation included University of North Texas (UNT) College of Music students performing in the Two O'clock Lab Band. Four subjects were selected according to their position within the ensemble. Measurements were taken during fifty-minute rehearsal periods over four consecutive days. A personal dosimeter was attached to the leader of the trombone, trumpet, and saxophone sections, and the percussionist.

Measurements included Lavg (average levels for the 50 minute period), time weighted average (projected levels for three and eight hour periods), peak level, maximum level, and daily percentage of allowable dose. Results indicated that levels exceeded the permissible exposure levels outline by OSHA thereby increasing the risk for hearing loss in this musician population.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Kris Chesky, Ph.D.**

Department: **Music and Medicine**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

UNT MUSICIAN HEALTH SURVEY: DATA ON MUSICIAN HEARING LOSS. Kris Chesky, Ph.D., & Miriam Henoch, Ph.D. College of Music and Department of Medicine, Department of Speech and Hearing Sciences, University of North Texas, Denton, Texas 76206.

There is very little information on the patterns of hearing loss problems of non-classical musicians. Research on classical musicians suggest a concern for problems with hearing loss. The purpose of this study was to examine the occurrence of problems with hearing loss within a heterogeneous group of musicians as a function of both primary performance area and primary instrument. The goal of this research is to ultimately direct additional investigative efforts that will address the needs of all musician populations that are at risk for hearing loss.

A heterogeneous sample of musicians (N= 3293) responded to the UNT-Musician Health Survey as of September (1998). The cohort was nationally distributed and represented a professionally experienced group and included a variety of musician types, both in terms of primary instrument and type of music performed. Data were generated from a UNT-MHS survey question that asked: "do you have a problem with hearing loss?" Response choices included, no; mild; or severe. Because only 1.5% percent of the respondents indicated severe problems, the response categories of mild and severe were collapsed into a single variable resulting in two categorical variables; no problem with hearing loss and problem with hearing loss.

The first analysis determined rates for problems with hearing loss as a function of the type of music performed. The second analysis compared problems with hearing loss as a function of primary instrument. The third analysis compared problems with hearing loss between classical and non-classical musicians that primarily play the same instrument. The classical group included musicians that reported classical or opera as their primary performance area. The non-classical group included all other primary performance categories except educator or composer.

Data clearly show that problems with hearing loss were associated with the type of music and the type of instruments played. A higher percentage of musicians performing in non-classical settings reported problems with hearing loss when compared to classical musicians. The data showed that, in most cases, fewer classical musicians reported problems with hearing loss when compared to non-classical musicians although they performed on the same instrument.

Trends suggest the need for further research to determine noise exposure levels, as well as other risk variables associated with non-classical musicians and musical settings. Together with the current lack of research related to non-classical musicians, these findings suggest and warrant further research into factors that effect hearing loss. These differences may be traced to physical proximity issues to other musicians, performance and vocational patterns, and pyschosocial factors.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Kris Chesky**

Department: **Music and Medicine**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MUSICIANS' PERCEPTIONS OF WIDESPREAD DRUG USE AMONG MUSICIANS. Kris Chesky, PH.D., & John Hipple, Ph.D. University of North Texas, Denton, Texas 76206.

From the beginning and throughout a musician's career, the perception of drug use among other musicians may be an important factor in developing attitudes and behaviors related to their own drug use. This study examined musicians' perceptions of the extent of widespread drug use among musicians.

A heterogeneous sample of musicians (N=3278) participated by filling out the University of North Texas Musician Health Survey. This survey is posted over the internet. Overall, subjects were mature adults (mean age = 34), musically educated (mean years of formal college instruction = 3.97), and professionally experienced (mean years as professional musician = 10.53). Non-parametric statistics, multiple regression with optimal scaling, and ordered probit analysis were used to determine patterns in the perception of drug use among musicians by gender, age, formal college instruction in music and musician type.

Results showed that approximately one third of this sample perceived that drug use among musicians is widespread. However, almost 40% indicated that they did not know if drug use is widespread among musicians. Gender and age characteristics were observed indicating that younger musicians and males were more likely to implicate drug use, whereas older musicians and females were more likely to indicate they don't know. The differences between groups based on amount of formal college instruction in music were less clear. The strongest link to the perception of drug use among musicians was musician type. Non-classical musicians were more likely to report widespread drug use compared to classical musicians. Results indicated that the linear combination of the predictor variables was significantly related to the perception of drug use, $R^2 = 0.10$; Adjusted $R^2 = 0.10$, $F(3, 3278) = 92.599$, $p < 0.000$). All four variables were significant at the $p < 0.000$ level. The musician type variable was most strongly related ($B = .249$) to the perception of drug use among musicians. Age was the second strongest, followed by gender then education level. Based on tolerance statistics, about 20 percent of the variance of the musician type predictor was shared with the other variables. Tolerance measures for the other variables were even higher and very high for the age variable indicating a negligible concern for multicollinearity between variables. The ordered probit model was significant (Log Likelihood = -3873.5471, $X^2(4) = 161.59$, Prob > X^2 = 0.0000) with significant estimates for age, gender, musician type and education.

The model provided evidence showing how variations in age, gender and musician type can lead to variations in the probabilities of how musicians perceive drug use among musicians. The specific drugs reported as used included marijuana, cocaine and amphetamines. Further research is needed to understand factors contributing to these perceptions and the relationship between perception and actual drug use among musicians.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Harbans Lal, Ph.D.**

Department: **Substance Abuse Institute of North Texas (SAINT)**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

SUBSTANCE ABUSE INSTITUTE OF NORTH TEXAS (SAINT)

Harbans Lal, Ph.D., Executive Director, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107

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 professionals 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 at Fort Worth campus to interact and perform research with members of SAINT.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **NancyEllen C. de Fiebre**

Department: **Pharmacology**

Place an "X" in the box to the left of your classification.

Graduate Student		Medical Student		Postdoctoral Fellow
Faculty	X	Staff		

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECTS OF NICOTINIC AGENTS ON LEARNING BEHAVIORS OF RATS CHRONICALLY TREATED WITH ETHANOL-CONTAINING LIQUID DIETS.
NancyEllen C. de Fiebre, Paul M. Seymour, Christopher M. de Fiebre, Ph.D., Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107-2699.

Alcoholics are almost invariably smokers; yet, little is known whether nicotine, via smoking, may modulate the neurotoxic properties of ethanol. In this study, the ability of nicotinic agents to modulate ethanol-induced deficits in learning and memory was examined. Male Long-Evans rats were treated for 24 weeks with either a Sustacal-based, ethanol-containing or isocaloric, sucrose-containing liquid diet as the sole source of nutrition and were given twice daily injections (i.p.) of saline, nicotine (0.2 mg/kg), mecamylamine, (0.2 mg/kg) or combined nicotine and mecamylamine. After 3 weeks of withdrawal from all drugs, one group of rats was tested in an active avoidance paradigm, a non-spatial learning task, while another group was tested in the Morris Water Maze, a spatial learning task. After completion of these paradigms, animals were subsequently tested on the alternative task. To test active avoidance learning, rats were tested in a shuttlebox. The CS consisted of a 7 sec light and tone followed by a UCS of a 2 sec, 0.6 mA footshock. Rats were given 15 trials daily with a intertrial interval of 30 sec. Avoidance behavior was measured for 21 days or until a rat reached a criterion of 13 avoidance for 3 consecutive days. Overall, the results demonstrated an ethanol-induced deficit. Among the ethanol-treated animals, those that were given twice daily injections of nicotine learned the task more quickly than did animals injected with saline. Concurrent injection of mecamylamine with nicotine blocked the protective effects of nicotine. In general, results from the Morris Maze demonstrated that all rats showed some spatial learning with sucrose treated animals performing better than ethanol treated animals. Among the ethanol-treated animals, animals treated with nicotine were able to learn a reversal paradigm following one day of reversal training while animals treated with saline could not. Again, the apparent protective effect of nicotine was blocked by concurrent administration of mecamylamine. These data suggest that nicotine may attenuate the neurotoxic effects of ethanol. Histological analyses are underway to confirm this.

(This project is supported by an ongoing 3-year grant from the National Institute on Alcohol Abuse and Alcoholism (AA-11597). Direct costs for the grant equal ~$155,000 annually.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Monica Yagle**

Department: **Pharmacology**

Place an "X" in the box to the left of your classification.

X	Graduate Student	Medical Student	Postdoctoral Fellow
	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

[^{3}H] ETHYNYLBICYCLOORTHOBENZOATE ([^{3}H] EBOB) BINDING IN RAT CEREBELLUM AND RECOMBINANT $GABA_A$ RECEPTORS.

Monica A. Yagle, Michael W. Martin, Christopher M. deFiebre, Nancy C. deFiebre, and Glenn H. Dillon. Dept. of Pharmacology, University of North Texas Health Science Center at Fort Worth, TX 76107

GABA is the predominant inhibitory neurotransmitter in the vertebrate CNS. $GABA_A$ receptors are ligand-gated chloride channels composed of multiple subunits (designated $\alpha, \beta, \gamma, \delta, \varepsilon$, and ρ), and are the site of action of convulsant agents and several therapeutics, including anxiolytics, hypnotics, anesthetics, and muscle relaxants. Analysis of ligands, such as tert-butylbicyclophosphorothionate (TBPS) and picrotoxin (PTX), which bind in the channel of the $GABA_A$ receptor, have proven useful for studying the modulation of this receptor. EBOB is a more recently developed ligand that appears to bind in the same region of the channel as TBPS, but with a higher affinity. Only a few studies have examined the binding of EBOB to vertebrate brain tissue, but none have examined potential subunit-dependent binding of EBOB. We have thus examined [^{3}H] EBOB binding in rat cerebellum and HEK293 cells stably expressing human $\alpha1\beta2\gamma2$ and $\alpha2\beta2\gamma2$ $GABA_A$ receptors. For comparison, [^{35}S] TBPS binding, which shows subunit selectivity, was also examined in $\alpha1\beta2\gamma2$ recombinant receptors. Dose-dependent studies on [^{3}H] EBOB binding by several known convulsant compounds, dieldrin, lindane, PTX, TBPS, and pentylenetetrazole (PTZ), were performed. In rat cerebellum, all compounds inhibited [^{3}H] EBOB binding at one site in a dose dependent fashion, with affinities in the μM range for dieldrin, lindane, PTX, and TBPS and in the mM range for PTZ. In human $\alpha1\beta2\gamma2$ and $\alpha2\beta2\gamma2$ receptors, lindane, PTX, and PTZ inhibited [^{3}H] EBOB binding at one site in a dose dependent manner, with lindane having the highest affinity and PTZ the lowest. Unexpected results were obtained with TBPS and dieldrin in both receptor subtypes. TBPS inhibited [^{3}H] EBOB binding at two sites, a high affinity site in the nM range and a low affinity site in the μM range. Dieldrin resulted in stimulation of [^{3}H] EBOB binding at high concentrations and dose dependent inhibition at lower concentrations. Studies of subunit selectivity of EBOB binding will be continued with other isoforms of $GABA_A$ receptors. Functional studies will also be performed to further examine dieldrin's stimulatory effect. (Support: NIH R29 ES07904)

AGING AND ALZHEIMER'S

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RESEARCH APPRECIATION DAY 1999

ABSTRACT

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

Department: **Geriatrics Education and Research Institute**

Place an "X" in the box to the left of your classification.

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

GERIATRICS EDUCATION AND RESEARCH INSTITUTE

Thomas J. Fairchild, Ph.D., 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.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Angel Rivera, MPH, DDS**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

IN-HOME HEALTH SCREENING AND ASSESSMENT PROJECT OF TARRANT COUNTY COMMUNITY-DWELLING OLDER ADULTS: A FOLLOW UP. Angel Rivera, DDS; Antonio Rene, Ph.D.; Janice Knebl, DO; Karen Godwin, Ph.D.; University of North Texas Health Science Center, Fort Worth, TX 76107

Purpose: This study examined three facets of the In-Home Health Screening and Assessment Project: 1) recommendations that made a difference in helping community-dwelling older adults remain independent, 2) the impact of community-based services on recommendations, and 3) the actions of personal physicians based on recommendations.

Methods: This is a retrospective chart review. To screen potential explanatory variables for predictors of nursing facility/ assisted living arrangements, logistic models were constructed. Follow-up life tables were used for the analysis of survival data. Significance levels were set at .05.

Results: The Follow-up life table analysis presents a 5.5 months median survival time of living independently for those that present positive health behaviors vs those that present negative health behaviors (i.e. smoking and drinking alcoholic beverages.) In the logistic model, community-dwelling older adults who need home health aide services and homemaker services but do not receive them were respectively over 2 times more likely and over 3.5 times more likely to be institutionalized than those who received the services.

Conclusions: Home health screening will prevent or at least postpone some disabling chronic conditions associated with aging, decreasing period of illnesses and institutionalization.

(Partially Funded by the Bureau of Health Professions)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Jon N. Swift, Jr., M.A.**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE EFFICACY OF ADJUNCTIVE OMT IN THE ELDERLY HOSPITALIZED WITH PNEUMONIA. D.R. Noll, D.O., J.H. Shores, Ph.D., R.G. Gamber, D.O., J.N. Swift, M.A., P.C. Slocum, D.O., University of North Texas Health Science Center at fort Worth, Department of Medicine. 3500 Camp Bowie Boulevard, Fort Worth, TX 76107.

Hypothesis: Adjunctive Osteopathic manipulative Treatment (OMT) is beneficial for reducing the duration of IV antibiotic use and length of hospital stay in elderly persons hospitalized with acute pneumonia.

Methods: We recruited patients age 60 and over hospitalized with acute pneumonia. The treatment group received OMT by a specialist in manipulative medicine and a standardized OMT protocol for 10 to 15 minute duration, twice daily. The control group received light touch sham treatments of the same duration and frequency as the treatment group. The attending physicians were blind to group assignment. Outcome measures were duration of IV and oral antibiotic use in the hospital and the length of hospital stay.

Results: Fifty-eight patients met inclusion criteria. We randomized 28 into the treatment group and 30 into the control group. There was no statistical difference between groups for age, sex or simplified acute physiology scores. The mean duration of IV antibiotic use was 5.25 days for the treatment group and 7.33 days for the control group ($p<0.005$). The mean duration of IV and oral antibiotic use in the hospital was 6.14 days for the treatment group and 8.13 days for the control group ($p<0.005$). The mean length of hospital stay was 6.61 days for the treatment group and 8.57 days for the control group ($p<0.005$).

Conclusion: Adjunctive OMT reduces the duration of IV antibiotic use and reduces the length of hospital stay for elderly persons hospitalized with acute pneumonia. *(supported by AOA Grant #96-11-390)*

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Amy Moss, DO**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

PREDICTORS OF LONGEVITY IN AN ALZHEIMER NURSING HOME FACILITY. Amy Moss, DO; Dawn Gonzalez, DO; Janice Knebl, DO, Angel Rivera, MPH; Karen Godwin, Ph.D. University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Purpose - The development of Alzheimer Special Care Centers is a phenomenon of the past decade. Providing effective medical and psychosocial care while individuals are challenged with behavioral, psychological, and communication difficulties is unique and costly. The cost of care is placed primarily upon the family. Increasingly, these Centers will be asked to predict life expectancy. *This study provides data on demographic characteristics, life expectancy, and predictors of longevity in one Alzheimer Care Center.*

Methods-187 charts were reviewed in this retrospective study. Cognitive, behavioral, functional status and disease states and medication were collected from resident charts. Chi Square and the Kaplan Meier Survival Analysis were used for analysis current residents and deceased residents. (=.05)

Results -Of the deceased residents, 76% expired within 24 months of admissions to the facility. Of the current residents, 57% had been residents for 24 months or longer. Analysis between these two groups show that predictors of longevity include the number of comorbid diseases, polypharmacy, and activities of daily living residents have upon admission.

Conclusions - Based on the results of this study, the identified predictors of longevity may serve as guidelines to families of patients of Alzheimer Disease as they seek answers to question such as "How long should I plan on this fiscal commitment?" The limitation of the study rests with the uniqueness of the facility.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DEMOGRAPHIC CHARACTERISTICS OF PATIENTS IN THE HOUSE CALL PROGRAM OF THE DIVISION OF GERIATRICS

Leslie Dunn, DO, Donald Noll, DO; University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Purpose -Three objectives are defined for this retrospective study. Determine the demographic characteristics of patients of the House Call Program, prevalent referral sources, health assessment at the point admission.

Methods-All current House Call Program patients of the Division of Geriatrics at the UNT Health Science Center at Fort Worth were included. To determine the health status at initial contact, assessment data collected included current medication use, prevalent medical conditions, Mini Mental Status Exam (MMSE), mobility, causes of death, admissions to nursing home facilities and hospitals.

Results - 48% of patients were 85+; 56% are referred by physicians; 88% lived with someone in the household. 44% of primary caregivers is a child; 16% was a spouse; and 24% a non-family member. 58% were moderately or severely impaired cognitively.

Conclusions - The age range of this cohort is older than anticipated. House Call patients live at home but appear to require someone living with them and depend on community-based ancillary services. The small sample size prohibits generalizations. However, trends relative to medical conditions and medication use may be reported.

(Partially funded by the Bureau of Health Profession.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Monica Mendez**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DEVELOPMENT OF A MOLECULAR-BASED ASSAY FOR EARLY DETECTION OF LATE-ONSET ALZHEIMER'S DISEASE Monica Mendez[1], Deipanjan Nandi[1], Craig Conrad[2], Ph.D., J.Mark Sherman[1], Ph.D., and Arthur Eisenberg[1], Ph.D. University of North Texas Health Science Center, [1]Department of Pathology, [2]Department of Molecular Biology and Immunology, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107

The apolipoprotein E (apo E) epsilon 4 allele, located on chromosome 19, has been identified as a risk factor for late-onset Alzheimer's Disease (AD). To date it is the only genetic polymorphism to be accepted as a risk factor. Mutations in several other loci have been identified as potential contributors in the development of AD. Among these genes are the presenilin 1 gene, located on chromosome 14, the low-density lipoprotein receptor-related protein (LRP) gene on chromosome 12, and the alpha-2 macroglobulin gene, also on chromosome 12. Mutations in the presenilin 2 gene on chromosome 1 and the amyloid B-protein precursor on chromosome 21 have been found to be linked to early-onset, but not late-onset AD. Currently, the assay used to detect the mutations in these genes requires PCR amplification, endonuclease digestion of the amplified products, followed by gel electrophoresis. These procedures can be time consuming and labor intensive and do not lend themselves to automation. In order to simplify and automate these procedures, we are developing a genetic bit analysis (GBA) system which can be used to detect single nucleotide polymorphisms. The PCR reaction utilizes a 5' phosphorothioate-modified primer, which acts as a protection group for one of the strands of the PCR products from an exonuclease digest. We can then hybridize this single-stranded product to another capture primer that has been immobilized in a 96-well microtiter plate. This hybridization occurs just adjacent to the polymorphism in question. Biotin-labeled and fluorescein-labeled dideoxynucleoside triphosphates are then added to the wells to extend the capture primers by one base and these extensions can then detected by colorimetric techniques. We have collected a total of 129 AD patient samples and age-matched controls. We will screen the genes suspected in AD using the GBA assay for the early detection of late-onset Alzheimer's Disease.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Craig Conrad Ph.D.**

Department: **Molecular Biology and Immunology**

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Graduate Student	Medical Student	X	Postdoctoral Fellow
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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

BLOOD SERA, CEREBRAL SPINAL FLUID, AND BRAIN TISSUE FROM ALZHEIMER'S DONORS HAVE INCREASED OXIDATIVE DAMAGE. Craig C. Conrad, Janos Kalman, Christina A. Malakowsky, Miriam N. Alame, Rachel Dawson, John M. Talent and Robert W. Gracy. *Molecular Aging Unit, Department of Molecular Biology and Immunology, UNTHSCFW.*

Oxidative damage has been proposed to be a mechanism in Alzheimer's disease (AD), and antioxidants such as vitamin E and melantonin appear to slow the disease progress. Animal studies have shown a direct correlation between the loss of the ability to perform cognitive tasks and the level of oxidative damage to brain proteins. Treatment of senile animals with free radical traps decreases this oxidative damage and restores short-term memory. Since the neurodegenerative changes of AD may begin years or decades before clinical symptoms, it is likely that *oxidized protein biomarkers* may be of extreme value in the early diagnosis and treatment of AD. We have utilized two-dimensional protein fingerprinting and immunostains to identify some specific oxidized proteins (potential biomarkers) in blood sera, cerebral spinal fluid and brain tissue from Alzheimer's patients. We have found that the number of oxidized proteins, as well as the level of oxidation, are increased in the sera, cerebral spinal fluid and tissue homogenate of Alzheimer's patients when compared to similar protein profiles from healthy, aged matched controls.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Craig C. Conrad, Ph.D.**

Department: **Molecular Biology and Immunology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

USING MT KOCKOUT MICE TO STUDY THE ROLE OF METALLOTHIONEIN FROM OXIDATIVE DAMAGE. Craig C. Conrad and Arlan Richardson. *Department of Molecular Biology and Immunology. University of North Texas Health Science Center at Fort Worth, and Univeristy of Texas Health Science Center in San Antonio.*

We have tested the role of metallothionein (MT) in cellular protection against oxidative damage using MT I- and -II deficient mice ($MT^{-/-}$). Both $MT^{-/-}$ and $MT^{+/+}$ mice were treated with either saline (control) or zinc (20μg $ZnCl_2$/g b.w.) 12 hours prior to an oxidative stress: whole body γ–irradiation (240Gy) or 2-nitropropane (2-NP, 4.0μmol/g b.w.) treatment. The level of hepatic MT was significantly increased (20-fold) in zinc treated $MT^{+/+}$ mice when compared to $MT^{-/-}$ mice. Zinc treatment of the mice did not alter the hepatic levels of superoxide dismutase, catalase, or glutathione peroxidase in either $MT^{-/-}$ or $MT^{+/+}$ mice. Oxidative damage to liver DNA, as measured by the increase in 8-hydroxydeoxyguanosine, increased over 3-fold when compared to saline treated controls in both $MT^{-/-}$ and $MT^{+/+}$ mice following treatment with 2-NP. The elevated levels of tissue MT in $MT^{+/+}$ mice, due to zinc pretreatment, did not ameliorate the amount of oxidative damage to DNA when compared to zinc treated $MT^{-/-}$ mice or 2-NP only treated mice. The amount of lipid damage, as measured by the level of TBA-reactive materials, also increased 3-fold following 2-NP treatment in both $MT^{-/-}$ and $MT^{+/+}$ mice compared with saline treated controls. Again, zinc pretreatment and elevated levels of hepatic MT in $MT^{+/+}$ mice did not protect liver lipids from oxidative damage when compared to zinc treated $MT^{-/-}$ mice or 2-NP only treated mice. In addition, hepatic MT in the $MT^{+/+}$ mice did not protect against protein oxidation as measured by a decrease in glutamine synthetase activity. Similar results were observed when mice were treated with γ-irradiation. These results indicate that elevated levels of MT in the liver do not play a protective role *in vivo* against reactive oxygen species generated by ionizing radiation or 2-NP. We also measured the survival of the $MT^{-/-}$ and $MT^{+/+}$ mice following exposure to whole body γ–irradiation (8.5Gy). Mean survival was similar for $MT^{-/-}$ and $MT^{+/+}$ mice; however, zinc pre-treatment significantly increased survival through a MT-independent mechanism.

ABSTRACT #16

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Rachel Dawson**

Department: **Molecular Biology and Immunology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

USING PROTEIN OXIDATION PATTERNS FROM ALZHEIMER'S AND CONTROL CULTURED FIBROBLASTS TO ELUCIDATE SUSCEPTIBLE PROTEIN BIOMARKERS FOR ALZHEIMER'S DISEASE.

Rachel Dawson, Janos Kalman, Christy Malowkowski, Robert W. Gracy, and Craig C. Conrad. *Molecular Aging Unit, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107*

Reactive oxygen species (ROS) are generated by a variety of sources from the environment (e.g., photo-oxidations & emissions) and normal cellular functions (e.g., mitochondrial metabolism & neutrophil activation). ROS include free radicals (e.g., superoxide & hydroxyl radicals), nonradical oxygen species (e.g., hydrogen peroxide & peroxynitrite) and reactive lipids and carbohydrates (e.g., ketoaldehydes, hydroxynonenal). Oxidation of proteins appears to play a causative role in many chronic diseases of aging including cataractogenesis, rheumatoid arthritis, and various neuro-degenerative diseases including Alzheimer's Disease (AD).

Our studies are designed to identify the proteins that are most susceptible to ROS damage and to use these as potential biomarkers for the early diagnosis of diseases such as AD. For example, separation of proteins from cells or tissues on one and two-dimensional gels followed by staining of both total protein and specifically oxidized residues (e.g., carbonyls, and nitrotyrosine) may allow identification of biomarkers for AD. Another goal is to elucidate the mechanism(s) where oxidative modification results in the disease. These studies have shown that (a) fibroblasts from AD individuals are more susceptible to oxidative damage than age matched controls or infant fibroblasts, (b) oxidative protein modification is not random; (c) some of the damage can be prevented by antioxidants. It is hoped that mechanistic insight into oxidative damage may allow intervention or prevention of cellular oxidative damage.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Bhalchandra J. Kudchodkar**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

SERUM PARAOXONASE RETARDS NEURODEGENARATION IN ALZHEIMER'S DISEASE.

Bhalchandra J. Kudchodkar, Janos, Kálmán, Walter, J. McConathy and Andras G. Lacko. Departments of Molecular Biology and Immunology and Internal Medicine. UNTHSC at Fort Worth, Fort Worth, TX. 76107.

Since serum lipoproteins have been implicated in the pathophysiology of Alzheimer's disease (AD), we have examined a number of parameters related to lipid metabolism and oxidation in patients with AD and with vascular dementia (VD). The findings obtained with AD and VD patients were compared with data from age matched control subjects in order to assess their value in differential diagnosis. No statistically significant differences were found in any of the parameters among the three groups. However, when subjects within each group were segregated into two subgroups, based on the levels of plasma high-density lipoprotein cholesterol (HDL): low (HDL-C <40 mg/dl) and high (HDL-C ≥ 40 mg/ dl) HDL groups, a number of striking differences emerged. Accordingly, the AD group with low HDL had significantly higher levels of lipid oxidation products (LOP) and lower levels of paraoxonase (PON) compared to the low HDL control and VD groups, ($p<0.05$). Among the three high HDL groups, serum LOP levels tended to be higher in AD but these differences were not statistically significant. Within the AD group, LOP were significantly higher and PON significantly lower in low HDL group compared to high HDL group. Furthermore within each group, patients with high plasma triglycerides (TG≥150 mg/dl) had higher levels of LOP in serum compared to the low TG group. Serum LOP levels correlated positively with plasma triglycerides ($r= 0.47$; $p<0.04$), plasma free cholesterol esterification rate ($r = 0.58$; $p< 0.005$) and negatively with HDL-C ($r = - 0.44$; $p<0.05$). Interestingly, while in the whole group, age correlated positively with LOP ($r= 0.47$; $p<0.04$) the same relationship was positive in hypertriglyceridemic patients ($r= 0.80$; $p<0.02$) and negative in normotriglyceridemic patients ($r = - 0.67$; $p<0.02$). Mini-Mental Status Exam scores correlated negatively with LOP ($r = - 0.41$; $p< 0.07$) and positively with serum PON activity ($r=0.57$;$p<0. 007$). These data suggest that (1) serum LOP may be involved in the pathophysiology of AD. (2) Hypertriglyceridemia exacerbates serum lipid oxidation (3) age per se may not increase oxidative stress and (4) The secretion and/or regulation of serum paraoxonase an antioxidant enzyme associated with HDL is abnormal in AD patients.

Since mini-mental status exam scores correlate with neurodegeneration, We suggest that serum paraoxonase plays an important role in retarding the neurodegeneration in patients with Alzheimer's disease.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Shelley McDonald**

Department: **Pharmacology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

COENZYME Q_{10} AND α-TOCOPHEROL ADMINISTRATION REVERSE AGE-RELATED LEARNING AND MEMORY IMPAIRMENTS IN MICE. Shelley McDonald, Brian Koller, and Michael J. Forster. Department of Pharmacology and Geriatrics Education and Research Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107

The most recognized physiological function of coenzyme Q (CoQ) is the transfer of electrons in the mitochondrial respiratory chan from complex I and II to complex III, and transfer of protons across the inner mitochondrial membrane. However, CoQ has also been postulated to act as a potent antioxidant and, most recently, has been shown to play a role in regeneration of α-tocopherol, a chain-breaking antioxidant, within the mitochondria (Lass & Sohal, 1998, *Arch. Biochem Biophys.* **352**-229). The objective of the current studies was to determine the potential functional significance of these antioxidant effects, *in vivo*. Separate groups of aged mice (22-24 months) received daily treatment (p.o.) with either CoQ_{10} (123 mg/kg/day), α-tocopherol acetate (200 mg/kg/day), combined CoQ_{10} and α-tocopherol treatment, or the vehicle (soybean oil). Two weeks following initiation of the treatments, the mice began receiving a battery of behavioral tests for assessment of learning, recent memory, and sensorimotor function. Previous studies had shown that performance of the aged mice was deficient, relative to performance of younger mice, when they were administered this testing battery. The combined treatment with α-tocopherol and CoQ_{10}, as well as treatment with CoQ alone, resulted in more rapid learning of a problem requiring recent memory capacity, when compared with the untreated controls or mice treated with α-tocopherol alone. The treatments were without out affect on the age-related impairments of sensorimotor performance. Measurement of α-tocopherol and CoQ in brain tissue, 13 weeks following initiation of the treatments, suggested that combined treatment was most effective in elevating levels of α-tocopherol in mitochondrial and synaptosomal brain fractions. Overall, the findings suggest that treatment with CoQ, alone or in combination with α-tocopherol, is effective in reversing brain dysfunctions responsible for age-related deficits in learning and memory performance. (Supported by grant AG07695 from the national institute on aging.)

ABSTRACT #19

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

AN ETS BINDING SITE IS CRUCIAL FOR TRANSCRIPTION OF THE HUMAN PRESENILIN-1 GENE Martine Pastorcic and Hriday K. Das, UNT Health Science Center at Fort Worth, Fort Worth, TX 76107

The presennilin-1 (PS1) protein is required for normal mammalian embryogenesis and particularly CNS development. This indicates the crucial importance of the mechanisms controlling the expression of the PS1 gene. Furthermore, structural mutations in the protein have been identified as the major cause of a most aggressive form of early onset familial Alzheimer's disease (FAD). In addition, PS1 appears to have an antiapoptotic role, and the downregulation of the gene is thought to contribute to the pathogenesis of FAD. However, the transcriptional control of the human PS1 gene has not been analyzed. In this paper, we have identified a promoter fragment (-22/+178) which promotes efficient transcription in human neuroblastoma SK-N-SH cells as well as hepatoma HepG2 cells. By a combination of transient transfection and DNase I footprinting we have identified a series of positive and negative promoter elements including ETS and Sp1 binding sites that are active in both cell lines. In particular, we have shown that a 16 base pair (-22/-6) fragment is required for over 90% of the expression of the gene. Using DNase I competition footprinting assays we have shown that this region includes a binding site for an ETS transcription factor. Furthermore, a mutation altering only two nucleotides of the ETS consensus recognition sequence reduces transcription of the gene by over 90%. This indicates further the crucial importance of this ETS target site. This ETS element overlaps with a putative binding site for the p53 protein. p53 has been shown to downregulate the expression of the PS1 gene. In our system we have shown that cotransfecting a vector expressing p53 together with a PS1 promoter construct reduces drastically transcription activity of the PS1 promoter. The present data should provide a tool to understand this control mechanism. The identification of the mechanisms controlling the expression of the PS1 gene should relate directly to further understanding of development and differentiation pathways, and of the pathogenesis of early onset familial Alzheimer's disease (FAD).

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **John M. Talent**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

TWO DIMENSIONAL FINGERPRINTS WITH POLYVINYLDIFLUORIDE: MEMBRANE DERIVATIZATION AND VISUALIZATION OF OXIDIZED PROTEINS. John. M. Talent, M.Sc.; Craig C. Conrad, Ph.D.; Christina A. Malakowsky, B.S; 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, the oxidation of proteins has been shown to be a causative factor in loss of cognitive abilities in Alzheimer's Disease. Identification of the specific proteins of the brain that are most susceptible to these modifications is key to understanding the disease mechanism and its therapy. Two dimensional protein fingerprint methods offer the analytical potential for resolution of thousands of individual proteins from tissues. 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 previously 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 of proteins pre-electrophoretically derivatized with dinitrophenyl hydrazine. We have now advanced the technique to include patterns from proteins derivatized directly on PVDF membranes after elecrophoresis/blotting. This aids identification of proteins by permitting comparision of sample electrophoretic profiles versus the large number of non-derivatized protein electrophoretic profiles already published in literature or on the Internet. The method has been applied to human sera, human cells grown in culture and tissue extracts human brain.

CARDIOVASCULAR

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RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Peter B. Raven, Ph.D.**

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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, cardioprotection 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.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Michael B. Clearfield, D.O.**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

LABILITY OF PLASMA LDL-CHOLESTEROL LEVELS IN SUBGROUP OF TEXAS CORONARY ATHEROSCLEROSIS PREVENTION STUDY (TEXCAPS) COHORT M.B. Clearfield, S.E. Weis, and W.J. McConathy, Department of Internal Medicine, UNTHSC, Fort Worth, Texas, 76133

In the enrollment phase of the Texas Coronary Atherosclerosis Prevention Study (TexCAPS), a number of participants in this heart-disease free population did not qualify because their LDL-cholesterol (LDL-C) levels fluctuated. Of 27,702 individuals (men 45-73 years, and women 55-73 years) tested at initial lipid screen; 4,257 (15.4%) qualified based on set lipid parameters (total cholesterol 180-264 mg/dL; LDL-C 130-190 mg/dL; HDL-C $\leq$ 45 mg/dL for men and $\leq$ 47mg/dL for women: and triglycerides $\leq$ 400 mg/dL). After 8 weeks on the AHA step 1 diet, lipid parameters were repeated, then at 10 weeks another lipid profile and a complete history/physical were performed. A number of individuals (n= 1389) were excluded for a variety of reasons. Of these, 1,070 (25.1% of those who qualified based on lipids) were excluded because of unacceptable lipid levels on the repeat evaluation at 10 weeks. This subpopulation was divided into 3 groups based on changes of LDL-C between 8 and 10 weeks on the step 1 AHA diet. One group had less than 15% variance in LDL-C (LN, n= 637, 15% of qualified cohort). The second group had a > 15% increase in LDL-C (LI, n=177, 4.2% of cohort), and the third had a > 15% decrease in LDL-C (LD, n=256, 6.0% of cohort). At week 8, TC and LDL-C were lower and HDL-C was higher in the LN group compared with both groups having labile lipids (LI and LD). Changes by gender showed similar trends, however, HDL-C was 5 mg/ml lower at 8 weeks in both female labile lipid groups (LI,LD) when compared to the female LN group ($p< 0.01$). The frequency of TG > 150 mg/dL was greater in males having labile LDL-C when compared to the control group with a similar trend for females. In assessing the incidence of coronary heart disease (CHD) in the nuclear family, parents of probands with labile LDL-C (LI and LD) had a higher frequency ($p= 0.0044$) of premature CHD than parents of probands with stable LDL-C (LN). The following conclusions can be drawn: 1) within the general population there is a substantial number (10%) of individuals with labile LDL-C levels; 2) labile LDL-C in the probands was found to be associated with an increased familial frequency of premature CHD in their parents. Definition of the molecular basis for this lability of LDL-C could reveal new opportunities to regulate plasma cholesterol levels and thus impact CHD morbidity and mortality in a substantial portion of the general population.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Karen R. Murray**

Department: **Molecular Biology and Immunology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

LIPOPROTEIN SUBSTRATE BINDING DOMAIN OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE Karen R. Murray*, Maya P. Nair*, P. Haydn Pritchard# and Andras G. Lacko*$.
Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Ft. Worth Texas* and Department of Pathology, University of British Columbia, Vancouver, CANADA#.

Lecithin:cholesterol acyltransferase (LCAT) catalyzes 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. LCAT was probed by three enzyme linked immunoassay models, utilizing three different antibodies to further characterize this putative binding domain. Two polyclonal antibodies, one against human plasma LCAT and the other against purified recombinant LCAT, and one site specific antibody, directed against 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. However, 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 solid phase immunoassay. These studies suggest that the 121-136 region of LCAT indeed represents a region with a high affinity for hydrophobic surfaces that could function as a lipoprotein substrate binding domain. Recombinant forms of three naturally occurring mutations of LCAT which occur within this functional domain have been prepared in order to further investigate the potential contribution of this region to substrate binding. A research strategy to study these mutants is presented.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Marcy Fitz-Randolph, D. O.**

Department: **Osteopathic Manipulative Medicine**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

A RANDOMIZED CONTROLLED STUDY TO COMPARE THE EFFICACY AND SAFETY OF OSTEOPATHIC MANIPULATIVE TREATMENT (OMT) VERSUS SHAM-TREATMENT IN PATIENTS UNDERGIONG CORONARY ARTERY BYPASS GRAFT (CABG) SURGERY BY MIDLINE STERNOTOMY APPROACH.

Marcy Fitz-Randolph, D.O., Jerry L. Dickey, D.O., Martin S. Weiss, D.O., Albert H. O-Yurvati, D.O., William Wallace, D.O.; UNT Health Science Center Departments of Osteopathic Manipulative Medicine, Internal Medicine, and Surgery, Fort Worth, TX 76107.

The primary purpose of this pilot study is to determine if osteopathic manipulative therapy (OMT) has risks or measurable benefits in patients having undergone median sternotomy for coronary artery bypass graft (CABG) surgery. This pilot study aims to look at objective outcomes in patients receiving OMT after CABG surgery in order to refine and justify a larger study. Approximately 30 (thirty) patients are being recruited for this pilot study. Patients will not be eligible if any of the following apply: no primary wound closure, ventricular-assist device (VADS) post-surgery, treated for infection in hospital in the 14 days prior to surgery, or peri-operative stroke. The patient is randomized to treatment or sham-treatment group upon arrival in the Coronary Care Unit after surgery. The patient then receives one (1) treatment daily, either OMT or sham-treatment, beginning 6 - 24 hours after arrival in the CCU. The OMT protocol used for this pilot study is broken into two sections: CCU (immediate post-operative) and Telemetry (recovery). These are based on the protocol by Jerry Dickey, D. O., with the modifications from the published form developed by Dr. Dickey and Marcy Fitz-Randolph, D.O. Objective outcomes for this study are generated from the patient chart and include: length of stay (CCU and hospital), length of time on mechanical ventilation, supplemental oxygen requirements, occurrence and duration of arrhythmias (atrial and ventricular), dosage and frequency of antiarrythmic medications, dosage and frequency of pain medication (oral and intravenous), amount of chest tube drainage, diet advancement, ambulatory distances, as well as occurrence of complications related to wounds (infection, dehiscence), lungs (atelectasis, pneumonia), peripheral vasculature (pain, edema; from graft sites), and devices (chest tube, IV line or pacemaker wire disruptions).

This pilot study was approved by the Institutional Review Board in the fall of 1998, and data collection begun February 1999. The study is ongoing at the time of this abstract.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **L. Don Roberts**

Department: **Cardiovascular Research Institute**

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X	Graduate Student	Medical Student	Postdoctoral Fellow
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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

AN INDUCIBLE CaMK-II EXPRESSION VECTOR SILENCES THE UP-REGULATION OF HYPERTROPHIC SENSITIVE GENES IN RAT CARDIOMYOCYTES

L. Don Roberts and Stephen R. Grant, PhD Laboratory of Cardiac and Vascular Molecular Genetics, CRI, University of North Texas Health Science Center at Ft. Worth, Texas 76147

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 the adult cardiomyocyte reverts to an embryonic genetic program during hypertrophy, and continuous up-regulation of cardiac hypertrophy-sensitive genes results in progression toward heart failure. Recent studies have documented a functional role for calcium signaling in initiating the hypertrophic response. Findings from our laboratory suggest that calcium/calmodulin-dependent kinases and phosphatase are principle regulators in the regulation of transcription during hypertrophy. Our laboratory has witnessed both CaM kinase-IV (CaMK-IV) and calcineurin (CaN), a phosphatase, both effectively up-regulate hypertrophic-sensitive genes to a maximum of 6-12 fold. In contrast, the presence of over-expressed CaM kinase-II (CaMK-II) silences this up-regulation. The purpose of this study is to utilize a newly constructed tetracycline inducible CaMK-II expression system that will allow inducible expression and modulation of CamK-II in the presence of induced hypertrophic responses by over-expression of CaN and/or CaMK-IV. The capacity therein allows for determining the relative potency of CaMK-II in its capacity to silence hypertrophic response against either CaN, CaMK-IV or a wide variety of chemical hypertrophic agonist. In the rat cardiomyocyte, hypertrophic response has been measured by the relative activity of the promoters driving classical cardiac hypertrophic sensitive genes (e.g. atrial natriuretic factor (ANF), cardiac-α-actin, skeletal-α-actin). Silencing of hypertrophic gene expression is accomplished by a second molecule, tTA (tet trans-activator) and is essential for activation of the minimal CMV promoter that drives the expression of CaMK-II, yet in the presence of doxycycline tTA undergoes a conformational change that impedes its binding affinity to the modified CMV promoter (tet consensus sites) and effectively stops expression of exogenous CaMK-II. Overall, the study, which in currently ongoing, will be able to elucidate mechanisms of cystolic regulation and action of CaMK-II and it's apparent dominant role in silencing the activity of hypertrophic sensitive genes.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Hong Zeng**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CALCIUM/CALMODULIN-DEPENDENT ENZYMES REGULATE CARDIAC HYPERTROPHY-SENSITIVE GENE EXPRESSION IN RAT NEONATE CARDIOMYOCYTES Hong Zeng, Thomas Valencia, Tom Taylor, Rebecca Sycks, and Stephen R. Grant. CRI, Laboratory of Cardiac and Vascular Molecular Genetics, University of North Texas Health Science Center at Fort Worth, TX 76107

A cultured neonate cardiomyocyte cell model was used to examine functional roles for calcium/calmodulin-dependent enzymes in regulating the expression of three cardiac hypertrophy-sensitive genes; atrial natriuretic factor (ANF), skeletal α-actin (SkA), and cardiac α-actin. Exogenous expression of a constitutively-active CaM kinase IV (CaMKIV) activated each of the three promoter-reporters up to a maximum of 12-fold. Furthermore, a constitutively-active form of the Ca^{2+}/calmodulin-dependent phosphatase, calcineurin (CaN), induced each of the three promoter-reporters up to 6-fold. Transactivation or silencing of these three genes was both dose and time dependent. Data presented here also suggest cross-regulation between CaM kinase II (CaMKII) and the other two enzymes. Co-expression of CaMKIV and CaN showed synergistic induction of the promoter activities for ANF, SkA, and cardiac α-actin. The induction of these three genes by either CaMKIV or CaN was reduced to a basal activity by co-expression of CaMKII. A series of Ca^{2+}/calmodulin-dependent enzyme inhibitors were employed to test the differential enzyme effects on the expression of the three hypertrophic genes. Each of the two immunosuppressant drugs, cyclosporin A (CysA) and FK506, inhibitors of CaN, completely blocked gene induction by CaN. CaMKIV-induced gene activation was reversed by KN-62 or KN-93, which selectively inhibit CaMKIV by binding directly to the calmodulin binding site of the enzyme. K-252a, a potent inhibitor of CaMKII, partially retrieved the gene activities inhibited by CaMKII. These data are the first to support evidence for regulation of hypertrophic gene expression through a Ca^{2+}-dependent kinases/phosphatase nuclear signaling cascade which may involve a transcriptional repression/derepression mechanism.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Tom Taylor**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

HYPERTROPHY-SENSITIVE ENHANCERS INDUCED OR SILENCED BY CONSTITUTIVE HETEROLOGOUS CA^{2+}/CALMODULIN-DEPENDENT ENZYMES IN RAT NEONATE CARDIOMYOCYTES Tom Taylor, Hong Zeng, Tom Valencia, and Stephen R. Grant, UNTHSC, Fort Worth, TX 76107

Myocyte Enhancer Factor-2 (MEF-2) and Nuclear Factor of Activated T Cells (NF-AT) are transcription factors and key activators of cardiac and skeletal muscle genes. In this study, we employed a primary neonate rat cardiomyocyte cell culture as our model. We used two enhancer-reporter constructs, one was engineered with multiple MEF-2 binding domains linked with a luciferase gene that reported the activity of endogenous MEF-2. The other construct contained NF-AT binding domains linked with a luciferase gene that reported the activity of endogenous NF-AT. Through exogenous expression, it was shown that both the calcium/calmodulin dependent enzymes, constitutively active CaMK IV and constitutively active calcineurin (CaN), upregulated MEF-2 and NF-AT respectively. Constitutively active CaMK II was shown to silence both the NF-AT and MEF-2 enhancer reporter activity. KN 93 and KN 62, two kinase inhibitors repressed the induction of MEF-2 by constitutively active CaMK IV. Cyclosporin A (Cys A) and FK 506, two immunosuppressive drugs specific for CaN, inhibited CaN-induced activation of NF-AT. The drug K252a, a serine-threonine kinase inhibitor, showed synergistic induction on both MEF-2 and NF-AT activity when used in combination of either constitutively active CaMK IV or CaN. This data provides evidence that enhancer elements which are located in most of the promoter regions and are responsive to hypertrophic signals of hypertrophy-sensitive genes can be regulated by calcium/calmodulin dependent enzymes.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Yun Bai, M.S.**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

REGULATION OF HYPERTROPHY-SENSITIVE GENE TRANSCRIPTION BY AN INDUCIBLE CALCINEURIN EXPRESSION SYSTEM. Y. Bai, H. Zeng, S.R. Grant, Laboratory of Cardiac Vascular Molecular Genetics, Cardiovascular Research Institute UNTHSC@ Ft.Worth, TX 76107

Calcium/calmodulin-dependent protein phosphatase, calcineurin (CaN) has recently been found to play a critical role in activating expression of hypertrophy-sensitive genes upon the initiation of cardiac hypertrophy. In order to quantitatively characterize the roles of CaN, we have established an inducible CaN expression system in which the expression of constitutively active form of CaN is under the control of a tetracycline-regulated transactivator (tTA). By using a reporter gene as a tTA target, we confirm that expression of the target gene can be dramatically induced by tTA in primary cardiomyocytes. Through transfecting cultured cardiac myocytes with tTA expression constructs, tTA-dependent constitutively active CaN expression plasmids as well as hypertrophic response gene promoter-reporter constructs, we indicated that transcription of hypertrophy-sensitive genes, including cardiac α-actin, ANF, and skeletal α-actin, was up-regulated by the induced expression of active CaN in cardiomyocytes. Transcription activation of these three hypertrophic response genes is dose and time-dependent with respect to active CaN plasmid DNA. Furthermore, CaN induction of hypertrophy-sensitive gene transcription can be significantly blocked by the specific CaN inhibitor, cyclosporin A (CsA). More importantly, expression of hypertrophy-sensitive genes mediated by active CaN can be regulated reversibly by removing CsA. In addition, expression of tTA-responsive target gene can also be inhibited by effector doxycycline, but doxycycline inhibition is not complete. These results suggested that transcription of hypertrophy-sensitive genes can be regulated conveniently by this inducible system. Therefore, the experimental manipulation of temporal and quantitative expression of CaN would be useful for further elucidating precise molecular mechanisms for CaN-dependent signal transduction during the onset of cardiac hypertrophy.

(This study was supported by Texas Advance Technology Program Grant.
ARP/ATP Project #: 003660-078b)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Don Selby**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ROLE OF TRANSCRIPTION FACTOR YY1 IN REGULATING GENES OF CARDIAC HYPERTROPHY Don Selby, Hong Zeng, and Stephen Grant, Ph.D. Laboratory of Cardiac and Vascular Molecular Genetics, Cardiac Research Institute, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107

Chronic stimulation of cardiac hypertrophy can lead to heart failure in humans. Models exist for upregulation of gene transcription, very little work has been done on pathways which downregulates gene transcription. YY1 as been shown to regulate transcription of many genes, both up and down. The current research that is being conducted is testing the effects of YY1 upon cardiac hypertrophy. Testing the effects of YY1 on the promoters of cardiac alpha actin, skeletal alpha actin, atrial natriuretic factor. All three of these promoters have shown repression in basal expression in the presence of YY1. In cardiomyocytes it appears that YY1's ability to repress transcription of hypertrophic genes is independent of a YY1 DNA binding motif. Data has shown that YY1 is capable of repressing the transcription of hypertrophic genes well below baseline promoter activity.

The ability to repress transcription in the absence of the YY1 DNA binding motif suggests that YY1 is capable of affecting transcription through a mechanism other than direct DNA interaction.

Nucleosomes exist as repeating units of organization of chromatin fibers in chromosomes, consisting of approximately 200 base pairs, and two molecules each of the histones H2A, H2B, H3, and H4. Most of this DNA, about 140 base-pairs wind around a core formed by the histones, the remainder of the base pairs join to the adjacent nucleosomes, forming a structure which resembles beads on a string.

The cell must unravel the DNA from this nucleosome complex in order to transcribe the DNA, this is done by the action of adding an acyl group to the histones which results in the unwinding of the nucleosome and allows transcription of the DNA.

Our research focuses on investigating the possibility that YY1 plays a role in function of upregulating cardiac hypertrophic gene transcription by modulating the nucleosome structure. These findings may assist in understanding how to turn down the transcription of cardiac hypertrophic genes and thus treat diseases which involve cardiac hypertrophy, such as heart disease.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **M. Isabel Tejero-Taldo**

Department: **Integrative Physiology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ANTIOXIDANT PROPERTIES OF PYRUVATE MEDIATE ITS POTENTIATION OF ß-ADRENERGIC INOTROPISM IN STUNNED MYOCARDIUM. M. Isabel Tejero-Taldo, Jie Sun, James L. Caffrey & Robert T. Mallet. Department of Integrative Physiology, Univ. North Texas Health Science Center, Fort Worth, TX 76107-2699

We tested whether pyruvate potentiation of ß-adrenergic stimulation in stunned myocardium is mediated by antioxidant mechanisms that restore adenylate cyclase activity. Isolated, ischemically stunned, working guinea pig hearts were treated at 15-30 min reperfusion with 2 nM isoproterenol alone (ISO) or combined with 5 mM pyruvate (PISO) or the antioxidant *N*-acetylcysteine (NISO). Cardiac power (mJ · g wet^{-1} · min^{-1}), cyclic AMP (cAMP: nmol/g dry), cytosolic energetics ([PCr]/[Cr][P_I], M^{-1}: ~ PCr) and reduced/oxidized glutathione ratio (GSH/GSSG) in these hearts were compared with values in time control (TC) and untreated stunned (STN) hearts (**Table**: means ± SEM, n = 8-15; *: p<0.05 *vs.* TC; †: p<0.05 *vs.* STN; ‡: p<0.05 *vs.* ISO).

	Power	cAMP	~PCr	GSH/GSSG
TC	114±7	2.0±0.1	263±23	49±7
STN	11±1*	2.0±0.2	244±40	16±3*
ISO	50±10*†	1.9±0.1	168±17*	29±2*†
PISO	160±19*†‡	2.6±0.2*†‡	217±17	45±3†‡
NISO	133±24†‡	4.0±0.3*†‡	98±12*†‡	66±5*†‡

Pyruvate and *N*-acetylcysteine similarly potentiated ß-adrenergic inotropism. Pyruvate moderately elevated cAMP, maintained cytosolic energy reserves and restored the intracellular antioxidant GSH/GSSG ratio. *N*-acetylcysteine also elevated cAMP and GSH/GSSG, but did not maintain energy reserves. **Conclusion**: Pyruvate potentiates ß-adrenergic inotropism in stunned myocardium at least in part by increasing GSH/GSSG, thus restoring adenylate cyclase activity. (NIH support: HL50441).

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Michael B. Williams**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECTS OF RECOVERY DRINKS AFTER PROLONGED GLYCOGEN-DEPLETION EXERCISE M.B. Williams, P.B. Raven and J.L. Ivy. Cardiovascular Research Institute, University of North Texas Health Science Center at Fort Worth, TX 76107, Department of Kinesiology and Health Education, University of Texas at Austin, TX 78712.

Purpose: Eight high-fit (bicycle VO_{2max} = 62.4 ± 1.10 ml•kg^{-1}•min^{-1}) male cyclists, aged 28.4 ± 1.65 yrs., performed a two-hour endurance bicycle exercise to achieve depletion of skeletal muscle and liver glycogen. During recovery, Endurox R^4 Recovery Drink, or Gatorade©, was ingested to investigate their relative restorative capacities to enable further exercise. **Methods**: Each subject performed two days of testing: one for each drink presented in random order. On each testing day, the twelve-hour fasted subject performed a two-hour cycling exercise bout at 75% VO_{2max} followed by one to three 5-minute sprints at 85% VO_{2max}. At the end of this exercise blood glucose concentrations were 3.98 ± 0.138 mmol/L. A four hour recovery period ensued in which the subject was given 24-ounces of the recovery drink. A performance test at 85% VO_{2max} to exhaustion was then conducted. Ventilatory responses were collected breath-to-breath, while venous blood samples were measured for oxidation products, glucose and insulin concentrations. **Results**: The recovery phase showed significant increases in both plasma glucose and insulin following Endurox R^4 Recovery Drink ingestion as compared to Gatorade©. There was a significant increase in time to exhaustion (+55.5%) following Endurox R^4 Recovery Drink during the performance ride compared to Gatorade©. Final oxidation products following Endurox R^4 Recovery Drink ingestion were significantly decreased as compared to Gatorade© ingestion, in that Thiobarbituric Acid Reactive Substrates (T-BARS) were significantly decreased. **Conclusions**: These data indicate that recovery from glycogen-depleting exercise was significantly enhanced by Endurox R^4 Recovery Drink when compared to Gatorade©. In addition, Endurox R^4 Recovery Drink decreased the formation of final oxidation products, when compared to Gatorade©. * [Sponsored by PacificHealth Laboratories, Inc., Woodbridge, NJ]

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

SIMULATION OF EXERCISE IN IN VITRO CULTURES. J. Storm Shirley, S.R. Grant, and P.B. Raven. Cardiovascular Research Institute & Dept. of Biomedical Sciences, The University of North Texas Health Science Center at Fort Worth. Fort Worth, Texas 76107

We have developed a first of its kind system capable of simulating the chronic exercise stimulus and subsequent downstream effects on gene expression in individual cells. By exploiting the cells innate ability to respond to an electrical stimulus, we have devised a unique electrode and tissue culture plate cover that permits electrical stimulation of media and cells in culture; thereby, initiating contraction of cardiac and smooth muscle myocytes. These plate covers allow us to control amplitude, train duration, voltage, delay and cycles per second (Hz). This system enables us to configure the electrical stimulus to mimic relevant physiologically relevant conditions. It is our belief that myocytes or other vascular cells are therefore exposed to an environment mirroring that seen *in vivo*. Reporter/Promoter and Western Blot experiments in Rat Pulmonary Artery Cells (PAC-1) demonstrate that the system is capable of inducing gene expression of hypertrophy and when applicable, hyperplasia sensitive genes.

(Supported in part by NIH Grant # HL45547 and ARP/ATP Project # 003660-078b)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

AEROBIC EXERCISE TRAINING AUGMENTS CREATINE KINASE CATALYTIC CAPACITY IN CANINE MYOCARDIUM

Steven R. Stuewe, Patricia A. Gwirtz, and Robert T. Mallet. Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, TX 76107.

The aim was to determine whether catalytic capacity of the myocardial creatine kinase (CK) energy shuttle is increased in response to aerobic exercise training. Mongrel dogs were conditioned by a 9 wk treadmill running regimen. Sedentary control dogs were cage-rested for 4 wk. Stop-frozen biopsies of left ventricular myocardium were obtained from sodium pentobarbital-anesthetized trained and sedentary dogs. CK in these biopsies was extracted in a phosphate buffer and assayed at 37°C. Exercise training increased total CK activity 46%, from 34.1 ± 5.91 to 50.8 ± 3.86 U · mg protein^{-1} ($P<0.05$). The CK_{MB} isoform was chromatographically isolated and measured by agarose gel electrophoresis. CK_{MB} activity determined from densitometry of the gel was increased 5-fold by training, from 0.35 ± 0.12 to 1.76 ± 0.43 U · mg protein^{-1} ($P<0.05$). CK_{MB} as a percentage of total CK activity was increased 3-fold, from 1.12 ± 0.44 to 3.37 ± 0.77 ($P<0.05$) by training. These training-evoked enhancements of the CK system could serve to increase energy supply to the contractile machinery and membrane ion pump during increased myocardial energy expenditure. (Support: NIH HL50441, HL59405; ACSM Foundation Research Grant.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ROLE OF CHEMICAL SENSITIVE MUSCLE RECEPTORS (CSMR) IN THE REGULATION OF BLOOD PRESSURE AND VENTILATION DURING DYNAMIC EXERCISE. P.J. Fadel, K.M. Gallagher, S.A. Smith, R.G. Querry, R.M.Welch-O'Connor, A.H. Olivencia-Yurvati and P.B. Raven. Department of Integrative Physiology, UNT Health Science Center, Fort Worth, Texas 76107.

The purpose of the present study was to examine the role of chemical-sensitive muscle receptors (CSMR) in the blood pressure and ventilation response to dynamic exercise. Five subjects performed incremental cycling exercise (40 Watts every 2 minutes) to exhaustion under two conditions: control and thigh cuff occlusion of +90 mmHg (Cuffs). The thigh cuff occlusion was used to impede venous outflow and cause the accumulation of metabolites in the legs to activate the CSMR. A femoral venous catheter was passed retrograde for blood sampling below the occlusion cuff. Heart rate (HR), oxygen uptake (VO_2), ratings of perceived exertion (RPE), ventilation volume (V_E), mean arterial pressure (MAP) and femoral venous blood lactate [La^-] were measured throughout each exercise session. No significant differences were found between control and Cuffs for HR, O_2, RPE and MAP. At workloads greater than 120 Watts [La^-] in the legs and $_E$ increased in response to Cuffs. Failure of this enhanced muscle [La^-] to elicit significant increases in MAP suggests that CSMRs do not play a major role in the regulation of blood pressure during dynamic exercise. However, the increase V_E in concurrence with the increase in muscle [La^-] suggests that the CSMRs were a potent stimulus of ventilation.

(Sponsored in part by NIH grant #HL45547)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECTS OF PARTIAL NEUROMUSCULAR BLOCKADE ON CAROTID BAROREFLEX FUNCTION DURING STATIC EXERCISE IN HUMANS

R.G. Querry, K.M. Gallagher, S.A. Smith, M. Strømstad, K. Ide, N.H. Secher, B. Saltin and P.B Raven. Cardiovascular Research Institute-UNTHSC, Ft. Worth, TX, 76107; Copenhagen Muscle Research Center, Rigshospitalet, Copenhagen, Denmark

We hypothesize that an increased effort of exercise (central command) induced by a partial neuromuscular blockade will augment cardiovascular responses and carotid-cardiac baroreflex function during low intensity steady state static exercise. Five subjects (26±2 yrs) performed static leg exercise at 20% maximal voluntary contraction for three minutes. Each subject performed the exercise bout under two conditions: control and partial neuromuscular blockade using Norcuron (Curare). Heart rate (HR; beats/min) and mean arterial pressure (MAP; mmHg) were measured throughout. Carotid baroreflex function (CBR) was recorded using a pulsed neck pressure / neck suction technique. Cardiac responses were used to model CBR function curves. Threshold (TH), saturation (SAT), gain, response range (RR) and minimum response (HRmin) were defined. Increased central command with curare significantly elevated HR (97.2±10) and RPE (16.2±2.4) from control HR (83.6±10) and RPE (13±2.6) during static exercise ($p<0.05$). There was a trend for an increase in MAP with curare ($p<0.09$)

Condition	RR (bpm)	HR min (bpm)	Gain (bpm/mmHg)	TH (mmHg)	Sat (mmHg)
Control	25.1 ± 4.7	67.7 ± 8.2	-0.74 ± 0.27	96.1 ± 8.1	139.2 ± 5.2
Curare	23.8 ± 5.9	76.1 ± 9.6	-0.64 ± 0.20	93.8 ± 10.5	142.7 ± 7.1

*Indicates significance from CON at $P<0.05$. Mean ± SE.

The results indicate that the feed-forward efferent neural input from central command augments cardiovascular responses and actively regulates the carotid baroreflex during low intensity static exercise.

(Supported by Copenhagen Muscle Research Center and Cardiovascular Research Institute.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CAROTID-CARDIAC BAROREFLEX RESPONSE TO STATIC EXERCISE DURING EPIDURAL ANESTHESIA IN MAN. S.A. Smith, R.G. Querry, M. Stromstad, K. Ide, K.M. Gallagher, N.H. Secher, and P.B. Raven. CRI-UNTHSC, Fort Worth, TX 76107; CMRC, Rigshospitalet, Copenhagen, Denmark.

We evaluated the influence of skeletal muscle sensory input on carotid baroreflex (CBR) function and blood pressure (MAP) regulation during 3 min of static exercise. Six healthy male subjects (24.0±0.4 yrs) performed one-legged knee extensor exercise at 20% of maximal voluntary contraction both without (CON) and with epidural anesthesia (EA) introduced at the level of L2-L3 (0.25% Marcain). CBR function was assessed using a pulsed neck pressure/neck suction technique. After 3 min of static exercise, MAP was significantly reduced during EA, 98.9±6.7 mmHg, compared to CON, 120.3±8.8 mmHg ($p<0.05$, mean±SE). The heart rate (HR) response was not different between the two exercise conditions (85.7±3.8 vs. 82.6±3.5 bpm, respectively). Utilizing the Kent Logistical Model, cardiac responses to carotid sinus stimulation were used to develop carotid-cardiac baroreflex function curves. The threshold and saturation pressures of the reflex were significantly reduced during EA exercise (85.0±7.6 and 125.7±5.7 mmHg, respectively) compared to CON exercise (113.2±11.8 and 152.3±11.3 mmHg, respectively) without a significant change in the gain, response range, or minimum HR response. The results indicate that reflex neural mechanisms within working skeletal muscle actively regulate CBR and vasomotor function during low intensity static exercise. Further, attenuation of this control with EA appears to reset the CBR function curve to operate at lower arterial pressures without affecting the HR response.

(Supported by the Copenhagen Muscle Research Center, Denmark, and the Cardiovascular Research Institute, UNTHSC, Fort Worth, TX.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Wendy Wasmund**

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INTERACTIVE EFFECTS OF MENTAL AND PHYSICAL STRESS ON CARDIOVASCULAR CONTROL. W.L. Wasmund, E.C. Westerholm, D.E. Watenpaugh, S.L. Wasmund, M.L. Smith University of North Texas Health Science Center, Fort Worth, Texas 76107.

Mental tasks and exercise often occur together. Physiological responses to each of these have been studied independently, yet interactive effects of these stressors are unknown. We hypothesized that combined mental and physical stress produce a synergistic interaction. We studied cardiovascular responses to 5 minutes of static left handgrip alone (25-35% of maximal grip strength), mental arithmetic alone, and combined stimuli in random order. Sympathetic nerve activity (SNA, micro-neurography), mean arterial blood pressure (MAP, Finapres), and heart rate (HR, ECG) were measured. All 3 stressors significantly increased MAP and HR. SNA and MAP responses to handgrip and the combined stimuli each exceeded responses to mental arithmetic alone, yet no significant difference existed between responses to handgrip alone and the combined stimuli. The 3 stimuli increased HR similarly.

PEAK CHANGE	HANDGRIP	MATH	COMBINED
SNA (bursts/min)	12 ± 3*	2 ± 1†	10 ± 3*
MAP (mm Hg)	26 ± 4*	8 ± 2*†	23 ± 3*
HR (beats/min)	15 ± 2*	10 ± 2*	13 ± 2*

SNA N = 8; MAP and HR N = 12. No significant difference existed between trial baselines ($P > 0.5$). *$P < 0.03$ vs. baseline; †$P < 0.03$ vs. grip and combined.

The data refuted our hypothesis: mental stimulation did not synergistically interact with or even add to the response elicited by handgrip exercise. In fact, a minor trend towards the opposite effect occurred: responses were slightly less when math accompanied the handgrip exercise vs. when handgrip was performed alone. (The National Aeronautics and Space Administration supported this research.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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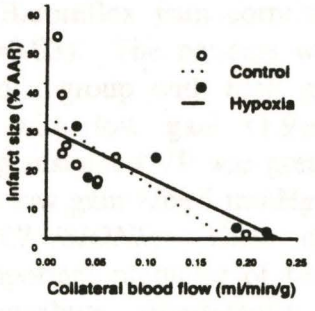
Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ONE SESSION OF WHOLE BODY HYPOXIA DOES NOT PROTECT MYOCARDIUM FROM SUBSEQUENT ISCHEMIA

Srinath Setty, Xiaoming Bian, Bradley J. Hart, Arthur G. Williams, Jr., and H. Fred Downey. Dept. Integ. Physiol., UNT Hlth. Sci. Ctr., Ft. Worth, TX 76107.

Perfusion with hypoxic blood protects myocardium against subsequent ischemia (*Cardiovas. Res.* 26: 534-542, 1992). The present investigation was conducted to determine if acute whole body hypoxia would have cardioprotective action. Following pentobarbital anesthesia, five free-breathing dogs were ventilated with hypoxic gas to reduced arterial $\%O_2Hb$ to 65-80%. The protocol consisted of five 10-min exposures to hypoxia; each followed by 5 min normoxia. After the last hypoxic cycle, the chest was quickly opened, and the LAD was occluded. Following 1 hr ischemia, the LAD was reperfused 4 hrs. At mid-ischemia, microspheres were injected to assess collateral flow. At completion of reperfusion, the heart was excised, dyed to delineate the risk area, and stained with tetrazolium chloride to identify infarcted myocardium. Infarct size is expressed as % of area at risk and examined as a function of collateral flow. Results are compared with control data from untreated animals. Results demonstrate no cardioprotective Benefit from this regimen of whole body hypoxia in anesthetized dogs (supported by NIH Grant HL-35027).



RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Stephen L Wasmund**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ARTERIAL BAROREFLEX GAIN IS AN IMPORTANT PREDICTOR OF ARTERIAL PRESSURE RESPONSES DURING SIMULATED VENTRICULAR TACHYCARDIA.

SL Wasmund, ML Smith, [†]RL Page, [†]JA Joglar, *MH Hamdan. Department of Integrative Physiology, University of North Texas Health Science Center, Ft Worth, TX 76107, [†]Division of Cardiology, UT Southwestern Medical Center, Dallas, TX, *VA Medical Center Dallas, TX.

Ventricular pacing (VP) used to simulate ventricular tachycardia causes an abrupt initial fall in mean arterial pressure (MAP) which is followed by some degree of recovery of MAP (increase from initial nadir) as VP is sustained. We have shown that the arterial baroreflex mediates a significant increase in sympathetic nerve activity (SNA) during VP in response to this hypotension (*Circ*, 93:1033, 1996; *Circ*, 96:I-633, 1997). The purpose of this study was to determine the relation of arterial baroreflex-SNA gain to the recovery of MAP. *METHODS*: We measured SNA, MAP and central venous pressure during VP at a cycle length of 400 ms in 14 patients referred for electrophysiologic evaluation. Arterial baroreflex gain was determined from the SNA/MAP during infusions of nitroprusside. The MAP recovery during sustained VP was correlated to baroreflex gain. *RESULTS*: Baroreflex gain correlated significantly with MAP recovery (r=0.48, P=0.03). The patients were then clustered based on baroreflex gain into a group with high gain (2.9±0.6 units/mmHg, n=8) and a group with low gain (1.9±0.4 units/mmHg, n=6). The MAP recovery during sustained VP was greater in the patients with high gain than those with low gain (26±5 mmHg vs 15±7 mmHg respectively, $p<0.05$). *CONCLUSIONS*: These data suggest that arterial baroreflex gain is an important predictor of MAP recovery during VP and thus, may contribute significantly to hemodynamic stability during tachyarrhythmias. Therefore, baroreflex function likely plays a role in patient's tolerance of tachyarrhythmias.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Nicolette K. Muenter**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

PERIODIC INSPIRATORY EFFORTS DURING APNEA ATTENUATE APNEA-INDUCED SYMPATHOEXCITATION. Nicolette K. Muenter, Donald E. Watenpaugh, Stephen L. Wasmund, Wendy L. Wasmund, and Michael L. Smith. University of North Texas Health Science Center, Fort Worth, TX 76107.

Obstructive sleep apnea increases sympathetic nerve activity (SNA) and may contribute to hypertension. Periodic inspiratory efforts occur during obstructive apneas. We previously demonstrated the surprising result that inspiratory efforts during apnea attenuate arterial oxygen desaturation. Therefore, we hypothesized that inspiratory efforts also reduce apnea-induced sympathoexcitation. *Methods:* Six healthy subjects held their breath at end-expiration (functional residual capacity) without and with concomitant periodic inspiratory efforts. The efforts of 1-2 s each reduced airway pressure 40 mm Hg every 3-4 s during apnea. Subjects breathed 12% O_2 plus 3% CO_2 for 1 min prior to each type of breath holding. Microneurography of the peroneal nerve yielded muscle SNA, which we quantified as a % of baseline levels. We quantified SNA over the same time period of apnea for each condition (apnea duration = 22.4 ± 2.9 s, mean ± SE). *Results:* SNA increased to 1346 ± 278 % of baseline levels during apnea with no inspiratory efforts ($P = 0.003$). When periodic inspiratory efforts accompanied apnea, SNA increased to 772 ± 210 % of baseline levels ($P = 0.012$). Therefore, inspiratory efforts during apnea reduced the sympathoexcitation associated with apnea by almost one half ($P = 0.005$). *Conclusions:* In conjunction with our previous oxygen desaturation results, the present findings suggest that inspiratory efforts may reduce sympathoexcitation by facilitating mobilization of pulmonary and circulatory oxygen stores during apnea. Alternatively, inspiratory efforts may more directly inhibit SNA via respiratory motor inputs to central sympathomotor centers. The results imply that greater sympathoexcitation may occur during central sleep apneas, which do not involve periodic inspiratory efforts, than during obstructive apneas.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DELTA OPIATE RECEPTOR ANTAGONIST BLOCKS THE VAGOLYTIC EFFECT OF MEAP IN THE SA NODE. Keith Jackson, Amber Stanfill, Martin Farias, and James L. Caffrey. Department of Integrative Physiology, University of North Texas Health Science Center Fort Worth, Texas 76107.

Met-enkephalin-arg-phe(MEAP) is an endogenous opiate derived from the C-terminal sequence of proenkephalin. This heptapeptide is abundant in the myocardium and has significant vagolytic activity when infused systemically. The peptide similarly interrupted vagal bradycardia when it was delivered directly into the SA node by local microdialysis. This study was conducted to determine the effective dose of nodal MEAP and if the resulting vagolytic effect could be blocked with the delta opiate antagonist, naltrindole. Microdialysis probes were placed in the SA node of mongrel dogs and perfused at 5 microliters per minute. Various doses of MEAP were included in the nodal perfusate and inhibition of vagal bradycardia was observed at 0.3nanomoles/microliter with the half-maximal response near 0.1nmoles/microliter. The maximal MEAP effect was reversed by the paired infusion of the delta opiate receptor antagonists, naltrindole. These data suggest that the vagolytic effect of MEAP may involve the activation of prejunctional delta opiate receptors within the SA node.(Supported by NIDA & Local Funds)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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RENOVASCULAR HYPERTENSION REDUCES BOTH ENDOTHELIAL-DEPENDENT AND INDEPENDENT CORONARY VASODILATION IN CONSCIOUS DOGS.

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

Patients with hypertension are known to have impaired coronary vasodilation; however, the mechanisms responsible for this are not fully understood. We examined the hypothesis that renovascular hypertension (RVH) reduces vasodilator responsivity by an endothelial dependent mechanism in conscious dogs. Six dogs were chronically instrumented to measure heart rate (HR), mean aortic pressure (MAP), and circumflex blood flow (CBF). Resting normotensive values were: MAP 91±2 (SEM), HR 83±8 bpm, and CBF 66±6 ml/min. After normotensive studies were concluded, RVH was induced by reducing left renal artery blood flow by 60%. This resulted in a rise in MAP to 113±5 mmHg ($p< .01$). After hypertension, resting HR and CBF were 65±8 bmp and 58±8 ml/min, respectively. Endothelial NO production was blocked with an intracoronary (i.c.) infusion of 65-75 mg nitro-L-arginine (L-NA). Responses to acetylcholine (ACh), adenosine (ADO) sodium nitroprusside (SNP)i.c, were examined before and after RVH. ACh and SNP were also given after LNA in the normotensive state. The following data are expressed as change in CBF (ml/min) from baseline.

Drug	Control	Control + LNA	RVH
ACh - 0.1 µg	72±4	47±10†	51±5*
- 0.5 µg	107±7	82±12*	74±11†
- 1.0 µg	122±14	99±7*	96±16*
SNP - 10 µg	79±16	107±18	50±9*
- 20 µg	100±17	128±19	69±17*
ADO - 50 µg	150±16		137±24*
- 70 µg	176±16		130±19*

*$p<0.05$ vs. Control; †$p<0.01$ vs. Control; n=6. CONCLUSIONS: L-NA reduced the responses the endothelial-dependent dilator ACh, in the normotensive state. After RVH, responses to ACh, SNP, and ADO were reduced at all doses. These studies indicate that RVH reduces both endothelial-dependent (ACh) vasodilation and endothelial-independent (SNP) vasodilation. RVH may reduce endothelial-dependent NO production and/or decrease vascular smooth muscle responsiveness to NO.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ATROPINE REDUCES THE TRANSFER FUNCTION MAGNITUDE OF SYSTOLIC BLOOD PRESSURE TO PULSE INTERVAL.

K. Formes, W. Wray, R. Welch-O'Connor, A. O-Yurvati, P. Reese, M. Weiss, and X. Shi,. Department of Integrative Physiology and the Cardiovascular Research Institute, UNT Health Science Center at Fort Worth, TX, 76107.

To investigate the contribution of vagal activity to blood pressure regulation, we analyzed the power spectral density of systolic blood pressure (SBP) and pulse interval (PI) variability and their transfer function (Welch method) before (B) and after (A) atropine administration in seven healthy young (24±1 yr. age) subjects. Atropine did not significantly change intra-radial arterial SBP (B vs A: 130±4 vs 133±4 mmHg), though it decreased PI from ($P<0.05$) both PI and SBP. However, ΔPI was greater in B than in A (-89±23 vs –39±11 ms, $P=0.078$), whereas ΔSBP tended to be less (-4.9±1.0 vs –10.1±2.5 mmHg, $P=0.084$). Table below summarizes the transfer function extracted from low frequency (LF, 0.04-0.12 Hz) and high frequency (HF, 0.20-0.28 Hz) spectra transformed from 6-min steady-state data. Both changes in LF and HF magnitude were significantly affected by the factors of drug and LBNP.

LBNP		Magnitude		Coherence		SBP		PI	
torr		LF	HF	LF	HF	LF	HF	LF	HF
B	0	7±0.7	3±0.7	0.59±0.07	0.52±0.07	4±1	1±0.1	379±99	19±8
	-40	5±0.6	2±0.4	0.57±0.04	0.55±0.05	4±1	1±0.3	218±41	10±7
A	0	1±0.2	0.3±0.1	0.58±0.04	0.25±0.05	2±1	1±0.2	10±4	0.2±0.1
	-40	1±0.2	0.2±0.1	0.72±0.06	0.29±0.04	11±5	1±0.4	13+3	0.2±0.1

We concluded that a diminished vagal activity with atropine significantly reduced baroreflex mediated transfer function gain. Subsequently, the function of blood pressure regulation is compromised as indicated by a greater drop is SBP associated with an augmented SBP variability in LF spectrum.

(Supported by UNTHSC Faculty Research Grant, NIH AG14219 and HL45547)

EYE RESEARCH

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RESEARCH APPRECIATION DAY 1999

ABSTRACT

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

Department: **North Texas Eye Research Institute**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

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 appoints to the NTERI. These faculty are located in three basic science departments (Anatomy and Cell Biology, Pharmacology, and Molecular Biology and Immunology) at the UNT Health Science Center, at 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 ehaling, aging, cell death, retina transplantation, glaucoma and diabetic complications. Broad technical areas of expertise are found through 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.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Kristi D. Henderson**

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THE EFFECTS OF SUBSTANCE P ON CORNEAL EPITHELIAL CELLS IN VITRO. Kristi D. Henderson, Dr. D. Dimitrijevich. University of North Texas Health Science Center, Fort Worth, TX 76107

The cornea, which is a major part of the anterior ocular surface of the eye, comes into contact with topical pharmaceuticals and consumer products intentionally or accidentally. Such exposures are sometimes irritating.

Little is known about the earliest events in the corneal inflammatory cascade in order to block irritation. It is therefore important to understand ocular irritation and the factors that initiate wound healing in the cornea. Of particular interest is the study of the cascade events resulting from the secretion of substance P (SP) and its effects on the human cornea.

Fluorescent microscopy was used to determine the presence of the neurokinin-1 (NK-1) SP receptor on human corneal epithelial cells (CEPI). The second messenger system of this receptor was determined using video imaging. SP effects on CEPI attachment and proliferation was determined using monolayer cultures in 6-well plates.

These experiments have shown that the NK-1 receptor is present on CEPI and calcium is the second messenger. Internalization of the receptor was shown with increasing temperatures. SP also increased CEPI attachment and proliferation.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Jami R. Kern**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MECHANISMS OF SCAR FORMATION USING NORMAL AND TELOMERASE TRANSFECTED FIBROBLASTS IN A THREE-DIMENSIONAL MATRIX Jami R. Kern and S.D. Dimitrijevich, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107

One of the final stages of wound healing is tissue contraction, which occurs in order to close the wound. Excessive wound contraction can result in severe scarring. This scarring, which may also involve overproduction of collagen, can interfere with normal tissue function. The major cellular component responsible for contraction and collagen synthesis is the activated fibroblast (myofibroblast). This myofibroblast phenotype develops and utilizes smooth muscle cell –like cytoskeletal apparatus to contract the wound through its interaction with the extracellular matrix. Significant increase in the expression of α-smooth muscle actin has been proposed to be characteristic of this phenotype. However, we have found that upregulation of myosin light chain may be an even more sensitive marker. Because there are numerous initiators of the activation of the fibroblast to myofibroblast phenotype, it has been difficult to rationalize points of intervention to prevent scar formation. Also, it is not known what the fate of the myofibroblast is once tissue contraction has closed the wound. The activated fibroblasts may return to their quiescent state within the tissue, or they may be eliminated via apoptosis. Furthermore, the question still remains, which cell is responsible for the prolonged matrix remodeling which occurs following scar formation. There is thus a need for a stable cellular model to examine these intricacies of the wound healing process. To address these questions, we are characterizing a telomerase transfected fibroblast cell line that is in an upregulated proliferative state *in vitro*. It has not been established how these cells respond to a three-dimensional tissue environment. It is possible that the transfected cells are insensitive or supersensitive to these conditions. We have shown arrested proliferation in a three-dimensional environment (Dermal Equivalent) and an altered cytoskeletal protein profile for these cells in a monolayer culture. We have also utilized tranfection of green fluorescent protein (GFP) as a marker to look at remodeling and the post contraction phase of wound healing by confocal scanning microscopy. Results from these and related studies will be useful in understanding the molecular mechanism of the scaring process, fibrotic diseases and their future therapies.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Rajnee Agarwal**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECTS OF DEXAMETHASONE (DEX) ON GROWTH FACTOR AND NEUROTROPHIN mRNA EXPRESSION BY CULTURED HUMAN TRABECULAR MESHWORK CELLS. R. Agarwal[1], W. Lambert[1], N. Agarwal[1], J. Seiler[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, Fort Worth, TX.[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX.[2]

Purpose: Glucocorticoids (GC) have been implicated in the pathogenesis of primary open angle glaucoma (POAG). Ocular hypertension following GC administration is due to increased resistance to aqueous humor (AH) outflow, which is associated with biochemical and morphological alterations in the trabecular meshwork (TM). Growth factors presented to TM cells via AH (paracrine) or produced locally by TM cells (autocrine) may be involved in maintaining the normal structure and/or function of the TM (Wordinger *et al.*, 1998; IOVS, 39: 1575-1589). However, in other cells it is known that gene expression of growth factors and neurotrophins can be altered by GC. The purpose of this study was to determine if dexamethasone (DEX) treatment of cultured human TM cells alters mRNA expression of growth factors and neurotrophins. **Methods**: Early passaged, previously characterized normal human TM cells from donors of 2 and 80 years were grown until confluent in Ham's F-10 media supplemented with 10% FBS. TM cells were treated with or without DEX (10^{-7}M) for 14 days. mRNA expression was examined by total cellular RNA isolation and a RiboQuant RNase Protection Assay (PharMingen, San Diego, CA.) for growth factors TGFβ-1, TGFβ-2, and TGFβ-3 and neurotrophins NGF, BDNF, NT-3, NT-4, CNTF, and GDNF. **Results**: DEX *decreased* mRNA expression of growth factors TGFβ-1, and TGFβ-2 and neurotrophin GDNF. mRNA expression for TGFβ-3 and other neurotrophins did not appear to be altered. **Conclusions**: To our knowledge, this is the first report that DEX *decreases* mRNA expression of growth factors and neurotrophins by human TM cells. Decreased growth factor and/or neurotrophin expression by TM cells may effect the microenvironment within this tissue and influence AH outflow. **CR**: E; **Support**: National Glaucoma Program of the American Health Assistance Foundation (Rockville, MD.) and Alcon Laboratories, Inc.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Patrick Cammarata, Ph.D.**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

A TRANSGENIC ANIMAL MODEL OF OSMOTIC CATARACT. *Patrick R. Cammarata,[1] Cheng Zhou,[1] Guoli Chen,[1] Inderpal Singh,[1] Rustin E. Reeves,[1] Jerome R. Kuszak,[2] and Michael L. Robinson[3] From the [1]Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth/North Texas Eye Research Institute, Fort Worth, Texas, the [2]Departments of Pathology and Ophthalmology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Il, and the [3]Children's Hospital Research Foundation, Columbus, Ohio.*

Purpose: Intracellular osmotic stress has been linked with the advancement of diabetic cataract. While the accumulation of organic osmolytes (myo-inositol, sorbitol, taurine) is held to protect the lens by maintaining osmotic homeostasis, the physiologic implication of osmotic imbalance on diabetic cataract formation is obscure. Studies from this laboratory have identified several osmotic compensatory mechanisms, which presumably afford the lens epithelium, but not the lens fibers, protection during periods of osmotic crisis. This presumption is founded on the supposition that the fibers of the lens are susceptible to damage by osmotic insult. To test this hypothesis, several mouse lines were developed which over express the bovine sodium/myo-inositol cotransporter (bSMIT) gene in lens fibers. ***Methods and Results***: *In situ* hybridization confirmed that the CPV14/bSMIT transgene was expressed at high levels in the differentiating fiber cells of the developing lens of the mouse line, MLR21, while expression was substantially lower in the family, MLR14. Embryonic day 15.5 MLR21 tg+ mice disclosed a marked swelling in the differentiating fibers of the bow region and subcapsular fibers of the central zone; the lens epithelium appearing morphologically normal. A coupled reverse transcription/polymerase chain reaction from total RNA isolated from individual MLR21 mouse lenses showed high CPV14/bSMIT expression in tg+ lenses relative to tg+ lenses of MLR14. Intralenticular myo-inositol from MLR21 tg+ mice was markedly higher compared with tg- littermates or MLR14 tg+ mice. The MLR21 tg+ mice develop severe bilateral nuclear cataract with normal rearing and diet of the birth mother. Transgene-positive pups of MLR14, unlike MLR21, display no lens opacity. Progression of nuclear cataract likely initiates during embryonic development but is most readily observed in neonates. ***Conclusions***: Lens fiber swelling and related cataractous outgrowth positively correlated to the degree of lens bSMIT gene expression and intralenticular myo-inositol content. The lens fibers appear to be unable to counteract the affect of accumulated myo-inositol.
National Health Public Service Award EY05570-12 (PRC)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Marcia Ong**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DIFFERENTIATION OF BOVINE LENS EPITHELIAL CELLS INTO LENS FIBER CELLS BY SERUM-STARVATION IN VITRO CULTURES Marcia D. Ong, Margaret H.Garner Department of Anatomy and Cell Biology, Graduate School of Biomedical Sciences, University of North Texas Health Science Center at Fort Worth, Texas 76107

Purpose. Previous studies have successfully shown differentiation of lens epithelial cells into lentoid bodies in vitro cultures. MIP26 is a marker for lens fiber cells. One study demonstrated expression of MIP26 in lentoid bodies (Arita, *Investigative Ophth. And Vis. Science,*31(11) 2395-2404, 1990), and another study showed that lentoid bodies failed to express MIP26 (Kidd, *Differentiation* 56:67-74, 1994). The goal of this project was to present a novel method of differentiation of a mammalian cell culture in which lens epithelial cells terminally differentiate into lens fiber cells. **Methods.** Primary cultures were grown from lens epithelium explants obtained from bovine lenses. The primary cultures were grown in Minimum Essential Medium containing 10 % calf serum for 1.5 weeks, then subsequently grown in either 5 %, 4%, 3%, or 1% calf serum for 1-2 weeks undisturbed. Cell morphology was observed under a light microscope, and indirect immunofluorescence for MIP26 (main intrinsic protein) was performed on desired cells. Alpha- actinin and normal rabbit serum were used as positive and negative controls, respectively. **Results.** Cells grown in 4%, 3%, and 1% calf serum elongated after 2 weeks, forming a net-like morphology devoid of organelles. Immunofluorescence results were positive for MIP26 in the cells grown in 3%, 4%, and 1% calf serum. Cells grown in 5% calf serum did not change morphology and were negative for MIP26. The cells from primary cultures were also negative for MIP26. **Conclusion.** Based on the immunofluorescence results and observations under light microscopy, in vitro cultured lens epithelial cells grown in serum- starved conditions differentiate into fiber cells and express MIP26.

(Supported by NIH grant EY07010)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Jonathan R. Matthews**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

A STUDY OF SODIUM,POTASSIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE IN LENS EPITHELIAL CELLS AND RETINAL PERICYTES USING A NOVEL COMPOUND – TEXAS RED®-OUABAIN. Matthews J.R., Garner M.H., Payne M. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.

In order to develop a procedure that allows for the determination of Na,K-ATPase (sodium potassium dependent adenosine triphosphatase) concentration using fluorescence, a novel compound known as TR-Ouabain (Texas Red®-Ouabain) was synthesized in this laboratory. Ouabain is a cardiac glycoside that specifically binds to the alpha subunit of Na,K-ATPase in a 1:1 complex, inhibiting enzyme activity; Texas Red Cadaverine is a fluorescent probe (mixture of two isomers) containing a reactive diamine linker. Reaction of Texas Red®-Cadaverine (Molecular Probes, Portland,OR) and oxidized ouabain yields two isomeric forms of TR-Ouabain. These products can be isolated using reverse-phase HPLC. TR-Ouabain binds to and inhibits Na,K-ATPase in renal microsome preparations as indicated by a decreased rate of ATP turnover following treatment. This finding is further supported by results obtained using competition binding studies and visualization of binding with fluorescence microscopy. Although procedures for the determination of Na,K-ATPase concentration already exist, these procedures require use of radioactive compounds and are therefore expensive and time-consuming. Measuring concentration by fluorescence is more efficient. When further studies have been completed to show the binding affinity of TR-Ouabain to Na,K-ATPase, this compound will be used in a study of the effects of diabetes mellitus on this enzyme. In addition, this method will increase the possibilities for dual fluorescence labeling studies of this enzyme within cells.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Ganesh Prasanna, Ph.D.**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ENDOTHELIN-1 DECREASES $^{86}RB^+$ UPTAKE IN HUMAN NON-PIGMENTED CILIARY EPITHELIAL CELLS BY INHIBITING NA^+/K^+-ATPASE. Ganesh Prasanna Adnan Dibas, and Thomas Yorio Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose. Endothelins (ETs; potent vasoactive peptides) may regulate intraocular pressure by enhancing aqueous humor outflow and by decreasing aqueous humor production. To determine if decreases in aqueous humor formation are a result of endothelin-1 effects on ion transporter activity, we measured the $^{86}Rb^+$ uptake in human non-pigmented ciliary epithelial (HNPE) cells. **Methods.** The $^{86}Rb^+$ uptake method was used to measure the activity of ouabain-sensitive Na^+/K^+-ATPase and bumetanide-sensitive $Na^+:K^+:Cl^-$ co-transport in the presence of ET-1. **Results.** ET-1 (1, 10, and 100 nM) decreased mean $^{86}Rb^+$ uptake in HNPE cells. ET-1's effects could not be prevented by BQ-610, an ET_A receptor antagonist, but was mimicked by sarafotoxin, an ET_B agonist. Isoproterenol, a β-adrenergic agonist that elevates cAMP concentrations, mimicked ET-1 effects. ET-1-induced reduction in $^{86}Rb^+$ uptake was still present following bumetanide treatment but was absent in the presence of ouabain. This suggests that ET-1-induced reduction in $^{86}Rb^+$ uptake is the result of an inhibition of Na^+/K^+-ATPase via an ET_B-like receptor. **Conclusions.** These findings suggest that ET-1 could affect aqueous humor production in an autocrine manner, by affecting ion-transport activity, specifically that of Na^+/K^+-ATPase and thus affect intraocular pressure.

(Texas Adv. Res. Grant)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Xiaochun Liu**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EXPRESSION OF CILIARY NEUROTROPHIC FACTOR (CNTF), GLIAL DERIVED NEUROTROPHIC FACTOR (GDNF) AND THEIR RECEPTORS BY CELLS OF THE HUMAN OPTIC NERVE HEAD

X. Liu[1], M. Talati[1], W. Lambert[1], R. Agarwal,[1] A. F. Clark[1,2], and R. 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: Ciliary neurotrophic factor (CNTF) and glial derived neurotrophic factor (GDNF) are polypeptide growth factors that provide trophic support and protection for neuronal and glial cells. We have previously demonstrated that astrocytes and lamina cribrosa cells of the human optic nerve head (ONH) express neurotrophins (NGF, BDNF, NT-3, NT-4 {Lambert et al., 1998; IOVS; 39 [4] S260}). The purpose of this study was to determine if, in addition to neurotrophins, neurotrophic factors and their signaling complexes are also expressed by cells of the human ONH. **Methods**: mRNA expression for CNTF, GDNF, and their respective receptor complexes was examined by total cellular RNA isolation, RT-PCR, and agarose gel electrophoresis using 6 well characterized LC cell lines, 2 ONH astrocyte cell lines, and 3 brain astrocyte cell lines. Specific PCR primers were designed using Oligo 4.0. **Results**: Using RT-PCR we detected mRNA's for CNTF and all 3 components of the signaling complex (eg. CNTFRα, LIFR, and gp 130) in LC cells, ONH astrocytes and brain astrocytes. In addition, mRNA for GDNF and GFRα-1 was expressed by all ONH cell types. However, while GFRα-2 was expressed by ONH astrocytes, it was not expressed by brain astrocytes and only variably expressed by LC cells. Ret mRNA was detected in ONH astrocytes and brain astrocytes. **Conclusions**: To our knowledge, this is the first report that CNTF, GDNF, and high affinity receptor complexes are expressed by cells of the human optic nerve head. This raises the possibility that paracrine/autocrine signaling using these neurotrophic factors exists within the human ONH. **CR**: E;
Support: The Glaucoma Foundation (New York, NY) and Alcon Laboratories, Inc.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Raghu Krishnamoorthy**

Department: **Anatomy and Cell Biology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CHARACTERIZATION OF A PUTATIVE CLONAL RETINAL GANGLION CELL LINE FOR A POSSIBLE IN VITRO MODEL OF GLAUCOMA.

Raghu Krishnamoorthy, Ph.D. and Neeraj Agarwal, Ph.D., UNT Health Science Center at Fort Worth, Fort Worth, TX 76107

Purpose. To establish a permanent rat retinal ganglion cell culture by transforming rat retinal cells with ψ2 E1A virus. These cells will be useful to study the mechanisms of retinal ganglion cell death in an in vitro model for glaucoma.

Methods. Rat retinal cells were isolated from post natal day 1 old rat retinas and transformed with ψ2 E1A virus. Single cell clones were picked at random from the transformed retinal cells. The expression of Thy-1, glial cell fibrilary acidic protein (GFAP, a positive marker for Muller Cells), HPC-1/syntaxin (a marker for amacrine cells), 8A1 (a marker for horizontal cells), and various neurotrophins were studied by using reverse-transcription polymerase chain reaction (RT-PCR), immunoblot analysis and immunocytochemistry to characterize prospective retinal ganglion cells. To establish the physiological relevance of RGC-5 cells as a possible in vitro model of glaucoma, we studied the effect of serum deprivation of these cells to test if "blocked axonal transport of neurotrophins" is one of the pathways leading to ganglion cell death in glaucoma. Apoptosis was studied by "terminal deoxynucleotidyl transferase mediated fluorescinated dUTP nick end labeling" (TUNEL) and DNA fragmentation by DNA laddering.

Results. Several clones were tested for Thy-1 and GFAP expression by RT-PCR analysis. One of the clones, RGC-5 was positive for Thy-1 expression and negative for GFAP, suggesting that it could represent a putative RGC clone. Upon further characterization by immunoblot analysis, the RGC-5 clone was positive for Thy-1, negative for GFAP, 8A1, and syntaxin. The results of RT-PCR analysis were further confirmed by immunocytochemistry for Thy-1 and GFAP. SCC 5 was also positive for the expression of various neurotrophins and their receptors. Serum deprivation of these cells resulted in their apoptosis.

Conclusions. Since clone RGC 5 expressed Thy-1, various neurotrophins, and not GFAP, 8A1, and syntaxin, it may represent a rat retinal ganglion like cell line. Since RGC-5 is sensitive to glutamate toxicity, and serum deprivation causes these cells to undergo apoptosis, these cells provide us with a rat retinal ganglion cell line which will be of immense value in performing in vitro manipulations in an experimental model of glaucoma.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Raghu Krishnamoorthy**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EXPRESSION OF RHAMM IN THE RETINA AND RPE. Raghu Krishnamoorthy, Anne-Marie Brun-Zinkernagel and Michael H. Chaitin, Department of Anatomy and Cell Biology and North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX.

Purpose. The goal of this study was to determine if RHAMM (receptor for hyaluronan-mediated motility) is present in the neural retina and RPE. **Methods.** Anti-RHAMM antibodies and Western Blot analysis were used to detect RHAMM protein in the rat retina and RPE. Immunoperoxidase labeling on paraffin embedded tissue sections and immunoelectron microscopy on ultrathin cryosections were done to determine the cellular localization of RHAMM. **Results.** Western blotting demonstrated one isoform of RHAMM at 70 kD and at least three isoforms in the 90-110 kD range. On tissue sections, RHAMM was immunocytochemically localized to the RPE at both the light and electron microscope levels. Label was also detected within the neural retina, in particular the inner plexiform layer. **Conclusion.** Cell surface RHAMM is one of the receptors for extracellular hyaluronan (hyaluronic acid), and the RHAMM detected within the retina may function in this capacity. Of particular interest is the presence of RHAMM in the RPE which provides this cell layer with a potential receptor for the hyaluronan within the interphotoreceptor matrix (IPM). The IPM is situated between the RPE and neural retina. CD44, another hyaluronan receptor, was previously localized to Müller cell apical microvilli which project from the neural retina into the IPM. Thus, CD44 molecules may mediate adhesion of the neural retina to the IPM. Recently, it was shown that both cell surface and intracellular RHAMM isoforms are involved in regulating extracellular signal-regulated kinase (ERK) activity. Thus, RHAMM in the retina and RPE may also function in this pathway. Supported by NSF grant BIR 9413907.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Tammy Hancock Nelson**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE ANTIBODY TO RPE-RETINA TROPHIC FACTOR (RPE-RTF) INHIBITS NEONATAL RAT PHOTORECEPTORS CELL MATURATION. T. Nelson, H. Sheedlo, J. E. Turner. Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center, North Texas Eye Research Institute, Ft. Worth, TX 76107.

Purpose: To demonstrate that RPE-RTF, a member of the cystatin super-family of protease inhibitor-like molecules found in retina, is important for the postnatal maturation of rat photoreceptor cells. **Methods**: Seven-day-old Long Evans rats received intravitreal injections of 1-2 ul of RPE-RTF antibody and retinas were analyzed by light and electron microscopy 7 days later. **Results:** Seven days after antibody treatment, there was a significant reduction in the thickness of the outer nuclear layer, as well as a reduction in the outer/inner segment length. Specifically, total outer/inner segment lengths were significantly reduced ($p<0.05$) throughout the entire retina 7 days after RPE-RTF antibody treatment. Similarly, the outer nuclear layer thickness was significantly decreased ($p<0.05$) in the equatorial and central retina. This degenerative process was corroborated by the presence of a two-fold increase in extruded photoreceptor cell nuclei found in the sub-retinal space. In addition, EM analysis revealed that outer segment maturation was significantly retarded in response to antibody treatment. Other areas of the postnatal retina appeared unaffected by RPE-RTF antibody treatment. **Conclusions**: RPE-RTF is important for the postnatal survival and complete maturation of rat photoreceptor cells. The mechanism(s) by which RPE-RTF influences photoreceptor development and survival is not clear; however, it is noted that this protein protected cells from apoptosis under both *in vitro* and *in vivo* conditions.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MÜLLER CELLS PROMOTE PHOTORECEPTOR CELL SURVIVAL IN DYSTROPHIC RETINAS AND IN RETINAL EXPLANTS. R.S. Roque, A.M. Brun-Zinkernagel, H.J. Sheedlo and J.E. Turner. Department of Anatomy and Cell Biology and North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107.

The wide distribution and extensive cell contacts of Müller cells with retinal neurons suggest a variety of functions including secretion of trophic factors that maintain the survival of photoreceptor cells in the normal retina. We have hypothesized that during the early stages of degeneration in the RCS retina, these trophic factors may not be available in concentrations sufficient to maintain photoreceptor cell survival. The effects of Müller cells were, therefore, tested on photoreceptor cells in the early dystrophic retina as well as in retinal explants. Fourteen 2-week old RCS rats were injected intravitreally using a dorsal approach with 100,000 Müller cells in 5 µl PBS or PBS alone and sacrificed after 8-10 weeks. The number of layers of photoreceptor cells in the outer nuclear layer (ONL) of paraffin-embedded retinas was then counted to determine the effects of Müller cell injections. Conditioned medium was also collected from Müller cell cultures (MCCM) in basal medium for 48 hrs. and tested for activity on retinal explants obtained from PN2 (postnatal day 2) normal rats. Retinal explants were observed for 2 weeks and compared with control explants grown in basal medium alone. Photoreceptor cell survival in the explants was determined using fluorescent probes and colorimetric assays. The ONL in RCS retinas that received PBS alone was 0-1 cell in thickness except at the injection site and in the inferior half of the retina where 1-2 layers of photoreceptor cells were detected. In RCS rats injected with cells, Müller cells had proliferated and occupied the vitreal cavity. The ONL was 2-3 cells thick in the superior half of the retina, however, the inferior half had a 6-8 cell thick ONL. MCCM-treated retinal explants survived longer and exhibited longer neurites as compared with those in basal medium. Our study shows that Müller cells may promote the survival of injured photoreceptors in the dystrophic rat retina. Furthermore, the growth promoting activity of Müller cells appears to be due to secreted factors.

Supported by NIH EY10766.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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

Department: **Anatomy and Cell Biology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

RPE-RETINA TROPHIC FACTOR (RPE-RTF) PROTECTS PHOTORECEPTOR CELLS FROM APOPTOTIC CELL DEATH. J. E. Turner, H. Sheedlo, T. Nelson, R. Krishnamoorthy, V. Doan, N. Agarwal, Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center, North Texas Eye Research Institute, Ft. Worth, TX 76107.

Purpose: To demonstrate that RPE-RTF, a novel cysteine protease inhibitor found in the retina, protects photoreceptor cells from apoptosis. **Methods**: A 661W transformed photoreceptor cell line was grown to sub-confluence. Cultures were exposed to 100-200 ng/ml of RPE-RTF for 16 hours, then exposed to light (4.5 milliwatts/cm ^{2} for two hours, which is sufficient to cause apoptotic cell death in unprotected cells. Control cells were exposed to light for 2 hours without RPE-RTF pre-treatment. Apoptotic cells were identified by a TUNEL technique. The presence of caspase-1 and -3 activity was determined by immunocytochemical localization. For *in vivo* analysis, intravitreal injections of RPE-RTF were administered at 7 days after birth. Ectopic photoreceptor cells in the inner nuclear layer were localized by opsin immunocytochemistry 7 days later, a time when most of these cells have undergone apoptotic cell death. **Results:** RPE-RTF significantly protected transformed retinal cells *in vitro* against light-induced apoptotic cell death. Caspase activity was elevated in light damaged cells but not under RPE-RTF treatment conditions. In addition, intravitreal injections of RPE-RTF significantly delayed apoptosis of ectopic photoreceptor cells located in the inner nuclear layer. **Conclusions**: RPE-RTF, a novel cysteine protease inhibitor found in the retina, is capable of protecting photoreceptor cells from apoptosis under both *in vitro* and *in vivo* conditions. This rescue is, at least partially due to inhibition of caspase activity.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Harold J. Sheedlo, Ph.D.**

Department: **Anatomy and Cell Biology**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

A NOVEL PROTEASE INHIBITOR (RPE-RTF) IS TEMPORALLY REGULATED IN THE RETINA. H. Sheedlo, T. Nelson, J. E. Turner. Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center, North Texas Eye Research Institute, Ft. Worth, TX 76107.

Purpose: To demonstrate the presence of RPE-RTF, a novel cysteine protease inhibitor, found in eye tissues and its temporal regulation in the retina. **Methods**: RPE and retina tissues were harvested from Long Evans rats at embryonic day 16-18 (E16-18), postnatal day 2 (PN2), PN5 and PN15. Tissue extracts were prepared for SDS-PAGE and Western blot analysis. In addition, RPE, retina, cornea, lens, ciliary body and optic nerves were processed for the immunocytochemical localization of RPE-RTF at the above mentioned time periods. **Results:** RPE-RTF was found in the corneal and ciliary epithelia, as well as in the retinal ganglion cells, optic nerve and photoreceptor cell inner segments of adult tissues. The greatest number of immunolabeled cells was noted in the neuroblast layer of early neonatal retinas. Western blot analysis confirmed that RPE-RTF was found at highest levels during early neonatal periods, particularly postnatal day 5. Lower levels of RPE-RTF were apparent in embryonic and young to older adult retinas. RT-PCR analysis confirmed a message for RPE-RTF only during this neonatal time period. **Conclusions**: RPE-RTF, a novel protease inhibitor found in the retina, appeared to be temporally regulated to where highest levels of protein and message were found in the neuroblast layer during early postnatal development. RPE-RTF was also found in the RPE, optic nerve and the epithelia of the cornea and ciliary body of adult retinas. A function for RPE-RTF has not yet been established; however, the cellular effects of intravitreal injections of RPE-RTF antibody in early postnatal retinas suggest a role in photoreceptor cell survival and maturation.

COMMUNITY AND PUBLIC HEALTH STUDIES

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POSTDOCTORAL SCHOLARS ASSOCIATION

69.	Samuel S. Chuang, Ph.D.	THE POSTDOCTORAL SCHOLARS ASSOCIATION

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Robert L. Kaman, J.D., Ph.D.**

Department: **PHPM**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DEVELOPMENT OF A COUNTY GOVERNMENT EMPLOYEE WORKSITE HEALTH PROMOTION PROGRAM. Robert L. Kaman, Claudia Coggin, Janet Helduser, Raven Green,* Bridget Lane,* Samer Nachawati, * Chang Pak,* Christopher Perkins,* Matt Richardson, * David Shaffer, * and Suzzane Whizin.* UNTHSC-FW.

Health promotion programs at the worksite have been shown to reduce health care costs, absenteeism, job-related injuries, and turnover, and increase productivity and employee morale. The Tarrant County, Texas workforce has experienced an increase in health care costs and illness-related absenteeism that has severely strained the county budget and the efficiency of the workforce. Actual health care costs have reached over $1 million per month for a workforce of 4200 employees, an expenditure that is 40% higher per capita than that spent by Dallas County for its employees (using the exact same health care provider system). In order to address this problem, a county-sponsored health promotion program was considered. A joint task force of county employees and Health Science Center faculty and students was formed to conduct a workforce assessment and a feasibility study for the introduction of such a program. The task force developed specific goals and objectives, and then implemented an analysis of need, based on a number of assessment instruments, and an inventory of available resources. A targeted program plan, including activities in exercise and fitness, nutrition, stress management, smoking cessation and health education was developed. The plan includes a facilities utilization and planning format, a staffing plan, including utilization of new and existing personnel positions, an opening/marketing plan, and an evaluation plan. A preliminary budget of $94,000 was proposed for the first year of operation, based on a program launch in May, 1999

* Members of the UNTHSC-FW Worksite Health Promotion Class of the MPH Program

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Tiiu Ford, M.S.**

Department: **Public Health and Preventive Medicine**

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	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CHURCHSITE HEALTH PROMOTION: A NEW WAY TO REACH PARTICIPANTS AWAY FROM THE WORKSITE. Tiiu Ford and Robert L. Kaman. UNT Health Science Center at Fort Worth, Fort Worth, TX, 76107.

Worksite health promotion practitioners have been successful in providing programs to their companies' employees, and many have achieved further success in extending their reach to employees' family members as well. Nevertheless, millions of self-employed Americans (and their families), or those who work in smaller companies without health promotion programs, remain out of the reach of practitioners and represent an enormous wellspring of people whose improved health may have dramatic effects on the overall health of the nation. Many of these individuals are members of religious organizations whose goals are to provide for as many aspects of life for their congregants as they can. Therefore, the churchsite may be examined for its potential as a vehicle to provide health promotion programs to its congregants, especially for those individuals not able to join programs at a worksite. A thorough needs assessment, interest survey of both management (the pastoral and administrative staff) and members, and an in-house and community resource survey was conducted in a moderate sized (450 members) church in a suburban location. Results of the assessments and surveys suggested that (1). there was a high level of interest within management (the pastors) and membership for provision of health promotion as part of the church program; (2) the pastors felt that members would participate without their encouragement; (3) there were several areas of program delivery that may be uniquely unsuitable for a churchsite (e.g. family planning); (4) there were significant differences among the pastors and the membership regarding favored programs; and (5) most of the members queried were willing to pay full or partial fees for their participation. The resource inventory indicated that there were both in-house and community facilities available for inclusion in the program, and several members of the staff were available to deliver program activities. A program implementation plan, including recommendations for budget support, based on the assessment and survey results was developed, and presented to the church at a general meeting.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Robert L. Kaman, J.D., Ph.D.**

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Place an "X" in the box to the left of your classification.

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DISTANCE LEARNING COURSES TO ENHANCE BRIDGE PARTNERSHIP CURRICULA Robert L. Kaman, J.D., Ph.D., Thomas Yorio, Ph.D., Suzzette Chopin, Ph.D., Diana Marinez, Ph.D. UNTHSC-FW, Fort Worth, TX 76107

As a result of the heavy teaching load at undergraduate institutions, faculty struggle to develop advanced courses in their discipline. As a result, science majors have fewer choices for advanced courses, and consequently, may be less well-prepared for the rigors of advanced graduate education. The development of distance learning resources, both through video-teleconferencing (V-tel) and the Internet, has created an opportunity to address this problem. A pilot program was developed between two Bridge partner institutions to offer an advanced (pharmacology) course taught by faculty at the senior institution to upper level biology majors at the undergraduate institution, principally through V-tel, with Internet support. Twenty four students, including two students at the home institution, took the course for three credit hours. The course, "The Biological Basis of Drug Action," was team-taught by the pharmacology faculty at the senior institution from the on-campus distance learning classroom/studio, into a classroom at the partner institution. The Course Director held scheduled "office hours" to interact with students via the Internet, responded to Email queries, and made one trip to conduct class in person at the partner institution campus. Exam grades and course evaluations suggest that this is an effective strategy for inclusion in a Bridge program to enhance graduate competitiveness.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Elizabeth Fawcett**

Department: **Public Health Program/Dept PHPM**

Place an "X" in the box to the left of your classification.

Graduate Student	Medical Student		Postdoctoral Fellow
Faculty	Staff	X	Alumnus

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

A SUPPORT GROUP DEVOLOPED ON THEORETICAL PRINICIPLES AND DESIGNED TO INCREASE KNOWLEDGE OF OSTEOPROROSIS Elizabeth Fawcett, MPH, CHES, American Cancer Society, Claudia Coggin, MS, CHES, Bernard R. Rubin, DO, University of North Texas Health Science Center, Fort Worth, TX.

Osteoporosis is one of the critical diseases facing the aging population today. Research suggests that social support is vital to the psychosocial well being of the person with this chronic disease. The Osteoporosis Awareness Group (OAG) was created to increase the awareness of osteoporosis risk factors, prevention, and treatment in high-risk individuals through education and support. This program was based on the Social Cognitive Theory and the Health Education Model. Increase in awareness was measured by knowledge pre-test/posttest and self-reported changes in behavior. At the end of the first three months of the OAG there was a twenty-six percent return rate of participants to group meetings. Posttest results indicated a general increase in knowledge about risk factors, prevention, and treatment of osteoporosis. According to the self-reported changes in behavior, there was little impact on the lives of those who participated. However, this pilot indicates a need for support groups to combat the negative psychosocial consequences of osteoporosis, as well as further research into methods of health education for older adults.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Henry R. Lemke, MMS, PA-C**

Department: **Physician Assistant Studies**

Place an "X" in the box to the left of your classification.

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X	Faculty	Staff	

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ESTABLISHING COMMUNITY-BASED PHYSICIAN ASSISTANT CLINICAL TRAINING SITES IN RURAL COMMUNITIES. Henry R. Lemke, MMS, PA-C; Carol F. Stehly, MS, Med., University Of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107

Purpose: This is part of a Department of Health and Human Services, Health Resources & Services Administration funded grant-project designed to identify, develop, implement, and evaluate new Physician Assistant clinical training sites in rural and medically underserved areas in hopes of increasing the number of graduates who will choose to practice in these communities. Methods: Potential clinical sites were screened using lists obtained from various medical & rural health interest associations based in Texas. Eliminating sites not in rural or medically underserved areas, & eliminating those with area codes outside a 200-mile radius of Fort Worth. The list of potential sites was narrowed down to 176. 34 rural clinics expressed interest in becoming a training site, 30 others were identified through word-of-mouth, and/or through contact via an Area Health Education Center. (AHEC). Preceptor credentialing & faculty-appointment processes were initiated after a successful site evaluation. Sites completing the credentialing process receive further analysis by the Curriculum Committee. Summary: Of 176 clinical sites, 64 (36%) expressed an interest in becoming a rural training site half of those from word of mouth. The on-site evaluation & review process eliminated 19 sites. 13 sites self-eliminated. Of the 32 remaining, 5 had a subsequent change in on-site preceptor, 18 are pending site visits, and 4 are pending completion of credentialing; leaving 5 sites that are approved. Conclusions: The most cost-effective means for identifying potential clinical sites in rural areas is through word-of-mouth contact or through AHEC. Previous relationships with potential preceptors strongly influence their desire to participate in the program. AHEC's can also play a crucial role in finding housing for students. Community involvement is essential to a successful training program.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Lynn F. Johnson, MIS**

Department: **Library**

Place an "X" in the box to the left of your classification.

	Graduate Student	Medical Student	Postdoctoral Fellow
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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

OSTEOPATHIC LITERATURE DATABASE PROJECT

Lynn F. Johnson, MIS; Ann Brooks, MLS, MBA, AHIP; Bobby Carter, MS; Craig Elam, MLS, AHIP, Dohn Martin, MSLS, MS; GIBSON D. LEWIS HEALTH CENTER LIBRARY, UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH, Fort Worth, Texas, 76017.

In July, 1997, the American Osteopathic Association (AOA) and the American Association of Colleges of Osteopathic Medicine (AACOM) awarded a five (5) year contract in the amount of $300,000.00 to the Gibson D. Lewis Health Center Library at the University of North Texas Health Science Center at Fort Worth, Texas (UNTHSC) to develop a comprehensive index to the osteopathic medicine literature. The project's goal is to meet the osteopathic profession's need for convenient access to its own research literature in support of regulatory and legislative objectives. This index, the only one devoted to the osteopathic medicine literature, will be in the form of a web-accessible database of bibliographic citations and abstracts. Its scope will include osteopathic principles, practices and philosophy, osteopathic manipulative treatment, the history of osteopathic medicine, and relevant literature from non-osteopathic manual medicine.

The Lewis Library will provide indexing of the current osteopathic literature as well as overall project development and administration, database design and indexing support and training. The database uses the STAR software from Cuadra Associates to provide structured index records, expanded searching capabilities, and abstracts. A key feature of STAR is its capacity to incorporate authority control. One of the major accomplishments of the project is the development of a unique thesaurus of osteopathic terms derived from the literature being indexed. This will include controlled indexing terms derived from the National Library of Medicine *Medical Subject Headings (MeSH)*, the AACOM Educational Council on Osteopathic Principles' *Glossary of Osteopathic Terminology*, and other appropriate headings and keywords.

The project is monitored by AOA and AACOM appointed Editorial and Advisory Boards. As part of the project, the KIRKSVILLE COLLEGE OF OSTEOPATHIC MEDICINE (KCOM) in Missouri is indexing and adding the historical (pre-1950) materials contained in the NATIONAL CENTER FOR OSTEOPATHIC HISTORY at the A.T. STILL MEMORIAL LIBRARY to the database.

(Sponsored by AOA and AACOM.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Michelle Bidaut-Russell, Ph.D.**

Department: **PHPM**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

IMPACT OF INSURANCE STATUS ON THE ATTITUDES AND HEALTH PRACTICES OF HISPANIC PATIENTS TOWARD DIABETES. M. Bidaut-Russell, Ph.D, MPH*; M. Marshal, DO, Dr.PH; G. Whiting, D.O. Dept. of Public Health/Preventive Medicine and Dept. of Family Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107-2699

Objectives of research: to assess the effect of lack of health insurance on the attitudes and health practices toward diabetes of 50 adult Hispanic patients recruited from a local community clinic. Information was culled using Schwab et al.'s questionnaire. Compared with patients with some insurance coverage (N = 21; 42%), patients with no insurance coverage (N = 29; 58%) were more likely to report: (1) having to be very ill before seeing a doctor; (2) that people around them encouraged them to eat the wrong food; (3) that they could eat anything they wanted as long as it was not sweet; (4) that they would take their medicine everyday if they had enough money to buy it; and (5) that they would have to change too many things in their lives in order to take their medicine.

Although none of these findings were statistically significant, lack of insurance coverage had a negative impact on several actions associated with reducing the threat of diabetes and its associated complications.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Sue Gena Lurie, Ph.D.**

Department: **Public Health/Preventive Medicine**

Place an "X" in the box to the left of your classification.

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

FAMILY VIOLENCE PREVENTION PROJECT SURVEY Sue Gena Lurie, Ph.D., Dept. Public Health/Preventive Medicine, UNT Health Science Center-Fort Worth, Tx. 76107 and Elizabeth Adair, R.N., Tarrant County Health Department.

The purpose of the survey was to determine the prevalence of domestic violence in a sample of pregnant women and new mothers in county Women, Infants and Children (WIC) and prenatal clinics, following clinic observations and self-reported abuse, to assess need for preventive intervention. The cross-sectional study used the "Abuse Assessment Screen" to measure prevalence of reported and threatened domestic abuse and use of support services in a sample of WIC and John Peter Smith Hospital prenatal clients in 1998. The instrument was piloted at selected WIC sites, revised to add date, age, race, zip code, and the body map for clients to self-identify injuries was eliminated. The survey was distributed in English, Spanish and Vietnamese by clinic staff to clients who consented, and confidentiality of respondents was protected. A total of 852 clients at 19 sites responded. The overall reported abuse rate was 14.90%. Prevalence was tabulated for each of 55 residential zip codes, race and ethnicity within each, although 28.52% omitted zip code. The findings confirm occurrence of domestic violence in county prenatal and WIC clinic clients and variations in prevalence across residential areas. Responses to threats and types of abuse may be analyzed. Data can be compared with data from the Fort Worth Health Department community neighborhood assessment and County Health Department "Behavioral Risk Factor Survey" in 1998. Findings support need for preventive health education to break the cycle of domestic violence; A Gentle Touch Program, a ten-step curriculum for parents to guide a child's behavior non-violently from birth to age five, is offered by Tarrant County Health Department. Future epidemiological studies using stratified sampling and longitudinal design could monitor sociodemographic variation and trends; community research could correlate family income, education, occupation, and household relationships with domestic violence.

(TITLE V grant to Tarrant County Health Department, Fort Worth, Tx.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Barbara D. Adams, MSA**

Department: **Family Medicine**

Place an "X" in the box to the left of your classification.

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

BORDERS WITHOUT BOUNDARIES: A BORDER HEALTH EXPERIENCE FOR STUDENTS IN THE HEALTH PROFESSIONS.

Barbara D. Adams, MSA[1], Claudia Coggin, MS, CHES[1], Arushi Sinha, [1] Muriel Marshall, DO, MPH, DrPH[1], Robert J. Hastings, MA[2], Carol Stehly, MEd[1], Henri Migala, MPH, MA[1], [1]UNT Health Science Center at Fort Worth, Fort Worth, TX and [2]Texas Tech University, Lubbock, TX.

Creating a foundation for development of a culturally sensitive health care professional requires an introduction to community as an environment and the cultural context of healthcare. Successful practice in a community involves understanding the community's social structure, its economics, culture and resources. Community-based experiences early in their education increase student awareness of the health care concerns and practices of underserved populations.

In 1997 and 1998 first- and second-year medical students and masters of public health students participated in an intensive field experience to learn about health care on the international border of the United States and Mexico.

Activities included presentations and lectures, visits to agencies, businesses and communities that deal with or illustrate border issues, and group discussions. Through these activities students explored the importance of culture and the realities of health care delivery in a multicultural setting.

Quantitative and qualitative evaluation and student reports show an increase in understanding of the complexity of border health issues and an appreciation of culture's impact on healthcare.

This presentation describes the experience and summarizes the results of evaluation conducted before and after the field trip.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Samuel Chuang, Ph.D., President of PSA**

Department: **Molecular Biology and Immunology**

Place an "X" in the box to the left of your classification.

Graduate Student	Medical Student	X	Postdoctoral Fellow
Faculty	Staff		

Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE POSTDOCTORAL SCHOLARS ASSOCIATION. University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107.

Postdoctoral training positions were originally designed to give new Ph.D. graduates 2-3 years of practical experience. The experience gained by the postdocoral researcher in these years would teach them to function independently in either an Academic or Industrial settings. Today, many postdoctoral researchers spend 7, 8, 9 or more years trying to figure out how to work independently from their mentors. Many postdocs do not know what is expected of them in order to write papers, obtain grants, and get permanent position in either Academia or Industry. For this reason, the Postdoctoral Scholars Association (PSA) was founded in 1997. The PSA is dedicated to educating postdocs on how to enhance their postdoctoral experience here at the University of North Texas Health Science Center. We believe the PSA will help postdoc strive to advance their career to the next level. The PSA provides a forum for postdocs to communicate and exchange ideas with each other. The PSA also encourages the university to follow fair and consistent policies for the management of post-graduate education. Although the primary members of PSA are postdoctoral researchers, others, such as faculty members, graduate students, and staff are encouraged to participate.

CANCER

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72.	Samuel S. Chuang, Ph.D.	CELL SPECIFIC TRANSCRIPTIONAL REGULATION OF THE 2B4 RECEPTOR GENE
73.	Myoung Kim, Ph.D.	ELUCIDATION OF MATRIX METALLOPROTEINASES (MMPS) AND TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES (TIMPS) PRODUCED BY RAT AND MOUSE IL-2 ACTIVATED NK (A-NK) CELLS
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79.	Sunitha Kumari	FUNCTIONAL INTERACTIONS OF P53 WITH POLY(ADP-RIBOSE) POLYMERASE (PARP) DURING APOPTOSIS FOLLOWING DNA DAMAGE: COVALENT POLY(ADP-RIBOSYL)ATION OF P53 BY EXOGENOUS PARP AND NON-COVALENT BINDING OF P53 TO THE Mr 85,000 PROTEOLYTIC FRAGMENT
80.	Pamela Lea Marshall	THE INVOLVEMENT OF CASPASES IN TUMOR NECROSIS FACTOR-α MEDIATED CELL DEATH
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RESEARCH APPRECIATION DAY 1999

ABSTRACT

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

Department: **Institute for Cancer Research**

Place an "X" in the box to the left of your classification.

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER INSTITUTE FOR CANCER RESEARCH. Ronald H. Goldfarb, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas 76107.

We have recently established an Institute for Cancer Research at the University of North Texas Health Science Center. 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. 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. (supported by grant from Bank One)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Gheath Al-Atrash**

Department: **Molecular Biology and Immunology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

INVESTIGATION OF RAT NATURAL KILLER CELL UROKINASE-TYPE PLASMINOGEN ACTIVATOR IN: ADHESION, INVASION, MIGRATION, AND CELL MEDIATED CYTOTOXICITY. Gheath Al-Atrash, Richard P. Kitson Ph.D., and Ronald H. Goldfarb Ph.D., Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX, 76107.

Urokinase plasminogen activator (uPA) is a neutral serine protease that has been shown to be involved in extracellular matrix degradation and migration for many cell types, including invasive tumor cells. Urokinase plasminogen activator receptor (uPAR) is a plasma membrane receptor that plays an important role in the localization of uPA to the cell surface and to the invadipodia of migrating cells. It is thought that uPAR also plays an important role as a signaling receptor that contributes to cell migration. IL-2 activated natural killer (A-NK) cells, upon their adoptive transfer, have been shown to accumulate within and control tumors in various *in vivo* models, including models of established tumor metastases. uPA has been found in rat NK cells and we hypothesize that it might contribute to the invasion and migration of NK cells. Fluorogenic assay employing a highly selective substrate for uPA, CBZ-Gly-Gly-Arg-7-Amino-4-Methylcoumarin, has also demonstrated the presence of uPA in rat NK (RNK) cells. The uPA is inhibited by Dansyl-Glu-Gly-Arg-CMK, a highly selective synthetic inhibitor of uPA, but not by TLCK, a selective tryptic inhibitor, or by TPCK, a selective chymotryptic inhibitor. Zymography has also been used to demonstrate the presence of uPA in the NK cell membranes. Inhibition of uPA in *in vitro* invasion assays prevents the invasion of the NK cells through extracellular matrices. To date our studies suggest that the uPA-uPAR system may play a major role in the invasion and migration of NK cells. Our studies will contribute to our understanding of how A-NK cells, including A-NK cells bearing anti-cancer drugs, accumulate within established cancer metastases.

(Supported by ATP grant)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CELL SPECIFIC TRANSCRIPTIONAL REGULATION OF THE 2B4 RECEPTOR GENE. S.S. Chuang, Y. Lee, P.R. Kumaresan and P.A. Mathew. Department of Molecular Biology and Immunology, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107.

Natural killer (NK) cells are bone-marrow-derived lymphocytes that have tumor cells and virally infected cells. The activation of NK cells is mediated positive signals from cell-cell interactions and from responses to cytokines. of NK cell recognition and activation by target cells is poorly understood. W cloned and characterized a receptor, 2B4, expressed on murine NK cells. 2B NK cells, but also on a subset of T cells which have NK-like killing propert indicated that 2B4 belongs to the CD2 subset of immunoglobulin superfami interact with CD48 with nine times more affinity than that of CD2-CD48 in understand the transcriptional regulation as well as the mechansims controll of the 2B4 gene, we obtained a genomic 2B4 clone system including the sec region. To define the start site of transcription, we performed primer extens assays and found that the 2B4 gene may be initiated at multiple start sites ar promoter. FACS analysis revealed 2B4 surface expression on CTLL-2 cells on YAC-1 cells, a mouse lymphoblast line. Transient transfections of neste promoter to drive CAT expression revealed tissue specific expression in CT fragment of 540 bases upstream from the first base of the mouse 2B4 cDNA maximal CAT activity in CTLL-2 cells. The presence of the region –654 to drastically reduced transcription. Sequence analysis of this promoter region recognition motifs for a number of lymphocyte-restricted and ubiquitous tra play a role in the transcriptional regulation of the mouse 2B4 gene. This wo from NIH (AI38938).

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ELUCIDATION OF MATRIX METALLOPROTEINASES (MMPS) AND TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES (TIMPS) PRODUCED BY RAT AND MOUSE IL-2 ACTIVATED NK (A-NK) CELLS

Myoung Kim, Richard P. Kitson, and Ronald H. Goldfarb. Dept. of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX.

We have previously documented that adoptively transferred A-NK cells accumulate within cancer metastases. Electron microscopic studies of pulmonary metastases have shown that adoptively transferred A-NK cells that accumulate within metastases can bind to endothelial cells and are able to traverse basement membranes. We have recently reported (J. Immunol 160:4248-53, 1998) that rat A-NK cells produce MMP-2 and MMP-9. In addition we have also found that rat A-NK cells can migrate through Matrigel, a model basement membrane-like extracellular matrix. This migration was partially inhibited by BB-94, a specific inhibitor of MMPs. Our results suggest that A-NK cells employ BB-94-inhibitable MMPs to degrade extracellular matrices which may play a role in the accumulation of A-NK cells within cancer metastases. We have recently performed RT-PCR with both rat and mouse A-NK cell cDNA. Our findings reveal the existence of mRNA for additional MMPs including membrane-type MMPs and TIMPs (MMP-2, MMP-7, MMP-9, MMP-11, MMP-13, MT-MMP-2, MT-MMP-2, TIMP-1, TIMP-2 in both rats and mice and MMP-10 in rats). The existence of MMP-2 and MMP-9 in mouse A-NK cells has been confirmed by zymography. Western blot analysis also confirmed the presence of membrane-type MMPs and TIMPs. These results further suggest that MMPs contribute to A-NK cell functions, perhaps including their accumulation as well as the accumulation of A-NK cells bearing anti-cancer drugs within cancer metastases following their adoptive transfer.

(ATP Grant)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

RAT NK CELLS EXPRESS TWO DIFFERENT ISOFORMS OF 2B4 RECEPTOR GENE. P.R. Kumaresan, S.E. Stepp*, S.S. Chuang, M. Bennett*, V. Kumar*, 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.

2B4 is a novel member of the immunoglobulin supergene family expressed on the surface of all murine NK cells and a subset of T cells that mediate NK-like killing. Functional studies indicate that ligation of 2B4 transduces an activation signal and is associated with increased target cell lysis. 2B4 is also expressed on dendritic epidermal T cells (DETC) and plays a functional role in killing of skin tumors. Recently we have shown that 2B4 is expressed on human NK cells and modulate NK cell cytolytic function. In both mouse and human, 2B4 interacts with CD48 with a tenfold higher affinity than the CD2-CD48 interaction. 2B4 is a membrane protein in both the mouse and human. Here, we report that in addition to a membrane bound form, rat NK cells express a soluble form of 2B4. The cDNAs corresponding to both transmembrane (p2B4.R15) and soluble (p2B4.R7) forms were isolated and characterized. The two mRNAs seem to be products of differential splicing of hnRNA. The p2B4.R15 clone encodes a protein of 311 amino acids with a characteristic transmembrane domain whereas p2B4.R7 encodes a protein of 205 amino acid residues without a transmembrane domain. This suggests that the protein encoded by p2B4.R7 is either a GPI linked membrane form or a secreted form. We generated polyclonal antibodies against rat 2B4 and using those antibodies, identified a protein in the media supernatant of RNK-16 cells as well as cell lysates on western blot analysis. Thus, rat NK cells express both transmembrane and secreted forms of 2B4.

Research was supported by NIH grant AI38938.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE EFFECT OF 2B4 ACTIVATION INTERACTION ON THE PRODUCTION OF HUMAN INTERFERON GAMMA IN NATURAL KILLER CELLS. L.A. Johnson, S.S. Chuang and P.A. Mathew. Department of Molecular Biology and Immunology, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107

Interferon gamma (IFNγ) is a cytokine shown to stimulate signaling cascades resulting in activation of gene transcription. This activity is important in anti-viral, anti-proliferative, and immunomodulatory processes. For instance, in antigen presenting cells, IFNγ has been shown to upregulate expression of major histocompatibility (MHC) Class II molecules and antagonize viral replication . IFNγ can also assist in the activation of macrophages, promote T cell differentiation, and promote expression of certain immunoglobulin (Ig) isotypes in B cells. The overall role of IFNγ in immune response is critical and it is believed to have potential in treating clinical diseases.

We previously identified a natural killer and T cell surface molecule, 2B4. 2B4 is involved in non-MHC-restricted lysis of virus-infected cells and some tumor cells. The interaction of 2B4 with an-2B4 monoclonal antibody has resulted in increased IFNγ secretion and granule exocytosis. The aim of the current study is to more closely examine the relationship between the observed increase in secreted IFNγ and the 2B4:ligand(s) interaction. Proposed methods would include the use of ELISA assays to measure IFNγ in response to 2B4 binding by various stimuli, including monoclonal antibodies against 2B4 and soluble CD48, 2B4's natural ligand. Transient transfection studies with nested promoter fragments of IFNγ promoter inserted into a CAT reporter vector will reveal promoter regions important to IFNγ transcription, particularly in response to 2B4 ligation. Northern analysis wil also be implemented to measure mRNA levels of IFNγ. Results from this study will allow us to better understand the importance of 2B4 in natural killer cell activation.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

NATURAL KILLER CELL PROTEASOME MAY CONTAIN MORE THAN ONE CHYMOTRYPTIC DOMAIN Min Lu, Richard P. Kitson, Ph.D. and Ronald H. Goldfarb, Ph.D. Department of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX 76107

The proteasome is a key enzyme in the degradation of proteins in all cells including eukaryotic cells, and has been implicated in numerous cellular processes such as cell-cycle control and cellular differentiation. The activity of proteasome in Interleukin-2 activated natural killer cells (A-NK cells) is also correlated with cell-mediated cytotoxicity. Although its subunits are arranged in a set of stacked rings in the form of $\alpha_7\beta_7\beta_7\alpha_7$, only the β subunit is catalytically active. Furthermore, of the seven β subunits in an individual eukaryotic proteasome, only three are proteolytically active. We have applied isopycnic sucrose gradient fractionation of postnuclear supernatants, molecular sieve chromatography, and heparin-Sepharose chromatography to purify the proteasome from the rat A-NK cells, liver cells and NK leukemic cell line CRNK-16 cells. Some characteristic biochemical similarities among the lymphocytes and the liver cells were observed. However, an examination of purified rat NK cell proteasomes by electron microscopy revealed unique proteasome concatemers that have not been reported for other proteasomes. SDS-PAGE and Western blot analysis using anti-rat liver proteasome polyclonal antibodies to the rat liver proteasome clearly indicated differences in the rat hepatic proteasome and the CRNK-16 derived proteasomal subunits. Moreover, CEP1612, a single synthetic selective chymotryptic proteasome inhibitor, had differential effects on two chymotryptic substrates in the studies with the Jurkat cell line. More recently, we investigated a series of unique chymotryptic inhibitors synthesized by Dr. J. C. Powers (Georgia Institute of Technology) for their proteasome inhibitory activities. Therefore, a conclusion was drawn that the lymphocyte proteasomes may contain more than one site for chymotryptic cleaving activity. (This study is supported by a grant from Robert A. Welch Foundation.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

FUNCTIONAL STUDY OF THE LECTIN-LIKE TRANSCRIPT (LLT) RECEPTOR ON HUMAN NK CELLS Hoang-Tuan K. Pham, Kent S. Boles, Samuel S. Chuang, and Porunelloor A. Mathew. Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Natural killer (NK) cells constitute the third major population of lymphocytes. They spontaneously kill tumor and virally infected cells and mediate the rejection of bone marrow grafts via the interaction of a repertoire of inhibitory and activatory NK receptors with target ligands. A group of NK cell receptors belongs to the C-type lectin superfamily and localizes to the NK gene complex on chromosome 6 and 12 in the mouse and human, respectively. The NK gene complex encodes type II receptors including the families of NKR-P1, Ly-49, and NKG-2 receptors. We recently identified and cloned a human lectin-like transcript 1 (LLT1) receptor expressed on NK, T, and B cells which localizes to the NK gene complex. The LLT1 cDNA encodes a predicted protein of 191 amino acid residues. The predicted protein contains a transmembrane domain near the N-terminus and an extracellular domain of 131 amino acid residues homologous to C-type lectins. Our goal is to characterize the LLT1 function. We PCR-amplified the LLT1 cDNA with a primer set designed to introduce a C-terminal histag epitope. Subsequently, it was subcloned into a mammalian expression vector (pCB6) and transfected into a human NK cell line, YT. We will conduct cytotoxic assays using anti-histag antibody on the transfected cells to observe the effects of LLT1 signaling on the effector function of NK cells. We are also subcloning LLT1 cDNA into a bacterial expression vector , pUR. Bacterially expressed LLT1 protein will be used to immunize rabbits and mice to produce polyclonal and monoclonal antibodies, respectively. These antibodies will be used to characterize LLT1 receptor function of the wild type receptor.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EXPRESSION OF C-JUN AND C-FOS PROTEINS IN APOPTOTIC HELA CELLS Sunitha Kumari and Rafael Alvarez-Gonzalez, Department of Molecular Biology & Immunology, University of North Texas Health Science Center, Fort Worth, TX-76107.

Programmed cell death was induced in HeLa cells by exposure to 50 ╡M MNNG (N-methyl-N'-Nitro-N-Nitroso Guanidine) for various time intervals (up to 120 min). Apoptotic death was confirmed by the microscopic observation of cell blebbing, cell granulation and cell aggregation. Cells also showed loss of phospholipid symmetry as judged by immunofluorescent microscopy with fluorescently labeled phosphatidlyserine-specific Annexin V. In addition, staining of cells with ethidium bromide showed the presence of genomic DNA apoptotic bodies. Gene expression levels of *c-jun* and *c-fos* increased in DNA damaged HeLa cells following MNNG treatment in a time-dependent fashion. While the levels of *c-fos* increased rapidly during the first 30 min and remained high for 2h, the increase in *c-jun* expression was more gradual and slower (60 -120 min) post-MNNG treatment. These results are consistent with the conclusion that *c-fos* is important in the initial stages (commitment phase) of apoptosis while *c-jun* is involved in the late stages (execution phase) of programmed cell death induced with DNA-damaging agents.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

FUNCTIONAL INTERACTIONS OF P53 WITH POLY(ADP-RIBOSE) POLYMERASE (PARP) DURING APOPTOSIS FOLLOWING DNA DAMAGE: COVALENT POLY(ADP-RIBOSYL)ATION OF P53 BY EXOGENOUS PARP AND NON-COVALENT BINDING OF P53 TO THE Mr 85,000 PROTEOLYTIC FRAGMENT Sunitha Kumari, Hilda Mendoza-Alvarez and Rafael Alvarez-Gonzalez, Department of Molecular Biology & Immunology, University of North Texas Health Science Center, Fort Worth, TX-76107.

Cultured HeLa cells were exposed to 50 uM MNNG(N-Methyl-N'Nitro-N-Nitroso guanidine) for 0, 10, 30, 60, 90 and 120 min to induce apoptosis. Cell death was followed by the i) oligonucleosomal fragmentation of chromatin DNA ii) increase in p53 expression iii) proteolytic degradation of poly(ADP-ribose) polymerase (PARP) into the 85 kDa (catalytic) and 29 kDa (DNA-binding) signature polypeptides of apoptosis. We also immunodetected p53 by western blotting in immunoprecipitates of PARP. By contrast, intact PARP co-immunoprecipitated with the p53 specific antibody only during the first 30 min of MNNG treatment. After 60 min, only the 85 kDa fragment of PARP co-immunoprecipitated with p53. Therefore, we conclude that PARP physically associates with p53 via its 85 kDa carboxyl-terminal domain. Next, we examined p53 as a potential target for endogenous poly(ADP-ribosyl)ation. Surprisingly, p53 was not poly(ADP-ribosyl)ated *in situ*. However, incubation of apoptotic HeLa cell extracts with exogenously added full length PARP and [^{32}P] ■NAD^+ resulted in the time-dependent covalent poly(ADP-ribosyl)ation of p53.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE INVOLVEMENT OF CASPASES IN TUMOR NECROSIS FACTOR-α MEDIATED CELL DEATH. P. Marshall and A. Basu, Dept. of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX, 76107.

We have investigated the signalling pathway involved in tumor necrosis factor-α (TNF)-mediated cell death in the MCF-7 breast cancer cell line. The binding of TNF to its receptors induces activation of caspases, a family of cysteine proteases which cleave after aspartate residues. Caspases are important for the execution of cell death by a variety of apoptotic stimuli, including TNF. A colorimetric MTT cytotoxicity assay was performed to examine the sensitivity of MCF-7 cells to TNF. Caspases are converted from their proform to the processed form upon activation. Activation of caspases was monitored by Western blot analysis. We have shown that TNF causes activation of Caspase-7 and Caspase-8 but not Caspase-2. In addition, caspase inhibitors, were used to see what caspases are important for MCF-7 cell death by TNF. Benzyloxycarbonyl-Ile-Glu-Thr-Asp-fluoromethylketone (Z-IETD-fmk), an inhibitor of Caspase-8, blocked TNF-induced cell death whereas an inhibitor of Caspase-1 had little effect. IETD also blocked TNF induced conversion of procaspase-8 to its active form. Protein kinase C (PKC), a group of isoenzymes critical in the regulation of cell growth, has been shown to regulate TNF sensitivity. We have shown that bisindolylmaleimide (BIM), a PKC inhibitor, enhanced sensitivity of MCF-7 cells to TNF. We purpose that PKC regulates caspase activation and hence cell death mediated by TNF. This research is supported by a grant CA71727 from NCI.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EXPRESSION CLONING OF THE HUMAN P53 CDNA INTO AN ECDYSONE INDUCIBLE MAMMALIAN VECTOR. P. C. Verrett[1], P. A. Mathew[1], and P. M. Muganda[2], Department of Molecular Biology and Immunology[1], University of North Texas Health Science Center, Fort Worth, TX, 76107 and Department of Biology[2], Southern University A&M College, Baton Rouge, LA, 70813.

The cellular p53 protein is classified as a tumor suppressor gene product. Wild type p53 regulates normal cell proliferation by arresting the cell cycle in G1 phase. Efforts to study the role of p53 in HCMV infected and transformed cells have been hampered by the fact that constitutive expression of p53 causes cessation of cell growth and/or apoptosis; this makes it difficult to obtain enough cells for experiments. In order to solve this problem, this study focuses on recloning the wild type and mutant p53 cDNAs into an inducible mammalian expression vector. The recloning process was initiated by first isolating the mutant and wild type p53 cDNAs from the plasmids pC53-Cx3 and pC53-Eco3, respectively. The cDNA fragments were then ligated to linearized inducible vector pIND. Three clones pINDp53wts56, pINDp53mts22, and pINDp53wtas13, expressing wild type p53, mutant p53, and wild type antisense RNA, respectively, were isolated for future studies. The constructed vectors once induced showed high levels of the expected proteins in a HCMV transformed cell line. These p53 expression vectors can now be used in studies to observe p53 function. Different ways that p53 function can be examined are monitoring phenotypic changes of host cells and viral infectivity of these cells under the conditions of transient and stable expression of protein.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

REGULATION OF CELL DEATH BY PROTEIN KINASE C. Basu, A., Miura, A., Akkaraju, G.R., and Johnson, D.E. University of North Texas Health Science Center, Fort Worth, TX 76107 and University of Pittsburgh Cancer Institute, Pittsburgh, PA 15261.

Protein kinase C (PKC), a family of phospholipid-dependent serine/threonine kinases, plays a critical role in signal transduction and cell growth regulation. We have shown that cell death mediated by a variety of apoptotic stimuli can be regulated by the PKC signal transduction pathway. The effects of PKC modulators, however, vary significantly depending on the apoptotic stimuli. While PKC activators potentiated cell death mediated by the DNA damaging agent cisplatin, they blocked tumor necrosis factor-α (TNF)-induced cell death. Both TNF and cisplatin caused cleavage of novel PKC isozymes, a substrate for caspase-3. Expression of anti-apoptotic proteins Bcl-2 and CrmA in HeLa cells blocked TNF-induced cell death and proteolytic cleavage of novel PKCδ. In contrast, Bcl-2 but not CrmA reversed cisplaitn-mediated cell death. Additionally, rottlerin, an inhibitor of novel PKCδ, enhanced PKCδ cleavage by TNF but blocked the effect of cisplatin. Although both TNF and cisplatin caused activation of caspases, PKC modulators had opposing effects on caspase activation. For example, rottlerin enhanced TNF-induced activation of caspase-3 and –7 but blocked cisplatin-induced activation of these caspases. Thus, PKC acts at distinct steps to regulate activation of caspases and hence cell death mediated by different apoptotic stimuli. (Supported by a grant CA71727 from NCI).

MOLECULAR BIOLOGY AND IMMUNOLOGY

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RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECT OF 25—HYDROXYCHOLESTEROL ON NUCLEAR PROTEIN BINDING TO REGULATORY REGION OF APOLIPOPROTEIN E GENE IN MOUSE MACROPHAGES. Peter Gargalovic, Ladislav Dory University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107

25-hydroxycholesterol (25-OH cholesterol) is a potent activator of apolipoprotein E (apoE) gene transcription in macrophages. In the present study we identify Activator Protein-1 (AP-1) binding site located –763 base pair (bp) upstream of mouse apoE gene. Although possible regulatory binding sites were up to –600 bp reported previously [Paik et al. (1995) Biochim. Biophys. Acta. **1262**: 124-132] the mechanism of 25-OH cholesterol mediated activation is not clear. Using Electro-mobility Shift Assay (EMSA) we examined the effect of 25-OH cholesterol treatment on nuclear protein binding to identified AP-1 binding region in mouse macrophages. Mouse thioglycolate induced peritoneal macrophages were incubated for 48 hours with 25-OH cholesterol in final concentrations 0.1; 0.5; 1.0 μg/ml of media in the presence of 10% Fetal Bovine Serum (FBS). Oligonucleotide 21 bp long corresponding to -770 ~ -750 bp upstream of mouse apoE gene containing the AP-1 binding site was synthesized [Integrated DNA Technologies®] and 5′ end-labeled with ^{32}P isotope. Electro mobility shift assay was performed on isolated nuclear protein extracts from control and 25-OH cholesterol treated macrophages using ^{32}P labeled DNA sequences. The reaction mixture was separated on 6 % polyacrylamide non-denaturing gel and detected by autoradiography. 25-OH cholesterol treatment increased binding of nuclear extracts to the AP-1 binding sequence (-TGAGTCA-) in dose dependent manner. The location of binding was confirmed by using mutated DNA sequence in points –758 bp (C→T) and –757 bp (A→G) which showed no binding reaction. Our preliminary data suggest that this binding region may be involved in the regulation of apoE gene transcription, possibly by 25-OH cholesterol mediated activation of AP-1 signaling pathway.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

REGULATION OF NOVEL PROTEIN KINASE C ISOZYMES. D. Sinha and A. Basu. Department of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX-76107

Protein Kinase C (PKC), a family of serine/threonine kinases, is a critical element in signal transduction pathway. Molecular cloning of PKC has revealed that it belongs to a multigene family of 12 isozymes that are divide into three groups-(a) conventional (α, βI, βII, and γ), novel (δ,ε, η, and μ) and atypical (ζ, and λ/ι) PKCs. All the different isozymes carry out distinct biochemical functions. The activity of PKC isozymes are highly regulated. The native enzyme remains loosely associated with the cyotplasmic side of the plasma membrane in an inactive conformation due to interaction of the regulatory domain with the catalytic domain through a pseudosubstrate sequence. This negative regulation is believed to be released by binding of allosteric PKC activators at the regulatory domain thereby inducing a reversible conformational change in the enzyme, which exposes the substrate binding site The consequence of PKC activation is its translocation to the membrane compartments. Prolonged cellular exposure to PKC activators causes it's downregulation.

Although considerable work has been done on cPKCs, little is known about regulation of nPKCs. Preliminary studies from our lab have shown that the regulation of nPKC is unique. For example, PKC activators actually caused an up-regulation of nPKCη instead of down-regulation. The objective of my studies. is to investigate the mechanism of activation of nPKC isozymes. Specifically, we propose to investigate whether phosphorylation and dephosphorylation influence nPKC activation or down-regulation.. Our studies should yield important new insights regarding the function and regulation of novel PKCs.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

DETERMINATION OF CYCLIC ADP-RIBOSE LEVELS IN CULTURED HUMAN CELLS. Paramjit K. Gill and Rafael Alvarez-Gonzalez. University of North Texas Health Science Center at Fort Worth, TX 76107-2699.

A fluorescent HPLC method for measuring the intracellular content of cADPR in cultured cells has been developed. The procedure involves harvesting of cells using a 20% (w/v) trichloroacetic acid (TCA) treatment to stop cellular metabolism. The TCA soluble extract was taken through a dihydroxyboronyl- Bio Rex column for affinity chromatography purification of nucleotides containing two or more riboses. Purified nucleotides were then treated with phosphodiesterase to hydrolyze all phosphoanhydride bonds. Under these conditions cADPR remains intact due to its cyclic structure. The enzymatic degradation products were isolated using dihydroxyboronyl -Bio Rex again. Next, we converted cADPR to ADPR using NAD glycohydrolase isolated from *Bungarus fasciatus* and the products generated were purified again using a third step of affinity chromatography with the dihydroxyboronyl – Bio Rex. ADPR was derivitized to the ε-ADPR fluorescent form via chloroacetylaldehyde at 60°C. The ε-ADPR was purified on a boronate PBA-60 column and quantified by HPLC on a Partisil 10-SAX followed by fluorescence detection.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Hilda Mendoza-Alvarez**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

BIOCHEMICAL CHARACTERIZATION OF MONO(ADP-RIBOSYL)ATED POLY(ADP-RIBOSE) POLYMERASE

Hilda Mendoza-Alvarez and *Rafael Alvarez-Gonzalez Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

Here, we report the biochemical characterization of mono(ADP-ribosyl)ated-poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30]. PARP was effectively mono(ADP-ribosyl)ated both in solution as well as via an activity gel assay following SDS-PAGE with 20 μM or lower concentrations of [^{32}P]3'-dNAD$^+$ as the ADP-ribosylation substrate. We observed the exclusive formation of [^{32}P]3'-dAMP and no polymeric ADP-ribose molecules following chemical release of enzyme-bound ADP-ribose units and high resolution-polyacrylamide gel electrophoresis. The reaction in solution; i) was time-dependent; ii) was activated by nicked dsDNA; and iii) increased with the square of the enzyme concentration. Stoichiometric analysis of the reaction indicated that up to four amino acid residues/mol of enzyme were covalently modified with single units of 3'-dADP-ribose. Peptide mapping of mono(3'-dADP-ribosyl)ated-PARP following limited proteolysis with either papain and/or α-chymotrypsin indicated that the amino acid acceptor sites for chain initiation with 3'-dNAD$^+$ as a substrate are localized within an internal 22 kDa automodification domain. Neither the amino-terminal DNA-binding domain nor the carboxy-terminal catalytic fragment became ADP-ribosylated with [^{32}P]3'-dNAD$^+$ as a substrate. Finally, the apparent rate constant of mono(ADP-ribosyl)ation in solution indicates that the initiation reaction catalyzed by PARP proceeds 232-fold more slowly than ADP-ribose polymerization.

This project was supported fully by Grant GM45451 from the NIH.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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REGULATORY MECHANISMS OF POLY(ADP-RIBOSE) POLYMERASE

Hilda Mendoza-Alvarez & Rafael Alvarez-Gonzalez Molecular Biology & Immunolo
University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 761(

The poly(ADP-ribosyl)ation of proteins catalyzed by poly(ADP-ribose) polymerase (PARP) [EC 2.4.2.30] is a multi-substrate enzymatic reaction where the ADP-ribose moiety of βNAD^+ is sequentially transferred to three distinct acceptor molecules: i) the protein target or initiation site, ii) the protein-distal ADP-ribose residue, as the chain elongation acceptor, and iii) a polymeric nicotinamide-derived ribose as branching site. Here, we first isolated and characterized the auto-mono(ADP-ribosyl)ation of PARP (initiation reaction) with 3'-$dNAD^+$ as the ADP-ribosylation substrate. ADP-ribose chain initiation was not only time-dependent but showed substrate saturation kinetics as a function of either nicked dsDNA or 3'-$dNAD^+$. These results are consistent with the notion that the number of DNA strand breaks present determines the total number of ADP-ribose polymers synthesized. The chain initiation rates increased with the square of PARP concentration indicating that this reaction occurs intermolecularly. By contrast, ADP-ribose elongation and branching appear to be homoallosterically regulated since the polymerization rates increased sigmoidally as a function of the log of βNAD^+ at three distinct concentrations of PARP (4.5, 9, and 18 nM). As expected for an autocatalytic event, substrate inhibition kinetics was also observed at 27 nM or higher concentrations of PARP. The average chain length of the polymers synthesized in these experiments was dependent on the concentration of βNAD^+. The rates of ADP-ribose polymerization as a function of nicked dsDNA at different constant levels of βNAD^+ were measured. The reaction rates increased with substrate saturation kinetics up to 20 μg/ml of nicked dsDNA. However, at higher concentrations of nicked dsDNA, the optimum dimerization of the enzyme, was lost due to increased ratio of DNA breaks/mol of PARP, thus resulting in lower enzymatic activity. In summary, these observations are consistent with the conclusion that nicked dsDNA is a heteroallosteric regulator of the poly(ADP-ribosyl)ation reaction catalyzed by PARP. Finally, we performed ADP-ribose polymerization assays with the 40 kDa carboxy-terminal fragment of PARP that contains the catalytic domain (CD) and lacks the DNA binding domain. Not surprisingly, the activity levels displayed by CD were identical to those observed with full length PARP in the absence of nicked dsDNA. Formation of a CD homodimer was a pre-requisite for catalytic activity at micromolar concentrations of CD. The length of the polymers was independent of the [βNAD^+]. In conclusion, the ADP-ribose polymerization reaction catalyzed by PARP involves homoallosteric and hetero-allosteric regulation and depends on the concentration of βNAD^+, PARP, and nicked dsDNA.

This project was supported fully by Grant GM45451 from the NIH.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

LONG-TERM STABLE EXPRESSION OF M-CSF IN COS-1 CELLS. Fu-mei Wu, Chuang-jiun Chiou* and Ming-chi Wu. Department of Molecular Biology and Immunology, UNT-HSC, Fort Worth, TX 76107. *Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

In addition to its role in macrophage proliferation, differentiation and maturation, Macrophage Colony-stimulating Factor (M-CSF) also plays important functions in placenta development, tooth eruption, bone formation and neuron development. Our lab has reported a series of studies on the expression of M-CSF from a pancreatic carcinoma cell line MIAPACA-2. In this study, attempt has been made to establish a long-term stable cell line expressing M-CSF in high yield. Four plasmids were constructed including: (1) pCJC 904: pEGFP-C3 (-EGFP, +DHFR). (2) pCJC 914a: pCJC 904-M-CSF. (3) pCJC 905: PEGFP-N1-DHFR. (4) pCJC 915a: pCJC 914-M-CSF. These plasmids are transfected separately into COS-1 cells. Initially, the results clearly indicated that plamid encoding M-CSF gene (PCJC 914a and PCJC 915a) expressed M-CSF as shown by Western blot and macrophage colony formation assay. The polycistron expression of M-CSF and GFP are then monitored for selection of the M-CSF producing cells in the presence of antibiotic genectin G418. As the selection process is continued, the number of green-fluorescence positive cells is increased and is in proportion to the increase of M-CSF activity. A greater than 90% GFP-positive cell population has been achieved after one month of continuous culture in the presence of G418. Expression of M-CSF in this culture has increased several folds from initial transfected cells. Further experiment is in progress to establish a high M-CSF expression permanent cell line.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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THE INVOLVEMENT OF MYOSIN LIGHT CHAIN PHOSPHORYLATION IN SECRATOGOGUE-INDUCED INSULIN SECRETION IN PANCREATIC β-CELLS. Harshika Bhatt, Barry P. Conner, Michael Lawrence and Richard A. Easom. Department of Molecular Biology and Immunology, UNTHSC at Fort Worth, Fort Worth, TX 76107.

It has been well documented 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. Besides calmodulin dependent kinase II, a second candidate 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. There is accumulating evidence in a number of nonmuscle cell types that these mechanisms regulate granule secretion processes. A hypothesis that we have begun to study is that myosin light chain kinase might function to regulate insulin secretion similarly as those signals as been recently suggested for other secreting secreting cell types. Our approach is to look for the activation of MLCK by the phosphorylation of endogenous MLC using urea glycerol gel electrophoresis and immunoprecipitation of myosin using antiplatelet myosin antibodies and to correlate this with insulin secretion in pancreatic β-cells and in islets. To elucidate the possible involvement of MLCK in the mechanism of insulin secretion, we studied the effects of MLCK inhibitors wortmannin, ML-9 and KT5926. Incubation of β-cells and islets with wortmannin, ML-9 and KT5926 inhibited both glucose-induced and high K^+-evoked MLC phosphorylation in the pancreatic β-cells. Further, these data was supported by the experiments with immunoprecipitation of myosin from ^{32}P-labeled cells and separation of MLC from myosin heavy chain by SDS-PAGE. Inhibitors wortmannin, ML-9 and KT5926 in their concentration ranges required to prevent MLCK activation completely inhibited insulin secretion and also inhibited MLC and MHC from immunoprecipitated myosin from ^{32}P-labeled cells. These results suggest that MLCK activation and MLC phosphorylation play central role in the Ca^{2+} -sensitive insulin secretion from the pancreatic β-cells.
(Supported by NIH grant DK-47925)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

IN VITRO EXPANSION OF PANCREATIC ISLETS. E.S. Ettinger, R.A. Easom, Ph.D., S.D. Dimitrijevich, Ph.D. UNTHSC, Fort Worth, Texas 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. An even more relevant approach would consider using islet implantation because the islet is the optimal glycemic control unit. The focus of this study is the in vitro maintenance and expansion of viable pancreatic islets and their expansion. We have shown that murine, porcine and human islets can be sustained in a three-dimensional culture for up to 6 weeks. We have also shown that murine, porcine and human islets can be a source of endocrine epithelial cells. When placed in a specific microenvironment these endocrine epithelial cells appeared to spontaneously aggregate into pseudo islets. Several culture substrates also have been studied to promote attachment and differentiation. Immunohistochemical studies demonstrated that these cells were composed of insulin, glucagon 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 identify the progenitor cell type and study the process of cytodifferentiation into functional islets. This approach will provide a plentiful source of good quality islets suitable for transplantation, thereby eliminating the need for exogenously administered insulin to diabetic patients.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Woo-Jin Chang**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

INTRACELLULAR GLYCOGEN IN *ESCHERICHIA COLI* STIMULATES STATIONARY-PHASE (ADAPTIVE) MUTAGENESIS at *ebgR*. Woo-Jin Chang and Tony Romeo
Department of Molecular Biology & Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107

α-1,4 glucan polymers such as glycogen, amylopectin, or amylose are primordial molecules present in modern archaebacteria, eubacteria, and eukaryotes. In *Escherichia coli*, endogenous glycogen is synthesized and accumulated in stationary-phase of bacterial growth. Stationary-phase bacteria are relatively resistant to stresses such as heat, hyperosmolarity, acid, or oxidation, and they concurrently exhibit the ability to mutate in selective environment. Stationary-phase (adaptive) mutation differs from neo-Darwinian (growth-dependent, random) mutation in that i) the mutation process occurs in non-dividing cells (hence stationary) and ii) the mutation appears to occur specifically in gene(s) related to the selective condition (hence adaptive). We previously isolated a glycogen-deficient *kan*R insertion mutant (TR3-1, *glgA*-) from *E. coli* K-12, and subsequently demonstrated biological functions of *E. coli* glycogen in the stationary-phase stresses. In the current study, we further expand the function of intracellular glycogen in *E. coli* by testing the mutant's ability to mutate in stationary phase environment. We used lactulose-negative *E. coli* strain SJ134 (F-, *glgA+*, *ebgA51*, *ebgR+*, *lacZ-*, *rpsL*) and its isogenic *glgA* mutant. In this system, a mutation in *ebgR* allows the bacteria to grow in a lactulose minimal medium. Stationary-phase bacteria were grown in a rich medium (Kornberg, 1.0% glucose), washed and resuspended in a minimal buffer, and plated on lactulose-Xgal minimal plate in the presence of 25-fold excess scavenger bacteria. Adaptive mutability in *ebgR* was monitored daily for two weeks by scoring lactulose-positive colonies appearing on the plates. Compared to glycogen-deficient TR3-1SJ134, glycogen-containing SJ134 gave significantly more Lac+ colonies. Experiments were also conducted using glycogen-overexpressing strain and similar results were obtained. Adaptive mutagenesis is thought to be an energy-dependent process, and we postulate that intracellular glycogen in *E. coli* provides energy charge for this process. In conclusion, glycogen in *E. coli* appears to promote bacterial evolution in a selective environment.

(Supported by NSF grant MCB9726197)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Katie A. Overheim**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE SUSCEPTIBILITY OF CATALASE-DEFICIENT VARIANTS OF *STAPHYLOCOCCUS AUREUS* TO MACROPHAGE KILLING

Katie A. Overheim and Mark E. Hart, UNT-HSC, 3500 Camp Bowie Blvd., Ft. Worth, TX 76107

Staphylococcus aureus is a gram-positive bacterium and a leading cause of nosocomial infections. Most strains of *S. aureus isolated* from nosocomial infections are resistance to multiple antibiotics and therefore, represent a major threat to human health. Given the seriousness of multiple-antibiotic resistant staphylococci it is imperative that new ways to treat staphylococcal infections be found. Catalase activity in *S. aureus* has been hypothesized to be a defense mechanism to prevent killing by the major phagocytes, polymorphonuclear leukocytes and macrophages. These phagocytes reduce oxygen to produce toxic oxygen radicals, such as superoxide and hydrogen peroxide, which are then used to facilitate killing of phagocytized bacteria. Catalase converts hydrogen peroxide to oxygen and water and thereby, eliminating one of the reactive oxygen products. Recently, we isolated variants of *S. aureus* S6C that were deficient in catalase activity as determined by their inability to generate oxygen and water from hydrogen peroxide. Due to this fact, we decided to examine the susceptibility of the catalase-deficient variants to intracellular killing by macrophages. Murine peritoneal macrophages were seeded in six-well tissue culture flasks at a concentration of 10^5 and allowed to grow overnight to form a confluent monolayer. Overnight cultures of catalase positive and negative variants were harvested by centrifugation, washed two times in sterile Phosphate-buffered solution, and finally suspended in Dulbecco's modified Eagle's media at a concentration of 10^7. One milliliter portions were inoculated onto the macrophage monolayers and were incubated for two hours. Macrophages were harvested by trypsinization and lysed with Triton-X-100 and briefly sonicated. The cell lysates were then serially diluted and plated on tryptic soy agar plates. Plates were incubated overnight prior to determining colony-forming units per milliliter. Results from these studies indicate that approximately half of the catalase positive and negative variants were phagocytosized with no appreciable decrease in cell number as a result of intracellular killing.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Matthew Tatarko**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

OPTIMIZATION OF AROMATIC AMINO ACID BIOSYNTHESIS THROUGH GLOBAL METABOLIC ENGINEERING. Matthew Tartarko and Tony Romeo, Department of Molecular Biology and Immunology, UNT Health Science Center at Fort Worth, Fort Worth, TX 76107.

Commercial viability of metabolite production is largely determined by the yield at which substrate is converted to end product. Successful optimization of yields has traditionally focused on improving metabolite-producing strains by genetically engineering the amounts and/or the intrinsic activities of enzymes of the respective biosynthetic pathways. Nevertheless, this strategy remains limited by the availability of biosynthetic precursors derived from central metabolism. We have attempted to genetically engineer a global regulatory system which controls central carbon flux, Csr (carbon storage regulator), as a means of improving yields of aromatic amino acids. Disruption of *csrA* has been shown to increase gluconeogenesis, with a concomitant increase in phosphoenol pyruvate, a central carbon precursor of aromatic compounds. A disruption of *csrA* (*csrA::kanR*) in an *Escherichia coli* strain engineered to produce high levels of phenylalanine lead to improved production by 167% (226 mg/L). A plasmid (pAT-1) encoding transketolase, which should enhance erythrose-4-phosphate, the second precursor of aromatic amino acids, slightly increased phenylalanine production (160 mg/L) over the parent strain. A strain containing pAT-1 and *csrA::kanR* produced 250% (338 mg/L) of the phenylalanine observed in the parent strain. Interestingly, levels of the aromatic precursor 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) in these strains were not indicative of phenylalanine yield. The optimal effect of the *csrA* mutation was observed to occur at a substrate glucose concentrations of < 0.2%. This work demonstrates the utility of "global metabolic engineering" in the production of economically important aromatic compounds.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Bangdong Wei**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

EFFECTS OF THE GLOBAL REGULATOR CSRA ON MOTILITY AND FLAGELLA BIOSYNTHESIS IN *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

Escherichia coli and many other bacteria can respond to variations in the environment by moving toward some substances and away from others. Cell motility of *E. coli* is through flagellar propulsion, which requires the expression of more that 40 genes organized in a 3-tier hierarchy. The master regulatory operon of the flagella hierarchy, *flhDC*, is itself subject to several global stress response networks. This study was designed to examine the effects of a global regulator, CsrA (carbon storage regulator A), on motility in *E. coli*. Previous studies demonstrated that CsrA functions as an mRNA binding protein in its role as a negative regulator. We compared the motility of *csrA* wild-type and mutant strains on semisolid tryptone agar plates and in liquid medium. Our results showed that *csrA* is required for cell motility. Negative staining electron microscopy further revealed that the *csrA* mutants are non-motile because they lack flagella. Using a *flhDC::lacZ* translational fusion, we observed that the *flhDC* operon was expressed 3- to 5-fold higher in wild-type versus *csrA* mutant strains. We subcloned the *flhDC'* genes into the plasmid vector, pUC18, which will be used to conduct *in vitro* S-30 coupled transcription and translation assay in the presence or absence of CsrA. This will allow us to determine whether CsrA directly or indirectly activates *flhDC* operon and will be followed by studies on the mechanism of positive control by this novel kind of bacterial regulatory factor. (Supported by the National Science Foundation)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

IDENTIFICATION OF A NOVEL GENE INVOLVED IN GLYCOG BIOSYNTHESIS IN *ESCHERICHIA COLI.* Thomas Weilbacher, Bangdong W and Tony Romeo. Department of Molecular Biology and Immunology, University North Texas Health Science Center at Fort Worth, Texas 76107-2699.

The presence of α-1,4-glucans, such as glycogen and starch, has been widely demonstrated in plant, animal and bacterial cells. These compounds are important reservoirs of intracellular carbon and energy. Three structural genes are essential for the production of glycogen in *E. coli*, *glgA, glgC*, and *glgB*, which encode glycogen synthase, ADP-glucose pyrophosphorylase, and glycogen branching enzyme, respectively. In addition, glycogen synthesis is intricately regulated, and is controlled by at least four global regulatory systems.

Studies in our laboratory have further implicated a role for at least two additional structural genes in glycogen synthesis. One of these novel gene is located at ~42.9 min on the *E. coli* chromosome, and has been shown to have a stimulatory effect on glycogen synthesis. A low copy plasmid clone containing this region of the chromosome, pMR261 was previously isolated due to its stimulatory effect on glycogen accumulation. Furthermore, a glycogen-deficient mutant of *E. coli* (M9-4) was isolated, which appeared to map to this same genetic locus. Transformation of M9-4 with pMR261 greatly increased glycogen production, providing further evidence that the plasmid clone and chromosomal mutation correspond to the same gene.

Sequence analysis indicated the presence of five open reading frames in pMR261. It is my hypothesis that the gene(s) responsible for phenotypic effects on glycogen levels will be attributable to one or more of these reading frames. I will attempt to test this hypothesis by transforming M9-4 with plasmid subclones of each individual reading frame, and monitoring for the desired change in phenotype. Once the responsible reading frame has been discerned, this will be confirmed by making appropriate deletions in pMR261, within that reading frame, and again monitoring for change in the glycogen phenotype of M9-4.

Once the location of the gene(s) has been determined, I will attempt to overexpress and purify the functional protein, and demonstrate its effects on glycogen synthesis in vitro. In conducting these experiments, I hope to establish a previously unknown gene and its protein product, as well as deduce its biochemical mechanism for enhancing bacterial glycogen synthesis. It is my intention that this research will contribute to the ongoing efforts to explore the intricacies of bacterial metabolism.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Debra L. White**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

THE GLOBAL REGULATOR *csrA* MODULATES BACTERIAL ADHERENCE Debra L. White, Mark E. Hart, and Tony Romeo. Department of Molecular Biology and Immnology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107

Many of the cellular components which promote virulence of bacterial pathogens are expressed when bacterial cultures enter the stationary phase of growth. Studies in *Escherichia coli* have identified a gene *csrA* (carbon storage regulator A), 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 pathogens. Furthermore, a homolog of *csrA* has been recently shown to regulate virulence properties of the plant pathogen *Erwinia carotovora*. A mutation in *csrA* alters a variety of suface properties, including mucoidy, adherence, and hydrophobicity, suggesting that this gene may likewise affect many virulence factors of mammalian pathogenesis. One such potential virulence factor known as curli fimbriae has been identified and implicated in the formation of biofilms. Under low salt, low temperature and in stationary phase, *E. coli* can express these thin, coiled surface structures. The curli polymers specifically interact with molecules such as congo red, and with human proteins such as fibronectin, laminin and MHC Class I which may facilitate colonization of host tissue. Understanding the regulation of curli fimbriae remains in the early stages. Since *csrA* has been shown to affect adherence, the current study was undertaken to determine the mechanism by which *csrA* affects adherence. We have demonstrated that a *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) media. In our laboratory, we have demonstrated that a *csrA* mutant is highly adherent to itself (aggregation) and to glass surfaces. An assay was developed to quantify binding to glass and plastic. Using this assay, we found that a *csrA* mutant is highly adherent over a 48 hour time span relative to *csrA*$^+$ or *rpoS* mutant. Negative staining transmission electron microscopy pictures revealed that a *csrA* mutant produces massive amounts of curli fimbriae. Preliminary data using transcriptional fusions for curli show that a *csrA* mutant is higher in activity in the stationary phase (National Science Foundation, Robert D. Watkins Minority Fellowship, ASM).

ABSTRACT #96

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ISOLATION AND CHARACTERIZATION OF THE SUPEROXIDE DISMUTASE GENE (*SOD*) OF *STAPHYLOCOCCUS AUREUS* Michelle L. Wright and Mark E. Hart, UNT-HSC, Fort Worth, TX 76107

Superoxide is a toxic reactive oxygen species generated during aerobic respiration by the partial reduction of oxygen. Reactive oxygen species have been implicated in DNA strand breakage and protein inactivation, as well as membrane lipid peroxidation. Superoxide is produced by professional phagocytes such as polymorphonuclear leukocytes (PMNs) and macrophages as part of the oxidative burst elicited by phagocytosis of bacteria and other antigens. Superoxide, in combination with other oxygen radicals, acts to kill bacteria by its intrinsic toxicity. Many bacteria have evolved superoxide dismutase as a defense against superoxide. This enzyme catalyses the conversion of superoxide to hydrogen peroxide and oxygen. As of yet, manganese, iron, and copper-zinc superoxides have been identified in bacteria. *S. aureus*, a gram positive facultative anaerobic pathogen, was previously shown to contain a manganese superoxide dismutase, though only a small portion of the gene was sequenced. PMNs have been shown to provide first line defense against staphylococcal invasion when the mucous membranes are breached. We therefore hypothesize that staphylococcal superoxide dismutase plays a role in the bacterium's pathogenicity. In an effort to assess the role manganese superoxide dismutase (Mn-SOD) plays in staphylococcal disease, we have isolated, cloned, and sequenced the full-length *sod* gene. Subsequently we have complemented an *E. coli* superoxide deficient mutant with the staphylococcal Mn-SOD and are in the process of generating a *sod* knock-out mutant in *S. aureus*. The *sod* mutant and its corresponding parent strain will be used to assess virulence in phagocytic assays with PMNs and in the mouse model of cutaneous infection. We believe that the results of these experiments will demonstrate that Mn-SOD activity is an important survival and/or virulence factor of *S. aureus*, and could potentially be a target for antimicrobial drug development.

STUDENT ORAL PRESENTATIONS

(1:30 PM)	Kent S. Boles	MOLECULAR CHARACTERIZATION OF A NOVEL HUMAN NATURAL KILLER CELL RECEPTOR HOMOLOGOUS TO MOUSE 2B4
(1:45 PM)	Lisa Hodge	ANTIBODY AND INFLAMMATORY RESPONSES AFTER INTRANASAL IMMUNIZATION
(2:00 PM)	Harlan Jones	TH1 and TH2 RESPONSE AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN
(2:15 PM)	Wendi Lambert	NEUROTROPHINS AND NEUROTROPHIN RECEPTORS IN THE HUMAN LAMINA CRIBROSA
(2:30 PM)	Michael C. Lawrence	CALCINEURIN ACTIVATES A NUCLEAR FACTOR OF ACTIVATED T-CELLS (NFAT) TO ENHANCE INSULIN GENE TRANSCRIPTION
(2:45 PM)	P. John Kamthong	CYCLIC AMP ATTENUATES INDUCED HUMAN M-CSF TRANSCRIPTION
(3:00 PM)	Dmitriy S. Ushakov	MGATP INDUCES CONFROMATIONAL CHANGE IN THE ESSENTIAL LIGHT CHAIN OF SKELETAL MUSCLE SUBFRAGMENT
(3:15 PM)	Bradley Joe Hart	HYPERLIPIDEMIA WITH HYPOGLYCEMIA REDUCES MYOCARDIAL OXYGEN UTILIZATION EFFICIENCY BUT NOT CONTRACTILE FUNCTION DURING CORONARY HYPO-PERFUSION
(3:30 PM)	Martin Farias	PREJUNCTIONAL OPIATE RECEPTORS IN THE SINOATRIAL NODE MODERATE VAGAL BRADYCARDIA
(3:45 PM)	Mohammed Dibas	SINGLE MUTATION UNCOVERS A LOW AFFINITYTIMULATORY SITE FOR THE CNS CONVULSANT PENTYLENETETRAZOLE (PTZ) ON GABAA RECEPTORS
(4:00 PM)	Stephen A. Stoffel	REWARD RECEPTOR MODULATORS FOR KETAMINE SELF-ADMINISTRATION
(4:15 PM)	D. W. Wray	MECHANISM OF AGE RELATED ORTHOSTATIC HYPOTENSION

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Kent S. Boles**

Department: **Molecular Biology and Immunology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MOLECULAR CHARACTERIZATION OF A NOVEL HUMAN NATURAL KILLER CELL RECEPTOR HOMOLOGOUS TO MOUSE 2B4

Kent S. Boles,* Marco Colonna,† Samuel S. Chuang,* and Porunelloor A. Mathew*. *Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX and †Basel Institute of Immunology, Basel, Switzerland.

Natural killer (NK) cells spontaneously detect and kill cancerous and virally infected cells through receptors that transduce either activating or inhibiting signals. The majority of well studied NK receptors are involved in inhibitory signaling. However, we have previously described an activating receptor, 2B4, expressed on all murine natural killer cells and a subset of T cells that mediate non-MHC restricted killing. Anti-2B4 monoclonal antibodies directed against IL-2 activated NK cells enhanced their destruction of tumor cells. Recently, we determined binding of 2B4 to CD48 with a much higher affinity than CD2 to CD48 in both the human and mouse. Here we describe the molecular characterization of a cDNA clone homologous to mouse 2B4, isolated from a human NK cell library. The cDNA clone contained an open reading frame encoding a polypeptide chain of 365 amino acid residues. The predicted protein sequence showed 70% similarity to murine 2B4. Additionally, it has 48, 45, and 43% similarity to human CD84, CDw150 (SLAM), and CD48, respectively. RNA blot analysis indicates the presence of 3 kb and 5 kb transcripts in T and NK cell lines. A single transcript of 3 kb is identified in poly(A)$^+$ RNA from human spleen, peripheral blood leukocytes, and lymph node, whereas, the level of expression in bone marrow and fetal liver was indeterminate. Preliminary functional data suggests that NK cell interaction with target cells via 2B4 modulates human NK cell cytolytic activity.

This grant was supported by NIH grant AI38938.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

ANTIBODY AND INFLAMMATORY RESPONSES AFTER INTRANASAL IMMUNIZATION L. Hodge and J. Simecka. Dept. Mol. Biol. & Immunol., UNT Health Science Center at Fort Worth, TX 76107

Mucosal immunity is the first line of defense in the upper respiratory tract. We propose that intranasal (IN) vaccination is an optimal approach to induce antibody production thus lowering the risks of respiratory infection. To determine the optimal dose of the mucosal adjuvant cholera toxin (CT), mice were IN immunized with varying doses of CT plus influenza (FLU) virus vaccine. Inclusion of CT enhanced generation of FLU serum antibody responses. FLU specific IgA was also found in nasal passages from mice IN immunized, but not after intraperitoneal (IP) immunization. To determine if IN immunization is more effective in priming immune responses in the upper respiratory tract, mice were immunized either IN or IP, followed by IN challenge. Mice given two IN immunizations produced the highest level of serum and nasal wash IgA. Serum IgG and IgM levels were high in both IN and IP immunized mice. Also, IgA antibody forming cells in nasal passages and other tissues were highest in mice given two IN immunizations, while IgG and IgM antibody forming cells were similar between IN:IN and IP:IN immunized groups. Importantly, IN immunization with antigen alone induced inflammatory reactions in lungs associated with IgE production. In conclusion, IN immunization is an effective approach to generate mucosal IgA responses in the upper respiratory tract; however, approaches avoiding adverse immunologic reactions need to be developed.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Harlan Jones**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

TH1 and TH2 RESPONSE AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN Harlan Jones, Lisa M. Hodge and J. W. Simecka, Dept. of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX.

Intranasal immunization (i.n.) is a promising route to protect against respiratory disease. BALB/c mice were i.n. immunized with influenza (Flu) vaccine alone or in combination with the mucosal adjuvant cholera toxin (CT) (0 and 7 days). 3 days later, there was an overall increase in the numbers of mononuclear cells, particularly $CD4^+$ T cells (as determined by FACS), isolated from the lungs of mice i.n. immunized with Flu plus CT. T helper cell cytokine mRNA expression in lungs was determined by RT-PCR and RNAse protection assays. We found that Th2 cytokine mRNAs were increased after immunization with antigen alone or in combination with CT. There was an increased expression of Th1 cytokines mRNA only after i.n. immunization with Flu plus CT. In vitro antigen stimulation of lung mononuclear cells from mice given Flu alone showed only Th2 cytokine (IL-4) production. Whereas, lung cells from mice given Flu plus CT produced both IFN-γ and IL-4 in response to in vitro stimulation with Flu antigen. These data indicate that $CD4^+$ T-cells are preferentially activated after i.n. immunization resulting in a Th2 cell activation which promotes mucosal IgA responses. However, the mucosal adjuvant, CT, appears to promotes Th1 and Th2 cell activation which may contribute to inflammatory reactions in the lung. This work is supported by the American Lung Association of Texas.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Wendi Lambert**

Department: **Anatomy and Cell Biology**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

NEUROTROPHINS AND NEUROTROPHIN RECEPTORS IN THE HUMAN LAMINA CRIBROSA. Wendi Lambert[1], Rajnee Agarwal[1], Ryan Betton[1], William Howe[2], Sherry English-Wright[2], Abbot F. Clark[1,2], and Robert 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, 76107[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX, 76134[2].

Purpose. Neurotrophins (NTs) constitute a family of polypeptide growth factors that promote the development, survival and differentiation of neurons. The expression of NTs has been detected in many non-neuronal cells that also express NT receptors (NTRs) suggesting the existence of paracrine and/or autocrine signaling pathways. Cells within the lamina cribrosa (LC) region of the optic nerve head are in close contact with retinal ganglion cell axons and could be a paracrine source of NTs for the axons. Alternatively, cells within the LC could signal each other via NTs. The purpose of this study was to determine which NTs and NTRs are expressed in cells isolated and cultured from the human LC. **Methods.** Synthesis of cDNA and the reverse-transcriptase polymerase chain reaction (RT-PCR) were conducted using total RNA from well characterized cell lines from the human LC. Western blotting and immunocytochemistry were used to evaluate NT and NTR protein expression. Immunoassay systems (ELISAs) specific for each NT were used to detect the secretion of NTs. **Results.** Lamina cribrosa (LC) cells and optic nerve head (ONH) astrocytes were isolated from human LC tissue and characterized using morphology and immunocytochemistry. Message and protein for each of the NTs was detected in both LC cells and ONH astrocytes. Similarly, message and protein for trk A, trk B, trk C and truncated trk B was detected in both cell types, as was message for truncated trk C. Neither cell type expressed message or protein for the low affinity NT receptor p75. Secretion of NGF, BDNF, and NT-3 was observed by both cell types. **Conclusions.** Cells isolated from the human LC express message and protein for NTs and trk receptors. In addition, these cells also secrete NTs suggesting paracrine and/or autocrine signaling exists within the LC. NT signaling may play an important role in the maintenance of the normal LC as well as in disease like glaucoma. (**Support.** The Glaucoma Foundation and Alcon Labs.)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Michael C. Lawrence**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CALCINEURIN ACTIVATES A NUCLEAR FACTOR OF ACTIVATED T-CELLS (NFAT) TO ENHANCE INSULIN GENE TRANSCRIPTION. M. Lawrence and R.A. Easom, UNTHSC, Fort Worth, TX 76107

There exist several cis-acting elements within the insulin gene promoter, which appear to work in synergy to upregulate insulin gene transcription when induced by glucose. It has been shown that selective pharmacological inhibitors of the Ca2+/calmodulin-dependent phosphatase, calcineurin, can diminish this response. Glucose metabolism initiates depolarization of β-cells and the subsequent rise in free intracellular Ca2+, and we therefore, have examined calcineurin and NFAT as a potential target for the upregulation of insulin gene transcription by this mechanism. In this study, we have identified five NFAT consensus sequences within the first 620 base pairs of the rat I insulin gene promoter, two of which their presence and location are conserved among other mammals, 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 or rat islet extracts and shifted with extracts pre-incubated in the presence of either anti-calcineurin or anti-NFAT antibodies. We have also detected the presence of NFAT by immunofluorescence in the INS-1 pancreatic β-cell line and pancreatic slices, in which the islets stained most intensely. Co-transfection experiments with NFAT-Luc and INS-CAT reporter constructs with a constitutively active form of calcineurin showed increased reporter activity over controls in β-cell lines. These studies establish the presence of NFAT in insulin-secreting cells, its ability to bind to elements within the insulin gene promoter, and suggest that calcineurin can enhance insulin gene transcription by activating NFAT in pancreatic β-cells. (Supported by the Advanced Research Program of the Texas Higher Education Coordinating Board)

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **P. John Kamthong**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

CYCLIC AMP ATTENUATES INDUCED HUMAN M-CSF TRANSCRIPTION P.John Kamthong, Ming-chi Wu Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107

The glycoprotein macrophage colony-stimulating factor (M-CSF) is produced by wide varieties of cells, such as fibroblasts, and endothelial cells. The functions of the protein do not only limit to promoting differentiation and survival of myeloid hematopoietic cells which are key effectors in cell-mediated immunity, but also involve in many biological processes including atherosclerosis and osteoporosis which are among the major public health problems. Nevertheless, most studies of the M-CSF emphasized on its biological functions and the signaling downstream of the receptor ligation, while the signal transduction pathways leading to the regulation of the gene expression are not thoroughly elucidated. We use human pancreatic cancer cell line MIA PaCa-2 and human lung fibroblast cell line CCL202 as the models for studying the regulation of human M-CSF gene expression in cells of supportive tissues. We and others have shown earlier that IL-1, TNF, phorbol ester, endotoxins, cGMP and calcium/calcium ionophore increase M-CSF production in MIA PaCa-2 and CCL202 cells. Contrarily, cAMP, forskolin, pertussis & cholera toxins and theophylline attenuated the IL-1-induced M-CSF expression as detected by biological activity assay. Changes in accumulated mRNA in the cells after treated with IL-1 or TPA or either in combination with Forskolin were determined by northern blot. Forskolin attenuated IL-1-induced M-CSF mRNA accumulation, but not TPA. Electromobility shift assay was used to initially delineate the roles of transcription factors pertinent to M-CSF gene enhancer activation; NF-kB and AP-1. NF-kB binding is elicited by IL-1 treatment, but not TPA. On the other hand, TPA strongly induces AP-1 binding, but not NF-kB. Meanwhile, only IL-1-induced NF-kB binding was affected by cAMP or cAMP elevating agents.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Dmitriy S. Ushakov**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MGATP INDUCES CONFROMATIONAL CHANGE IN THE ESSENTIAL LIGHT CHAIN OF SKELETAL MUSCLE SUBFRAGMENT 1 D.S. Ushakov, R. Moreland, L.D. Saraswat[#], S. Lowey[#], O.A. Andreev, J. Borejdo University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Blvd, Fort Worth, TX, 76107; # University of Vermont

The mechanism of muscle contraction is believed to involve rotation of the "neck" part of the heavy chain of myosin head (S1). This part of S1 is occupied by the essential light chains (either LC1, or LC3). The ELC's belong to a calmodulin family of proteins. These proteins consist of the N- and C-terminal lobes, each bearing two helix-loop-helix motifs (so-called EF-hands). Crystal structures of S1 revealed interactions of the C-terminal lobe of ELC with the converter domain of the heavy chain, which is thought to relay conformational changes associated with ATP hydrolysis to the neck region. We therefore hypothesized that ELC changes its conformation during the power stroke. We studied the effect of MgATP on the mobility of an extrinsic fluorophore attached to a single Cys 178 located near the C-terminus of LC1. The LC1 was isolated, labeled with rhodamine and exchanged with the native ELC of chymotryptic skeletal muscle S1. The steady state anisotropy (ρ) of S1 in rigor was 0.195. Addition of F-actin caused only a slight decrease in mobility, suggesting that ρ reflects largely the mobility of LC1 and not of the heavy chain. Addition of MgATP dramatically decreased the mobility of a probe. No such decrease was seen when the heavy chain was labeled with rhodamine at Cys 707, nor when MgADP or MgPPi was used. Addition of MgATP to acto-S1 decreased the mobility to the extent characteristic of S1+MgATP. Control experiments ruled out the possibility that LC1 binds to actin in the presence of MgATP. Further, we exchanged rhodamine labeled LC1 with the native ELC in skeletal muscle fibers and myofibrils. The diffrenece between the perpendicular and parallel polarization ($\Delta\mathbf{p}$) in rigor was close to 0, indicating considerable disorder of the probe. In the relaxed muscle $\Delta\mathbf{p}$ was large, indicating considerable order. Since LC1 can also bind to actin through its N-terminal amino acid residues, we speculated that a probe located near the N-terminus would be immobilized in rigor and freely rotating in relaxation. To check this we used a mutant LC1 in which cysteine was moved from near the C-terminus, to near the N-terminus. Mutant was labeled with rhodamine and exchanged with native ELC of skeletal muscle fibers and myofibrils. As expected, $\Delta\mathbf{p}$ was large in rigor and small in relaxation. These results suggest that binding/hydrolysis of MgATP induces conformational change in LC1. Supported by NIH.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Bradley Joe Hart**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

HYPERLIPIDEMIA WITH HYPOGLYCEMIA REDUCES MYOCARDIAL OXYGEN UTILIZATION EFFICIENCY BUT NOT CONTRACTILE FUNCTION DURING CORONARY HYPOPERFUSION. Bradley J. Hart, Xiaoming Bian, Robert T. Mallet, and H. Fred Downey, UNT Health Science Center at Fort Worth, Fort Worth, TX 76107.

This study was designed to determine changes in myocardial contractile function and metabolic substrate selection during moderate coronary hypoperfusion in the presence of elevated plasma fatty acid (FFA) and reduced glucose concentrations. Coronary perfusion pressure (CPP) was sequentially lowered from 100 to 60, 50, and 40 mmHg in the left anterior descending coronary artery (LAD) of anesthetized, open-chest dogs. Regional glucose uptake (GU), fatty acid uptake (FAU), percent segment shortening (%SS), and oxygen consumption (MVO_2) were determined with normal arterial plasma FFA concentrations (Group 1) or with elevated FFA concentrations (Groups 2 and 3). In Group 3, glucose in the coronary perfusate plasma was reduced from 3.53 ± 0.36 to 0.15 ± 0.03 mM by hemodialysis. In Group 1, FAU fell by 85% when CPP was lowered to 60 mmHg and remained depressed as CPP was reduced further. The hyperlipidemia of Group 2 did not alter glucose uptake at any CPP, but maintained FAU at baseline levels until CPP was lowered to 40 mmHg. At 40 mmHg CPP, myocardial function and metabolic variables were similar in Groups 1 and 2. In Group 3, FA uptake increased four fold ($p<0.05$), glucose uptake decreased fell to zero ($p<0.05$), and MVO_2 doubled ($p<0.05$), but %SS was unchanged relative to Group 2. Addition of glucose to the dialysate prevented the metabolic effects of Group 3. Thus, glucose utilization provides an increase in the oxygen utilization efficiency during hypoperfusion. Blocking this increase, however, does not contribute to a reduction in myocardial contractile function.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

PREJUNCTIONAL OPIATE RECEPTORS IN THE SINOATRIAL NODE MODERATE VAGAL BRADYCARDIA. Martin Farias, Keith Jackson, Amber Stanfill, and James L. Caffrey. University of North Texas Health Science Center, FortWorth, TX 76107.

Met-enkephalin-arg-phe(MEAP) interrupts vagal bradycardia when infused into the systemic circulation. This study was designed to locate the opiate receptors functionally responsible for this inhibition. Previous observations suggested that the receptors were located in either the intracardiac parasympathetic ganglia or the prejunctional nerve terminals innervating the sinoatrial node. In this study 10 dogs were instrumented with a microdialysis probe inserted into the substance of the sinoatrial node. MEAP was infused directly into the sinoatrial node via this microdialysis probe. Vagal stimulations were conducted at .5, 1, 2, and 4 Hz during all treatments (infusions). Saline ascorbate (control), diprenorphine (opiate antagonist), and diprenorphine with MEAP were also infused into the sinoatrial node via the microdialysis probe. Cardiac responses during vagal stimulation were recorded online. MEAP introduced into the sinoatrial node via the microdialysis probe reduced vagal bradycardia by more than one half. Local nodal blockade of these receptors with diprenorphine eliminated the effect of MEAP demonstrating the participation by opiate receptors. Systemic infusions of MEAP were administered while the opiate receptors of the sinoatrial node were blocked locally with diprenorphine. In this treatment, the effect of MEAP was blocked as well. The opiate receptors responsible for the inhibition of vagal bradycardia are most likely located on prejunctional nerve terminals innervating the sinoatrial node with few if any extra-nodal or ganglionic receptors.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Mohammed Dibas**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

SINGLE MUTATION UNCOVERS A LOW AFFINITYTIMULATORY SITE FOR THE CNS CONVULSANT PENTYLENETETRAZOLE (PTZ) ON GABAA RECEPTORS. Mohammed I. Dibas and Glenn H. Dillon Department of Pharmacology, University of North Texas Health Science Center at Fort Worth.

γ-AMINOBUTYRIC ACID type-A (GABAA) receptors are the major sites of fast synaptic inhibition in the brain. They form a pentameric heterozygous receptor/chloride ion channel, which is composed of combinations of α, β, γ, and δ subunits. Picrotoxin (PTX), U93631, and pentylenetetrazole (PTZ) are convulsant drugs that reduce the GABA activated current, presumably by blocking the channel lumen. Previous studies have shown that mutation of threonine 246 to phenylalanine in β2 subunit abolishes PTX convulsant effect. Therefore, we generated this mutation in the β2 subunit to test the hypothesis that this single mutation would also abolish the inhibitory effect of PTZ and U93631. Whole-cell patch clamp recordings were obtained from HEK293 cells transiently expressing the wild type α3β2γ2 and the mutant α3β2(T246F)γ2 GABAA receptor. The GABA EC_{50} was shifted two-fold suggesting that the affinity of GABA was slightly decreased by the mutation. In addition, the modulatory effect of benzodiazepine was not changed suggesting that the integrity of the channel was still maintained. PTZ inhibitory effect was abolished; the inhibitory effect of U93631 was also greatly reduced. Surprisingly, at high concentration PTZ now enhanced GABA activated current (up to 400%). This result suggests that this mutation revealed a low affinity stimulatory site for PTZ. This site could be novel or it may overlap with other stimulatory sites such as the barbiturate or benzodiazepine site. In conclusion, T246F mutation abolishes the convulsive effect of PTZ and U93631, as it did previously to PTX. In addition, further characterization of the newly discovered stimulatory site of PTZ should be pursued in the future.

(Support: NIH ES 07904).

RESEARCH APPRECIATION DAY 1999

ABSTRACT

First Author: **Stephen A. Stoffel**

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

REWARD RECEPTOR MODULATORS FOR KETAMINE SELF-ADMINISTRATION. Stoffel, S.A., Egilmez, Y., Oglesby, M.W., Wallis, C., Lal, H., Forster, M. Department of Pharmacology, University of North Texas Health Science Center at Fort, Fort Worth, TX.

Ketamine is a dissociative anesthetic derivative of PCP. Usually, it is used only for sedation or anesthesia in geriatric and pediatric patients. However, more recently it has become a problem as a drug of abuse. Drugs of abuse typically cause an increase in dopamine at nucleus accumbens and the N-methyl-D-aspartate (NMDA) receptor causes an increase in dopamine at nucleus accumbens when stimulated. The classification of ketamine as an antagonist for the NMDA receptor makes it an unusual drug of abuse. This experiment delves into the receptor mechanisms that mediate the rewarding properties of ketamine. Utilizing the technique of electrochemistry and receptor agonists and antagonists for nicotine, dopamine, sigma receptors and opiates, this experiment examines the patterns of ketamine self-administration. The animal model of drug abuse has an established pattern of self-administration whereby an increase in concentration, the addition of an agonist, or the decrease in metabolism of the rewarding drug results in a prolonged interval between responses. The action of antagonists, the decrease in concentration, or the increase in metabolism of the rewarding drug diminishes the interval between responses. One would expect that ketamine self-administration would follow this pattern since it results in the increase in dopamine at nucleus accumbens like other drugs of abuse. However, The antagonists for nicotine, dopamine, mu opiates and the sigma receptor caused a significant increase in the interval between responses for ketamine self-administration. This is in direct opposition to the present theory on drug reward. The NMDA receptor is a mediator for glutamatergic pathways. It has been hypothesized that glutamate is the primary excitatory transmitter for the central nervous system. Many rewarding drugs are mediated through inhibitory pathways, dopamine is just one of them. This experiment provides evidence for excitatory neural pathway mediated reward.

RESEARCH APPRECIATION DAY 1999

ABSTRACT

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Use only the space below for your abstract. Do not exceed the space within the borders indicated. Do not alter the formatting, font or font size.

MECHANISM OF AGE RELATED ORTHOSTATIC HYPOTENSION.

W.Wray, K.Formes, R.Welch-O'Connor, A.O-Yurvati, P.Reese, M.Weiss, and X.Shi. Department of Integrative Physiology and the Cardiovascular Research Institute, UNTHSC, Fort Worth, Texas, 76107.

The aim of this study was to test the hypothesis that vagal dysfunction was responsible for a greater hypotension observed at the onset of orthostatic stress in the elderly. We compared the responses of arterial blood pressure (BP) and pulse interval (PI) of younger adults (Y, <30 yr-old, N=10), with and without muscarinic cholinergic (MC) antagonists, to older healthy adults (O, >60 yr-old, N=10), to orthostatic stress simulated by lower body negative pressure (LBNP) -40 torr. Baseline PI and systolic BP (SBP) were not significantly different between the Y and O, see TABLE. However, at the onset of LBNP the Y responded with a significant tachycardia without hypotension, whereas an orthostatic hypotension was present in O without tachycardia. Atropine (A), which blocks both central and peripheral MC receptors, and glycopyrrolate (G), a peripheral MC antagonist, similarly decreased PI without altering the SBP. Both A (40 μg/kg) and G (16 μg/kg) abolished tachycardiac responses and elicited systemic hypotension in the Y.

		C	ΔP1	ΔP3	ΔP5	ΔP10
PI	Y	1081±58	-125±35†	-156±40†	-172±41†	-189±45†
ms	O	1112±35	-44±25*	-57±23*	-54±23*	-51 ±21*
	A	604±22*	-10±8*	-21±8*	-24±8*	-31±8*
	G	567±19*	24±21*	32±28*	0±12*	-18±7*
SBP	Y	122±4	4±2	0±2	0±2	-3±2
mmHg	O	129±6	-6±2*	-17±5*†	-22±6*†	-19±6*†
	A	126±3	-9±3*	-12±3*†	-13±4*†	-16±4*†
	G	119±3	-10±2*	-13±2*†	-15±4*†	-18±7*†

C is averaged from 1-min data prior to LBNP. ΔP1-ΔP10 is the 1st to 10th pulse response to LBNP. * & † denote significant difference compared to Y and compared to C, respectively.

***Conclusion & Implication*:** Orthostatic hypotension occurs in the elderly at the onset of LBNP, which may explain why they are more prone to dizziness upon assumption of the upright posture. The underlying mechanism is the result of age related vagal dysfunction, mainly at the periphery.

(Supported by UNTHSC Faculty Research Grant, NIH AG14219 & HL45547)

FIRST AUTHOR INDEX

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