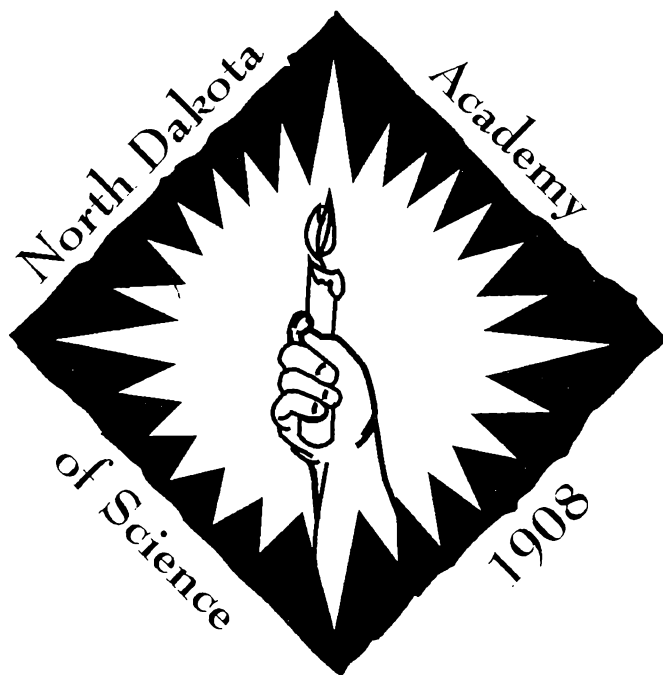


North Dakota Academy of Science

Proceedings of the 96th Annual Meeting

Ramada Plaza Suites
Convention Center
Fargo, ND

April 2004
Volume 58



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NORTH DAKOTA ACADEMY OF SCIENCE

Volume 58

April 2004

NORTH DAKOTA ACADEMY OF SCIENCE
(Official State Academy; Founded December 1908)

2003-2004

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96th Annual Meeting
April 29–30, 2004
Ramada Plaza Suites Conference Center
Fargo

HISTORY

The *Proceedings of the North Dakota Academy of Science* (NDAS) was first published in 1948, with Volume I reporting the business and scientific papers presented for the 40th annual meeting, May 2–3, 1947. Through Volume XXI, the single yearly issue of the *Proceedings* included both abstracts and full papers. Commencing with Volume XXII, the *Proceedings* was published in two parts. Part A, published prior to the annual meeting, contained an abstract of each paper to be presented at the meeting. Part B, published later, contained full papers by some of the presenters.

In 1979 (Volume 33) the *Proceedings* changed to the present 8 ½ x 11-inch format. It was produced from camera-ready copy submitted by the authors and issued in a single part to be distributed initially at the annual meeting. Commencing with Volume 51 all submissions were on computer disk; the entire *Proceedings* was then assembled by desktop publishing software. This approach allowed the Editor control over all formatting; many of the papers are reformatted in order to give the *Proceedings* a more consistent look. Also, incorporating all of the submissions on computer allowed production of an electronic copy of the *Proceedings* for the first time. Thus, the current Secretary-Treasurer has the capability to generate electronic copies of issues 55 through the present.

VOLUME 58 ORGANIZATION

This year the council of the NDAS decided to accept for publication the abstracts of all presentations scheduled for the 96th Annual Meeting. This meant that the communications of this volume did not undergo the standard level of peer review, but rather were chosen to give an accurate and up-to-date reflection of the material presented before the membership of the Academy at the Annual Meeting in Fargo. As a result, the presentations featured in this year's *Proceedings* are presented in four major sections. The first section contains the communications presented as part of the Symposium of Biomedical Research Infrastructure Network (BRIN) researchers and their projects. The second section comprises the undergraduate communications presented as part of the A. Rodger Denison Student Research Competition. The third section contains Graduate Denison Competition papers, and the final section comprises professional communications presented by faculty members of the Academy. Readers may locate communications by looking within the major sections of these Proceedings (see table of contents) or by referring to the author index on page 90.

Symposia Communications

Commencing with the 88th Annual Meeting [Volume 50], presenters of Symposia annual meetings have been given the opportunity to contribute an expanded or full-length article consisting of a multiple-page contribution, thus providing a presentation of much greater depth and scope than possible in a single-page communication.

This approach has allowed speakers to present more educationally-oriented lectures or workshop-type discussions and still provide a rigorous and/or more technical professional paper to the Proceedings. In a few cases, a speaker does not have a written communication. Again, this approach was taken to allow the symposia convenors the greatest flexibility possible in organizing speakers for the benefit of the audience.

Collegiate and Professional Communications

Each Collegiate and Professional presentation at the annual meeting is represented by a full-page communication that is more than an abstract, but less than a full paper. The communications contain results and conclusions, and permit the sharing of important data and conclusions. The communication conveys much more information to the reader than does an abstract, and yet still provides the advantages of timeliness and ease of production.

Constitution and Bylaws

This issue of the Proceedings also contains the Constitution and Bylaws of the Academy, a list of officers and committee members, a list of all dues-paying members of the Academy as of FALL 2002 (we'd appreciate your help in pruning and weeding this list, as well as fertilizing it with names of new and prospective members), a listing of past presidents of the Academy, and an index of presenters and paper authors. Copies of the financial statement and the unapproved minutes from last year's annual business meeting will be available at the meeting as appendices A & B, respectively.

IN APPRECIATION

The Academy wishes to acknowledge current and emeritus members of the Academy who have supported the mission of the North Dakota Academy of Science Research Foundation through their special gifts. A listing of these supporters is found on page 85 of these Proceedings. The Academy also wishes to express its thanks to the presenters of papers at the Annual meeting, the session chairs, as well as all who have helped in organizing spaces and places, soliciting manuscripts, and compiling of this year's communications. The President of the Academy also wishes to sincerely thank Drs. Dale Redmer and Cherlie Robinson, who served as honored guest speakers at this year's meeting.

Jon A. Jackson
Secretary-Treasurer
Proceedings Editor

Anna Grazul-Bilska
President

Feature – BRIN and the North Dakota Research Enterprise	5
AGENDA: BRIN-sponsored workshop (Friday April 30): Grant writing	6
Symposium of BRIN-supported Research	7
Communications – Undergraduate	25
Communications – Graduate	45
Communications – Professional	65
Constitution of the North Dakota Academy of Science	77
Minutes (Unapproved) of the 2002 Annual Business Meeting	appendix A
Academy Officers and Committees	83
Past Presidents and Locations of the Annual Meetings	84
Donors to the North Dakota Academy of Science Research Foundation	85
Statement of Financial Status	appendix B
Directory of Members (WE NEED YOUR HELP UPDATING THIS!)	86
Author Index	90

So what is ND BRIN, anyway?

North Dakota BRIN's purpose is to build biomedical research capacity within the state. Networking and human resource development are the watchwords for this collaborative effort between the two North Dakota research universities, four baccalaureate institutions in the North Dakota University System and five tribal community colleges.

Objectives

North Dakota BRIN has established numerous meaningful and productive collaborative interactions between participating institutions in the realm of biomedical research capacity building. Long-term outcomes include an increase in biomedical research competitiveness and an increase in the number of college graduates entering into biomedical research careers.

Core Areas

Four core areas are key to accomplishing the BRIN goals.

- A Bioinformatics Core - establishing a statewide consortium of libraries that coordinates acquisition of and access to electronic biomedical literature, databases and bioinformatics software. It is also building a computational chemistry and biology network to serve biomedical research in the state.
- A Start-up Core has enhanced recruitment packages needed to attract new talented biomedical scientists to the two research universities.
- A Tribal College/Baccalaureate Science Core has focused largely on enlarging the pipeline of science graduates seeking careers in biomedical research. Tribal colleges have strengthened science instruction through curriculum development and utilization of videoconferencing for distance learning. Baccalaureate institutions have also encouraged student-oriented research projects, assisted in science faculty recruitment and expansion, and participated in a scientist exchange program with the research universities. A graduate teaching internship program has provided intensive teaching experiences for advanced graduate students in the biomedical sciences while generating release time for selected tribal college and baccalaureate college faculty to do other BRIN-related activities.
- The Administrative Core provides overall project management and oversees program evaluation. It has also provide outreach and educational services through the sponsoring of grant-writing and bioinformatics workshops, enhancement of state-wide scientific conferences, and publication of newsletters and a website highlighting biomedical science in the state.

History

In October 2001, the National Institutes of Health (NIH) awarded 24 grants totaling \$45 million to biomedical research institutions located in 23 states and Puerto Rico that have not fully participated in NIH grant funding in the past. The University of North Dakota School of Medicine and Health Sciences received a three-year, \$6 million grant.

The awards, funded through the Institutional Development Award (IDeA) Program, enhance biomedical research capacity among academic institutions and research institutions within the states. The National Center for Research Resources (NCRR), the NIH component, administers the IDeA Program.

BRIN's Purpose

The grants enable each institution to establish a BRIN, a subcomponent of the IDeA Program. Through BRIN, the grantee institutions will develop areas of potential research through staff development and access to research resources. The program provides funding to:

- Bring together institutions within a state to establish the network;
- Make institutional alterations and renovations;
- Improve laboratory equipment; and
- Assist in the recruitment of new faculty.

Each BRIN program has unique characteristics depending on a state's infrastructure needs. However, the ultimate purpose of a network is to build an effective research base that will eventually lead to competitive research applications from multidisciplinary research teams.

BRIN's EPSCoR Roots

The IDeA program, established in 1993, is administered by the National Center for Research Resources (NCRR). The program's intent is similar to the National Science Foundation's (NSF) Experimental Program to Stimulate Competitive Research (EPSCoR). IDeA was designed to broaden the geographic distribution of NIH funding for health research. As authorized by Congress, the program's intent is to enhance the competitiveness for research funding of institutions located in 23 states and Puerto Rico with historically low aggregate success rates for grant applications to the NIH.

ND BRIN-sponsored workshop

“Write winning grants”

presented by GRANT WRITERS SEMINARS AND WORKSHOPS L.L.C.

April 30, 2004
 Ramada Plaza Suites
 Fargo, North Dakota

Presenter: David C. Morrison, Ph.D.

8:30	Introduction to the Seminar
8:40	How to develop a fundable idea for a grant application
9:45	The fundamentals of a grant
10:15	Coffee break
10:30	The review process
11:15	Before you begin to write
12:00	Lunch – provided by ND BRIN
1:00	How to write for reviewers
1:30	Specific Aims page
2:15	Afternoon break
2:30	Specific Aims page-practical exercises
3:00	The Narrative of your proposal
3:30	Background and Significance Section
3:45	Biographical sketch, Resources and Preliminary Data
4:15	Title and Abstract
4:30	Submission, Review and (Potential) Resubmission
4:45	General Discussion and Wrap up

ND BRIN Professional Session

Crystal Ballroom

Session I**Chair: Dr. John Shabb, University of North Dakota**

- 9:10 - 9:30 am Mary Ann Sens* **METALLOTHIONEIN AND BREAST CANCER** Department of Pathology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND
- 9:30 - 9:50 am 14 Jonathan J. Sheng*, Andrea H. Greiff and Michael W. Duffel **HYDROXYSTEROID/ALCOHOL METABOLISM BY HUMAN SULFOTRANSFERASE SULT2A1** Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND and Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA
- 9:50 - 10:10 am Lynn C. Burgess* **BRIN AND THE UNDERGRADUATE RESEARCH PROGRAM AT DICKINSON STATE UNIVERSITY** Department of Natural Sciences, Dickinson State University, Dickinson, ND
- 10:10 - 10:30 am Min Wu*, Daniel Foster, Shibi Kannan, Jessica Knittel, Kieran Miller, Neal Bansal, and Jenny Guido **DNA REPAIR PROTEINS MODULATE MITOGEN-ACTIVATED PROTEIN KINASE DURING HYPEROXIA** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND

Session II**Chair: Dr. Don Schwert, North Dakota State University**

- 10:50 - 11:10 am Jonathan Geiger* **BNIP3, A POTENTIAL MARKER FOR AND REGULATOR OF NECROTIC AND/OR ATYPICAL NEURONAL CELL DEATH** Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, ND
- 11:10 - 11:30 am Christopher P. Keller* **LEAF INTACTNESS IS REQUIRED FOR AUXIN-INDUCED GROWTH INHIBITION OF *PHASEOLUS VULGARIS* (COMMON BEAN) LEAF MESOPHYLL** Department of Biology, Minot State University, Minot, ND
- 11:30 - 11:50 am Hilde E. van Gijssel*, Andre W. Delorme and Leslie E. Wong **BIOMEDICAL RESEARCH AT VCSU: PROGRESS, CHALLENGES AND FUTURE** Department of Science, Valley City State University, Valley City, ND
- 12:00 - 12:45 pm Lunch - NDAS Business meeting

Session III**Chair: Dr. Mark Sheridan, North Dakota State University**

- 1:00 -1:20 pm Brij B Singh* **TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS ARE REGULATED VIA PROTEIN-PROTEIN INTERACTION** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 1:20 -1:40 pm Bin Guo* **BCL-G IN APOPTOSIS AND CHEMORESPONSE OF PROSTATE CANCER** Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58105
- 1:40 -2:00 pm Thomas P. Gonnella* **NOVEL FLUORESCENCE METHODOLOGY FOR UNDERGRADUATE BIOMEDICAL RESEARCH** Mayville State University, Mayville, ND
- 2:00 -2:20 pm Mikhail M. Bobylev* and Robin Gonzalez **GREEN CHEMISTRY: ONE POT SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES** Department of Science — Chemistry, Minot State University, Minot, ND
- 2:20 -2:40 pm John Watt* **CILIARY NEUROTROPHIC FACTOR IS UPREGULATED DURING AXONAL SPROUTING IN THE RAT MAGNOCELLULAR NEUROSECRETORY SYSTEM** Department of Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND

Session IV**Chair: Dr. Kathy Sukalski, University of North Dakota**

- 3:00 -3:20 pm Shirley Cole-Harding*, Alana Tergeson & Clare Pettis **INDIVIDUAL DIFFERENCES IN HUMAN ALCOHOL RESPONSES** Department of Addiction Studies, Psychology & Social Work, Minot State University, Minot, ND
- 3:20 -3:40 pm Chris Kelland Friesen* **ATTENTIONAL ORIENTING EFFECTS OF GAZE DIRECTION CUES** Department of Psychology, North Dakota State University, Fargo, ND
- 3:40 -4:00 pm Clayton Neighbors* **CORRECTING NORMATIVE MISPERCEPTIONS AS A STRATEGY FOR REDUCING HEAVY DRINKING AMONG COLLEGE STUDENTS** North Dakota State University, Fargo, ND

METALLOTHIONEIN AND BREAST CANCER

Mary Ann Sens

Department of Pathology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND

Metallothioneins (MT) are a family of cysteine-rich, low molecular weight, intracellular proteins that bind transition metals. There are 4 classes of very similar MT proteins, designated MT-1 through 4, defined on the basis of small differences in sequence and charge characteristics. MT-3, initially thought to be confined to the brain, has a limited tissue distribution in normal, non-CNS tissue. It is not present in normal human breast, however is increased in a sub-set of breast carcinomas and may be related to a poor outcome in this disease. This talk illustrates the role of pathology in research and how the visual assessments used for diagnosis give clues and insights into the pathogenesis of a disease using investigations into the role of MT-3 in breast cancer and potential for prognostic information needed by patients and physicians.

HYDROXYSTEROID/ALCOHOL METABOLISM BY HUMAN SULFOTRANSFERASE SULT2A1Jonathan J. Sheng^{1*}, Andrea H. Greiff¹ and Michael W. Duffel²¹Department of Pharmaceutical Sciences, College of Pharmacy, North Dakota State University, Fargo, ND 58105;²Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA 52242

Sulfoconjugation is an important pathway in the phase II biotransformation of many endogenous and exogenous compounds. The enzymes that catalyze these reactions are a superfamily of cytosolic sulfotransferases. Chemically, the reactions catalyzed by sulfotransferases can often increase the water solubility of substrate compounds, including hormones, neurotransmitters, drugs, and carcinogens. The reactions catalyzed by these enzymes involve the transfer of a sulfonyl group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to an acceptor molecule to produce adenosine 3', 5'-diphosphate (PAP) and a sulfuric acid ester. The long-term objective of our research work is to enhance our understanding of the structure/function of cytosolic steroid sulfotransferases and their roles in steroid-associated cancer development.

Hydroxysteroid (alcohol) sulfotransferases (SULT2A) catalyze the sulfation of several endogenous steroids and many exogenous xenobiotics. The substrate stereoselectivities of sulfotransferases may be critically important in determining their overall roles in metabolism of drugs, carcinogens, and other xenobiotics. In the present work, stereoselectivity of the human hydroxysteroid sulfotransferase SULT2A1 was examined through analysis of its catalytic activities with the enantiomers of 1-naphthyl-1-ethanol and 2-naphthyl-1-ethanol. The k_{cat}/K_m value for sulfation of the *R*-(+)-enantiomer of 1-naphthyl-1-ethanol catalyzed by SULT2A1 was 3.3 min⁻¹mM⁻¹, while any sulfation of the *S*-(-)-enantiomer was below the detection limits of the assay. This stereospecificity was not present with the enantiomers of 2-naphthyl-1-ethanol, since both were substrates for SULT2A1. Such differences between the sulfation of 1- and 2-naphthyl-1-ethanol are consistent with previous studies on a rat hydroxysteroid sulfotransferase, ST2A2, wherein the importance of steric interactions between the ethanol group and a hydrogen atom at the *peri*-position (C8) on the naphthyl ring combines with the topology of the enzyme's active site to determine stereoselectivity.

Cancers of the prostate tend to respond differently to hormone signals than normal prostate tissue. One explanation for this phenomenon is that hormones are processed differently in cancer cells. To explore the roles of SULT2A1 catalyzed sulfation in human steroid-associated cancer development, we quantitatively assessed the expression pattern of hydroxysteroid sulfotransferase SULT2A1 in a pair of human steroid hormone-associated prostate cell lines, PZ-HPV-7 and CA-HPV-10, using quantitative real-time PCR techniques. Data indicated expression of SULT2A1 is elevated in the CA-HPV-10 prostate cancer cell line as compared to the non-malignant PZ-HPV-7 cell line. Growth assays performed on this pair of cell lines in the presence of DHEA, a substrate of SULT2A1, also showed a significant difference in the response of malignant versus non-malignant cells to physiologically relevant concentrations of hormone. CA-HPV-10 cells survived higher doses of DHEA than PZ-HPV-7 cells, suggesting a greater hormone processing ability exists in the cancer-derived cells. Increases in intracellular steroid metabolism by enhanced steroid-related sulfotransferase expression in cancer cells could lead to reduced hormone signal transduction.

BRIN AND THE UNDERGRADUATE RESEARCH PROGRAM AT DICKINSON STATE UNIVERSITY

Lynn C. Burgess

Department of Natural Sciences, Dickinson State University, Dickinson, ND 58601

The BRIN program was funded at \$68,000 for a biomedical undergraduate research program that started June 2002. Our current research project is to study the anticarcinogenic mechanisms of Lycopene and possible changes to the expression of gap junction communication proteins. Before BRIN, there was no research in our department. The university wanted to develop a research program for the students but we had no space, no release time, and no equipment. The administration was able to provide funding of \$500-\$1000 per project but this was inadequate without a large investment for equipment.

While waiting for our lab to be remodeled, I started a summer program for field research. This program engages the students, enhances their classroom experience, and provides chances to conduct short-term research projects. They get the chance to visit and study very different ecosystems that do not exist in North Dakota. We also visit museums, universities and other important sites on our trips.

BRIN funded the construction of a class II cell culture lab and some additional equipment for DNA and protein studies. Funds have been severely limited, so I purchased much of the equipment used from eBay, then repaired or adapted it to our use. Students are paid to conduct research, construct facilities, train others students, manage the budget, and to assist in my workload so I can have more time for research.

Getting students into the program has been a challenge, since most fear research. I recruited students by talking to science classes, using the good reputation of the summer program and by employing students. However, finding students early enough in their education is important, so they are not lost too soon after being trained.

The most difficult problem that has arisen during our project is if I don't remember, understand or just forgot something, and then I don't have anyone else to ask about what could have gone wrong. This is compounded by the years I was away from research, meaning many of my skills are outdated.

Research, despite its problems (and many times because of the problems), has benefited our department. We now have students who are not scared of research, they see many new possible careers, students have learned to work independently and understand that science isn't neatly packaged and fits into 2-hour labs. Additional benefits are available from research. We have additional equipment to use in other classes that we never could have purchased, students are more involved with their learning and they learn things that can't be taught in a class. Every time I do something with research, I usually have students available to learn, but each student as to be carefully instructed in each lab technique. They start out without the background or confidence to learn methods independently. I have had some success in using the more experience students to train the new students. This is very promising but requires a long-term investment in time. The biggest drawback to this is with limited funds, the students cannot use large amount of disposables to practice the method or skills, plus the chance of costly repairs to the equipment is very possible.

Most students have greatly benefited from this exposure to research. The students learn best when we cut the apron strings and they figure out things for themselves. Research is very attractive for use in regular classes but the grading stigma and the time constraints of semesters limits its effectiveness.

Problems continue for us. They include funding, space, equipment, and university and the support of other faculty. Research should involve as many students as possible but with ten students working at a time is more than one person can handle. Recruiting students early in their education and keeping them involved until graduation will require a long-term funded project. The packaging of projects into semesters for grading purposes and having research results ready for when the administration or funding agents want to examine our output is difficult. It is very difficult to keep students available for presentations if anytime has expired since they did the project. Additionally, in every case when it has been an issue, research and academics has always lost out to athletics and outside student employment.

Many questions about students research program need to be answered; is it my research project or is it the students; do they assist or do I act as their coach? At a small teaching college, do I conduct research to produce papers or do I teach students to do research? Do I supply trained, talented students to research institutes or do I compete with these institutes for grants and papers? Do I need to conduct peculiar type of research to engage and train my students or will any type of project assist these students into moving into additional scientific education and research careers? Supported by P20-16471 from the NCRR.

DNA REPAIR PROTEINS MODULATE MITOGEN-ACTIVATED PROTEIN KINASE DURING HYPEROXIA

Min Wu*, Daniel Foster, Shibi Kannan, Jessica Knittel, Kieran Miller, Neal Bansal, and Jenny Guido
Department of Biochemistry & Molecular Biology, University of North Dakota, Grand Forks, ND 58203

High concentrations of oxygen can cause severe toxicity to lung cells, particularly to DNA. Recent advances indicate DNA base excision repair (BER) protein, *i.e.*, 8-oxoguanine DNA glycosylase (hOgg1), can reverse oxidative DNA damage. Mitogen activated protein kinases (MAPK) have been linked to hyperoxia toxicity in both cell cultures and animal models. The main goal of this study is to examine what the interaction is between MAPK and DNA repair proteins during the oxidative pressure. This will provide new insight into the mechanism of DNA repair signaling in oxidation and ultimately new therapeutic approaches for reducing hyperoxia toxicity.

Methods: We have transduced A549 cells to express hOgg1 using the retroviral vector pSF91 for studying the signaling transduction of MAPK. The main method was the MAP kinase activity assay, using Western blotting with specific antibodies against p38 and ERK1/2 following immunoprecipitation of phosphorylated p38 and ERK1/2 molecules.

Results: Phosphorylation of ERK1/2 in A549 cells was increased by exposure to 95% O₂, compared with the control (in room air). p38 activity in A549 cells was also enhanced by exposure to 95% O₂ compared with the control. Furthermore, we have transduced A549 cells with human enzyme 8-oxoguanine DNA glycosylase (hOgg1) using retroviral vector pSF91 and tested the regulatory function of hOgg1 on the MAP kinase. Our results demonstrate that over-expression of hOgg1 decreased the p38 activation, but increased ERK1/2 activity in the presence of 95% O₂.

Indications: This confirms that MAP kinases are related to the cell cycle arrest. When oxygen damages DNA, cells are halted at the G1 or S phase. With the repair of damaged DNA, cells may resume their cycle and progress into the M phase.

Conclusions: Base excision repair proteins along with regulating MAPK may be useful for repairing oxygen damage in lung cells.

Acknowledgements: This work is supported by ND BRIN and EPSCoR faculty start-up funds.

BNIP3, A POTENTIAL MARKER FOR AND REGULATOR OF NECROTIC AND/OR ATYPICAL NEURONAL CELL DEATH

Jonathan D. Geiger*

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University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58203

Neuronal cell death has been shown to occur by apoptotic, necrotic and so-called atypical cell death mechanisms. Genetic factors have been identified that cause apoptotic cell death and markers for apoptosis continue to be discovered. However, to date genetic markers for and causative agents of necrotic and/or atypical neuronal cell death have yet to be identified. Previously, it was reported that BNIP3 induced cell death in a necrotic-like manner in non-neural cells. BNIP3 is a member of the Bcl-2 family of mitochondrial proteins that is an adenovirus E1B – Nineteen kD Interacting Protein that is a member of an unique subfamily of death inducing mitochondrial proteins that includes NIX and a *C. elegans* ortholog, ceBNIP3. This gene-regulated cell death pathway involves opening of the mitochondrial permeability transition pore without caspase activation and cytochrome *c* release. Here, we tested the hypothesis that BNIP3 may be a gene that when activated in brain is capable of inducing atypical apoptotic nerve cell death. BNIP3 was not detectable in rat brain and in primary cultures of rat hippocampal neurons under normal conditions. However, BNIP3 expression was increased dramatically *in vivo* when kainic acid, a structurally rigid analog of the excitatory amino acid glutamic acid was injected into rat striata, or when traumatic brain injury was introduced. BNIP3 expression was also increased when primary cultures of rat hippocampal neurons were treated with excitotoxic compounds. Expression of full length BNIP3 in primary hippocampal neurons induced an atypical cell death that required protein synthesis but the cell death pattern was largely independent of caspase activation. Neuronal survival rates after 5 days of transfection with full length BNIP3 plasmid were decreased compared to cells transfected with truncated BNIP3 or the plasmid alone. In neurons expressing full length BNIP3, 81% showed DNA condensation. In contrast, DNA damage was observed in only 24% of BNIP3-positive neurons transfected with truncated BNIP3. Expression of the dominant negative (truncated) form of BNIP3 that lacked the functional transmembrane domain of BNIP3 protected against glutamate-induced neuronal cell death. Thus, BNIP3 activation and expression appears to be both necessary and sufficient for atypical neuronal apoptosis in excitotoxicity. These results suggest that BNIP3 may be a marker for and possibly a regulator of atypical neuronal cell death and as such may be a new target for neuronal rescue strategies.

(Supported by the Canadian Institutes of Health Research and NIH Grant Number P20 RR-16471-03 from the BRIN Program of the National Center for Research Resources.)

**LEAF INTACTNESS IS REQUIRED FOR AUXIN-INDUCED GROWTH INHIBITION OF
PHASEOLUS VULGARIS (COMMON BEAN) LEAF MESOPHYLL**

Christopher P. Keller*

Department of Biology, Minot State University, Minot, ND 58707

Development in plants is controlled by a number of morphogenic hormones including the auxins. The principle naturally occurring auxin in all plants is indole acetic acid (IAA). It is primarily synthesized in young leaves and is transported downward in the plant where it has a controlling role in diverse aspects of development including stem elongation, tropisms, and generation of lateral roots. The role of IAA in the control of leaf growth, however, was long thought to be limited to control of vein elongation. Auxin has since been shown to be important in the initiation of new leaves in tomato, in leaf vascular development in *Arabidopsis* and in the cell-division phase of leaf expansion in *Arabidopsis*. Most recently, I and my colleagues have reported that auxin also plays a role in the control of the post-cell division/cell-expansion phase of leaf growth (which accounts for most final leaf size) in both *Arabidopsis* and *Phaseolus* (1). We found that that increased leaf auxin (either through exogenous auxin application or through inhibition of auxin transport at the leaf petiole) *inhibited* long-term leaf expansion and final leaf size in the common bean and in *Arabidopsis*. We also reported that this auxin-induced inhibition is not mediated by auxin-induced synthesis of ethylene, another plant growth hormone, the production of which is known to be auxin-induced.

These results were somewhat surprising in that auxin functions primarily in plants to stimulate rather than inhibit growth. Indeed shorter term experiments have found increased leaf growth following auxin treatment. For example, excised tobacco leaf tissues grew more rapidly incubated in auxin over a 20 hour period resulting in epinastic (downward) curvature (2, 3) and one-time application of auxins to expanding common bean (*Phaseolus vulgaris*) leaves produced transient (multi-hour) hyponasty (upward curvature) due to increased abaxial (underside) cell expansion (3).

In an attempt to reconcile the longer term inhibitory effect of auxin on bean leaves with the possibility of shorter term stimulatory effects, I re-examined, in high resolution continuous recordings, the effect auxin on leaf expansion of bean leaves. Also, examined was the effect of IAA on growth of excised leaf strips.

For these experiments *Phaseolus vulgaris* L cv. Contender were grown under greenhouse conditions for 10-13 days. Plants with monofoliolate leaves having midribs 30-40 mm were selected for experimentation. For high resolution recordings, a linear displacement transducer was connected to a leaf margin by means of a mobile clamp separated by 15 mm from a second immobile clamp so that growth of the intervening leaf tissue could be recorded. For leaf strip experiments, 1.5 mm X 10.0 mm strips were excised from non-veinal mid-leaf leaf regions by means of double bladed cutters. The strips were subsequently incubated in complex media with or without auxin.

In high resolution recordings, elevation of leaf auxin (1 mM IAA applied directly to the leaf by cotton swab) resulted in a biphasic growth response. Increased leaf auxin initially induced a growth surge that was complete in about 6 hours (1.02 +/- 0.15mm S.E. increase in length versus 0.47 +/- 0.07mm for a benzoic acid control n=10). This auxin-induced growth increase was followed by the sustained leaf growth inhibition described earlier (1). Analysis of epidermal cell areas indicated that sustained auxin-induced leaf growth inhibition could be entirely attributed to inhibition of cell expansion and not to inhibition of cell division.

Excised bean leaf strips, incubated up to 48 hours in 10 μ M IAA, experience only the initial auxin-induced growth phase seen in the intact plant tissue. Auxin-induced growth was sustained, however, so that at 48 hours auxin treated strips had increased in length 34.9 +/- 0.6 % versus 26.6 +/- 0.4 % for the minus auxin controls (n = 12). The apparent requirement for leaf intactness, or probably attachment to the plant, for the subsequent auxin-induced growth inhibition suggests a model of leaf auxin response in which auxin alters the transport or function of other growth signals in the leaf.

The project described was supported by NIH Grant Number P20 RR-16471-02 from the BRIN Program of the National Center for Research Resources.

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BIOMEDICAL RESEARCH AT VCSU: PROGRESS, CHALLENGES AND FUTURE

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In 2002 Valley City State University (VCSU) hired Dr. Hilde E. van Gijssel as an Assistant Professor of Science. The position was partly funded the North Dakota Biomedical Research Infrastructure Network (ND BRIN). One of the main objectives of the ND BRIN is to increase the biomedical research capacity in North Dakota and make North Dakota more competitive for biomedical research funding. VCSU received money from ND BRIN to start a biomedical research program for undergraduate students. In 2002 none of the faculty present at VCSU had significant biomedical research experience. The VCSU science faculty were at their maximum capacity with regards to teaching load. In order to create the research opportunity, the ND BRIN funds were used to hire a new person in stead of using it for equipment.

Dr. van Gijssel's main objectives have been to build a research program for the students at VCSU, to make the students more familiar with research, and stimulate career choices in research. During the last two years van Gijssel has designed and furnished a lab space. Equipment is currently purchased and the first students have started experiments. More research has been integrated in the curriculum at VCSU. An example of this is the following; students in microbiology design and perform experiment to test microbial activity of a soap produced by a local company as part of their lab. Dr van Gijssel has been the moderator for the Students Research Seminar Series. This seminar series uses the existing Interactive Video Network (IVN) to connect university to expose students to research seminars. On average the seminar draws the attendance of around 20 faculty and students per seminar. Students and faculty of the various ND universities have been presenters with as a highlight the presentation of Luis Herrera, a professor of the Universidad Autonoma de Baja California Sur (UABCS) in La Paz, Mexico

Research has become more visible at VCSU during the last 2 years. Two students successfully applied for the Advanced Undergraduate Research Award (AURA) from EPSCoR. Three students and Dr van Gijssel were part of the Frank Low Research Day at the UND medical school in April 2003. In April 2004 five students will be part of the Frank Low Research Day which has extended into the Rail program. During their three day visit students will spend two days in the lab with faculty or students and will have an opportunity to experience research. A Capstone Research class, which will award students 16 credits towards graduation for a semester of research instead of taking regular classes, has been added to the curriculum. Dr. van Gijssel's presence at VCSU also gives other faculty release time for their research efforts. Which means that one faculty member can finish her doctorate and another faculty member can extend his research efforts. Both Dr. Delorme and Dr. van Gijssel are involved in the renewal of BRIN with a project. Taken together, joint efforts by science faculty at VCSU in the last three years, supported by funding from BRIN, have led to increased visibility of our research efforts and more interest in careers in research at VCSU.

The project described was supported by NIH Grant Number P20 RR-16471-03 from the BRIN Program of the National Center For Research Resources.

TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS ARE REGULATED VIA PROTEIN-PROTEIN INTERACTION

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Members of the TRP superfamily of putative ion channel proteins have been suggested as components of Ca^{2+} influx channels. These channels are activated in response to stimulation of the G-protein coupled receptors that, leads to the hydrolysis of PIP_2 and generation of two key intracellular second messengers, IP_3 and DAG. The mechanism(s) involved in the regulation of these TRP proteins is still illusive. We show here that both N and the C terminus of TRPC1 protein are involved in protein-protein interaction, which not only regulates the channel activity, but also regulates its plasma membrane localization and multimerization. N-terminus of TRPC1 interacts specifically with caveolin-1 protein and disruption of TRPC1-Cav1 interaction, significantly reduces TRPC1 staining in the plasma membrane and calcium influx across the membrane. C-terminus of TRPC1 interacts specifically with a calcium sensor protein "calmodulin". Expression of the C-terminus truncated TRPC1 protein attenuated Ca^{2+} dependent inactivation. These data demonstrate that calmodulin interaction with TRPC1 is required for feedback inhibition of the TRPC1 channel. To understand the mechanism of activation of these TRP channels, we show here that TRPC3 (another member of the TRP superfamily) interacts with VAMP2 and ?SNAP proteins both in neuronal and epithelial cells. Imaging of GFP-TRPC3 demonstrated localization in the plasma membrane and mobile intracellular vesicles. There was significant recovery of GFP-TRPC3 after photobleaching in the plasma membrane region which was blocked by brefeldin-A, BAPTA and tetanus toxin. These treatments also decrease surface expression of TRPC3 protein. Importantly, activation of TRPC3 channel by G-protein coupled receptor agonist increased surface expression of TRPC3 protein which was attenuated by tetanus toxin suggesting that VAMP2-dependent mechanism is involved in generation of functional TRPC3 channels in the plasma membrane. Thus overall we can conclude that regulation of TRP proteins is mediated via specific interaction with key proteins. Disruption of any of these interactions significantly alters its function as well as its regulation.

BCL-G IN APOPTOSIS AND CHEMORESPONSE OF PROSTATE CANCER

Bin Guo

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Background: Cytogenetic deletions on chromosome 12p12.3 are common, recurring alterations found in prostate cancer patients, with loss of heterozygosity (LOH) at this region identified in 47% of patients who died of prostate carcinoma. LOH in the same region in a variety of other solid tumors suggests the presence of a tumor suppressor gene. Fluorescence in situ hybridization analysis further refined the commonly deleted segment to a 600 kb region, which encodes a total of seven putative genes. Bcl-G is a novel pro-apoptotic member of the Bcl-2 family proteins. This principle investigator (PI) has cloned the Bcl-G gene and performed initial characterization of its function in apoptosis regulation. The PI has also located Bcl-G gene to chromosome 12p12.3, right in the middle of the 600 kb LOH region. The pro-apoptotic activity of Bcl-G suggests that it is a good candidate for the tumor suppressor in chromosome 12p12.3.

Objective/Hypothesis: The pro-apoptotic function of Bcl-G may be responsible for its potential tumor suppressing activity in prostate cancer. My hypothesis is that Bcl-G is a key regulatory protein responsible for apoptosis induction in prostate cancer cells. Loss of Bcl-G function renders prostate cancer cells resistant to apoptotic signals. Prostate cancer cells may employ different strategies to abolish the function of Bcl-G and evade apoptosis.

Specific Aims: (1) To determine the function of Bcl-G in apoptosis of prostate cancer cells. (2) To investigate the molecular mechanism by which BCL-G regulates apoptosis in prostate cancer cells. (3) To investigate how prostate cancer cells abrogate the pro-apoptotic function of Bcl-G. (4) To determine whether Bcl-G can be used as biomarker to predict clinical response of prostate cancer.

Study Design: The function of Bcl-G will be investigated by establishing and studying prostate cancer cell lines stably expressing Bcl-G and Bcl-G targeting siRNA, respectively. To investigate the apoptosis mechanism of Bcl-G, a yeast-based system including the use of functional cloning and two-hybrid screening methods will be employed to identify proteins that are involved in Bcl-G's proapoptotic action. The Bcl-G inactivating mechanism will be examined by analysis of clinical prostate cancer samples for mutations in Bcl-G gene (by SSCP) and DNA methylation in the Bcl-G promoter (by methylation-specific PCR). Other potential inactivating mechanisms such as Bcl-G sequestering proteins and post-translational modifications of Bcl-G will also be explored. Clinical patient samples will be examined by immunohistochemistry to evaluate the correlation between Bcl-G expression and clinical response to chemotherapy.

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Acknowledgement

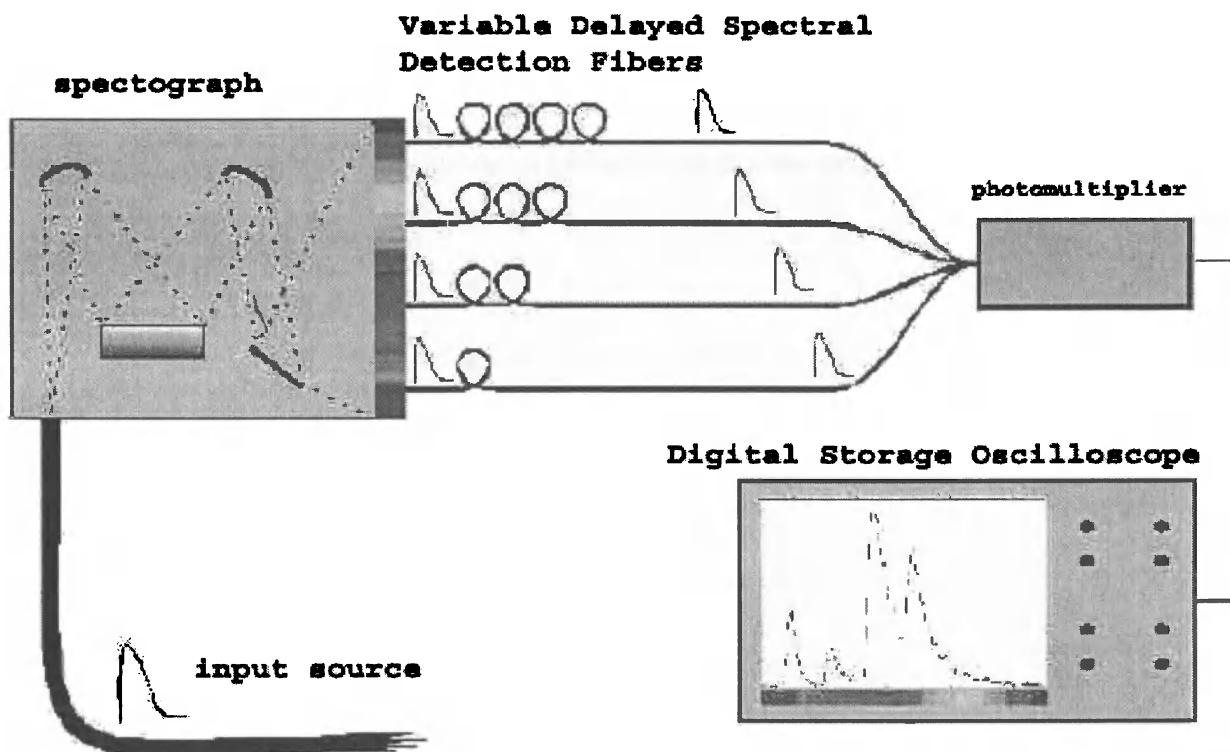
The project was supported by NIH Grant Number P20 RR-16471-03* from the BRIN Program of the National Center For Research Resources

NOVEL FLUORESCENCE METHODOLOGY FOR UNDERGRADUATE BIOMEDICAL RESEARCH

Thomas P. Gonnella
Mayville State University, Mayville, ND

Our research group has implemented novel fluorescence measurement technology developed by our collaborators at Dakota Technologies, Inc. Fluorescence emission is excited by short pulses of light from a microchip laser and complete fluorescence decay waveforms are recorded for every excitation pulse. A wavelength-time matrix (WTM) consists of a series of fluorescence decay waveforms at multiple emission wavelengths. The information contained within the WTMs is relevant to many biomedical research applications, including DNA sequencing, SNP detection, multiplex PCR, and drug-target binding.

Near-term goals of the Mayville State research group are demonstrating the value of WTMs for mixture analysis and fluorescence background suppression. The schematic shown below illustrates our initial approach to rapidly generating WTMs. In this presentation, I will present preliminary data acquired with an improved and more versatile hardware design that is better suited for undergraduate research. During the past year, we have characterized the fluorescence lifetime properties of a set of commercially available fluorescence probe dyes. Lifetime accuracy and precision are substantially improved over our results from a year ago. We are now generating fluorescence polarization data, which provides an excellent way to monitor binding of small molecules to proteins and nucleic acids. I will conclude the presentation by summarizing lessons learned during BRIN and our transition plan into INBRE. The project described was supported by NIH Grant Number P20 RR-16471-03 from the BRIN Program of the National Center for Research Resources.



GREEN CHEMISTRY: ONE POT SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES

Mikhail M. Bobylev * and Robin Gonzalez

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Formamides are a novel group of fungicides discovered by Bobylev et al at the All-Union Research Institute of Plant Protection Chemicals, Moscow, Russia. One of the leading candidates among novel formamide fungicides appeared N-[1-t-butyl-3-(2,4-dichlorophenyl)-2-propenyl-1]-formamide (**I**). The method of obtaining **I** comprised a two-step process that included aldol condensation followed by reductive amination via Leuckart reaction. In this work a new low waste one pot process for the synthesis of **I** is developed. In this process aldol condensation is carried out without solvent, and the reaction mixture is immediately subjected to Leuckart reaction. The new one pot process produced significantly higher yield of **I** compared to the two step process. The process can be used for the production of large quantities of **I** necessary for biological tests, and is suitable for the preparation of structurally diverse series of analogs.

CILIARY NEUROTROPHIC FACTOR IS UPREGULATED DURING AXONAL SPROUTING IN THE RAT
MAGNOCELLULAR NEUROSECRETORY SYSTEM

John Watt*

Department of Anatomy & Cell Biology, University of North Dakota, School of Medicine and Health Sciences, Grand
Forks, ND 58202

The response of the magnocellular neurosecretory system (MNS) to partial denervation is characterized by an initial period of axonal degeneration followed by the near complete restoration of axon density within the neural lobe (NL) by 30 days post-denervation (PD). We have utilized this preparation to investigate the expression of neurotrophins during the well defined periods of cellular and axonal degeneration and axonal sprouting. In this regard, ciliary neurotrophic factor (CNTF) has been reported to act as a survival and sprouting factor for axotomized magnocellular neurons *in vitro*. These observations have led us to investigate the potential role for CNTF, and its cellular sources, during collateral axonal sprouting in the MNS of the rat *in vivo*. Within the axotomized hypothalamic supraoptic nucleus (SON), a significant increase in the intensity and extent of CNTF immunoreactivity (CNTF-ir) was observed between 1 and 10 days PD. In the contralateral non-axotomized SON a significant but less pronounced increase was also observed over the same period. Subsequent dual-label fluorescence confocal microscopy demonstrated that the CNTF-ir within the SON is localized exclusively to astrocytes. *In situ* hybridization histochemical analysis (ishh) confirmed an upregulation of CNTF message had occurred concomitantly with the increased CNTF-ir. additionally, ish studies show a distinct upregulation of the CNTF receptor alpha in the non-axotomized, sprouting SON. Within the denervated NL where axonal sprouting occurs a similar increase in CNTF-ir was observed. Subsequent stereometric assessment of the numbers of CNTF-ir cells demonstrated a highly significant increase by 3 days PD reaching 130% of control values by 10 days post-denervation. The cell numbers remained elevated at 30 days PD suggesting that CNTF may play a role in the sprouting process within the axon terminal field. However, in the NL the CNTF-ir cells did not co-localize with GFAP indicating CNTF is not expressed by resident pituicytes. The morphological characteristics of the CNTF-ir cells in the NL suggests a perivascular phenotype. Studies are currently underway to unequivocally establish the phenotype of these cells. These studies were supported by BRIN-NCRR- P20-RR16471 and NIH RO3-MH64171-01 to JAW.

INDIVIDUAL DIFFERENCES IN HUMAN ALCOHOL RESPONSES

Shirley Cole-Harding*, Alana Tergeson & Clare Pettis

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Robinson and Berridge (1) defined incentive sensitization as an increasingly heightened “desire” for a drug after exposure to that specific drug. This idea was based on cocaine research using rats. The main purpose of this study is to test incentive sensitization to alcohol in humans.

Variability in the degree of incentive sensitization was observed in the rat studies. We are exploring factors, such as family history, drinking history and their interaction, that may cause variation in the degree of incentive sensitization in a human sample. Our hypothesis is that the incentive value of alcohol will be positively correlated with binge drinking and heavier drinking, especially in those who have family histories of alcoholism. Specifically, we predict that the greatest proportion of variance in measures of incentive value such as mood change, measures of liking the alcohol effects and wanting more, as well as changes in physiological measures, will be explained by the interaction of drinking history variables with family history of alcoholism.

This study’s design is distinctive in several ways:

- A heterogeneous sample of social drinkers is being tested. This allows a multiple regression analysis to explore the influence of family history and drinking history on alcohol responses.
- A naturalistic environment is used. Many previous studies have attempted to control the environment to the point that the responses to alcohol have no environmental validity, i.e., they do not resemble the real world of social drinking.
- Both genetic factors (family history) and experiential factors (drinking history) are included in the same study. Most research looked at one or the other.
- A variation on the balanced placebo design is used, along with a within-subjects design, in order to estimate the effects of conditioned tolerance and expectancy, compared to unconditioned alcohol responses and a control placebo condition. These conditions have usually been measured with between-subjects designs, which do not allow responses to be corrected with (have partialled out) the appropriate control conditions.
- Low doses of alcohol (.4 g/kg) are used. Many studies have used doses of alcohol that are high enough to produce intoxication. Normal social drinkers do not regularly drink that much, and measures of “liking” the effects are not meaningful.
- A battery of dependent variables is being used, in order to assess responses in as many behavioral and physiological domains as is practical within the 15 minute window necessitated by changing blood alcohol levels. We will measure the alcohol responses immediately after the subjects finish drinking in order to catch conditioned alcohol responses such as expectancies, as well as at other time periods during the blood alcohol curve.

In this preliminary report, we will present the results from 9 subjects. Although the sample is not large enough to do a complex regression analysis, we will present comparisons of the different conditions of the balanced placebo design. Correlations among predictor variables and selected dependent variables will also be presented.

The project described was supported by NIH Grant Number P20 RR-16471-03 from the BRIN Program of the National Center For Research Resources.”

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ATTENTIONAL ORIENTING EFFECTS OF GAZE DIRECTION CUES

Chris Kelland Friesen

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My research has demonstrated that people will shift their attention automatically to a location gazed at by a cartoon face presented on a computer screen — even when the face’s gaze direction provides no meaningful or useful information. This automatic orienting may occur because information from faces and eyes is so fundamentally important to humans as social creatures, and it may be a unique form of attentional orienting. Indeed, I have found that social orienting exhibits some important differences from the types of orienting traditionally studied by attention researchers using stimuli that are not socially or biologically relevant. In this talk I will briefly describe several studies with adults, children, and colostomy (“split-brain” patients) that investigated the properties of gaze-triggered orienting and explored its relationship to traditional reflexive and voluntary orienting, as well as to face processing. The primary goal of my research program is to continue to investigate the attentional effects of directional social cues, and to identify the brain mechanisms that subserve this form of orienting. For example, currently underway is a follow-up to a study that established that nonpredictive gaze direction cues can trigger an attention shift *even when participants are unaware of having seen a gazing face*. This finding provides strong evidence for the reflexivity of the attentional effects of gaze, and it also suggests that social orienting is not occurring by way of the cortical brain pathways that underlie consciously directed voluntary orienting. I plan to continue to with this line of research using a variety of techniques, including eye movement monitoring, electrophysiological recording, and neuropsychological patient testing. I anticipate that my research will lead to a refinement of our theories of human spatial attention in general, and will contribute to our understanding of the neural processes that underlie shifts of spatial attention in response to biologically and socially relevant directional information in the visual environment.

The project described was supported by NIH Grant Number P20 RR-16471-03 from the BRIN Program of the National Center For Research Resources and the Natural Sciences and Engineering Research Council of Canada.

CORRECTING NORMATIVE MISPERCEPTIONS AS A STRATEGY FOR REDUCING HEAVY DRINKING AMONG COLLEGE STUDENTS

Clayton Neighbors
North Dakota State University

Heavy drinking among college students continues to be an epidemic problem. Approximately 45% of college students nationwide report heavy episodic drinking, which has been linked to numerous consequences including academic problems, sexual assault, and death. In North Dakota, heavy drinking rates have been consistently higher than the national rates, making this a particularly salient issue in our region. This research evaluates a brief computer based intervention designed to reduce consumption among heavy drinking college students. The intervention incorporates a social influence model and is based on the finding that college students, especially heavy drinking college students, overestimate the alcohol consumption of their peers. Overestimating the prevalence of peers' consumption has in turn been associated with heavier drinking and negative alcohol related consequences. This research evaluates the efficacy of computer delivered personalized normative feedback in correcting misperceptions of drinking norms and thereby reducing consumption. Personalized normative feedback consists of information regarding one's own drinking, one's perceptions of others' drinking, and others' actual drinking. Data will be presented from two longitudinal studies evaluating the efficacy of this intervention. Participants were randomly assigned to receive or not receive personalized normative feedback immediately following a baseline assessment. Results indicate that personalized normative feedback has large effects on normative misperceptions and small but significant effects on alcohol consumption for up to six months. Merits and limitations of the approach will be discussed.

The project described was supported by NIH Grant Number P20 RR-16471-03* from the BRIN Program of the National Center For Research Resources."

A. Rodger Denison Student Research Competition

COMMUNICATIONS

UNDERGRADUATE DIVISION

Undergraduate Denison Competition**Session Chair: Kim Vonnahme, NDSU**

Denison Award Judges: **Dr. Kim Vonnahme, NDSU**
 Dr. Holly Brown-Borg, UND
 Dr. Hilde van Gijssel, VCSU

- 10:50-11:10 am April Tepfer*, Mary Lynn Johnson, Kimberly Petry, Ewa Borowczyk, Pawel Borowicz, Justin S. Luther, Disha Pant, Joan D. Beckman, Kimberly A. Vonnahme, Dale A. Redmer, Lawrence P. Reynolds, and Anna T. Grazul-Bilska **IN VITRO EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR (FGF-2) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ON PROLIFERATION OF SKIN FIBROBLASTS FROM DIABETIC AND NON-DIABETIC MICE: IMPLICATIONS FOR WOUND HEALING.** Department of Animal and Range Sciences¹and Cell Biology Center², North Dakota State University, Fargo, ND
- 11:10-11:30 am Katherine M. Splichal*, Shane M. Meyer, Krysta Mann, and Garl K. Rieke **AB₄₂ HAS POTENTIAL DIRECT AND INDIRECT EFFECTS ON MITOCHONDRIA LEADING TO ALZHEIMER'S DISEASE** Department of Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND
- 11:30-11:50 am Mikhail M. Bobylev, Christopher L. Aaron*, Marisa A. Upton **OPTIMIZATION OF LEUCKART REACTION FOR THE SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES** Department of Chemistry, Minot State University, Minot, ND
- 1:00-1:20 pm Jessica D. Hamilton* and Heidi J. Super **MOLECULAR ANALYSIS OF THE MLL BREAKPOINT REGION IN LEUKEMIA: A STUDY OF BINDING OF DNA-TOPOISOMERASE II AND/OR OTHER NUCLEAR PROTEINS TO SPECIFIC DNA BREAKPOINT FRAGMENTS** Department of Biology, Minot State University, Minot, ND
- 1:20-1:40 pm Brad D. Hohnadel* and Andre W. DeLorme **EXPLORING THE POSSIBILITY OF AQUATIC MACROINVERTEBRATE REFUGIA IN THE TRIBUTARIES OF THE UPPER SHEYENNE RIVER** Department of Biology, Valley City State University, Minot, ND
- 1:40-2:00 pm Cindy M. Anderson, Faye Lopez*, Hai-Ying Zhang, Kristin Pavlish and Joseph N. Benoit **INTRAUTERINE FETAL GROWTH RESTRICTION AND MATERNAL HYPERTENSION IN A RAT MODEL OF REDUCED PLACENTAL UTERO-PLACENTAL PERFUSION** Department of Pharmacology, Physiology and Therapeutics, University of North Dakota, Grand Forks, ND
- 2:00-2:20 pm Joshua Steffan*, Tyler Burgess, and Lynn Burgess **CHANGES TO THE TIMBERLINE AND THE GROWTH OF SUBALPINE TREES AT HIGHER ELEVATIONS IN RESPONSE TO GLOBAL CLIMATIC CHANGE** Department of Natural Sciences, Dickinson State University, Dickinson, ND
- 2:20-2:40 pm Joan D. Beckman*, Larry P. Reynolds, Anna T. Grazul-Bilska, James D. Kirsch, Kim C. Kraft, Kimberly D. Petry, Corrie B. Redmer, Mary Lynn Johnson, and Dale A. Redmer **ANGIOGENIC FACTOR EXPRESSION IN PERICYTES ISOLATED FROM OVINE CORPUS LUTEUM** Department of Animal and Range Sciences¹and Cell Biology Center², North Dakota State University, Fargo, ND

Undergraduate Denison Competition**Session Chair: Kim Vonnahme, NDSU**

Denison Award Judges: **Dr. Kim Vonnahme, NDSU**
 Dr. Holly Brown-Borg, UND
 Dr. Hilde van Gijssel, VCSU

- 3:00-3:20 pm Mary L. Jaros-Gourneau* and Vasyl Tkach **MOLECULAR SYSTEMATIC STUDY OF NEASCUS-TYPE METACERCARIAE FROM THE PHOXINUS EOS-NEOGAEUS GYNOGENETIC CYPRINID COMPLEX IN VOYAGEURS NATIONAL PARK, MINNESOTA** Department of Biology, University of North Dakota, Grand Forks, ND
- 3:20-3:40 pm Ryan Klapperich* and Scott Korom **DENITRIFICATION IN THE AQUIFER ZONE: CORRELATION OF ¹⁵N ISOTOPIC ENRICHMENT AND FIRST-ORDER RATE CONSTANTS** Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND
- 3:40-4:00 pm Rachel Duerre*, Mary Lynn Johnson, Kimberly Petry, Pawel Borowicz, Justin S. Luther, Joan D. Beckman, Robert M. Weigl, Dale A. Redmer, Lawrence P. Reynolds and Anna T. Grazul-Bilska **PROLIFERATION OF SKIN CELLS DURING WOUND HEALING IN DIABETIC AND NON-DIABETIC MICE: EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR (FGF-2) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) TREATMENT IN VIVO** Department of Animal and Range Sciences and Cell Biology Center, North Dakota State University, Fargo, ND
- 4:00-4:20 pm Shanna A. Mazurek*, Richard R. Barkosky, Christopher P. Keller **THE EFFECT OF HYDROQUINONE ON ROOT MEMBRANE POTENTIALS AND GROWTH OF THE COMMON BEAN (*PHASEOLUS VULGARIS*)** Department of Biology, Minot State University, Minot, ND
- 4:20-4:40 pm John Totenhagen*, Bruce Wheeler, and Dan Ewert **EFFECTS OF SIMULATED MICROGRAVITY ON CARDIAC VISCOELASTIC PARAMETERS IN RHESUS MONKEYS** Department of Electrical and Computer Engineering North Dakota State University, Fargo, ND
- 4:40-5:00 pm Michele Fronk* and Andre DeLorme **SURVEY OF MICROCADDISFLIES IN MERCER COUNTY, NORTH DAKOTA** Department of Science, Valley City State University, Valley City, ND

IN VITRO EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR (FGF-2) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ON PROLIFERATION OF SKIN FIBROBLASTS FROM DIABETIC AND NON-DIABETIC MICE: IMPLICATIONS FOR WOUND HEALING

April Tepfer¹, Mary Lynn Johnson^{1,2}, Kimberly Petry¹, Ewa Borowczyk¹, Pawel Borowicz¹,
Justin S. Luther¹, Disha Pant¹, Joan D. Beckman¹, Kimberly A. Vonnahme¹,
Dale A. Redmer^{1,2}, Lawrence P. Reynolds^{1,2}, and Anna T. Grazul-Bilska^{1,2}

Department of Animal and Range Sciences¹, Cell Biology Center², North Dakota State Univ., Fargo, ND 58105

The wound healing process involves interactions of several cell types with extracellular matrix components and chemical mediators. Wound healing is divided into three parts: inflammation, proliferation and maturation. During the proliferative phase, several cell types including fibroblasts (the primary connective tissue cell type), keratinocytes (epidermal skin cells) and endothelial cells (the primary vascular cell type) proliferate dramatically and are crucial in promoting healing of the wound. Fibroblasts synthesize key extracellular components (e.g., collagens, proteoglycans, fibronectin and elastin) that form a scar to replace the damaged tissue. Fibroblast function is regulated by numerous growth factors including FGF-2 and VEGF. To evaluate the effects of these growth factors on fibroblast proliferation, skin was collected from diabetic (n=6) and non-diabetic (n=5) mice. Skin tissue was cut into pieces, incubated for 30 min with collagenase, and isolated cells were cultured in DMEM media containing 20% serum for about 2-3 weeks. Media were changed every 2-3 days, and when confluent cells were transferred from smaller (35 mm) to larger (60 mm) petri dishes. After the third or fourth passage, cells were collected, counted and frozen. For proliferation assay, frozen cells from each mouse were thawed and cultured in serum-containing media for 7-14 days in T-25 Falcon dishes. Cells were trypsinized, counted and plated in 96-well plates at a concentration 4×10^3 cells/100 ml of serum-containing media. A standard curve was included on each 96-well dish consisting of several concentrations of cells (0 , 1×10^3 , 2×10^3 , 4×10^3 , 6×10^3 , 8×10^3 , and 10×10^3 cells/well). After 24 h, medium was changed to serum-free medium containing high (5 mg/ml) or low (1 mg/ml) concentration of glucose and with or without 0.1, 0.3, 1, 3, 10, 30 or 100 ng/ml FGF-2, VEGF or FGF-2 + VEGF (Biosource, CA). After 72 h incubation with treatments, proliferation cell reagent (Roche, Germany) was added, and 3 h later absorbance was measured by using a microplate reader. Data were then analyzed statistically. Overall, the rate of proliferation was less ($P < 0.01$) for fibroblasts from diabetic than non-diabetic mice, and proliferation was reduced ($P < 0.01$) in media containing high glucose concentration for both mouse types. FGF-2 and FGF-2 + VEGF, but not VEGF, increased ($P < 0.05$) proliferation of diabetic and non-diabetic fibroblasts in low glucose media, but not in high glucose media. These data demonstrate that 1) the rate of proliferation of diabetic fibroblasts is reduced compared with non-diabetic fibroblasts, 2) a high glucose level reduces FGF-2 stimulatory effects on fibroblast proliferation, and 3) VEGF does not affect fibroblast proliferation. These data suggest that the FGF-2 receptor-mediated cascade that leads to cell proliferation is suppressed by high glucose level. Identifying the mechanisms of FGF-2 effects on fibroblast function may improve the clinical response of the diabetic wound to FGF-2. *Supported by grants from NDSU Research Development Support Program and ND EPSCoR.*

AB₄₂ HAS POTENTIAL DIRECT AND INDIRECT EFFECTS ON MITOCHONDRIA
LEADING TO ALZHEIMER'S DISEASE

Katherine M. Spichal, Shane M. Meyer, Krysta Mann, and Garl K. Rieke
Department of Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND 58202

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases impacting the elderly; therefore, it is imperative to find out what factors may be the cause. From pathologic reviews in human brain, hallmarks of AD include extracellular plaques and intracellular neurofibrillary tangles (breakdown of cytoskeleton) in parts of the brain involved with cognition and memory. The extracellular plaques are formed in part from aggregates of the amyloid beta protein (AB) secreted by nerve cells, which may play a causative role in AD. Plaques and tangles seen in AD may be secondary to the actual intracellular events induced by AB fragments within cells which lead to cell death and AD.

AB originates from the amyloid precursor protein (APP), which is synthesized in the rough endoplasmic reticulum (RER). Enzymes, specifically the beta and gamma secretases, cleave APP to a number of short fragments, primarily 40 or 42 amino acids in length. The 42 amino acid long fragment (AB₄₂) is believed to be the neurotoxic form. Research has shown that interaction of AB₄₂ with intracellular membranes affords the peptide opportunity to induce apoptotic cell death, particularly in hippocampus and cerebral cortex. Such pathology leads to changes commonly manifested in AD including memory loss, personality changes, and cognitive deterioration.

Using an AD rat model, our experiments have demonstrated the presence of AB₄₂ in the endoplasmic reticulum, on mitochondria and the nucleus. The focus was to determine how AB₄₂ interacts with these various intracellular membranes. Of particular interest are mitochondria, which regulate metabolic respiration via ATP production, and control apoptosis by either facilitating or inhibiting this form of cell death.

Chloroquine challenge was used to raise the intracellular levels of AB₄₂ in rat brain, and control brains were used to compare results. Differential centrifugation of brain homogenates on a percoll gradient was used to isolate mitochondria. Mitochondria were stained with Mitotracker red (chloromethyl-X-rosamine, CMX), then viewed by confocal microscopy to detect CMX fluorescence. High CMX fluorescence indicates the viability of mitochondria (an indirect measure of normal transmembrane potential). Reduction in CMX response under experimental conditions (increased intracellular AB₄₂) suggests that AB₄₂ damages the mitochondria (loss of transmembrane potential) a condition that favors apoptosis.

To test for the induction of apoptosis by intracellular AB₄₂, similarly isolated mitochondrial preparations were fixed in 4% paraformaldehyde/0.5% glutaraldehyde and embedded in Lowicryl. Thin sections (60nm) cut from the experimental and control mitochondrial pellets were immunolabeled with antibodies that target antigens active in apoptotic cascades. The antigens Bax, cytochrome c and caspase 3 were labeled with primary antibodies and secondary antibodies conjugated to gold particles (18nm) and viewed with the electron microscope (EM immunogold technique). Gold particles observed in electron micrographs of experimental mitochondria suggest that AB₄₂ acts on mitochondria to induce activation of these apoptotic favoring compounds. AB₄₂ induces lipid peroxidation and one product of this process, 4-Hydroxynonenal (4-HNE), directly attacks mitochondria, and thereby plays a central role in oxidative stress and cell death.

The outcome of this research thus far has shown that the AB₄₂ fragment is found inside of hippocampal pyramidal cells, specifically on mitochondria, and induces apoptosis through various means. The research is discovering possible mechanisms of how AB₄₂ kills these cells, whose loss in humans contributes to the profound memory impairments of AD.

OPTIMIZATION OF LEUCKART REACTION FOR THE SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES

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Formamides are a novel group of fungicides discovered by Bobylev *et al.* Some of these novel fungicides were obtained by reductive amination via Leuckart reaction, where formamide was used both as a reagent and solvent. During the early development stage it was discovered that the reaction proceeded faster and produced better quality products with low grade technical formamide than with highly purified reagent grade formamide. Since technical grade formamide may contain rather substantial amounts of water, ammonia, and formic acid, it was assumed that a certain amount of either of these components should be used as an additive to the reagent grade formamide to facilitate the reaction. In this work the optimal concentrations of the additives were determined and the reaction procedure was made suitable for the preparation of a structurally diverse series of analogs, and for the production of large quantities of the samples necessary for further testing.

MOLECULAR ANALYSIS OF THE *MLL* BREAKPOINT REGION IN LEUKEMIA: A STUDY OF BINDING OF DNA-TOPOISOMERASE II AND/OR OTHER NUCLEAR PROTEINS TO SPECIFIC DNA BREAKPOINT FRAGMENTS

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A common phenomenon of leukemia cells is specific, non-random, reciprocal chromosome translocations, in which two non-homologous chromosomes exchange pieces of DNA with specific breakpoints in each chromosome. As a result of the translocation, the regulation of the gene at the breakpoint is either altered, creating too much or too little of the gene product, or a whole new fusion gene can be created, producing a new fusion gene product, with altered activity. Either of these scenarios can set off malignant transformation of cells and contribute to the development of leukemia. Many chromosomal translocations correlate with a specific subtype of leukemia. The *MLL* gene is located at the chromosome breakpoint in translocations on the long arm of chromosome 11 (band q23). The *MLL* gene is involved in translocations with 45 other chromosomal regions including specific identified genes on chromosomes 4, 6, 9, 19, X. In all studies so far the 5' end of the *MLL* gene becomes fused to the 3' end of another gene creating an oncogenic *MLL* fusion gene and protein. The *MLL* translocation breakpoint is 8000 base pairs in length and contains exons 5-11 of the *MLL* gene. *MLL* translocations occur in both acute childhood leukemias and in adult leukemias that arise following treatment of a primary tumor. Therapy-related leukemias develop following treatment with a cellular enzyme DNA-topoisomerase II (topo II) inhibiting drug.

The restricted size of the *MLL* breakpoint region, only 8kb, suggests that the mechanism of the translocations involve a specific DNA sequence(s) in the region. Several studies have suggested that the *MLL* breakpoint region is prone to cleavage by topo II, but the region has not yet been analyzed for direct binding of topo II or other nuclear proteins. We have used a standard gel shift mobility assay to determine if topo II protein and/ or nuclear proteins normally bind to the *MLL* breakpoint region. Using Polymerase Chain Reaction (PCR) we amplified three 200 base pair DNA fragments from the breakpoint region. Two of the fragments, probes A and C, lie at the extreme 5' and 3' ends of the breakpoint region, respectively. Our third probe, B, is located at a proposed cleavage site for topo II in the *MLL* gene. Probes A, B, and C were labeled for chemiluminescent detection and then incubated with nuclear proteins from a human leukemia cell line, REH, in separate experiments. The binding of nuclear proteins to the probes was determined by retarded migration (shifting) of probes during polyacrylamide gel electrophoresis.

Our analysis indicates that probe A specifically binds one or more nuclear proteins from the REH cell line. In a modified "supershift" assay we tested the binding of the topo II antibody by adding a polyclonal antibody to topo II to the reaction. We preliminarily noted additional shifting of the probe, suggesting that the protein topo II is binding to probe A. Since topo II antibody binding was inconclusive and suitable antibody controls were not available, we took a more direct approach of using the purified topo II protein in the electrophoretic mobility shift assay. No shifting was detected using probe A and purified topo II, indicating a different nuclear protein is binding this region of the *MLL* breakpoint. From previous studies we had predicted that probe B would possibly bind topo II directly, but after many trials we did not detect binding of REH nuclear proteins to either probes B or C.

The correlation of topo II binding and *MLL* translocations is still a possibility. There are many more regions of the 8kb *MLL* breakpoint region yet to be analyzed for binding of topo II and/or nuclear proteins. Future studies will also focus on identification of the nuclear protein binding probe A.

EXPLORING THE POSSIBILITY OF AQUATIC MACROINVERTEBRATE REFUGIA IN THE TRIBUTARIES OF THE UPPER SHEYENNE RIVER

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INTRODUCTION

In discussions of the effects of the proposed Devils Lake outlet on the Sheyenne River, the possibility of eradication of much the present macroinvertebrate community is a concern (1). In response, the idea of re-colonization from tributaries, once the outlet is closed, has been raised. In this scenario the tributaries of the Sheyenne would act as areas of refugia for macroinvertebrates. To determine the viability of this refugia concept in the upper Sheyenne river system we sampled the macroinvertebrates of the tributaries of the Sheyenne River and the Sheyenne River itself. We propose if the river and its tributaries share the same taxa, the tributary may well serve as a refuge. However, if Sheyenne River taxa are absent from the tributaries, it seems unlikely that they could displace the existing tributary organisms and use it as refugia.

METHODS

Six pairs of sites were sampled in the Sheyenne River system. Each pair of sites was composed of a tributary near its confluence with the Sheyenne River and a spot in the Sheyenne River near the tributary. Sampling was completed in July and August of 2003. Aquatic D-frame nets were used as part of sampling techniques laid out in Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (2). Samples were preserved in 70% ethanol. After identification of the macroinvertebrates, the taxa list from each tributary was compared with the taxa list from the corresponding Sheyenne River site.

RESULTS

An average of 43% of Sheyenne River macroinvertebrates were also found in the tributary nearest the sampling site (values ranged from 24% to 60%). If only EPT taxa are considered, the average drops to 38% (ranging from 13% to 60%). One group of macroinvertebrates of special concern are the freshwater Unionoid mussels. Unionoid mussels were collected in 4 out of 6 Sheyenne River sites, but were not found in any of the tributaries.

DISCUSSION

The refugia concept seems in doubt for a majority of the macroinvertebrates in the Sheyenne River. An average of 57% of the macroinvertebrates from the Sheyenne do not currently live in the tributaries. If water conditions change unfavorably in the Sheyenne River, these organisms are unlikely to displace the current residents of the tributaries. The absence of these organisms in the tributaries indicates they are not well suited to those conditions, or they are unable to compete successfully with the current macroinvertebrates in the tributaries.

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INTRAUTERINE FETAL GROWTH RESTRICTION AND MATERNAL HYPERTENSION IN A RAT MODEL OF
REDUCED PLACENTAL UTERO-PLACENTAL PERFUSION

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Pregnancy is a physiological process that requires a synchronized adaptation of multiple organ systems. Increased blood flow to the uterus and cardiac output are hallmarks of the characteristic hemodynamic alterations in normal pregnancy [1]. Inadequate remodeling of the resistance arteries supplying the uterus and placenta results in a reduction of perfusion to these organs through the establishment of a high resistance/low perfusion system [2]. Intrauterine fetal growth restriction as a result of decreased perfusion to the fetal/placental unit is a frequent consequence. Decreased utero-placental perfusion in the early days of pregnancy has also been implicated in the etiology of preeclampsia [3]. Reduction in tissue perfusion systemically precedes clinical manifestations of preeclampsia [4]. Clinical signs of preeclampsia from endothelial-derived vasospasms become evident in later pregnancy most often during the third trimester, and include hypertension and proteinuria [5].

Individual female Sprague-Dawley rats weighing between 250-300 g were paired with a single male and mated overnight. Identification of the vaginal plug was designated as gestational day 1. Systolic blood pressures were measured in conscious, restrained rats using an automated system with a photoelectric sensor (IITC, Woodland Hills, CA) linked to a dual channel recorder (Linseis, IITC, Woodland Hills, CA), tail cuff and sphygmomanometer which previously have been demonstrated to be closely correlated with direct arterial measurements [6]. Reduced uterine perfusion pressure (RUPP) was surgically induced in female rats from the experimental group (n=18) on gestational day 14. After preemptive analgesia, an abdominal midline surgical incision was made under anesthesia with 2% isoflurane delivered by an anesthesia apparatus. Following the midline incision, silver clips were placed on the lower abdominal aorta and both left and right ovarian arteries to reduce uterine perfusion by ~40%. Sham operations were conducted on normal pregnant rats in which the vessels were isolated but not clipped, serving as the control group (n=18). Six days after initial surgery, rats were anesthetized and euthanized. Fetal pups and placentas were removed and weighed.

The systolic blood pressure in the RUPP group (mean = 128 ± 3 mmHg; * p < 0.05) was significantly less as compared to sham-operated animals (mean = 137 ± 4 mmHg) during the pregestational period. During days 1-14 of gestation, there were no differences between the groups. Five days after reduction of utero-placental perfusion on gestational day 19, the RUPP group had a significant increase in systolic blood pressure (mean = 150 ± 3 mmHg; *** p < 0.001) as compared to control (mean = 117 ± 4 mmHg). The total maternal weight gain was determined for the entire pregnancy in both the RUPP (n=16) and sham-operated groups (n=16). Significant decreases in weight gain were evident by day 20 in the RUPP group (16.25 ± 5.1 g; *** p < 0.001) as compared to control (47 ± 3.8 g) and were reflected in total weight gain as well (RUPP 73.5 ± 6.4 g, ** p < 0.01; Sham 99.06 ± 4.7g). Weights and litter size were determined in RUPP (n=13) and sham-operated (n=15) animals on gestational day 20. Significant reductions in placental weight (p < 0.01), fetal weight (p < 0.01) and litter size (p < 0.05) were evident in the RUPP group as compared to control, verifying the existence of significant intrauterine fetal growth restriction secondary to reduced utero-placental perfusion.

The results of this study support the use of the RUPP model as an authentic representation of intrauterine fetal growth restriction and preeclampsia. Future directions will include the investigation of the long-term consequences of fetal exposure to reduced utero-placental perfusion.

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CHANGES TO THE TIMBERLINE AND THE GROWTH OF SUBALPINE TREES AT HIGHER ELEVATIONS
IN RESPONSE TO GLOBAL CLIMATIC CHANGE

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Global climatic changes have been in the news for several years and may be producing warmer annual temperatures. Trees are important in many aspects of our lives and are very temperature dependent. The timberline is the elevation at which temperature is the limiting factor for tree growth and development; therefore, our hypothesis was that global climatic temperature increases the growing season. Because of this, we predict that the timberline will increase in elevation due to warmer growing conditions. We found a mountain slope with adequate soil above the present timberline, little or no rockslides or late season snow accumulation, and was protected from any major wind damage, thus making temperature the greatest limiting factor. Our research location was in the Spirit Lake drainage in the Uinta Mountains, Ashley National Forest, in Daggett County, Utah, USA. This area was chosen because it is away from sources of urban, agricultural, or industrial pollution and heating, for these activities affect local environments. Starting at the highest tree, transient lines were ran every 30 meters down the mountain slope and 100 meters across on which the height, girth, and approximate age of the trees encountered were taken. Twenty replications were taken until mature, full-grown trees were encountered. It was found that very young trees, less than 12 years of age, comprised the top 100 meters of mountain slope. Comparing this elevation data to a forest service map revised in 1993, we was concluded that the timberline has increased in elevation 100 meters in the last 10 to 12 years, which is a very significant increase. However, further study must be done in other areas to examine whether this is occurring in multiple localities. Limiting the possibilities of other influences on tree growth remains a concern and must be managed to ensure accurate results and conclusions. This work was supported by the Explorer's Club of New York and by P20-16471 from the NCRR.

ANGIOGENIC FACTOR EXPRESSION IN PERICYTES ISOLATED FROM OVINE CORPUS LUTEUM

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The corpus luteum (CL) is a highly vascularized tissue, in which 50-70% of the tissue comprises microvascular pericytes and endothelial cells. Angiogenesis, the formation of new blood vessels, requires several key processes that are mediated by growth factors. Capillary pericytes express one of the major angiogenic growth factors, vascular endothelial growth factor (VEGF). Angiogenesis is associated with vasodilation, or relaxation of the vascular wall, a process that is mediated by nitric oxide (NO). In the CL, the microvascular endothelium has been shown to express endothelial NO synthase (eNOS), the enzyme responsible for the production of NO. Our previous research has demonstrated strong expression of VEGF in the capillary pericytes of the developing CL (Redmer *et al.*, Biol. Reprod. 65:879-889, 2001). VEGF targets endothelial cells to initiate angiogenesis and has also been shown to stimulate NO production through eNOS. Conversely, NO has been shown to upregulate VEGF expression by perivascular cells, including vascular smooth muscle cells and pericytes.

In order to investigate the relationship between angiogenic factors and eNOS, microvascular pericytes and endothelial cells were isolated from CL collected from superovulated ewes (n=5) nine days following the estrus cycle. The CL from each ewe were pooled and digested with collagenase. Endothelial cells were isolated by using BS-1 lectin-coated magnetic beads, and cultured in a selective medium containing DMEM, endothelial cell growth supplement, D-valine, heparin, and plasma-derived horse serum. Pericytes were isolated using Percoll gradients and were cultured in DMEM containing fetal bovine serum. Cell types were identified by using immunofluorescent staining for the pericyte cell marker smooth muscle cell alpha actin (SMCA), the endothelial cell marker Factor VIII, and the fibroblast cell marker Type IV collagen, as well as morphological comparisons with pure cultures of aortic vascular smooth muscle cells (AVSM) and aortic endothelial cells. Cell lines for luteal endothelial cells and luteal pericytes/vascular smooth muscle cells were successfully established.

Isolated pericytes were incubated with or without doses ranging from 0 to 10 mM of the NO-donor stock solution (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazene-1-ium-1,2-diolate (DETA-NO) or a control of diethylenetriamine (DETA) for 8 hr. Expression of mRNA for VEGF, fibroblast growth factor-2 (FGF-2), soluble guanylate cyclase (sGC, NO receptor), and the angiopoietins (ANG) 1 and 2 by these luteal pericytes was evaluated using real time RT-PCR. NO caused a twelve to thirteen fold increase ($p < 0.001$) in VEGF expression, a five-fold increase ($p < 0.01$) in FGF-2 expression, and a twelve to thirteen fold increase ($p < 0.06$) in ANG-2 expression over the DETA control treatments. The expression of sGC and ANG-1 in luteal pericytes was not affected by NO treatment. Our work using a microvascular model of luteal development, along with work by others using various tissues, indicates that there is a potentially complex set of paracrine interactions that occur between the endothelium and pericytes, perhaps including a series of positive feedback loops between NO and VEGF, NO and FGF-2, FGF-2 and VEGF, and VEGF and ANG-2. Combined, these data provide strong evidence that NO plays a role in the regulation of angiogenic factor expression by luteal microvascular pericytes.

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MOLECULAR SYSTEMATIC STUDY OF NEASCUS-TYPE METACERCARIAE FROM THE PHOXINUS EOS-NEOGAEUS GYNOGENETIC CYPRINID COMPLEX IN VOYAGEURS NATIONAL PARK, MINNESOTA

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INTRODUCTION

Fish are suitable hosts for a diverse fauna of parasites, which have been subjects of numerous studies investigating different aspects of their morphology, systematics, geographic distribution and host-parasite interactions. Diplostomid metacercariae (Platyhelminthes, Digenea, Diplostomiodea) that form *Neascus-type* cysts or “black spots” on the skin, fins and mouth of fish, are among the most common fish parasites found in North America. Because these so-called “black spots” are obvious and easy to count, they represent ideal subjects for population studies, and thus, allow for the processing of a large number of samples in a timely manner.

In this study, black spot parasites of the *Phoxinus eos-neogaeus* gynogenetic cyprinid complex, in Voyageurs National Park, were used to re-test the *Red Queen Hypothesis*, which suggest that sexual fish have an advantage over clonal fish regarding parasite loads. Until recently, reliable identification of these metacercariae was not possible. By using DNA analysis, conscientious results, for assessing precisely what metacercariae target the complex of interest, has been performed.

METHODS

Fish from the Gynogenetic Cyprinid complex were collected from 3 drainages in Voyageurs National Park, transported to the lab in oxygenated coolers, and examined. Following excystment of metacercariae, the specimens were placed in guanidine lysis buffer. If more than one specimen (of the same type) per fish was available, they were heat-killed, fixed in 70% EtOH, and prepared as whole mounts for the use of morphological examination. Specimens were processed for molecular analysis in the following steps: DNA extraction, PCR, sequencing, assembling and comparison of obtained sequences using assembling/phylogenetic software (Tkach & Pawlowski, 1999).

Complete ITS region and partial 28S gene sequences were obtained from each sample. These regions have been demonstrated to be among most suitable for differentiation among closely related taxa of digeneans (Littlewood & Johnston, 1995; Jousson et al., 1998; Tkach et al., 2000).

RESULTS AND DISCUSSION

Our results, contrary to some previous studies involving *Neascus-type* digenean larvae, or so-called “black spots,” which suggested homogeneity, prove these parasites to be heterogeneous. Because of this, data that constituted the basis of past research should be revised and re-evaluated. When distinguishing between different types of metacercariae, by morphological characteristics alone, three types of parasites were identified. However after DNA analysis, there proved to be four genetically different metacercariae that prey on the *Phoxinus eos-neogaeus* gynogenetic cyprinid complex in Voyageurs National Park.

By obtaining DNA sequences from adult digeneans and matching them with sequences from metacercariae we have obtained, we will be able to provide reliable taxonomic identification of the diplostomid larvae causing “black spots” on the skin of the *Phoxinus eos-neogaeus* gynogenetic cyprinid complex. Studies of *Neascus-type* larvae, from many more fish species in different regions, is necessary in order to better understand the diversity and distribution of various diplostomid species in the Northern part of the United States.

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AQUIFER DENITRIFICATION: CORRELATION OF ^{15}N ISOTOPIC ENRICHMENT AND FIRST-ORDER RATE CONSTANTS

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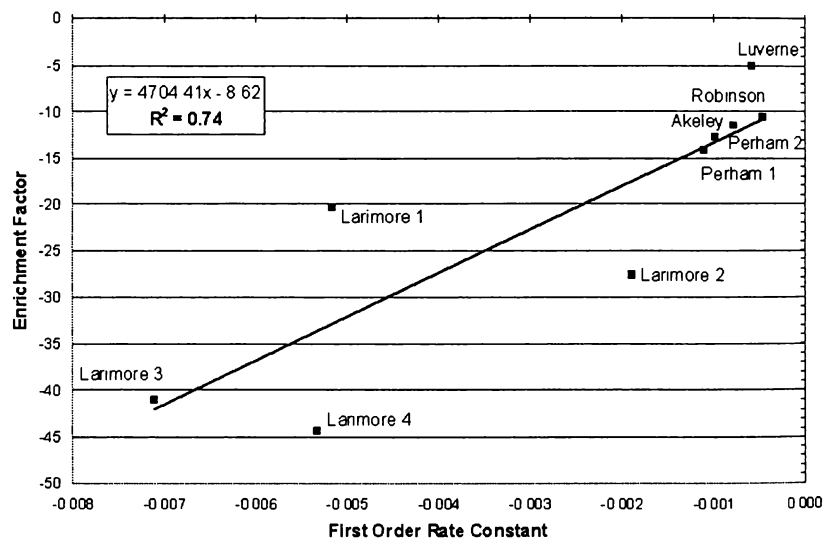
Denitrification is the process by which potentially dangerous nitrate is biogeochemically reduced into harmless nitrogen gas. This process is mediated by bacteria in the presence of suitable electron donors, such as organic carbon, sulfide, and ferrous iron. Due to its potential utility in the remediation of contaminated environments, the process of denitrification has received considerable attention. However, a relationship between the rate constant of the reaction and isotopic enrichment of ^{15}N , which occurs as a direct result of the chemical reaction, has received little consideration. A strong correlation between these two factors could lead to a techniques to estimate denitrification rates based on ^{15}N isotopic enrichment.

Mariotti, et al. (1) were the first researchers to investigate this relationship. After observing lab experiments, they proposed the hypothesis that a positive correlation exists between ^{15}N enrichment and first-order reaction rates. That is, higher rates of denitrification result in higher (less negative) values of isotopic enrichment and lower rates result in lower (more negative) values of isotopic enrichment. This hypothesis was tested using data from bromide tracer tests conducted since 1997 by Korom and his students.

Data were collected by use of a network of in situ mesocosms (ISMs) in eight aquifers across western Minnesota and eastern North Dakota. The ISMs are large hollow chambers which are emplaced into the aquifer sediments and allow for direct observation of in situ sediments during tracer testing. Experiments were run for a period of one to two years, while samples were analyzed about monthly for nitrate concentrations as well as isotopic composition. Values of isotopic enrichment were calculated, and first-order and zero-order rate constants were calculated, for each test. The isotopic enrichment values were then plotted against the first-order and zero-order rate constants and the resulting correlations were analyzed

Results showed that a good correlation exists between enrichment and first-order reaction rate constants with an R^2 value of 0.74 (see Fig. 1). A majority of the test sites correlated better using the first-order rate model, lending support to the enrichment vs. first-order rate constant correlation. Three of the tests did not fit this model, as they displayed a higher correlation with a zero-order rate constant. However, when enrichment was plotted versus zero-order rate constant a lower correlation was found. These three tests occurred at the Larimore site where denitrification occurs at much higher rates than the other sites analyzed.

When applied to the sites considered for this study, the hypothesis purposed by Mariotti, et al. (1) seems to be correct, as a good correlation exists between ^{15}N isotopic enrichment and first-order reaction rates. However, significant variability, particularly that of the Larimore site, remains unexplained. Further research, including investigation of bacteria types, variations due to temperature, and an increased frequency of similar studies to expand the data set is suggested.



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PROLIFERATION OF SKIN CELLS DURING WOUND HEALING IN DIABETIC AND NON-DIABETIC MICE: EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR TREATMENT IN VIVO

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During wound healing, an ordered sequence of events takes place that promotes the repair of injured tissues. Wound healing has three phases: inflammation (day 1 to 2 after injury), proliferation (approximately day 2 to 10 after injury), and maturation or remodeling (approximately day 11 to years after injury). In diabetes, there are multiple alterations in the local wound environment with abnormalities in all phases of wound healing. Numerous growth factors, including FGF-2 and VEGF, have been demonstrated to enhance the wound healing process. The purpose of this project was to determine the rates of cell proliferation throughout the three phases of wound healing in the skin of diabetic and non-diabetic mice, and after FGF-2 and VEGF treatment during the inflammatory and proliferative phases.

Diabetic (n=50) and non-diabetic (n=48) mice were wounded and then sutured with 6-7 stitches. One hour before tissue collection, mice were injected with BrdU (125 µg/g), a marker of proliferating cells. In Experiment 1, samples of the entire wound were collected on days 1, 3, 5, 8, 14, and 21 (n=4-5 mice/day) after wounding. In Experiment 2, FGF-2, VEGF or FGF-2+VEGF (Biosource, CA) were injected once daily (1 µg/50 µl/injection) around the wound for 7 days starting on the day of wounding (n=4-5 mice/treatment). The wounded skin tissues were fixed in Carnoy's solution for immunolocalization of BrdU followed by fast red staining to visualize cell nuclei. The number of proliferating cells and total number of cells in the tissues were determined using image analysis. Labeling index (%) was calculated as the number of BrdU-positive cells expressed as a percentage of the total number of cells. Overall, the rate of proliferation for diabetic and non-diabetic mice was similar (P>0.2), but differed (P<0.01) at several days after wounding (Exp. 1) and after growth factor treatment (Exp. 2). For diabetic mice, the labeling index in connective tissues of the wounded area increased (P<0.05) from day 3 until day 5, 8 and 14 and then decreased. Labeling index in the epidermis of the wounded area was similar on days 1 and 3 and increased (P<0.05) on day 5, 8 and 14 after wounding. For non-diabetic mice, the labeling index in connective tissues increased (P<0.05) on days 3, 5 and 8 and then decreased. In the epidermis the labeling index of the wounded area increased (P<0.05) on days 3 and 5 and then decreased. For diabetic mice, FGF-2+VEGF tended (P<0.2) to increase the labeling index in connective tissue, and FGF-2 tended (P<0.2) to increase labeling index in the epidermis. For non-diabetic mice, FGF-2 and FGF-2+VEGF increased (P<0.05) the labeling index in connective tissue, and FGF-2, VEGF and FGF-2+VEGF increased (P<0.05) labeling index in the epidermis.

These data demonstrate that 1) cellular proliferation is delayed during wound healing in diabetic mice compared to non-diabetic mice, and 2) the effects of FGF-2 and VEGF on cell proliferation are greater in non-diabetic mice than diabetic mice. Understanding the mechanism of FGF-2 and VEGF action on cellular proliferation during the wound healing will help to improve clinical application of growth factors for diabetic and non-diabetic wound treatment. *Supported by grants from NDSU Research Development Support Program and ND EPSCoR.*

[WITHDRAWN]

THE EFFECT OF HYDROQUINONE ON ROOT MEMBRANE POTENTIALS AND GROWTH OF THE COMMON BEAN
(*PHASEOLUS VULGARIS*)

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Allelopathy is a term that describes biochemical interactions between plant species. These interactions are most commonly manifested as reductions in growth of the target plant species. While hydroquinone (HQ) has been implicated as an interfering allelochemical in natural and agroecosystems, the mechanism of this interference remains poorly understood. We conducted experiments to test the hypothesis that growth interference upon exposure to HQ begins with a disruption of normal root membrane function which then leads to changes in photosynthetic activity which ultimately effects plant growth.

Seeds were germinated in vermiculite and seedlings transferred to nutrient solutions after 13 days and allowed to equilibrate for 2 days prior to treatment. For treatment (day 1) with HQ, nutrient solutions were amended with HQ in various amounts representing millimolar (mM) concentrations ranging from 0.001 to 0.25 mM. Plants were exposed to HQ for 14 days. A subset of 6 plants were harvested on day 1 in order to determine any changes in relative growth rate (RGR) of treated plants compared to controls (plants grown in nutrient solution without HQ). At harvest (day 14), stems were separated from leaves and roots and oven-dried at 104°C for 48 hours. Plants tissue was then weighed and data analyzed using one-way analysis of variance with means separated with Duncan's Multiple Range Test.

For determination of the effect of HQ on photosynthesis, measurement of chlorophyll fluorescence was conducted on day 14 prior to harvest. Leaves were dark-adapted for 10 minutes and chlorophyll fluorescence was measured using an Opti-Sciences OS-30 fluorometer. Fluorescence variables were analyzed using one-way analysis of variance with means separated with Duncan's Multiple Range Test.

The effect of HQ upon root membrane function was tested through measures of the acute effects on bean root membrane potential. For these experiments root tips (2-3 cm in length), excised from approximately 13 day old seedlings, were secured within a small flow through chamber and subjected to a constant irrigation with 0.1 mM KCl, 1 mM Mes/Btp pH 6.0, and 1 mM Ca₂CL. Membrane potential recording was then achieved by impaling root tip cortical cells with a conventional microelectrode filled with 300 mM KCl. Once a stable recording (-100 mV or lower and changing less than 1 mV/2 minutes) was established the flow through medium was changed to one also including HQ.

Treatment with as little as 0.01 mM HQ resulted in significant ($p < 0.05$) reductions in plant growth variables including relative growth rate, leaf area, leaf weight, root weight, stem weight, root weight/length ratio, and leaf weight ratio (leaf weight/plant weight). Plants treated with 0.25 mM HQ experienced changes in photosynthetic activity as indicated by a reduced Fv/Fm ratio.

Preliminary results indicate the membrane potential of cortical cells exposed to various concentrations of HQ invariably responded with an immediate small (2-4 mV) transient (2-3 minute) hyperpolarization. This was followed for the higher concentrations of HQ by a larger (10-40 mV) sustained depolarization lasting at least 30 minutes. Root tips exposed to the lowest concentration of HQ so far tested (1 μ M) depolarized only 7.7 mV +/- 5.5 mV (S.D., n=5).

Results of these experiments suggest a mechanistic relationship between root membrane potential, photosynthesis, and plant growth. While exposure to HQ clearly interferes with the growth of the common bean, the exact nature of the physiological mechanisms of action remain elusive.

EFFECTS OF SIMULATED MICROGRAVITY ON CARDIAC VISCOELASTIC PARAMETERS IN RHESUS MONKEYS

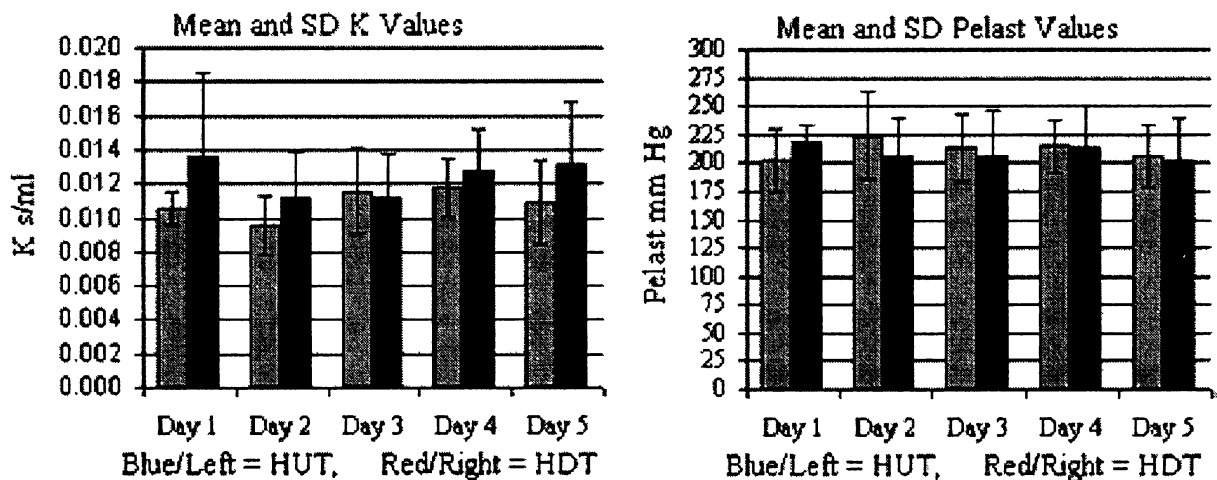
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Data were analyzed from a previous study in which invasively instrumented rhesus monkeys underwent periods of simulated microgravity. Left ventricular pressure (LVP), aortic pressure (AoP), and aortic flow (AoF) measurements were studied to determine cardiac viscoelastic parameters Pelast and K for five monkeys during the microgravity condition. These results were then compared to values at normal gravity conditions over the course of five days to find any relation between the viscoelastic parameters and gravity. No major effect of simulated microgravity on Pelast and K was found.

Future space exploration and research depends on the ability of humans to survive and operate during and after long-term exposure to a microgravity environment. This research was intended to add to the knowledge of the effects of microgravity on the cardiovascular system by examining relations between gravity and viscoelastic cardiac parameters Pelast and K.

The data used in this study was collected during experiments done several years ago at Brooks Air Force Base in San Antonio, Texas (1). These experiments were performed on rhesus monkeys and included the chronic invasive recording of hemodynamic signals during simulated microgravity via prolonged head down tilt (HDT). The ten-degree HDT produced cardiac conditions similar to those which would occur during long duration human space flight. The animals were randomly assigned to begin in either head-up tilt (HUT) or HDT condition, and this was maintained for five days, with data recorded during each day. The animals were then allowed to recover and were switched to the other gravity condition for five days, with data recorded each day and saved in separate computer data files.

Matlab software was used to extract the data from the studies and obtain properly calibrated LVP, AoP, and AoF signals. The equations for finding Pelast and K from AoP, LVP, and AoF were developed at NDSU and are described in detail by Ewert (2). Matlab scripts were developed to determine Pelast and K values for single ejecting beats. The results of the analysis are illustrated in the figures below.



Statistical analysis was performed at the NDSU Statistics Consulting Laboratory to bring to light any possible relationships between the viscoelastic parameters and gravity condition (HUT or HDT). The analysis of K and Pelast values showed no consistent significant relationships to HUT or HDT states.

- 1) Convertino VA, Koenig SC, Krotov VP, Fanton JW, Korolkov VI, Trambovetsky EV, Ewert DL, Truzhennikov A, and Latham RD. (1998) *Acta Astronautica*, Vol 42, No 1-8, pp 255-263.
- 2) Ewert D, Wheeler B, Doetkott C, Constantine I, Pantalos G, and Koenig SC. The Effect of Heart Rate, Preload and Afterload on the Visco-elastic Properties of the Swine Myocardium. Submitted for Publication.

Research funded by North Dakota Space Grant Consortium

SURVEY OF MICROCADDISFLIES IN MERCER COUNTY, NORTH DAKOTA

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The purpose of this project was to take a survey of the microcaddisflies in Mercer County North Dakota. The microcaddisfly is a small insect that belongs to the Family Hydroptilidae, Order Trichoptera. It has an aquatic larval and pupal stage and a winged, terrestrial adult stage. At this time there are four species of Hydroptilidae on record in North Dakota. Harris et. al (1980), reported the following species from North Dakota; *Agraylea multipunctata*, *Hydroptilia waubesiana*, *Ochrotrichia stylata*, and *Oxyethira serrata*.

In 2000, a sample in Mercer County, North Dakota revealed microcaddisfly larvae that current keys identified as genus *Metrichia*. This genus' known habitat is in Central America and Southwestern United States. Suggesting, either these larvae are out of the known habitat range, they are an undescribed larvae of a similar genus, or that they are a larvae for a new species for this region. In order to determine which of the above possibilities is correct, samples were taken both in 2002 and 2003 from sites in Mercer County, North Dakota. Samples were taken from Otter Creek, Bush Creek and Coyote Creek. The purpose was to collect, rear, and identify species of microcaddisflies in artificial microcosms. In 2002 our collections yielded adults of *Ithytrichia clavata* (a new record for North Dakota), larvae of a species of *Hydroptilia*, and additional samples of the larvae in question. However we were not able to raise the unknown larvae to the adult stage. We returned to the sites in Mercer County in 2003 to collect the unknown larvae to rear in lab, and to collect the adults in their natural habitat.

Two trips were taken for collection in 2003, one in early July and the other at the end of July. Samples were obtained from Otter and Coyote Creeks located near Beulah, North Dakota in Mercer County. The early July collection produced two reared adult samples of the genus *Hydroptilia*. These specimens were not *Hydroptilia waubesiana*. Late July collection was more successful. From these samples we were able to rear adults of *Hydroptilia*, *Ochrotrichia*, and *Ithytrichia*. Using the criteria of Blickle (1979) and Ross (1944), we were able to identify the unknown larva as *Ochrotrichia tarsalis*.

Out of these two summer surveys we have two new records for North Dakota, *Ithytrichia clavata* and *Ochrotrichia tarsalis*. However, we have yet to identify the unknown *Hydroptilia sp.*

This project was funded in part by a grant from the North Dakota Academy of Science.

Harris, S.C., Lago, P.K., and Carlson, R.B. 1980. Preliminary Survey of the Trichoptera of North Dakota. Proc. Entomol. Soc. Wash. 82(1). pp. 39-43.

Blickle, R.L. 1979. Hydroptilidae (Trichoptera) of America North of Mexico. University of New Hampshire, Agricultural Experimental Station Bulletin 509.

Ross, H.H. 1944. The Caddis Flies, or Trichoptera, of Illinois. Bulletin Illinois Natural History Survey. Vol. 23

A. Rodger Denison Student Research Competition

COMMUNICATIONS

GRADUATE DIVISION

Graduate Denison Competition**Session Chair: Dale Redmer, NDSU****Denison Award Judges: Dr. Dale Redmer, NDSU;
Dr. Richard Barkosky, Minot State;
Dr. Ronald Jyring, Bismarck State
Dr. John Watt, UND**

- 9:10 - 9:30 am Ewa Borowczyk*, Mary Lynn Johnson, Dale A. Redmer, Lawrence P. Reynolds, Pawel P. Borowicz, Justin S. Luther, Disha Pant, Robert M. Wiegler, Jerzy J. Bilski and Anna T. Grazul-Bilska **GAP JUNCTIONAL PROTEINS CONNEXIN (Cx) Cx26, Cx32 AND Cx43 mRNA EXPRESSION IN THE CORPORA LUTEA DURING THE ESTROUS CYCLE AND PROSTAGLANDIN F₂ (PGF)-INDUCED LUTEAL REGRESSION IN SHEEP** Department of Animal and Range Sciences and Cell Biology Center, North Dakota State University, Fargo, ND
- 9:30 - 9:50 am Balachandra Gorentla* and Roxanne A. Vaughan **THE ROLE OF PKC IN DOPAMINE TRANSPORTER PHOSPHORYLATION** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 9:50 - 10:10 am Sunitha Bollimuntha* and Brij B Singh **VAMP2 DEPENDENT ACTIVATION AND EXOCYTOSIS OF TRPC3 CA²⁺ CHANNEL PROTEIN** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 10:10 - 10:30 am Pawel P. Borowicz*, Mary Lynn Johnson, Anna T. Grazul-Bilska, Sergio A. Soto-Navarro, Magdalena A. Borowicz, Dale A. Redmer and Lawrence P. Reynolds **CORRELATION BETWEEN VASCULARIZATION PARAMETERS AND EXPRESSION OF mRNA FOR VASCULAR ENDOTHELIAL GROWTH FACTOR, ANGIOPOIETINS, AND THEIR RECEPTORS** Department of Animal and Range Sciences and Cell Biology Center, North Dakota State University, Fargo, ND
- 10:50 - 11:10 am Christopher W.D. Jurgens*, James E. Porter and Van A. Doze **ADRENERGIC MODULATION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY** Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, ND
- 11:10 - 11:30 am Xuesong Chen*, Hai-Ying Zhang, Kristin Pavlish, David Machado-Arando¹, David A. Dean and Joseph N. Benoit. **IMPAIRED VASCULAR CONTRACTILE FUNCTION IN PORTAL HYPERTENSION: ROLE OF SMALL HEAT SHOCK PROTEINS** Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, ND
- 11:30 - 11:50 am M. Jumbo* and M.J. Carena **EVALUATION OF MAIZE POPULATION AND SINGLE-CROSS HYBRIDS ACROSS EASTERN NORTH DAKOTA ENVIRONMENTS** Department of Plant Sciences, North Dakota State University, Fargo, ND

- 1:00 - 1:20 pm Mark A. Cervinski* and Roxanne Vaughan **PSYCHOSTIMULANT AND SUBSTRATE-INDUCED DOPAMINE TRANSPORTER PHOSPHORYLATION: A TRANSPORT AND PROTEIN KINASE C DEPENDENT MECHANISM** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 1:20-1:40 pm Jon D. Gaffaney* and Roxanne A. Vaughan **UPTAKE BLOCKER-INDUCED CONFORMATIONAL CHANGES OF THE DOPAMINE TRANSPORTER REVEALED BY PROTEOLYSIS** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 1:40 - 2:00 pm Lata Balakrishnan*, Ryan Clauson, Tim Weiland, Michelle Bianco, Barry Milavetz **SEXUALLY TRANSMITTED HPV SEROTYPE VARIATIONS RESULTING IN HIGH-GRADE CERVICAL DYSPLASIA IN NORTH-EAST NORTH DAKOTA AND NORTH-WEST MINNESOTA** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 2:00 - 2:20 pm M. Laura Parnas*, Jon D. Gaffaney, Amy H. Newman, Mu-Fa Zou, John R. Lever and Roxanne A. Vaughan **EXPLORING THE COCAINE BINDING SITE ON THE DOPAMINE TRANSPORTER** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 2:20 - 2:40 pm Lei Ding*, D.P. Guo, B. Singh, and G.A. Homandberg **OSTEOARTHRITIC-LIKE CARTILAGE DAMAGE BY FIBRONECTIN FRAGMENTS OCCURS THROUGH ELEVATED INTRACELLULAR KINASE ACTIVITY AS TRIGGERED BY CHANGES IN DISTRIBUTION OF RECEPTOR, ACTIN, VINCULIN AND C-SRC KINASE** Department of Biochemistry & Molecular Biology, University of North Dakota, Grand Forks, ND
- 3:00 - 3:20 pm Erica Swanson*, Jan Stube, and Paul Edman **FUNCTION AND BODY IMAGE LEVELS IN INDIVIDUALS WITH TRANSFEMORAL AMPUTATIONS USING THE C-LEG® PROTHESIS** Department of Occupational Therapy, University of North Dakota, Grand Forks, ND
- 3:20 - 3:40 pm Lynn Swanson,* and Sonia Zimmerman **FACILITATING OCCUPATIONAL PERFORMANCE IN AT-RISK RURAL MIDDLE SCHOOL STUDENTS** Department of Occupational Therapy, University of North Dakota, Grand Forks, ND
- 3:40 - 4:00 pm Brandi Johnson*, Kim Anderson, and Janet Jedlicka **POSITIONING AND ERGONOMICS FOR PREGNANT WOMEN WHILE PERFORMING DAILY TASKS** Department of Occupational Therapy, University of North Dakota, Grand Forks, ND
- 4:00 - 4:20 pm Mike Davis* and Phil Gerla **CALIBRATION OF PARAMETRIC EQUATIONS TO PREDICT PRAIRIE POTHOLE BASIN GEOMETRY AND AREA-DEPTH-VOLUME RELATIONSHIPS** Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND

GAP JUNCTIONAL PROTEINS CONNEXIN (Cx) Cx26, Cx32 AND Cx43 mRNA EXPRESSION IN THE CORPORA LUTEA DURING THE ESTROUS CYCLE AND PROSTAGLANDIN F₂α (PGF)-INDUCED LUTEAL REGRESSION IN SHEEP

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Corpora lutea (CL) exhibit extremely rapid growth, differentiation and regression during each estrous cycle. In fact, the transient nature of CL is one of its most intriguing features and makes it an outstanding model for studying the regulation of tissue growth and regression. Gap junctions are involved in control of cell growth, differentiation and regression. Gap junctions are present in most tissues and organs in mammals and other species and seem to play an important role in the regulation of luteal function. They allow for transfer of regulatory molecules <1000 daltons from cell to cell. Gap junctions are encoded by a multi-gene family known as the connexins. Each connexin exhibits a unique pattern of expression that can be metabolically, hormonally or developmentally regulated. In addition, multiple connexins can be expressed within a single cell. Expression of Cx26, Cx32 and Cx43 was evaluated in CL obtained from superovulated ewes. Superovulation was induced by twice-daily injections of follicular stimulating hormone (FSH) on days 13, 14 and 15 of the estrous cycle. CL collected on days 5, 10 and 15 of the estrous cycle and at 0, 4, 8, 12 and 24 h after induction of luteal regression were snap-frozen for isolation of total cellular RNA (tcRNA). Luteal regression was induced by one injection of Estrumate (clorostenol sodium, analog of PGF, 2 ml/injection; 250 mg/ml) on day 10 of the estrous cycle. The quantity and quality of the tcRNA were evaluated using an Agilent 2100 bioanalyzer (Agilent Technologies, Wilmington, DE) and mRNA expression for Cx26, Cx32 and Cx43 was quantified and normalized to expression of the housekeeping 18s ribosomal gene by real time RT-PCR using the ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Expression of Cx26 was ~70% greater (p<0.06) on day 10 than on days 5 or 15 of the estrous cycle, which were similar. In contrast, expression of Cx43 was the greatest (p<0.01) on day 5 and then decreased by ~40% on days 10 and 15 of the estrous cycle. The PGF-treatment decreased (p<0.001) Cx26 expression during luteal regression; at 4 h after PGF treatment Cx26 expression decreased by ~50%, at 8 h by ~80%, and at 12 and 24 h by ~90%. PGF-treatment tended (p<0.2) to increase Cx43 expression transiently by ~80% 8 h after induced luteal regression. Expression of Cx32 was 50-fold less than Cx26 and Cx43 during the estrous cycle and during PGF-induced regression and was not affected by stage of luteal development. This study indicates that the expression pattern of Cx26 and Cx43 depends on the stage of luteal development, differentiation and regression. Furthermore, these observations indicate that regulation of Cx26 expression may be of critical importance in luteolysis. Knowledge of the pattern of connexin expression, coupled with studies of the functional consequences of regulating connexin expression (e. g., RNAi studies), will provide a better understanding of the regulation of cell growth and function in the corpus luteum as well as rapidly growing tissues in general.

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THE ROLE OF PKC IN DOPAMINE TRANSPORTER PHOSPHORYLATION

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Dopamine transporters (DAT) are neuronal proteins that clear dopamine from the synaptic cleft and thus regulate synaptic transmission. In addition they act as a target for psychostimulants like cocaine and amphetamine. DAT is a PKC dependent phosphoprotein. Phosphorylation of DAT may play a role in regulation of forward and reverse transport of dopamine, subcellular distribution of DAT, and interaction of DAT with binding partners. These studies suggest that regulation of DAT by phosphorylation may occur by multiple molecular mechanisms. Numerous potential sites for phosphorylation including consensus PKC sites are present on the N-terminus of the DAT, but we do not know whether DAT is directly phosphorylated by PKC or if other kinases are involved. The goal of this study is to examine the ability of PKC to phosphorylate DAT *in vitro*. In one approach phosphorylation was performed by reconstituting purified rat striatal DAT with lipid lamellae and incubating with purified PKC, and γ -³²ATP. The samples were analyzed on SDS-PAGE followed by autoradiography to assess the phosphorylation of DAT. In the second approach rat striatal membranes were treated under similar conditions followed by extraction and immunoprecipitation of DAT before analysis. The activation of PKC is shown by autophosphorylation. These experiments will indicate the potential role of PKC in phosphorylation of DAT. If PKC does not phosphorylate the DAT *in vitro* it will suggest that PKC may act upstream to activate other kinases that might be playing a role in the regulation of DAT.

Supported by NIH Grant DA 13147

VAMP2 DEPENDENT ACTIVATION AND EXOCYTOSIS OF TRPC3 Ca^{2+} CHANNEL PROTEIN.

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Transient receptor potential channel (TRP) proteins are putative plasma membrane Ca^{2+} channels which are activated upon intracellular Ca^{2+} store depletion. The mechanism via which these channels are activated is unclear. In the present study we report that TRPC3 a member of the TRP super family are present in intracellular vesicles and get fused to the plasma membrane upon activation. Using yeast two-hybrid technique we had identified interaction between TRPC3, VAMP2, and aSNAP proteins. The TRPC3- VAMP2 interaction was mediated via the N-terminus of TRPC3 protein. Further the interacting domain was identified using successive deletions in the N terminal domain. Carbachol stimulation of receptor coupled phospholipase C in the TRPC3 expressing cells increased TRPC3 surface expression, but was attenuated in cells where VAMP2 protein was degraded via tetanus toxin. These cells also showed decreased Ca^{2+} influx upon stimulation. Confocal and Immunoprecipitation studies also reveal the interaction of TRPC3 with VAMP2, aSNAP, NSF and SYNTAXIN 3 -proteins involved in vesicular trafficking. GFP tagged TRPC3 and CFP tagged VAMP2 was also used to show that both the proteins co-localize. To further establish our point, a dominant negative VAMP2 (DNVAMP2) construct was made, interestingly, expression of CFP-DNVAMP2 in HEK 293 cells showed a diffused staining of the VAMP2 protein in the cytoplasm. Further, expression of GFP-TRPC3 along with CFP-DNVAMP2 showed a significant decrease in the plasma membrane staining of GFP-TRPC3. Taken together these data suggests that VAMP2 dependent mechanism is involved in the activation and generation of functional TRPC3 channels in the plasma membrane.

CORRELATION BETWEEN VASCULARIZATION PARAMETERS AND EXPRESSION OF mRNA FOR VASCULAR ENDOTHELIAL GROWTH FACTOR, ANGIOPOIETINS (ANG), AND THEIR RECEPTORS

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All of the respiratory gases, nutrients, and wastes that are exchanged between the maternal and fetal systems are transported via the placenta. It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on the dramatic growth of the placental vascular beds and the resultant large increases in uterine and umbilical blood flows. Thus, factors that influence placental vascular development have a dramatic impact on fetal growth and development, and thereby on neonatal survival and growth. Failure in the development of healthy and fully functional placenta can lead to intrauterine growth retardation of the fetus or cause pregnancy loss. Angiogenesis in the placenta is a complex process tightly regulated by a number of promoters and inhibitors. The search for potential regulators of angiogenesis has led to the identification of the major angiogenic growth factors which are involved in development and organization of the fetal and maternal placental vasculature. These angiogenic growth factors include the vascular endothelial growth factor family (VEGF) and its receptors (VEGFR-1 and VEGFR-2), the angiopoietins (Ang-1 and Ang-2) along with their common receptor Tie-2. We have shown that these major angiogenic factors, as well as their receptors are expressed in a time- and cell-specific fashion throughout gestation. Comparing their expression patterns along with the pattern of placental blood vessel growth will be crucial in understanding the mechanisms of placental angiogenesis; in other words, by establishing a model of vascular growth and correlating it with the expression of various angiogenic factors we can understand their influence on placental vasculature development at any given time. To provide a quantitative description of placental vascular growth and evaluate vascular growth factor gene expression, gravid uteri were obtained from ewes on days 50, 70, 90, 110, 130, and 140 after mating (n=4 ewes/day; length of gestation approx. 145 days). For each ewe, separate placentomes were fixed with Carnoy's solution by vascular perfusion of the caruncular (CAR; maternal portion of the placenta), or cotyledonary (COT; the fetal portion of the placenta) tissues. After fixation, the placentas were embedded in paraffin, sectioned and stained (hematoxylin and periodic acid-Schiff's). Vascularity was then determined by image analysis (Image-Pro Plus[®]). For modeling purposes we evaluated for CAR and COT: capillary area/unit tissue area (capillary area density, CAD), capillary no./unit tissue area (capillary no. density, CND), capillary surface area/unit tissue area (capillary surface density, CSD) and area/capillary (APC). To evaluate placental expression of some major angiogenic factors, CAR and COT from the same ewes were snap frozen and VEGF, VEGFR-1, VEGFR-2, ANG-1, ANG-2 and Tie-2 mRNA concentrations were measured by real-time RT-PCR (ABI Prism 7000[®]). After statistical analysis the pattern of capillary growth was correlated with expression of the angiogenic factors. As we previously reported, CAR angiogenesis was reflected by increased CAD and APC (3.3- and 2.2-fold increases from day 50-140, respectively), which are strongly correlated ($P < 0.01$) with VEGFR-1 ($r = 0.54$ and $r = 0.46$, respectively) and ANG-2 ($r = 0.50$ and $r = 0.58$); CAD, CND, and CSD are also correlated ($P < 0.03$) with VEGFR-2 ($r = 0.36$). As we previously reported, COT angiogenesis was reflected primarily by increased CAD, CND, and CSD (6.2-, 12.3-, and 6.0- fold increases from day 50-140, respectively) but decreased APC (1.9-fold decrease, day 50-140). For COT tissues, CAD, CND, and CSD are correlated with mRNA for VEGF ($P < 0.04$; $r = 0.45$, $r = 0.53$, and $r = 0.46$) and ANG-2 ($P < 0.05$; $r = 0.54$, $r = 0.45$, and $r = 0.60$); both VEGF and ANG-2 mRNAs are negatively correlated ($P < 0.05$) with APC ($r = -0.40$, $r = -0.34$). For COT, ANG-1 mRNA is correlated ($P < 0.06$) with CSD and CND ($r = 0.36$ and $r = 0.32$) but negatively correlated ($P < 0.05$) with APC ($r = -0.33$); CAD, CND and CSD are also correlated ($P < 0.07$) with VEGFR-1 ($r = 0.36$, $r = 0.31$, and $r = 0.42$). These data demonstrate the potential role of the VEGF and ANG systems in various aspects of placental angiogenesis in sheep.

Supported by NIH HL64141 grant to DAR and LPR.

ADRENERGIC MODULATION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY

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INTRODUCTION

Epilepsy, or recurring uncontrolled seizures is a common neurological disorder. Despite many decades of research and several newer antiepileptic drugs, many people still suffer from incompletely controlled seizures or debilitating adverse effects of current treatment modalities. Norepinephrine (NE), an endogenous neurotransmitter, has been shown to be potently antiepileptogenic (i.e., prevents seizures) *in vivo*, even against some of the most intractable epilepsies. However, the mechanism of this action has not been fully characterized. Working with *in vitro* models and using electrophysiological recording techniques, we present evidence that this effect is mediated via an α_2 adrenergic receptor (AR) pathway.

METHODS

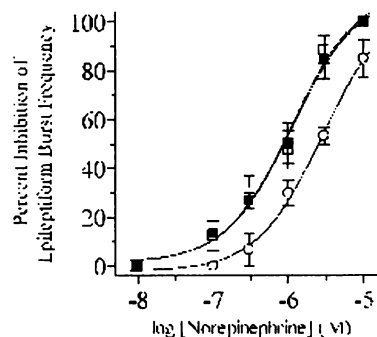
In this study, we utilized pharmacological analysis of electrophysiological methodologies to characterize the effects of NE on spontaneous epileptiform discharges induced by the GABA_A receptor antagonists in the CA3 region of neonatal rat hippocampal slices. Field potential recordings of epileptiform activity were studied in the presence of subtype specific AR agonists and antagonists to determine functional effects of α AR activation.

RESULTS

Increasing concentrations of NE were used to generate concentration-response curves associated with the inhibition of epileptiform discharge frequency for rat hippocampal slices in the absence (open squares) or presence of the selective α_1 antagonist, prazosin (1 nM, closed squares) or the selective α_2 antagonist RS79948 (1 nM) plus 1 nM prazosin (open circles). The EC₅₀ calculated from these curves showed no differences for the potency of NE in the presence of prazosin ($1.0 \pm 0.3 \mu\text{M}$) when compared to control ($1.2 \pm 0.4 \mu\text{M}$). Conversely, there was a significant ($P < 0.05$, $N = 3$) difference in the EC₅₀ calculated for NE ($2.7 \pm 1.3 \mu\text{M}$) in the presence of RS79948 and prazosin when compared to control. Measurements are presented as the mean \pm S.E.M. Similar results were observed using the α_1 antagonist terazosin and the α_2 antagonist RX 821002 (data not shown).

DISCUSSION

The present findings may provide insight into possible mechanisms underlying the antiepileptogenic effects of NE observed *in vivo*. The identification of the specific α AR type mediating the antiepileptic properties of NE is important for determining exactly how α AR activation modulates epileptiform activity, and more importantly, delineates a specific therapeutic target for future pharmacological exploitation.



Supported by the NIH BRIN, NIH COBRE, NSF ND EPSCoR and various UND SEED grant programs.

IMPAIRED VASCULAR CONTRACTILE FUNCTION IN PORTAL HYPERTENSION:
ROLE OF SMALL HEAT SHOCK PROTEINS

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Studies have shown that impaired vascular contractile responses in chronic portal hypertension (CPH) are mediated via cAMP dependent events (1). Recent data indicated that sustained vasorelaxation induced by cAMP is independent of myosin light chain phosphorylation (2). Two small heat shock proteins (HSP), namely HSP20 and HSP25/27, have been implicated in the regulation of vascular tone without changing myosin light chain phosphorylation. Phosphorylation of HSP20 is associated with vasorelaxation while phosphorylation of HSP25/27 is associated with vasoconstriction (2). Both HSP20 and HSP25/27 are shown to be actin-associated proteins, which depend on the phosphorylation status of HSP (3, 4). We hypothesized that alterations in the expression and/or phosphorylation of small HSPs may play a role in impaired vasoconstriction in CPH. **Methods** A rat model of prehepatic chronic portal hypertension induced by partial ligation of portal vein was used (14 days). Mesenteric resistance vessels were harvested. The mRNA levels of HSP20 and HSP25/27 in mesenteric resistance vessels were determined by GeneChip® expression analysis. HSP expression and phosphorylation in mesenteric resistance vessels were examined by western blot and by isoelectric focusing, respectively. To examine the role of cAMP in the regulation of HSP expression and phosphorylation, we measured the expression and phosphorylation in the presence of the PKA inhibitor Rp-cAMPS. **Results** HSP 20 and HSP27 mRNA levels in mesenteric resistance vessels from CPH rats were significantly decreased by 33.33% and 23.33% respectively, compared with Normal (P<0.05). HSP20 expression in mesenteric resistance vessels from CPH rats was significantly decreased, which normalized by Rp-cAMPS (**Figure 1**). The expression of HSP25/27 in mesenteric resistance vessels remained unchanged in CPH. HSP20 phosphorylation in mesenteric resistance vessels was significantly increased in CPH, which was normalized by Rp-cAMPS (**Figure 2**). The phosphorylation of HSP25/27 in mesenteric resistance vessels remained unchanged in CPH. **Conclusion** Data from the present studies demonstrate a role for PKA mediated events in the modulation of HSP20 in chronic portal hypertensive condition. We suggest that altered vasoconstrictor function may be partially explained by modulation in the expression and/or phosphorylation of HSP20 by cyclic AMP dependent pathways. (Supported by NIDDK-51430)

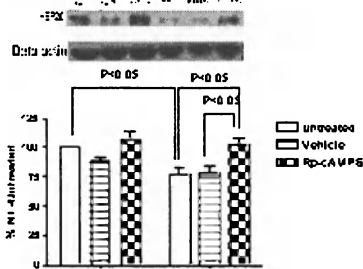


Figure 1: The expression of HSP20 in the presence of PKA inhibitor Rp-cAMPS (µM for 30 min, n=4) was measured. There were no vehicle effects in either group. cAMPS did not alter HSP20 expression in normal vessels but did increase HSP20 expression in portal hypertensive vessels.

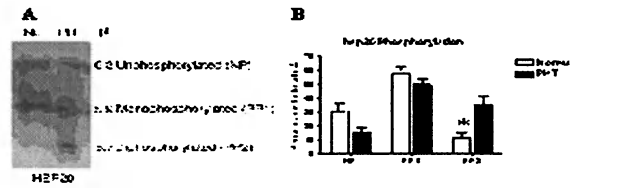
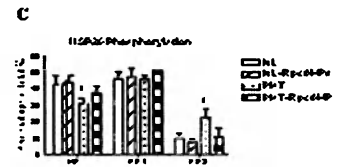


Figure 2: HSP20 phosphorylation in mesenteric resistance vessels from NL and PH rats. The phosphorylation of HSP20 was measured by Western blotting. The phosphorylation of HSP20 was significantly increased in PH rats compared to NL rats. This increase was normalized by Rp-cAMPS. *P<0.05 compared to Untreated.



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EVALUATION OF MAIZE POPULATION AND SINGLE-CROSS HYBRIDS
ACROSS EASTERN NORTH DAKOTA ENVIRONMENTS

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Maize (*Zea mays* L.) is the third top commodity in North Dakota. North Dakota is one of the few states in the United States that almost exclusively uses very short season corn hybrids. Hybrids currently grown in the area are not well adapted to such environments. Improved maize population hybrids can be both, a profitable alternative to commercial F_1 hybrids as well as good elite sources of diverse inbred lines for the region. The objective of this research was to evaluate the grain yield and agronomic potential of early maturing maize population hybrids. Maize populations and their crosses (S_0 generations) were evaluated in experiments arranged in partially balanced lattice designs across 7 ND environments in 2002 and 2003. Data across years showed that top population hybrids were not different statistically ($P \leq 0.05$) from single-cross hybrids for grain yield performance and for root and stalk lodging percentages. Eight population hybrids have been identified at North Dakota State University (NDSU) with above average grain yield and agronomic performance. These are BS21(R)C7 x CGSS(S_1 - S_2)C5, BS21(R)C7 x CGL(S_1 - S_2)C5, NDSCD(M)C10 x BS22(R)C7, NDSAB(MER)C12 x BS21(R)C7, NDSAB(MER-FS)C13 x BS21(R)C7, BS22(R)C7 x CGSS(S_1 - S_2)C5, BS22(R)C7 x CGL(S_1 - S_2)C5, LEAMING(S)C4 x BS22(R)C7, and LEAMING(S)C4 x CGSS(S_1 - S_2)C5. Breeding efforts toward population hybrids have demonstrated that germplasm improvement is extremely valuable and deserves public funding. These efforts should be addressed to not only develop sources of inbred lines but also to develop improved cultivars for specific markets. Corn producers could, therefore, benefit by reducing the cost associated with seed and technology fees and by growing population hybrids at a lower cost. Further research will evaluate the potential of population hybrids in western North Dakota.

PSYCHOSTIMULANT AND SUBSTRATE-INDUCED DOPAMINE TRANSPORTER PHOSPHORYLATION:
A TRANSPORT AND PROTEIN KINASE C DEPENDENT MECHANISM.

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Introduction: The dopamine transporter (DAT) is an integral membrane protein located in dopamine producing neurons and is responsible for clearing the synapse of secreted dopamine (DA) following neural depolarization and synaptic release. As well as regulating synaptic levels of DA, DAT is also the target for many psychostimulant and therapeutic drugs such as cocaine, amphetamines, the antidepressant bupropion (Wellbutrin™) and the ADHD treatment methylphenidate (Ritalin™). DAT undergoes functional down regulation and increased phosphorylation and internalization in response to PKC activation. Similarly, treatments with the DAT substrate DA and the potentially neurotoxic psychostimulant substrates amphetamine (AMPH) and methamphetamine (METH), but not with the uptake blocker cocaine, also induce down regulation and internalization of DAT.

Methods: We have investigated DAT phosphorylation in response to treatments of AMPH, METH, DA and (-) cocaine. LLC-PK₁ cells expressing rat DAT (rDAT LLC-PK₁) and rat striatal tissue were metabolically labeled with ³²PO₄ followed by treatment with AMPH, METH, DA or (-) cocaine. The cells and tissue were solubilized and DAT was immunoprecipitated and analyzed by SDS-PAGE and autoradiography.

Results: DAT phosphorylation was increased in cells and in striatal tissue by the application of DA, METH and/or AMPH. *In vivo* injection of rats with METH also increased striatal DAT phosphorylation, implicating the physiological significance of the effect. The METH effect was dose and time dependent reaching peak phosphorylation at 10 minutes. In contrast to the amphetamines, cocaine produced no discernable increase in DAT phosphorylation, but when co-incubated with METH, prevented the METH-induced increase in DAT phosphorylation. METH-induced phosphorylation was also prevented by the addition of the PKC blocker, bisindolylmaleimide I (BIM).

Conclusions: These results demonstrate that the transported substrates, AMPH, METH and DA but not transport blockers, are capable of inducing DAT phosphorylation and show that the uptake of the substrates by DAT and PKC activity is necessary to produce this effect. These results also suggest that the substrate-induced effect may be related to substrate-induced internalization in a manner similar to that found by PKC activation. This process may thus represent part of a homeostatic mechanism controlling DA clearance or a neuroprotective mechanism limiting the accumulation of potentially neurotoxic substances.

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UPTAKE BLOCKER-INDUCED CONFORMATIONAL CHANGES OF THE DOPAMINE TRANSPORTER REVEALED BY PROTEOLYSIS

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Introduction. The dopamine transporter (DAT) is a membrane bound protein responsible for the clearance of dopamine from the synapse. Psychostimulant drugs such as cocaine and methamphetamine target DAT resulting in increased synaptic levels of dopamine. Understanding the mechanism of action for uptake blockers such as cocaine will aid in the design of novel therapies for drug addiction and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. DAT is a membrane-bound, polytopic, glycoprotein protein that co-transporters dopamine, Na⁺, and Cl⁻. Computer generated models predict twelve transmembrane domains (TMs) with intracellular N- and C-termini. Current experimental evidence suggests the TM domains are critical for uptake blocker binding and substrate transport, however relatively little is known about the role of the extracellular loops (ELs) in these processes. EL2 is a large 78 amino acid loop connecting TM3 and TM4, a region associated with uptake blocker binding and substrate translocation. In this study we examine the role of EL2 in uptake blocker and substrate binding by monitoring its sensitivity to proteolysis.

Methods. Membranes or synaptosomes were prepared from rat striatum, treated with trypsin or Asp-N in the presence or absence of blockers/substrates, and DAT proteolysis analyzed by SDS-PAGE and immunoblotting. To examine the effect of EL2 proteolysis on binding membranes were digested with protease and incubated on ice with [³H]CFT, a cocaine analog, for 1 hour. Similarly, to examine the effect of proteolysis on uptake synaptosomes were digested with protease, and incubated with [³H]dopamine for 5 minutes at 37 °C in modified Krebs-phosphate buffer containing.

Results. Proteolysis of DAT with trypsin produces a 45 kDa glycosylated fragment visualized by immunoblotting with mAb 16. This fragment is consistent with proteolysis at R218 located on the C-terminal region of EL2. In the presence of DAT uptake blockers but not substrates DAT sensitivity to trypsin is reduced 100-1000 fold. Blocker-induced protease resistance was Na⁺-dependent and was not observed in the presence of imipramine or desipramine, serotonin (SERT) and norepinephrine transporter (NET) blockers, indicating it was specific for uptake blocker binding. Striatal membranes or synaptosomes proteolyzed with trypsin display decreases in [³H]CFT binding and [³H]dopamine transport, suggesting the integrity of EL2 is required for binding and transport activity. Proteolysis with Asp-N produces 20 and 21 kDa fragments when immunoblotted with mAb16. These fragments correspond to potential protease sites at D174, D191, or D199, located in the N-terminal region of EL2. As observed with trypsin, DAT uptake blockers but not substrates reduced Asp-N proteolysis by at least 30%. Digestion of EL2 with Asp-N prior to [³H]CFT binding and [³H]dopamine uptake resulted in a loss of DAT function consistent with the extent of proteolysis as monitored by immunoblotting.

Discussion. These data provide evidence for conformational changes that occur in DAT during binding of uptake blockers but not substrates. Uptake blocker-induced protease resistance is apparent in both N- and C-terminal regions of EL2 implying that substantial conformational changes occur over a large portion of EL2. All uptake blockers tested affected the protease sensitivity of EL2 similarly, supporting the hypothesis that they use a single overlapping binding pocket. Moreover, this data also implies uptake blockers are not static molecules that simply block a permeation pathway, but instead actively induce movements in DAT. The finding that substrates do not induce protease resistance in EL2 indicates they may utilize a separate binding mechanism distinguishable from blockers. This study provides novel data for the role of EL2 in DAT function.

This work is supported by F31 DA 14857-01

SEXUALLY TRANSMITTED HPV SEROTYPE VARIATIONS RESULTING IN HIGH-GRADE CERVICAL DYSPLASIA IN
NORTH-EAST NORTH DAKOTA AND NORTH-WEST MINNESOTA

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Human papillomaviruses (HPV) are double stranded DNA viruses consisting of a small circular genome of approximately 8kb. Around 100 different sub-types of HPV have been identified, of which one-third have been shown to spread through sexual contact. Low-risk sub-types of HPV are known to cause benign warts whereas many of the high-risk sub-types have been implicated in the pathogenesis of cervical cancer. Review of Pap smear diagnoses from a reference laboratory in Grand Forks, North Dakota over a 3-year period (6/1/2000 to 5/31/2003) revealed a two-fold higher rate of high grade squamous intraepithelial lesion in a community in northwest Minnesota (Roseau, 0.519%) as compared to that in northeast North Dakota (Grand Forks, 0.256%), in spite of having similar rates of low-grade squamous intraepithelial lesion (1.31% vs. 1.26%). In order to determine whether the difference in proportion of patients with high-grade dysplasia was a consequence of the HPV sub-type we analyzed pathological specimens for the presence of HPV viral DNA. Formaldehyde-fixed, paraffin embedded cervical tissue samples from 17 age-matched patients who displayed a high-grade cervical dysplasia from northeast North Dakota and north-west Minnesota were used in order to study the presence of HPV serotypes. DNA from the tissue samples was extracted by the thermal deparaffinization, chelex-100 purification method. Extracted preparations were first subjected to Polymerase chain reaction (PCR) targeting a 155 base pair fragment (GP 5+/GP6+) of the L1 open reading frame (ORF) of the human papillomavirus (HPV).

HPV- positive tissue samples were subsequently characterized by PCR amplification using primer sets type specific for HPV type 16, 18, and 31. DNA from HeLa cells and CaSki cells were used as a positive control for HPV type 18 and HPV type 16 respectively. Of the 17 cases studied from Grand Forks (north east North Dakota), 82.35% were positive for the general primers. 11.8% and 17.6% were positive for only HPV serotype 16 and 18 respectively. There were no cases with single infection with HPV serotype 31. 5.9% showed dual infections with both HPV 16 and 18, 5.8% were doubly infected with HPV 16 and 31 and 23.5% were doubly infected with HPV 18 and 31. 11.8% showed triple infections with HPV 16, 18 and 31. Of the 17 cases studied from Roseau (north west Minnesota) 82.3% were positive for the general primers. Single infections of HPV 18 were not detected. 5.9% had single infections with HPV 16 and 11.8% with HPV 31. 11.8% were doubly infected with HPV 16 and 18, 17.64% with HPV 16 and 31 and 5.9% with HPV 18 and 31. Triple infections with HPV 16, 18 and 31 were detected in 41.17% cases. Our results show that differences in 1) high-risk HPV serotypes, 2) relative serotype frequencies, and 3) rates of multiple infections can be present among patients with high-grade dysplasia from separate communities within the same geographic area, and may explain the differing rates of high-grade dysplasia between these groups.

EXPLORING THE COCAINE BINDING SITE ON THE DOPAMINE TRANSPORTER

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INTRODUCTION: The dopamine transporter (DAT) is a neuronal transmembrane protein that mediates the removal of dopamine from the synaptic cleft, modulating synaptic dopamine concentration and accessibility. Cocaine binds to DAT and abolishes its transport activity, but the localization of the drug binding site and its relationship to the dopamine translocation pathway still remain unclear. These studies examine the incorporation site on DAT of a novel cocaine photoaffinity label, [¹²⁵I]MFZ-2-24, in comparison to known sites of other DAT inhibitors.

METHODS: Rat striatal dopamine transporters were photoaffinity labeled with [¹²⁵I]MFZ-2-24 and analyzed by proteolysis followed by epitope-specific immunoprecipitation using antisera 16 and 5, originated to N-terminal and extracellular loop 2 (EL2) amino acids respectively.

RESULTS: These approaches generated photolabeled fragments of 45 and 14 kDa that immunoprecipitated with antiserum 16, corresponding to N-terminal transmembrane domains (TMs) 1 and 2. Smaller amounts of a 32 kDa fragment were immunoprecipitated with antiserum 5, belonging to TMs 4-6. Both fragments showed specificity of antiserum recognition through peptide block analysis. The TMs 1-2 region has been previously identified as a site for binding of non-cocaine-like photoaffinity labels, whereas a closely related cocaine analog, [¹²⁵I]RTI-82, is known to bind to TMs 4-6.

CONCLUSION: The current studies suggest that TMs 1-2 and 4-6 are in close three-dimensional proximity and may comprise different faces of the cocaine binding site. More precise identification of the ligand binding sites is currently under investigation by subjecting peptide fragments labeled with [¹²⁵I]MFZ-2-24 or [¹²⁵I]RTI-82 to analysis by HPLC and mass spectrometry.

Supported by NIDA DA 13147, DA 15175, and NIDA-IRP.

OSTEOARTHRITIC-LIKE CARTILAGE DAMAGE BY FIBRONECTIN FRAGMENTS OCCURS THROUGH ELEVATED INTRACELLULAR KINASE ACTIVITY AS TRIGGERED BY CHANGES IN DISTRIBUTION OF RECEPTOR, ACTIN, VINCULIN AND C-SRC KINASE.

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Fibronectin (Fn) fragments (Fn-f), which are elevated in cartilage and synovial fluids in osteoarthritis, can greatly augment cartilage destruction through upregulation of catabolic cytokines and matrix metalloproteinases (MMPs). The precursor, native Fn, is inactive. How Fn-f cause changes in signal transduction that lead to enhanced chondrolytic activities is unknown. Since receptor-binding Fn peptides decrease focal contacts, disturb cytoskeletal elements such as actin and decrease Fn receptor aggregation in fibroblasts, we have proposed that Fn-f have similar effects in chondrocytes and that these changes in receptor and cytoskeletal elements are the cause of altered signal transduction that eventually leads to upregulation of cytokines and MMPs. Our first objective was to investigate whether Fn-f alter actin and focal contacts and disrupt the Fn receptor, $\alpha 5\beta 1$, in chondrocytes and to compare with native Fn. Our second objective was to test whether specific kinases, which are involved in integrin signaling, cytokine upregulation and MMPs expression, are upregulated by Fn-f. To investigate, fluorescent confocal microscopy was used to visualize changes caused by added Fn-f or Fn. Bovine chondrocyte monolayer cultures were treated with unlabeled or with FITC-labeled Fn or Fn-fs for 4 hrs or with BSA as a negative control. Cells were then fixed by paraformaldehyde, permeabilized and probed with antibody against $\alpha 5$ integrin subunit to test for changes in Fn receptor or with rhodamine phalloidin to test for changes in actin or with antibody to vinculin to test for changes in focal contacts. Changes in intracellular kinase activity were examined by Western Blotting by use of antibodies specific to total and phosphorylated kinase forms. The targets included c-src which can activate focal adhesion kinase (FAK), PYK2, a soluble form of FAK, and the MAP kinases ERK1/2, p38 and SAPK/JNK, which can upregulate cytokines that upregulate MMPs. The transcription factor, NF- κ B, involved in cytokine upregulation was also investigated. The effects of Fn and Fn-fs on kinetics and effects as a function of concentration were examined. The effects of several kinase inhibitors on upregulation of MMPs were also tested. We found that Fn-f disrupted cortical actin, caused diffusion and internalization of the Fn receptor and appeared to alter distribution of the focal contact protein, vinculin. There are dramatic effects of increasing concentrations of Fn-f (0-200 nM) on actin and on c-src (p-src is phosphorylated form) kinase. The Fn-f enhanced diffusion of cortical actin and that this movement was associated with movement of p-src away from the plasma membrane. Images obtained using confocal microscopy emphasize this association. The Fn-f also activated c-src, PYK2, ERK1/2, p38 and SAPK/JNK and NF- κ B while native Fn only enhanced ERK1/2 and FAK. For c-src, Fn appeared to enhance phosphorylation at an inactivation site of c-src, while the 29-kDa Fn-f did not but had a weak effect on enhancing phosphorylation at the activation site. This is consistent with our tests of the c-src inhibitor, PP2, which blocked Fn-f mediated upregulation of MMP-13. Our data suggest that Fn-f disrupt Fn receptor and enhance diffusion of cortical actin, the latter of which is associated with movement of c-src away from the plasma membrane where it can be inactivated. Active c-src might then activate PYK2, which may lead to activation of the inflammatory kinases (p38 and SAPK/JNK) and transcription factor NF- κ B, and eventually cause cartilage damage. In contrast, we propose that exogenous Fn inactivates c-src and thus, cannot activate p38 and SAPK/JNK. Since the Fn-f system is a model of osteoarthritis, further knowledge of the mechanism may be applicable to therapeutic intervention in catabolic processes. Future work will more precisely delineate the mechanism and test additional kinase inhibitors for their interventive potential. Supported by ND EPSCoR and National Science Foundation Grant (#01322899).

FUNCTION AND BODY IMAGE LEVELS IN INDIVIDUALS WITH TRANSFEMORAL AMPUTATIONS
USING THE C-LEG® PROSTHESIS

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Purpose: It was purposed that individuals using the Otto Bock C-Leg®, a microprocessor controlled prosthetic knee joint, may experience an enhanced level of functional independence. Secondly, it was hypothesized that with increased functional abilities and independence from using the C-Leg® comes a positive body image.

Methods: Following IRB approval, a purposive sampling method was used to recruit 8 adult volunteers from a regional rehabilitation hospital. Inclusion criteria for participants in this study included individuals who had a transfemoral amputation, were currently using the C-Leg®, were over the age of 18 years, and without cognitive limitations. Subjects were asked to complete a series of three surveys: the Reintegration to Normal Living Index (RNL), the Situational Inventory of Body-Image Dysphoria (SIBID), and the C-Leg® Function & Body Image Survey (CFBIS). Survey questions pertained to personal satisfaction with the C-Leg®, functional independence, role performance, and body image.

Results: Response categories of functional role performance and body satisfaction were correlated to test the hypotheses. A Spearman's rho of $-.434$ was calculated, showing a fair but not statistically significant relationship. Significant relationships were found between functional role performance and social integration ($r_s = .743$), self-efficacy ($r_s = .863$), personal relationships/sexuality ($r_s = .711$), and psychological distress ($r_s = -.772$). This relationship was supported by responses from the CFBIS indicating that the C-Leg® expands a client's level of function, self-esteem, and motivation.

Conclusions: There was a fair correlation between functional role performance and body satisfaction in individuals using the Otto Bock C-Leg®. Individuals using the C-Leg® were found to exhibit patterns of improvement regarding improved lifestyle, activity performance, motivation, and self-confidence. The most common improvements in activity performance were found with walking, walking up and down stairs, participating in sports (i.e. basketball, hiking, and skating), work/employment activities, and decreased fatigue due to low requirement of energy expenditure. Body image was found to be improved due to the fact that individuals were able to walk with a more natural gait, and also felt more secure in public places because of the stability the C-Leg® offers.

FACILITATING OCCUPATIONAL PERFORMANCE IN AT RISK MIDDLE SCHOOL STUDENTS

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Purpose: Adolescents in today's society are making the transition from childhood to adulthood in very turbulent times. Adolescents in rural areas have the unique experience of transitioning during a fast-changing rural landscape, with the weakening economic structure and weakening of the traditional family structure. The rates of adolescent depression, substance abuse, eating disorders, suicide, and anxiety disorders are rising nationally, and the impact is felt in rural areas.

Methods: An extensive literature review was conducted on the topics of adolescents, the problems they face, and existing preventative programming. The information established the need and foundation for a new program, Leadership Development.

Summary of Results: This is a preventative program, aimed at grades 6 through 8 (12 to 14 years old), focusing on facilitating occupational success by increasing self-efficacy, healthy lifestyle, and interpersonal skills. An approach that incorporates preparatory, purposeful, and occupation-based activities is used in the Leadership Development program. This includes using occupations and activities related to schoolwork tutoring, music, athletics (basketball, volleyball, and other areas of client interest), healthy lifestyle including diet and exercise, fieldtrips (to be decided on and planned by clients), groups to develop healthy and effective communication skills using role-play, and community volunteerism of clients choice and plans.

Conclusion: The goal of this program is for the adolescents to increase their occupational performance success by improving perceptions of self-efficacy, performance of a healthy lifestyle, and developing interpersonal skills. The purpose of the staff is to facilitate the positive skills and interactions, assisting the adolescents in generalizing the skills to their own lives. The expected outcome of this program is the participating adolescents are less likely to experience any of the previously mentioned problems, benefiting themselves, their families, schools, and communities by coping positively with the changes of adolescence and gaining independence.

POSITIONING AND ERGONOMICS FOR PREGNANT WOMEN WHILE PERFORMING DAILY TASKS.

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Pregnant women frequently experience low back pain and difficulties carrying out daily activities at work and in the home environment. This project was designed to address these issues by developing an educational packet for pregnant women and employers. The literature supports addressing these areas in order for women to remain active and employed for a longer duration throughout pregnancy, thus decreasing unnecessary medical leaves, maintaining productivity, and providing continued revenue for the employer and the woman. There is a need for increased information for employers regarding ergonomics and positioning needs unique to pregnant employees. In addition, the women may be able to continue to pursue other occupations including daily living tasks and leisure activities.

Information was obtained and synthesized from journal articles, books and websites into an extensive literature review. The data gathered assisted in developing an easy-to-read educational packet with photographs of a woman in her third trimester displaying the proper positioning and body mechanics necessary to carry out daily activities at work and in the home. The educational packet is divided into four sections: activities of daily living, including self care and sexual activity; instrumental activities of daily living, including home tasks, cleaning, caring for others, and exercise; and work activities. The summary section includes: general guidelines, employer considerations, recommendations, restrictions and a list of additional resources to access. The goal of our project is to increase the knowledge of proper positioning and ergonomics for pregnant women and their employers. The educational packet provides information to assist in promoting the health and safety of the pregnant woman and her fetus.

CALIBRATION OF PARAMETRIC EQUATIONS TO PREDICT PRAIRIE POTHOLE BASIN GEOMETRY
AND AREA-DEPTH-VOLUME RELATIONSHIPS

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The basin geometry of 15 prairie pothole wetlands in the upper Turtle River watershed has been measured. These potholes are all ephemeral and, although similar in size and shape, each exhibits different geometrical character. A few are asymmetrical rather than the more typical symmetrical shape. This suggests a different research direction, which will focus on testing a hypothesis about the cause for the difference. We propose that wind may be an important mechanism that causes the asymmetrical shape, but do not yet know why only a small subset of the potholes shows this characteristic.

Continued research into their geometry will allow for a better understanding of the reasons behind their shapes. A rapid method for their measurement has been devised and implemented, allowing us to increase the rate that potholes can be surveyed, thus creating a better database. Also, sampling different types of potholes will increase the amount of knowledge. Currently, we lack good data on larger, more permanent potholes, as these are more difficult to measure. We expect that the shape of these will differ significantly from those already measured. Basic equations to estimate the volume of symmetrical potholes have been applied, but modifications to the equations need to be made to accommodate the asymmetrical shapes.

Each wetland is surveyed using a telescopic auto-level to determine relative elevation and a GPS unit to establish spatial coordinates. Data are input into SURFER software to estimate volumes and provide calibration of parametric volume-area and depth-volume relationships. For each wetland, a cross-section is created to characterize the degree of symmetry and to judge whether an asymmetric version of the existing equations needs to be applied.

Our intent will be to continue the measurement of these potholes throughout the entire watershed, so that a consensus can be reached about the general character and distribution pothole geometry. These data should provide us with an enhanced knowledge on the distribution of pothole shape, shoreline characteristic, and volume-area relationship, and information on the physical factors that lead to these features. Based on data already extracted from the U.S. Fish and Wildlife Service National Wetlands Inventory, we believe it will be possible to characterize the variability and degree of asymmetry for wetlands throughout the prairie pothole region.

COMMUNICATIONS

PROFESSIONAL

NDAS Professional Session (I)

Moderator: Dr. Siegfried Detke, University of North Dakota

- 9:10-9:30** Siegfried Detke* **MAPPING OF THE FUNCTIONAL DOMAINS OF TOR, PROGENITOR OF AN NEW ATYPICAL MULTIDRUG RESISTANCE MECHANISM IN LEISHMANIA** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 9:30-9:50** Cindy M. Anderson*, Faye Lopez, Hai-Ying Zhang, Kristin Pavlish and Joseph N. Benoit **REDUCED UTERO-PLACENTAL PERFUSION INDUCES ALTERED LEPTIN SIGNALING AND VASCULAR DYSFUNCTION** Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, ND
- 9:50-10:10** James McAllister*, John Kirby, and Phil Gerla **ICHTHYOFAUNAL SURVEY OF AN AGRICULTURALLY DISTURBED PRAIRIE-WETLAND COMPLEX IN POLK COUNTY, MINNESOTA** Department of Natural Science, Dickinson State University, Dickinson, ND and the Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND
- 10:10-10:30** James D. Foster*, Randy D. Blakely, and Roxanne A. Vaughan **ANALYSIS OF DOPAMINE TRANSPORTER PHOSPHORYLATION SITES BY CHEMICAL AND ENZYMIC DIGESTION AND MUTAGENESIS** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND

NDAS Professional Session (II)

afternoon session begins at 4:15!

- 4:20-4:40** Joseph Hartman*, Marron Bingle, and Paige Baker **INTERPRETING THE EXISTENCE OF A PAIR OF CLOSELY RELATED SPECIES OF CRETACEOUS HELL CREEK FORMATION FRESHWATER MUSSELS** Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND
- 4:40-5:00** Hai-Ying Zhang*, Kristin M. Pavlish, Xuesong Chen, David Machado-Arando, Cindy M. Anderson, David A. Dean, Joseph N. Benoit **EFFECT OF CHRONIC PORTAL HYPERTENSION ON RHOA/RHO-KINASE MEDIATED VASCULAR SMOOTH MUSCLE SIGNALING TRANSDUCTION** Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, ND
- 5:00-5:20** Barry Milavetz*, L. Balakrishnan **LOCALIZATION OF HYPERACETYLATED HISTONE H4 ON THE SV40 GENOME DURING THE SV40 LIFE-CYCLE** Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND
- 5:20-5:40** Paul S. Hardersen* **PLANS FOR A 1-METER-CLASS PROFESSIONAL ASTRONOMICAL OBSERVATORY FOR THE STATE OF NORTH DAKOTA** Department of Space Studies, University of North Dakota, Grand Forks, ND

MAPPING OF THE FUNCTIONAL DOMAINS OF TOR, PROGENITOR OF A NEW ATYPICAL MULTIDRUG RESISTANCE MECHANISM IN LEISHMANIA

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Leishmania are protozoan parasites present in large areas of the world and are responsible for Leishmaniasis. It is estimated that 350 million people live in endemic areas and 1.5 to 2 million will be infected each year. There may be as many as 12 million infected people worldwide. TOR was originally discovered by its ability, when present in high levels, to render Leishmania resistant to toxic nucleosides. It also affected the sensitivity of Leishmania to a number of other toxins, some of which are used at the clinical level. TOR brings about resistance to toxic nucleosides by "shutting down" the ability of these cells to transport purine nucleosides and nucleobases. In addition, TOR elicits a rapid internalization and degradation of these permeases. Truncation mutants showed that the domain responsible for inhibiting permease activity resided at the N terminal portion of TOR. A clathrin binding domain was found in the C terminal half. Deletion of this clathrin binding domain by site specific mutagenesis abolished TOR's ability to internalize the adenosine transporter. These data indicate that TOR is the progenitor of a new class of endocytotic accessory proteins.

REDUCED UTERO-PLACENTAL PERFUSION INDUCES ALTERED LEPTIN SIGNALING AND VASCULAR DYSFUNCTION

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Preeclampsia, a form of pregnancy-induced hypertension complicating 6-8% of pregnancies, plays a major role in fetal growth restriction and premature birth as well as infant and maternal morbidity and mortality worldwide [1]. The pathology of preeclampsia is believed to develop during early pregnancy, and eventually manifests as endothelial dysfunction leading to hypertension and decreased perfusion to major organ systems. Reductions in utero-placental perfusion lead to impaired growth in the fetus resulting from decreased placental size as well as reduced delivery of oxygen and nutrients [2-4].

Leptin is a protein product of the obesity (ob) gene, with the placenta serving as the major site of production during pregnancy. Leptin's effects are exerted through binding to the leptin receptor, inducing isoform-specific signal transduction pathways. Placental secretion follows a gestational pattern in both humans and in Sprague-Dawley rats [5] believed to be representative of leptin's role as a fetal growth factor [6-8]. While data regarding the implications of leptin in preeclampsia have often been conflicting, the description of leptin's potential influence on the progression of pathology in preeclampsia and fetal growth restriction may provide important insights. The present study was designed to determine plasma leptin concentrations and to characterize leptin signaling in small uterine arcuate arteries in a rat model of reduced uterine perfusion pressure. The reduced uterine perfusion pressure (RUPP) model was utilized to mimic the underlying pathology of preeclampsia.

The uterine arteries and abdominal aortae of pregnant Sprague-Dawley rats were surgically constricted on day 14 of gestation. The rats were euthanized on gestational day 20. Plasma was collected for determination of plasma leptin levels. Placental tissue was removed and immediately frozen in liquid nitrogen until assayed. The uterus was removed, uterine arcuate arteries dissected free from surrounding tissue and either mounted on a small vessel wire myograph or frozen in liquid nitrogen until analysis. Endothelium-dependent relaxation response was measured in precontracted uterine arteries exposed to increasing concentrations of leptin. Western Blot analysis was used to determine leptin receptor expression and signaling in uterine arteries. Polymerase chain reaction was used to determine leptin receptor subtypes.

Reduction in utero-placental perfusion induced a significant decrease in plasma leptin levels (1.48 ± 0.13 vs. 2.43 ± 0.35 ng/ml). A truncated form of leptin receptor, Ob-Ra, was predominant in placenta and vascular tissues. Signaling pathways producing generation of endothelial nitric oxide synthase were altered in the RUPP model, with deficient production of endothelial nitric oxide synthase evident in the uterine arteries. Endothelium-dependent relaxation response was reduced in the uterine arteries of the RUPP group.

This research provided the first detailed characterization of plasma leptin levels, leptin-related signaling pathways and leptin functional response of the uterine artery in a rat model of reduced utero-placental perfusion. The data suggest that reduced utero-placental perfusion induces alterations in leptin levels and signal transduction pathways, contributing to a hyper-responsive uterine vasculature and the development of fetal growth restriction.

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ICHTHYOFAUNAL SURVEY OF AN AGRICULTURALLY DISTURBED PRAIRIE-WETLAND COMPLEX IN POLK COUNTY, MINNESOTA

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Introduction. The ichthyofauna was surveyed within and adjacent to The Nature Conservancy's 10,000 ha Glacial Ridge prairie restoration project, near Crookston, MN. Six ditches extend the headwaters of the surrounding stream system into gently sloping agricultural land that lies within the beach ridges of glacial Lake Agassiz. The resulting fish data allow comparison of habitat use between natural streams and the ditch system.

Materials and Methods. Seventeen sites were sampled between May 24 and May 28, 2003. The fishes were collected with a Cofelt Mark 10 electroshock unit, netted, identified, breeding characteristics noted (colors, tubercles, behaviors, gravid specimens), and released. The shocked stream lengths, widths, and fish counts provide data to calculate fishing effort (FE): $FE(\text{Site}) = \# \text{ fishes collected at a site/local stream length shocked} \times \text{stream width}$; $FE(\text{species}) = \# \text{ fish collected for a species at all sites/total stream length shocked} \times \text{stream width}$. The substrate (mud, sand, pebble, cobble) and stream morphology (pool, riffle, channel, dam) were characterized at each sample site. Physical characteristics of water, including conductivity, were measured when fishes were sampled.

Results. Most sites consist of mud substrate, low gradient channels interspersed with pools and emergent vegetation. Conductivity ranged between 200 and 1,000 $\mu\text{S}/\text{cm}$, generally increasing downstream from southeast towards the west. One site is distinct in fishes and habitat. This site crosses a beach ridge and provides a rocky spawning substrate with pools and riffles. The spawning site is one of only two sampled sites with significant riffle habitat. There were 876 total fishes from these 17 sites (499 fishes from the spawning site). Shocked stream areas ranged from 13-514 m^2 , and averaged 157.8 m^2 . The range and average FE (site) at the 16 sites is 0.0 to 1.7, and 0.34 fishes/ m^2 . The spawning site extends the range and average to 6.4 and 0.70 fishes/ m^2 .

Twelve species in six families were collected. The species names are listed with the total number of specimens, the number of specimens from the spawning site (if applicable), and the FE (species) in fish/ m^2 : Catostomidae: white sucker, *Catostomus commersoni* (42, 25, 0.016); Cyprinidae: creek chub, *Semotilus atromaculatus* (113, 21, 0.042); blacknose dace, *Rhinichthys atratulus* (23, 8, 0.009); longnose dace, *Rhinichthys cataractae* (3, 0.001); northern redbelly dace, *Phoxinus eos* (28, 0.010); fathead minnow, *Pimephales promelas* (111, 19, 0.041); bigmouth shiner, *Notropis dorsalis* (428, 424, 0.160); common shiner, *Luxilus cornutus* (31, 0.012); Esocidae, northern pike, *Esox lucius* (2, 0.001); Gasterosteidae, brook stickleback, *Culaea inconstans* (33, 0.012); Percidae, johnny darter, *Etheostoma nigrum* (16, 2, 0.006); Umbridae, central mud minnow, *Umbra limi* (46, 0.017). Spawning fishes include white suckers, creek chub, blacknose dace, fathead minnows, and bigmouth shiners.

Discussion. The surveyed fishes are typical residents of small natural streams within the Red River basin. The fishes are expected to be natural migrants or recent descendants with established reproduction in the ditches, except for northern pike. As adults, pike are found in larger streams or lakes and were likely stocked in the gravel quarry (pers. comm. Jason Ekstein, 2003). Bigmouth shiners congregating at the spawning site skew the species proportions; almost 50% of the sample fishes were bigmouth shiners versus 1% if the spawning site is excluded. White suckers were also in spawning congregations at this site.

Conclusions. The ichthyofauna is dominated by cyprinids in species richness and total numbers. These fishes are prairie natives, common in headwaters, and typically tolerant of variable oxygen concentration and temperature. The species diversity reflects small stream habitat. Within the study area, there is a distinct difference of habitat use between the beach ridge riffles and the more typical mud substrate channels. A northern pike population is sustained by the presence of an active quarry refuge.

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ANALYSIS OF DOPAMINE TRANSPORTER PHOSPHORYLATION SITES BY
CHEMICAL AND ENZYMATIC DIGESTION AND MUTAGENESIS

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Introduction. Dopamine transporters (DATs) terminate dopaminergic neurotransmission by the reuptake of dopamine (DA) from the synapse into presynaptic neurons. DATs are phosphorylated and down-regulated in response to protein kinase C (PKC) activators and the protein phosphatase inhibitor, okadaic acid (OA). Previously, we identified a cluster of six serines in the N-terminal tail as the primary sites of DAT phosphorylation in rat striatum, but the precise sites utilized are not known.

Methods. Mutagenesis: To address the issue of which DAT N-terminal serine residues are phosphorylated, we constructed stably transfected LLC-PK₁ cell lines expressing rat DATs with serine to alanine mutations at residues 2, 4, 7, 12, 13, and 21. Stably transfected mutant rat DAT expressing LLC-PK₁ cell lines were generated in the laboratory of Dr. Randy Blakely by Michelle Mazei. The LLC-PK₁ cell line expressing wild type rat DAT was obtained from Dr. Gary Rudnick and Dr. Howard Gu. Metabolic Phosphorylation of DAT: Cell cultures were incubated with phosphate-free medium for one hour followed by ³²PO₄-containing medium (0.5 mCi/ml) for 2 hours. Cultures received vehicle or test compounds (OA and PMA) for an additional 30 min prior to the solubilization of cells and extraction of DAT. Immunoprecipitation: Immunoprecipitation of phosphorylated samples was performed with rabbit polyclonal antisera 16 against N-terminal amino acids 42-59 (peptide 16) of the rat DAT protein sequence. Proteolysis/Chemical Cleavage: ³²PO₄-labeled DAT was purified by immunoprecipitation followed by SDS-PAGE and electroelution. Purified DAT was incubated with either leucine aminopeptidase (20 µg/ml) at 37°C for 2 h or pfu aminopeptidase 1 (20 µg/ml) at 75°C for 2 h. Cyanogen bromide (CNBr) cleavage of DAT (50% formic acid, 0.25 M CNBr) was performed at room temperature with an overnight incubation.

Results. All single point mutants retained significant phosphorylation in response to PMA plus OA suggesting that phosphorylation occurs on more than one residue. Digestion of ³²PO₄-labeled DAT with Pfu aminopeptidase I, an exo-type aminopeptidase that does not hydrolyze the peptide bond at the ?-amino residue side of proline, showed no removal of radiolabel from DAT which contains a proline at residue 10. On the other hand, radiolabel was removed efficiently from DAT in the presence of leucine aminopeptidase, a nonspecific exo-type aminopeptidase. CNBr cleavage at the C-terminal side of methionine 11 would generate a 975 Da DAT peptide fragment containing serines 2, 4, and 7, but no radiolabeled fragment of this mass was generated in the presence of CNBr. Furthermore, manual N-terminal sequencing of phosphorylated DAT by Edman degradation did not liberate any phosphorylated amino acid residues through seven cycles.

Conclusion. These results suggest that serines 2, 4 and 7 may not be the major phosphorylation sites in DAT.

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INTERPRETING THE EXISTENCE OF A PAIR OF CLOSELY RELATED SPECIES OF CRETACEOUS HELL CREEK FORMATION FRESHWATER MUSSELS

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INTRODUCTION

The Upper Cretaceous biota is replete with interesting fossils, not the least of which are the freshwater mussels (Unionoidea) living in the rivers and lakes between the Sevier and Laramide Mountains to the west and the Western Interior Seaway to the east. During the last 2 Ma of the Cretaceous, the species richness of these mussels reached new levels. The cause of this diversity and its subsequent demise is the main interest in ongoing investigations. This contribution addresses two surface-sculptured taxa, assigned provisionally to the modern genus *Plethobasus*, from the type area of the Hell Creek Formation in Garfield County, Montana.

PALEONTOLOGY

Plethobasus aesopiformis and *P. biesopoides* were both part of the inaugural description of the Hell Creek molluscan fauna in 1903 and 1907 (1, 2). As was true for any toothed mussel of the time, they were assigned to *Unio*. These taxa were subsequently reassigned to *Plethobasus* by Russell (2). We consider the assignment to *Plethobasus* provisional as these fossil taxa did not survive the K/T boundary. Thus there is only convergent superficial resemblance to extant taxa, which likely have their origins in the eastern United States. We consider *P. aesopiformis* and *P. biesopoides* to be sister species. They were initially thought to be only distinguishable by the number of rows of irregularly shaped nodes that curve posteroventrad from the umbo. *P. aesopiformis* was recognized as having one row of nodes, while *P. biesopoides* has two. Both species possess comparable beak placement, the number of umbonal corrugations that curve ventrad around the umbo, disc convexity, and node shape, node prominence, and number of nodes. However, further inspection permits discrimination based upon other character traits. *P. biesopoides* is slightly larger and appears more robust. It also possesses more robust and more prominent pseudocardinal teeth. The umbonal cavity is also deeper than found in *P. aesopiformis*. Another noticeable difference between these two species occurs in the disc outline shape. Traditionally, the genus *Plethobasus* is recognized as having an orbicular outline, and *P. aesopiformis* generally conforms to this ideal. However, *P. biesopoides* is more trigonal in marginal outline, with a more inflated umbo.

STRATIGRAPHY AND OCCURRENCE

Both *Plethobasus aesopiformis* and *P. biesopoides* are distinctive elements in the assemblage of species in the Hell Creek fauna and are not easily overlooked during collecting or subsequent taxon sorting. Thus their geographic and stratigraphic distribution within the collections made since 1903 can more easily be taken as valid than some other taxa. Both range through most of the Hell Creek Formation. Both are known from the lower but not lowest part of the formation (basal 27 m) and, likewise, neither are known from the uppermost part of the formation (top 10 m). Both are known from about the same number of localities and may co-occur. Typically, however, *P. aesopiformis* is much more abundant in number of specimens at a locality. In total, between 4 and 5 times as many specimens of *P. aesopiformis* have been collected. Although not widely reported, in contrast to Russell's (3) restricted distribution, *P. aesopiformis* is also known from the Lance Formation of the Powder River Basin, Wyoming, and the Hell Creek Formation in the North Dakota part of the Williston Basin. *P. biesopoides* is also known from the Hell Creek fauna of the eastern Crazy Mountains Basin, Montana.

DISCUSSION

Current studies indicate that *Plethobasus aesopiformis* and *P. biesopoides* are distinct and part of radiation of unionoids that we are only beginning to fully understand. They are found associated with other sculptured mussels, none of which survive past the K/T boundary. In fact, both *P. aesopiformis* and *P. biesopoides* were likely extinct before the end of the Cretaceous, and we suggest that this is due to the loss of diversified lotic habits with the eustatic rise of the Cannonball Sea, the last transgressive-regressive cycle of the Western Interior Seaway.

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EFFECT OF CHRONIC PORTAL HYPERTENSION ON RHOA/RHO-KINASE MEDIATED VASCULAR SMOOTH MUSCLE SIGNALING TRANSDUCTION

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Functional studies from our lab suggested that portosystemic shunting other than elevated portal venous pressure is responsible for the altered vascular responses presented in hyperdynamic circulation in chronic portal hypertension (1). Although the physiological behavior of the resistance vasculature in portal hypertension has been well studied, the cellular and molecular mechanisms underlying the physiological changes remain undefined. The present study was designed to examine the effect of both elevated portal venous pressure and portosystemic shunting on RhoA/Rho-kinase mediated vascular smooth muscle signaling pathway.

Two animal models, partial portal vein ligation (pre-hepatic portal hypertension, PHT) and portacaval shunt (PCS), were used and the small mesenteric arteries were harvested 14 days after the initial surgery. Splenic pulp pressures, which represent portal venous pressures, were measured in the three groups. PCS animals had the lowest pressures (4.10 ± 1.39 mmHg) and PHT animals had the highest pressures (14.00 ± 1.80 mmHg) when compared with sham operated controls (9.40 ± 1.29 mmHg). To this end the PCS is a high shunting low portal pressure model and the PHT is a high shunting high portal pressure model. RhoA and Rho-kinase beta expression were down-regulated at transcriptional levels in both PHT and PCS groups ($P < 0.01$). Membrane associated RhoA and Rho-kinase beta protein levels were decreased in PHT and PCS when compared with controls ($P < 0.01$) while the cytosolic fractions remained unchanged. Using GeneChip expression analysis, we confirmed the down-regulation of Rho-kinase beta mRNA expression in PHT vessels. Protein kinase C delta expression was down-regulated at the transcriptional level in both PHT and PCS groups ($P < 0.05$). Myosin phosphatase phosphorylation (MYPT1 Thr696) was decreased in both groups ($P < 0.05$). CPI-17 phosphorylation (Thr38) remained unchanged. Phenylephrine (10^{-4} M) induced myosin regulatory light chain (RLC₂₀) phosphorylation was impaired in both PCS ($36.75 \pm 4.40\%$ vs. $48.10 \pm 2.70\%$, $P < 0.05$) and PHT (34.26 ± 1.18 vs. $48.10 \pm 2.70\%$, $P < 0.05$) groups.

MLCP is a trimeric enzyme, which is composed of a 110/130 kDa regulatory subunit (MYPT1), a 37 kDa catalytic subunit (PP1c) and a 20 kDa subunit of unknown function. It has been extensively studied that a small monomeric GTPase RhoA plays a key regulatory role in calcium sensitization. Independent studies suggested that RhoA activates Rho-kinase, leading to the inhibition of MLCP by phosphorylation of MYPT1 at Thr696. It is suggested that the activation of Rho-kinase by RhoA depends on the recruitment of both RhoA and Rho-kinase to plasma membrane (2). It is also proposed that MLCP inhibition is not limited in the phosphorylation of MYPT1 subunit. CPI-17, a 17 kDa potent phosphatase inhibitor when phosphorylated at Thr38, is thought to inhibit the PP1c directly. And both PKC and Rho-kinase are responsible for the phosphorylation of CPI-17 at Thr38 (3,4). Our results suggest the target of RhoA/Rho-kinase pathway is MYPT1 in portal hypertensive resistance vasculature, but not CPI-17.

These results indicate that chronic vasodilatation condition in portal hypertensive hyperdynamic circulation alters vascular smooth muscle contraction related gene expression. A down-regulated RhoA/Rho-kinase pathway may underlie the reduced vasoconstrictor effectiveness in portal hypertensive resistance vasculature. We further suggest that these changes are the result of portosystemic shunting, not elevated portal venous pressure.

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LOCALIZATION OF HYPERACETYLATED HISTONE H4 ON THE SV40 GENOME DURING THE SV40 LIFE-CYCLE

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In order to investigate the role of histone acetylation in establishing an infection by simian virus 40 (SV40), we have analyzed formalin fixed and unfixed SV40 chromosomes obtained 30 minutes and 3 hours post-infection for the presence of tetraacetylated histone H4 and di-acetylated histone H3 using a combination of chromatin immunoprecipitation (ChIP) techniques including quantitative ChIP assays with competitive PCR, depletion analyses, and competitive ChIP analysis. From this analysis we have determined that there are two distinct classes of SV40 chromosomes containing hyperacetylated histones present at 30 minutes post-infection. One class is specifically hyperacetylated on only histone H4, while the other class is hyperacetylated on both histone H4 and histone H3. During the first three hours of infection we observed the deacetylation of the class of chromosomes which contain only hyperacetylated histone H4 but little if any effect on the other class of chromosomes.

Because our analysis were performed on intact SV40 chromosomes it was not possible to determine if the chromosomes contained hyperacetylated histones uniformly distributed on the SV40 genome or whether there were preferred positions for the modified histones. To address this question we are analyzing SV40 chromosomes for the location of the hyperacetylated histones in a two-step approach. In the first step chromosomes are bound to protein agarose using antibody recognizing a form of hyperacetylated histone as in the regular ChIP analysis. In the second stage this immune-selected chromatin bound to agarose is subjected to non-specific fragmentation either using sonication or mild micrococcal nuclease digestion. The residual bound chromatin and solubilized chromatin are then analyzed by PCR using sets of primers recognizing different regions on the SV40 genome. Our preliminary results suggest that hyperacetylated H4 is distributed randomly on the SV40 genome.

PLANS FOR A 1-METER-CLASS PROFESSIONAL ASTRONOMICAL OBSERVATORY
FOR THE STATE OF NORTH DAKOTA.

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Introduction: The Department of Space Studies at the University of North Dakota (UND) is proposing to build and operate a 1-meter-class professional astronomical observatory in the State of North Dakota. Currently, no professional observatories exist within the state. The proposed facility, which will be operated and maintained by the Department of Space Studies, will serve state-wide research, education, and public outreach goals through collaborative outreach efforts with North Dakota's colleges, universities, and public and private schools. Educators, students, and researchers will have access to the observatory either on-site or via the Internet. Research efforts will primarily focus on different types of photometry due to the low-altitude location of the observatory.

Facilities Overview: The observatory will be located on UND land ~13 miles west of Grand Forks, near Emerado, ND. The plot of land for the observatory is already maintained by the Department of Space Studies and includes ACIT, a 16-inch Meade Schmidt-Cassegrain Internet-controlled telescope that is used for undergraduate-level classes. Additionally, an 18-inch Newtonian telescope is housed in a 24-foot-diameter dome. Three permanent telescope piers will be installed during summer 2004 for the Department's small 8-inch and 10-inch telescopes.

Construction of the new observatory will involve removal of the 24-foot-diameter dome and a trailer that has been used for equipment storage.

The new observatory will consist of a ~1-meter telescope housed in a traditional dome. The dome will be connected to a large, 70' x 30' multipurpose building via a walkway. The attached building will serve a variety of purposes such as telescope control, maintenance and repair, and as a meeting place for classes, community groups, and public events. The first floor of the building includes a large open area that will facilitate group events.

Site: The observatory site is located in the Red River Valley of eastern North Dakota and sits at an elevation of ~200 meters. As is typical of low-altitude observatories, the number of clear nights is variable. In the past ~3 years, the number of clear nights has averaged ~50%. Light pollution is moderate. Sky glow from Grand Forks is visible ~20° above the eastern horizon; similar sky glow from Grand Forks Air Force Base is visible ~10° above the northwestern horizon. Although the sky conditions are not ideal, they are acceptable and will allow substantive astronomical research and educational activities to occur.

The site includes access to power and water infrastructure that will not require significant upgrading in anticipation of the new observatory.

Research: Due to its low-altitude location, most of the research activities will involve different types of photometry. Examples of research activities include variable star photometry; asteroid light-curve photometry; extrasolar planet transit photometry; and search programs for near-Earth asteroids, supernovae, gamma ray bursts, and lunar flashes.

Establishment of an asteroid light-curve photometry project will complement the work of UND's researchers who conduct near-IR reflectance spectroscopy of asteroids [1,2]. Although light-curves exist for ~2000 asteroids [3], more than ~150,000 are present in the main asteroid belt. Deriving asteroid rotation rates from light-curve data will complement spectroscopic measurements of asteroid surfaces and studies of their mineralogical compositions.

Extrasolar planetary transits have been detected by small telescopes [4]. Since the first extrasolar planet detection in 1992 [5], more than 120 extrasolar planets have thus far been detected. Photometric transits offer an independent detection technique that can verify detections by other methods, such as Doppler shifts from a star orbiting its system's center-of-mass [6].

Many types of variable stars exist that vary their photometric output in a systematic manner [7]. Long-term observation programs, such as those conducted by the American Association of Variable Star Observers (AAVSO) [8], offer researchers and students the ability to study particular stars, their modes of variability, and the underlying physical mechanisms.

Education: Efforts are underway to inform the North Dakota educational community about the capabilities of the proposed observatory. State-wide mailings have been conducted to introduce educators to this project. Thus far, positive responses have been received from colleges, school districts, individual schools, and teachers from across North Dakota. Letters of support have been received from Minot State University, Bottineau Campus; Turtle Mountain Community College, Belcourt; Lake Region State College, Devils Lake; Minnesota State University, Moorhead, Minnesota; Jamestown Public Schools; Grand Forks Public Schools; Sawyer School District #16; Lakota Public School District #66; Beulah Public School District #27; Edinburg Public School District #106; St. Mary's Central High School, Bismarck; Woodrow Wilson Community High School,

Fargo; Hope-Page High School, Hope; Century High School, Bismarck; Des Lacs Burlington High School, Des Lacs; Midway Public Schools, Inkster; and Northwood Public Schools, Northwood. In addition, a letter of support has been received from the City of Grand Forks.

Internet-control of the observatory, with staff overseeing telescope operations on-site, will allow educators and students from across the state to utilize a high-quality telescope without the necessity of traveling to the Grand Forks area. Due to winter weather hazards and long distances, travel to the observatory during the winter months would not usually be feasible. Internet-control, however, will provide access for educators and students from across the state and will truly make the observatory a state-wide resource.

Collaborations are planned with interested schools and colleges that will allow researchers, educators, and students access to the telescope. Educators, as well as researchers, must submit proposals that will be competitively reviewed by a telescope allocation committee (TAC). Successful proposals will then be allocated observing time.

Public Outreach: Due to the lack of any large astronomical facilities within North Dakota and the planned observatory design presented here, significant efforts will be made to develop and implement public outreach programs in the Grand Forks area as well as throughout the state.

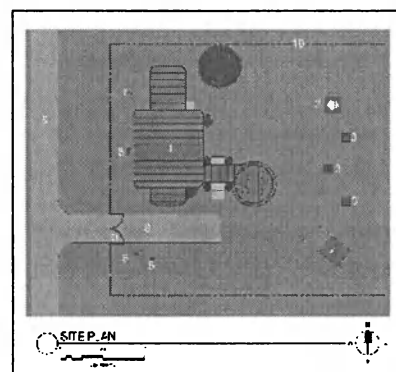
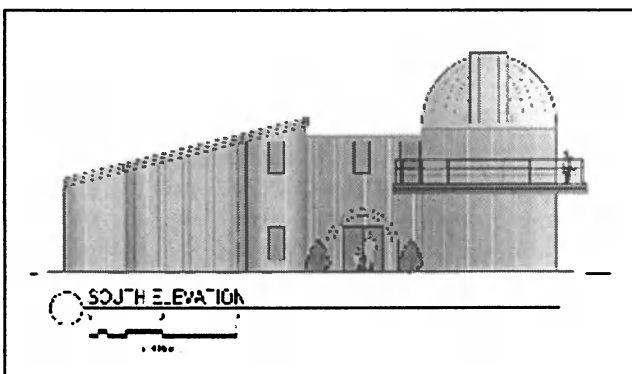
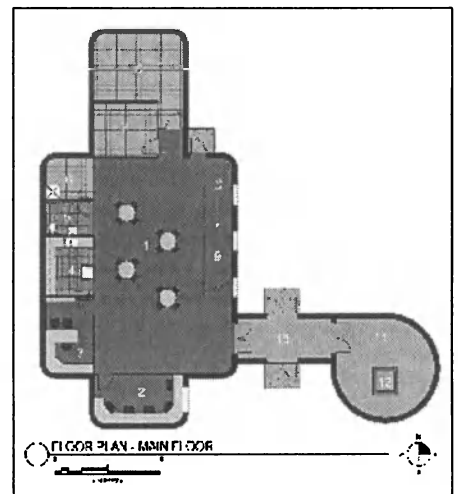
Events such as Astronomy Day, Space Week, Space Day, and regularly-scheduled star parties will serve as ways to promote astronomy and science education in the Grand Forks area and throughout the state. Unique astronomical events will also be used to inform and educate the general populace about astronomy, the night sky, and the scientific method.

Current Status: The architectural firm of Widseth-Smith-Nolting (WSN) completed a feasibility study of the observatory for UND in September 2003 [9]. Estimated capital costs for the telescope, multipurpose building, and all associated furnishings is ~\$2 million in 2006 dollars. An endowment of ~\$1.2 million is also planned for the observatory to pay for most annual operating expenses.

Efforts are currently underway to raise funds from public and private sources. Proposals are currently being submitted to private foundations in North Dakota and throughout the United States. A private giving program to encourage North Dakotans to become active participants in the observatory project will begin soon. Other efforts will be made to obtain funds from additional private foundations, the National Science Foundation, the North Dakota state legislature, and the federal government.

References:

- [1] Hardersen P. S. et al. (2004) *Icarus*, 167, 170.
- [2] Hardersen P.S. et al. (2002) *LPSC XXXIII*, Abstract #1148.
- [3] Lagerkvist C.-I. et al. (1989) *Asteroids II*, 1162.
- [4] www.transitsearch.org.
- [5] Wolszczan A. and Frail D.A. (1992) *Nature*, 355, 145.
- [6] Marcy G.W. and Butler R.P. (1998) *Annu. Rev. Astron. Astrophys.*, 36, 57.
- [7] Good G.A. (2003) *Observing Variable Stars*, Springer-Verlag, London.
- [8] www.aavso.org.
- [9] Hardersen P.S. (2003). Feasibility Study for Observatory Facility, Emerado, North Dakota. Project #780G153. Widseth-Smith-Nolting.



CONSTITUTION of the NORTH DAKOTA ACADEMY OF SCIENCE*Founded 1908, Official State Academy 1958***ARTICLE I - Name and Purpose**

Section 1. This association shall be called the NORTH DAKOTA ACADEMY OF SCIENCE.

Section 2. The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

ARTICLE II - Membership

Membership in the Academy shall be composed of persons who share the stated purpose of the Academy and who are active or interested in some field of scientific endeavor.

ARTICLE III - Council

The officers of the Academy shall be a President, a President-Elect, and a Secretary-Treasurer. The Council, consisting of the officers, the retiring President, and three elected Councilors, shall be responsible for the fulfillment of the scientific and business obligations of the Academy.

ARTICLE V - Dissolution and Limits of Action

Section 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for education and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c)(3) of the Internal Revenue Code of 1954.

Section 2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in or intervene in, any political campaign on behalf of any candidate for public office.

Section 3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

ARTICLE VI - Amendments

Section 1. This Constitution may be amended at any annual Business Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary -Treasurer who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.

Section 2. Bylaws may be adopted or repealed at any regular business meeting by a two-thirds vote.

BYLAWS**BYLAW 1. Meetings**

Section 1. *Scientific Meetings.* The Academy shall hold at least one annual scientific meeting each year at a time and place determined by the Council. Other scientific meetings, regional, state, or local, may be held at times and places determined by the Council. The Council shall establish regulations governing the presentation of papers at Academy sessions. Such regulations shall be made available to members at least three months before any meeting at which they are to apply.

Section 2. *Business Meetings.* A Business Meeting of the membership shall be scheduled at the regular, annual scientific meeting of the Academy. Ten percent of the active members shall constitute a quorum at the annual business meeting.

Section 3. *Special Meetings.* Special meetings shall be called by the President upon the request of ten percent of the active members and require twenty percent of the active members for a quorum. Notice of the time and place of such meetings shall be sent to all members of the Academy at least four weeks in advance of the meeting. Only matters specified in the call can be transacted at a special meeting.

Section 4. *Procedure.* Parliamentary procedures to be followed in all business meetings shall be those specified in "Standard Code of Parliamentary Procedure" by Alice F. Sturgis.

BYLAW 2. *Financial*

Section 1. *Dues and Assessments.* The annual dues and assessments may be changed from time to time by the Council, subject to approval by a two-thirds vote of the members at an annual Business Meeting. The student member dues shall be one-third (to nearest dollar) of the regular member dues. These dues are payable 1 December of each year.

Section 2. *Supporting Members.* Council shall maintain a program to encourage members to voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

Section 3. *Sustaining Members.* Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at \$50 or greater may be invited by the President or designee to become a Sustaining Associate.

Section 4. *Audit and Reports.* The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year. The Secretary-Treasurer shall report on the financial affairs of the Society, including the results of an annual audit, as may be requested by the Council.

BYLAW 3. *Membership*

Section 1. *Membership Categories.* Classes of membership shall include the following: (a) Regular, (b) Student, (c) Emeritus, (d) Honorary, (e) Supporting, (f) Sustaining, and (g) Lifetime Members.

Section 2. *Eligibility and Procedure for Membership.* Candidates for membership, except Sustaining Member, may be proposed by any regular or emeritus member of the Academy by submitting the candidate's name to the chairman of the Membership Committee.

(a) *Regular Members.* Any person who is active or interested in some field of scientific endeavor shall be eligible for regular membership. A majority vote of Council shall elect to regular membership.

(b) *Student Members.* Any student who is an undergraduate or graduate student in some field of science shall be eligible for student membership. A majority vote of Council shall elect to regular membership.

(c) *Emeritus Members.* Any member in good standing upon formal retirement is eligible for emeritus membership. A majority vote of Council shall elect to emeritus membership.

(d) *Honorary Members.* The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. A two-thirds vote of members attending the annual business meeting shall elect to honorary membership.

(e) *Supporting Members.* Regular or student members may voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

(f) *Sustaining Associates.* Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at \$50 or greater may be invited by the President or designee to become a Sustaining Associate.

(g) *Lifetime Members.* Any regular member in current good standing for at least one year may become a Lifetime Member by paying an assessment equal to 18 times the current annual dues in one lump sum or in two equal payments over the current and following year.

Section 3. *Privileges of Membership.*

(a) Voting at the annual business meeting is permitted of regular and emeritus members.

(b) Members of all categories may attend business meetings of the Academy.

(c) The Secretary-Treasurer and members of Council must be regular members in good standing.

(d) Regular, student, and emeritus members may submit abstracts or communications for scientific meetings of the Academy.

(e) Emeritus and Honorary Members shall be exempt from payment of dues.

(f) A Sustaining Member is provided a display area at the annual scientific meeting of five linear feet per \$50 donation up to a maximum of 20 linear feet.

(g) Every member in good standing shall receive a printed copy or an electronic copy (if available and of equal or lesser cost than the printed copy) of the annual *Proceedings of the North Dakota Academy of Science*, the form to be determined by the member.

(h) Special offices such as Historian may be created by the unanimous vote of the regular members at the annual Business Meeting.

(i) All student research participants shall receive a properly inscribed certificate.

Section 4. *Forfeiture of Membership.*

(a) *Nonpayment of dues.* Members shall be dropped from the active list on 31 November following the nonpayment of dues during the membership year commencing the previous 1 December. A member may return to the active list by paying the current year dues.

(b) *Expulsion for Cause.* Membership may be terminated for conduct injurious to the Academy or contrary to the best interests of the Academy. The accused member shall be given an opportunity for a hearing before the Council. If a majority of the Council votes to expel the member, the action must be ratified by at least two-thirds of the members present at the next annual business meeting of the Academy. An expelled member shall forfeit all paid dues and assessments.

BYLAW 4. *Duties and Responsibilities of the Council and Council Members*

Section 1. *Council.* The Council shall meet, at the call of the President, at least twice a year. The Council shall:

- (a) be the governing board of the Academy, responsible only to the membership.
- (b) arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the Academy.
- (c) determine the location of the annual meeting three years in advance.
- (d) annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science.
- (e) shall appoint and may compensate a Secretary-Treasurer.
- (f) shall appoint and may compensate an Editor of the PROCEEDINGS and other publications.
- (g) shall be empowered to charge a publication fee of authors on a per page basis.
- (h) shall control all activities of the Academy including grant applications.

Section 2. *President.* The President shall preside at meetings of the Council and over the annual business meeting of the Academy at the close of the regular term of office. The President shall vote only to break a tie. Unless otherwise specified, the President shall, with the approval of the Council, appoint members to serve on Standing Committees and *ad hoc* Committees, designate the chair of each Committee, and appoint representatives to other organizations. The President serves as Coordinator of the Local Arrangements Committee for the annual meeting that occurs at the end of the President's term.

Section 3. *President-Elect.* The President-elect shall be considered a vice president and shall serve as such in the absence of the President.

Section 4. *Past-President.* The retiring President shall serve as Past-President and chair of the Nominating Committee. The Past President shall serve *ex officio* on those committees designated by the President and shall serve in the absence of the President and President-elect.

Section 5. *Secretary-Treasurer.* The Secretary-Treasurer shall:

- (1) Assist Council in carrying on the functions of the Academy including the receipt and disbursement of funds under the direction of Council.
- (2) Manage the Academy Offices under Council's general supervision.
- (3) Serve as Managing Editor of the *Proceedings of the North Dakota Academy of Science*.
- (4) Prepare a summary of the most recent audit and a report of the Academy's current financial status. This information shall be shared with the membership at the annual business meeting and published in the PROCEEDINGS following the business meeting.
- (5) Perform all other duties of the Secretary-Treasurer listed in the Bylaws.
- (6) Serve as archivist and be responsible for all official records, archives, and historic material which shall be in deposit with the Secretary-Treasurer.

BYLAW 5. *Appointment, Nomination and Election of Members of Council*

Section 1. *Eligibility for Office.* All candidates for election or appointment to the Council must be regular members in good standing. Nominees for President-elect must be members who reside within easy commuting distance of the site of the annual meeting selected by the Council that occurs when the President-elect serves as President.

Section 2. *Nomination Procedures.* The Nominating Committee shall be responsible for all nominations to elective office, shall determine the eligibility of nominees, shall ascertain that nominees are willing to stand for office, and shall be required to advance to the Secretary-Treasurer at least two names for each open position as needed. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report.

Section 3. *Election Procedures.* Election shall be by secret mail ballot. The Secretary-Treasurer shall prepare a printed ballot that bears all names submitted by the Nominating Committee, that contains a brief biography of each candidate, and that has space for write-in candidates for each office. This ballot is to be mailed to all members no later than 1 November. Each member wishing to vote must return the marked ballot in a sealed signed envelope to the Secretary-Treasurer postmarked not more than thirty days after the ballots were mailed out to members. The President shall appoint tellers who shall count the ballots which have been received by the Secretary-Treasurer and the tellers shall present the results in writing to the President. A plurality of the votes cast shall be necessary to elect and in the case of a tie vote, the President shall cast the deciding vote. The results of the election shall be announced at the annual Business Meeting.

Section 4. *Term of Office.* A President-Elect shall be elected annually by the membership and the following years shall succeed automatically to President and Past President to constitute a three year nonrenewable term. Three Councilors shall be elected by the membership to three-year, non-renewable terms on a rotating basis. All elected Council members shall take office at the end of the next annual Business Meeting following election and shall continue until relieved by their successors. Council is empowered to appoint and compensate a Secretary-Treasurer to successive three-year terms that commence with the beginning of the fiscal year.

Section 5. *Removal from office or position* If for any reason any elected member of Council is unable to fulfill his/her duties, the Council member may be removed from office by two-thirds vote of Council. If for any reason the Secretary-Treasurer is unable to fulfill his/her duties, the Secretary-Treasurer may be relieved of all duties by a majority vote of Council.

Section 6. *Interim vacancies.* Should a vacancy occur in the Presidency, the Council by a majority vote shall appoint a member of the Academy able to coordinate the next annual meeting to fill the unexpired term. A retiring interim President shall succeed automatically to Past President. Should a vacancy occur in the Presidency-elect, the Council shall reassess and change the location of the coinciding annual meeting as necessary and then call for a special election by mail ballot. An interim vacancy in the Past-Presidency shall be filled by the most recently retired Past-President able to fill the duties of the Past-President. Persons appointed to fill the unexpired term of Secretary-Treasurer are expected to remain in the position for a minimum of three years. A vacancy in the office of Councilor shall be filled by a majority vote of Council until the following election at which time the interim Councilor may stand for a full three year nonrenewable term.

BYLAW 6. *Committees*

Section 1. *Standing Committees.* Standing committees shall include but not be limited to, the following: Editorial, Education, Denison Award, Necrology, Nominating, Resolution, Membership, and Audit Committees. The President shall appoint members of committees other than the Nominating and Audit Committees.

Section 2. *Editorial Committee.* The Editorial Committee shall consist of three regular members appointed to three year terms. The duties are explained in BYLAW 7 (Publications).

Section 3. *Education Committee.* The Education Committee shall consist of five regular members and two high school teachers appointed to five year terms. The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.

Section 4. *Denison Awards Committee.* The Denison Awards Committee shall consist of six regular members appointed to three year terms. The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors.

Section 5. *Necrology Committee.* The Necrology Committee shall consist of three regular members appointed to three year terms. The Necrology Committee shall report to the annual meeting on those deceased during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

Section 6. *Nominating Committee.* The Nominating Committee shall consist of the five most recent past-presidents. The major duties of the Nominating Committee are listed in BYLAW 5 (*Appointment, Nomination and Election of Members of Council*). The Nominating Committee will also administer the selection process, develop a separate funding source for a monetary award, and develop, for Executive Committee approval, the criteria for the North Dakota Academy of Science Achievement Award.

Section 7. *Resolution Committee.* The Resolution Committee shall consist of three regular members appointed to three year terms. The Resolution Committee shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.

Section 8. *Membership Committee.* The Membership Committee shall consist of unlimited numbers of regular members appointed annually.

Section 9. *Audit Committee.* The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year.

Section 10. *State Science Advisory Committee.* The State Science Advisory Committee (SSAC) shall consist of five regular or emeritus members appointed to four year terms. The SSAC shall serve to direct questions of a scientific nature to the appropriate expert as requested, shall inform regional granting agencies and state and national science policymakers of its expertise and availability and shall counsel those agencies and persons upon their request. The SSAC shall adhere in particular to the guidelines described in Article V, Section 2 of the Constitution.

Section 11. *Ad hoc Committees.* The President may appoint such additional committees as may be needed to carry out the functions of the Academy. Ad hoc committees serve only during the tenure of the president who appointed them. Reports of ad hoc committees shall be presented to Council or to the annual meeting.

BYLAW 7. *Publications*

Section 1. *Editorial Committee.* Three regular members are appointed to the Editorial Committee for renewable three year terms. The Editorial Committee shall develop and recommend the Academy publication program and policies to the Council. It will assist the Editors of each official publication in reviewing manuscripts for those publications that include the *Proceedings*. Chairs of symposia will review manuscripts written for relevant symposia.

Section 2. *Managing Editor.* The Secretary-Treasurer shall serve as the Managing Editor of all Academy publications and as such shall oversee each Editor.

Section 3. *Editor.* Editors shall serve three year terms. The Editors shall edit all official publications of the Academy including the *Proceedings*.

BYLAW 8. *Memorial Fund*

The Council of the Academy shall establish a J. Donald Henderson Memorial Fund and administer this fund so that the proceeds will be used to promote science in North Dakota.

BYLAW 9. *Fiscal Year*

The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December.

BYLAW 10. *Achievement Award*

The Academy establishes the North Dakota Academy of Science Achievement Award to be given periodically to an Academy member in recognition of excellence in one or more of the following:

- a. Nationally recognized scientific research.
- b. Science education.
- c. Service to the Academy in advancing its goals.

The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Council approval, the criteria for the award.

BYLAW 11. *Research Foundation*

The **North Dakota Science Research Foundation** is established as an operating arm of the Academy. The purposes of the Foundation are:

- (1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and
- (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota.

Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the Academy and shall be separately accounted for annually.

The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President of staggered five year terms. The chairperson of the Board shall be

appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral report to the membership of the Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

BYLAW 12. *Affiliations*

The Academy may affiliate itself with other organizations which have purposes consistent with the purposes of the Academy. Such affiliations must be approved by the Council and by a majority of those attending a regularly scheduled business meeting of the membership.

BYLAW 13. *Indemnification*

Section 1. Every member of the Council or employee of the North Dakota Academy of Science shall be indemnified by the Academy against all expenses and liabilities, including counsel fees, reasonably incurred or imposed upon him/her in connection with any proceedings to which he or she may be made part, or in which he or she may become involved, by reason of being or having been a member of the Council, or employee at the time such expenses are incurred, except in such cases wherein the member of the Council or employee is adjudged guilty of willful misfeasance or malfeasance in the performance of his or her duties. Provide, however, that in the event of a settlement of the indemnification herein shall apply only when the Council approves such settlement and reimbursement as being for the best interests of the Academy.

The foregoing right of indemnification shall be in addition to and not exclusive of all other rights to which such members of the Council or employee may be entitled.

ACADEMY OFFICERS AND COMMITTEES - 2002-2003

Executive Committee

Membership:

Past-President
 President
 President-Elect
 Secretary-Treasurer
 Councilors (three-year terms)

President-Elect

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Committees of the North Dakota Academy of Science**Executive Committee****Necrology Committee*****Editorial Committee*****Nominating Committee****Education Committee*****Resolution Committee*****Denison Awards Committee*****Membership Committee***

**North Dakota Research Foundation
 Board of Directors***

* indicates available openings

PAST PRESIDENTS AND LOCATIONS OF THE ANNUAL MEETING

NORTH DAKOTA ACADEMY of SCIENCE

1909	M A Brannon	Grand Forks	1957	W E Cornatzer	Grand Forks
1910	M A Brannon	Fargo	1958	W C Whitman	Fa: go
1911	C B Waldron	Grand Forks	1959	Arthur W Koth	Minot
1912	L B McMullen	Fargo	1960	H J Klosterman	Fargo
1913	Louis VanEs	Grand Forks	1961	Vera Facey	Grand Forks
1914	A G Leonard	Fargo	1962	J F Cassel	Fargo
1915	W B Bell	Grand Forks	1963	C A Wardner	Grand Forks
1916	Lura Perrine	Fargo	1964	Fred H Sands	Fargo
1917	A H Taylor	Grand Forks	1965	P B Kannowski	Grand Forks
1918	R C Doneghue	Fargo	1966	Paul C Sandal	Fargo
1919	H E French	Grand Forks	1967	F D Holland, Jr	Grand Forks
1920	J W Ince	Fargo	1968	W E Dinusson	Fargo
1921	L R Waldron	Grand Forks	1969	Paul D Leiby	Minot
1922	Daniel Freeman	Fargo	1970	Roland G Severson	Grand Forks
1923	Norma Preifer	Grand Forks	1971	Robert L Burgess	Fargo
1924	O A Stevens	Fargo	1972	John C Thompson	Dickinson
1925	David R Jenkins	Grand Forks	1973	John R Reid	Grand Forks
1926	E S Reynolds	Fargo	1974	Richard L Kiesling	Fargo
1927	Karl H Fussler	Grand Forks	1975	Arthur W DaFoe	Valley City
1928	H L Walster	Fargo	1976	Donald R Scoby	Fargo
1929	G A Talbert	Grand Forks	1977	Om P Madhok	Minot
1930	R M Dolve	Fargo	1978	James A Stewart	Grand Forks
1931	H E Simpson	Grand Forks	1979	Jerome M Knoblich	Aberdeen, SD
1932	A D Wheedon	Fargo	1980	Duane O Erickson	Fargo
1933	G C Wheeler	Grand Forks	1981	Robert G Todd	Dickinson
1934	C I Nelson	Fargo	1982	Eric N Clausen	Bismarck
1935	E A Baird	Grand Forks	1983	Virgil I Stenberg	Grand Forks
1936	L R Waldron	Fargo	1984	Gary Clambey	Fargo
1937	J L Hundley	Grand Forks	1985	Michael Thompson	Minot
1938	P J Olson	Fargo	1986	Elliot Shubert	Grand Forks
1939	E D Coon	Grand Forks	1987	William Barker	Fargo
1940	J R Dice	Fargo	1988	Bonnie Heidel	Bismarck
1941	F C Foley	Grand Forks	1989	Forrest Nielsen	Grand Forks
1942	F W Christensen	Fargo	1990	David Davis	Fargo
1943	Neal Weber	Grand Forks	1991	Clark Markell	Minot
1944	E A Helgeson	Fargo	1992	John Brauner (elect)	Grand Forks
1945	W H Moran	Grand Forks	1993	John Brauner	Jamestown
1946	J A Longwell	Fargo	1994	Glen Statler	Fargo
1947	A M Cooley	Grand Forks	1995	Carolyn Godfread	Bismarck
1948	R H Harris	Fargo	1996	Eileen Starr	Valley City
1949	R B Witmer	Grand Forks	1997	Curtiss Hunt	Grand Forks
1950	R E Dunbar	Fargo	1998	Allen Kihm	Minot
1951	A K Saiki	Grand Forks	1999	Joseph Hartman	Grand Forks
1952	Glenn Smith	Fargo	2000	Mark Sheridan	Moorhead, MN
1953	Wilson Laird	Grand Forks	2001	Ron Jyring	Bismarck
1954	C O Clagett	Fargo	2002	Jody Rada	Grand Forks
1955	G A Abbott	Grand Forks	2003	Richard Barkosky	Minot
1956	H B Hart	Jamestown	2004	Anna Grazul-Bilska	Fargo

Contributors to the North Dakota Academy of Science Research Foundation

Virgil Carmichael (Bismarck)
James Dogger (Gore, VA)
Van A. Doze (Grand Forks)
J. Mark Erickson (Canton, NY)
Jon Jackson (Grand Forks)
W. Thomas Johnson (Grand Forks)
Glenn Lykken (Grand Forks)
Douglas Munski (Grand Forks)
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Katherine Sukalski (Grand Forks)

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 Bingle, M. 71
 Blakely, R.D. 70
 Bollimuntha, S. 51
 Bobylev, M.M. 20, 31
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 Borowicz, M.A. 52
 Borowicz, P.P. 29, 39, 49, 52
 Burgess, T. 35
 Burgess, L.C. 12, 35

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 Clauson, R. 58
 Cole-Harding, S. 22
 Combs, C. 40

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 Detke, S. 67
 Ding L., 60
 Doze, V.A 53
 Duerre, R. 39
 Duffel, M. W. 11

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 Ewert, D. 42

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 Foster, D. 13
 Friesen, C.K. 23
 Fronk, M. 43

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 Greiff, A.H. 11
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 Guo, B. 18
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 Jedlicka, J. 63
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 Jurgens, C. W.D. 53

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 Kirby, J. 69
 Kirsch, J.D. 36
 Klapperich, R. 38
 Knittel, J. 13
 Korom, S. 38
 Kraft, K.C. 36

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Lever, J.R.	59	T	
Lopez, F.	34, 68	Tepfer, A.	29
Luther, J.S.	39, 49	Tergeson, A.	22
M		Tkach, V.	37
Machado-Arando, D.	54, 72	Totenhagen, J.	42
Mann, K.	30	U	
Mazurek, S.A.,	41	Upton, Marisa A.	31
McAllister, J.	69	V	
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Milavetz, B.	58, 73	Vaughan, R.A.	50, 56, 57, 59, 70
Miller, K.	13	Vonnahme, K.A.	29
N - O		W	
Neighbors, C.	24	Watt, J.	21
Newman, A.H.	59	Weiland, T.	58
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Stube, J.	61		
Super, H.J.	32		
Swanson, E.	61		