Abstract
This application requests matching funds to purchase in situ brain perfusion equipment. The in situ perfusion system will be used as a core, shared instrument for research activities in the College of Pharmacy and the School of Medicine through the Brain Barriers Research Center (BBRC). In situ brain perfusion technology will be utilized in collaborative research activities and training. This specialized equipment will be used to study hormone and drug transport in the rodent brain. We anticipate that in addition to supporting specific current research projects, the availability of in situ brain perfusion equipment will stimulate development of new research protocols involving drug penetrance of the blood-brain barrier and help garner new sources of research funding. In addition, we anticipate offering this equipment and methodology to other interested University researchers through the BBRC.

Dr. Anderson will work with Mr. Kevin Viken to develop the in situ brain perfusion methodology. Mr. Viken is a technical support staff member supported by the BBRC. He will train faculty, students and staff in the use of the in situ brain perfusion equipment. Funds from the College of Pharmacy will pay for service contracts used to ensure proper maintenance and support of the equipment.

Grant W. Anderson (Department of Pharmacy Practice and Pharmaceutical Sciences, College of Pharmacy): Principal investigator/primary user. Dr. Anderson’s research focuses on studying the role of transporters expressed at the BBB in transporting hormones and drugs into the brain.

Bjoern Bauer (Department of Pharmacy Practice and Pharmaceutical Sciences, College of Pharmacy): Primary user. The focus of Dr. Bauer’s research program is to identify mechanisms to control efflux transporter expression at the BBB and thereby enhance penetrance of CNS active drugs into the brain.

Lester R. Drewes (Department of Biochemistry and Molecular Biology, School of Medicine Duluth): Primary user. Dr. Drewes’ research is to discover and to apply new knowledge on the development, expression, and regulation of nutrient, hormone, protein and drug transporters in the blood-brain barrier.

William F. Elmquist (Department of Pharmaceutics, College of Pharmacy): Primary user. The Elmquist lab examines physiologic and biochemical determinants of drug delivery to the CNS; currently this research is focused on the influence of drug efflux proteins in the blood-brain barrier on drug distribution to the brain.
Title: In Situ Brain Perfusion Equipment

Principal Investigator: Grant W. Anderson, Pharmacy Practice and Pharmaceutical Sciences

1. Category justification: Category 2. Matching support is requested for purchasing in situ brain perfusion equipment to be shared by Duluth and Twin Cities campus investigators. An in situ brain perfusion system is not available at the University of Minnesota. Purchase of this instrument system will increase the likelihood of obtaining external research funds.

2. Present Status of Knowledge:

The in situ brain perfusion equipment we are planning to purchase is not a single piece of equipment but rather is comprised of multiple individual instruments. Together the instrumentation is used to assess solute transport across the rodent BBB (1). This is accomplished by taking over the circulation to the brain through the direct infusion of solute carrying perfusate into the carotid artery for defined intervals of time. At specific timepoints the perfusion is stopped and solute concentrations in the brain are measured. These measurements allow the researcher to then determine the kinetics of solute transport across this barrier. Importantly, the kinetics of both influx (blood to brain) and efflux (brain to blood) transport can be determined. Additional major advantages of this system in studying brain barrier transport include measurement of transport over broad intervals of time, limiting solute exposure to organs that may metabolize the substrate (e.g. liver, lung and kidneys), and assessing the direct effects of specific inhibitors to the solute transport process.

The steps involved in the method involve surgical preparation, perfusion of the test solute, brain tissue harvest, and measurement of test solute in the brain. The surgical preparation of the rodent is fairly extensive and requires several pieces of specialized surgical equipment. These include a Homeothermic Blanket System with a flexible rectal probe to maintain constant rodent body temperature throughout the experiment, a rodent surgical board, a binocular dissecting microscope and a fiber optic light illuminator to dissect and visualize the small carotid artery during cannulation, an electrosurgical/cautery unit, and numerous small surgical instruments not included in this equipment request. Together the surgical equipment is used to cannulate one carotid artery on an anesthetized animal. Next, the animal is perfused with perfusate containing the test solute. Specialized equipment required for this step include glass infusion syringe and syringe warmer, a jacketed buffer reservoir with aeration frit to aerate the perfusate, a circulating water bath to maintain holding temperature of perfusion fluid, a variable rate infusion pump, a physiologic pressure transducer with amplifier and chart recorder to monitor the arterial perfusion pressure, and an electroencephalograph (EEG) to monitor brain activity during the procedure. At the end of the perfusion procedure the compound trapped in the brain blood vessels is removed by a short washout, the brain is harvested, and the amount of test compound that has entered the brain tissue is measured.


3. Plan of work

Grant W. Anderson PhD, Assistant Professor
Principal Investigator

Dr. Anderson’s research program is presently focused on studying the role of thyroid hormones (THs) in regulating brain development. The developing mammalian brain is a well-recognized target of TH. In the absence of TH, the brain develops asynchronously. In humans this ultimately results in profound mental retardation as seen in endemic cretinism. The developing mammalian brain is transiently sensitive to TH. TH is required for normal brain development
during only a discrete window of time. It is hypothesized that the developing brain engages specific physiologic mechanisms designed to control TH action during discrete phases of development (1).

Thyroid hormones are synthesized in the thyroid gland. The predominant synthesized thyroid hormone is thyroxine (T4). Metabolism of thyroxine to the active form (triiodothyronine (T3)) involves deiodination by specific deiodinase enzymes found in multiple tissues including the developing brain. To enter and exit the brain, the THs (T4 and T3) must cross the blood-brain-barrier. Recent reports have revealed the existence of several thyroid hormone transporters (2). These transporters transport THs with high affinity and specificity. Data from our laboratory demonstrate that the expression of TH transporters in the rodent brain is controlled in a developmental and thyroid hormone-dependent fashion. Additionally, we have observed that specific TH transporters are expressed in cells found in the BBB and likely mediate transport of this important hormone into the brain.

TH transporter expression levels are regulated by developmental age and thyroidal status in the brain. Thus, we hypothesize that these parameters also control TH flux in the developing brain (3). To test these hypotheses we will measure in vivo TH transport at various stages of development by in situ brain perfusion. We will determine the kinetic parameters of thyroid hormone uptake and through the use of pharmacologic inhibitors, we will identify the specific transporters responsible for the observed uptake. We are also using quantitative structure activity relationship (QSAR) studies to identify novel substrates for the thyroid hormone transporters expressed in the BBB. These studies are designed to reveal the structural motifs required for substrate recognition of TH transporters and use this knowledge to rationally design drugs to penetrate the BBB. Access to in situ brain perfusion equipment will allow us to directly assess our hypotheses in vivo. Thus, we will extensively use the equipment requested in this application.


**Bjoern Bauer PhD, Assistant Professor**

**Primary User**

Dr. Bauer’s research program is concerned with the regulation of drug efflux transporters at the blood-brain barrier to improve treatment of brain disorders. Over 1.5 billion people worldwide suffer from brain disorders, including depression, epilepsy, stroke, brain tumors, neuro-AIDS, and Alzheimer’s disease. Thus, there is a huge demand for effective treatments. However, pharmacotherapy of brain disorders is greatly impaired by the blood-brain barrier. The molecular basis for active barrier function is a group of drug efflux transporters such as P-glycoprotein, multidrug resistance proteins (MRPs) and breast cancer resistance protein (BCRP). These efflux transporters limit therapeutic drugs from getting into the brain and are a major obstacle for effective treatment of brain disorders. One strategy to increase drug levels in the brain for better treatment of brain disorders is to manipulate the regulation of efflux transporters at the blood-brain barrier. However, despite the importance of these transporters for the therapy of brain disorders, little is known about their intracellular regulation.

We have addressed this fundamental and clinically relevant problem and have described several signaling mechanisms by which drug efflux transporters are regulated at the blood-brain
barrier, both under physiologic as well as pathophysiologic conditions (1). Several of the mechanisms we have identified provide potential molecular targets for therapy. We are now interested in translating these mechanisms into clinical applications to improve treatment of brain disorders. Currently, we are working on three projects, including i) regulation of P-glycoprotein in drug-resistant epilepsy (2, 3), ii) regulation of P-glycoprotein in Alzheimer’s disease, and iii) modulation of BCRP to treat brain tumors. We are currently limited in our ability to directly assess the functional role of these efflux transporters in vivo. Acquisition of in situ brain perfusion equipment will allow us to determine whether manipulating efflux transporter expression at the BBB alters brain penetration of drugs targeting the central nervous system.


William F. Elmquist PhD, Professor
Primary User
The Elmquist group will use the in situ perfusion technique to examine the influence of plasma protein binding, active drug transport into and out of the brain, and the saturability of those transporters, in both the rat and mouse model on the delivery of novel tyrosine kinase inhibitors that are being tested for malignant glioma. We will do this in both normal mice and in a genetically engineered model of glioma. This technique will allow us to further understand how drug delivery across the blood-brain barrier can be different in normal brain vs. the tumor core. This is critical in understanding the impact chemotherapy has on the invasive glioma cells that are far from the tumor core, in a region of brain that is likely to have a very different BBB permeability. The in situ perfusion technique is the ideal way to examine these regional differences in BBB permeability.


Lester R. Drewes PhD, Professor
Primary User
The long-term goal of our research is to characterize the functional properties and molecular features of the monocarboxylic acid transporter known as MCT1 (SLC16A1)(1). MCT1 is highly expressed in the blood-brain barrier (BBB) and functions in both the influx and efflux of substrates such as lactate and b-hydroxybutyrate, metabolites critical for brain energy metabolism in neonates, fasting adults, hibernating animals and in certain human subjects such as brain tumor patients (2). A major hypothesis is that selected and specific inhibitors of MCT1 will enable major advances for understanding the mechanistic features of this key protein and
may lead to pharmacologic agents that target MCT1 to combat disease or improve pathological conditions. We have established in vitro methods for characterizing MCT1 in cultured brain endothelial cells (3). This enables the determination of transporter kinetics and activity, and also facilitates the screening of large chemical libraries for specific inhibitors and transport regulators of this BBB membrane carrier. The identification of transport inhibitors or modifiers will then require the testing or evaluation of these compounds in vivo. To measure MCT1 transport activity in the BBB of the intact brain will require the experimental equipment and facility as described in this application. The set up will enable the quantitative measurement of blood-brain transport of the substrate (i.e. lactate, β-hydroxybutyrate) in the absence or presence of lead compounds identified in our in vitro studies and will be invaluable in the development of potential therapeutics drugs.


4. BUDGET JUSTIFICATION

This proposal is a request for funds to purchase in situ brain perfusion equipment. In situ brain perfusion is one of the most widely used methods to assess solute transport into the brain. Currently, neither the equipment nor the methodology is established at the University of Minnesota. Recently, the Brain Barriers Research Center has been established at the University of Minnesota to bring together University of Minnesota scientists specializing in studying this important barrier system. The establishment of this Center recognizes our unique position in this field as very few research institutions have more than a single brain barrier investigator. Development of the in situ brain perfusion technique will allow the University of Minnesota brain barrier researchers to add this important method to their research armamentarium and will facilitate synergistic research activities. Our research programs have a demonstrated need for the methodology requiring this equipment and we are confident that obtaining in situ brain perfusion equipment will benefit the University research capacity. The total cost of the in situ perfusion equipment is $28,657. A commitment from the College of Pharmacy towards the purchase of this equipment totals $9,000. Thus, we are requesting $19,657 from the Graduate School to complete this purchase. The recent formation of the BBRC is a collaboration between the College of Pharmacy and the School of Medicine and demonstrates a commitment towards an integrative and collaborative research enterprise between these schools. In this spirit the purchased equipment will also be made available to other University of Minnesota researchers interested in substrate transport at the BBB.

5. NEED JUSTIFICATION

The principal investigator has expended his start-up package. His other funded studies do not have a sizable equipment budget. The other primary users of this equipment do not currently have funding lines dedicated in their grants for an equipment purchase of this magnitude. The College of Pharmacy recognizes the need and importance of this equipment and will contribute towards the purchase of this instrument.

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