# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 8. Influence of Brain Astrocyte Glutamate Concentrations on Neurotransmission

7. **Start and End Date of Research Project:** 1/1/2010 - 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Kathryn F. LaNoue, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 46,530

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Somenhali, Kavitha | Research Tech | 100 | $28,660 (sal+fr) |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project |
|---|---|---|
| LaNoue, Kathryn | PI | 5% |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| None | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| Role of Branched Chain Amino Acids and Glutamate in Diabetic Retinopathy | ☐NIH<br>☐ Other federal (specify:_)<br>☒ Nonfederal source (specify:_ PA Lions Site Conserv & Res Foundation__) | 10/2010 | $50,000 | $39,483 |
| Astrocytic Glutamate Metabolism in the Central Nervous System | X NIH<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify:_) | 6/2011 | $927,000 | $0 |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

The PI retired and is no longer with Penn State.

**12. Future of Research Project.** What are the future plans for this research project?

The PI retired and is no longer with Penn State.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____X_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | |
| Female | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | | | | |
| Black | | | | |
| Asian | | | | |
| Other | | | | |
| Unknown | | | | |
| **Total** | | | | |

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No___X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X_____ No____ _____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    The preliminary studies that were conducted under this award enabled the PI to obtain an externally funded foundation grant that supported capstone studies in her laboratory in the final years of an exceptionally productive research career.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

The two specific aims involve investigations of cataplerosis in the brain. Cataplerosis refers to a process that irreversibly converts glutamate to pyruvate. It is particularly active in brain astrocytes and acts to balance de novo synthesis of glutamate. Pyruvate carboxylase is the rate limiting enzyme in de novo glutamate synthesis, which is called anaplerosis, and it is relatively well studied. However, neither the rate limiting enzyme nor the exact pathway for cataplerosis is known. We propose that cataplerosis is an exceptionally important process because low concentrations of glutamate in astrocytes may facilitate removal of glutamate from the synaptic spaces. Moreover, excess synaptic space glutamate can cause glutamate excitotoxicity, neuronal death and a variety of central nervous system ailments.

In specific aim 1 we will measure the influence of the concentrations of intracellular glutamate in cultured astrocytes on transport of glutamate into the astrocytes from the external media.

In specific aim 2 we will assess the roles of two enzymes, malic enzyme and phosphoenol pyruvate carboxykinase on conversion of [$U^{14}C$] glutamate to [$U^{14}C$] lactate in cultured astrocytes. These will be done by silencing (one at a time and/or simultaneously) the genes for malic enzyme and phosphoenolpyruvate carboxykinase using commercially available siRNAs.

Glutamate in the brain acts as a source of nutrient energy and as an excitatory neurotransmitter. This project focuses on mechanisms that ensure optimal use of glutamate as a neurotransmitter, by providing optimal maintenance of low glutamate concentrations in astrocytes. Astrocytes form part of the boundaries of the synaptic spaces and one of their important functions is to remove the excitatory glutamate from the space where it activates post synaptic glutamate receptors. Removal stops the signal after it has been sent.

We plan to test the hypothesis that excess concentrations of glutamate in the astrocytic cytosol may interfere with regulation of neurotransmission. We want to develop techniques to vary astrocytic cytosolic glutamate and determine the influence of intracellular glutamate on influx of glutamate from the external media. Preliminary data suggested that glutamate levels within the astrocytes have more influence on removal of glutamate from the cytosol than glutamate outside the astrocytes.

Therefore, we have initiated studies of intracellular astrocyte metabolism. The activities of malic enzyme (ME1) and phosphoenolpyruvate carboxykinase are key factors in regulation of glutamate concentrations in astrocytes. These enzymes are expressed predominantly in brain astrocytes and they irreversibly convert malate to pyruvate. Since glutamate is rapidly converted to malate in astrocytes, these enzymes act as glutamate drainage sinks. The activities of ME1 and PEPCK2 probably play a significant role in maintaining low astrocyte glutamate levels after the glutamate excitatory signal is sent.

Once we have found effective ways to alter glutamate concentrations within astrocytes over a wide range, we intend to measure the rate of [$U^{14}C$] glutamate transport into isolated cultured astrocytes as a function of intracellular glutamate. Therefore, preliminary studies have been aimed at regulation of ME1 and PEPCK2 activities. These two astrocytic enzymes are likely key factors in regulating astrocytic intracellular glutamate. Since both enzymes consume malate and oxaloacetate and produce pyruvate and $CO_2$, we expect that reduction in their activities will result in higher than normal levels to citric acid cycle intermediates and glutamate compared to control cells.

We are currently using gene silencing techniques to lower expression of these two enzymes. We have obtained the siRNA for ME1 from Qiagen. We used the same siRNA sequence as that which was successfully used by Pongratz (Pongratz et al, 2007) who used it to decrease ME1 expression in INS1 cells. Currently we are transfecting the primary astrocytes with siRNA in order to halt transcription of the ME1.

## Materials and Methods

*Methods/Primary Astrocyte culture*

We have successfully learned to culture and study primary astrocytes. Primary astrocytes were prepared by a modification of the method of McCarthy and de Vellis (1978, 1980) from the newborn rats. All animals were treated in accordance with Institutional Animal Use and Care Committee of Pennsylvania State University College of Medicine. Briefly, 12-18 hour old Sprague-Dawley rat pups were anesthetized and then decapitated. The brain was removed and placed in CMF-HBSS solution (Gibco, Rockville, MD, USA). The meninges were carefully removed, and cells were separated by dicing the tissue and treating it with 0.25% trypsin-EDTA (Gibco) for 30 min. Following incubation in DNAse for 5 min, the brain homogenate was passed through 75µm Nitex screens (SEFAR America, Depew, NY, USA) and resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). Cells were plated in poly-D lysine (Sigma)-coated flasks. The medium was changed after 24 hours and every other day thereafter. Cells were allowed to grow for 9 days, and astrocytes were removed by shaking at 260 rpm for 18h to loosen contaminating cell types.

*Results/Cell Transfection*

Initial studies of cell transfection using siRNA purchased from Qiagen have commenced. 21-Nucleotide siRNA sequences (Qiagen) targeting the specific cytosolic malic enzyme genes were used for transfection. siRNA were synthesized by Qiagen with 19- base pair ribonucleic acid sequences with 5/ dideoxythymidine overhangs of the following sequences. ME1a and ME1b are isoforms of siRNA for cytosolic malic enzyme with the sequences "AACCAGGAGATCCAGGTCCTT" and "AAGCCAAGAGGCCTCTTTATC" respectively. The siRNA duplex consisted of the Sense strand and its complimentary antisense strand to provide a 2-nucleotide dithymidine overhang at each 3'terminus. siRNA constructs (scramble, ME1a, ME1b) were introduced into primary astrocytes using Lipofectamine 2000 and OptiMEMI reduced serum medium (Invitrogen) at a different concentration. For each transfection sample siRNA – Lipofectamine 2000 complexes were made in Opti-MEM I reduced serum medium.

Next we will begin the studies to determine if ME1 levels in the cultured astrocytes can be lowered with the siRNA constructs and what the influence of lowered ME1 is on intracellular glutamate.

Studies of glutamate metabolism in the central nervous system (brain and retina) were initiated to evaluate the role of glial cells in prevention of glutamate excitotoxicity. Glutamate is the most abundant neurotransmitter in the brain and retina but it has dual roles because it acts both as a neurotransmitter and as an energy generating metabolite. Glutamate can enter neuronal and glial cells (astrocytes in brain, Müller cells in the retina) where it is transaminated to α-ketoglutarate. When glutamate increases outside the cells due to rapid neurotransmission or anoxia, the extracellular glutamate can excite post synaptic neurons, produce excessive calcium in the cytosol, hydrolyze cell membrane lipids and cause neuronal death. Central nervous systems glial cells act as safeguards preventing glutamate excitotoxicity by removing glutamate from the extracellular fluid and converting it to glutamine. Our studies show that glutamate levels in astrocytes remain surprisingly constant when challenged with increasing amounts of extracellular glutamate. We have published a report of glutamate metabolism in control and diabetic retinas, (Gowda, K., Zinnati, WJ and LaNoue, KF, 2011) which was supported by funds from this grant. We demonstrated in that report the existence of an important glutamate buffering system in Müller cells of the retina. The buffer prevents accumulation of intracellular glutamate in Müller cells which would otherwise slow influx of glutamate.

Studies Undertaken and Completed

To monitor the influence of diabetes on glutamate metabolism, we measured 1). Glutamatergic neurotransmission, 2). Glutamate synthesis from $^{14}CO_2$ and pyruvate (anaplerosis) and 3). [U-$^{14}$C] glutamate conversion back to $^{14}CO_2$ and $^{14}$C-pyruvate cataplerosis. The data suggested the existence of a glutamate buffering anaplerotic/cataplerotic metabolic cycle in astrocytes which is uncoupled by diabetes. In retinal Müller cells, anaplerosis is initiated by a small pyruvate pool which is also the product of cataplerosis. In cells from diabetic rats the cataplerotic pathway, (glutamate conversion to α-ketoglutarate and then to pyruvate) is reduced by 90% because glutamate transamination by branched chain aminotransferase is competitively inhibited by elevated branched chain amino acids (BCAAs). Branched chain amino acids, but not the ketoacids, were almost twice as high in diabetic rat retinas compared to euglycemic rat retinas. The data suggest that glutamate levels in retinal Müller cells from diabetic rats are elevated due to the presence of excess branched chain amino acids, and that the elevated glutamate in Müller cells causes glutamate excitotoxicity.

In order to monitor glutamate formation and pool sizes in the retina and to understand the metabolic changes in retinas of diabetic rats, we measured three metabolic pathways for glutamate in excised control and diabetic retinas. These metabolic processes were neurotransmission, de novo anaplerotic glutamate synthesis from pyruvate and $CO_2$ and cataplerotic, irreversible disposal of glutamate to form pyruvate and $CO_2$. Glutamate neurotransmission was quantitated by measuring flux through the glutamate/glutamine cycle as we have done previously (Xu et al. 2007). We also measured rates of de novo synthesis of glutamate from $^{14}CO_2$ and pyruvate which is catalyzed by enzymes expressed in retinal Müller cells and is dependent on ATP and pyruvate carboxylase (PC). We traced the carbon flow of anaplerosis by incubating excised retinas with $^{14}CO_2$ and measuring incorporation of carbon 14

into the products. Fig 1A illustrates the pathways. Most of these enzymes are expressed in Müller cells in the retina and in brain they are expressed predominantly in astrocytes (Vogel et al. 1998; Vogel et al. 1998; Waagepetersen et al. 2002). Malic enzyme but not pyruvate carboxylase is also active in brain neurons (Olstad et al. 2007). Fig 1A illustrates glutamate entry into the Müller cells from the neurons and the return of the glutamate carbon (as glutamine) to the neurons. Finally, we measured the reversal of anaplerosis, (i.e. cataplerosis). This is the process that converts glutamate and citric acid cycle intermediates to pyruvate and $CO_2$. Cataplerotic carbon flow was monitored by including [U-$^{14}$C] glutamine in the medium and following appearance of carbon 14 (as a function of time) into pyruvate/lactate. The pathways of cataplerosis, including activities in both Müller cells and neurons and the flow of labeled metabolites between neurons and Müller cells are illustrated in Fig 1B. Cytosolic malic enzyme (ME1) and phosphoenolpyruvate carboxykinase (PEPCK2) both catalyze cataplerosis. These enzymes are expressed in Müller cells in the retina and astrocytes in the brain (Schmoll et al. 1995; Cruz et al. 1998; Vogel et al. 1998). Because anaplerosis and cataplerosis are likely to be present in the same cell type (Müller cells in the retina), and have overlapping substrates (pyruvate), they can catalyze a futile cycle. Still the site of expression of malic enzyme remains unresolved (Olstad et al. 2007). Cataplerotic/anaplerotic cycling together are sometimes referred to as pyruvate recycling (Cruz et al. 1998). Interaction between the two processes (anaplerosis and cataplerosis) can only occur when they are present in the same cell type. They influence each other, and the anaplerotic and cataplerotic fluxes are similar in magnitude. We focused on glutamate metabolism in the Müller cells because Müller cells remove glutamate from synaptic spaces. They express $Na^+$ linked glutamate transporters which remove glutamate from the extracellular synaptic spaces.

To monitor glutamate de novo synthesis, we measured rates of anaplerosis in the retinas. Pyruvate carboxylase (PC) is the first and rate limiting enzyme in the pathway of anaplerosis. PC initially converts pyruvate and $CO_2$ to oxaloacetate. The oxaloacetate enters the citric acid cycle and increases the citric acid cycle intermediates including α-ketoglutarate and glutamate (Fig 1A). Excessive activity of this pathway causes glutamate excitotoxicity, because an increase in glutamate in the Müller cells interferes with uptake of glutamate into the Müller cells from the extracellular spaces. However the pathway was not increased, it was decreased in the diabetic retinas.

We also evaluated cataplerosis which catalyzes the reversal of anaplerosis. If cataplerosis was decreased it could cause glutamate excitotoxicity. To do so we incubated retinas with [U-$^{14}$C] glutamine. The rate limiting enzymes of cataplerosis are cytosolic malic enzyme (ME1) and mitochondrial phosphoenol pyruvate carboxykinase (PEPCK2). The carbon pathway is shown in Fig 1B. The anaplerotic pathway (Fig 1A) increases citric acid cycle intermediates and glutamate starting with $CO_2$ and pyruvate. The cataplerotic pathway (Fig 1B) decreases citric acid cycle intermediates and glutamate starting with glutamine. The products of the cataplerotic pathway are the same as the substrates of the anaplerotic pathway (i.e. $CO_2$ and pyruvate). Measurements of anaplerosis showed that de novo synthesis of citric acid cycle intermediates and glutamate were low in the diabetic retinas. The initial rate of cataplerosis in control retinas was 1.29 ± 0.08 nmoles/mg⊙min compared to the rate of anaplerosis (1.00 ± 0.09 nmoles/mg⊙min). Thus the rates of anaplerosis and cataplerosis were similar in controls and both rates reduced in the diabetic retinas. The similarity of the rates of anaplerosis and cataplerosis in control retinas suggest the existence of a cycle where pyruvate connects the two

pathways. The initial rate of anaplerosis was decreased from 1.00 ± 0.09 nmoles/mg⊙min in controls to 0.58 ± 0.06 nmoles/mg⊙min (~ 42%) in the diabetic.

The initial rate of cataplerosis was decreased from 1.29 ± 0.08 nmoles/mg⊙min to 0.26 ± 0.13 nmoles/mg⊙min (~90%) in the diabetic retinas. We conclude that the low amounts of [U-$^{14}$C] lactate generated by diabetic retinas, incubated with [U-$^{14}$C] glutamine is due to a decrease in the carbon 14 products of glutamate following inhibition of Müller cell glutamate transamination. [U-$^{14}$C] glutamate conversion to α-ketoglutarate is likely inhibited by BCAAs. Branched chain amino acids compete with glutamate for transamination in Müller cells. The BCAAs compete with glutamate for the amino acid binding site on BCATm (Hall et al. 1993). The Km of leucine for BCATm is ~0.8mM, for isoleucine it is 0.6mM, whereas Km for glutamate is 12mM (Conway et al. 2004).

In Müller cells, α-ketoglutarate is produced not only by transamination of glutamate entering the cells, but also by pyruvate carboxylase. The source of nitrogen in the brain for conversion of α-ketoglutarate to glutamate is debated, but in most studies it is either BCAAs or alanine (Sakai et al. 2004; Bak et al. 2005; Yudkoff et al. 2005). Therefore, our data and that of Frayser and Buse (1978) suggest that neither alanine nor aspartate is the source of the excess nitrogen used for glutamate synthesis in diabetic retinas.

Since BCAAs provide much of the amino nitrogen for retinal transamination of α-ketoglutarate to glutamate (LaNoue et al. 2001), increased levels of BCAAs will increase the pool size of glutamate in the Müller cells, just as they do in cultured brain astrocytes (McKenna et al. 1998), and perhaps interfere with uptake of glutamate from the synaptic spaces.

In the diabetic retinas, the decrease in conversion of [U-$^{14}$C] glutamine to [U-$^{14}$C] lactate and pyruvate (cataplerosis), ~90%, was accompanied by a decrease in anaplerosis ~42%. This may be because the products of cataplerosis are the substrates of anaplerosis and the enzymes are in the same cell type. When cataplerosis was inhibited the rate of anaplerosis declined, suggesting recycling. We define recycling here as the combination of anaplerosis and cataplerosis. For pyruvate recycling to influence Müller cell metabolism and buffer glutamate excitotoxicity, it must involve PC and ME1 in the same cell type. If anaplerosis and cataplerosis are active in the same cell and there is only a very small pool of pyruvate, increases in glutamate pool size will be determined by availability of pyruvate entering from the extracellular fluid. If cataplerosis did not supply most of the pyruvate needed by the anaplerotic pathway, anaplerosis and cataplerosis would be independent. The data show that the two pathways are dependent on each other. Our previous studies and those of others show that pyruvate is not generated glycolytically in the Müller cells and therefore is not present in excess in Müller cells as it is in neurons. In Müller cells, pyruvate is not the product of glycolysis because Müller cells do not express a malate aspartate cycle (LaNoue et al. 2001; Ramos et al. 2003; McKenna et al. 2006; Xu et al. 2007; Contreras et al. 2009). Therefore, in Müller cells, NADH generated glycolytically is not consumed by mitochondria (LaNoue et al. 2001; Xu et al. 2007) but instead is used to reduce pyruvate to lactate and the lactate is transported into the extracellular fluid (Pellerin 2008).

Thus we speculate that the two pathways anaplerosis and cataplerosis are present in the same cell types and are tightly linked by a small pool of pyruvate. We believe the cycle acts as a buffer to prevent large swings in the concentration of a Müller cell cytosolic glutamate. The two arms of

the buffer are equal in the control retinas but not equal in the diabetic retinas. This may reflect loss of buffer capacity and the existence of glutamate excitotoxicity in the diabetic retinas.

It has been puzzling to us for some time that the glycolytic pathway from glucose to pyruvate is inactive in brain and retinal glial cells. Glycolysis is active in the glia but it only produces lactate which is exported to the neurons. The inability of the glia to oxidize lactate to pyruvate is due to the lack of a malate aspartate cycle in the glia. The "electrogenic" malate aspartate cycle provides a pathway to reduce cystolic NAD+ to NADH. Otherwise, the conversion is energetically unfavorable because the NADH/NAD ratio in the mitochondria is so much higher than in the cytosol. Thus, the glial cells sacrifice the energy they could get from oxidation of pyruvate in order to provide the proposed glutamate buffering system.

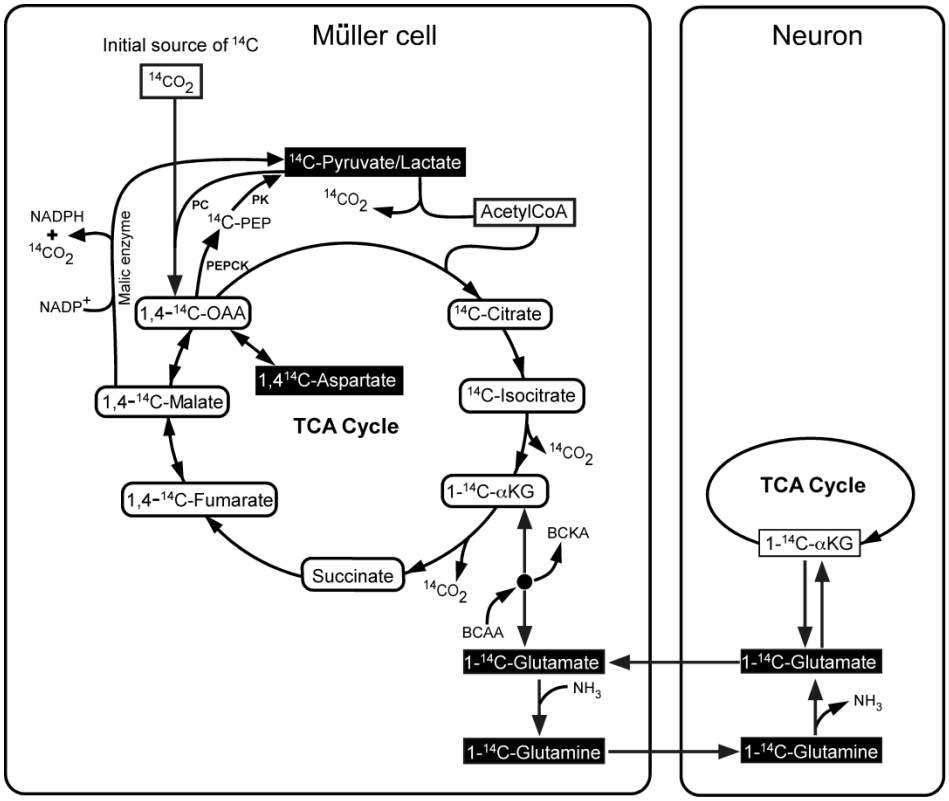


Figure 1A. Flow chart of metabolism in Müller cells involved in $^{14}CO_2$

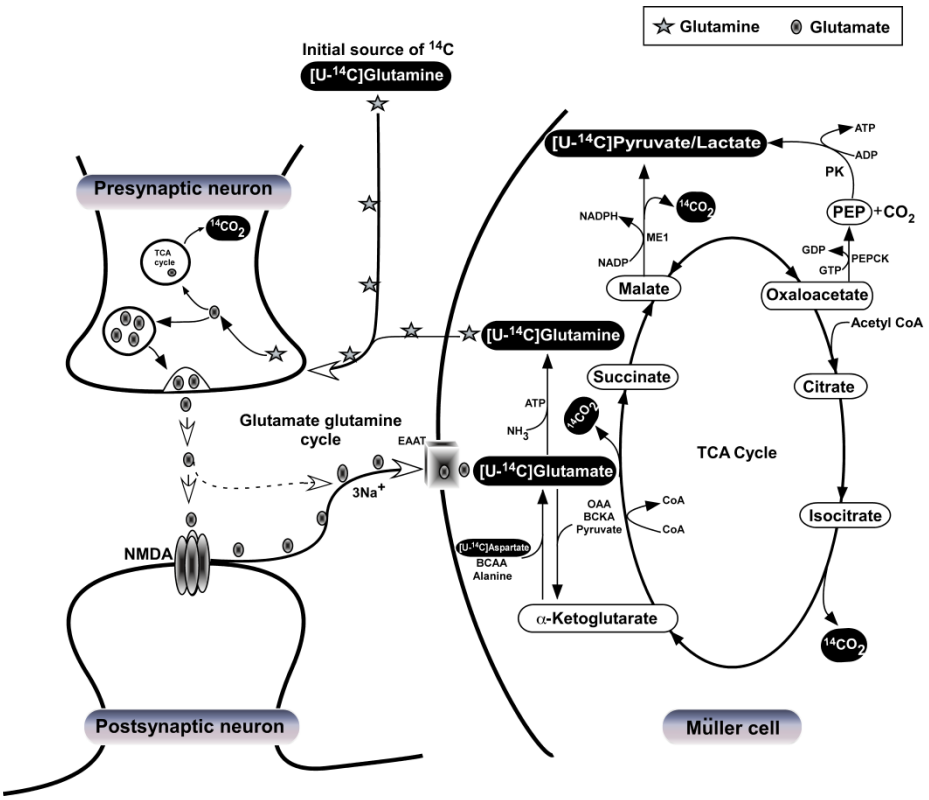


Figure 1B. Flow chart of retinal metabolism of glutamine including reactions that are catabolic.

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. The influence of diabetes on glutamate metabolism in retinas | Gowda, K., Zinnati, WJ and LaNoue, KF | Journal of Neurochemistry | 6/2010 | ☐Submitted<br>☐Accepted<br>X Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___X_____

If yes, please describe your plans:

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

**Kathryn F. LaNoue, MD, PhD**

| | |
|---|---|
| **Title** | Professor Emeritus |
| **Institution** | College of Medicine |
| **Department** | Cellular and Molecular Physiology |
| **Address** | 500 University Drive<br>Hershey PA 17033<br>Mailbox: H166 |


PREFERRED TITLE/ROLE: Professor Emeritus of Cellular and Molecular Physiology

GRADUATE PROGRAM AFFILIATIONS:

Cell and Molecular Biology, MD/PhD Degree Program, Physiology, Neuroscience

EDUCATION:

Ph.D., Yale University, 1961

Postdoctoral Training, University of Pennsylvania Medical School, 1968-1970

NARRATIVE:

Dr. LaNoue and her colleagues are engaged in an evaluation of factors that control the de novo synthesis and utilization of glutamate in the brain and retina. Glutamate is the most important neurotransmitter in the brain. About half the total cerebral pool of glutamate is used and re-synthesized every 10 minutes. Several drugs, which are known to alter the rate of resynthesis of glutamate specifically in neural tissues, have important influences on epilepsy, chronic pain, and, possibly neurodegeneration. Dr. LaNoue's laboratory is using radioisotopic techniques to map pathways of neuronal glutamate synthesis and degradation and to identify new drug targets for altering glutamate turnover.

Dr. LaNoue's laboratory is also involved in studies of diabetes and insulin resistance in a strain of genetically obese rats. The laboratory has observed that an A2b adenosine receptor antagonist is able to partially reverse the insulin resistance characteristic of these obese animals without inducing a change in their percent body fat. By independently varying insulin levels, fatty acid levels, and A2b adenosine receptor antagonist levels while measuring radiolabeled glucose uptake in the muscles of these animals, Dr. LaNoue hopes to identify the cause of the insulin resistance and to learn how the A2b adenosine receptor antagonist ameliorates the problem. Measurements of the extent of activation of components of the insulin signaling pathway such as insulin receptor kinase activity, insulin receptor substrate 1 activation, and phosphatidylinositol-3-kinase activity will aid in interpretation of the data obtained.

**Publications listed below are automatically derived from MEDLINE/PubMed and other sources, which might result in incorrect or missing publications. Faculty can login to make corrections and additions.**


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2. Ola MS, Hosoya K, LaNoue KF. Influence of insulin on glutamine synthetase in the Müller glial cells of retina. Metab Brain Dis. 2011 Sep; 26(3):195-202. View in: PubMed
3. Gowda K, Zinnanti WJ, LaNoue KF. The influence of diabetes on glutamate metabolism in retinas. J Neurochem. 2011 Apr; 117(2):309-20. View in: PubMed
4. Figler RA, Wang G, Srinivasan S, Jung DY, Zhang Z, Pankow JS, Ravid K, Fredholm B, Hedrick CC, Rich SS, Kim JK, LaNoue KF, Linden J. Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. Diabetes. 2011 Feb; 60(2):669-79. View in: PubMed
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6. Freeman WM, Bixler GV, Brucklacher RM, Lin CM, Patel KM, VanGuilder HD, LaNoue KF, Kimball SR, Barber AJ, Antonetti DA, Gardner TW, Bronson SK. A multistep validation process of biomarkers for preclinical drug development. Pharmacogenomics J. 2010 Oct; 10(5):385-95. View in: PubMed
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9. Abcouwer SF, Shanmugam S, Gomez PF, Shushanov S, Barber AJ, Lanoue KF, Quinn PG, Kester M, Gardner TW. Effect of IL-1beta on survival and energy metabolism of R28 and RGC-5 retinal neurons. Invest Ophthalmol Vis Sci. 2008 Dec; 49(12):5581-92. View in: PubMed
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11. Berkich DA, Ola MS, Cole J, Sweatt AJ, Hutson SM, LaNoue KF. Mitochondrial transport proteins of the brain. J Neurosci Res. 2007 Nov 15; 85(15):3367-77. View in: PubMed

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