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- 2. Academic Rank: Associate Professor**
- 3. Department: Neurology**
- 5. Name(s), Academic Rank, and Department(s) of any Co-Investigators: N/A**
- 6. Project Title: A novel rat model of hypoxic/hypoglycemic seizures.**
- 7. Brief Summary/Description of Project: (Limit this section to one page)**

Stroke and cerebral hypoxia frequently result in seizures or myoclonus, disorders of excessive neuronal excitability, which may be linked to dysfunction of inhibitory GABA_A receptors (GABA_ARs). GABA_ARs are transmembrane chloride channels activated by the inhibitory neurotransmitter, GABA, and composed of multiple protein subunits from several subunit families. The pharmacology of the receptor is determined by which subunits are present in the assembled receptor, hence factors that regulate the expression or assembly of GABA_AR subunits into receptors can affect GABA_AR pharmacology and function. We have previously found that exposing NT2-N neuronal cells or cortical neurons to 1% O₂ for 8 h reduces expression of specific GABA_AR subunits and lowers maximal currents with peak effect 48 h after exposure, but the mechanisms underlying this regulation remain poorly understood. Hypoxia also transiently increased levels of the hypoxia-induced basic helix-loop-helix (bHLH) transcription factor, hypoxia-inducible factor-1 α (HIF-1 α), which stimulates expression of a number of hypoxia-induced genes involved in angiogenesis, glycolysis and erythropoiesis, including vascular endothelial growth factor (VEGF) which has been associated with neuroprotection. Whether HIF-1 α is involved in hypoxia-induced GABA_AR changes is unclear, as chemical agents like cobalt and deferoxamine that also induce HIF-1 α have the opposite effect of inducing GABA_AR subunit expression and increasing GABA_AR currents 48 h after exposure. Moreover the connection between hypoxia and seizures remains tenuous, as hypoxia can induce seizures in rats only at a specific developmental stage 10 days after birth.

The concept that seizures result from an imbalance of excitatory and inhibitory neurotransmission is well established, but the underlying mechanisms that trigger this imbalance are often unknown. We hypothesize that seizure induction by hypoxia is related to a neuronal energy deficit that leads to abnormal regulation of GABA_ARs. To test this hypothesis, it is first necessary to generate an animal model of seizures induced by a low energy state. Prior hypoxic seizure models have combined hypoxia with carotid ligation, which creates a unilateral stroke-like injury. Hypoglycemia has also been used to generate seizures, though the time course for seizure generation is slow (2-4 h). This project will determine whether a new seizure model can be generated in which the combination of hypoxia with hypoglycemia induces a consistent pattern of seizures, and then to determine the effect of these seizures on GABA_AR expression and function. This model may not only help us understand one particular trigger for seizures, but it may also be relevant for understanding how spontaneous seizures are triggered in epileptic patients. If seizures are generated, it will be important to find out 1) whether they begin in the cortex or in subcortical structures like the amygdala and hippocampus that are known to be both predisposed to seizures and sensitive to hypoxia, 2) whether HIF-1 α expression is stimulated by hypoglycemia, hypoxia or the combination, 3) whether there is significant apoptosis or seizure-induced changes in hippocampal cytoarchitecture (e.g. axonal reorganization/sprouting) or other cellular changes, and 4) whether there are associated changes in inhibitory neurotransmission and GABA_AR function.

Animal protocols: Adult Male Sprague Dawley rats (P35-40, ~ 150g) will be implanted with bilateral ventral hippocampal (AP -4.7; L 5; V -8) and basolateral amygdala (P-2.2, L-4.7, V 8.2) bipolar wire electrodes and cortical screws under pentobarbital anesthesia, with wires terminating in a connector mounted to the skull with dental cement, and allowed to recover for 7 days. Rats will be connected to an EEG monitoring connector in a specialized plexiglass chamber that allows control of oxygen while monitoring for seizures using video and EEG recording. Rats are then injected with either insulin (5, 10, or 20 IU/kg) or saline control and then subjected to either normoxia or 5 % oxygen for up to 20 min or until a seizure occurs. After seizure induction, the chamber will be reperfused with air. If seizures do not terminate spontaneously within 10 minutes,

diazepam (10 mg/kg) will be administered and repeated at 10 minute intervals as needed until seizures terminate. Rats will be anesthetized with isoflurane and euthanized after 0, 1, 2 or 7 day recovery.

Immunohistochemistry. After transcardiac ice cold saline (0.9%) perfusion followed by 4% paraformaldehyde, rat brains will be embedded in paraffin and thin sectioned at 4-5 mm in the parasagittal or coronal orientation. Slide-mounted sections will be deparaffined, washed in Tris buffer then blocked with 10% serum prior to overnight incubation with a primary HIF-1 α , GABA_AR β 2/ β 3 subunit or calcineurin antibody. After washing, slides will be incubated with a biotinylated secondary rabbit anti-IgG antibody, followed by incubation with avidin-HRP and chromogen reaction with diaminobenzidine, or fluorophore-linked secondary antibodies for confocal imaging. Sections are then dehydrated through ascending alcohol concentrations and coverslipped with Permount. Control sections will exclude the primary antibody. Quantitation of immunostaining intensity will use NIH-image software. Immunoblots of proteins from dissected brain regions will be performed and compared to actin to quantitate regional changes in expression.

8. Describe Student's Role and Responsibilities.

The student will learn to handle the animals, perform drug injections and electrode implantation. The student will be particularly involved in performing and monitoring the animals during the hypoxic seizure protocol. The student will participate in the biochemical and histological techniques (immunohistochemistry, light and confocal microscopy, Western blots). Experiments will be performed under the guidance of the mentor, lab technician, post-doc and graduate students, but it is expected that the student will be able to perform some of these experiments independently later in the course of the summer. The student is expected to spend "full time" effort on this project, to keep meticulous records of results in a laboratory notebook, and to ask questions and seek direction when needed. The student will view training tapes to learn animal handling techniques and is expected to carefully follow animal protection and safety guidelines. The student will attend weekly lab meetings with other lab personnel and the mentor, and participate in other lab activities. As required of the program, the student will present a short talk on his/her results and experience, and will have the opportunity to "rehearse" this presentation at a lab meeting. Some background reading (review papers) will be helpful to introduce the student to the concepts and techniques that will be used over the course of the summer.

9. Special Qualifications Required

The student should have prior experience in a research laboratory and be thoroughly familiar with use of laboratory balances, pH meters, pipetters, the preparation of solutions, laboratory etiquette, data recording and manipulation, etc. Prior experience with laboratory animals, molecular biological and histological techniques is highly desirable but not essential. Enthusiasm and a strong interest in scientific research are the most important requirements. Willingness to use animal models in research is required.