Capillary Electrophoretic Determination of Citrulline and Ornithine in Rat Brain Microdialysate during Epileptic Seizures
Y. Wang 1, M. W. Ducey 2, C. E. Lunte 1
1 University of Kansas, 2 Missouri Western State University

Purpose
Epileptic seizures cause an important secondary effect, oxidative stress. Oxidative stress is an imbalance between the body’s reactive oxygen species (ROS)/reactive nitrogen species (RNS) and endogenous antioxidants. Nitric oxide (NO) is an abundant RNS in the brain, which is produced from arginine through the nitric oxide synthase (NOS) pathway. Citrulline is a biomarker of this pathway. Meanwhile, the arginase pathway competes with the NOS pathway for arginine, and ornithine is a biomarker of this pathway. Our goal was to develop a CE-LIF method to monitor the change in citrulline and ornithine, as markers of NO production in rat brains during epileptic seizures.

Methods
The fluorescence derivatization reagent was 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F), whose excitation and emission wavelengths are 450 and 490 nm, respectively. Its advantage was that DBD-F could derivatize ornithine, the diamine, without resulting in self-quenching. The separation method was cyclodextrin-modified capillary electrophoresis. The background electrolyte consisted of 20 mM lithium tetraborate, 20 mM (2-hydroxypropyl)-beta-cyclodextrin, and 30% ethanol. In order to monitor citrulline and ornithine, a microdialysis probe was implanted into the rat hippocampus. 10 mM 3-mercaptopropinoic acid (3-MPA) was perfused to induce seizures for 50 min. Microdialysate were collected over a 10 min interval and then analyzed by the CE-LIF method.

Results
The reproducibility test showed that % R.S.D. was less than 10%. The regression coefficients ($R^2$) from the calibration curve (0.2 µM-10µM) were over 0.99. The detection limits (S/N=3) of ornithine and citrulline were 9 nM and 23 nM, respectively. This method could also be used to analyze 13 other common amino acids. The in vivo animal studies indicated that the concentrations of citrulline and ornithine both increased after the 3-MPA dosage, which proved that both NOS and arginase pathways occur, and that NO was produced during epileptic seizures.

Conclusion
A cyclodextrin-modified CE-LIF separation method with good linearity and reproducibility was developed for ornithine and citrulline. The method allowed these two arginine metabolites to be monitored in rat brain microdialysate.