Finite Element Analysis of Induced Electroosmotic Flow in Brain Tissue and Application to Ex Vivo Determination of Enzyme Activity

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Abstract

According to the American Stroke Association, stroke is the No. 4 cause of death and the leading cause for adult disability in the United States. Some of the complications of stroke are a direct result of inflammatory responses in the brain. Studies have shown that many neuropeptides display neuroprotective behavior by ameliorating neuronal damage caused by such a response. Although its mechanism is not yet established, we hypothesize that one such neuropeptide, galanin, may exhibit anti-inflammatory effects as shown in Figure 1. One important checkpoint of this proposed mechanism is the hydrolysis of galanin by ectopeptidases, an enzymatic reaction that is also not well understood. To elucidate this process, we are developing the technique of electroosmotic sampling to study ectopeptidase activity in the CA3 region of the p7 rat hippocampus. However, the overall picture of galanin hydrolysis and the resulting biological significance cannot be constructed without knowing various immeasurable parameters. We are thus using COMSOL Multiphysics to determine, for example, the potential drop in the tissue, the velocity of the fluid, the concentration profile of the peptide at specific times, and the residence-time distribution of the neuropeptide itself. The setup consists of two electrolyte-filled capillaries in electrical contact with an organotypic hippocampal slice culture (Figure 2). Applied voltages and the resulting current are the primary driving force for bulk fluid movement through the tissue, which has a zeta-potential of about -0.023 V. The three physics we use are Electric Currents, Free and Porous Media Flow, and Nernst-Planck Equations. The equations solved apply most straightforwardly in COMSOL to a uniform porous medium. However, because we direct fluid through the brain tissue from open capillary tubes, the coefficients in the existing equations must take into account the porosity as well as the tortuosity of the tissue. We do this by providing COMSOL with volume-averaged parameters derived from known microscopic parameters (e.g. electroosmotic mobility). Figure 3 shows the geometry of the electroosmotic sampling setup. The electric field, voltage drop, and fluid velocity (Figure 4) across the domains have been determined. Using values of porosity and tortuosity, correction factors were applied to both the Navier-Stokes as well as the Nernst Planck equations in COMSOL to suit the macroscopic problem at hand. Soon, we expect to have the concentration profile as well. Eventually, we also expect to obtain the distribution of times galanin molecules reside in the tissue, which is an important parameter in enzyme kinetics.By combining numerical calculations from COMSOL with analytical results (capillary HPLC, MALDI tandem MS sequencing), we can derive the enzyme activity and kinetics of the ectopeptidases responsible for the hydrolysis of galanin into

its many fragments. By knowing what peptides are formed and in what quantity, we can determine whether galanin and/or its fragments bind to receptor GalR2. We hypothesize that it is through the interaction with this receptor that the concentration of anti-inflammatory cytokines are increased to combat the oxidative environment introduced under inflammatory (and thus stroke) conditions.

Reference

1. Hongjuan Xu et al., Electroosmotic Sampling. Application to Determination of Ectopeptidase Activity in Organotypic Hippocampal Slice Cultures, Anal. Chem., 82, 6377-6383 (2010).
2. Amy Hamsher et al., Minimizing Tissue Damage in Electroosmotic Sampling, Anal. Chem., 82, 6370-6376 (2010).
3. Yifat Guy et al., Determination of Zeta-potential and Tortuosity in Rat Organotypic Hippocampal Cultures from Electroosmotic Velocity Measurements under Feedback Control, Anal. Chem., 81, 3001-3007 (2009).
4. Malin Stridh et al., Enhanced Glutathione Efflux from Astrocytes in Culture by Low Extracellular Ca2+ and Curcumin, Neurochem. Res., 35, 1231-1238 (2010).
5. Yaming Su et al., Galanin Down-regulates Microglial Tumor Necrosis Factor-alpha Production by a Post-transcriptional Mechanism, J. Neuroimmunol., 134, 52-60 (2003).
6. S. Hobson et al., Galanin Acts as a Trophic Factor to the Central and Peripheral Nervous Systems, Cell Mol. Life Sci., 65, 1806-1812 (2008).
7. N. Innamorato et al., Role of Microglial Redox Balance in Modulation of Neuroinflammation, Curr. Opin. Neurol., 22, 308-314 (2009).

Figures used in the abstract

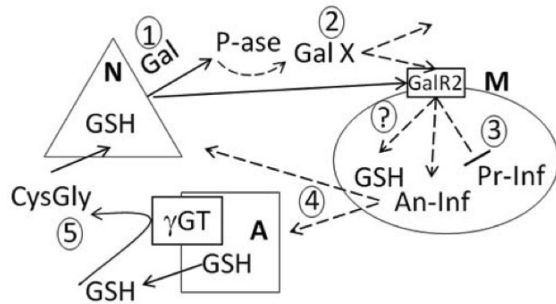

Figure 1: Figure 1 shows the potential pathway for galanin's role in the anti-inflammatory response. After an injury (due to stroke, for example), the neuron releases galanin into the extracellular space, where it's hydrolyzed by ectopeptidases (P-ase) to some fragments (Gal X). Either galanin (Gal) and/or Gal X bind to the GalR2 receptor, which increases the concentration of anti-inflammatory cytokines (An-Inf) while decreasing the concentration of pro-inflammatory (Pr-Inf) ones. The An-Inf then increases the reducing environment of the microglia (M) as well as that of the neuron (N) and astrocytes (A) by increasing the concentration of glutathione (GSH). The reducing environment can then combat the oxidative stress introduced by stroke/inflammatory conditions.

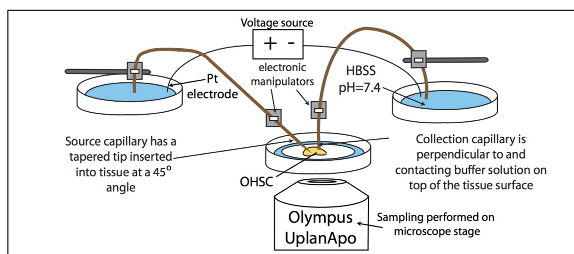

Figure 2: Figure 2 shows the setup for the electroosmotic sampling experiment. There are two fused-silica capillaries that are connected via an electrical circuit of HBSS buffers, platinum electrodes, a voltage source, and the tissue itself. The application of a voltage drives bulk fluid movement from the source capillary (pulled tip) through the tissue into the sampling capillary, a process that can be monitored by a fluorescent marker.

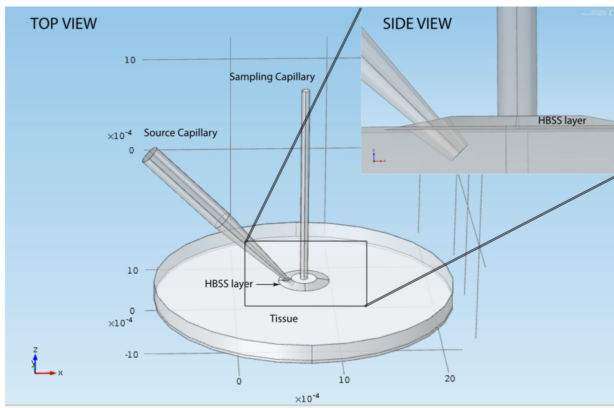

Figure 3: Figure 3 shows the setup for electroosmotic sampling in COMSOL Multiphysics. The geometry has been reduced to focus only on the region near the tissue.

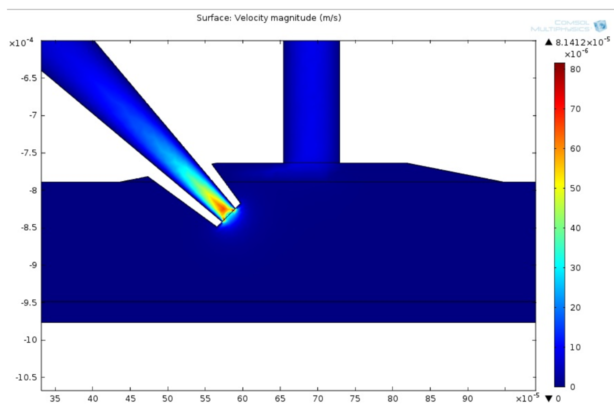

Figure 4: Figure 4 shows the calculated result for fluid velocity (Free and Porous Media Flow). The average linear velocity from the tip of the source capillary to the tip of the sampling capillary (through the tissue) was determined to be 6.8 microns/sec.