Tuning Sensitivity to Ectopeptidase Rates in the Rat Hippocampus Using Numerical Simulations

Y. Ou[1], S. Weber[1]

[1]University of Pittsburgh, Pittsburgh, PA, USA

Abstract

We have created and optimized a multiphysics model in COMSOL Multiphysics® software that incorporates the Electric Currents, Free and Porous Media Flow, and Species Transport in Porous Media interfaces, as well as the Particle Tracing Module to study sensitivity to ectopeptidase rates in the rat hippocampus. Ectopeptidases are membrane-bound enzymes whose catalytic domains face the extracellular space (ECS) (Figure 1). They have traditionally been accepted to inactivate peptides from the ECS[1]. Recently, not only can these enzymes activate peptides, some have also been shown to modulate peptides critical to many important physiological processes, including Alzheimer's and stroke[2,3]. Conventionally, measurements of ectoenzyme activity in tissue or cell are done by incubating whole cells or membrane fractions isolated from homogenization in media with exogenously added substrates. Our group has successfully developed two generations of sampling techniques to study enzyme activity in intact tissues, which, as far as we know, was the first time it has been done[4,5].

Using the calculations as a guide, we varied different parameters (i.d. of the sampling capillary and applied current) of our second-generation sampling tool (electroosmotic push-pull perfusion, EOPPP, Figure 2) to tune the residence time of the substrate peptide. This residence time allows us to "view" a specific range of hydrolysis rates. Longer residence times are optimal for slow kinetic processes while shorter residence times are for rapid ones. In addition, we used the model to achieve a better understanding of the physics of our technique, such as mapping out where transport is diffusion-dominated and where it's advection-dominated (Péclet number, Figure 3), quantifying the collection efficiency of our technique, and understanding the effect of porous properties (e.g. porosity, permeability, tortuosity, etc) on transport efficiency, to name a few. Through a combination of calculations and experiments, we were able to determine the Vmax (and an estimation of Km) for membrane-bound aminopeptidases that hydrolyzes Leu-enkephalin, an important endogenous opioid neurotransmitter, in the Cornu Ammonis 3b (CA3b) subregion of the rat hippocampus.

Reference

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Figures used in the abstract

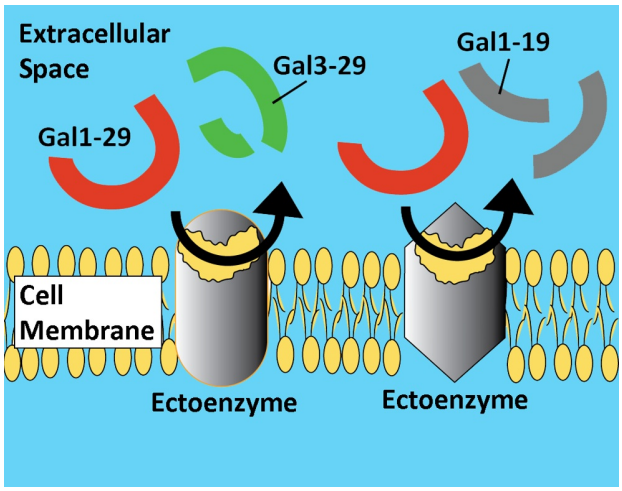

Figure 1: An example of ectopeptidases cleaving the neuroprotective peptide galanin into different fragments, whose affinities for the galanin receptors may alter the apparent activity of the parent peptide [5].

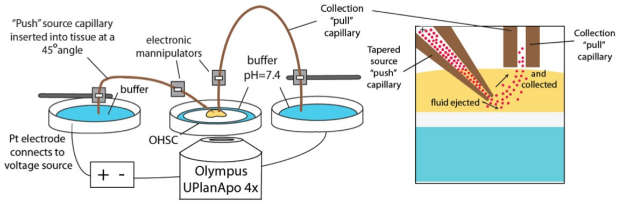

Figure 2: Setup for electroosmotic push-pull perfusion. A fused silica capillary (source) is pulled to a fine tip that is then inserted into a certain depth of organotypic hippocampal slice cultures. A second capillary (sampling) is positioned above the tip of the first, above the tissue at a specified distance. Both distal ends of the capillaries are in buffer solution, in electrical contact with Pt electrodes that completes a full circuit. An application of an electric field parallel to the walls of the capillaries induces electroosmotic flow, which drives transport of exogenous peptides from source to the tissue and to the sampling capillary [6].

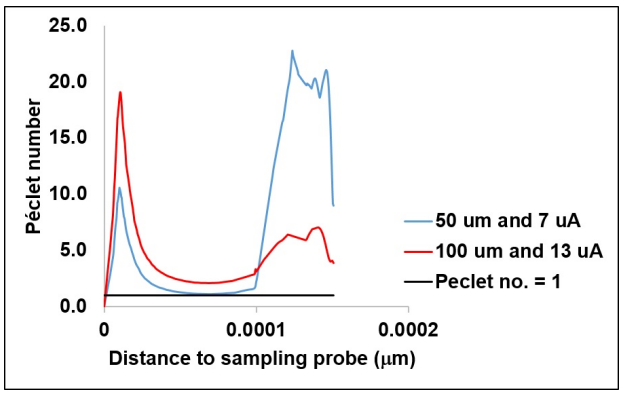

Figure 3: Changes in the Peclet number, an indication of whether advection or diffusion dominates, are mapped in the tissue under two sampling conditions.