Enhanced Microdialysis Sampling of Neuropeptides using Affinity Agents

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Abstract

Neuropeptides, such as opioids, are involved in complex and coupled neural networks in addiction. One way to gain insight and investigate how the intact and poorly understood networks regulate neurotransmission is to measure neuropeptide concentrations. The most commonly used and available technique to sample and monitor the release of neuropeptides is microdialysis sampling. However, this technique results in low amounts of neuropeptide recovered across the probe membrane due to their size and low basal concentrations. Therefore, improvement of this technique would allow higher recoveries of the neuropeptide to be obtained. By adding affinity agents into the perfusion fluid, which passes through the probe, an enhanced recovery of the neuropeptide can result, thus preconcentrating the sample collected and ultimately allowing for easier detection and quantitation.

The affinity agents, cyclodextrins, antibodies and antibody-immobilized beads, were perfused through microdialysis sampling probes (20 kDa and 100 kDa molecular weight cut-off membranes) to improve the mass transport of the neuropeptides, met- and leu-enkephalin, dynorphin A, neuropeptide Y and various rat endocrine hormones (amylin, GLP-1, glucagon, insulin and leptin). Cyclodextrins resulted in the least amount of recovery enhancement (2-fold) followed by antibodies (2.5-fold) and then antibody-immobilized beads (2 to15-fold). Probe membranes and bead densities were tested for their effect on recoveries. *In vivo* studies conducted in rat striatum and subcutis space of rats showed this method is applicable.

To allow direct quantitation of captured analytes on antibody-immobilized beads, immunoassays suitable for the Luminex platform were created for leu-enkephalin, dynorphin A and neuropeptide Y. These assays allow for quantitation of low volumes of sample that no other assay provides (besides mass spectrometry). Dissociation buffers (chaotropic salts) and organic/acid solvents were tested for LE release from antibody-immobilized beads for mass spectrometric analysis to be able to identify if inactive forms of the neuropeptide cross-react with the antibody immobilized to the bead.

The binding between the neuropeptides, leu-enkephalin, dynorphin A and neuropeptide Y, and their respective antibodies used (on the beads) were studied using SPR and equilibrium dialysis. The K_D values and k_{on}/k_{off} rates were determined to

further investigate why different enhancements occur for different analytes during microdialysis sampling. The data suggests that these parameters may play a major role in the microdialysis enhancement process. The data presented in this thesis shows an applied quantitative enhanced microdialysis sampling method that increases relative recovery of neuropeptides and provides insight into understanding the parameters that may be optimized for a potential affinity agent.