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Online glutamate measurement during brain altered function with sub seconds time resolution. A new alternative to measure biological compounds with laser induced fluorescence detection and the use of enzymatic reactors

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Abstract

Glutamate (Glu) constitutes the principal excitatory neurotransmitter in the brain. To understand the Glu neurotransmitter functions and their alteration in disease, methods with high temporal resolution are needed. This feature will allow correlating Glu concentrations with normal or brain altered functions. Unfortunately, available methodologies do not have the temporal resolution needed. The microdialysis technique is the most used method to measure the extracellular Glu concentrations. In this procedure, the samples are collected periodically (minutes resolution) and quantified mainly by High Performance Liquid Chromatography (HPLC). In this work, it is showed a new methodology with high temporal resolution to measure Glu. This method uses glutamate oxidase and Amplex Red, generating a fluorescent derivate (resorufin) that can be optically detected and measured. Microdialysate coming from a probe is mixed with a similar volume of an enzymatic reactor prepared to measure Glu concentration. The mixture is introduced into silica tubing placed inside of an incubation chamber at 37 oC to carry out the reaction. A fluorescence cell, located at the end of this tubing, measures fluorescence at 590 nm every two hundred milliseconds, resorufin is excited at 561 nm by a laser beam. Calibration curves were carried out immersing the probe in different Glu concentrations (50-500 uM) a linear regression analysis of 0.998 was obtained. The following experiment was carried out to validate this method. A microdialys was inserted into the hippocampus in adult awake rat. Epileptiform activity was induced administering 4-aminopyridine by reverse microdialysis procedure. Results show that abnormal electroencephalographic activity and glutamate concentrations can be correlated. These results demonstrate that this method has the appropriate sensitivity and temporal resolution to measure Glu in brain structures. This methodology could be easily adapted to measure other neurotransmitters and compounds using enzymatic reactors that generate fluorescence derivate.

Biography

Alberto Morales-Villagrán has a PhD in Basic Biomedical Research (UNAM, México) and he works for the University of Guadalajara since 1987. He is the PI of the Neurophysiology and Neurochemistry Laboratory at the Molecular and Cellular Department, CUCBA, University of Guadalajara. His research is mainly focused in searching new methods and device to monitor brain neurotransmitters. He has published more than 20 papers related with neurotransmitter alterations during epileptiform activity and has a patent granted related in this topic.

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