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An amperometric glutamate biosensor for monitoring glutamate release from brain nerve terminals and in blood plasma

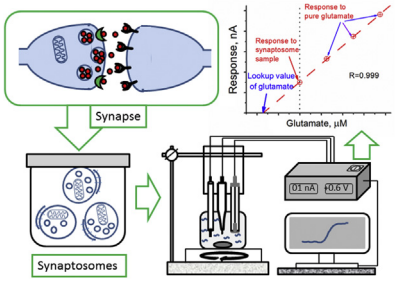
T. Borisova [a], D. Kucherenko [b, c], O. Soldatkin [b, c, *], I. Kucherenko [b], A. Pastukhov [a], A. Nazarova [a], M. Galkin [a], A. Borysov [a], N. Krisanova [a], A. Soldatkin [b, c], A. El`skaya [b]

[a] The Department of Neurochemistry, Palladin Institute of Biochemistry, NAS of Ukraine, 9 Leontovicha Street, Kyiv, 01601, Ukraine
[b] Laboratory of Biomolecular Electronics, Department of Translation Mechanisms of Genetic Information, Institute of Molecular Biology and Genetics, NAS of Ukraine, 150 Zabolotnogo str., Kyiv, 03143, Ukraine
[c] Institute of High Technologies, Taras Shevchenko National University of Kyiv, 64, Volodymyrska Str., Kyiv, 01003, Ukraine

HIGHLIGHTS

- A biosensor-based approach for the analysis of glutamate transport was developed.
- Isolated rat brain nerve terminals (synaptosomes) were used for the studies.
- Tonic, exocytotic and transporter-mediated glutamate release rates were determined.
- The biosensor results were confirmed by traditional methods of glutamate analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

An excess of the excitatory neurotransmitter, glutamate, in the synaptic cleft during hypoxia/ischemia provokes development of neurotoxicity and originates from the reversal of Na^+-dependent glutamate transporters located in the plasma membrane of presynaptic brain nerve terminals. Here, we have optimized an electrochemical glutamate biosensor using glutamate oxidase and developed a biosensor-based methodological approach for analysis of rates of tonic, exocytotic and transporter-mediated glutamate release from isolated rat brain nerve terminals (synaptosomes). Changes in the extracellular glutamate concentrations from 11.5 ± 0.9 to 11.7 ± 0.9 μM for 6 min reflected a low tonic release of endogenous glutamate from nerve terminals. Depolarization-induced exocytotic release of endogenous glutamate was equal to 7.5 ± 1.0 μM and transporter reversal was 8.0 ± 1.0 μM for 6 min. The biosensor data correlated well with the results obtained using radiolabelled L-[^{14}C]glutamate, spectrofluorimetric glutamate dehydrogenase and amino acid analyzer assays. The blood plasma glutamate concentration was also tested, and reliability of the biosensor measurements was confirmed by glutamate dehydrogenase assay. Therefore, the biosensor-based approach for accurate monitoring rates of tonic, exocytotic and transporter-mediated release of glutamate in nerve terminals was developed and its adequacy was confirmed by independent analytical methods. The biosensor measurements provided precise data on changes in the concentrations of endogenous glutamate in nerve terminals in response to stimulation. We consider that the glutamate biosensor-based approach can be applied in clinics for neuromonitoring glutamate-related parameters in brain samples, liquids and blood plasma in stroke, brain trauma,

* Corresponding author. Zabolotnogo Street 150, 03143, Kyiv, Ukraine.
E-mail address: alex_sold@yahoo.com (O. Soldatkin).

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therapeutic hypothermia treatment, etc., and also in laboratory work to record glutamate release and uptake kinetics in nerve terminals.

1. Introduction

Glutamate is a key excitatory neurotransmitter in the central nervous system because of its involvement in almost all aspects of normal brain functioning. The main mechanism of glutamate release from presynaptic nerve terminals to the synaptic cleft is stimulated exocytosis. Neuronal injury and death in stroke, cerebral hypoxia/ischemia, hypoglycemia, traumatic brain injury, etc., are mainly provoked by an increase in the concentration of extracellular glutamate in the synaptic cleft that overstimulates the glutamate receptors and initiates an excessive calcium entry. Under these pathological conditions, excessive extracellular glutamate originates from the neuronal cytoplasm and is released through the membrane Na^+-dependent glutamate transporters operated in a reverse mode [1]. Beside the stimulated exocytotic release of glutamate and pathological glutamate transporter reversal, unstimulated tonic release from nerve terminals also deserves attention. This release occurs permanently via several mechanisms and is an important constituent that balances the ambient level of glutamate in the synaptic cleft between the episodes of exocytosis [2,3].

Recently, we have revealed that alterations in the extracellular glutamate level during therapeutic hypothermia can be unique for each patient [4]. Therefore, the test parameters and clinical criteria for continuous glutamate monitoring and evaluation of individual hypothermia-induced effects should be developed for personalized medicine practice.

Excessive extracellular glutamate can be removed from brain interstitial fluids to the blood plasma for the maintenance of proper extracellular glutamate homeostasis in the mammalian central nervous system [5–7]. The glutamate concentration in the blood plasma increases in case of ischemic stroke and other neurological disorders [8].

The traditional methods of glutamate determination include high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and spectrophotometry. These techniques are sensitive and powerful, but they require very expensive and complex equipment that limits their application in the laboratory work and monitoring kinetics of neurotransmitter release/uptake in clinics [9]. The electrochemical biosensors for the glutamate determination are faster, more user-friendly and cheaper than the traditional methods [10]. Furthermore, the biosensors can be miniaturized for the detection of glutamate in living tissues that cannot be achieved by other methods [11]. Thus, the development and application of the glutamate-sensitive biosensors is a new perspective for simplifying analysis procedure and decreasing its price that can result in their wider involvement in the neurochemical research.

Currently, a number of glutamate-sensitive biosensors were developed. They are based on glutamate oxidase (GluOx) [12,13] or glutamate dehydrogenase (GLDH) [14,15]. Both enzymes oxidize glutamate to ketoglutarate, although the first enzyme also generates hydrogen peroxide, whereas the second one reduces a cofactor (NAD^+). Both biosensor types appeared to be efficient in the determination of glutamate concentration in biological and food samples. However, GLDH requires the addition of the factor to the working buffer or its co-immobilization with the enzyme. This fact makes the GLDH-based biosensor more complex in comparison with the GluOx-based one. Furthermore, the stability of the GluOx-based biosensor is much better [16–19].

In our previous study, we developed a biosensor-based method for monitoring the rate of glutamate uptake that takes into consideration the extracellular level of endogenous glutamate in the preparations of nerve terminals [20].

The aim of this study was to upgrade the recently constructed amperometric glutamate oxidase-based biosensor and develop a methodological algorithm for precise monitoring the rates of exocytotic glutamate release and the glutamate transporter reversal (pathological ischemia-related glutamate transport mechanism) in nerve terminals. The experimental data obtained with the glutamate biosensor were confirmed by independent analytical methods.

2. Materials and methods

2.1. Design of amperometric transducers

In this work, we used in-lab made platinum disc electrodes as amperometric transducers (Fig. 1A and B). The electrodes were produced according to the following algorithm. First, 3 mm long platinum wire of 0.4 mm in diameter was sealed in the terminal part of a glass capillary of 3.5–5 mm in outer diameter. An open end of the wire served as a working surface of the transducer. Then the platinum wire was connected by fusible Wood alloy to the conductor placed inside the capillary. A contact pad was attached to the other end of the conductor for connection with the measuring setup. The working electrode surface was obtained by grinding with alumina powder (particles of 0.1 μm and 0.05 μm) and treated with pure ethanol before the bioselective element immobilization. The electrode surface was periodically restored using the same grinding procedure. During the operation of the amperometric biosensor, we used a three-electrode scheme of the amperometric analysis (Fig. 1, C). The working amperometric electrodes, an auxiliary platinum electrode and an Ag/AgCl reference electrode were connected to the PalmSens potentiostat (Palm Instruments BV, The Netherlands).

2.2. Modification of amperometric transducer with phenylenediamine

The proposed biosensor operation is based on the measurement of the oxidation current flowing to the working electrode at applied potential (+0.6 V vs Ag/AgCl). The current is generated due to the decomposition of hydrogen peroxide, a product of the enzymatic reaction of glutamate oxidation, on the working electrode. Biological samples contain numerous substances (ascorbic acid, cysteine, dopamine, etc.) that can be also oxidized at the electrode resulting in the errors in glutamate measurements. To improve the selectivity of the biosensor, we placed a permselective membrane onto the electrode surface (below the enzyme layer). This membrane was based on poly(*m*-phenylenediamine) (PPD) and contained pores that allowed the access of hydrogen peroxide molecules to the electrode surface, but blocked the molecules of a larger size. The membrane was prepared by a method described in Ref. [21]. Briefly, a three-electrode system with a bare working electrode was