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Abstract

In vitro L-Glutamate detection in different brain regions by GluOxRGO/Pt biosensor †

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Abstract: In this study we developed and validated a biosensor that is sensitive to quantify L-glutamate in different regions of rat's brain. We have proposed the biosensor consisting of semipermeable membrane with L-Glutamate oxidase immobilized in to albumin/reduced graphene oxide (RGO) matrix and Pt electrode (GluOxRGO/Pt). The GluOxRGO/Pt biosensor was validated to quantify L-glutamate in brain tissues, namely the cerebellum, brainstem, hippocampus, striatum, mesencephalon and prefrontal cortex of the wild type (WT), dopamine transporter knock out (DAT KO) and DAT KO heterozygous (HET) rats. By this study we prove different L-glutamate distribution in separate regions of brains determined by type of rats.

Keywords: L-glutamate; amperometric biosensor; brain regions; WT and mutated rats

1. Introduction

L-glutamate is known as the most common excitatory neurotransmitter in the mammalian central nervous system [1]. Abnormal transmission of L-glutamate can cause neurological diseases such as communication dysfunction, cognitive impairments, schizophrenia, Parkinson's disease, stroke and epilepsy [2]. However, it is a known fact that there are very low concentrations of L-glutamate (5–15 mmol/kg brain tissue) and it's depending on the region [3]. Moreover, the changes in L-glutamate caused by various pathologies are within the limits of extremely low concentrations. Thus, investigations of L-glutamate release in different brain regions are very complex, but on the other hand, would be particularly informative as it would allow the identification of, L-glutamate-related pathophysiological pathways. Nowadays, a growing body of literature examines *in vivo* L-glutamate biosensors which helpful in understanding the physiology of neurotransmitters in the brain [4]. However, *in vivo* sensors are extremely invasive, short-term and can lead to further complications such as infections. Consequently, there is still gap for essential improvement of L-glutamate biosensors while fabricating biosensors for *in vitro* monitoring.

2. Materials and Methods

2.1. Materials

GluOx *from Streptomyces sp.* (EC 1.4.3.11), Merck KGaA, Germany. WT, DAT KO and DAT KO HET brain samples were obtained from University of Mons, Belgium. Semi-permeable terylene membrane (thickness 12 μm, pore diameter 0.4 μm), Joint Institute of Nuclear Research, Russia. Other chemical reagents were obtained from Sigma–Aldrich (analytical grade). RGO was synthesized according to the protocol reported by J. Gaidukevic doctoral dissertation (Vilnius University).

2.2. L-Glutamate Biosensor Construction

The biosensor consists of semipermeable membrane with immobilized GluOx, working Pt electrode as a contact zone and isolating corps. Aiming to design enzymatic membranes a mixture containing GluOx, bovine serum albumin, glutaraldehyde and RGO paste, which was prepared by mixing of RGO powder with the pasting liquid consisting of 10% polyvinyl dichloride in acetone, was deposited on the inner surface of the ring-fixed membrane (Ø 2.4 mm) and then left at 4 °C overnight. After that membrane was mechanically attached to the surface of the Pt electrode.

3. Discussion

In this study we have proposed biosensor (GluOxRGO/Pt) that is sensitive to quantify the concentration of L-glutamate in samples obtained from different regions of rat's brain. The biosensor action is based on detection of current generating during electrooxidation of H_2O_2 released in L-glutamate converting to alfa-ketoglutarate reaction. The GluOxRGO/Pt biosensor was validated to quantify L-glutamate in the brain tissues, namely the cerebellum (CVT), brainstem (BS), hippocampus (HYP), striatum (STR), mesencephalon (MES) and prefrontal cortex (PFC). The samples were taken from different brain regions of WT, HET and DAT KO type of rats and the weight of these ranged from 0.02 to 0.33 g. The study of L-glutamate distribution in different regions of the rat brain samples demonstrated that HET is practically always among WT and DAT KO. The largest amounts of L-glutamate were found in PFC area, when in HYP and in STR tissues. The highest differences in L-glutamate between DAT KO and WT rats also were found in PFC and HYP regions. Taking into account that weight of PFC, HYP and STR tissues are about 10-15 or 3.4 times smaller comparing with the weight of the CVT, it is evidence that in these zones the role of L-glutamate becomes crucial. The fact that the STR and the PFC is important for behavior, especially in the development of adolescents, has been shown by Steinberg [5]. In the area of MES it was found the opposite dependence since concentration of L-glutamate in DAT KO were higher than in WT. These facts open up the possibility of future studies to explore the potential of L-glutamate in the management of neurological diseases. Furthermore, results revealed that GluOxRGO/Pt can be used for sensitive measurements of L-glutamate distribution in different regions of brains in order to study mechanisms of various diseases.

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