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Research Report

In vivo monitoring of extracellular glutamate in the brain with a microsensor

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ABSTRACT

Recent discoveries have revealed that glutamatergic neurotransmission in the central nervous system is mediated by a dynamic interplay between neurons and astrocytes. To enhance our understanding of this process, the study of extracellular glutamate is crucial. At present, microdialysis is the most frequently used analytical technique to monitor extracellular glutamate levels directly in the brain. However, the neuronal and physiological origin of the detected glutamate levels is questioned as they do not fulfil the classical release criteria for exocytotic release, such as calcium dependency or response to the sodium channel blocker tetrodotoxine (TTX). It is hypothesized that an analytical technique with a higher spatial and temporal resolution is required. Glutamate microsensors provide a promising analytical solution to meet this requirement. In the present study, we applied a 10 μm diameter hydrogel-coated glutamate microsensor to monitor extracellular glutamate levels in the striatum of anesthetized rats. To explore the potential of the microsensor, different pharmacological agents were injected in the vicinity of the sensor at an approximate distance of 100 μm. It was observed that KCl, exogenous glutamate, kainate and the reuptake inhibitor DL-threo-β-benzyloxyaspartate (DL-TBOA) increased the extracellular glutamate levels significantly. TTX decreased the basal extracellular glutamate levels approximately 90%, which indicates that the microsensor is capable of detecting neuronally derived glutamate. This is one of the first studies in which a microsensor is applied in vivo on a routine base, and it is concluded that microsensor research can contribute significantly to improve our understanding of the physiology of glutamatergic neurotransmission in the brain.

1. Introduction

L-glutamate is the predominant excitatory neurotransmitter in the central nervous system (CNS) and is involved in most aspects of normal brain functioning, such as cognitive processes, the formation of memory and the development and plasticity of the CNS. Consequently, glutamate is involved in the pathophysiology of many neurological, neurodegenerative and psychiatric disorders, such as epilepsy, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, depression, stroke and schizophrenia (Danbolt, 2001; Coyle et al., 2002; Javitt, 2004).

Recent discoveries have revealed that the physiology of glutamatergic neurotransmission in the CNS is mediated by

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an intimate partnership between neurons and astrocytes (Haydon, 2001; Nedergaard et al., 2002; Volterra and Meldolesi, 2005). To further improve our understanding of these processes, the assessment of extracellular glutamate in the brain is crucial. Several analytical techniques are currently being applied to monitor glutamate in the brain, e.g. microdialysis (Ungerstedt, 1991), push–pull perfusion (Gaddum, 1961) or direct sampling by fused silica tubing in combination with capillary electrophoresis (Kennedy et al., 2002). Of these techniques, microdialysis is by far the most frequently used. However, the physiological origin of glutamate in dialysate is uncertain (Westerink et al., 1987; Miele et al., 1996; Timmerman and Westerink, 1997; Drew et al., 2004). It appears that glutamate detected by microdialysis does not fulfil the classical release criteria for exocytotic release as it does not show calcium dependency or response to sodium channel blockade. Detection of extracellular glutamate with a higher spatial and temporal resolution is apparently required to facilitate sampling of the synaptic pool (Rossel et al., 2003; Drew et al., 2004).

Evidence is provided that microsensors may give a closer approach to the glutamatergic physiology in the brain due to their analytical properties (Hu et al., 1994; Kulagina et al., 1999; Burmeister and Gerhardt, 2001; Rahman et al., 2005; Day et al., 2006). However, until now glutamate microsensors are hardly used on a routine base. It appears that the use of microsensors is often hampered by technical difficulties in their construction and application.

To overcome these technical difficulties, we gave much attention to the construction and characterization of a 10 μm diameter hydrogel-coated glutamate microsensor (Oldenziel et al., 2004; Oldenziel and Westerink, 2005; Oldenziel et al., 2006a), which was initially developed by Kulagina et al. (1999). In addition, we have evaluated the microsensor both in vivo and in vitro (Oldenziel et al., 2006b, in press). In the present study, the glutamate microsensor is applied on a routine base for monitoring extracellular glutamate in the striatum of anesthetized rats. The influence of different pharmacological agents, which are known to affect the release of glutamate, was investigated.

2. Results

2.1. *Microsensor recordings in the striatum*

The experiments in the present study were conducted by placing both a glutamate and background microsensor together with a micropipette in the striatum of anesthetized rats. The current output of both microsensors was monitored, and the difference in current was considered to represent the extracellular glutamate concentration ($[Glu]_0$). Different pharmacological agents were applied via a micropipette, and their influence on the microsensor recordings was monitored.

The difference in current output between the glutamate and background microsensor after drug administration was divided by the difference prior to administration (set at 100%) and expressed as percentage (%). The placement of the microsensor in the brain was investigated retrospectively by histological examination. A typical example of a histological section is presented in Fig. 1. Visualized are the implantation sites of the micropipette and of a microsensor in the striatum. Only one microsensor is visible as the other one was situated more anterior. To follow the track of the micropipette and sensor as good as possible, slices were prepared in a semi-coronal way (oblique).

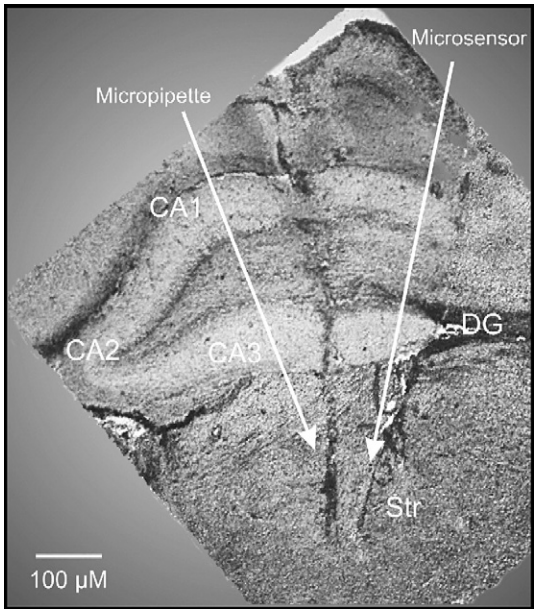

Fig. 1 – An example of a histological section. Visualized are the track of the micropipette and of one microsensor. Note: the slice was prepared in a semi-coronal way (oblique) to follow the track of the microsensor and micropipette.

2.2. *Basal extracellular glutamate levels*

In a total of 85 experiments the average current output of the glutamate microsensor was 53.9±5.1 pA (mean±SEM), and of the background sensor 41.3±4.1 pA. When the difference in current between the glutamate and background microsensor for each individual experiment was examined, a statistically significant difference of 10.1±5.3 pA was observed ($p<0.05$; Mann–Whitney Rank Sum Test). To correlate this current to final glutamate concentrations, the microsensor needs to be calibrated at conditions which are encountered in vivo. The average sensitivity of the microsensor under these conditions was approximately 0.55 pA/μM (Oldenziel et al., 2006b), which indicates that the difference between both sensors represents a $[Glu]_0$ of 18.2 μM±9.3 μM. This sensitivity is displayed as a footnote in the figures.

During the experiments we observed that the depth of anesthesia affected the current output of the microsensors, i.e. the deeper the level of anesthesia, the lower the current output of the microsensors. In particular, when the anesthetic tended to wear off, which was monitored by hind-limb compression, an increase in the current output of the microsensors was observed (results not shown). Throughout this study, the anesthetic equitensine was used as this compound is known to have lesser effect on the extracellular glutamate concentrations in comparison to other anesthetics (Lada et al., 1998; Rozza et al., 2000; Bo et al., 2003). In addition,