Sensors and Actuators B 237 (2016) 298–307

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Microelectrode array biosensor for high-resolution measurements of extracellular glucose in the brain

Cátia F. Lourenço[a], Ana Ledo[a], João Laranjinha[a,b], Greg A. Gerhardt[c], Rui M. Barbosa[a,b,*]

[a] Center for Neuroscience and Cell Biology, University of Coimbra, Portugal
[b] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[c] Center for Microelectrode Technology, University of Kentucky, Lexington, USA

ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 3 June 2016
Accepted 14 June 2016
Available online 18 June 2016

Keywords:
Glucose oxidase
Microbiosensor
Microelectrode arrays
Glucose measurement
Brain *in vivo*

ABSTRACT

Direct measurement of glucose in the brain extracellular space with high spatial and temporal resolution is needed for understanding of brain energy metabolism. In this work we developed and extensively characterized an amperometric glucose microbiosensor using ceramic-based platinum multisite microelectrode arrays (MEAs) for measuring extracellular glucose in the rat brain *in vivo*. Glucose-oxidase (GOx) was immobilized at the microelectrode surface by cross-linking with glutaraldehyde in the presence of BSA. The *in vitro* evaluations supported the high analytical performance of these microbiosensors for application in the brain, including high selectivity and sensitivity, adequate linear range and limit of detection, good operational stability and response time, minimal oxygen-, pH- and temperature-dependencies. The microbiosensors were successfully used for measurements of glucose with high temporal and spatial resolution in the hippocampus of anesthetized rats in response to systemic, local glucose changes and upon glutamatergic stimulation. Overall, the results support the suitability of these microbiosensors for measuring rapid changes of extracellular glucose in the rat brain.

1. Introduction

The brain has a very high energetic demand and is dependent on a continuous supply of glucose that serves as a primary source of energy under physiological conditions [1]. Metabolic disturbances have been described in several brain pathological conditions such as epilepsy and Alzheimer's Disease [2–4].

Direct measurement of the flux of glucose and other metabolites between cells, with high spatial and temporal resolution, is critical for the understanding of brain energy metabolism [5]. However, measuring metabolic substrates in real-time in the brain extracellular space is challenging, mainly due to the complexity of the chemical environment, the heterogeneous cellular organization of the brain and the rapid release and uptake processes associated with energy metabolism [6]. Brain microdialysis has been widely used to monitor metabolic substrates and other neurochemicals [6,7]. However, while allowing very specific analyte detection, the real-time monitoring is compromised by the relative low temporal and spatial resolution of the technique [8].

Microbiosensors associated with fast electrochemical techniques (*e.g.* amperometry, fast cyclic voltammetry) are an attractive approach for the real-time detection of neurochemicals due to their high sensitivity, selectivity, response time and minimal damage to tissue [9,10]. A rational design of microbiosensors for measuring non-electroactive neurochemicals has been accomplished by immobilizing oxidase enzymes in a suitable polymeric film onto the electrode surface combined with the amperometric detection of hydrogen peroxide as a reporter molecule. Platinum or Pt alloys, namely Pt/Ir (90:10), have been widely used as electrode surface materials due to the well-known electrocatalytic activity of Pt towards the oxidation of H_2O_2 [11,12].

One of the most successful biosensing strategies has been achieved by immobilizing glucose oxidase (GOx) using the cross-linking agent glutaraldehyde (GA) in the presence of BSA onto the surface of Pt-wire microelectrodes [13–15]. The use of such wire-type microelectrodes has been challenged by the use of planar microelectrode arrays (MEAs) based on ceramic [16], silicon [17,18] or polyimide substrates [19]. In opposition to the hand-made fabricated wire-type Pt microbiosensors, microfabricated MEAs exhibit well-defined and highly reproducible geometrical configurations that are key features to accomplish reproducible measurements. Also, they have unique electrochemical properties which confer significant advantage for a variety of analytical appli-

* Corresponding author at: Faculty of Pharmacy, University of Coimbra, Health Sciences Campus, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.
E-mail address: rbarbosa@ff.uc.pt (R.M. Barbosa).

http://dx.doi.org/10.1016/j.snb.2016.06.083
0925-4005/

cations, such as investigation of reaction mechanisms and detection of redox species at very low concentrations [20,21]. Furthermore, these platforms are of particular interest in the construction of microbiosensors by differential coating of the Pt sites with the enzyme cocktail or the inactive protein matrix, allowing for "self-referencing" recordings [22]. The use of a ceramic platform as the support material is valuable due to its positive impact on biocompatibility of these microbiosensor arrays, in addition to its strength and electrical inertness [23,24].

Ceramic-based MEAs have been widely used for measuring tonic and phasic glutamate levels in anesthetized [25] and awake animals [26]. Interestingly enough, MEAs can also be configured for multi-analyte detection, as described for choline and acetylcholine [27]. This is of utmost importance considering the relevance of monitoring glucose simultaneously with other metabolites (*e.g.* lactate and pyruvate) for the understanding of brain metabolism. The ceramic-based MEA platform was successfully used to develop a lactate microbiosensor array to measure lactate in brain tissue [28]. However, a thorough *in vitro* characterization and *in vivo* validation for MEA-based glucose biosensor is still lacking. Hence, in this work we have used MEAs to develop an amperometric glucose microbiosensor array. The MEA-based biosensor was extensively characterized *in vitro* and used for measurements of glucose with high temporal and spatial resolution in the hippocampus of anesthetized rats.

2. Materials and methods

2.1. Chemicals and solutions

D-(+)-Glucose, Glucose Oxidase (EC 1.1.3.4, Type VII) from *Aspergillus niger* in powder form, *meta*-phenylenediamine (*m*-PD), ascorbic acid, dopamine, uric acid, hydrogen peroxide, L-glutamic acid, bovine serum albumin (BSA), glutaraldehyde solution (25%), urethane and human insulin were obtained from Sigma-Aldrich. All other reagents were purchased from Merck, unless specified. All solutions were prepared in bi-deionized MilliQ water with resistivity ≥18 MΩ cm (Millipore Corporation, USA). The electrolyte for *in vitro* analytical evaluation of microbiosensors was phosphate buffer saline (PBS) 0.05 M (pH 7.4) with the following composition (mM): 100 NaCl, 10 NaH_2PO_4 and 40 Na_2HPO_4. Stock solutions of glucose (1 M) were allowed to equilibrate the β/α anomeric ratio for 24 h before use. The solutions used for intracranial pressure-ejections were prepared in saline (0.9% NaCl), adjusted to pH 7.4, and filtered prior to use (0.2 μm pore size filter).

2.2. Preparation and modification of glucose microbiosensor arrays

Ceramic-based MEAs were obtained from Center for Microelectrode Technology, University of Kentucky, US. The S2 design configuration containing two pairs of side-by-side sites (15 μm × 333 μm) was used as the microelectrode platform for construction of the glucose microbiosensor arrays. Glucose oxidase was immobilized onto the Pt surface using a coating procedure essentially as described [22] (Fig. 1). A cocktail solution containing either 1 mg/mL (0.1%, 200 U/mL) glucose oxidase (GOx) or 5 mg/mL (0.5%, 1000 U/mL) GOx, BSA (1%) and glutaraldehyde (0.125%) in water was applied to the surface using a microsyringe mounted on micromanipulator under a stereomicroscope onto the bottom pair of recording sites. The remaining pair of sites (top) was coated with the inactive protein matrix solution containing BSA (1%) and glutaraldehyde (0.125%) using the same procedure (Fig. 1). This pair of recording sites is insensitive to glucose and as such act as sentinel or null sites. Glucose was thus measured in a self-referencing recording mode achieved by subtracting the current from the sentinel sites from that of the active sites coated with glucose oxidase [22]. The microbiosensors were stored dry in the dark and protected from dust, at room temperature, for at least three days to allow for curing and stabilization of the active and non-active matrices. To minimize access of undesirable electrochemically active compounds to the Pt recording sites, the MEAs-based GOx biosensors were modified, before use, with an exclusion layer of *m*-PD. Briefly, the *m*-PD solution (10 mM) was freshly prepared in deoxygenated PBS and electropolymerized onto the MEA Pt surfaces using the FAST16 mkII high-speed electrochemical system (Quanteon, Nicholasville, KY, USA) by cyclic voltammetry between +0.25 and +0.75 V *vs* an Ag/AgCl reference electrode at a scan rate of 50 mV/s during 20 min.

2.3. In vitro *evaluation and characterization*

The *in vitro* evaluation of MEA-based GOx biosensors for measurement of glucose was performed by amperometry at +0.7 V *vs* Ag/AgCl (RE-5, BAS Inc., USA) using the FAST16 mkII high-speed electrochemical system in a two-electrode configuration mode. The experiments were carried out in 40 mL PBS 0.05 M at 37 °C under gentle stirring after a 30 min period of current stabilization. Analytical and kinetic parameters were determined after baseline stabilization by adding aliquots of the stock glucose solution to obtain final concentrations in the range of 0.125–28 mM. The sensitivity to glucose, selectivity against major interferents and the sensitivity to the reporter molecule were determined by three additions of glucose 1 mM in the presence of ascorbic acid 0.5 mM, followed by dopamine 10 μM, uric acid 100 μM and H_2O_2 10 μM. The stability of the MEA-based GOx biosensors was evaluated two weeks after the first calibration and at the end of the acute brain implantation.

To evaluate the oxygen dependence of the MEA-based GOx biosensors, the response to glucose was measured under variable oxygen concentrations. Glucose (0.5, 1 or 2 mM) was added to PBS at 37 °C saturated with argon (purity >99.99%, <0.1 ppm O_2), and the MEA-based GOx biosensor response was continuously monitored while the oxygen concentration in solution equilibrated with atmospheric air. The oxygen concentration was simultaneously monitored with a carbon fiber microelectrode polarized at −0.8 V *vs* Ag/AgCl essentially as previously described (Lourenço et al. [59]). To evaluate the effect of temperature, the response to glucose (0.5 mM) was measured in PBS pH 7.4, while varying the temperature between 30 and 40 °C. The tests for the effects of pH were carried out at 37 °C in PBS with pH ranging from 5.5 to 8.5. For each condition, the sensitivities to glucose and hydrogen peroxide were evaluated.

2.4. Surgical procedures *and* in vivo *experiments*

All the procedures used in this study were performed in accordance with the European Union Council Directive for the Care and Use of Laboratory animals, 2010/63/EU and were approved by the local ethics committee (ORBEA). *In vivo* studies were carried out on adult male Wistar rats (8–10 weeks, n = 6) maintained in controlled environmental conditions: temperature of 22–24 °C, relative humidity of 45–65%, 15 air exchanges per hour and a 12:12 light/dark cycle. Animals were housed in filter-topped type III Makrolon cages on an individually ventilated caging system (VentiRack Bioscreen™). Rats were fed with a standard rat chow diet (4RF21-GLP Mucedola, SRL, Settimo Milanese, Italy) and were provided with chlorinated water, both *ad libitum*.

Rats were anesthetized with urethane (1.25–1.50 g/kg, i.p.) and placed in a stereotaxic frame. The skin overlying the brain surface was reflected and the region of interest was exposed by drilling a hole in the skull. The meninges were removed from