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Simultaneous *in vivo* ascorbate and electrophysiological recordings in rat brain following ischemia/reperfusion

Hanjun Cheng [a], Tongfang Xiao [a,b], Dalei Wang [a], Jie Hao [a,b], Ping Yu [a,b], Lanqun Mao [a,b,*]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, the Chinese Academy of Sciences (CAS), Beijing 100190, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Simultaneous recording of neurochemical signal and electrophysiological activity in the same brain region remains vitally essential to understanding the essences of physiological and pathological events because of the intrinsic chemical and electrical nature of communication between the neurons. However, the difficulty in selectively recording chemical signal with a high temporal resolution in chemically complex central nervous system presents a great challenge for achieving this goal. In this study, we demonstrate a method for simultaneous and real-time recording of ascorbate and electrical signals in live brain by using an integrated dual-mode microelectrode (IDMME), in which a single-walled carbon nanotube (SWNT)-modified carbon fiber microelectrode (CFME) was efficiently integrated with a glass microcapillary electrode. The SWNT-based CFME was served as an electrochemical recording channel to *in vivo* monitor cerebral ascorbate with a high selectivity and an improved temporal resolution, while the glass microcapillary electrode was employed as an electrophysiological recording channel for single-unit recording in the cerebral system. We found that no reciprocal interference between the two recording channels with the IDMME when constant-potential amperometry was employed for *in vivo* monitoring of ascorbate. The validity of the IDMME in neurochemical studies was illustrated by concurrently and real-time recording the dynamic changes of cortical ascorbate level and electrophysiological signal in the same brain region of live rats following ischemia/reperfusion. This study essentially offers an effective approach to *in vivo* monitoring of neurochemical and electrophysiological events in live brain, which is envisaged to be useful for understanding physiological processes involved in brain functions.

1. Introduction

Understanding the essence in brain functions is a long-standing goal because, as the most complex and elaborate system in human body, brain is composed of *ca.* 100 billion neurons that are connected into circuit through *ca.* 100 trillion synapses [1–5]. Neurons receive information from other cells through dendrites and transmit it in the form of electrical impulses, or action potentials, along the axon to other nerves. At the end of the axon, the message is transmitted to other cells at synapses, where the electrical message causes the release of neurotransmitters that diffuse across a small gap to another neuron. The neurotransmitters bind to and activate receptors on the second neuron, finally causing another action potential. Repeating such cycles of chemical and electrical transformations substantially enables various brain functions [6–15]. Due to the chemical and electrical nature of neuron-to-neuron communications, information on the simultaneous changes of neurochemical and electrical signals involved in the physiological and pathological processes remains very essential to understanding the neurotransmission mechanism in brain functions [16–26]. This is because the change in the levels of chemical species from the extracellular space surrounding neurons largely facilitates understanding of neurochemically associated physiological and pathological processes on a molecular basis, while the electrophysiological changes can provide immediate information on the condition of the tissue and offer additional prognostication of neurological outcome in varied neurological disorders [19,21,24,27–31].

As one kind of the most important neurochemicals, ascorbate exists as a water-soluble small molecule that has been demonstrated to play critical roles in many physiological and pathological processes [32–34]. For example, ascorbate has been demonstrated to be neuroprotective toward global cerebral ischemia and salicylate-induced tinnitus [35–37]; loss of ascorbate and other low-molecular-weight antioxidants during ischemia or other injury leaves cells vulnerable to oxidative damage [38,39]. On the other hand, ascorbate has been considered as a neuromodulator, which appears to modulate striatal function by

* Corresponding author at: Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, the Chinese Academy of Sciences (CAS), Beijing 100190, China.
E-mail address: lqmao@iccas.ac.cn (L. Mao).

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enhancing neuronal responsiveness to glutamate [40,41]. Typically, the release of ascorbate was suggested to promote the reuptake of glutamate transmission *via* hetero-exchange at the site of glutamate transmission transport, which finally changes the neural activity by blocking *N*-methyl-D-aspartate (NMDA) receptors [42,43]. Since NMDA receptors participate in fundamental brain functions, such as synaptic plasticity and learning, concurrently *in vivo* recording neural activities and ascorbate levels involved in the same brain regions can offer information that not only directly reflects the properties of single ion channels in neurotransmission process but also indirectly displays ascorbate-modulated neurochemical changes in the central nervous system. In previous attempts, Ewing et al. and Crespi et al. successfully combined electrochemical methods with electrophysiology to concurrently record ascorbate level and electrophysiological signal in the central nervous system, in which ascorbate was *in vivo* monitored with potential-pulsed amperometry and differential pulse voltammetry, respectively [16–19,21]. This information was envisioned useful in understanding the neurotransmission mechanisms and chemical essence involved in physiological and pathological processes such as ischemic injury.

In this study, we demonstrated a method that could continuously record cerebral ascorbate and neuronal activity with an improved temporal resolution and less technical demand for *in vivo* monitoring of ascorbate. In the early attempts, we have observed that the electron transfer for the oxidation of ascorbate could be remarkably facilitated at carbon nanotubes (CNTs) [44–46]. Although the mechanism underlying this observation has been currently investigated in our group, the facilitated electron transfer process observed previously has been successfully employed to constitute novel electrochemical methods for selective and (near) real-time monitoring of ascorbate through both *in vivo* amperometry [46,47] and online electrochemical system composed of *in vivo* microdialysis and online selective electrochemical detection [45,48–50]. So far, these methods have been used in various physiological studies [35–37,47,51]. On the other hand, glass microcapillary electrodes have been used as effective tools to record neuronal electrical signals, which allow us to directly monitor the communication of neurons at an extremely high signal-to-noise ratio [28,52,53]. Inspired by these early investigations, we developed here one kind of integrated dual-mode microelectrodes (IDMMEs) by efficiently combining the single-walled carbon nanotube (SWNT)-modified CFMEs with glass microcapillary electrode, which enable simultaneously and real-time monitoring of extracellular cerebral ascorbate and neuronal activity in live brain. When the IDMMEs are implanted into the brain of live rats, no reciprocal interference between the two independent technologies is observed when constant-potential amperometry was used for *in vivo* monitoring of ascorbate. The validity of this parallel approach in neurochemical studies is further illustrated by concurrent and real-time monitoring of the dynamic changes of cerebral ascorbate and the single-unit electrical activity in the same brain region of cortex following ischemia/reperfusion. The strategy demonstrated here is technically simple and has an improved temporal resolution and could thus be useful for understanding the molecular basis of physiological and pathological events.

2. Experimental

2.1. Chemicals and reagents

Single-walled carbon nanotubes (SWNTs, with an average diameter less than 2 nm and a length of about 50 μm) were purchased from Shenzhen Nanoport Co. Ltd. Prior to use, SWNTs were purified by refluxing in 2.6 M nitric acid for 5 h followed by centrifugation, resuspension, filtration, and air-drying to evaporate the solvent. Sodium ascorbate was purchased from Sigma and used as received. Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) into water, and the solution pH was adjusted to 7.4. All aqueous solutions used in the experiments were prepared with Milli-Q water (18.2 MΩ, Millipore).

2.2. Preparation of IDMMEs

Carbon fiber microelectrodes (CFMEs) for *in vivo* electrochemical measurement of cerebral ascorbate were fabricated as reported previously [46]. Briefly, a glass capillary (i.d. 1.5 mm, length 100 mm) was pulled on a microelectrode puller (WD-1, Chengdu Instrument Factory, Sichuan, China) into two capillaries with the fine tip of 30–50 μm in diameter. The pulled capillary was used as the sheath of CFMEs. A single carbon fiber (7 μm in diameter, Tokai Carbon Co., Tokai, Japan) was attached to a copper wire with silver conducting paste and air-dried. Then, the fiber was carefully inserted into the capillary with the fiber exposed to the fine open end of the capillary and Cu wire exposed to the other end of the capillary. Both open ends of the capillary were sealed with epoxy resin with 1:1 ethylenediamine as the harder and the excess epoxy on the fiber was carefully removed with acetone. After that, the obtained CFMEs were first dried at 100 °C for 1 h and then exposed carbon fiber was cut to a length of 500–1000 μm under a microscopy. Prior to modification with SWNTs, the fabricated CFMEs were first sequentially sonicated in acetone, 3.0 M HNO_3, 1.0 M KOH, and Milli-Q water each for 10 s. Then, the electrodes were subject to electrochemical activation, first with potential-controlled amperometry at +2.0 V for 30 s, at −1.0 V for 10 s, and then with cyclic voltammetry in 0.5 M H_2SO_4 within a potential range from 0 to 1.0 V at a scan rate of 0.1 V s^{-1} until a stable cyclic voltammogram was obtained.

To prepare the SWNT-modified CFMEs, SWNTs was dispersed into *N*,*N*-dimethylformamide (DMF), and the mixture was sonicated to give an almost homogeneous dispersion (1 mg mL^{-1}). One drop of the dispersion was applied onto a clean glassy plate, and SWNTs were confined onto the CFMEs by carefully immersing and rolling the CFMEs into the droplet for 1 min under a microscope. Close attention should be paid not to break the carbon fibers during this process. The SWNT-modified CFMEs were then taken out from the droplet, air-dried, and thoroughly rinsed with Milli-Q water before use.

Glass microcapillary electrodes for electrophysiological recordings were drawn from fiber-filled borosilicate glass tubing with an inner diameter of 0.68 mm and an outer diameter of 1.5 mm. These glass microcapillaries were cleaned by immersing the capillaries into aqua regia solution (3:1 HCl/HNO_3) for 12 h, followed by thoroughly rinsing with Milli Q-water (*Caution, aqua regia is a strong acid and is highly corrosive; it should be handled with extreme care*). After drying, the capillaries were pulled on a vertical puller (WD-1, ChengDu Instrument Factory, China) and then filled with 3 M KCl solution.

For simultaneously monitoring of cerebral ascorbate and electrophysiological signals, IDMMEs were fabricated by carefully in parallel attaching the SWNT-modified CFME and the glass microcapillary electrode with epoxy resin with 1:1 ethylenediamine as the harder under a microscope. Close attention should be paid to keep the carbon fibers to be close to the fine tips of the glass microcapillary electrodes.

2.3. Apparatus and measurements

Electrochemical measurements were performed with a computer-controlled electrochemical analyzer (CHI650, Shanghai, China) with the as-developed IDMMEs as working electrode and a platinum wire as counter electrode. For both *in vitro* and *in vivo* electrochemical measurements, an implantable micro-sized Ag/AgCl electrode was used as reference electrode. The reference electrode was prepared by first polarizing Ag wire (diameter, 1 mm) at +0.6 V in 0.10 M hydrochloride acid for 30 min to produce an Ag/AgCl wire. The as-prepared Ag/AgCl wire was then inserted into a pulled glass capillary, in which aCSF was sucked from the fine end of the capillary and used as the inner solution for