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Rational Design of Surface/Interface Chemistry for Quantitative in Vivo Monitoring of Brain Chemistry

MEINING ZHANG, PING YU, AND LANQUN MAO*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, the Chinese Academy of Sciences, Beijing 100190, China

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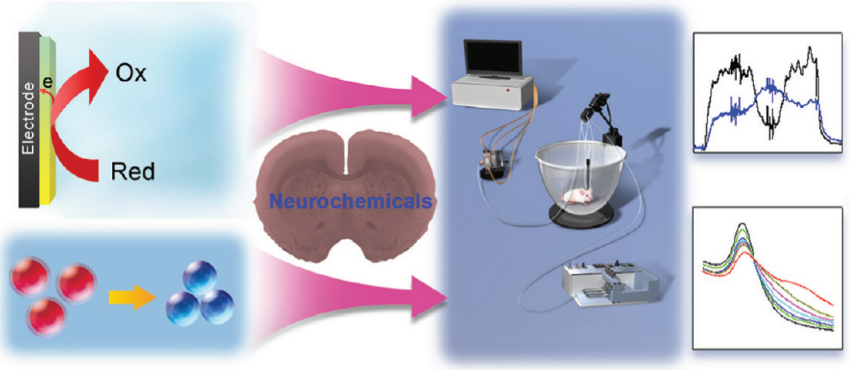

To understand the molecular basis of brain functions, researchers would like to be able to quantitatively monitor the levels of neurochemicals in the extracellular fluid in vivo. However, the chemical and physiological complexity of the central nervous system (CNS) presents challenges for the development of these analytical methods. This Account describes the rational design and careful construction of electrodes and nanoparticles with specific surface/interface chemistry for quantitative in vivo monitoring of brain chemistry.

We used the redox nature of neurochemicals at the electrode/electrolyte interface to establish a basis for monitoring specific neurochemicals. Carbon nanotubes provide an electrode/electrolyte interface for the selective oxidation of ascorbate, and we have developed both in vivo voltammetry and an online electrochemical detecting system for continuously monitoring this molecule in the CNS. Although Ca^{2+} and Mg^{2+} are involved in a number of neurochemical signaling processes, they are still difficult to detect in the CNS. These divalent cations can enhance electrocatalytic oxidation of NADH at an electrode modified with toluidine blue O. We used this property to develop online electrochemical detection systems for simultaneous measurements of Ca^{2+} and Mg^{2+} and for continuous selective monitoring of Mg^{2+} in the CNS.

We have also harnessed biological schemes for neurosensing in the brain to design other monitoring systems. By taking advantage of the distinct reaction properties of dopamine (DA), we have developed a nonoxidative mechanism for DA sensing and a system that can potentially be used for continuously sensing of DA release. Using "artificial peroxidase" (Prussian blue) to replace a natural peroxidase (horseradish peroxidase, HRP), our online system can simultaneously detect basal levels of glucose and lactate. By substituting oxidases with dehydrogenases, we have used enzyme-based biosensing schemes to develop a physiologically relevant system for detecting glucose and lactate in rat brain. Because of their unique optical properties and modifiable surfaces, gold nanoparticles (Au-NPs) have provided a platform of colorimetric assay for in vivo cerebral glucose quantification. We designed and modified the surfaces of Au-NPs and then used a sequence of reactions to produce hydroxyl radicals from glucose.

Introduction

Brain functions including physiology and pathology are basically encoded by neurotransmitters, neuromodulators, and other kinds of neurochemicals among neurons.[1] Neurochemicals relay messages between synapses on each neuron through making direct electrical contacts and converting action potential into chemical signals. Brain chemistry represents a complex system that allows brain

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to function efficiently with the use of neurochemicals and variations in brain chemistry may therefore explain how those neurochemicals affect brain functions. Therefore, analytical methods that can be used for quantitatively monitoring release and uptake of neurochemicals in the extracellular space surrounding neurons are very essential for probing brain chemistry and understanding the molecular basis of brain functions.[2−5]

In general, analytical methods capable of quantitative in vivo monitoring of the dynamic changes of neurochemicals can fall into two categories. One is in vivo sensing and biosensing that employs microsized electrochemical or optical probes directly implanted into the brain regions to real-time record the dynamic change in the levels of neurochemicals in the central nervous system (CNS).[2,6] The other is in vivo sampling-based neurochemical analysis that generally in vitro analyzes neurochemicals sampled in vivo from the brain regions.[3−5,7,8] The latter analysis is carried out through combination of in vivo sampling either with sample separation and offline detection for simultaneous multiple neurochemical analysis or with selective online detection for continuous monitoring of one/two kind(s) of neurochemicals.

These analytical methods actually complement each other in most cases. For example, as the main part of in vivo sensing and biosensing techniques, in vivo voltammetry has been well studied over the last several decades[9−17] since its establishment in 1973.[18] This kind of method has a high temporal and spatial resolution and minimal tissue damage, both benefiting from the small size of the probes employed and has been used for real-time monitoring the levels of catecholamines, serotonin, glucose, and other kinds of neurochemicals.[2,10,13,19] In the sampling-based neurochemical analysis, in vivo microdialysis has been proven particularly useful for extracellular fluid sampling because of less damage to brain tissue and ease-of-use in most investigations.[20] By combining in vivo microdialysis with sample separation and offline detection, one may simultaneously determine multiple neurochemicals that have almost the same optical and electrochemical properties. As well described in recent several excellent reviews,[4,7,21−23] these methods have been demonstrated to be particularly useful for quantification of neurotransmitters, although they require several procedures and much instrumentation for microdialysate collection and storage, pretreatment, separation, and detection. Compared with in vivo voltammetry, these methods have a lower temporal and spatial resolution, of which the temporal resolution could be greatly enhanced with the elegant strategies systematically developed by Kennedy and co-workers.[4,8,23] The other sampling-based neurochemical analysis is essentially conducted with online detecting systems established through efficient integration of in vivo microdialysis with selective detection. This kind of system directly and continuously monitors neurochemicals in the dialysates sampled from freely moving animals with a near real-time nature.[5,24−27] Compared with the existing methods, the online detecting systems emerge as new analytical tools for neurochemical quantification with simplicity in both experimental procedures and instrumentation and easy physiologist-adaptability as well as an improved temporal resolution. Moreover, these systems can be potentially developed for multiple neurochemical monitoring with combination of the technologies such as microfluidics and microelectrode array.

As demonstrated above, the merge of the techniques of microelectrode and in vivo microdialysis with the mechanisms effective for selective neurochemical detection, in principle, offers a platform for establishing in vivo voltammetry and online detecting systems for neurochemical quantification. The difficulties in developing the mechanisms for selective neurochemical detection, unfortunately, put such a pursuit into a challenge. This Account mainly focuses on the mechanistic development for selective sensing and biosensing of neurochemicals in the CNS through rational design and construction of electrodes and nanoparticles with specific surface/interface chemistry.

Carbon-Nanotube-Based Ascorbate Monitoring

Ascorbic acid (AA) is a water-soluble, hexonic sugar acid and has two dissociable protons with pK_a values of 4.2 and 11.8. At physiological pH, AA exists as a form of monovalent anion, ascorbate. Although it is not one kind of neurotransmitter directly involved in neurotransmission process, effort so far has revealed that ascorbate has multifunctional physiological functions.[28] Typically, as an electron donor, ascorbate serves as one of the most important small-molecular-weight antioxidants and free radical scavengers and, as such, is normally neuroprotective. Moreover, ascorbate acts as one kind of neuromodulator of both dopamine- and glutamate-mediated neurotransmission in the CNS.[28]

In electrochemistry, ascorbate is oxidized through a two-electron and one-proton pathway followed by an irreversible hydrolysis process to produce an electroinactive 2,3-diketogulonic acid with a quick rate constant of 1.2×10^3 s^{-1}. This reaction consequence is responsible for the irreversible electrochemical redox process of the ascorbate

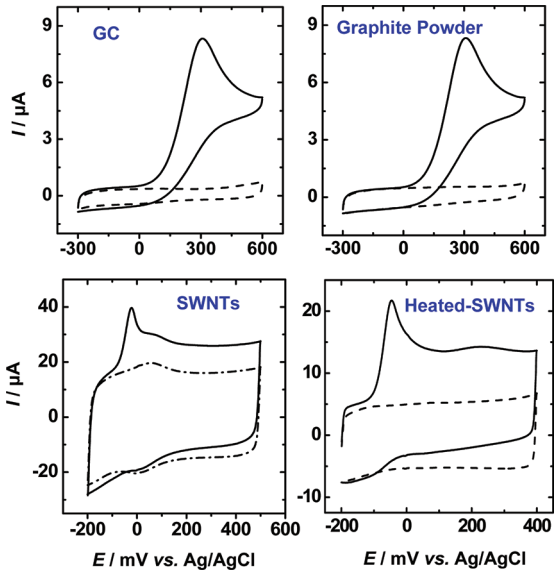

FIGURE 1. Typical cyclic voltammograms (CVs) obtained at bare GC, graphite-modified, SWNT-modified, and heat-treated SWNT-modified GC electrodes in 0.10 M phosphate buffer (pH 7.0) in the absence (dotted lines) and presence (solid lines) of 0.5 mM ascorbate. Scan rate, 50 mV s^{-1}. The SWNT-modified and heat-treated SWNT-modified electrodes were prepared by dip-coating 2 μL of 2 mg/mL SWNT or heat-treated SWNT dispersion in DMF onto the electrodes and allowing the electrodes to be air-dried to evaporate the solvent.

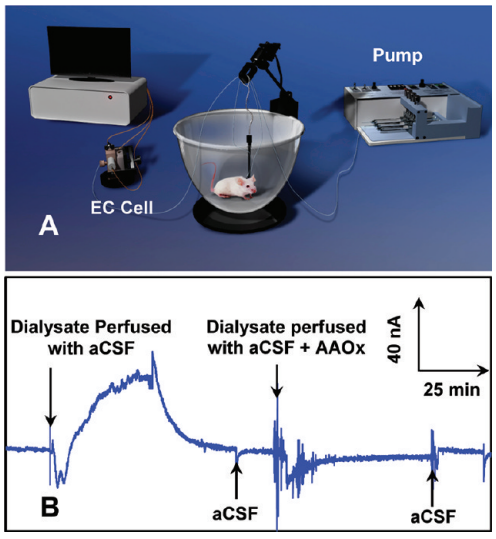

FIGURE 2. (A) Schematic diagram of the online electrochemical detecting system for continuous monitoring of ascorbate in the CNS with heat-treated SWNT-modified GC electrode as the detector. (B) Current responses recorded for the dialysate continuously sampled from the rat striatum with pure artificial cerebral spinal fluid (aCSF) and aCSF containing 47.2 U mL^{-1} of AAOx as the perfusion solutions. The electrode was polarized at +30 mV. Flow rate, 3 μL/min.

oxidation with a high overpotential at most kinds of electrodes.[29] The high overpotential essentially invalidates selective electrochemical detection of ascorbate because of the great interference from other kinds of electroactive species coexisting in the CNS.

From fundamental electrochemistry point of view, ascorbate represents one kind of inner-sphere redox species at carbon electrodes with redox property sensitive to the nature of electrode surface.[30,31] This property stimulated our investigation on the oxidation of ascorbate at carbon nanotubes (CNTs), since, as one member in the carbon family, CNTs have distinct electronic and structural properties from other kinds of carbon materials. Moreover, other groups and we have found that CNTs have excellent electrode reactivity and could be used to construct electrode/electrolyte interface for both fundamental electrochemical studies and electroanalytical applications.[32−37] After carefully comparing the ascorbate oxidation at different carbon electrodes including glassy carbon (GC), graphite, single-walled carbon nanotubes (SWNTs), and heat-treated SWNTs (Figure 1), we found that ascorbate oxidation was largely accelerated on the heat-treated SWNTs, which might be elucidated with the oxidation at the edge planelike carbon on the heat-treated SWNTs.[27] To prepare an electrode for ascorbate detection, 4 μL of 2 mg/mL SWNT dispersion in *N,N*-dimethylformamide was dip-coated onto GC electrode (6 mm diameter) and the electrode was then air-dried. The SWNT-modified electrode was used as the selective detector to develop an online detecting system for continuous selective monitoring of cerebral ascorbate by directly integrating with in vivo microdialysis (Figure 2A). In vivo selectivity test through comparing the current responses for the microdialysates sampled with pure artificial cerebrospinal fluid (aCSF) and aCSF containing ascorbate oxidase (AAOx) as the perfusion solutions revealed that SWNT-based electrochemical sensing of cerebral ascorbate was virtually interference-free (Figure 2B). Moreover, the online detecting system with SWNT-based electrochemical detector was relatively stable and reproducible for quantitative monitoring of cerebral ascorbate.

With the online detecting system, we were able to continuously monitor the dynamic changes in regional ascorbate induced by two-vessel occlusion (2-VO).[38] As displayed in Figure 3, in 1 h after cerebral ischemia induced by 2-VO, the striatum ascorbate was slowly decreased to 86 ± 6% of the basal level. While the ascorbate levels in the cortex, dorsal hippocampus, and ventral hippocampus were increased to 550 ± 168%, 168 ± 42%, and 261 ± 65%, all in relative to their own basal levels, respectively. The dynamic changes of regional ascorbate were likely associated with the richness in neuron, abundance in glutamatergic

neurotransmitter, and vulnerability to the anoxia of the different brain regions and might reflect the different extents of the anoxia depolarization, glutamate toxicity, glutamate reuptake, neural damage, and oxidative stress induced by the 2-VO cerebral ischemia. On the other hand, by using the same online detecting system, we studied the dynamic changes of the striatum ascorbate during the acute periods of different cerebral ischemia models including 2-VO ischemia and ischemia/reperfusion, left middle cerebral artery occlusion (LMCAO) ischemia, and ischemia/reperfusion, as demonstrated in Figure 4.[39] The observed different changes in the striatum ascorbate might be the synergic consequences of the neurochemical processes such as anoxia depolarization and glutamate-ascorbate hetero-exchange, cell necrosis, and overproduction of reactive oxygen species.

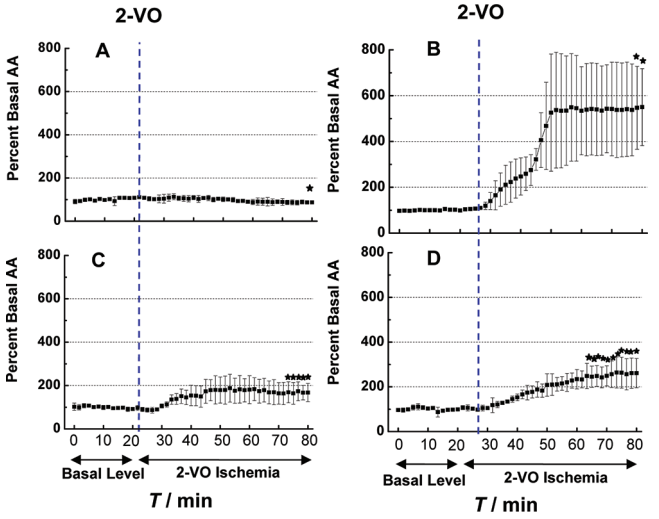

FIGURE 3. (A) Statistic regional differences of ascorbate levels before and after global cerebral ischemia induced by two-vessel occlusion (2-VO) in (A) striatum, (B) cortex, (C) dorsal hippocampus, and (D) ventral hippocampus.

The use of electrochemically favorable CNTs to construct electrode/electrolyte interface further enabled us to develop in vivo voltammetry with the CNT-modified carbon fiber microelectrode (CFME) as the in vivo probe for real-time monitoring ascorbate in rat brain, as depicted in Figure 5A.[40] The application of the in vivo voltammetric method was preliminarily demonstrated for in vivo observation of homeostatic regulation of striatum ascorbate with exogenous infusion of ascorbate into the brain (Figure 5B). Gonon et al. used electrochemically pretreated CFMEs for in vivo voltammetric monitoring cerebral ascorbate,[10] and such a method was used for physiological investigations.[16,17] The use of CNTs to construct and electrode/electrolyte interface for selective oxidation of ascorbate virtually provides a new method for in vivo monitoring cerebral ascorbate.

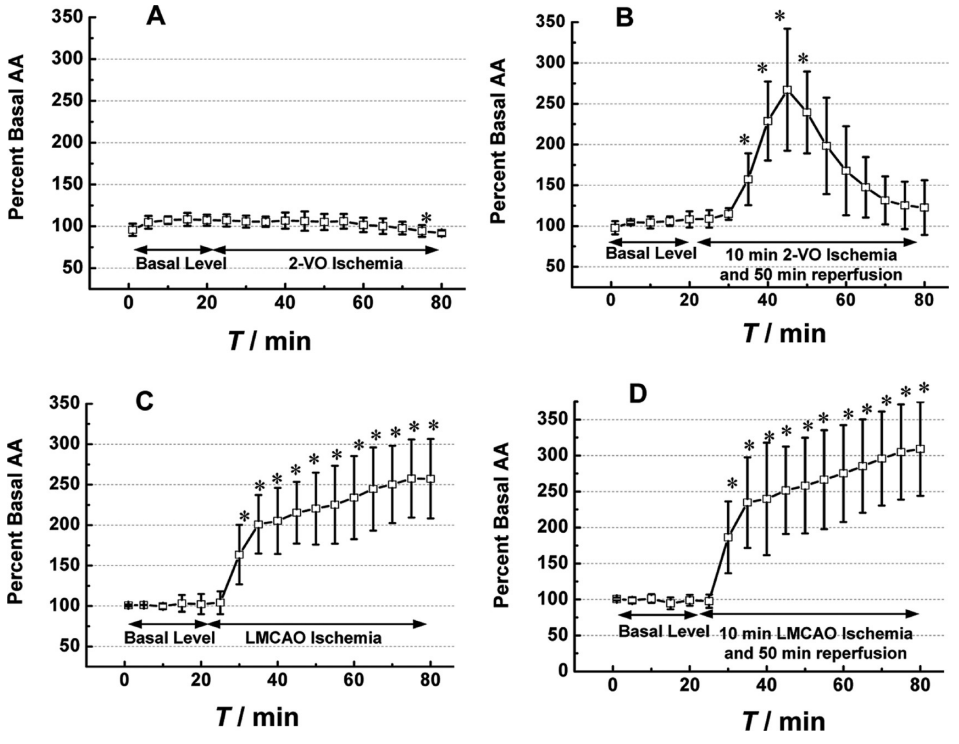

FIGURE 4. Statistical results of the level of striatum ascorbate in different global and focal ischemia/reperfusion models. (A) two-vessel occlusion (2-VO) ischemia, (B) 2-VO ischemia/reperfusion, (C) left middle cerebral artery occlusion (LMCAO) ischemia, and (D) LMCAO ischemia/reperfusion.

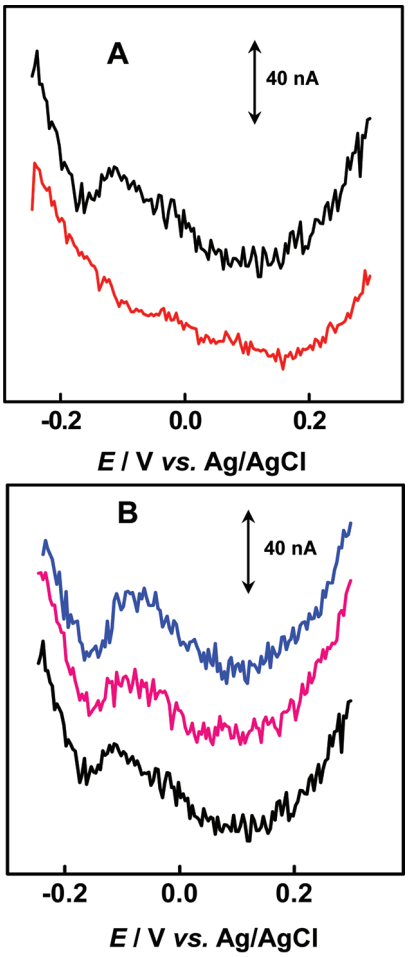

FIGURE 5. (A) Differential pulse voltammograms (DPVs) at the MWNT-modified CFME in the striatum of the anesthetized rat before (black) and after (red) exogenous infusion of AAOx (39.3 U mL^{-1}) into the striatum. (B) DPVs recorded at the MWNT-modified CFME in the striatum of the anesthetized rat after continuous infusion of 1.0 mM ascorbate for 6 min with 3 μL min^{-1} (blue) and 10 min after stopping ascorbate infusion (pink). Black curve represents DPV recorded with the MWNT-modified CFME in the striatum under normal physiological conditions. The MWNT-modified CFME was prepared by carefully immersing and rolling the CFME into one droplet of MWNT dispersion in DMF (2 mg/mL) for 1 min under a microscope. The electrodes were taken out from the droplet, air-dried, and rinsed with distilled water.

NADH Oxidation Enhancement for Monitoring of Mg^{2+} and Ca^{2+}

In the CNS, Ca^{2+} and Mg^{2+} are involved in many physiological processes. For example, Ca^{2+} is an important signal transduction element and is required for many functions including gene expression, neurotransmitter release, and synaptic transmission.[41] Meanwhile, Mg^{2+} is an important mediator and regulator of Ca^{2+} signaling and plays a classical role in defining the properties of adenosine triphosphate.[42] While some methods have been reported for in vitro measurements of Ca^{2+} and Mg^{2+}, effective measurements of both species in the CNS remain a challenge.

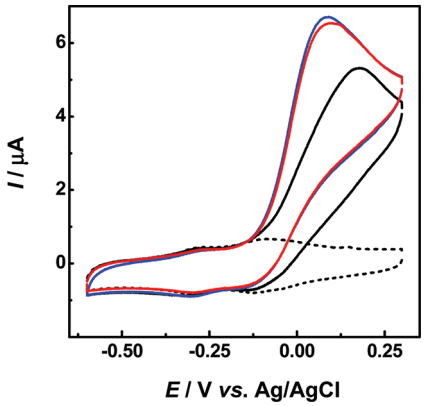

FIGURE 6. CVs at the poly(toluidine blue O)-modified GC electrode in 0.10 M Tris-HCl buffer (pH 7.0) containing 2 mM NADH in the absence (black) and presence of 20 mM Ca^{2+} (blue) or 20 mM Mg^{2+} (red). Dashed black curve represents CV obtained at the electrode in Tris-HCl buffer containing no NADH. Scan rate, 20 mV/s.

Based on the enhancement of divalent cation toward the inner-sphere electrocatalytic oxidation of β-nicotinamide adenine dinucleotide (NADH), we have recently developed an electrochemical mechanism for effective monitoring of electrochemically inactive Ca^{2+} and Mg^{2+}.[43] As depicted in Figure 6, the presence of Ca^{2+} or Mg^{2+} into solution clearly enhances the electrocatalytic oxidation of NADH at the electrode modified with toluidine blue O (TBO). The enhancement, which was elucidated with the formation of a ternary complex among TBO, NADH, and the divalent cation,[44] was used to develop an online detecting system for Ca^{2+} and Mg^{2+} by coupling with in vivo microdialysis. The system was responsive to Ca^{2+} and Mg^{2+} with a high selectivity against other kinds of neurochemicals endogenously existing in the CNS. Moreover, the system also bears a high tolerance against the changes in the chemical environments co-occurring following global ischemia including the fluctuation in levels of extracellular pH and O_2, ascorbate, and NADH.

To validate the system for online simultaneous monitoring of Ca^{2+} and Mg^{2+} in rat brain, we used ethyleneglcol-bis(2-aminoethylether) tetraacetic acid (EGTA) as the masking reagent to selectively chelate Ca^{2+} so as to differentiate the net current response for Ca^{2+} ($I_{Ca^{2+}}$) and Mg^{2+} ($I_{Mg^{2+}}$) from the total response for both Ca^{2+} and Mg^{2+} ($I_{Ca^{2+}+Mg^{2+}}$). Simultaneous measurements of Ca^{2+} and Mg^{2+} in rat brain were performed by first measuring $I_{Ca^{2+}+Mg^{2+}}$ with pure NADH solution in the Tris-HCl buffer as the perfusion solution (S2, Figure 7A), and then measuring $I_{Mg^{2+}}$ with NADH

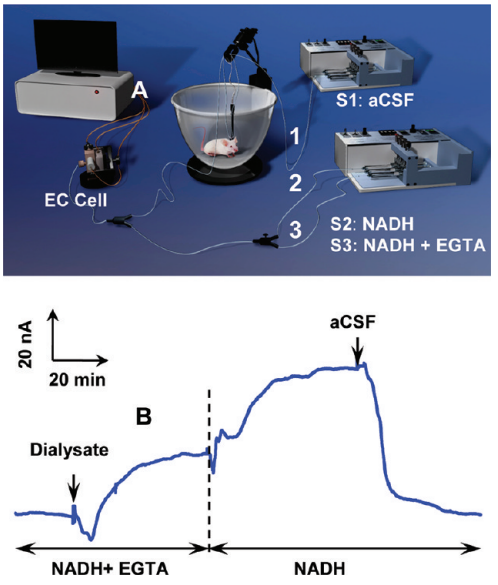

FIGURE 7. (A) Schematic illustration of the online electrochemical detecting system for simultaneous monitoring of Ca^{2+} and Mg^{2+} in the CNS. (B) Current–time responses obtained with the system for the dialysate continuously sampled from the cortex of the rat brain. The brain dialysate was continuously sampled from syringe 1 (S1) and online mixed with pure NADH solution (1 mM) in Tris-HCl buffer (pH 7.0) in syringe 2 (S2) or a mixture of NADH (1 mM) + EGTA (2 mM) in syringe 2 (S3). The perfusion rates for both pumps were 1 μL/min. The electrode was polarized at 0 V.

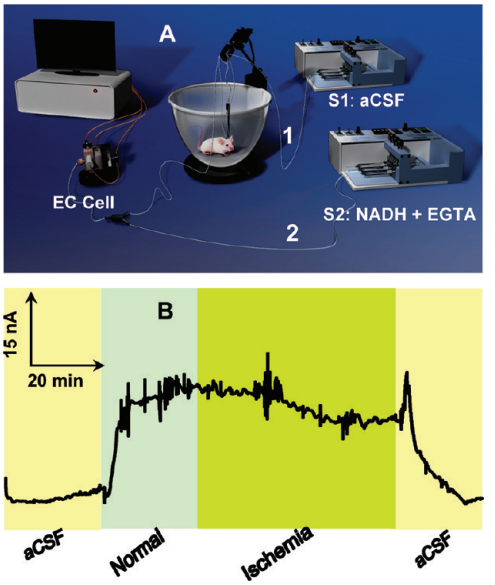

FIGURE 8. (A) Schematic illustration of the online electrochemical detecting system for continuously monitoring Mg^{2+} in the CNS. (B) Current–time responses obtained for the cortex dialysate continuously sampled from the rats following global ischemia. The brain dialysate was continuously sampled from syringe 1 (S1) and online mixed with a mixture of NADH (1 mM) + EGTA (2 mM) in syringe 2 (S2). The perfusion rates for both pumps were 1 μL/min. The electrode was polarized at 0 V.

solution in the Tris-HCl buffer containing EGTA as the perfusion solution (S3, Figure 7A). $I_{Ca^{2+}}$ was thus able to calculate by subtracting $I_{Ca^{2+}+Mg^{2+}}$ with $I_{Mg^{2+}}$. With such a system, the basal levels of Ca^{2+} and Mg^{2+} in the dialysates from the rat cortex were determined to be 285 ± 106 μM and 240 ± 92 μM, respectively, which were quite close to those determined with a traditional method, ICP-AES. The concentration of Ca^{2+} was lower than that in brain tissue slices determined with ion selective electrode, presumably because of the low sample recovery of microdialysis probe.[45]

The mechanism for electrochemical detection of Ca^{2+} and Mg^{2+} was further extended to develop an online detecting system for continuously selectively monitoring of Mg^{2+} in the CNS following global ischemia by using a Ca^{2+}-masking agent (i.e., EGTA) to eliminate the interference from Ca^{2+}, as demonstrated in Figure 8A. The level of Mg^{2+} in the cortex microdialysate was decreased by 26 ± 3%, following global brain ischemia induced by occluding the bilateral common carotid arteries of the rats (Figure 8B).

Nonoxidative Mechanism for Selective Biosensing of DA Release

As one of the most important neurotransmitters, DA has been widely accepted to be involved in many physiological processes in mammalian CNS and as a prognostic biomarker for several kinds of diseases, such as Parkinson's disease.[46] While electrochemical methods have been proven to be very valuable for in vivo and real-time measurements of DA in the CNS, the achievement of selectivity for DA measurements was relatively difficult. Such selectivity could not be achieved with the uses of enzymes or electrochemically functional nanostructures,[11,15,34] as employed for the quantification of other kinds of neurochemicals such as glucose/lactate and ascorbate.

Unlike the strategies employed for DA detection with the oxidative pathway through the oxidation of DA into dopamine-*o*-quinone, we developed a nonoxidative pathway for selective detection of DA.[47] The mechanism was based on the chemical reaction properties of DA including oxidation (1), deprotonation (2), intramolecular cyclization (3), and disproportionation reactions (4), to finally give 5,6-dihydroxyindoline quinone (Figure 9A). The formed 5,6-dihydroxyindoline quinone undergoes a two-electron and two-proton reduction pathway at a potential well separated from those for the redox processes of ascorbate and 3,4-dihydroxyphenylacetic acid (DOPAC). To establish an online detecting system for selective quantification of DA, laccase was immobilized into a fused-silica capillary to form a magnetic microreactor to catalyze the oxidation of DA into its

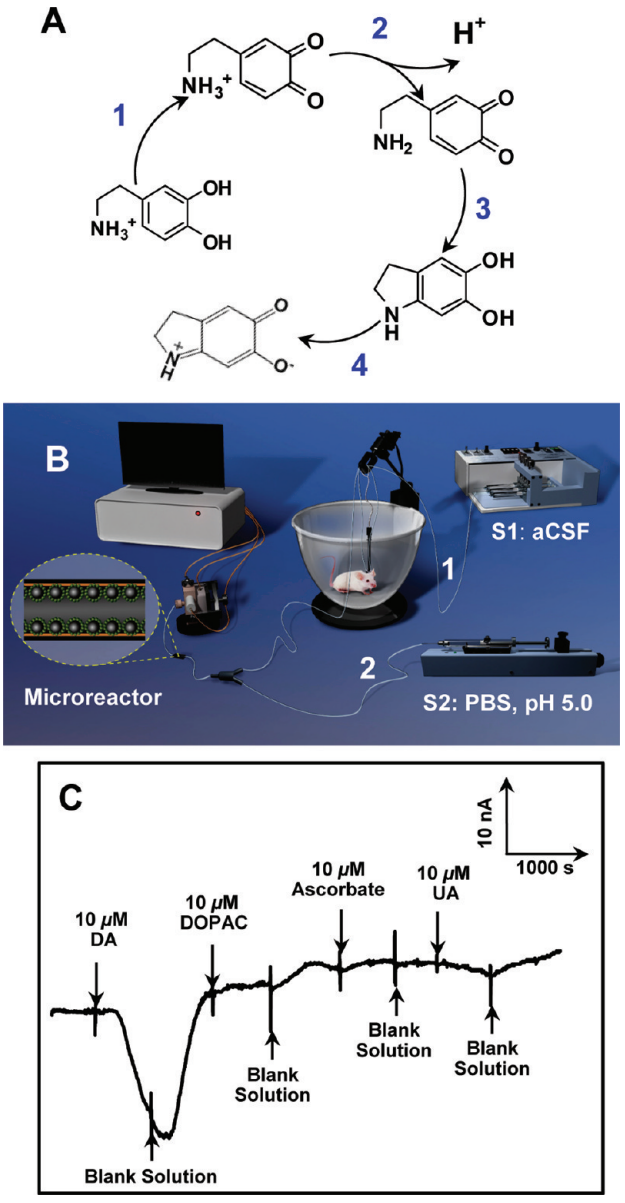

FIGURE 9. (A) Sequential reactions for conversion of DA into 5,6-dihydroxyindoline quinone. (B) Illustration of online electrochemical detecting system for DA based on a nonoxidative electrochemical approach with integration of microdialysis, laccase-immobilized magnetic microreactor, and electrochemical detector. (C) Typical amperometric response recorded for DA, DOPAC, ascorbate, and UA in the online system with bare GC electrode as the detector and with a laccase-immobilized microreactor in the upstream of the thin-layer radial flow cell. The electrode was polarized at −0.30 V vs Ag/AgCl electrode. Pure aCSF and standards in aCSF were pumped from pump 1 at 1.2 μL/min and online mixed at a T-joint with 0.10 M phosphate buffer (pH 5.0) that was pumped at 0.8 μL/min from pump 2.

quinonoid form and thereby initialize the sequential reactions to finally give 5,6-dihydroxyindoline quinone (Figure 9B).[48] The online detecting system was stable, reproducible, and interference-free from DOPAC, uric acid (UA), and ascorbate (Figure 9C) and could thus be potentially applicable for continuously recording DA release in the CNS.

Enzyme Reaction Regulation for Selective Biosensing of Glucose and Lactate

Information on the levels of glucose and lactate in the CNS is of great physiological and pathological importance because this information has been used as an indicator of brain activity and as a diagnostic tool in acute human brain insults, such as stroke and head trauma.[49] Although the pressing need of diabetes diagnosis has greatly activated intensive interests in in vitro biosensing of glucose,[50,51] the complexity of the cerebral environments, which becomes even more complicated in some pathological conditions, substantially makes the effective monitoring of glucose and lactate under pathological conditions a challenge for most kinds of the electrochemical biosensors reported so far.

Usually, electrochemical measurements of glucose and lactate could be accomplished through the utilization of enzymes such as oxidases and dehydrogenases. While various types of biosensing mechanisms have been reported for electrochemical biosensors with oxidases as the recognition elements, the biosensors based on the detection of hydrogen peroxide (H_2O_2) produced from the enzymatic reactions (i.e., first-generation biosensors) remain relatively useful for the neurochemical biosensing in the CNS. In this kind of biosensors, the acceleration of H_2O_2 reduction at a potential more positive than O_2 reduction becomes essential. However, such a process was thermodynamically difficult and was not readily achieved with the electrocatalysts frequently used.[24,25] The use of horseradish peroxidase (HRP) could accomplish an efficient electrocatalysis. Such a strategy was, unfortunately, technically complicated, requiring coimmobilization of redox mediators and HRP onto the electrode surface. More importantly, the use of HRP might result in interference from ascorbate since HRP catalyzes the reaction between ascorbate and H_2O_2.[52]

We have recently demonstrated a new mechanism by rationally designing the reaction schemes for catalyzing H_2O_2 reduction through a non-HRP pathway.[53] In this case, so-called "artificial peroxidase", Prussian blue (PB), was used as the electrocatalyst for H_2O_2 reduction because of its almost highest electrocatalytic activity toward H_2O_2 reduction among the electrocatalysts reported so far. By using PB to substitute the "natural peroxidase" (i.e., HRP), we have successfully developed an online detecting system for simultaneous monitoring glucose and lactate in the brain of living rats.

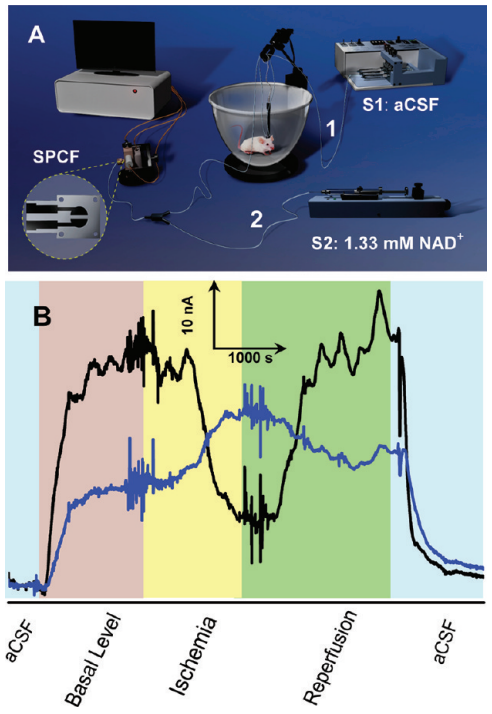

FIGURE 10. (A) Diagram of online electrochemical detecting system for simultaneous monitoring of striatum glucose and lactate following global cerebral ischemia/reperfusion. (B) Current−time response obtained for the dialysate continuously sampled from the rat striatum following the global cerebral ischemia/reperfusion. The detectors were composed of two dehydrogenase-based biosensors fabricated on dual split-disk plastic carbon film (SPCF) electrodes. The perfusion rate of aCSF with syringe 1 (S1) was 1 μL min^{-1}. The perfusion of aCSF containing 1.33 mM NAD$^+$ with syringe 2 (S2) was 3 μL min^{-1} (A). The biosensors were polarized at 0.0 V vs Ag/AgCl.

While the PB-based online detecting system could be used for quantitative measurements of the basal levels of glucose and lactate, such a system was actually O_2-dependent because of the O_2-dependent property of the oxidase-based catalytic reaction schemes. This feature unfortunately made it difficult to continuously monitor glucose and lactate under some kinds of pathological conditions, for typical example, brain ischemia/reperfusion, since brain ischemia/reperfusion normally occurs with a large fluctuation in the brain anoxia, resulting in a change in the O_2 level in the brain. To address this problem, we further designed the enzyme reaction schemes for interfacing biosensing with dehydrogenases as the biocatalysts because the dehydrogenase-based catalytic chemical oxidation of glucose and lactate is virtually O_2-independent.[54] To construct an online detecting system, glucose- and lactate-dehydrogenase-based electrochemical biosensors were fabricated on the dual split-disk plastic carbon film (SPCF) electrodes with methylene green (MG) adsorbed onto SWNTs as the

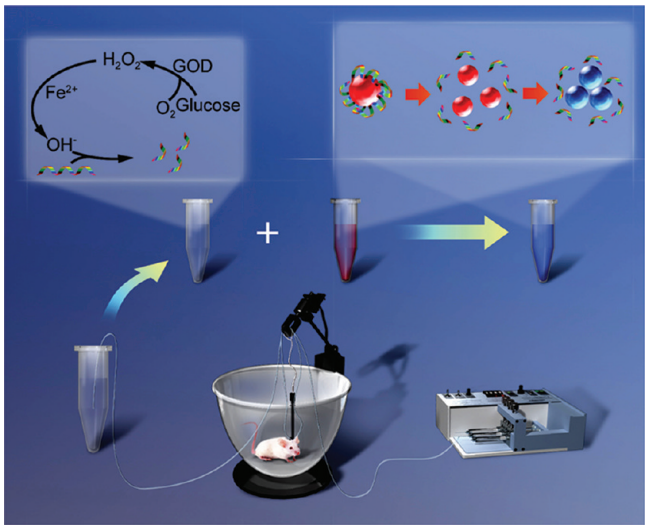

FIGURE 11. Illustration of colorimetric mechanism for simple detection of glucose in rate brain using gold nanoparticles (Au-NPs) through rational design of surface chemistry of Au-NPs and utilizing the reactions to produce hydroxyl radicals from glucose.

electrocatalyst for the oxidation of NADH (Figure 10A). aCSF containing NAD$^+$ cofactor was externally perfused (S2) and online mixed with the brain microdialysates (S1) to minimize the pH variation that occurred following the cerebral ischemia/reperfusion and to supply NAD$^+$ cofactor for the dehydrogenases, respectively. The system was found to be effective for continuously monitoring the dynamic changes in the levels of glucose and lactate in the striatum microdialysates following cerebral ischemia/reperfusion (Figure 10B) and might be used as a platform for the studies on the energy metabolism in physiological and pathological processes of ischemic stroke.

Colorimetric Biosensing of Cerebral Glucose

Combination of the unique optical properties of Au nanoparticles (Au-NPs) with excellent surface recognition ability through rationally designing the surface chemistry of Au-NPs potentially makes the Au-NP based colorimetric assays particularly attractive for neurochemical measurements.[55,56] To explore this potentiality, we have recently developed a colorimetric assay for selective biosensing of cerebral glucose by carefully designing the surface chemistry of Au-NPs and utilizing the cascade reactions including glucose-oxidase-catalyzed oxidation of glucose, Fe^{2+}-catalyzed Fenton reaction of H_2O_2, and cleavage of single-stranded DNA sequence with ·OH radical (Figure 11).[57] In addition to its good linear response toward glucose, the colorimetric method was essentially interference-free from the other kinds of neurochemicals and was effective for

simple quantification of the basal level of glucose in the microdialysate from the rat brain. The simplicity of this assay makes it very attractive for quantitative monitoring of brain chemistry in a simple fashion.

Conclusions and Outlook

Effective quantification of brain chemistry remains a hot topic in the interdisciplinary research fields ranging from chemistry, life sciences, physics, and materials science because of its great role in understanding of the molecular basis of brain functions. While some elegant methods and techniques have been demonstrated to be effective for probing brain chemistry at the molecular level, it is fair to say that there is now only limited availability of this kind of method. This is because, as mentioned above, the chemical and physiological complexity of the CNS substantially keeps almost all kinds of in vitro analytical methods far from application in continuous monitoring of molecular events in the CNS. Therefore, development of new mechanisms for reliable and durable measurements of neurochemicals remains very imperative.

In this Account, we have described how rational design and smart construction of surface/interface chemistry provides a straightforward route to accomplishing such a purpose. This route is believed to become more effective especially with the ever increasing development in molecular sciences, molecular biology, and materials science. While this Account is not a comprehensive review and only summarizes a small fraction of research activities mainly from our group on the mechanistic development for selective quantification of brain chemistry, we have demonstrated that the strategy is a powerful and general platform for developing new mechanisms for neurochemical quantification. It is reasonable to expect that, in the future studies, by rationally designing the surface/interface chemistry,[58] one may potentially extend the mechanisms for neurochemical monitoring from the current stage mainly with electrochemistry to UV−vis spectrometry, chemiluminescence, fluorescence, surface plasma resonance, mass spectrometry, quartz crystal microbalance, and so forth. This is the case, since not all kinds of neurochemicals are electrochemically active or can be changed to be electrochemically detectable through either a chemical or biochemical approach. As a result, the methodology for neurochemical quantification should be further developed in future studies not only continuously based on electrochemical mechanism but also on other mechanisms beyond electrochemistry. New mechanisms for neurochemical quantification can be presumably developed through exploitation of the chemical, optical, and electrochemical reaction features of the neurochemicals,[59] micro/nanostructures including CNTs, metal nanoparticles, and metal−organic frameworks that have been demonstrated to possess rich surface chemistry and excellent optical and electrochemical properties.[60,61] Mechanisms can also be established through exploitation of new kinds of synthetic biorecognition elements such as aptamers to construct surface/interface chemistry for specific biosensing. As one kind of artificial oligonucleotide with specific binding affinity toward a variety of targets ranging from small molecules, proteins, and even to cells, aptamers have been used as the recognition elements for biosensing applications.[62]

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BIOGRAPHICAL INFORMATION

Meining Zhang received her B.Sc. and M.Sc. in Chemistry from Shaanxi Normal University in 2001 and 2003, respectively. She received her Ph.D. degree under the supervision of Prof. Lanqun Mao in the Institute of Chemistry at the Chinese Academy of Sciences (2006). She is currently an associate professor in the Department of Chemistry at Renmin University of China, working on in vivo microdialysis and in vivo electrochemistry.

Ping Yu received her B.Sc. and M.Sc. in Chemistry from Yantai Normal University (2001) and Xiangtan University (2004), respectively. She then moved to Institute of Chemistry, the Chinese Academy of Sciences and received her Ph.D. in 2007 under the supervision of Prof. Lanqun Mao. She is currently an associate professor in Key Laboratory of Analytical Chemistry for Living Biosystems at Institute of Chemistry, the Chinese Academy of Sciences. Her ongoing work focuses on fundamental interface electrochemistry and colorimetric biosensors.

Lanqun Mao is currently a professor of Key Laboratory of Analytical Chemistry for Living Biosystems at Institute of Chemistry, the Chinese Academy of Sciences. He has been working on the interface of electrochemistry and life sciences, including bio/nanoelectrochemistry, electrocatalysis, sensors and biosensors, biofuel cells, in vivo microdialysis, and electroanalytical methodologies to understanding brain chemistry. He obtained his Ph.D. in East China Normal University in 1998 and then worked in BAS Inc. Japan as a research scientist (1998−2000) and pursued postdoctoral studies at Department of Electronic Chemistry at

Tokyo Institute of Technology (2000-2002). He is a recipient of the "Hundred Distinguished Young Scholars" from the Chinese Academy of Sciences (2002) and the "National Distinguished Young Scholars" from National Natural Science Foundation of China (2006).

FOOTNOTES

*To whom correspondence should be addressed. Telephone: 86-1-62646525. E-mail: lqmao@iccas.ac.cn.

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