2010 International Conference on Biology, Environment and Chemistry
IPCBEE vol.1 (2011) © (2011) IACSIT Press, Singapore

In Vivo Microdialysis Coupled With On-line Electrochemical Detection for Continuous Monitoring Of Ascorbic Acid in Striatum During Exhaustive Exercise

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***Abstract*—In vivo microdialysis coupled with on-line electrochemical detection was used to continuous monitor the extracellular levels of ascorbic acid in striatum of rats during exhaustion exercise. 20 male rats were used. We found that there was a continuously increase in the concentration of extracellular ascorbic acid in rats striatum during exhaustion exercise. At the beginning of exercise there were no significant changes in the concentration of extracellular ascorbic acid. During the later period of the exercise there was a significant change in the concentrations of extracellular ascorbic acid ($P<0.01$) compared with that at rest. During the recovery period, the concentration of extracellular ascorbic acid begin to fall-off but still higher than that at rest ($P<0.05$). In vivo microdialysis coupled with on-line electrochemical detection for continuous monitoring the extracellular levels of ascorbic acid is a feasible method. The extracellular levels of ascorbic acid in striatum of rat show dynamic changes during exhaustive exercise. The reason for the increase of extracellular ascorbic acid maybe concerned with a large number of free radicals generated during exercise.**

Keywords-rat; exhaustive exercise; striatum; ascorbic acid; on-line monitor

I. INTRODUCTION

Ascorbic acid (vitamin C, AA) is a vital antioxidant molecule in the brain and is differentially compartmentalized between neurons and glia, with an average intracellular concentration in neurons of 10mM, with slightly less than 1mM in glia[1]. So it shows a strong, linear dependence on neuron density (the ratio between neuron and glia). It is derived from the plasma by specific carrier systems located in the choroid plexus and in cerebral capillaries. When plasma concentrations are high, it can also enter the brain by simple diffusion[2].

Besides it's antioxidant function, it also has a number of other important functions, participating as a cofactor in several enzyme reactions[3]or regulation the energy metabolism of the central nervous system[4](CNS) and electrical activity in neurons[5]. More recent studies show that the primary mechanism for ascorbate release appears to be by heteroexchange with glutamate via the glutamate transporter, such that ascorbate release accompanies and reflects glutamate release[6].

In our previous research, we found that the striatum plays an important role in the process of centre fatigue caused by exercise. We observed the changes of the glutamate in rat's striatum during exhaustive exercise and found it significantly higher than the basal level. So how changes in ascorbic acid concentrations of rat's striatum during exhaustive exercise will be solved in this article. In the present study a new method for the determination of extracellular ascorbic acid concentration was used; this consisted of selective electrochemical detection with a thin-layer electrochemical flow cell and in vivo microdialysis for continuous monitoring of ascorbic acid in striatum of rats during exhaustive exercise.

II. MATERIALS AND METHODS

A. Animals and stereotaxic surgery

Male Wistar rats (2 months of age, weighing 200±50g at the time of surgery) purchased from Beijing Vital River Laboratories were served as subjects. The rats were housed individually with food and water available ad libitum and maintained with a 12-h light/dark cycle. After being anesthetized with Pentobarbital (50mg/kg), the rats were placed in a stereotaxic frame with the incisor-bar set at 3.3 mm below the interaural line for the flat skull position. A stainless steel guide cannula was lowered into the left striatum (P:0.2, L:3.0, H:3.2), according to standard stereotaxic procedures[7]. Three screws were placed in the skull surrounding the cannula and cemented in place with dental acrylic. The body temperature of the rats was maintained at 37℃ with a heating pad during the experiments.

B. In vivo microdialysis

In vivo microdialysis was performed by implanting microdialysis probe (BAS, MD-2204) into the striatum through the guide cannula. After equilibrating for 90 min by continuously perfusing with artificial cerebrospinal fluid (aCSF)[8] (126 mM NaCl, 2.4 mM KCl, 1.1 mM $CaCl_2$, 0.85 mM $MgCl_2$, 27.5 mM $NaHCO_3$, 0.5 mM Na_2SO_4, 0.5 mM KH_2PO_4, pH 7.0) at a flow rate of 2μL/min driven by a microinjection pump (BAS, MD-1001), the brain microdialysate was directly delivered into a thin-layer radial electrochemical flow cell through tetrafluoroethylene

hexafluoropropene (FEP) tubing for the continuous measurements of AA.

C. On-line electrochemical measurements of AA

On-line electrochemical method used for continuous monitoring of striatum AA during exhaustion exercise consists of selective electrochemical detection with a thin-layer electrochemical flow cell and in vivo microdialysis. The thin-layer electrochemical flow cell consists of a thin layer radial flow block equipped with a glassy carbon electrode as working electrode, an Ag/AgCl electrode as reference electrode and a stainless steel as counter electrode. The thickness of the gasket used was 50 mm. To achieve the specificity for AA measurements, the working electrode was modified with the heat-treated single-walled carbon nanotubes (SWNTs)[9].

D. Exercise Protocol

During the experiment, animals ran on a treadmill at a speed of 8.2m/min for 15min, 15 m/min for 15min and 20 m/min until to exhaustion[10].Electric shocks were used sparingly to motivate the animals to run. The exhaustion defined as the animal touching the electrified grid at the rear of the treadmill five times in 2min[11].

E. Statistical analysis

The data were presented as the mean±standard deviation. The AA levels in the striatum microdialysate during exercise and recovery stage were compared with the basal level (resting state) with Repeated measures ANOVA. "＊"means $P<0.05$ and"＊＊"means $P<0.01$.

III. RESULTS

A. The Current response recorded for AA standard solutions in aCSF

The current response recorded for AA standard solutions in aCSF with the on-line electrochemical method was indicated. The online electrochemical method shows a linear response toward the ascorbic acid, within the concentration range from 0.5 μM to 100 μM (C_{AA} (μM)=0.2071 I(nA)-7.1024) with a linear coefficiency of 0.9985.

B. The typical trendgraph of striatum ascorbic acid changing during the exhaustive exercise

Using in vivo microdialysis coupled with on-line electrochemical detection for continuous monitoring the extracellular levels of ascorbic acid in striatum of rats during exhaust exercise. We get the typical trendgraph of striatum extracellular ascorbic acid changing as shown in Fig.1. Y-axis for the current of ascorbic acid in single-walled carbon nanotubes modified working electrode(unit: nA). X-axis for the experiment time(unit: S).

C. Changes in extracellular ascorbic acid level in striatum during exhaustion exercise

Because of the time for exhaustive exercise has different varies widely between rats. So in order to observe the changes in extracellular ascorbic acid level in striatum during exhaustive exercise, we divided the whole experimental procedure into four stages: resting state(0-30min), exercise state Ⅰ(30-60min), exercise state Ⅱ (60min before exhaustion-exhausted), and recovery phase (exhausted-recovery for 90min). In order to exclude the error caused by the recovery rate of microdialysis probe, we set up the average concentrations of AA in the dialysate at resting state as the basal level, set the average level of all the testing value per 5 minutes as a monitoring point, the all monitoring points of AA concentrations in brain dialysate compared with the basal level, and take the percentage to reflect the concentration of striatal extracellular ascorbic acid changes during exhaustive exercise.

The changes of rat's striatum extracellular ascorbic acid level during exhaustion exercise are shown in Fig.2. The results showed that there was a continuously increase in the concentration of extracellular ascorbic acid in rats striatum during exhaustive exercise. At the beginning of exercise there was no significant change; During the later period of exercise (exercise state Ⅱ) there was a significant change in the concentration of extracellular ascorbic acid, as compared with that at rest ($P<0.01$); During the recovery phase, the concentration of extracellular ascorbic acid begin to fall-off but still higher than that at rest ($P<0.05$).

IV. DISCUSSION

Ascorbate acid is a vital antioxidant molecule in the brain. It is transported into the brain and neurons via the sodium-dependent vitamin C transporter 2 (SVCT2), which causes accumulation of AA within cells against a concentration gradient[3]. The highest concentrations of AA in the body are found in the brain and in neuroendocrine tissues[12]. AA is distinctly compartmentalized between neurons and glia, with an average intracellular concentration of 10mM in neurons and 1mM in glial cells[1]. These facts suggest an important role for AA in the brain. The application of in vivo microdialysis coupled with on-line electrochemical detection for effective and continuous measurements of extracellular ascorbic acid in striatum of rats during exhaustive exercise is very feasible. The results shows that the concentrations of extracellular ascorbic acid continues to rise during exercise and early recovery phase; during the whole recovery phase it begin to fall-off but still higher than that at rest. The reasons for this phenomenon maybe related to the generated of the free radical and the released of the glutamate (a kind of excitatory amino acids)during exhaustive exercise.

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals [13]. Free radicals (particularly oxygen- and nitrogen-centered radicals), and related reactive oxygen and nitrogen species, are generated in cells and tissues during exercise[14]. However endurance exercise of extreme duration and extreme intensity appears to generate much higher levels of free radicals that overwhelm cellular antioxidant defenses, and cause tissue damage[14]. It can "attack" important macromolecules leading to cell damage and homeostatic disruption. Free radicals also can react with the lipid bilayer of cell membranes and alter their membrane

fluidity characteristics, and also lead to release of potentially toxic byproducts and react with proteins leading to enzyme inactivation and disruption of cellular function[13]. Free radicals are elusive and hard to detect because of their highly reactive and extremely short-lived. So in order to confirm their production or clarify their function, researchers often have to search for end products or by-products of radical-induced reactions. Indeed, two of the most common markers used to detect free radical production are products of lipid peroxidation and glutathione oxidation which produced by free radical-associated damage. Hara et al. reported that swimming-exposed rats suffered significant increase in lipid peroxidation, and glutathione oxidation was also increased[15]. So the concentrations of extracellular ascorbic acid continues to rise during exercise and early recovery phase as shown in Fig.2, indicated that generated lots of free radicals during exhaustive exercise. The rising of the concentrations of extracellular ascorbic acid maybe reveal the powerful antioxidant capacity of the central nervous system.

In addition to its actions as an antioxidant, ascorbate has been shown to be a neuromodulator of both dopaminergic and glutamatergic neurotransmission, as reviewed by Grunewald[16] and Rebec[17]. Predominant localization of ascorbate in neurons is consistent with such neuromodulatory function. In addition, ascorbate is concentrated in synaptosomes[18] and is released in the CNS by depolarizing stimuli [19]. The primary mechanism for ascorbate release appears to be by heteroexchange with glutamate via the glutamate transporter, such that ascorbate release accompanies and reflects glutamate release. As glutamate is taken up after release, intracellular ascorbate is released from cells by a glutamate–ascorbate heteroexchange mechanism[20]. John X. Wilson and his colleague observed in primary cultures of rat cerebral astrocytes that glutamate increased ascorbate efflux significantly within 30 min and the results are consistent with activation by glutamate of ascorbate-permeant channels in astrocytes[20]. George V. Rebec monitored striatal glutamate transients evoked by electrical stimulation of cerebral cortex in anesthetized rats tested with varying concentrations of ascorbate by reverse dialysis and indicated that the level of extracellular ascorbate plays a critical role in regulating corticostriatal glutamate transmission[5]. In our previous research we found that the concentrations of extracellular glutamate began to rise from the beginning of the movement, arriving at topmost 60 minutes before exhausting, and then reversed to drop, arriving at lowest 30 minutes after stopping movement, and then reversed to rise again. So the concentrations of extracellular ascorbic acid increase during exercise stage Ⅰ maybe caused by the rising of the concentrations of extracellular glutamate.

ACKNOWLEDGMENT

This research was supported by Grant No. 30771050; 30971416 from the National Natural Science Foundation of China.

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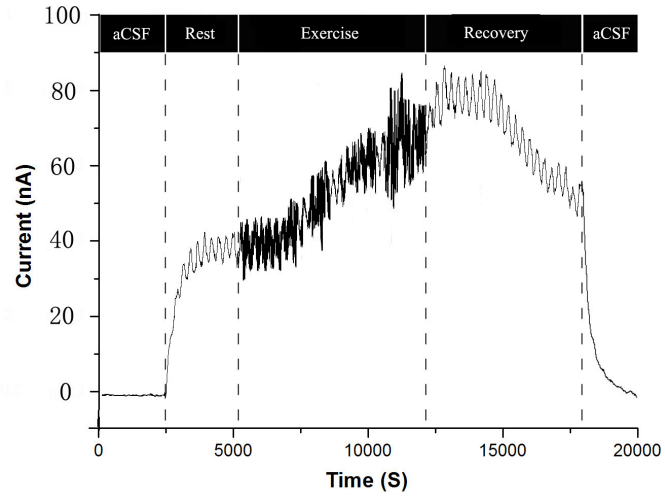

Figure 1. The typical trendgraph of striatum ascorbic acid changing during the exhaustive exercise

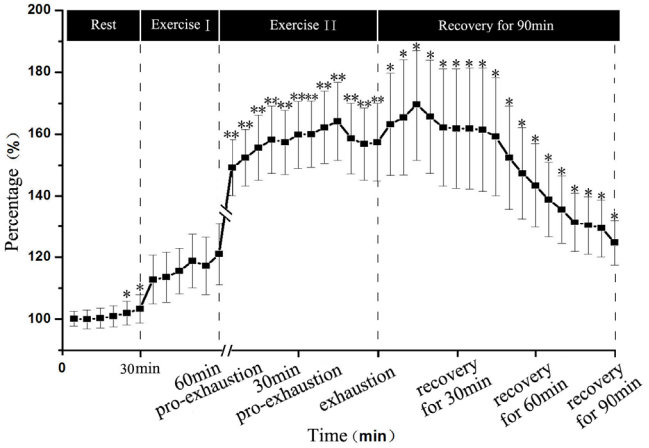

Figure 2. Changes in extracellular ascorbic acid level in striatum during exhaustive exercise.