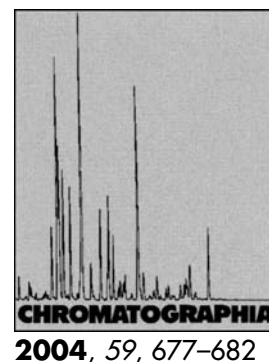


In Vivo Determination of Tetrahydrobiopterin and Monoamine Neurotransmitters in Rat Brain by Liquid Chromatography with a Nano Crystalline Mn-Doped Lead Dioxide Film Modified Electrode



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Abstract

The fabrication and application of a novel electrochemical detector (ED) with nano crystalline Mn-doped lead dioxide film chemically modified electrode (CME) for liquid chromatography (LC) were described. The Mn-doped PbO₂ film was characterized by scanning tunnel microscope. The electrochemical behaviors of tetrahydrobiopterin, monoamine neurotransmitters and their metabolites at the CME were investigated by cyclic voltammetry and differential pulse voltammetry. It was found that the CME exhibited efficiently electrocatalytic effect on the current response of the seven analytes and the linear ranges of them were over three orders of magnitude with the detection limits being 5.0×10^{-10} mol L⁻¹ for tetrahydrobiopterin, 2.5×10^{-10} mol L⁻¹ for dopamine, 2.0×10^{-10} mol L⁻¹ for norepinephrine, 5.0×10^{-10} mol L⁻¹ for serotonin, 4.0×10^{-10} mol L⁻¹ for 3,4-dihydroxyphenylacetic acid, 2.0×10^{-9} mol L⁻¹ for homovanillic acid, 1.0×10^{-9} mol L⁻¹ for 5-hydroxyindoleacetic acid. For its stability, sensitivity, convenience in preparing and long-life of activity, the Mn-doped PbO₂ electrode is therefore suitable for determination of real samples. Coupled with microdialysis sampling, the application of this method for the analysis of tetrahydrobiopterin, monoamine neurotransmitters and their metabolites in rat brain was satisfactory.

Keywords

Column liquid chromatography
Electrochemical detection
Mn-doped PbO₂ modified electrode
Monoamine neurotransmitters
Tetrahydrobiopterin

Introduction

Tetrahydrobiopterin (BH₄) is an essential cofactor of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine (DA) from tyrosine [1, 2], and also a cofactor of tryptophan hydroxylase, the rate-limiting enzyme in

the biosynthesis of serotonin (5-hydroxytryptamine;5-HT) from tryptophan [3, 4]. Early research showed that peripherally administered BH₄ do enter the brain [5] and increase the concentration of some neurotransmitters in the brain [6]. It has also been reported that the administration of BH₄ can improve

the clinical symptoms of some diseases which are caused by abnormal monoamine metabolism, such as Parkinson's disease [7] and depression [8]. The relationship between BH₄ and monoamine neurotransmitters [9] can be described as in Fig. 1.

To make even more clear the therapeutic mechanism of BH₄, it should be studied how the neurotransmitters and their metabolites change after the administration of BH₄. A method that can fast separate and sensitively determine these compounds is therefore needed.

The determination of BH₄ and monoamine neurotransmitters has been carried out by using ultraviolet [10], fluorescence [11], chemical luminescence [12], voltammetry [13] and mass spectrometry [14]. However, because the monoamine neurotransmitters and BH₄ are absent of spectrum group, the applications of fluorometric or other photometric methods for the determination of them are limited.

High performance liquid chromatography (HPLC) is the most commonly employed method for analyzing complex samples in vivo and in vitro. HPLC combined with electrochemical detection (ED)[15–18] has many advantages in the analysis of biological samples, such as simplicity, rapidity, low cost and high sensitivity. Moreover, for HPLC-ED, the sensitivity and selectivity can be further enhanced by using chemically modified electrode (CME).

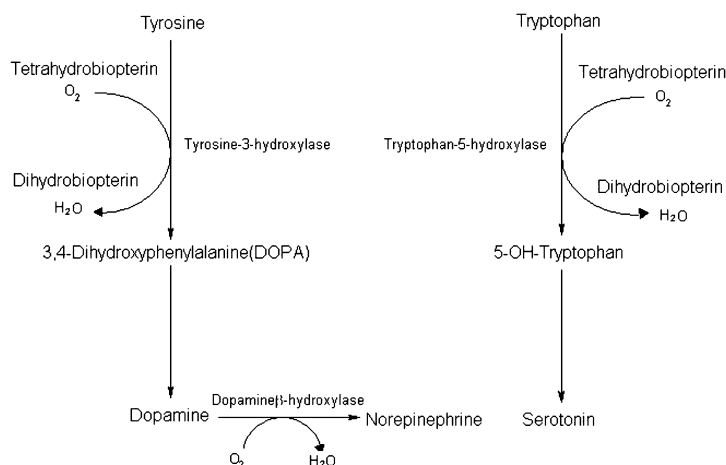


Fig. 1. The role of BH₄ in the pathway of monoamine neurotransmitters

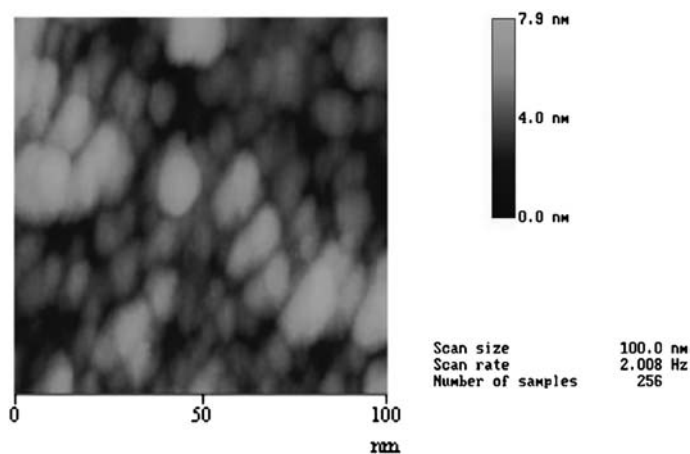


Fig. 2. STM micrograph of Mn-doped PbO₂ films

(STM). Both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) showed that the Mn-doped PbO₂ CME catalysed BH₄ and monoamine neurotransmitters. When the CME was used as an amperometric detector for LC, it had very stable electrochemical behaviors and high sensitivity. Coupled with microdialysis sampling, the employment of this method for the simultaneous determination of monoamine neurotransmitters, their metabolites and BH₄ in rat brain was satisfactory.

Experimental

Chemicals and Reagents

Pb(NO₃)₂ and Mn(NO₃)₂ were of analytical grade and purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Tetrahydrobiopterin (BH₄), Dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were of analytical grade and purchased from Sigma (St. Louis, USA). All buffer components were of analytical grade or better quality. Double-distilled deionized water was used for all solutions. Prior to use, all solutions were degassed with purified nitrogen for 20 min.

Apparatus

Electrochemical experiments were performed with a CHI832 electrochemical system (CHI Co. USA). The three-electrode system consisted of a Mn-doped PbO₂ modified electrode or a glassy carbon electrode as working electrode, a Ag/AgCl electrode as reference electrode and a gold wire electrode as counter electrode.

Liquid chromatographic experiments were conducted on a model 510 pump and a U6K injector (Waters Assoc., USA). The injection volume was 20 μL. The column was Luna 5u C₁₈ (4.6 mm×25 cm) (Phenomenex Company, USA) directly attached to a C₁₈ precolumn (1.0 mm×15 mm). The detector consisted of a laboratory-made thin-layer cell and a CH-I Potentiostat (Jiangsu Electrochemical Instruments Works, Jiangsu, China). The working electrode was a Mn-doped PbO₂ CME or a glassy carbon electrode. The mobile phase was 0.2 mol L⁻¹ phosphate buffer

There has been much interest in a kind of CME which is based on the electrodeposition of metal or metal oxide on insert surface, such as zinc electrodeposited on nickel disk electrode [19], silver electrodeposited on carbon paste electrode [20], lead dioxide electrodeposited on Au electrode [21] and on glassy carbon electrode [22]. These CMEs provide a good physical dispersion of the catalytic centers and lead to highly active electrode surfaces. Because of their efficient electrocatalysis, they have been widely applied for the determination of many important analytes with low concentrations in various samples [23, 24]. Among these CMEs, electrodes modified with lead dioxide have some advantages as analytical sensor. Firstly, lead dioxide can be easily obtained by anodic deposition from the low valence lead ions solution. Secondly, the electrocatalytic activity of PbO₂ electrode, as well as their stability, can often

be considerably enhanced by the incorporation of some foreign ions added to the electrodeposition solution and formed "doped PbO₂ electrode", such as Fe-doped PbO₂ electrode [25], As-doped PbO₂ electrode [26] and Bi-doped PbO₂ electrode [27]. If the PbO₂ particles are in nano scale, the electrode will show high catalytic activity towards analytes and the application will be highly extended. To our best knowledge, there is no report on the biological analysis by using the nano scale PbO₂ modified electrode as HPLC electrochemical detector.

In this paper, a novel nano crystalline Mn-doped PbO₂ film modified electrode was fabricated by simultaneous electrodeposition of Pb(II) and Mn(II) at a glassy carbon electrode and was successfully used as an amperometric detector in biological samples analysis. The Mn-doped PbO₂ film was characterized by scanning tunnel microscopy

solution (pH = 5.1, 5% methanol) which was delivered at a constant flow rate of 1.0 mL min⁻¹. All the experiments were performed at room temperature (25 °C) and the pH value was calibrated with pH meter (Horiba Ltd., Japan).

Microdialysis was accomplished by using a CMA 101 microdialysis pump (CMA Microdialysis AB, Stockholm, Sweden) and a CMA 12 microdialysis probe (dialysis length, 3 mm; diameter, 0.24 mm, BAS Co., Japan). The probe was perfused with the Ringer solution (147 mmol L⁻¹ Na⁺, 4.0 mmol L⁻¹ K⁺, 2.2 mmol L⁻¹ Ca²⁺) at a flow rate of 1.0 μL min⁻¹.

Scanning tunnel micrograph was obtained by using AJ-I scanning tunnel microscope (Shanghai Aijian Nanoscience Development Co.Ltd., China).

Preparation of Mn-doped PbO₂ Modified Electrode

Prior to preparation of the Mn-doped PbO₂ CME, the surface of the glassy carbon was polished with 0.3 μm alumina on a polishing micro-cloth and rinsed with deionized water. Subsequently, the electrode was ultrasonicated thoroughly with acetone, NaOH solution (1.0 mol L⁻¹), HNO₃ (1.0 mol L⁻¹) and doubly distilled water.

After pretreating, the glassy carbon electrode was cycled in 1.0 mol L⁻¹ HClO₄ solution with the potential range from 0.00 V to 1.80 V at 100 mV s⁻¹ until the reproducible background was obtained. Then, the Mn-doped PbO₂ film was deposited in 1.0 mol L⁻¹ HClO₄ solution containing 1.0 mmol L⁻¹ Pb(NO₃)₂ and 1.0 mmol L⁻¹ Mn(NO₃)₂ with the cycling potential from 1.20 V to 1.80 V at 100 mV s⁻¹ for 10 cycles. After electrodeposition, the Mn-doped PbO₂ CME was rinsed with doubly distilled water and then stored in buffer solution.

In Vivo Microdialysis Experiment

Animal care was in accordance with the Guide for the Care and Use of Laboratory Animal (NIH Publication No. 86-23, 1985, Bethesda, MD, USA). Experiments were performed on Sprague-Dawley (SD) male rats weighing 250–300 g. The animals were anesthetized by 20% urethane (1.25 g kg⁻¹) and

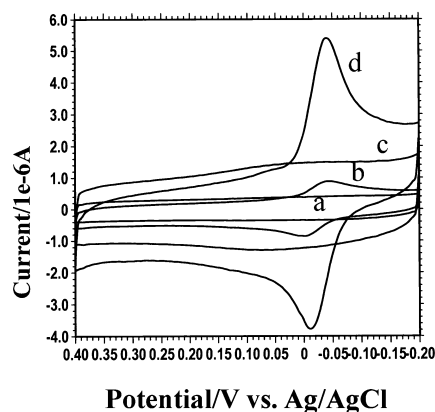


Fig. 3. Cyclic voltammograms of BH₄ at bare glassy carbon electrode and Mn-doped PbO₂ electrode: (a) bare glassy carbon electrode in the blank solution; (b) bare glassy carbon electrode in 1.0 × 10⁻⁶ mol L⁻¹ BH₄ solution; (c) Mn-doped PbO₂ electrode in the blank solution; (d) Mn-doped PbO₂ electrode in 1.0 × 10⁻⁶ mol L⁻¹ BH₄ solution. Electrolyte: 0.20 mol L⁻¹ phosphate solution (pH = 5.1). Scan rate: 0.1 V s⁻¹

placed in a stereotaxic frame. A small hole was drilled into the skull and then the microdialysis probe was implanted into the striatum. It was stereotaxically implanted into the striatum at coordinates 0.2 mm posterior to the bregma, 4.0 mm lateral from midline and the middle of the probe 4.5 mm below the dura [28]. The microdialysis samples were collected at the microdialysis rate of 1.0 μL min⁻¹ every 30 min and analyzed on the Mn-doped PbO₂ CME. The dialysates samples collected over the first 60 min were discarded to allow recovery from the acute effects of the surgical procedure.

Results and Discussions

Physical Characterization of Mn-Doped PbO₂ Film

The STM micrographs of undoped PbO₂ (A) and Mn-Doped PbO₂ (B) films are shown in Fig. 2. It can be found that the crystallites of Mn-doped PbO₂ film was regular and well oriented and of a nano scale (20–30nm), which helped improving the electrocatalytic activity of the electrode.

Electrocatalysis of Tetrahydrobiopterin at Mn-Doped PbO₂ CME

Figure 3 shows the cyclic voltammograms of BH₄ at the bare glassy carbon electrode and at the Mn-doped PbO₂

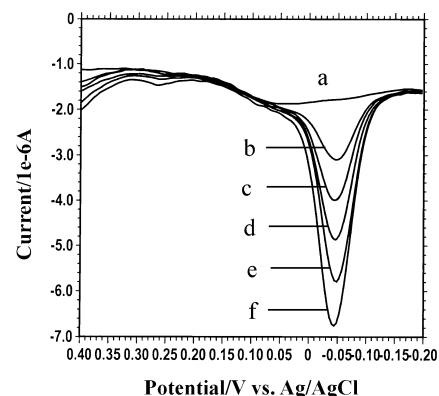


Fig. 4. Differential pulse voltammograms of BH₄ at the Mn-doped PbO₂ electrode: (a) blank solution; (b) 5 × 10⁻⁷ mol L⁻¹; (c) 1.0 × 10⁻⁶ mol L⁻¹; (d) 1.5 × 10⁻⁶ mol L⁻¹; (e) 2.0 × 10⁻⁶ mol L⁻¹; (f) 2.5 × 10⁻⁶ mol L⁻¹ BH₄. Electrolyte: 0.20 mol L⁻¹ phosphate solution (pH = 5.1). Scan rate: 0.1 V s⁻¹

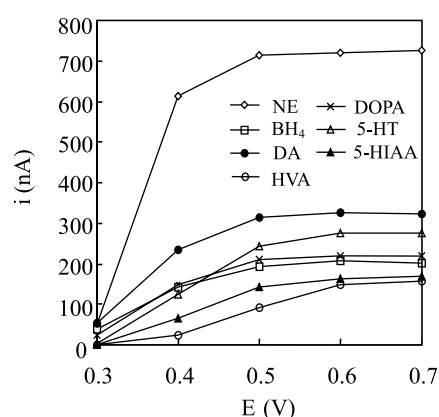


Fig. 5. Hydrodynamic voltammograms of a mixture of 1.0 × 10⁻⁶ mol L⁻¹ BH₄, NE, DA, DOPAC, HVA, 5-HT and 5-HIAA at the Mn-doped PbO₂ electrode in LC-ED. Column was Luna 5u C₁₈ (4.6 mm × 25 cm) and directly attached to a C₁₈ precolumn (1.0 mm × 15 mm); injection volume: 20 μL; mobile phase: 0.20 mol L⁻¹ phosphate solution, pH = 5.1, 5% methanol; flow rate: 1.0 mL min⁻¹

CME in 0.2 mol L⁻¹ phosphate buffer solution (pH = 5.1), respectively. BH₄ had a pair of redox peaks in the buffer solution at the electrodes. Compared with those at the bare electrode, the peak currents of BH₄ were increased significantly and the reversibility was also improved at the Mn-doped PbO₂ CME. This was due to the nano crystalline structure of the Mn-doped PbO₂ film, which could increase the effective area of the electrode and make better electrocatalysis.

The DPV responses of BH₄ with different concentrations at the Mn-doped PbO₂ CME are shown in Fig. 4. It can be observed that the oxidation currents had

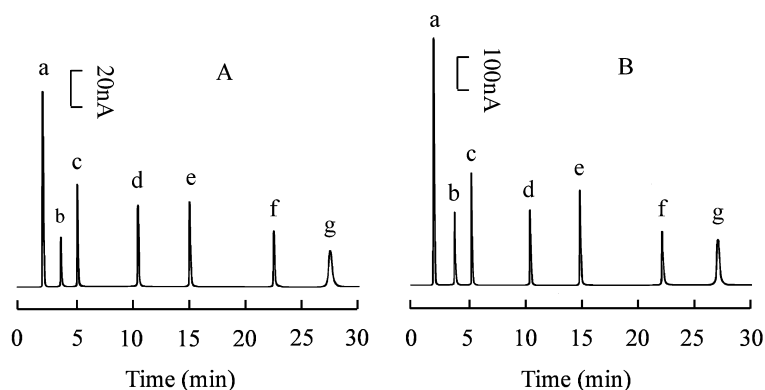


Fig. 6. Chromatograms of $1.0 \times 10^{-6} \text{ mol L}^{-1}$: **a** = NE; **b** = BH_4 ; **c** = DA; **d** = DOPAC; **e** = 5-HT; **f** = 5-HIAA; **g** = HVA at the bare glassy carbon electrode and the Mn-doped PbO_2 electrode. Applied potential: +0.60V; other conditions were as in Fig. 5

good linear correlation with BH_4 concentrations. Besides the electrochemical behaviors of BH_4 , the responses of monoamine neurotransmitters and their metabolites at the glassy carbon electrode and at the Mn-doped PbO_2 CME were also investigated. It was found that the Mn-doped PbO_2 CME had a good catalysis function to all these analytes and when it was used as electrochemical detector, it could enhance the sensitivity greatly.

Liquid Chromatography Conditions

Hydrodynamic Voltammetry

Hydrodynamic voltammetry is a method suitable for selecting the appropriate potential applied on HPLC-ED. In this study, standard solutions of the seven analytes were repetitively injected while the HPLC-ED operating potential was increased from 0.00 V–0.70 V in 0.10 V increments. Figure 5 are the hydrodynamic voltammograms of the mixed sample containing $1.0 \times 10^{-6} \text{ mol L}^{-1}$ BH_4 , DA, NE, 5-HT, DOPAC, HVA and 5-HIAA on the Mn-doped PbO_2 CME. When the applied potential was higher than +0.20 V, the current responses of BH_4 , NE increased apparently and reached the high value at +0.50 V without obvious increase after that and the responses of DA, DOPAC and 5-HT increased significantly after +0.30 V and reached the maximum value at +0.60 V. When the potential increased continuously, the current responses of 5-HIAA and HVA increased continuously but slowed down after +0.60 V. Moreover, if the applied potential was higher than +0.60 V, the baseline current became high and other substances may respond

on the electrode. In order to obtain the best selectivity and signal/noise ratio, +0.60 V was chosen as the optimum detection potential.

pH Effect of the Mobile Phase

The pH value of the mobile phase has great effects on the separation and determination of BH_4 and the monoamine neurotransmitters in LC-ED. At $\text{pH} < 4.0$, the peaks of DA and BH_4 had little overlap although the retention time of the analytes was short. At $\text{pH} > 6.0$, the 5-HT, 5-HIAA and HVA showed tailing peaks and baseline separation could not be reached. Furthermore, the mobile phase pH could influence the amperometric responses of the analytes. The experiments showed that all the analytes had sensitive responses between $\text{pH} 4.5$ and $\text{pH} 5.5$. Finally, the mobile phase $\text{pH} 5.1$ was selected in the experiment as the optimum pH value.

Liquid Chromatograms on Glassy Carbon Electrode and Mn-Doped PbO_2 CME

Fig. 6 shows the current responses of BH_4 , monoamine neurotransmitters and their metabolites at the glassy carbon electrode and at the Mn-doped PbO_2 CME in HPLC-ED. It was found that the current responses of these analytes were much larger at the Mn-doped PbO_2 CME. It was due to PbO_2 particles in nano scale on its surface that enlarged the surface of efficient electrocatalysis.

Linearity, Detection Limits and Repeatability

To determine the linearity for BH_4 , DA, NE, 5-HT, DOPAC, HVA and 5-HIAA

on the Mn-doped PbO_2 CME in LC-ED, a series of mixed standard solutions of these analytes ranging from $1.0 \times 10^{-10} \text{ mol L}^{-1}$ to $1.0 \times 10^{-5} \text{ mol L}^{-1}$ were tested. The ranges of the linear relationships observed between currents and concentrations were over three orders of magnitudes and all the coefficients were more than 0.999. The detection limits of these analytes at the CME were investigated and the data were shown in Table 1.

The repeatability was estimated by making repetitive injection (eight times) of a standard solution containing a $1.0 \mu\text{mol L}^{-1}$ mixture for the seven analytes under the same condition every 30 min. The relative standard deviation of the peak currents were found to be 1.3% for BH_4 , 1.2% for DA, 1.4% for NE, 1.5% for 5-HT, 1.8% for DOPAC, 2.1% for HVA and 1.8% for 5-HIAA.

In addition, the long-term stability of the Mn-doped PbO_2 CME stored at 4°C in PBS was examined by checking its relative activity periodically. No apparent change in the current responses of these analytes was observed over 3 weeks. The results indicated that the Mn-doped PbO_2 CME had good stability and reproducibility when it was used as the HPLC detector for monoamine neurotransmitters, their metabolites and BH_4 .

Relative Recovery of Microdialysis Sampling Experiment

The relative recovery of microdialysis probe for an analyte is equal to the ratio of its concentration in the microdialysate, i.e. the outlet from the probe (C_{out}) to its concentration in the medium surrounding the probe (C_{in}). Therefore the recovery is $C_{\text{out}}/C_{\text{in}}$. The relative recovery of a microdialysis probe is affected by the microdialysis rate. At low microdialysis rates, there will be high relative recoveries. However, during the long collection time with low microdialysis rates, endogenous compounds may be also dialyzed and BH_4 , which is not quite stable, may be partly oxidised. In this paper, several microdialysis rates were investigated for a proper relative recovery. In order to detect the analytes rapidly and accurately, $1.0 \mu\text{L min}^{-1}$ was selected as the optimum microdialysis rate. The microdialysis relative recovery was found to be 40.2% for BH_4 , 41.8% for DA, 43.3%

Table 1. Analytical data of the seven analytes by LC-ED at the Mn-doped PbO₂ CME^a

Analytes	Regression equation ^b	Correlation Coefficient	Range (mol L ⁻¹)	Detection limit (mol L ⁻¹) ^c
BH ₄	Y = 0.2073X - 0.0364	0.9996	1.0 × 10 ⁻⁹ ~ 1.0 × 10 ⁻⁵	5.0 × 10 ⁻¹⁰
DA	Y = 0.3184X + 0.0052	0.9995	5.0 × 10 ⁻¹⁰ ~ 1.0 × 10 ⁻⁵	2.5 × 10 ⁻¹⁰
DOPAC	Y = 0.2158X + 0.0397	0.9995	1.0 × 10 ⁻⁹ ~ 1.0 × 10 ⁻⁵	4.0 × 10 ⁻¹⁰
HVA	Y = 0.1483X - 0.0453	0.9993	5.0 × 10 ⁻⁹ ~ 1.0 × 10 ⁻⁵	2.0 × 10 ⁻⁹
NE	Y = 0.7215X - 0.0229	0.9997	5.0 × 10 ⁻¹⁰ ~ 1.0 × 10 ⁻⁵	2.0 × 10 ⁻¹⁰
5-HT	Y = 0.2758X - 0.0497	0.9995	1.0 × 10 ⁻⁹ ~ 1.0 × 10 ⁻⁵	5.0 × 10 ⁻¹⁰
5-HIAA	Y = 0.1637X - 0.0230	0.9994	2.0 × 10 ⁻⁹ ~ 1.0 × 10 ⁻⁵	1.0 × 10 ⁻⁹

^a LC-ED conditions as in Fig.6.

^b Where Y and X represent the peak current (nA) and the concentration of the analytes (nmol L⁻¹), respectively.

^c The detection limits of the analytes were investigated using a signal-to-noise ratio of 3 (S/N = 3).

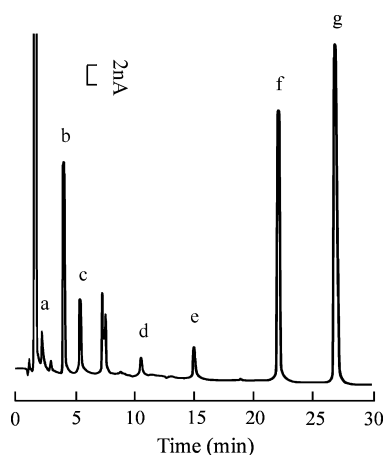


Fig. 7. Chromatogram of a = NE; b = BH₄; c = DA; d = DOPAC; e = 5-HT; f = 5-HIAA; g = HVA at the striatum of the rat brain 240 min after the administration of BH₄. Applied potential: +0.60 V; 20 mg L⁻¹ EDTA and 150 mg L⁻¹ dithioerythritol (DTE) in the mobile phase and other conditions were as in Fig. 5

for NE, 39.6% for 5-HT, 40.9% for DOPAC, 30.7% for HVA and 35.1% for 5-HIAA under the conditions.

In Vivo Experiment

To explore the effect of administration of BH₄ on the neurotransmitters levels in the rat brain, we injected BH₄ at a dose of 50 mg kg⁻¹ body weight intraperitoneally 60 min after commencement of dialysate-collection. Figure 7 shows the chromatogram of BH₄ and monoamine neurotransmitters and their metabolites 240 min after BH₄ administration. Fig. 8 shows the time course of BH₄ effects on the concentration of analytes at the striatum of rat brain. Following BH₄ administration, the concentration of BH₄ immediately increased to a high level (232.5 ± 6.4 nmol L⁻¹) and then dropped continually until 150 min later the decline slowed down. The changes of DA was similar to those of BH₄. The DA con-

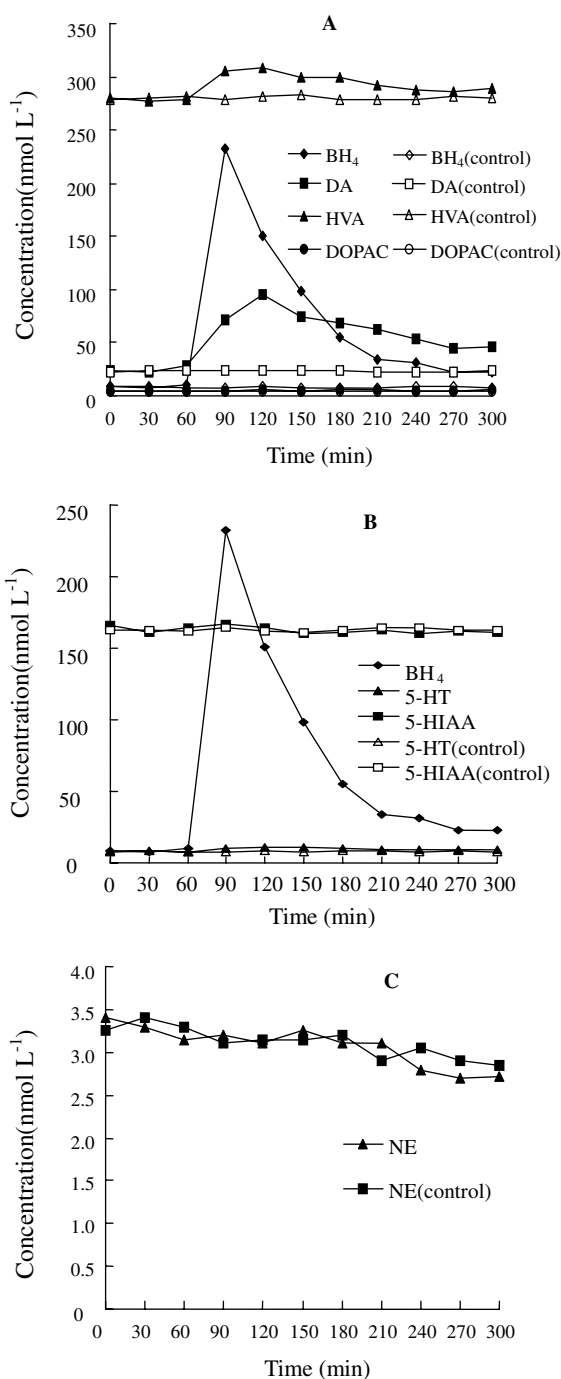


Fig. 8. The time course of BH₄ effects on the concentration of analytes at the striatum of rat brain. BH₄ effects on (A) DA and its metabolites; (B) 5-HT and its metabolites; (C) NE. n = 4 in BH₄ administrated group and n = 3 in control group

centration reached the highest value ($96.3 \pm 2.7 \text{ nmol L}^{-1}$) 60 min after injection and then descended slowly. At the end of microdialysis, DA still had a 2-fold concentration compared with the control group. 5-HT also obtained the maximum concentration 60 min after injection and at the end of microdialysis, the concentration increased about 30% comparing with control. As the metabolites, HVA was observed to have the similar change of concentration to DA. These results accorded with early reports [16, 29, 30]. However, we did not observe obvious increase in DOPAC, 5-HIAA and NE, one possibility is that the concentration of DA or 5-HT has not a decisive influence on them and there are other factors affecting the metabolism of DA to DOPAC and of 5-HT to 5-HIAA. Further studies are proceeding now in our laboratory.

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References

- Brennan AR, Kaufman S (1964) *Biochem Biophys Res Commun* 17:177–183
- Nagatsu T, Levitt M, Udenfriend S (1964) *J Biol Chem* 239:2910–2917
- Friedman P, Kappelman A, Kaufman S (1972) *J Biol Chem* 247:4165–4173
- Kato T, Oka K, Nagatsu T (1980) *Biochim Biophys Acta* 612:226–232
- Kapatos G, Kaufman S (1981) *Science* 212:955–956
- Sumi-Ichinose C, Urano F, Kuroda R, Ohye T, Kojima M, Tazawa M (2001) *J Biol Chem* 276:41150–41160
- Dissing IC, Güttler F, Pakkenberg H (1989) *Acta Neurol Scand* 79:493–499
- Koch R, Güttler F, Blau N (2002) *Molec Genet Metab* 75:284–286
- Blau N, Thny B, Spada M (1996) *Turk J Pediatr* 38:19–35
- Parsons LH, Kerr TM, Weiss F (1998) *J Chromatogr B* 709:35–45
- Smadja C, Le Potier I, Chaminade P, Jacquot C, Trouvin JH, Taverna M (2003) *Chromatographia* 58:79–85
- Barnett NW, Hindson BJ, Lewis SW (1998) *Anal Chim Acta* 362:131–139
- Downard AJ, Roddick AD, Bond AM (1995) *Anal Chim Acta* 317:303–310
- Dahlgren RL, Page J S, Sweedler JV (1999) *Anal Chim Acta* 400:13–26
- Candito M, Nagatsu T, Chambon P, Chatel M (1994) *J Chromatogr B* 657:61–66
- Ishida Y, Todaka K, Kuwahara I, Hashiguchi H, Ishizuka Y (1998) *Neurosci Lett* 253:45–48
- Wolf WA, Anastasiadis PZ, Kuhn DM, Levine RA (1990) *Neurochem Int* 16:335–340
- Giese RW (2003) *J Chromatogr A* 1000:401–412
- Lin YF, Sun IW (1999) *Electrochim Acta* 44:2771–2777
- González-García MB, Costa-García A (2000) *Biosensors Bioelectron* 15:663–670
- Velichenko AB, Gerenko TV, Danilov FI (1995) *Electrochim Acta* 40:2803–2807
- Gonzía JG, Sáez V, Iniesta J, Montiel V, Aldaz A (2002) *Electrochem Commun* 4:370–373
- Hayes ET, Bellingham BK, Galal A (1996) *Electrochim Acta* 41:337–344
- Zhu M, Liu M, Shi GY, Xu F, Jin LT, Jin JY (2002) *Anal Chim Acta* 455:199–206
- Velichenko AB, Amadelli R, Zucchini GL, Girenko DV, Danilov FI (2000) *Electrochim Acta* 45:4341–4350
- Yeo IH, Johnson DC (1987) *J Electrochem Soc* 134:1973–1977
- Popovic ND, Cox JA, Johnson DC (1998) *J Electroanal Chem* 445:153–160
- Bao XM, Su QY, (1991) *The Stereotaxic Atlas of the Rat Brain*, Renmin Weisheng Publisher, China
- Koshimura K, Miwa S, Lee K, Fujiwara M, Watanabe Y (1990) *J Neurochem* 54:1391–1397
- Mataga N, Imamura K, Watanabe Y (1991) *Brain Research* 551:64–71