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Semi-differential voltammetry with carbon fiber electrodes for *in vivo* determination of monoamine metabolites and ascorbic acid in rat corpus striatum[1]

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ABSTRACT Carbon fiber electrodes combined with semi-differential voltammetry were used to determine endogenous monoamine metabolites and ascorbic acid (AA). These electrodes treated by a new electrochemical procedure (30 μA for 30 s, then -2 V for 10 s) showed a significant improvement on the sensitivity and selectivity. In the rat corpus striatum, these electrodes allowed a distinct separation and continuous detection of AA, 3, 4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) simultaneously. The normal striatal levels of AA, DOPAC, and 5-HIAA were found to be 168.2 $\pm$ 19.5, 21.4 $\pm$ 3.9, and 3.3 $\pm$ 0.5 μmol $\cdot$ L^{-1}. Our method is credible for detecting the changes of monoamine metabolite contents induced by pharmacologic manipulations.

KEY WORDS reserpine; haloperidol; electrochemistry; corpus striatum; ascorbic acid; 3,4-dihydroxyphenylacetic acid; hydroxyindoleacetic acid

In vivo voltammetry for direct and continuous measurement of monoamines and their metabolites in the central nervous system is an exciting research area [1]. It is now realized that the voltammetric signals recorded from brain probably reflect the oxidation of several substances at a given potential. The high levels of ascorbic acid and uric acid (UA) in brain would interfere with the measurement of monoamines and their metabolites[2,3]. Gonon's group first reported that pretreated carbon fiber electrodes combined with differential pulse voltammetry successfully separated AA and catechols, their pretreatment mainly consisted of triangular-wave-form potential (0 to +3 V, 70 Hz for 30 s, -0.8 V for 5 s, then +1.5 V for 5 s) [4,5]. However, we failed to obtain clear separation of AA and catechols when carbon fiber electrodes combined with semi-differential voltammetry, although our electrodes were treated according to above same procedure, these facts suggested that the optimal treatment conditions be rather critical for different voltammetric techniques. Here we explored a new electrochemistry procedure to treat carbon fiber electrodes for the purpose of distinguishing AA from catechols and indoleamines by semi-differential voltammetry instead of pulse differential voltammetry. Specific details for electrode preparation and treatment and voltammetric technology were presented. The effects of reserpine and haloperidol on the striatal levels of AA, DOPAC, and 5-HIAA were observed.

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MATERIALS AND METHODS

Materials Dopamine (DA), DOPAC, 5-hydroxytryptamine (5-HT), 5-HIAA, and AA were purchased from Sigma and dissolved in $HClO_4$ 1 mol $\cdot$ L^{-1} solution prior to use. The supporting electrolyte was phosphate buffer solution (PBS). Reserpine and haloperidol were obtained from the Pharmaceutical Factory of Shanghai Medical University and the Shanghai Haipu Pharmaceutical Factory, respectively.

Electrode preparation The working electrodes were prepared with carbon fiber (8 μm o d, Serofim Genevilliers, France), graphite powder (1 μm particles, Ultra Carbon, Shanghai Graphite Factory), glass tube (1 mm i d, GG−17), and 502 resin (Zhejiang Jiao Jiang Chemical Factory). A glass tube was drawn using the pipet puller (DKI 700C) to obtain a tip diameter of 1.5 μm. A carbon fiber (50 mm in length) was threaded into the capillary (40 mm in length) until it was blocked by the tip. The capillary was cut at the level where the fiber was blocked. A suitable length of carbon fiber was exposed, then the mixture of the graphite powder and resin was inverted to the capillary. Electric contact was made by pushing a copper wire down the neck of the capillary. Prior to pretreatment the exposed fiber was cut to a length of 1 mm under a microscope. The reference electrode was constituted by a micro Ag / AgCl electrode. A platinum wire *in vitro* or stainless steel screw *in vivo* was used as the auxiliary electrode.

Electrochemical treatment Carbon fiber electrodes were immersed in PBS solution and the electrochemical treatment was performed by using JH2C−Semiconduct Constant Potential Instrument. The first treatment was applied constant current 30 μA to working electrode for 30 s. Then, the second was applied reverse constant potential −2 V for 10 s.

***In vivo* voltammetric technique** Sprague−Dawley rats (250± *s* 25 g) were anesthetized with chloral hydrate (400 mg · kg^{-1}) and fixed in a David Kopf Stereotaxic Instrument. Holes were drilled through the skull and the dura mater. The working electrode was implanted in the corpus striatum (AP 1.8, L 2.5, DV−5.1 mm). Reference and auxiliary electrodes were placed in burr holes to make contact with the brain surface. The 3 electrodes were connected to a CV−37 Voltammograph(BAS), the output of which was displayed on an X−Y recorder. The parameters used *in vivo* were as follows: linear potenial sweep from −200 mV to +600 mV and scan rate 60 mV · s^{-1}. Before implantation, all carbon fiber electrodes were calibrated in the mixed solutions contained AA 100 μmol · L^{-1}, DOPAC 20 μmol · L^{-1}, and 5−HIAA 5 μmol · L^{-1} to mimic brain extracellular conditions. Drugs or saline was injected when the peak height was stable during the 60 min period of the control. The results were expressed as the real extracellular concentration of AA, DOPAC, and 5−HIAA. Electrode placement was verified histologically at the end of the experiment.

RESULTS

In vitro In the voltammograms obtained from the mixed solution of AA (100 μmol · L^{-1}), DOPAC(10 μmol · L^{-1}), and 5−HIAA (2.5 μmol · L^{-1}), 3 well−defined peaks exhibited at the potentials of 20, 180, and 350 mV, respectively (Fig 1a). A loss of sensitivity of electrodes (10−30%) appeared after the *in vivo* experiment (Fig 1b). The amplitude of this loss depended on the quality of the surgery and the duration of *in vivo* detecting. The calibration curves of AA, DOPAC, and 5−HIAA *in vitro* were shown in Fig 2. All data were the mean of the peak heights measured from 10 electrodes. In order to mimic the *in vivo* situation, the starting PBS solution for AA calibration curve contained DOPAC 20 μmol · L^{-1} + 5−HIAA 5 μmol · L^{-1} and similarly, AA 200 μmol · L^{-1} + 5−HIAA 5 μmol · L^{-1} for DOPAC calibration curve, and AA 200 μmol · L^{-1} + DOPAC 20 μmol · L^{-1} for 5−HIAA calibration curve.

In vivo In the voltammograms obtained from the corpus striatum of anesthetized rats, the 3 stable well−defined peaks were recorded, which lasted 6−8 h (Fig 1c). These peak positions were consistent with those we

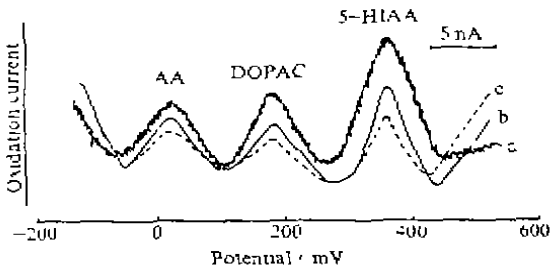

Fig 1. Voltammograms recorded *in vitro* from phosphate buffer solution (PBS) containing AA 100 μmol · L^{-1}, DOPAC 10 μmol · L^{-1}, and 5-HIAA 2.5 μmol · L^{-1} before (a) and after (b) *in vivo* implantation and *in vivo* from the rat corpus striatum (c).

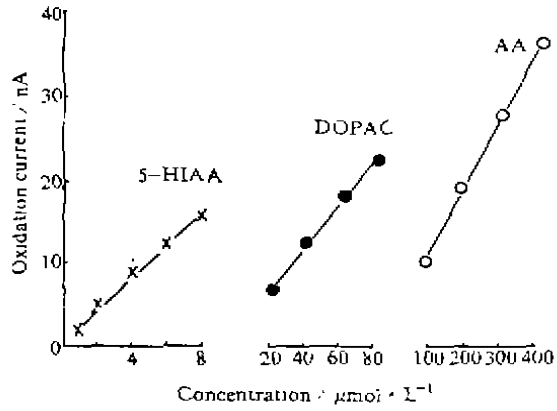

Fig 2. Calibration curves of AA, DOPAC, and 5-HIAA *in vitro*.

obtained *in vitro*. Although DA and DOPAC oxidized at 180 mV and 5-HT, 5-HIAA, and UA oxidized at 350 mV, the extracellular levels of DA (10 nmol · L^{-1}) and 5-HT (5 nmol · L^{-1}) in the rat corpus striatum were below detection limits. The contribution of UA to peak 3 was negligible on carbon fiber electrodes. The striatal concentrations of AA, DOPAC, and 5-HIAA in the extracellular space were found to be 168.0 ± 19.0, 21.4 ± 3.9, and 3.3 ± 0.5 μmol · L^{-1}, respectively. Reserpine (5 mg · kg^{-1}, ip) induced marked increases of DOPAC (214.7%) and 5-HIAA (103.5%). While haloperidol (1 mg · kg^{-1}, ip) only caused an increase of DOPAC level (62.1%). 5-HIAA remained unchanged (Fig 3). Both drugs produced no effects on the AA level.

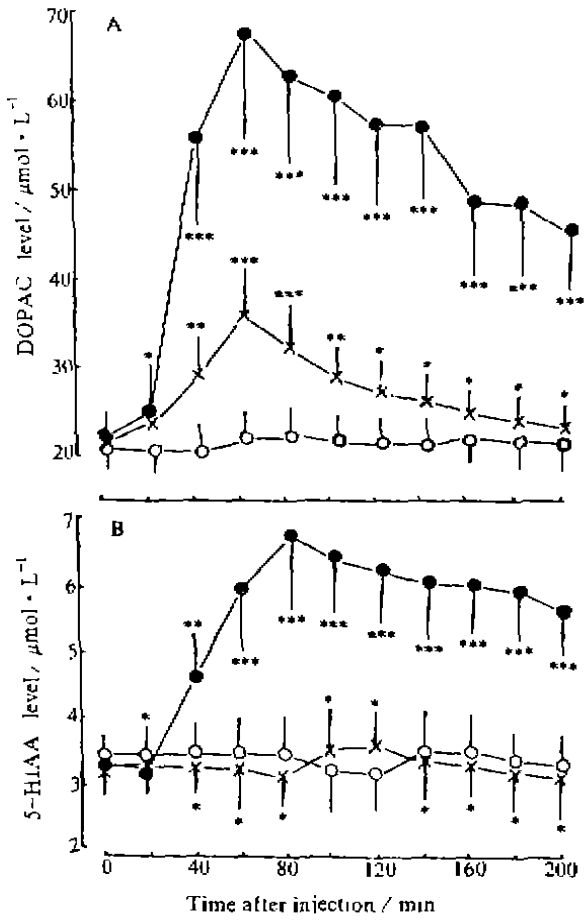

Fig 3. Effects of reserpine (●, 5 mg · kg^{-1}) and haloperidol (×, 1 mg · kg^{-1}) on striatal level of DOPAC and 5-HIAA. $n=6$. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control (○).

DISCUSSION

Here we first reported that carbon fiber electrodes combined with semi-differential voltammetry instead of differential pulse voltammetry were used as chemical sensors of monoamine metabolites. In order to meet the requirements of semi-differential voltammetric detection, we explored a novel

electrochemical procedure (30 μA for 30 s, and −2 V for 10 s) to treat carbon fiber electrodes, which was more simple and convennient than that reported by Gonon. Our treated electrodes offered a selectively measure of DOPAC in the presence of AA. Furthermore, the superiority of our method allowed a distinct separation and continuous detection of DOPAC and 5−HIAA *in vivo* simultaneously[6,7]. However, carbon fiber electrodes treated by applying a triangular−wave−form potential could not detect 5−HIAA, although these electrodes could separate AA from catechols[8]. In addition, carbon paste electrodes treated by either nafion or sterate only eliminated the interference of AA to catechols rather than detecting AA itself[9,10]. A substantial improvement of the methodology provided an effective analytical approach for selective measuring monoamine metabolites and explorating the roles of AA in brain functions.

It was known that reserpine rapidly depletes the DA and 5−HT in the corpus striatum which oxidized by MAO to form DOPAC and 5−HIAA and therefore a marked increase in the height of DOPAC and 5−HIAA voltammetric peaks was recorded. Haloperidol could block dopaminergic receptor and enhance the DA turnover in the rat corpus striatum and an increase of DOPAC level was also exhibited. The results demonstrate that the changes of monoamine metabolite contents induced by pharma−cological manipulations could be monitored *in vivo* by this technique.

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半微分伏安法结合碳纤维电极体内测定大鼠纹状体中单胺代谢物及抗坏血酸

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提要 本文首次报道用碳纤维电极结合半微分伏安法在体内测定单胺代谢物及抗坏血酸. 这些电极经一新的电化学程序处理，选择性和灵敏性显著提高. 在大鼠纹状体能完全分辨和同时连续测定 AA，DOPAC 和 5−HIAA，三者浓度分别为 168.2，21.4 和 3.3 μmol · L^{-1}. 用此方法测定由药物引起的单胺代谢物含量变化结果可靠.

关键词 利血平；氟哌啶醇；电化学；纹状体；抗坏血酸；3,4−二羟苯乙酸；5−羟吲哚乙酸