All Sciences Proceedings

5th International Conference on Applied Engineering and Natural Sciences

July 10-12, 2023 : Konya, Turkey

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A Homemade Electrochemical System for Local Analysis of Dopamine Released by PC12 Cells

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Abstract **–** Dopamine (DA) is a catecholamine-based neurotransmitter that functions in synaptic communication between cells. Imbalance in the amount of DA can cause many diseases ranging from Parkinson's disease to depression. In this study, local electrochemical measurement of DA released by PC12 cells after chemical stimulation was performed. First, the production of needle-tipped carbon-fiber microelectrodes was carried out by micropulling method. Carbon-fiber electrodes are frequently used for effective and high sensitivity detection of dopamine. Afterwards, cell clusters of PC12 cells were obtained for measurement of DA release. For the measurement process, a microcontroller unit integrated to a inverted microscope was used to precisely position the needle-tipped electrodes. DA release was successfully measured as a result of chemical stimulation with K^+ ions. Basically, a homemade system was developed to analyze DA release triggered by chemical or physical stimuli.

Keywords – Carbon-Fiber Electrode, Electrochemical Measurement, Dopamine, Local Analysis, PC12 cells

I. INTRODUCTION

Dopamine (DA) is a naturally produced catecholamine neurotransmitter in the body. DA provides neurotransmission in the central nervous system and plays an important role in the regulation and control of movements, motivation and cognitive development. Abnormalities in DA levels in the brain cause psychiatric problems. Increased DA level indicates cardiotoxicity leading to rapid heartbeat, hypertension, heart failure, and drug addiction [1]. However, a decrease in DA levels can cause stress, Parkinson's disease, schizophrenia, Alzheimer's disease and depression [2]. Therefore, it is necessary to monitor DA levels in vivo and in real time to understand its physiological role and biological functions [3]. In many studies, rat pheochromocytoma cells known as PC12 are used as a mature dopaminergic neuron (source of dopamine). PC12 cells are preferred mainly because of their extreme versatility, ease of culture, and large amount of background information on proliferation and differentiation. PC12 cells synthesize and store DA and are a suitable model for in vitro catecholaminergic neurotoxicity studies. Dopaminergic drugs, which function to increase DA levels, are frequently used in the treatment of many diseases caused by low DA levels. An in vitro model system has the potential to contribute to the testing and development of these types of drugs in different aspects such as cost and convenience. Within the scope of this study, a homemade system was developed to analyze DA release triggered by chemical or physical stimuli. For this purpose, a needle-tipped carbon-fiber microelectrode with a very low detection limit was produced and DA release from an adherent cluster of PC12 cells via stimulation with K^+ ions was successfully measured with the help of a microcontroller integrated to an inverted microscope.

II. MATERIALS AND METHOD

A. *Fabrication and Characterization of Carbon-fiber Microelectrodes*

First, the carbon-fibers were cut to the appropriate length and connected to copper wires with the help of a conductive silver paste. The silver paste was heated to 120°C (10-15 minutes) for hardening and thus to make the connection permanent. The carbon-

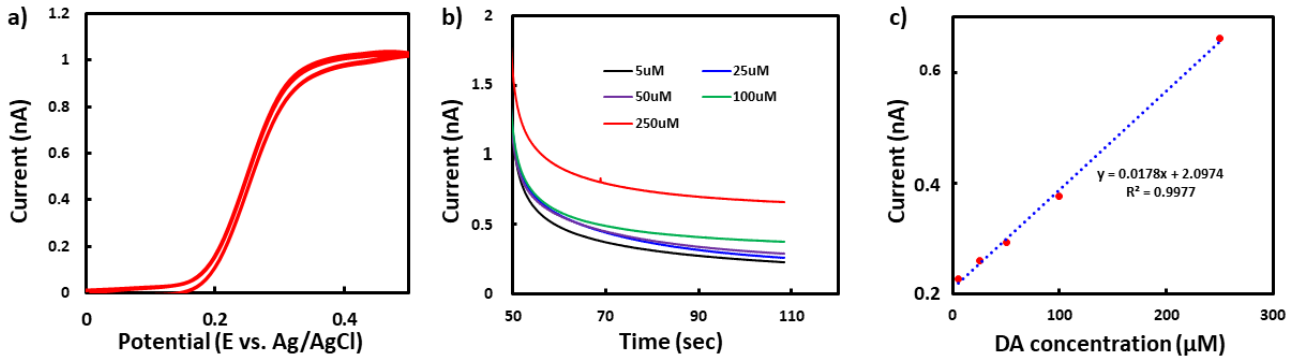

Fig. 1. CV curve of a carbon-fiber microelectrode obtained in 1 mM $FcCH_2OH$ (a). Chronoamperometric curves obtained in PBS containing varying concentrations of DA (b) along with a calibration curve (c).

fibers were then placed in the glass capillary tubes the help of copper wires, which were then fixed to the glass capillary tubes with a heat shrinking tube to ensure electrical connection and fixation of the carbon-fibers. The prepared glass tubes were pulled with the help of a micro puller (Narishige PC-10) to cover and insulate carbon-fibers with glass [4-7]. In the final stage, the tip of the microelectrodes was ground with a microgrinder to obtain microdisk electrodes. The electrochemical behavior of the electrodes was checked with CV curves obtained by sweeping the potential between 0 and +0.5 V (vs Ag/AgCl) at a scan rate of 50 mV/s in 1 mM $FcCH_2OH$ containing PBS. The performance of carbon-fiber electrodes in the measurement of DA was tested by chronoamperometry [8]. Briefly, current values in DA solutions prepared at different concentrations (5, 25, 50, 100 and 250 µM) were recorded at +0.3 V (vs. Ag/AgCl) for 60 sec and the final current value was used for analysis.

B. *Real-time Electrochemical DA measurement from PC12 cells*

PC12 cells were cultured in collagen-coated flasks containing RPMI 1640 (10% DHS + 1% FBS + 1% L-Glutamine + 0.1% Gentamicin) medium in an incubator at 37 °C with 5% CO2. PC12 cell clusters required for measurement were obtained in 35 mm collagen-coated petri dishes. DA measurement was carried out in PBS by means of a microcontroller integrated to an inverted microscope. Briefly, the carbon-fiber microelectrode was placed 40 µm away from the cells through the integrated system and the measurement was carried out by chronoamperometry at +0.3 V. After the current value reached a steady state, DA was released from PC12 cells through chemical stimulation with K+ ions and the amount of DA released was measured in real time through the electrodes.

III. RESULTS & DISCUSSION

The microelectrodes were successfully fabricated using the present strategy. The CV curves obtained in $FcCH_2OH$ (Fig. 1a) clearly show that the electrode displayed an excellent microelectrode behavior. As can be seen in Fig. 1b, the DA

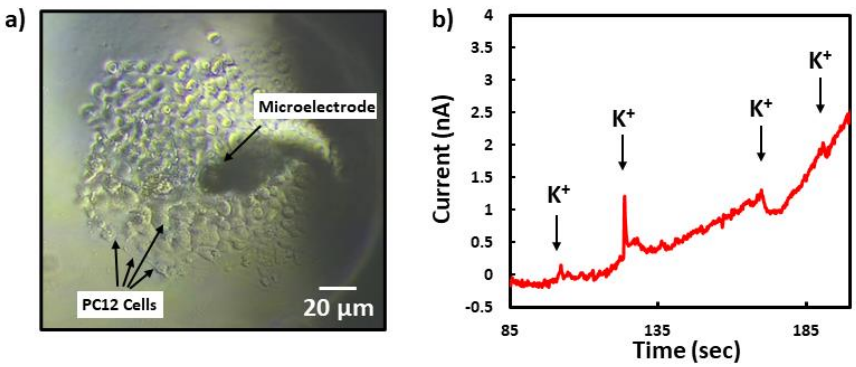

Fig. 2. An optical image of a carbon-fiber microelectrode closely positioned near a PC12 cell cluster (a). Chronoamperometric curve showing the real-time change in DA oxidation current as a result of chemical stimulation of the PC12 cell cluster with K^+ ions (b).

oxidation current level increased proportionally with increasing DA level. The current value of each concentration level at 60 sec was used to obtain a calibration curve to demonstrate the relationship between DA and electrical current signal. Fig. 1c clearly shows that the relationship was linear in the range of 5 to 250 μM of DA with an R^2 value of 0.998. The limit of detection value (LOD) of the carbon-fiber microelectrode was calculated as 1.03 μM using the formula of LOD = 3.3 σ/slope. Next, the homemade system integrating a microcontroller unit to an inverted microscope was used for real-time measurement of DA release from PC12 cells after chemical stimulation with K+ ions. The microcontroller system can be automatically controlled in x-, y- an z-axis with a precision close to 1 μm. In order to measure DA release from PC12 cell clusters in PBS filled collagen coated petri dishes, the carbon-fiber microelectrodes were approached to cells slowly with the help of the microcontroller unit. The motion of the electrode was monitored through the camera of the inverted microscope throughout the whole process. The electrode was positioned approximately 20 μm away from the cells. Prior to stimulation of DA release from PC12 cell clusters, the electrical current was allowed to reach a steady state and then K^+ ions were injected four times into the petri dish through a micropipette. As can be seen in Fig. 2, the DA oxidation current increased every time the cells were chemical stimulated.

IV. CONCLUSION

Dopaminergic drugs, which function to increase DA levels, are frequently used in the treatment of many diseases caused by low DA levels. An in vitro model system has the potential to contribute to the testing and development of these types of drugs in different aspects such as cost and convenience. Within the scope of this study, a model homemade system was developed to measure DA release in real-time from PC12 cell clusters exposed to a chemical stimulus. Carbon-fiber microelectrodes showed high sensitivity in the measurement of DA and thus sensitively measured DA released from PC12 cell clusters. The home-made system can be useful in investigating the effects of dopaminergic drugs.

ACKNOWLEDGMENT

The study was supported by supported by the Scientific and Technical Research Council of Turkey (TUBITAK 2209-a).

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