Simultaneous Monitoring of Electrochemical and Unitary Neuronal Activities by a Single Carbon Fiber Microelectrode

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Abstract—Simultaneous measurements of electrochemical and electrophysiological changes in the substantia nigra pars compacta of the rat were tried with a single carbon fiber microelectrode. Electrochemical detection of catecholamines was done by differential pulse voltammetry. The effects of haloperidol on the level of catecholamines in extracellular spaces and on dopaminergic neuronal discharges were investigated. Haloperidol induced an increase in unitary discharges parallel to the elevation of the catecholamine level. With this technique, direct information can be obtained on the relationship between released catecholamines and unitary neuronal activity.

A recently developed method for simultaneous measurements of unitary neuronal discharges and released catecholamines in the rat brain is based on a combination of in vivo voltammetry and electrophysiological techniques (1). This procedure employs a pair of electrodes, a tungsten wire for unitary activity and a carbon fiber for detecting released catecholamine. The electrode tips are placed 500 μm apart from each other. Here we report a method for detecting these two kinds of biological signals with the same carbon fiber electrode. Furthermore, the usefulness of this method for biochemical and electrophysiological studies was confirmed by investigating the effects of haloperidol on the release of catecholamines in extracellular spaces and on dopaminergic neuronal discharges in the substantia nigra pars compacta (SNC) of rats. Thus, our method can provide direct information on the relationship between released catecholamines and unitary activity. Part of the data has been reported elsewhere (2).

Male Wistar rats (Shizuoka Laboratory Center), weighing 240–260 g, were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted on a stereotaxic instrument (Type B-2, Takahashi Shoten, Japan). A microelectrode for electrochemical detection and recording of unitary discharges was fabricated from a carbon fiber by a reported method (2) and inserted into the SNC according to the coordinates of König and Klippel (3) (anterior, 2180 μm; lateral, 2 mm; vertical 3 mm). In brief, a carbon fiber (7 μm in diameter, type T-300, TORAYCA, Japan) was encased in a glass microcapillary filled with epoxy resin, and then the tip was blunted with sandpaper to be a disc-shape. Such an electrode has an impedance of between 2 and 4 megohms at 1 KHz and can be used as a working electrode either for electrochemical detection or as a microelectrode for unitary discharge recordings by switching the intervening circuit to either an electrophysiological recording system (ATAC-350, Nihon Kohden Co., Ltd., Japan) or a device for differential pulse voltammetry (modified PAR-174A) (Fig. 1). An Ag/AgCl wire (250 μm in diameter) and a silver wire (200 μm in diameter) were used as a reference and an auxiliary electrode, respectively. They were placed on the dura surface of the frontal cortex area. Single-unit discharges were counted with a sequential pulse-count program (ATAC-350). Recording parameters for the differential pulse voltammetry were as follows: potential range, −200 mV to +400 mV; pulse amplitude, 25 mV; scan rate, 50
mV/sec; pulse frequency, 10 Hz; and pulse duration, 50 msec. SNC neurons were identified according to the method described by Tsai et al. (4), i.e., spontaneous single-unit discharges of the SNC neurons (3 to 9 spikes/sec) were decreased in frequency about 50% during application of repetitive sciatic nerve stimulations (square wave pulses, 4–8 v, 0.1 msec, 1 Hz). After establishing the recording of stable electrochemical and electrophysiological signals, electrochemical measurements were performed for 12 sec at 10–20 min intervals, and the resting time was assigned to unitary activity measurements. Haloperidol (Shionogi) was given intraperitoneally at 10 min after starting the recordings.

Voltammograms obtained in the SNC showed a distinct oxidation peak at +120 mV. On the basis of electrochemical and pharmacological manipulation, we have already shown that the oxidation peak appearing around +120 mV corresponds to the released dopamine (DA) and/or 3,4-dihydroxyphenylacetic acid (DOPAC) (2, 5). The voltammograms recorded from the SNC yielded unstable sizes and shapes for about 1 hr after electrode implantation. However, after that, the stable amplitude of the oxidation peak was monitored at intervals of 10 min for 3 hr or more. From the pharmacological studies, DA and DOPAC appeared to be responsible for the electrochemical signal to the same degree. Homovanillic acid (HVA) also presented an oxidation peak at +380 mV, but this peak was not included in this study. A representative result following i.p. injection of 2 mg/kg of haloperidol is shown in Fig. 2. Haloperidol produced a rapid increase in unitary discharges with parallel enhancement of the peak in the voltammogram. The dose of haloperidol employed here produced about a 100% increase in both of the unitary discharge and the electrochemical signal over their basal levels within 60 min.

Dopaminergic autoreceptors in the SNC seem to play an important role in regulating the dopaminergic cell activity and dopamine metabolism (6, 7). A receptor blockade by haloperidol leads to compensatory increases in the activity of the dopaminergic cells and dopamine synthesis via a neuronal feedback mechanism (8, 9). Our results clearly proved the haloperidol-induced compensatory activation of dopaminergic neurons in the SNC via the above-mentioned mechanism.
Thus, this technique can be used as a convenient tool to study the meaning of neuronal activity changes in a limited area of the brain in connection with dynamic biochemical changes in the local area around the monitored cells.

References
1 Ewing, A.G., Alloway, K., Curtis, S.D., Dayton, M.A., Wightman, M. and Rebec, G.V.: Simultaneous electrochemical and unit recording measurements: characterization of the effects of d-amphetamine and ascorbic acid on neostriatal neurons. Brain Res. 261, 101–108 (1983)
2 Ikeda, M. and Matsushita, A.: Simultaneous measurements of catecholamine release and unitary neuronal discharges by carbon fiber microelectrode. Japan. J. Pharmacol. 32, Supp. 72P (1982)
3 König, J.F.R. and Klippel, R.A.: The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore (1963)
4 Tsai, C.T., Nakamura, S. and Iwama, K.: Inhibition of neuronal activity of the substantia nigra by noxious stimuli and its modification by the caudate nucleus. Brain Res. 195, 299–311 (1980)
5 Miyazaki, H., Ikeda, M. and Matsushita, A.: In vivo voltammetry: Characters of carbon fiber microelectrode and direct measurements of dopamine in the rat brain. Rev. Polarography 28, Supp. 72 (1982)
6 Nieoullon, A., Cheramy, A. and Glowinsky, J.: Release of dopamine in vivo from cat substantia nigra. Nature 266, 375–377 (1977)
7 Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G.K.: Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J. Pharmacol. Exp. Ther. 185, 560–571 (1973)
8 Carlsson, A. and Lindqvist, M.: Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. 20, 140–144 (1963)
9 Haubrich, D.R. and Pflueger, A.B.: The auto-receptor control of dopamine synthesis. An in vitro and in vivo comparison of dopamine agonists. Mol. Pharmacol. 21, 114–120 (1981)