In vivo Simultaneous Monitoring by Pt-disk Microelectrodes of Intracerebral Hydrogen Peroxide and Dopamine in Rats

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A technique was developed for simultaneous monitoring of temporal changes in hydrogen peroxide (H_2O_2) and dopamine (DA) levels by using Pt-disk microelectrodes (ϕ 30 μm). H_2O_2 and DA were determined by differential double-pulse amperometry (for H_2O_2; 1st step, 750 mV, 1000 ms; 2nd step, 1100 mV, 1000 ms: for DA; 1st step, 150 mV, 600 ms; 2nd step, 250 mV, 50 ms). The electrode for H_2O_2 or DA is capable of determining each target substance and these substances do not interfere with each other. For an *in vivo* application, the electrodes were implanted into the striatum of rats and used successfully to monitor intrastriatal changes in H_2O_2 and DA simultaneously after intraperitoneal injection of methamphetamine while the animals were moving freely.

Recently, we fabricated a Pt-disk microelectrode to conduct differential double-pulse amperometry (DDPA) to detect hydrogen peroxide (H_2O_2), one of the active oxygen species, in the brain of a freely moving animal.[1] Using this electrode, we detected H_2O_2 directly in the rat's striatum after intraperitoneal (i.p.) administration of methamphetamine (MAP), which, it is well-known, increases dopamine (DA)-release in the striatum.[2-4]

In theory, H_2O_2 is produced during the course of DA metabolism. It is believed that simultaneous monitoring of H_2O_2 and DA in the brain is essential for neurochemical research because the intracerebral dynamism of H_2O_2 and DA are interrelated. Determining intracerebral DA by employing DDPA has previously been described by Akiyama et al.[5,6] If our electrochemical technique for detecting H_2O_2 can be combined with their method, it should be possible to determine both H_2O_2 and DA simultaneously. In this study, a technique was developed in which Pt-disk microelectrodes were used to monitor the temporal changes in H_2O_2 and DA levels simultaneously. For an *in vivo* application, the electrodes were implanted into the striatum of rats, and intrastriatal changes in the amount of H_2O_2 and DA were monitored after i.p. injection of MAP while the animals were in freely moving states.

A Pt-disk microelectrode was constructed as follows. A Pt wire (30 μm in the diameter) was electrically welded to a copper wire. This was insulated with melted lead glass and enclosed in a glass capillary tube (1 mm in the outer diameter) by using epoxy resin. The tip of the electrode was then polished to make a flat disk surface. To detect H_2O_2 and DA, two Pt-disk microelectrodes were employed. An Ag/AgCl electrode served as a reference/counter electrode.

In the both measurements of H_2O_2 and DA, DDPA was applied. The DDPA uses stepped pulses where the 1st pulse is lower and the 2nd pulse higher than the characteristic oxidation potential of a target substance. The concentration of the target substance can be obtained from the difference between the current values derived from both pulses. The response is emphasized for the target with the onset oxidation potential in the region between 1st and 2nd pulses. In addition, the capacitive and residual currents are suppressed as do the conventional pulse electrochemical measurements.[1,5,7] The pulse parameters of DDPA for H_2O_2 detection were: 1st step, 750 mV, 1000 ms; 2nd step, 1100 mV, 1000 ms.[1,7] Those for DA were: 1st step, 150 mV, 600 ms; 2nd step, 250 mV, 50 ms.[5] The pulse parameters

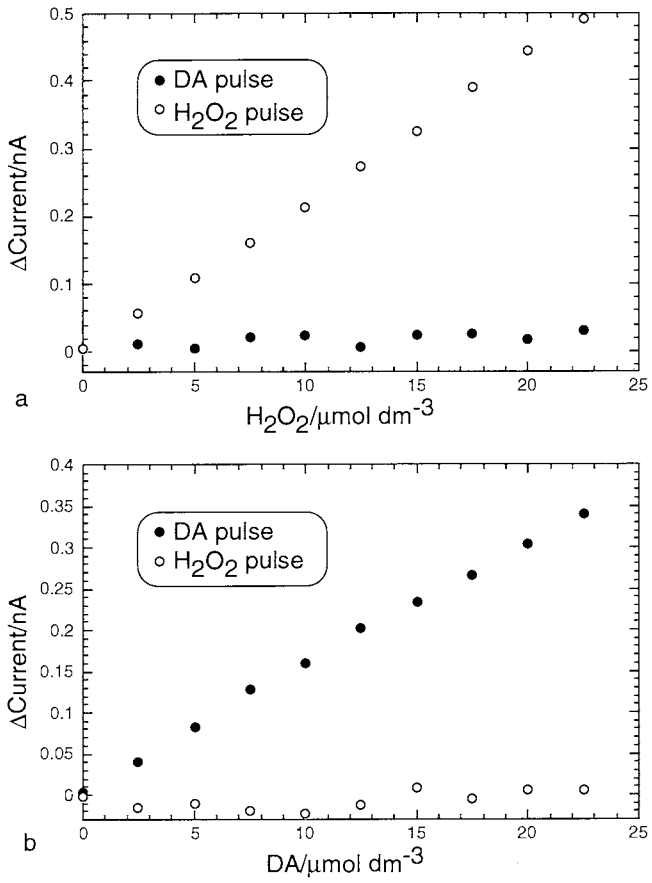

Figure 1. The changes in current through the Pt-disk microelectrode plotted against the concentrations of H_2O_2 (a) or DA (b). (a) The electrode through which the pulses for H_2O_2 detection were applied responded linearly to the concentration of H_2O_2 (open circles); no response was observed from the electrode through which the pulses for DA detection were applied (closed circles). (b) The electrode through which pulses for DA detection were applied responded linearly to the concentration of DA (closed circles); no response was observed from the electrode through which pulses for H_2O_2 detection were applied (open circles).

for two analytes were controlled by a personal computer (PC9-801BA, NEC) via two channels of the D/A converter (DAJ98, Canopus). Oxidation currents of the two analytes were amplified by two current-amplifiers (CEZ-2300 and CEZ-2400 with some modification, Nihon Kouden) and collected via two channels of the A/D converter (ADJ98, Canopus), using the same computer. Data sampling period for H_2O_2 detection is 160 or 20 ms at the 1st or 2nd pulse, respectively. That for DA is 30 or 3 ms.

The performance of the microelectrodes by DDPA to detect H_2O_2 or DA which were dissolved in a phosphate buffer solution (pH 7.4) at various concentrations was investigated. Figure 1 shows the change in the current values at the Pt-disk microelectrode plotted against the concentration of H_2O_2 and DA. The electrode through which the pulses for H_2O_2 detection were applied responded linearly to the concentration of H_2O_2 (Figure 1a, open circles; correlation coefficient, 0.999); but no response was observed from the electrode through which the pulse for DA were applied (Figure 1a, closed circles). On the other hand, the microelectrode for DA detection responded linearly to the concentration of DA (Figure 1b, closed circles; correlation coefficient, 0.999); but no response was observed from the electrode for H_2O_2 detection (Figure 1b, open circles). The concentrations ranged from 2.5 to 22.5 μ mol dm^{-3}. These results show that the electrode for H_2O_2 or DA can determine each target substance and that these substances do not interfere with each other. At low concentration (0.25 to 1.25 μ mol dm^{-3}) of H_2O_2 or DA, the electrode also responded linearly (correlation coefficient for H_2O_2 = 0.999; for DA = 0.996). The detection limit for H_2O_2 or DA was estimated to be about 0.02 or 0.08 μ mol dm^{-3}, respectively, at a signal-to-noise ratio of 3. For each fabricated electrode, the responses were plotted against H_2O_2 or DA concentrations to obtain calibration curves. With repeated calibrations for the same electrode after a cyclic potential scan (scan width, 0-700 mV; scan speed, 50 mV/s), the reproducibility of the current response was confirmed.

Male Wistar rats (200 g) were anesthetized with pentobarbital (50 mg/kg, i.p.) and the microelectrode for detecting H_2O_2 or DA was stereotaxically implanted into the right or left striatum, respectively. The reference electrode was placed on the dura over the parietal cortex. The electrodes were firmly anchored to the skull with dental cement, which also served to seal the wound. Before the implantation, a cyclic potential scan (the above mentioned conditions) of each microelectrode in the phosphate buffer solution had been performed to estimate the capacitive current. One day later, the value of the capacitive current of the electrode that had been implanted in the rat's brain was obtained by a cyclic potential scan in the freely moving states and was compared with the value before the implantation. The rats in which there was a difference in capacitive currents smaller than 10% were deemed minimum fouling of their electrodes and suitable for use in the following experiments.

The animals were divided into two groups: the control (n=6) and MAP (n=6). The later group received 8 mg/kg of MAP (via i.p.) that had been dissolved in a physiological saline solution (0.9 ml/kg). The animals in the control group received an identical volume of the physiological saline solution, also through the i.p. route. In each group, the oxidation current of H_2O_2 and DA were measured continuously for 45 min by DDPA while the behaviors of the freely moving animals were observed. The behavioral changes were evaluated, using Ujike's rating scale.[8] For the animals in the control group, the physiological saline solution injections caused no behavioral change. However, in the MAP groups, MAP injections caused hyperactivity from 4 to 10 min after injection, followed by a change in the animals' behavior, i.e., MAP-induced stereotypy.[8,9] The changes in current recorded for each animal were converted to those in H_2O_2 or DA concentration by using each calibration curve. In theory, DDPA is selective for the substances whose oxidation potential are sufficiently different (such as H_2O_2 and DA), but not for ones showing similar potential. DA level which is presented in the *in vivo* study includes DA and its metabolized products because their potential are similar. The physiological saline solution did not influence the H_2O_2 (Figure 2, open squares) and DA levels (Figure 2, open circles); however, MAP injection significantly increased both (Figure 2, closed squares and circles).

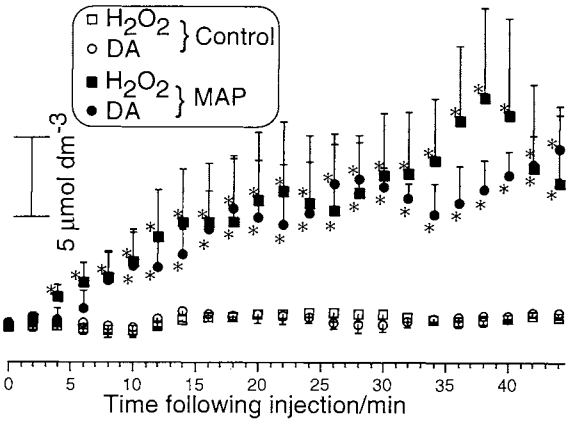

Figure 2. Temporal changes in the H_2O_2 or DA level in the striatum after injection of physiological saline solution (H_2O_2, open squares; DA, open circles; n=6) or 8 mg/kg of MAP (H_2O_2, closed squares; DA, closed circles; n=6) in freely moving rats. Ordinate indicates degree of elevation (difference between post- and pre-injection concentrations). Values are means ± standard error (Student's *t*-test: $p<0.05$, *)

In vivo electrochemical determination for each amount of H_2O_2 and DA in the brain has previously been demonstrated[1,6] but to our knowledge, simultaneous monitoring of both has not been performed. The present study is the first *in vivo* simultaneous monitoring of intracerebral H_2O_2 and DA in freely moving animals.

References

1 H.Yokoyama, N.Tsuchihashi, N.Kasai, T.Matsue, I.Uchida, N.Mori, H.Ohya-Nishiguchi, and H.Kamada, *Biosens. Bioelectron.*, **12**, 9(1997).
2 T.Zetterstrom, T.Sharp, C.A.Marsden, and U.Ungerstedt, *J. Neurochem.*, **41**, 1769(1983).
3 T.Sharp, T.Zetterstrom, T.Ljungberg, and U.Ungerstedt, *Brain Res.*, **401**, 322(1987).
4 H.Suzuki, T.Shishido, Y.Watanabe, H.Abe, M.Shiragata, K.Honda, R.Horikoshi, and S.Niwa, *Prog. Neuro-Psychoph.*, **21**, 359(1997).
5 A.Akiyama, T.Kato, K.Ishii, and E.Yasuda, *Anal. Chem.*, **57**, 1518 (1985).
6 T.Nakazato and A.Akiyama, *J. Neurochem.*, **51**, 1007(1988).
7 E.Tamiya, Y.Sugiura, A.Akiyama, and I.Karube, *Ann. N.Y. Acad. Sci.*, **613**, 396(1990).
8 H.Ujike, K.Akiyama, and S.Otsuki, *Psychopharmacology*, **102**, 459(1990).
9 A.Randrup and I.Munkvad, *J. Psychiatr. Res.*, **11**, 1(1974).