Monitoring of extracellular dopamine levels in the dorsal striatum and the nucleus accumbens with 5-minute on-line microdialysis in freely moving rats

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Abstract: We report a reliable 5-min on-line monitoring of dopamine released from the dorsal striatum and the nucleus accumbens of rats using in vivo brain microdialysis. The detection limit for dopamine was approximately 20 fg in a 10-µl injection sample using high-performance liquid chromatography with electrochemical detection set-up. Basal levels of dopamine in the dorsal striatum and the nucleus accumbens 4 h after probe insertion were 2.65 ± 0.30 pg/5 min and 1.57 ± 0.31 pg/5 min, respectively, whereas those of 20 h after probe insertion were lower: 0.97 ± 0.21 pg/5 min and 0.51 ± 0.09 pg/5 min, respectively. Infusion of the sodium channel blocker, tetrodotoxin (TTX; 2 µM), essentially suppressed levels of dopamine in both brain areas. At 4 h after probe insertion, TTX perfused for 4 h via dialysis probe reduced levels of dopamine to 0.47 ± 0.08 pg/5 min (80% reduction) in the dorsal striatum and to 0.56 ± 0.19 pg/5 min (65% reduction) in the nucleus accumbens. At 20 h after probe insertion, a similar TTX perfusion more rapidly reduced levels of dopamine to 0.05 ± 0.01 pg/5 min (95% reduction) in the dorsal striatum and to 0.08 ± 0.01 pg/5 min (85% reduction) in the nucleus accumbens. These results suggest that relatively fast changes in extracellular dopamine levels in these two brain areas can reliably be followed by this in vivo microdialysis technique. (J. Oral Sci. 43, 129-134, 2001)

Key words: extracellular dopamine; rapid sampling; in vivo microdialysis; tetrodotoxin; dorsal striatum; nucleus accumbens.

Introduction

Intracerebral microdialysis has become a well established and a widely-used in vivo measurement of extracellular levels of neurotransmitters such as dopamine. The responses of dopamine to many drugs and to some behavioral changes in awake animals are of particular interest. However, most microdialysis studies use sample times of 10-25 min (1-7). In order to follow relatively rapid changes, a shorter sampling interval is necessary, whereas more frequent sampling inevitably leads to smaller samples with lower quantities of transmitters. High-performance liquid chromatography with electrochemical detection (HPLC-ECD) has been used almost universally for this purpose, and efforts have been made to improve the technique enabling lower detection limits and higher sampling frequency. In the present study, a new in vivo microdialysis system (HTEC-500, Eicom), an on-line combination of HPLC-ECD consisting of a short octadecysilane column (4.6 × 30 mm, 2 µm particle), that showed high sensitivity was employed. This system therefore allowed us to determine dopamine levels in 5-min dialysate samples. By applying this system, we compared basal levels of...
extracellular dopamine in the dorsal striatum with those in the nucleus accumbens at both 4 h and 20 h after probe insertion. Moreover, in order to ensure that the measured dopamine was dependent on neuronal release, we also examined the effects of the sodium channel blocker tetrodotoxin (TTX) on extracellular dopamine concentrations in these two brain areas.

Materials and Methods

Animals

Male Sprague-Dawley rats (NRC Haruna, Japan) weighing between 200 and 220 g at the start of the experiment were used. These rats were housed in a temperature-controlled environment on a 12-h light-dark cycle (light period 07:00-19:00 h) with free access to food and water.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The anesthetized animals were placed in a stereotaxic apparatus, and a guide cannula was implanted just above the left dorsal striatum or the nucleus accumbens according to the atlas of Paxinos and Watson (8) [dorsal striatum: antero-posterior (AP) 9.2 mm, medio-lateral (ML) 3.2 mm, dorso-ventral (DV) 7.2 mm; nucleus accumbens: AP 10.6 mm, ML 1.5 mm, DV 4.0 mm from interaural line]. To avoid the ventricular system, cannulae directed at the nucleus accumbens were angled 18° from the mid-sagittal plane. After completion of surgery, rats were allowed to recover for 7 to 10 days before experiments were carried out. Guide cannulae were kept patent by stainless steel inserts. Each animal was used only once.

These experiments were performed in accordance with institutional guidelines in the care and use of experimental animals that are in compliance with the U.K. Animals (Scientific Procedures) Act, 1986, and all efforts were made to minimize animal suffering, and to reduce the number of animals used.

Dialysis and neurochemical measurements

A commercially available I-shaped removable-type dialysis probe (2 mm length cellulose membrane, 0.22 mm o.d., 50000 mol. wt. "cut-off", Eicom A-I-8-02 type, Kyoto, Japan) was used. The experiment was started by removing the stylet from the guide cannula and inserting the dialysis probe so that only the dialysis tubing protruded from the tip. The probe was secured to the guide cannula by a screw. Each rat was then placed in a plexiglass box (30 × 30 × 35 cm), and inlet and outlet tubes were connected to a swivel located on a counterbalanced beam to minimize discomfort. The probe was perfused at a rate of 2.0 μl/min with modified Ringer solution (NaCl 147 mM, KCl 4 mM, CaCl2 1.2 mM, MgCl2 1.1 mM; pH 7.4) and the outflow connected by Teflon tubing to a high-performance liquid chromatography system (HTEC-500; Eicom, Kyoto, Japan).

Dopamine was separated on an Eicompak PP-ODS column (particle size, 2 μm, 4.6 × 30 mm; Eicom, Kyoto, Japan) maintained at 25°C, using phosphate buffer (0.1 M) containing decanesulfonic acid (2.0 mM), EDTA (0.13 mM) and 1% methanol (pH 6.0) as the mobile phase at a flow rate of 0.5 ml/min. Compounds were quantified by electrochemical detection using a glassy carbon working electrode set at +400 mV against a silver-silver chloride reference electrode (WE-3G; Eicom, Kyoto, Japan). The probes had an in vitro recovery of approximately 12% for dopamine, but the reported concentrations were not adjusted for recovery in vivo because these estimations are inaccurate (9, 10). Perfusion samples were taken every 5 min for quantification of dopamine.

Drugs

The sodium channel blocker, TTX (Sigma), was dissolved in the modified Ringer solution to be used for perfusions, and was administered intracerebrally through the dialysis probe either 4 h or 20 h after probe insertion. The concentration of TTX (2 μM) was based on the outcome of our previous studies (11-14).

Histology

At the end of the experiment, rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and perfused transcardially with 10% formaldehyde solution. The brain was removed, sectioned (50 μm) and stained with cresyl violet to permit assessment of probe location.

Statistical analysis

All values are expressed as means ± S.E.M. Comparison of time-course data was performed using one-way analysis of variance (ANOVA) (within group) and two-way ANOVA for repeated measures with the factors treatment and time (between groups), when appropriate. Statistical significance was considered to be P < 0.05.

Results

Histology

Placements of the dialysis probes in the nucleus accumbens and in the dorsal striatum are given in Fig. 1.
Under the present chromatographic conditions, dopamine eluted after 2.0 min (Fig. 2A–E). Using a full-scale setting of 280 pA standard solution containing 1 pg/10 μl produced a peak of 68 pA with an amplitude of noise that was 0.4 pA or less (Fig. 2A). The limit of detection was about 20 fg/10 μl which is the amount of dopamine producing a peak height of 3.4 times the noise. This provides a linear calibration curve for dopamine (using area value) even over a range (20–90 fg) that is close to the detection limit (Fig. 3). Related catecholamines as norepinephrine and acid metabolites like 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyphenylacetic acid were eluted in the front. The chromatograms of dialysates from the dorsal striatum (Figs. 2B, D) and the nucleus accumbens (Figs. 2C, E) 4 h (Figs. 2B, C) and 20 h (Figs. 2D, E) after probe insertion showed rather similar profiles with peaks of dopamine that appeared at 2.0 min.

Effects of TTX perfusion on dialysate dopamine levels in the dorsal striatum and the nucleus accumbens 4 h after probe insertion

Concentrations of dopamine in dialysates from the dorsal striatum and the nucleus accumbens 4 h after probe insertion reached relatively stable baseline values of 2.65 ± 0.30 pg/5 min (mean ± S.E.M., n = 7) and 1.57 ± 0.31 pg/5 min (n = 5), respectively. The basal levels of dialysate dopamine for 60 min before TTX perfusion in the dorsal striatum was significantly higher than that in the nucleus accumbens (F1,120 = 72.3, P < 0.0001, two-way ANOVA) (Fig. 4).

TTX (2 μM) perfused for 4 h via the dialysis probe significantly reduced levels of dopamine to 0.47 ± 0.08

Fig. 1 Schematic illustration showing the locations of the probe in the nucleus accumbens and in the dorsal striatum. The diagrams are taken from the atlas of Paxinos and Watson (8) and represent anterior distance (mm) from the interaural line.

Fig. 2 Chromatograms obtained by an HPLC-ECD system with PP-ODS column (HTEC-500, Eicom) of (A) standard dopamine (1 pg/10 μl injection sample) and of 5-min (10 μl) dialysates from the dorsal striatum (B) 4 h and (D) 20 h after probe insertion, and of 5-min (10 μl) dialysates from the nucleus accumbens (C) 4 h and (E) 20 h after probe insertion. Retention time of dopamine (DA) was 2.0 min in each case.

Fig. 3 Standard curve for dopamine (DA; 20–90 fg) in a 10-μl injection volume detected by an HPLC-ECD system with PP-ODS column (HTEC-500, Eicom).
pg/5 min (approximately 80% reduction) in the dorsal striatum \( (F_{39,360} = 19.2, P < 0.0001, n = 7, \text{ one-way ANOVA}) \) and to \( 0.56 \pm 0.19 \text{ pg/5 min} \) (approximately 65% reduction) in the nucleus accumbens \( (F_{59,239} = 2.76, P < 0.0001, n = 5, \text{ one-way ANOVA}) \) (Fig. 4).

Effects of TTX perfusion on dialysate dopamine levels in the dorsal striatum and the nucleus accumbens 20 h after probe insertion

Concentrations of dopamine in dialysates from the dorsal striatum and the nucleus accumbens 20 h after probe insertion reached stable baseline values of \( 0.97 \pm 0.21 \text{ pg/5 min} \) \((n = 6)\) and \( 0.51 \pm 0.09 \text{ pg/5 min} \) \((n = 7)\), respectively. The basal levels of dialysate dopamine for 60 min before TTX perfusion in the dorsal striatum was significantly higher than that in the nucleus accumbens \((F_{1,132} = 55.4, P < 0.0001, \text{ two-way ANOVA}) \) (Fig. 5). The levels of dopamine in the both brain areas were significantly smaller than those of 4 h after probe insertion in the respective areas (nucleus accumbens: \( F_{1,132} = 173.2, P < 0.0001 \); dorsal striatum: \( F_{1,132} = 236.5, P < 0.0001 \), two-way ANOVA).

TTX \((2 \mu M)\) perfused for 4 h via the dialysis probe significantly reduced basal levels of dopamine to \( 0.05 \pm 0.01 \text{ pg/5 min} \) (approximately 95% reduction) in the dorsal striatum \((F_{59,297} = 12.9, P < 0.0001, n = 6, \text{ one-way ANOVA}) \) and to \( 0.08 \pm 0.01 \text{ pg/5 min} \) (approximately 85% reduction) in the nucleus accumbens \((F_{59,360} = 14.9, P < 0.0001, n = 7, \text{ one-way ANOVA}) \) (Fig. 5). Such reductions in both areas occurred more rapidly when compared to those that occurred 4 h after probe insertion.

**Discussion**

HPLC-ECD showed a detection limit for dopamine of approximately 20 fg in a 10-μl (5 min) dialysate sample. Such a detection limit is considerably lower than that reported previously by using a microbore or a smallbore chromatography, which had detection limits of around 100 fg \((15-17)\). Because of the detection limit a dopamine uptake inhibitor was required in some studies to elevate basal levels \((16)\), thereby producing readily detectable dopamine levels. Moreover, for frequent sampling, manual injections of samples are commonly employed \((15, 18)\). In contrast, the present experiment, due to the low detection limit, a dopamine uptake inhibitor was not necessary. Further, we introduced an on-line system to easily perform a frequent sampling by shorting a retention time of dopamine.

Previous experiments in which we have used a conventional HPLC-ECD technique and procedure have shown that dopamine efflux is more or less stabilized 4 h after probe insertion, and that levels seen at that time are largely dependent on neuronal release, as 65-80% of the release is TTX-sensitive \((11-14)\). The present experiment also showed that basal levels of dopamine were almost stabilized 4 h after probe insertion, which levels being \( 2.65 \pm 0.30 \text{ pg/5 min} \) in the dorsal striatum and \( 1.57 \pm 0.31 \text{ pg/5 min} \) in the nucleus accumbens. Perfusion of TTX, which began 4 h after probe insertion, decreased rapidly the levels of dopamine, which then reached a similar relatively stable concentration (approximately 0.5 pg/5 min). Reduction rates were 80% in the dorsal striatum and 65% in the nucleus accumbens. At 20 h after probe insertion, basal levels of dopamine were \( 0.97 \pm 0.21 \text{ pg/5 min} \) in the dorsal striatum and \( 0.51 \pm 0.09 \text{ pg/5 min} \) in the nucleus accumbens. Perfusion of TTX more rapidly
decreased the levels of dopamine, which then reached stability at very low concentrations irrespective of the brain sites (<0.08 pg/5 min). Reduction rates were 95% in the dorsal striatum and 85% in the nucleus accumbens. Such a large TTX-induced reduction indicates that the detection limit for dopamine in this study was sufficient to reliably detect not only enhancement, but also reduction, of dopamine from the baseline level.

These results demonstrated that there exists a regional difference in extracellular levels of dopamine between the dorsal striatum and the nucleus accumbens at both 4 h and 20 h after probe insertion; namely dopamine levels in the dorsal striatum are significantly higher than those in the nucleus accumbens.

The current findings are in agreement with previous studies using conventional HPLC-ECD showing that dopamine levels that reflect neuronal activity are best observed approximately one day after probe insertion (19). The present results demonstrated that, at 20 h after probe insertion, TTX perfusion very rapidly decreased extracellular dopamine levels in both the dorsal striatum and the nucleus accumbens, which then reached a stable level of 0.05-0.08 pg, which is about 3 times as much as the detection limit. This is comparable to that observed in the experiment done at 4 h after probe insertion, in which TTX reduced, to lesser degree, dopamine levels 4 h after perfusion. The remaining dopamine levels were 0.4-0.5 pg in both areas, and particularly in the dorsal striatum the levels of dopamine were still somewhat reduced. In either case, levels of dopamine were similar in these two brain areas. This indicates that the effects of TTX (2 µM) reached a maximum, hence apparent differences seen in reduction rate of dopamine in both areas are largely attributable to their pre-infusion levels.

In conclusion, we demonstrate the routine use of relatively rapid on-line sampling of extracellular dopamine in the dorsal striatum and the nucleus accumbens without the presence of an uptake inhibitor in freely moving rats. Moreover, dopamine measured in this way reflects neuronal release in both brain areas, particularly 20 h after probe insertion.

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References
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