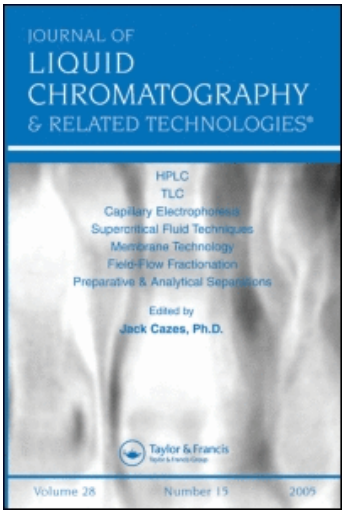


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# Analysis and Pharmacokinetics of Apomorphine in Rat Brain by Microdialysis Coupled with Microbore HPLC Electrochemical Detection

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# ANALYSIS AND PHARMACOKINETICS OF APOMORPHINE IN RAT BRAIN BY MICRODIALYSIS COUPLED WITH MICROBORE HPLC ELECTROCHEMICAL DETECTION

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## ABSTRACT

The feasibility of an on-line microdialysis coupled with a sensitive microbore high performance liquid chromatography with electrochemical detection (HPLC-ED) system for the direct analysis of apomorphine was investigated. A microdialysis probe was inserted into the right striatum of male Sprague-Dawley rats, which had been administered apomorphine (10 mg/kg, i.v.).

Brain dialysates were automatically injected into a cyano microbore column with an electrochemical detector through an on-line injector. Samples were eluted with a mobile phase containing 0.1 M monosodium dihydrogen phosphate-methanol (84:16, v/v, pH 3.0 adjusted with orthophosphoric acid) at a flow rate of 0.05 mL/min. A monophasic phenomenon with a elimination phase was observed from the brain apomorphine concentration-time curve. The results indicate that the brain pharmacokinetics of apomorphine appears to conform to a one-compartment model.

## INTRODUCTION



Apomorphine is well known for its dopaminergic effects.[1-2] However, it exhibits dual effects, where a low dose causes a decrease in dopamine neurotransmission and function by the stimulation of inhibitory pre-synaptic D1 receptor.[3] A larger dose causes direct stimulation of post-synaptic D2 receptor, resulting in an increase in the functional activity of dopamine.[4]

For the determination of apomorphine, a number of analytical methods have been developed including gas chromatographic,[5] fluorometric[6] and mass fragmentographic.[7] Recently, high performance liquid chromatography (HPLC) coupled with ultraviolet,[8,9] fluorometric[10] and electrochemical detection (ED)[11,12] have been reported. HPLC-ED is the most sensitive method.

Currently, microbore columns instead of conventional columns coupled with ED have advantages of very high sensitivity and a relatively small sample introduction.

For the sample processing, apomorphine is a very labile compound in solution. In order to warrant the stability of apomorphine, we used an automatic on-line injection method to inject dialysate directly.[13]

Recent advances in brain microdialysis techniques have enabled the direct measurement of various neurotransmitters in the brain, but pharmacokinetic investigations of psychotropic drugs by this method are limited.[14,15] Since drug monitoring is crucial for the rational therapeutic use of drugs, the feasibility of employing the brain microdialysis method for pharmacokinetic studies is of particular importance.

Of interest in elucidating the central disposition of apomorphine in the brain, a precise and sensitive method using an on-line microdialysis system coupled with microbore HPLC-ED was developed to measure apomorphine in brain dialysates. In addition, the central pharmacokinetics of apomorphine in rat brains was also investigated.

## MATERIALS AND METHODS

### Materials and Reagents

Apomorphine was purchased from Research Biochemical International (RBI, Natick, MA, USA). Methanol and orthophosphoric acid were obtained from E. Merck (Darmstadt, Germany). Triple de-ionized water (Millipore Corp., Bedford, MA, USA) was used for all preparations.



### Chromatography

The HPLC-ED system consisted of a chromatographic pump (BAS, PM-80, Bioanalytical System, West Lafayette, IN, USA) at flow-rate 0.05 mL/min for apomorphine analysis using a cyano microbore column (BAS, SepStik CN-5μ, 150 x 1 mm i.d., particle size 5 μm) in series after an on-line injector (Fig. 1). The mobile phase consisted of 0.1 M monosodium dihydrogen orthophosphate-methanol (84:16, v/v, pH 3.0 adjusted with orthophosphoric acid). The mixture was filtered with a 0.22 μm Millipore membrane and degassed by helium. The injection volume was configured with a 10 μL sample loop on an on-line injector (CMA-160, CMA/Microdialysis AB, Stockholm, Sweden). Apomorphine was measured using an electrochemical detector (BAS 4C). The potential for the glassy carbon working electrode was set at + 0.8 V with respect to a Ag/AgCl reference electrode. The output from the electrochemical detector was recorded using Waters Millennium 2020 software.[16-18]

### Microdialysis

Adult, male Sprague-Dawley rats (250-320 g) were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The rat was cannulated with a PE-50 tube at the right femoral vein for drug administration. After the femoral vein cannulation, the rat was placed in a Kopf stereotaxic frame and its body temperature was maintained at 37°C with a heating pad. A microdialysis probe

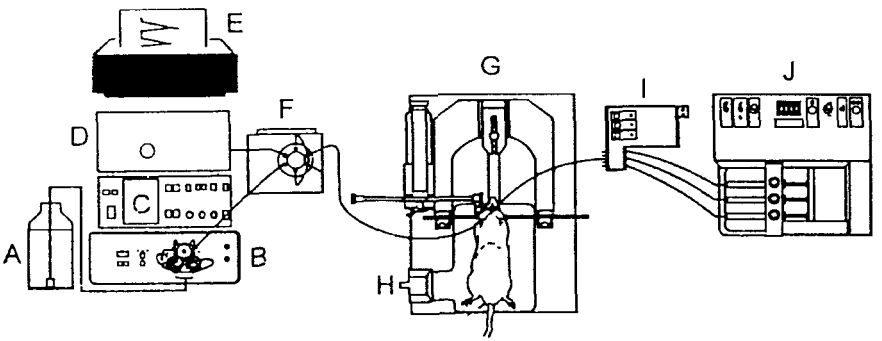


**Figure 1**. The schematic diagram of an on-line microdialysis coupled with the microbore HPLC-ED system. A: mobile phase; B: chromatography pump; C: electrochemical detector; D: column cabinet; E: data system; F: on-line injector; G: stereotaxic device; H: heating pad; I: solvent selector; J: microinjection pump.



(CMA-12; CMA/Microdialysis AB) with a tip length of 4 mm and an outer diameter of 0.5 mm was implanted into the right striatum with its tip located at AP 0.4 mm, ML -3.0 mm, DV -7.0 mm, from the bregma and dura surface, respectively.[19] The probe was perfused with Ringer solution (147 mM $Na^+$, 4.0 mM $K^+$, 2.2 mM $Ca^{++}$) at a flow-rate of 1 μL/min, by a microinjection pump (CMA-100). The outflow from the dialysis probe was connected to an on-line injector (CMA-160) and HPLC-ED.[13] The samples were automatically injected every 10 min for 160 min after drug administration.

## Recovery

The recovery of the dialysis probe for apomorphine is the ratio of its concentration in the dialysate, i.e. the outlet from the probe ($C_{out}$) to its concentration of apomorphine in the medium surrounding the probe ($C_{in}$).

$$\text{The recovery}_{\text{in vitro}} = C_{out} / C_{in}$$

## Pharmacokinetic Analysis

Calibration curves were constructed based on the analysis by HPLC-ED of various concentrations of apomorphine (0.5-100 ng/mL) and were used to determine the concentrations of apomorphine in rat brain dialysates. Following a 2-h period for stabilization, dialysates were automatically injected every 10

min for 160 min after drug administration (10 mg/kg, i.v.). The volume of i.v. apomorphine (10 mg/mL) solution administered was 1 mL/kg. After the administration of the drug, the catheter was then immediately flushed with 0.5 mL normal saline.

All brain dialysate concentration-time data were processed by the computer program "PCNONLIN" (SCI Software Inc. Lexington, KY, USA), with reciprocal concentration weights (1/C) for the calculation of pharmacokinetic parameters.

The data were compared with pharmacokinetic models (one- vs two-compartment) according to the criteria of Akaike's information criterion (AIC)[20] and Schwartz criterion (SC),[21] with minimum AIC and SC values being regarded as the best representation of the concentration-time course data. The following equation applies into a one-compartment pharmacokinetic model:

$$C = Ae^{-\alpha t} \quad (1)$$

In equation 1, A is the concentration (C) intercepts and $\alpha$is disposition rate constant for the disposition phase. The elimination phase half-life ($t_{1/2}$) of apomorphine in brain dialysate were defined as $0.693/\alpha$.

The noncompartmental method for calculating disposition parameters of apomorphine in the brain is based on the theory of statistical moments.[22] The area under the concentration-time curve (AUC) of a plot from time zero to infinity is often referred to the area under the moment curve (AUMC).[22] The ratio of AUMC to AUC for apomorphine in the brain is a measure of its mean residence time (MRT).[23]

## RESULTS

Under the conditions described above, the retention times of apomorphine was found to be 6.5 min (Fig. 2). Figure 2(A) shows a standard sample of apomorphine (10 ng/mL). Figure 2 (B) shows a chromatogram of a blank brain dialysate. No discernible peaks were observed within the time frame in which apomorphine was detected. Figure 2(C) shows a chromatogram of a dialysate sample containing apomorphine (27.92 ng/mL) obtained from brain microdialysis 40 min after apomorphine (10 mg/kg, i.v.) administration. The in vitro recovery of apomorphine of the microdialysis probe based on a 10 ng/mL standard, was 32%.

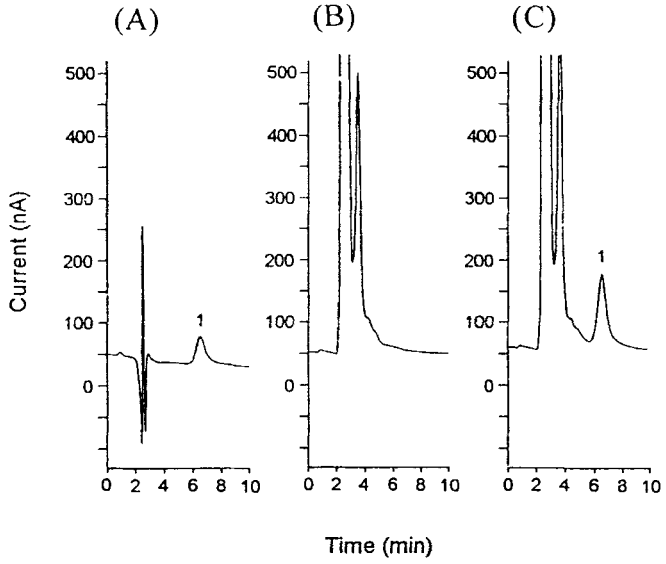


**Figure 2**. Typical chromatograms of (A) a standard sample containing 10 ng/mL apomorphine, (B) a blank brain dialysate, and (C) a dialysate sample containing apomorphine (27.92 ng/mL) collected from a rat after apomorphine (10 mg/kg, i.v.) administration. 1: apomorphine.



The limit of quantitation is defined as the lowest concentration on the standard curves which can be measured with acceptable accuracy and precision. The limit of quantitation was 0.5 ng/mL for apomorphine. However, the detection limits for apomorphine, at a signal-to-noise ratio of 3, was 0.1 ng/mL.

A one-compartment open model in rat brain with individual animal data after apomorphine i.v. administration was proposed by the computer program "PCNONLIN". Analysis of data in Fig. 3 yields equation 2.

$$C = 55.53e^{-0.021t} \quad (2)$$

The brain pharmacokinetic parameters, as calculated by PCNONLIN program and derived from these data, are shown in Table 1.

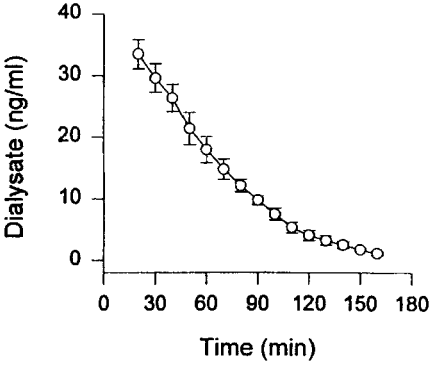


**Figure 3**. The brain dialysate concentration-time curve after apomorphine (10 mg/kg, i.v.) administration.



## Table 1

**Brain Pharmacokinetic Parameters of Apomorphine (10 Mg/Kg, I.V.) in Rats**

| Parameters | Estimate |
|---|---|
| A, ng/mL | 55.53 ± 6.03 |
| $\alpha$, 1/min | 0.021 ± 0.002 |
| AUC, ng min/mL | 2666 ± 201 |
| $t_{1/2}$, min | 33.92 ± 2.37 |
| AUMC, $\mu g\ min^2/mL$ | 129.97 ± 11.86 |
| MRT, min | 48.94 ± 3.41 |

Data are expressed as mean±SEM (n=5).

## DISCUSSION

Compared to conventional HPLC systems, microbore columns decrease band broadening of analytes so that sharper peaks are obtained. Furthermore, the slow flow-rates in a microbore HPLC-ED system provides a smoother baseline to achieve lower detection limits.[16,17] It also prolongs the time of analytes contact with the working electrode and results in higher coulometric yields.[16,24] Hence, microbore HPLC-ED systems can enhance detection sensitivity and achieve optimum detection limits. Furthermore, the microbore HPLC-ED

system requires only small quantities of samples which is compatible to microdialysis sampling methods. In addition, the on-line analysis improves analytical reproducibility and obviates the need of preservatives in the samples and other tedious manual procedures.

In the present study, a microbore HPLC-ED system was applied to the determination of apomorphine in rat striatal dialysates from an on-line microdialysis system in rats receiving an i.v. administration of apomorphine. The limit of quantification and the detection limit of apomorphine were 10 ng/mL and 1 ng/mL, respectively. A monophasic phenomenon with a first-order elimination rate constant for apomorphine was observed from the brain dialysate concentration-time curve. The results indicate that the brain pharmacokinetics of apomorphine appears to conform to an one-compartment model.



Noncompartmental methods for calculating disposition parameters of apomorphine in brain dialysate are based on the theory of statistical moments.[22] After administration of apomorphine (10 mg/kg, i.v.), MRT and $t_{1/2}$ were 48.94 and 33.92 min, respectively. MRT is a function of both distribution and elimination. Elimination half-life ($t_{1/2}$) is the time required to eliminate 50% of the dose, whereas $MRT_{iv}$ is the time required to eliminate 63.2% of the dose.[22] The disposition of apomorphine in rat brain might process from blood through the blood-brain-barrier. Our results suggest that the disposition of apomorphine in brain exhibit one disposition phase.

In conclusion, the present results recommend that the brain microdialysis method may be applicable to further pharmacokinetic studies of psychotropic or neurotropic agents in the brain.

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