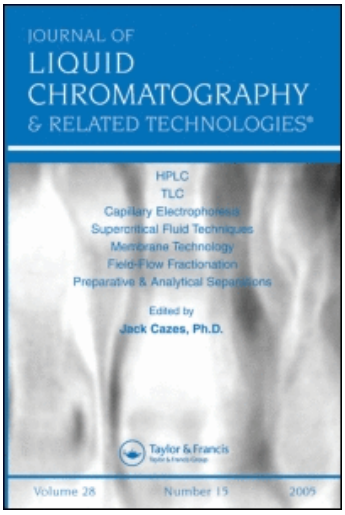

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Rapid Determination of Apomorphine in Brain and Plasma Using High-Performance Liquid Chromatography

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RAPID DETERMINATION OF APOMORPHINE IN BRAIN AND PLASMA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A new method for quantitative determination of apomorphine in mouse brain and rat plasma is described. The drug was extracted utilizing SEP-PAK C_{18} cartridge, and quantified by high performance liquid chromatography with electrochemical detector. The average recovery was 92 $\pm$ 2.8% with a day-to-day coefficient of variation of 10.2%. Apomorphine concentration in mouse brain and in rat plasma, as a function of dose and time, after injection with apomorphine-HCl were determined. The results indicate that the method is adequate for pharmacokinetic studies.

INTRODUCTION

Apomorphine (APO) is a drug that has been used for treatment of Parkinson's disease (1), Huntington's chorea (2), tardive dyskinesia (3), spasmodic torticollis (3), Gilles de la

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Tourette's syndrome (4), schizophrenia (5,6) and thalamic pain (7). It is also a direct dopamine receptor agonist in the central nervous system and an important tool in biochemical investigations of dopamine receptor function (8). Because relatively small doses of APO have been used, it has been difficult to measure this compound in biological fluids. Previously reported techniques for determining APO in biological matrixes include: spectrofluorimetry (9), gas chromatography (GC) (10-12), high-performance liquid chromatography (HPLC) (13,14), an enzymatic-radioisotopic method (15), and selected ion monitoring (16,17). All these methods have either a limitation in sensitivity, selectivity or simplicity.

This paper describes a new method for the determination of APO in rodent plasma and brain tissue using SEP-PAK C_{18} extraction procedure and HPLC with electrochemical detector. The method is simple, rapid, sensitive, and suitable for pharmacokinetic studies.

MATERIALS AND METHOD

Chemicals

Apomorphine hydrochloride was obtained from Merck & Co. Inc., Rahway, NJ. N-n-Propylnorapomorphine hydrochloride (PNAPO) was obtained from Sterling Winthrop Research Institute (Rensselaer, NY). SEP-PAK C_{18} cartridges were purchased from Waters Assoc. Inc., (Milford, MA). All

solvents, buffer components and chemicals were of analytical-reagent grade. Water was deionized and then double-distilled in glass.

Apparatus

The high performance liquid chromatography was constructed from four components: M45 solvent delivery system (Waters Assoc., Inc.); LC-3 electrochemical detector with a TL-5 glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN); Model 7125 injection valve (Rheodyne Inc., Cotati, CA); Model LS-44 recorder (Linseis, Inc., Princeton Jct., NJ). A μBondapak C_{18} reverse phase column (Waters Assoc., Inc.) was used with an in-line guard column of 5 μm RP-18 (Brownlee Labs., Santa Clara, CA). The mobile phase was methanol -0.02 M Na_2HPO_4/0.03 M citric acid, pH 3.2 (34:66, v/v). The flow rate was fixed at 0.7 ml/min at ambient temperature and detector potential was set at 0.7 volts vs. the Ag/AgCl reference electrode.

Procedure

Brain. Mouse brain was rapidly removed following decapitation and immediately frozen at -70°C. The whole brain sample was weighed and put into a polypropylene tube containing 4 ml of 0.4 M perchloric acid, 2 μg of PNAPO, as (an internal standard) and 2 mg of sodium bisulfite The mixture was homogenized using Ultra-Turrax, (Janke and Kunkel, West Germany) for 30 seconds, vortexed, and then centrifuged at 27,000 RPM for 10 min (4°C). Extraction was performed using SEP-PAK C_{18} cartridge. The cartridge was

prepared by flushing with 5 ml of H_2O, 5 ml of MeOH through a glass syringe followed by 10 ml of H_2O. One ml of the supernatant of the homogenate was throughly mixed with 2 ml of 0.5 M Na_2HPO_4 buffer (pH 7.0) and then passed through the C_{18} cartridge at a flow rate not greater than 2 ml/min. The cartridge was washed with 5 ml of H_2O followed by 0.2 ml of a mixture prepared by mixing 1 part of MeOH and 1 part of 0.02 M Na_2HPO_4 + 0.03 M citric acid solution of (pH = 3.2). APO and PNAPO were eluted from the cartridge with 1.5 ml of the above mentioned MeoH-Na_2HPO_4-citric acid solution. The eluate (40 µl) was analyzed by HPLC.

Plasma. 0.5 µg of PNAPO was added to 1 ml of rat plasma, mixed thoroughly and passed through SEP-PAK C_{18} cartridge, which was then washed and eluted as described above.

With each set of brain or plasma samples, a standard curve was prepared by adding different amounts of APO and the same amount of PNAPO as samples into a drug-free brain or plasma. The standard samples were analyzed by HPLC as described above. The peak height ratio, APO/PNAPO, was calculated for the concentration of APO in samples. The cartridge was regenerated by flushing with 5 ml of MeOH and 10 ml of H_2O.

RESULTS AND DISCUSSION

Figures 1 and 2 show representative chromatograms of mouse brain and rat plasma. APO and PNAPO have retention

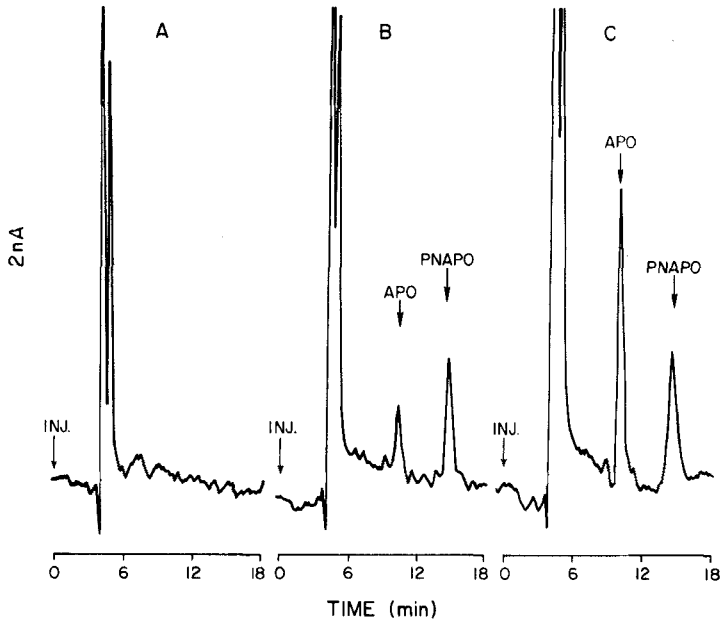

Fig. 1 Chromatograms of mouse brain samples:
(A) brain sample from a drug-free animal;
(B) brain sample after administration of 2 mg/kg of APO;
(C) brain sample after administration of 8 mg/kg of APO.
Conditions were as given in the Materials and Method section.

times of 9.9 and 14.4 min, respectively. No significant interference was found in samples from components not identifiable as APO and PNAPO.

To determine the optimum potential for the assay, a current-potential curve was generated (Fig. 3). The optimum operating potential for this assay was chosen to be 0.7 v. The use of the SEP-PAK C_{18} cartridge for extraction had several advantages over solvent extraction methods (9,14, 16-18). It provided chromatographically cleaner extracts in a single and

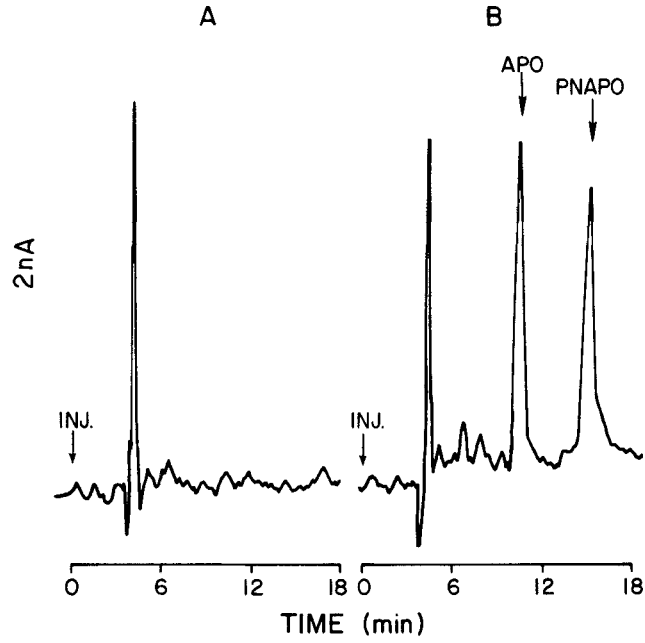

Fig. 2 Chromatograms of rat plasma samples:
(A) plasma sample from a drug-free animal;
(B) plasma sample, 10 minutes after administration of 10 mg/kg of APO.

quick step. Recovery utilizing this method was determined by comparing the current response of spiked drug-free brain homogenate or plasma extracts to that of a standard solution of APO. The average recovery was 92 $\pm$ 2.8% (M $\pm$ SD, N=20) over a concentration range of 0.3 - 3.0 µg/ml. The within-day and day-to-day precision, coefficient variation (CV) were 6.8% and 10.2% (n=10) respectively for samples spiked with 2 µg/ml of APO. The minimum detection, using a signal-to-noise ratio of 3:1, was determined to be 1 ng injected, corresponding to a concentration of 40 ng/ml of plasma and 500 ng/g of brain

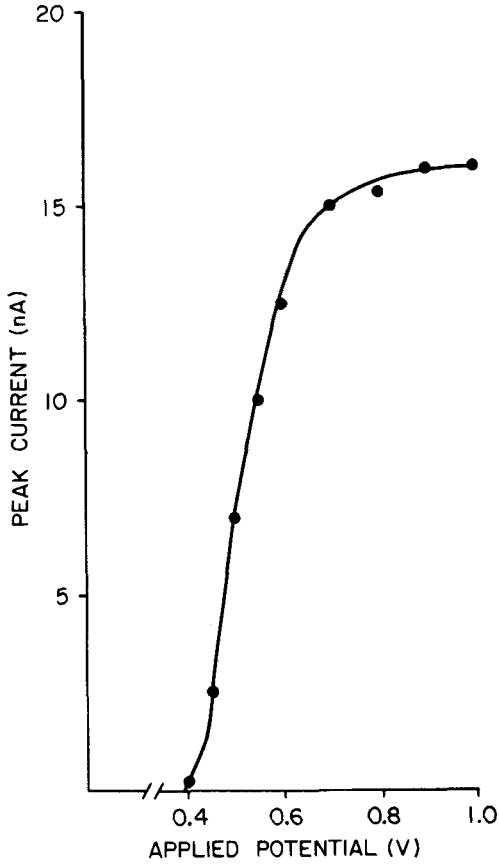

Fig. 3 Current-potential curve for APO.

tissue. Lower levels could be measured by eluting APO from SEP-PAK C_{18} cartridge with 1 ml of MeOH-Na_2HPO_4 buffer, and/or injecting of larger samples into the injection valve. The present method approaches the sensitivity found using a GC/MS method which selected ion monitoring technique (16,17).

As an application of the method to an APO diposition study, mouse brain APO levels 10 minutes after the

TABLE 1. Concentration of APO in the whole brain of two strains of mice after intraperitoneal injection.

Dose of APO/kg	Mouse brain APO concentration* AKR/J	DBA/2J
Control	0	0
2 mg	1.65±0.37	1.33±0.23
8 mg	4.03±1.51	4.01±1.65

*Values are expressed in ug/g wet tissue and mean ± S.D. from 7 animals in each group.

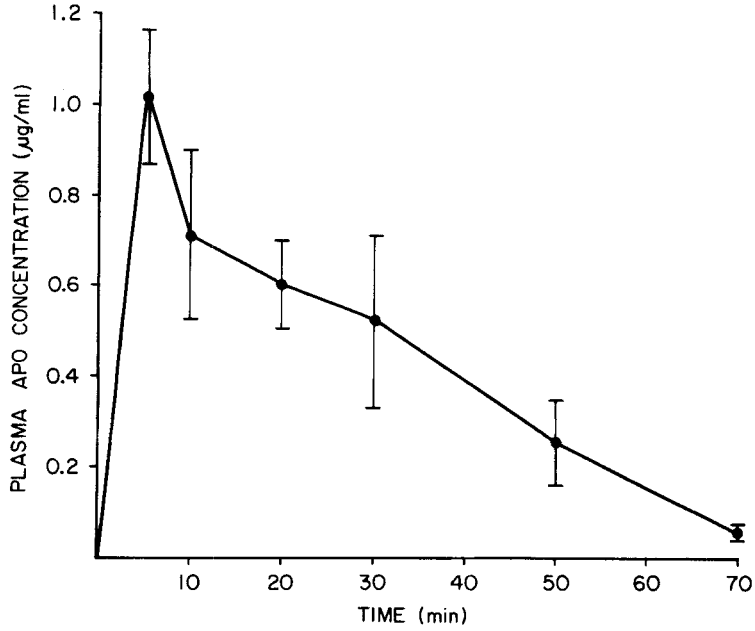

Fig. 4 Plasma APO concentration-time course in rats after intraperitoneal application of the drug 10 mg/kg. Each point represents the mean ± S.D. of four samples.

intraperitoneal administration of 2 mg/kg and 8 mg/kg of APO HCL were examined in different mouse strains. As shown in Table 1, there is no strain difference in APO concentrations, but clear dose effects. Figure 4 shows the time course of rat plasma APO levels following intraperitoneal injection of 10 mg/kg of this drug. These data are consistent with those reported by R.V. Smith et al (19), and H. Watanabe et al (16,20) who used HPLC with UV detector and a selected ion monitoring procedure, respectively. This essay is sensitive enough to detect brain and plasma levels of APO after administration of APO in doses commonly used in animal pharmacology ($\geq$2.0 mg/kg). For clinical doses ($\leq$0.1 mg/kg). Without modification, it is therefore unlikely that this assay will have the requisite sensitivity to detect APO levels in man after clinical doses.

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