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Note

Determination of apomorphine and N-*n*-propylnorapomorphine in plasma using high-performance liquid chromatography and fluorescence detection

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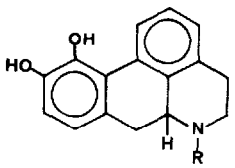
Apomorphine (I) is a drug that has been used as an emetic for many years. There is an increasing interest in I and related aporphines such as N-*n*-propylnorapomorphine (II) in the treatment of neurological disorders (e.g. Gilles de la Tourette's syndrome, Huntington's chorea, and Parkinsonism) which result from dopamine imbalances in the brain [1—7]. These studies have stimulated the development of gas chromatographic (GC) [8—10], spectrophotometric [11], thin-layer chromatographic [12], fluorometric [13], radioenzymatic [14—16], radioreceptor assay [17, 18], and high-performance liquid chromatographic (HPLC) (UV detection [19—21] and electrochemical detection [22]) methods for the determination of I in biological fluids. It was felt, however, that an HPLC procedure using fluorescence detection might provide advantages in sensitivity and/or convenience relative to methods developed earlier.

This paper describes a sensitive and selective procedure for the determination of I and II in plasma using HPLC with fluorometric detection. The devised method provides good accuracy and precision in the concentration range of 100—1000 ng/ml.



(I) Apomorphine: R = CH_3
(II) N-*n*-Propylnorapomorphine: R = $CH_2CH_2CH_3$

MATERIALS AND METHODS

R-(—)-Apomorphine hydrochloride hemihydrate was obtained from McFarland-Smith (Edinburgh, Great Britain). *R*-(—)-N-*n*-Propylnorapomorphine

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was supplied by the Sterling Winthrop Research Institute (Rensselaer, NY, U.S.A.). The aporphines were essentially homogeneous following HPLC [19, 21] and found to have greater than 98% purity based on UV spectroscopy [23]. Organic solvents were distilled-in-glass grade. Water was distilled and deionized. All other chemicals were reagent-grade or better. The diethyl ether used for extraction was distilled on the same day in which analyses were performed. Recently outdated human plasma was obtained through a local blood bank.

HPLC

The HPLC system used throughout consisted of an Altex 100A pump and Altex 210 injector, Schoeffel FS 970 LC fluorometric detector, and Altex integrator (Model C-RIA). The integrator was operated with the chart speed set at 2 cm/min and attenuation at 4. The detector was set at an excitation wavelength of 281 nm and fitted with filters 7-54 (bandpass; entrance) and 418 nm (cut-off; emission). Separations were achieved with an Altech μBondapak-phenyl column (15 cm × 4 mm I.D., particle size 10 μm) (Arlington Heights, IL, U.S.A.). The mobile phase consisted of 40% methanol in buffer (0.02 *M* sodium acetate—0.03 *M* acetic acid, pH 3.25) and was filtered through a Millipore PTFE-filter and degassed by ultrasonication before use. The flow-rate was set at 1.0 ml/min.

Extraction and recovery studies

In a 10-ml screw-capped culture tube, a mixture of 1 ml of human plasma and 100 μl of standard solution (I and II) was adjusted to pH 2.0 using 100 μl of 1 *N* hydrochloric acid. A 3-ml portion of toluene was added and the mixture vortexed for 30 sec at minimum speed (Vortex mixer, Scientific Industries, Bohemia, NY, U.S.A.) and centrifuged at ca. 800 *g* for 10 min. The toluene layer was discarded. A 200-μl portion of 1 *M* $NaHCO_3$ and a 100-μl portion of 15 m*M* of Tris—HCl [24] were added to adjust the pH to 7.0. The mixture was vortexed for 15 sec and a 3-ml portion of diethyl ether was added. After vortexing for 1 min at minimum speed, the mixture was centrifuged at ca. 800 *g* for 10 min to separate the layers. The diethyl ether was taken to dryness under a nitrogen stream at room temperature and the residue dissolved in 1 ml of mobile phase containing 0.5 mg/ml of $NaHSO_3$ [25]. A 100-μl portion of the solution was injected into the HPLC system.

Plasma standard curve

Apomorphine assay. Solutions of 0.1 mg/ml of I and 0.1 mg/ml of II (internal standard) were freshly prepared in 0.1 *N* hydrochloric acid containing 100 mg/ml of ascorbic acid [25]. These solutions were used to prepare standard solutions containing 1.0, 2.5, 7.5, 10.0 μg/ml of I and 5.0 μg/ml of II in 0.1 *N* hydrochloric acid.

N-n-Propylnorapomorphine assay. Solutions of 0.1 mg/ml of II and 0.1 mg/ml of I (internal standard) were freshly prepared in 0.1 *N* hydrochloric acid containing 100 mg/ml of ascorbic acid. These solutions were used to prepare standard solutions containing 1.0, 2.5, 7.5, 10.0 μg/ml of II and 5.0 μg/ml of I in 0.1 *N* hydrochloric acid.

Four 1-ml portions of plasma are spiked with drug (I or II; 100, 250, 750, and 1000 ng/ml) and internal standard (II or I; 500 ng/ml) by addition of 100-μl portions of aporphine standard solutions. Peak area ratios (drug/internal standard) are determined for each injection and plotted versus the concentration of drug (I or II).

Accuracy and precision of assay

Plasma test samples containing an equivalent of 150, 300, 450, 900 ng/ml of I or II and 500 ng/ml of I or II (Serving as internal standard) were extracted and analyzed in replicate as described above. Peak area ratios (drug/internal standard) were determined for each injection. Concentrations of I and II were calculated using a standard curve generated from the standards for each day of analysis. The means and relative standard deviations of these values were calculated.

RESULTS AND DISCUSSION

A satisfactory separation of I and II was achieved using a solvent system consisting of 40% methanol in acetate buffer, pH 3.25 (40 : 60) and a μphenyl reversed-phase HPLC column (capacity factor, k', I = 2.3; II = 3.3). The mobile phase is similar to ones previously described [19, 21] for these aporphines but is less complicated and permits complete chromatographic development within 10 min. A typical chromatogram resulting from the analysis of I in plasma is depicted in Fig. 1.

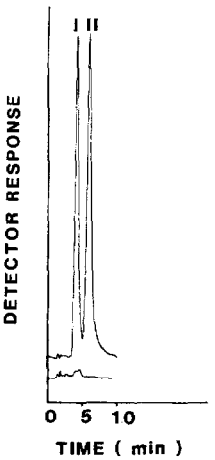

Fig. 1. Chromatograms of blank human plasma (lower trace) and human plasma spiked with apomorphine (I), 300 ng/ml and N-*n*-propylnorapomorphine (II), 500 ng/ml (upper trace).

The cleanup procedure incorporates a toluene pre-extraction of acidified plasma which removes potentially interfering fluorescent endogenous substances. After pre-extraction, the plasma is extracted with diethyl ether at pH 7.0 which has been shown previously to give quantitative recoveries of I and II in the μg/ml range [20].

The absolute mean recovery of I from plasma was 86.0 ± 10.1% (n = 15) at

levels of 250—1000 ng/ml; recovery of II averaged 83.6 ± 12.8 (n = 15) for samples containing 500 ng/ml. Consistently linear calibration curves for I/II peak area ratios (determination of I) in the concentration range of 100—1000 ng apomorphine per ml were typically obtained (slope = 0.021, y intercept = 0.059, r = 0.997) along with the II/I peak area ratios (determination of II) in the concentration range of 100—1000 ng N-n-polynorapomorphine per ml (slope = 0.002, y-intercept = —0.045, $r > 0.999$).

The results of replicate analyses of I and II in plasma are given in Tables I and II. The accuracy and precision of the assays for I and II are good.

Attempts to measure levels of I below 100 ng/ml revealed apparent endogenous interferences which affected accuracy adversary though R.S.D. values were about ± 10%. The detection limit of the method is approximately 50 ng/ml of I or II (signal-to-noise ratio ≈ 7).

The HPLC method described provides convenient measurement of nanogram quantities of I and II in plasma and improved sensitivity by a factor of approximately ten compared to previous chromatographic methods [19—21]. The method shows complete chromatographic development within 10 min and good accuracy and precision in the concentration range of 100—1000 ng/ml. This compares favorably with all literature HPLC procedures for apomorphine in plasma [19—21]. Westerink and Horn [22] report higher sensitivities for determinations of apomorphine in brain tissue using electrochemical detection, however, their method has not been extended to plasma. No comparable methods have been published for N-n-propylnorapomorphine.

TABLE I

ACCURACY AND PRECISION OF HPLC ASSAY FOR APOMORPHINE (I) IN HUMAN PLASMA

I concentration prepared (ng/ml)	Mean recovery (%)	R.S.D. (%)	n
150.0	104.4	8.2	8
300.0	105.0	7.0	8
450.0	102.4	6.6	7
900.0	92.9	8.7	8

TABLE II

ACCURACY AND PRECISION OF HPLC ASSAY FOR N-n-PROPYLNORAPOMORPHINE (II) IN HUMAN PLASMA

II concentration prepared (ng/ml)	Mean recovery (%)	R.S.D. (%)	n
150.0	104.8	4.9	6
300.0	97.2	4.2	5
450.0	102.8	5.6	6
900.0	102.8	4.1	6

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REFERENCES

1 F.C. Colpaert, W.F.M. Van Bever and J.E.M.F. Leysen, Int. Rev. Neurobiol., 19 (1976) 225.
2 C.A. Tamminga, M.H. Schaffer, R.C. Smith and J.M. Davis, Science, 200 (1978) 567.
3 I. Shoulson and T.N. Chase, Ann. Rev. Med., 26 (1975) 416.
4 E.S. Tolosa, G.C. Cotzias, P.G. Burckhardt, L.C. Tang and K.E. Dahl, Exp. Neurol., 55 (1977) 56.
5 G.C. Cotzias, P.S. Papavasiliou, E.S. Tolosa, J.S. Mendez and M. Bell-Midura, New Engl. J. Med., 294 (1976) 567.
6 M.K. Menon, W.G. Clark and J.L. Neumeyer, Eur. J. Pharmacol., 52 (1978) 1.
7 W.H. Riffee, R.E. Wilcox and R.V. Smith, Eur. J. Pharmacol., 54 (1979) 273.
8 R.V. Smith and A.W. Stocklinski, Anal. Chem., 47 (1975) 1321.
9 J.R. Miller, J.W. Blake and T. Tobin, Res. Commun. Chem. Path. Pharmacol., 15 (1976) 447.
10 D.M. Baaske, J.E. Keiser and R.V. Smith, J. Chromatogr., 140 (1977) 57.
11 P.N. Kaul, E. Brochmann-Hanssen and E. Leong Way, J. Amer. Pharm. Assoc., Sci. Ed., 48 (1959) 638.
12 P.W. Erhardt, R.V. Smith, T.T. Sayther and J.E. Keiser, J. Chromatogr., 116 (1976) 218.
13 W.K. Van Tyle and A.M. Burkman, J. Pharm. Sci., 60 (1971) 1736.
14 R.J. Baldessarini, in E. Usdin, J.D. Barchas and I.J. Kopin (Editors), Catecholamines: Basic and Clinical Frontiers, Vol. 2, Pergamon Press, New York, 1979, pp. 1596—1598.
15 A. Campbell, N.S. Kula, B. Jeppson and R.J. Baldessarini, Eur. J. Pharmacol., 67 (1980) 139.
16 J. Kebabian, J. Neurochem., 30 (1978) 1143.
17 A. Campbell, M. Herschel, B.M. Cohen and R.J. Baldessarini, Life Sci., 27 (1980) 633.
18 B.M. Cohen, B. Herschel, E. Miller, H. Mayberg and R.J. Baldessarini, Neuropharmacology, 19 (1980) 663.
19 R.V. Smith, J.C. Glade and D.W. Humphrey, J. Chromatogr., 172 (1979) 520.
20 R.V. Smith, D.W. Humphrey, S. Szeinbach and J.C. Glade, Anal. Lett., 12 (1979) 371.
21 R.V. Smith, A.E. Klein and D.O. Thompson, Mikrochim. Acta, I (1980) 151.
22 B.H.C. Westerink and A.S. Horn, Eur. J. Pharmacol., 58 (1978) 39.
23 W.H. Soine, P. Salgo and R.V. Smith, J. Labelled Compd. Radiopharm., 16 (1979) 597.
24 R.V. Smith and S.P. Sood, Anal. Lett., 5 (1972) 273.
25 R.E. Wilcox, D.W. Humphrey, W.H. Riffee and R.V. Smith, J. Pharm. Sci., 69 (1980) 974.