Journal of Chromatography, 231 (1982) 473–477
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1325

Note

Determination of prenalterol in plasma by high-performance liquid chromatography with fluorescence detection

C.J. ODDIE, G.P. JACKMAN* and A. BOBIK

Clinical Research Unit, Alfred Hospital, Commercial Road, Prahran, Victoria 3181 (Australia)

(Received January 19th, 1982)

Prenalterol, S-(−)-(4-hydroxyphenoxy)-3-isopropylamino-2-propanol, is a relatively new beta-adrenoceptor agonist with positive cardiac inotropic effects in man [1] making it of possible use in the treatment of congestive heart failure [2—6] or in overcoming beta-blockade produced by oxprenolol or metoprolol [4—7]. Some pharmacokinetic information has been obtained using gas—liquid chromatographic (GLC) assays using electron-capture detection [8] or mass spectrometric detection [9]. However, these assays while being of satisfactory sensitivity for most purposes involve time-consuming extraction and derivatisation steps and have not been evaluated for use in patients who may be taking other medication. We describe a relatively simple, yet sensitive, assay for prenalterol using high-performance liquid chromatography (HPLC) which is relatively free of interference from many common cardiovascular drugs which may be administered in addition to prenalterol. The detection of prenalterol is by its endogenous fluorescence on low wavelength excitation, a technique which we have successfully employed to detect catecholamines, serotonin and a beta-adrenergic blocking drug, pindolol [10—12].

EXPERIMENTAL

Reagents

Prenalterol hydrochloride was obtained from Ciba-Geigy (Basle, Switzerland). Stock solutions (1 g/l) were prepared in 0.1 *M* hydrochloric acid containing 0.3 m*M* EDTA and stored at 4°C for up to one month. More dilute solutions were prepared fresh daily. Acetonitrile 190 nm HPLC grade was obtained from Waters Assoc. (Milford, MA, U.S.A.). Diethyl ether, analytical grade was

0378-4347/82/0000—0000/$02.75

washed with 1 *M* sodium hydroxide, 1 *M* hydrochloric acid and water before use. Water was redistilled from alkaline potassium permanganate before use. All other reagents were of analytical grade.

Chromatographic system

A 5000 series liquid chromatograph with universal loop injector (Varian, Palo Alto, CA, U.S.A.) was used with a Spherisorb 5 μm ODS reversed-phase column (250 X 4.6 mm I.D.) (Laboratory Data Control, Riviera Beach, FL, U.S.A.). The detector was a Schoeffel FS970 fluorimeter (Schoeffel, Westwood, NJ, U.S.A.) with a deuterium arc source. Maximum prenalterol fluorescence was found on excitation at 220 nm while emission was selected with a glass filter (bandpass 320—400 nm).

Plasma samples

Drug-free venous blood was obtained from healthy human subjects receiving no other medication, and from volunteers receiving prenalterol 2.5 mg intravenously as a 5-min infusion. Plasma used to test for possible interferences was obtained from out-patients receiving medication for cardiac disorders, but not taking prenalterol. Blood was collected into plastic tubes containing lithium heparin and centrifuged for 10 min at 1000 *g* in a refrigerated centrifuge. Plasma was separated and stored at —20°C in plastic tubes until assayed.

Extraction of prenalterol and HPLC estimation

Plasma (2 ml) in stoppered 25-ml glass tubes is extracted with diethyl ether (10 ml) by 2 min shaking after the addition of sodium chloride (200 mg) and 2 *M* sodium carbonate (200 μl). The phases are separated by brief centrifugation and the ethereal phase transferred to clean tubes containing 100 μl of 0.1 *M* phosphoric acid adjusted to pH 3.0 with sodium hydroxide into which prenalterol is extracted by 2 min shaking. The diethyl ether is aspirated after brief centrifugation. Phase separation is simplified at each step by freezing the aqueous phase in a dry ice—ethanol bath. Aliquots (50 μl) of the extract are injected directly onto the chromatographic column. The mobile phase was 0.01 *M* perchloric acid—acetonitrile (4:1) at a flow-rate of 2 ml/min. Calibration is by the assay of known amounts of prenalterol (1 to 40 ng/ml) added to control plasma. The mean calibration curve from nine assays was $y = 8.14$ (S.D. 1.50)$x + 0.77$ (S.D. 3.65) where y is peak fluorescence intensity in nA and x is plasma concentration in ng/ml.

RESULTS AND DISCUSSION

Prenalterol isolated from plasma chromatographed with a retention time of 5.8 min and was well resolved from peaks present in control plasma (Fig. 1). Recovery of prenalterol in nine assays, assessed by the injection of known amounts of prenalterol onto the chromatographic column, averaged 70 ± 5% S.D. The standard curve from these assays is linear and shows only slight variation between assay days. The linearity has been found to extend to at least 200 ng/ml. Precision of the assay was assessed by replicate assays of known standards added to control plasma (Table I). The coefficient of variation (C.V.)

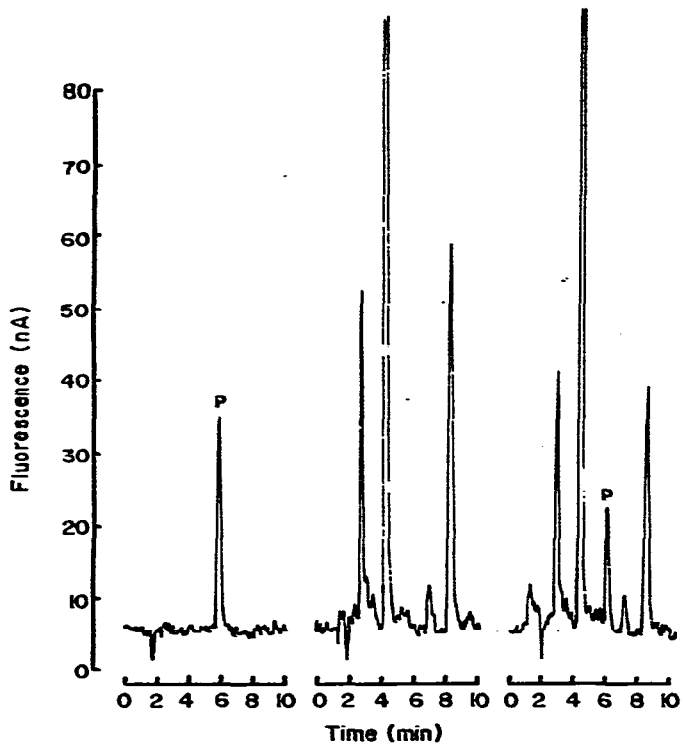

Fig. 1. Chromatographic traces. From left to right; 5 ng prenalterol standard (P) injected; extract of control plasma; extract of plasma containing 2.8 ng/ml prenalterol (P).

TABLE I

PRECISION OF THE ASSAY

Prenalterol (ng/ml)		No. of assays	C.V. (%)
Added	Found ± S.D.		
1	1.1 ± 0.16	4	15
2	2.1 ± 0.23	5	11
5	5.0 ± 0.22	6	4
10	10.1 ± 0.40	6	4
25	26.8 ± 2.1	6	8
100	110.5 ± 6.3	6	6

of 15% at 1 ng/ml is an improvement over the GLC assays, C.V. 20% at 5 ng/ml with electron-capture detection [8] and 14% at 2.5 ng/ml with mass spectrometry [9].

Selectivity was assessed by analysing plasma from patients likely to be taking a variety of cardiovascular drugs (see *Plasma samples*). Drugs known to have been prescribed in this group were methyldopa, amiloride, chlorothiazide, cyclopenthiazide, diazepam, digoxin, frusemide, glycerol trinitrate, metoclopramide, naproxen, pindolol, quinidine, sorbide nitrate and thyroxine. Interfering peaks were observed in only one sample from a patient taking nitra-

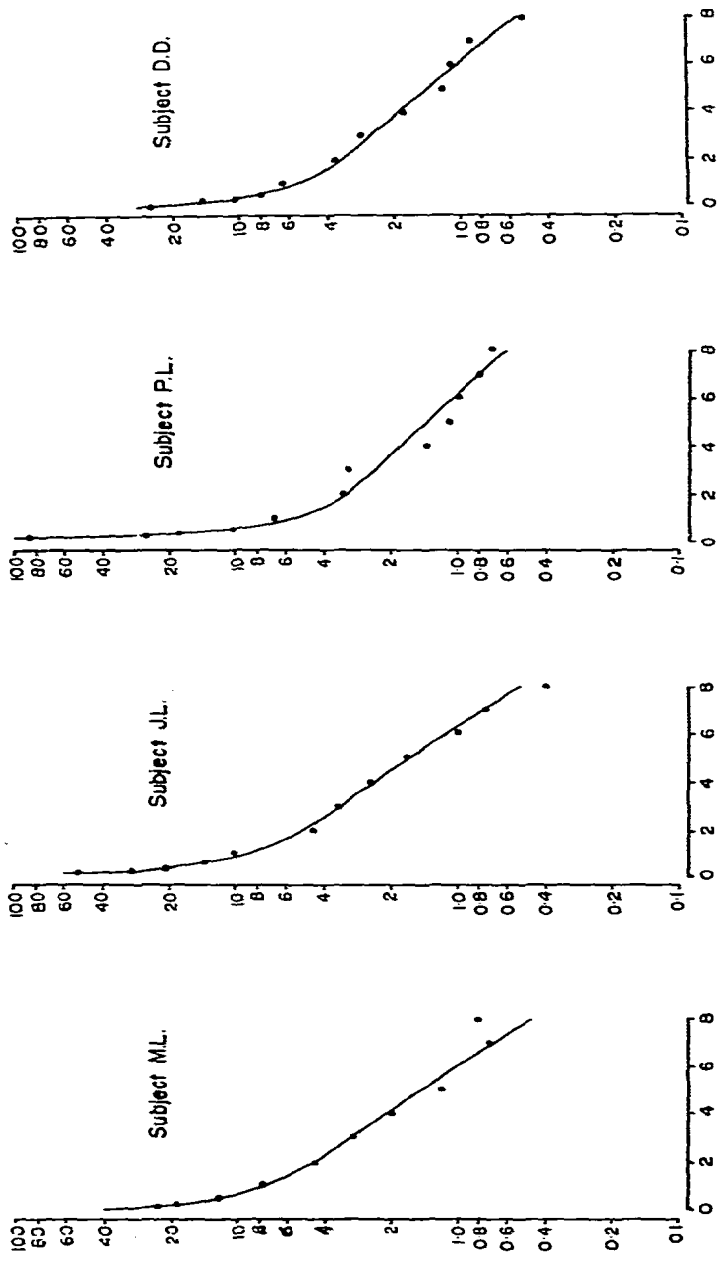

Fig. 2. Plasma levels of prenalterol following 2.5 mg given as a 5-min intravenous infusion, in four normal subjects.

zepam. The assay was used initially to determine the plasma concentrations of prenalterol in four normal subjects administered 2.5 mg intravenously. The plasma concentration—time curves in these subjects (Fig. 2) are similar to those previously reported using GLC assays [3, 6, 7, 9].

In summary, the assay described has the advantage over the GLC assays of employing a simpler extraction procedure and improved sensitivity without derivatization. While internal standardisation may be included in the assay as with the GLC assay [8] it is not necessary and may lead to drug interference. The relative freedom from interference by commonly used cardiovascular drugs makes the assay suitable for patient monitoring.

REFERENCES

1 E. Carlsson, C.G. Dahlöf and A. Hedberg, Naunyn Schmiedebergs Arch. Pharmacol., 300 (1977) 101—105.
2 G. Johnsson, L. Jordö, P. Lundberg, O. Rönn, I. Welin-Fogelberg and J. Wikstrand, Eur. J. Clin. Pharmacol., 13 (1978) 163—170.
3 O. Rönn, E. Fellenius, C. Graffner, G. Johnsson, P. Lundberg and L. Svensson, Eur. J. Clin. Pharmacol., 17 (1980) 81—86.
4 T. Lysbo Svendsen, O.J. Hartling and J. Trap-Jenssen, Eur. J. Clin. Pharmacol., 18 (1980) 219—223.
5 A. Weiss, B. Pfister, P. Imhof, P.H. Degen, D. Burckhardt and U.C. Dubach, Eur. J. Clin. Pharmacol., 18 (1980) 383—390.
6 D.H.T. Scott, G.R. Arthur, R.N. Boyes and D.B. Scott, Brit. J. Clin. Pharmacol., 7 (1979) 365—370.
7 O. Rönn, C. Graffner, G. Johnsson, L. Jordö, P. Lundberg and J. Wikstrand, Eur. J. Clin. Pharmacol., 15 (1979) 9—13.
8 P.H. Degen and M. Ervik, J. Chromatogr., 222 (1981) 437—444.
9 C. Graffner, K.J. Hoffman, G. Johnsson, P. Lundberg and O. Rönn, Eur. J. Clin. Pharmacol., 20 (1981) 91—97.
10 M. Bangah, G. Jackman and A. Bobik, J. Chromatogr., 183 (1980) 255—259.
11 G.P. Jackman, V.J. Carson, A. Bobik and H. Skews, J. Chromatogr., 182 (1980) 277—284.
12 G.P. Jackman, Clin. Chem., 27 (1981) 1202—1204.