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Note

Determination of prenalterol in plasma and urine by liquid chromatography with electrochemical detection

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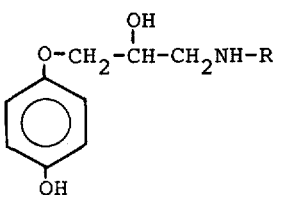
Prenalterol is a selective beta-1-adrenoceptor agonist in man [1] and animals [2]. Prenalterol is structurally related to beta-adrenoceptor blocking agents (Fig. 1) and has been assayed in biological fluids after perfluoroacylation by gas chromatography with electron-capture [3] or mass spectrometric [4] detection. The latter technique allows determination of plasma concentrations down to less than 5 nmol/l. Recently, a liquid chromatographic method



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Prenalterol	$-CH(CH_3)_2$
H 133/12 (internal standard)	$-C(CH_3)_3$

Fig. 1. Chemical structures of prenalterol and internal standard (H 133/12).

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offering equivalent sensitivity with fluorometric detection has been suggested by Oddie et al. [5].

In the present paper, liquid chromatography was combined with electrochemical detection for the determination of prenalterol in plasma samples in concentrations as low as 2 nmol/l.

EXPERIMENTAL

Reagents

Prenalterol and the internal standard, H 133/12, were used as hydrochlorides and obtained from the Department of Organic Chemistry, AB Hässle (Mölndal, Sweden). Stock solutions (100 μmol/l) were prepared in 0.01 *M* hydrochloric acid and could be stored at 4° C for more than a month. More dilute working standards, 2 μmol/l for prenalterol and 4 μmol/l for H 133/12, were prepared weekly from the stock solutions. HPLC grade methanol was used in the mobile phase (E. Merck, Darmstadt, F.R.G., or Fisons, Loughborough, U.K.). Diethyl ether (May and Baker, p.a. quality) was distilled before use. Hexane was HPLC grade from Rathburn Chemicals (Walkerburn, U.K.). All other reagents were prepared from analytical grade chemicals.

All glassware was washed in an automatic dishwasher with detergent at pH 12, rinsed with phosphoric acid solution (pH 2) and repeatedly with distilled water and finally dried at 70° C before use.

Chromatographic system

The liquid chromatograph consisted of an LDC Model 71147 pump with an extra pulse-dampener or a Waters M6000 pump, a Rheodyne injection valve with a 150-μl sample loop, a stainless-steel column (150 × 4.5 mm) and a BAS LC-4 electrochemical detector (Bioanalytical Systems, Indiana, U.S.A.) with a glassy carbon working electrode, operated at +0.70 V. The separation column was packed with LiChrosorb RP-8, 5-μm average particle diameter (Merck). The mobile phase was a citrate—acetate buffer (pH 3.5) containing 15% methanol and 10^{-2} mol/l propylamine. The composition of the buffer was sodium acetate 106 mmol/l, sodium hydroxide 59 mmol/l and citric acid 200 mmol/l. The flow-rate was 1 ml/min, giving a retention time of about 6 min for prenalterol and 9 min for the internal standard H 133/12.

Analytical procedure

The frozen plasma sample was allowed to thaw at room temperature and was mixed and centrifuged. A 1.00-ml volume of the sample or reference sample (100 nmol/l) was transferred to a 15-ml centrifuge tube, 0.4 g of sodium chloride and 100 μl of carbonate buffer were added (final pH 9.5—10.0) and prenalterol was extracted with 6.00 ml of diethyl ether by shaking for 10 min.

After centrifugation for 5 min, 5.00 ml of the organic phase were transferred to a 5-ml tapered centrifuge tube and prenalterol was back-extracted with shaking for 10 min into 250 μl of orthophosphoric acid 0.10 mol/l. After centrifugation the ether phase was sucked off and the aqueous phase was shaken twice with 1.5 ml of hexane for 2 min for removal of dissolved diethyl ether. After centrifugation 100 μl of the aqueous phase were injected via the injection valve onto the chromatographic column.

Calibration and accuracy

Calibration was effected by adding 50 μl of working standard (2 μmol/l) of prenalterol to 1 ml of blank plasma and taking these standards (n = 4) through the analytical procedure. The peak heights for prenalterol in the reference samples in the chromatograms were measured and used to calculate the drug concentratation in the authentic samples. The recovery was determined by comparison with a directly injected reference solution of prenalterol. When internal standard (H 133/12) was used to compensate for minor variations in detector response and for volume changes, 50 μl of the working standard (4 μmol/l) were added to the plasma sample. The ratios between the peak heights of prenalterol and the internal standard in the chromatograms from the reference samples were then used to evaluate the content of prenalterol in the authentic samples.

Assay of prenalterol in urine

In urine unchanged prenalterol was determined, using a similar method to that in plasma; 0.5 ml of sample was taken and 0.5 ml of sodium carbonate 0.5 mol/l was used to buffer the sample to pH 9.5. A lower potential (+0.55 V) was used to decrease the influence from interfering endogenous compounds.

RESULTS AND DISCUSSION

Extraction

The extraction of prenalterol from aqueous solution was thoroughly investigated in a previous paper [4]. Due to the zwitterionic character of the compound, maximum extraction is achieved at pH 9.5. Different organic solvents were tested and the extraction recovery was found to be improved significantly by addition of sodium chloride to the aqueous phase. The purification of the final phosphoric acid extract by mixing with hexane was found to be necessary since diethyl ether dissolved in the aqueous phase interfered severely in the chromatographic procedure.

Liquid chromatography

Acetate—citrate buffer was preferred to phosphate buffer in the mobile phase, since about 50% higher detector response was obtained. Propylamine was included as modifier in the mobile phase to improve the chromatographic behaviour of prenalterol. A chromatogram from a blank plasma sample is shown in Fig. 2 and from the same sample after addition of prenalterol (Fig. 3A), at a concentration in the proximity of the sensitivity of the method. A chromatogram from an authentic plasma sample is illustrated in Fig. 3B.

Recovery and precision

The recovery of prenalterol from spiked plasma samples was 100% at the 4 μmol/l level and 97% at the 15 nmol/l level, the same figure as that calculated from distribution constants. The repeatability (S.D., %) on analysing replicate samples (n = 8) was 2.4 and 1.6, respectively, at these two concentration levels. The minimum determinable concentration (S.D. $\leq$ 10%) was 2 nmol/l.

In urine the recovery was 100% and 103%, respectively, with relative

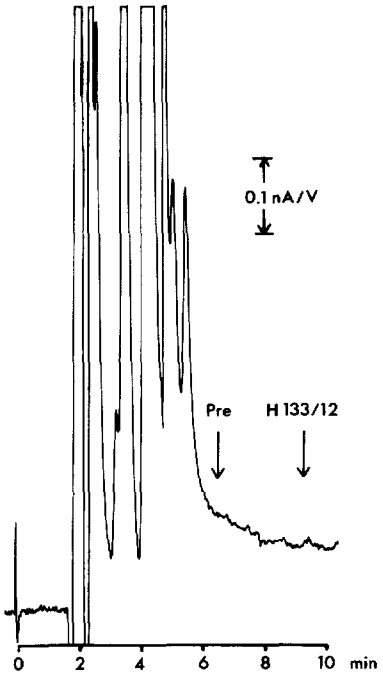

Fig. 2. Chromatogram from blank plasma sample. Stationary phase: LiChrosorb RP-8, 5 μm. Mobile phase: acetate—citrate buffer, pH 3.5, containing 10^{-2} *M* propylamine and 10% methanol. Potential: +0.70 V.

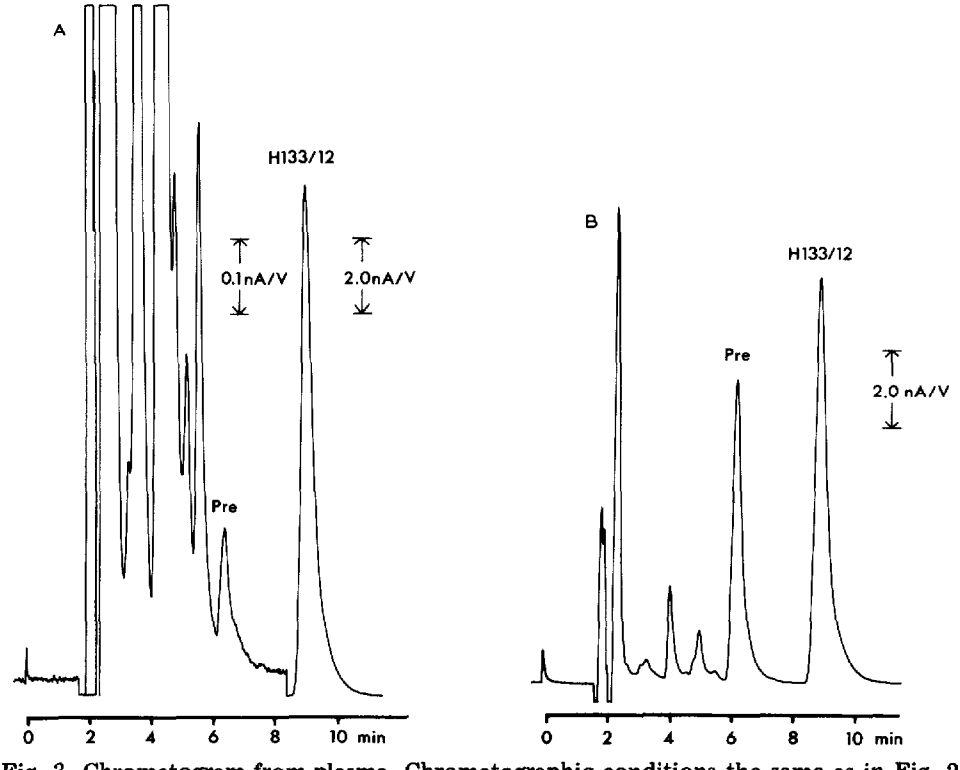

Fig. 3. Chromatogram from plasma. Chromatographic conditions the same as in Fig. 2. (A) Spiked plasma sample containing 2 nmol/l prenalterol. (B) Authentic plasma sample containing 80 nmol/l prenalterol.

standard deviations of 2.5% at concentration levels of 0.2 and 2 μmol/l of urine. The minimum determinable concentration (S.D. ⩽ 10%) was 20 nmol/l.

Stability

The chromatographic system and the electrochemical detector showed good long-term stability. Intermittently the upper part (1—2 mm) of the column packing was replaced with new material. No interference from plasma constituents or drugs expected to be co-administered was observed. This analytical method was evaluated against a gas chromatographic—mass spectrometric method as reported previously [4] and showed a good correlation. The stability of prenalterol in plasma samples on storage at −18°C was also examined [4] and no changes were found over a period of six months.

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