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The gas chromatographic determination of atenolol in biological samples

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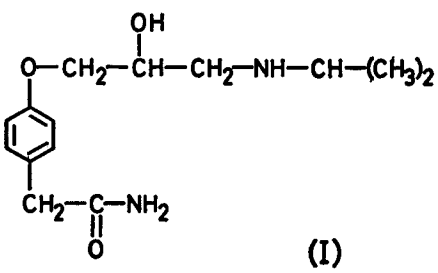
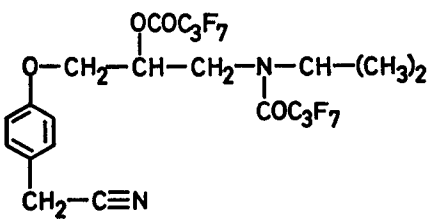
Methods are described for the gas chromatographic determination of the β-adrenoceptor blocking agent atenolol (ICI 66,082 Tenormin) in whole blood, serum, tissue and urine. The method involves the extraction of the free base from the prepared biological sample into an organic solvent mixture. Further purification is done by back-extracting the base into dilute acid from which, after basification it is extracted again into organic solvent. Any atenolol in the residue remaining after solvent evaporation is converted into a heptafluoro-butyrate derivative ready for gas chromatography. Use of an electron-capture detector enables concentrations as low as 0·01 μg ml^{-1} body fluid and 0·04 μg ml^{-1} tissue to be measured.

Atenolol [4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide, I] is a cardio-selective β-adrenoceptor blocking agent, which possesses neither intrinsic sympathomimetic activity nor membrane stabilizing properties (Barrett, Carter & others, 1973). The compound could be valuable as a antihypertensive agent in man (Hansson, Åberg & others, 1973; Amery, Billiet & others, 1973).

A fluorescence assay for this compound (Kaye, 1974) is rapid and reasonably specific for the unchanged drug but suffers from a lack of sensitivity.



(I)



(II)

The gas chromatographic method described below is sensitive to 0·01 μg ml^{-1} and enables plasma concentrations to be measured for at least 5-half lives after the peak concentration is obtained with a therapeutic oral dose of 100 mg per total body weight. This is the method already referred to in the clinical studies of Graham, Littlejohns & others (1973).

MATERIALS AND METHODS

Gas chromatography

A Hewlett-Packard Model 402B was used, fitted with an automatic sample injector model 7670A/71A. A glass column (1·50 m × 6 mm i.d.) packed with 3·8% UCW 98 on Gas-Chrom Q, 80–100 mesh was used at an oven temperature of 185°, the flash

heater at 200° and the detector at 330°. The electron capture detector (^{63}Ni) was operated with a pulse interval of 150 μs and a purge gas of 10% methane in argon at 30 ml min^{-1}. The carrier gas was argon at 60 ml min^{-1}. Under these conditions the retention time of the heptafluorobutyrate derivative (II) was about 6 min.

Gas chromatography—mass spectrometry. The structure of the heptafluorobutyrate of the drug was examined using both the direct inlet and the coupled gas chromatograph of an LKB 9000. Results obtained with the isolated derivative and with derivatized extracts from biological samples confirmed the results of elemental analysis, which suggested the structure as (II).

Reagents

Cyclohexane, ethyl acetate and n-butanol AR were used without further purification. Diethyl ether was distilled and stored in the dark over sodium wire. Other solvents were used as purchased. Commercially available HFB was unsuitable; it was prepared from the free acid by treatment with a large excess of phosphorus pentoxide (16 h at room temperature followed by 2 h refluxing). The product was distilled through a 12 inch Vigreux column, and the HFB (b.p. 107–108°) was satisfactory for at least 8 weeks if kept under anhydrous conditions.

Standard solutions of atenolol, 0·01 and 1·0 mg ml^{-1} in methanol, were prepared weekly. These were used to prepare known concentrations of drug (0·01 to 10·0 μg ml^{-1}) in body fluids or tissue homogenates from undosed control animals; from these, calibration curves of concentration versus peak height were prepared, and used to obtain the concentration of drug in the unknown test samples.

Oxprenolol added at 1·0 μg ml^{-1} body fluid or g^{-1} tissue, was a satisfactory internal standard, with a retention time of about 3 min.

Biological samples

Venous blood samples were oxalated, and the blood and prepared plasma were stored at 0–4° for no more than 3 weeks before assaying. Urine and tissue samples were kept at −15°.

Procedure for body fluids

Oxalated whole blood, plasma or dilute urine (1·0 ml) is added to dilute ammonia (2·0 ml, 0·1 N) in stoppered glass tubes and the mixture shaken mechanically (5–10 min) with n-butanol containing 30% cyclohexane (8·0 ml). A 7·5 ml portion of the organic layer is extracted with 2·0 ml of 0·1 N HCl and the acid extract then washed by inversion with clean solvent mixture (3 ml), which is then aspirated as completely as possible. The solvent-free acid layer is basified with ammonia (2·0 ml, 1·0 N) and extracted with the butanol–cyclohexane solvent mixture (8·0 ml). A 7·5 ml aliquot of the organic solvent is then transferred to a 15 ml glass stoppered tube and evaporated to dryness at 80° under a stream of nitrogen. The dry residue is dissolved in diethyl ether (0·3 ml) and reacted for 10 min at room temperature with HFB (0·1 ml). After evaporation in a stream of dry nitrogen (20 to 40 min) the residue is dissolved in ethyl acetate (1·0 to 10·0 ml) and a 1 μl portion containing 0·0 to 1·0 ng drug injected onto the gas chromatograph.

Tissue samples. These were prepared as 25% homogenates in 0·5 N ammonium hydroxide before extraction of a 1·0 ml aliquot with n-butanol containing 30% cyclohexane (8·0 ml). The procedure was then identical with that for body fluids.

RESULTS AND DISCUSION

The gas chromatographic peak of the heptafluorobutyrate derivative (II) is well defined, and the relation between peak height or area, and the weight of drug is linear over the range 0 to 1·0 ng injected onto the chromatograph.

The extraction of drug from biological samples was optimized by using n-butanol containing 30% cyclohexane; it was pH-stable, ensured good extraction efficiency (80% from alkali, and 98% back into 0·1 N HCl) and it had a low water capacity (Fig. 1) resulting in minimum interference from contaminating materials present in whole blood, brain and muscle.

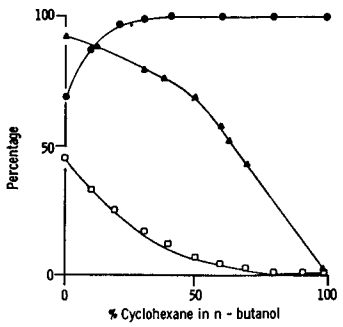


FIG. 1. The effect of different cyclohexane to n-butanol ratios on the percentage extraction of atenolol from (▲) 1·0 ml plasma plus 2·0 ml 0·1N NH_4OH into 8·0 ml of solvent mixture, and from (●) 8·0 ml solvent mixture into 2·0 ml 0·1N HCl. (□) The percentage of the alkaline aqueous phase dissolving in the solvent mixture.

The overall throughput of drug from body fluids and tissues was about 55%, the sensitivity is such as to allow concentrations as low as 0·01 μg ml^{-1} body fluid and 0·04 μg g^{-1} tissue to be measured. In a complete assay of 14 separate 1·0 ml-blood samples, each fortified with 1·0 μg drug (1·00 μl of a 1·0 mg ml^{-1} standard), the coefficient of variation was $\pm$3·5%.

The drug is slightly unstable in oxalated whole blood, decomposing at about 10% per month at 0–4°.

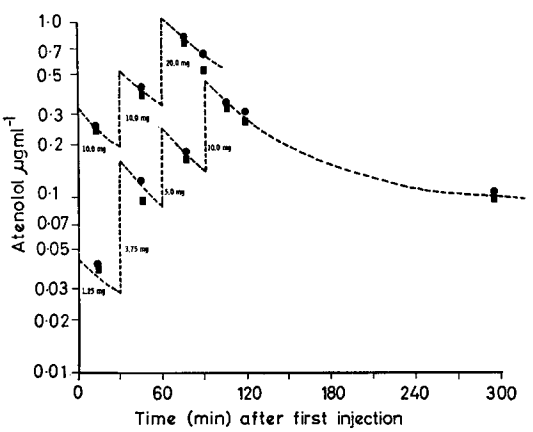


FIG. 2. Blood (●) and plasma (■) concentrations of atenolol in two volunteers after multiple intravenous administration. Doses are mg per total body weight. Lines are lines of best fit.

The method is specific for the drug. No g.l.c. peaks were obtained from frusemide, chlorthalidone, hydrochlorthiazide, α-methyldopa, hydralazine and diazepam. Additional peaks were obtained with practolol, propranolol, oxprenolol, timolol, tolamolol and chlordiazepoxide, but they did not interfere.

Whole blood and plasma concentrations of atenolol

Preliminary results of blood and plasma concentrations in 2 volunteers given incremental 1 min-intravenous infusions, at 30 min intervals are illustrated in Fig. 2. Venous blood was sampled at 15 min after each dose and additionally at 30 and 200 min after the last dose. On average, the concentration in whole blood is approximately 10% higher than in plasma, suggesting that the red cell concentration is about 20% higher than that of plasma. After each infusion the increase in circulating drug is strictly proportional to the dose. The lines of best fit, show that there is probably an initial distribution phase with a half-life of 20 to 30 min, but that by 1 to 2 h after the dose the half-life has increased to 5 to 6 h.

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