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Note

Simultaneous determination of the propranolol enantiomers in biological samples by gas–liquid chromatography

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Propranolol, a well known beta-adrenoceptor antagonist, is a mixture of equal amounts of its laevo and dextro isomers. The two enantiomers have been reported to have different pharmacological effects[1–3] and different elimination kinetics[4]. It is, however, useful to determine both enantiomers after administration of racemic propranolol, in order to compare the disposition of the enantiomers, since only the *l*-isomer affects hepatic blood flow, thereby decreasing hepatic clearance[5]. This has recently been achieved using stereospecific antibodies for *d,l*-propranolol and *l*-propranolol[6].

We report an alternative gas chromatographic method for the resolution and quantitative determination of propranolol enantiomers after administration of the racemic form. The method was used to investigate enantiomer distribution in the rat.

MATERIALS AND METHODS

Standard and reagent

d- and *l*-propranolol hydrochloride and *d,l*-propranolol hydrochloride were kindly supplied by I.C.I. (Macclesfield, Great Britain). N-Heptafluorobutyryl anhydride and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Fluka (Milan, Italy) and Pierce (Rockford, Ill., U.S.A.), respectively. The resolving reagent N-heptafluorobutyryl-1-prolyl chloride was prepared as previously described[7] and stored at −20° until used.

Apparatus

A Carlo Erba gas chromatograph with a ^{63}Ni electron-capture detector was used. The chromatographic column was a glass tube (2 m × 4 mm I.D.) packed with Chromosorb W HP (80–100 mesh) and 3% OV-225 (Pierce). Column temperature, 250°; injector port temperature, 325°; detector temperature, 300°; carrier gas (nitrogen) flow-rate, 60 ml/min.

Animals

Female Charles River rats (175 ± 10 g) were treated with racemic propranolol (10 mg/kg, i.v.) and killed at various times after injection. The brain, heart and lungs were removed immediately after death and stored at −20° until assay. Blood was analyzed immediately.

Extraction procedure

To 0.5–2.0 ml of water or blood were added, various amounts of the two propranolol enantiomers (25–300 ng) together with 1 ml of borate buffer (pH 9.5) and 0.5 g of NaCl. The anantiomers were extracted twice with 4 ml of ethyl acetate[8], and centrifuged to separate the layers after each extraction.

Tissues were homogenized (6 mg/g) in cold acetone–1 *N* formic acid (85:15). After centrifugation at 4°, the supernatant was mechanically shaken twice with *n*-heptane–chloroform (4:1). The organic phase was discarded and the aqueous phase (previously adjusted to pH 9.5 with 2 *N* NaOH) was used for propranolol extraction as described for blood.

Derivative formation

To form diastereoisomers, 100 μl of N-heptafluorobutyryl-l-prolyl chloride were added to the ethyl acetate extract. After 60 min, the solution was briefly shaken with 7 ml of 0.05 *M* sodium hydroxide. The aqueous phase was re-extracted with ethyl acetate (after addition of NaCl) and the combined extracts were evaporated to dryness *in vacuo*. To make the derivatives more volatile, the hydroxyl groups of the two diastereoisomers were esterified with 25 μl of BSTFA in the presence of 75 μl of benzene after heating at 50° for 30 min. The derivatives were then diluted to 0.5–2 ml benzene, containing diazepam (0.5 ng/μl) as an internal marker, and 1 μl of the final solution was injected into the gas chromatograph for analysis.

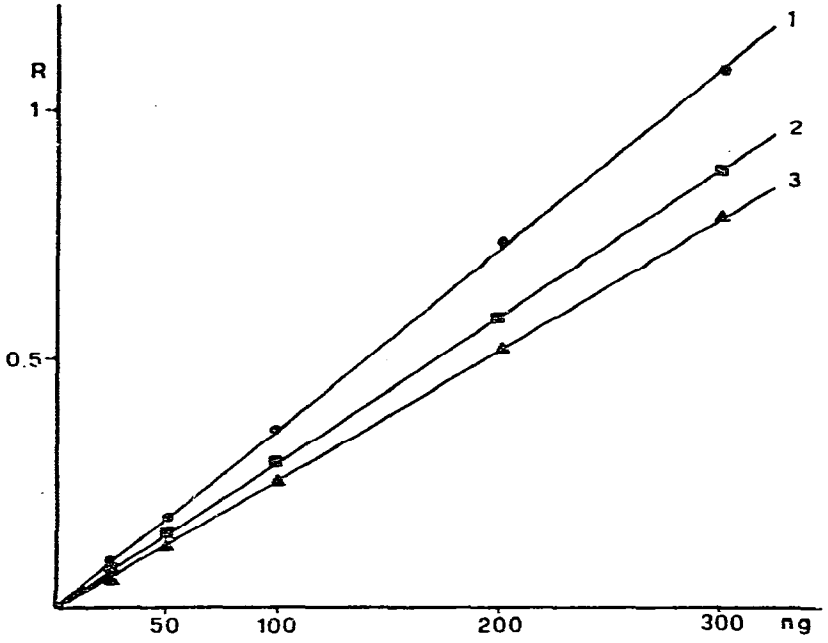

Fig. 1. Calibration curves of the propranolol enantiomers from water (1) blood (2) and tissues (3). The curves of the *d*- and *l*-enantiomers coincide.

RESULTS AND DISCUSSION

Derivatization of enantiomers with an optically active reagent is known to yield diastereoisomers which can often be separated by gas–liquid chromatography (GLC)[9–14]. We have previously reported the GLC resolution of some beta-adrenoceptor antagonists[7] with N-trifluoroacetyl-l-prolyl chloride (TPC)[9] and N-heptafluorobutyryl-l-prolyl chloride[13] as chiral reagents.

In this assay procedure the propranolol enantiomers were resolved as N-heptafluoro-l-prolyl derivatives and silyl esters and measured by electron capture detection.

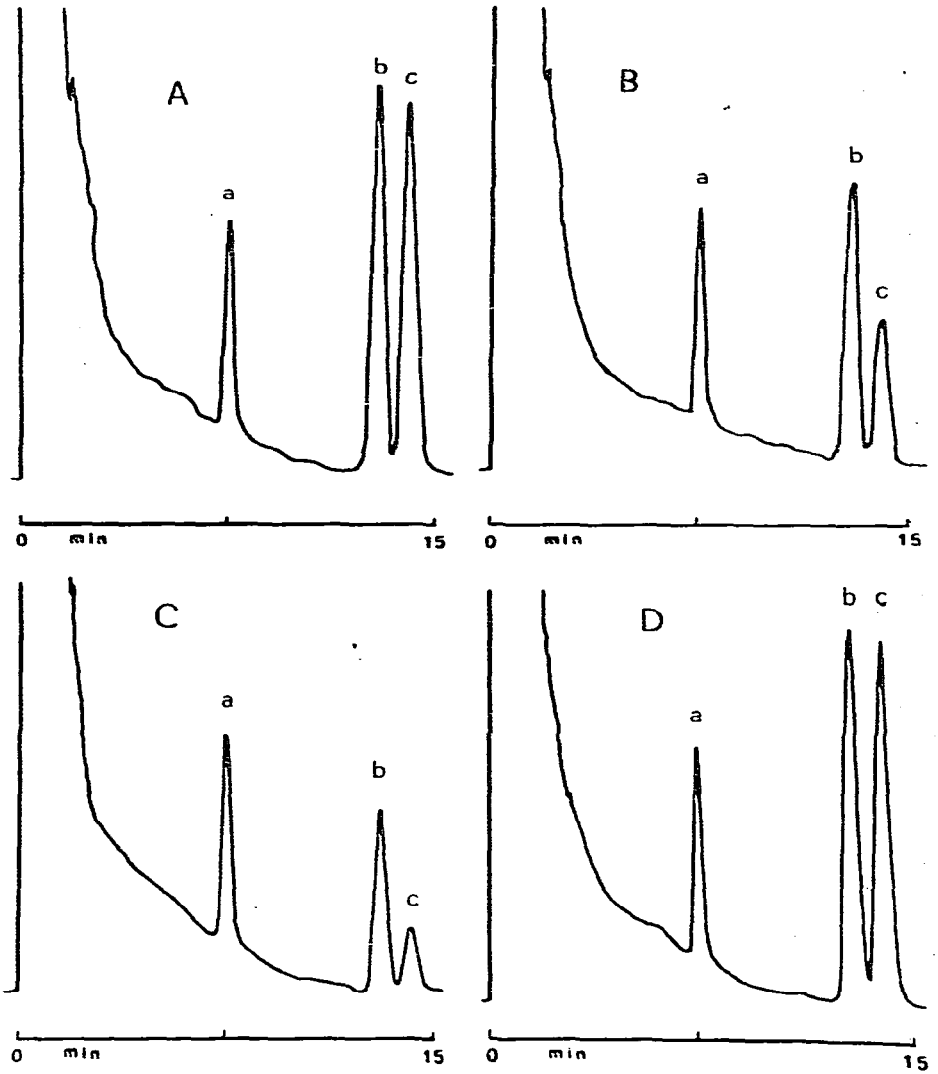

Fig. 2. Gas chromatograms of heart extracts obtained from rats 30 (A), 120 (B) and 240 min (C) after administration of *d,l*-propranolol (10 mg/kg, i.v.), and of heart extract (D) with *d,l*-propranolol added (internal standard sample). Peaks: a = diazepam (internal marker); b = *l*-propranolol; c = *d*-propranolol.

The diastereoisomers were sufficiently separated, with a resolution factor[15] of 1.4, and were stable for at least one week at room temperature. The peak area ratio of an equal mixture of the anantiomers was 1.0 ± 0.2. Any column producing a different ratio was discarded.

The calibration graphs for *d*- and *l*-propranolol added to blood and tissues are illustrated in Fig. 1 and coincide completely. The graphs obtained by plotting the ratio of the peak areas of the diastereoisomers to that of the internal marker against known concentrations of propranolol enantiomers are linear in the range from 25 to 300 ng per sample. The recovery from blood was 80 ± 3.5% and from tissues 70 ± 5%. The minimum detectable amount was 25 ng per sample. Fig. 2 shows typical chromatograms obtained from heart extracts of rats treated 30 (A), 120 (B) and 240 min (C) before death with racemic propranolol hydrochloride (10 mg/kg, i.v.); (D) is the chromatogram of an internal standard sample (*d,l*-propranolol added to the homogenized heart).

The pharmacokinetics of *d*- and *l*-propranolol were followed in rats after administration of racemic propranolol (10 mg/kg, i.v.). The curves of blood concentration against time (Fig. 3) show a biphasic decline for both enantiomers, with an initial rapid phase during the first 30 min followed by a second slower phase. The half-life of the slow phase was 44 min for *d*-propranolol and 64 min for *l*-propranolol. The apparent volume of distribution (V_β) was 4.9 l/kg and 7.15 l/kg for *d*- and *l*-propra-

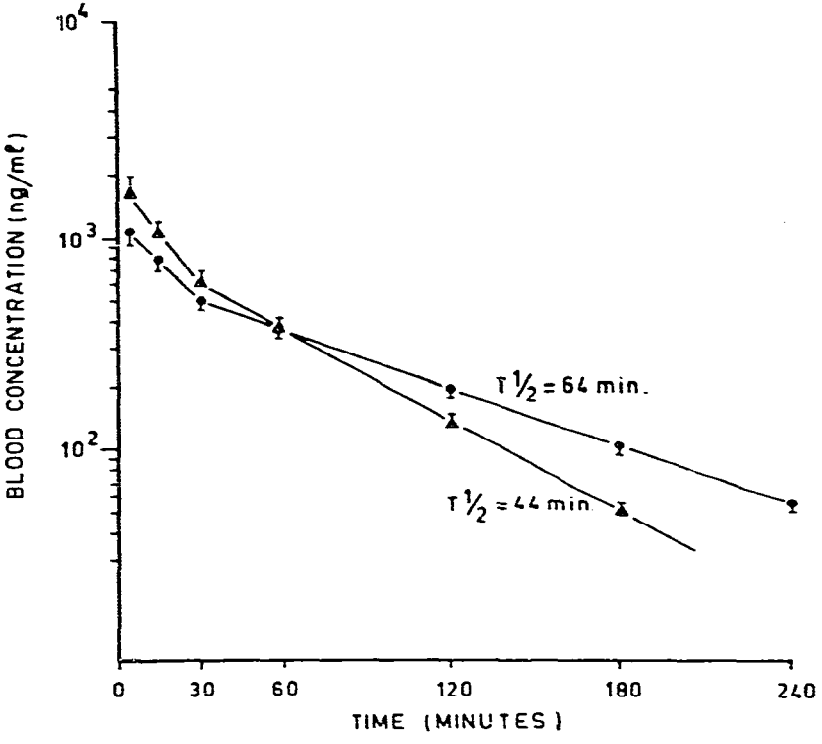

Fig. 3. Curves of blood concentration against time for the enantiomers after administration of racemic propranolol hydrochloride (10 mg/kg, i.v.). ●—●, *l*-Propranolol; ▲—▲, *d*-propranolol. Each point represents the average from four animals.

nolol respectively. Systemic clearance was practically the same for both enantiomers and was calculated to be 77.5 and 77.1 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively. The curves in Fig. 3 reflect the distribution of the enantiomers into various tissues.

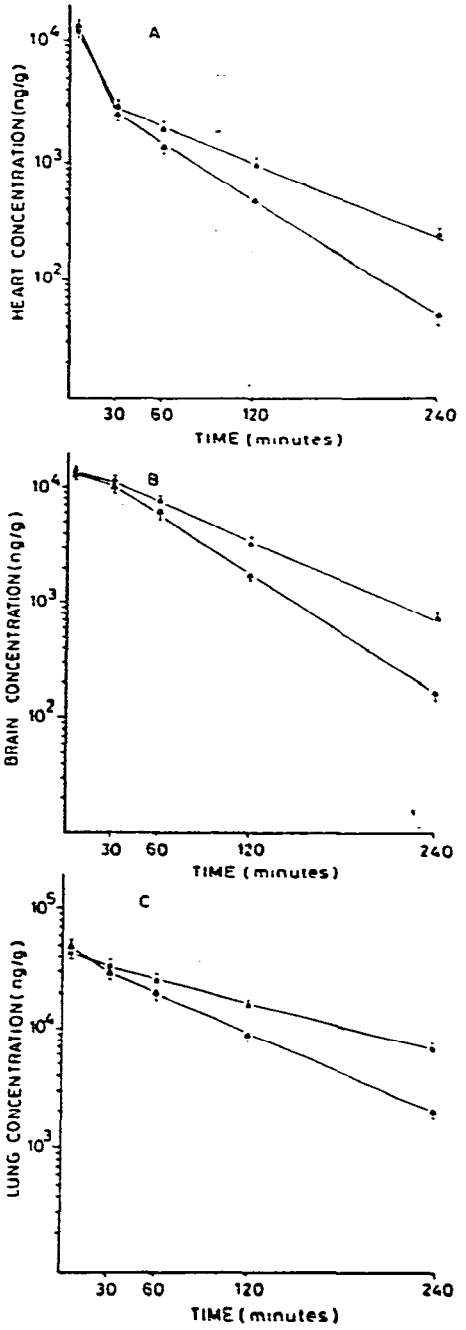

Fig. 4. Disappearance curves of *d*- and *l*-propranolol from heart (A), brain (B) and lungs (C) after i.v. injection of 10 mg/kg *d,l*-propranolol hydrochloride. ●—●, *l*-Propranolol; ▲—▲, *d*-propranolol. Each point represents the average from four animals.

Fig. 4 shows the disappearance curves of the enantiomers from heart (A), brain (B) and lungs (C). The concentration ratio of the enantiomers (*l*:*d*) rises gradually from 1.0 at 30 min to 4.0 at 240 min after drug administration. As reported by other investigators[16–18], the lungs gave the highest concentration of propranolol compared to the other tissues.

These results are in agreement with previous studies which reported differences in the disposition of the two enantiomers[4,17]. The differences observed may be significant in explaining the different pharmacological activity of the two enantiomers.

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