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QUANTITATIVE DETERMINATION OF PROPRANOLOL, PROPRANOLOL GLYCOL AND N-DESISOPROPYLPROPRANOLOL IN BRAIN TISSUE BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

A method for the quantitative determination of propranolol and two of its active metabolites, 3-(α-naphthoxy)-1,2-propanediol (propranolol glycol) and N-desisopropylpropranolol, in brain tissue of mice is described. Tissues are homogenized in perchloric acid–acetonitrile. Propranolol and its metabolites are isolated from the supernatant by solvent extraction and separated and detected as their trifluoroacetyl derivatives by electron capture gas chromatography. Chemical structures of the derivatives were confirmed by gas chromatography–mass spectrometry. The electron capture detector response of all three compounds is high, 0.7–2.0 $\times 10^{-16}$ moles/sec. Brain levels of 10–250 ng/g can be detected of all three compounds with high specificity and good precision.

INTRODUCTION

Studies of the metabolism of propranolol in man and laboratory animals have resulted in the identification of thirteen phase I metabolites[1–6]. Recent investigations in this laboratory have shown[7] one of these metabolites, 3-(α-naphthoxy)-1,2-propanediol (propranolol glycol), to be twice as potent as the parent drug against strychnine induced convulsions in mice, suggesting a possible role for this metabolite in the anticonvulsant effects observed after propranolol administration. N-desisopropylpropranolol, an intermediary metabolite between propranolol and propranolol glycol[2,8], has also been shown to be an effective anticonvulsant against pentylenetetrazol seizures[9].

A rigorous examination of the possible role of these metabolites in the central nervous system (CNS) effects exerted by propranolol requires sensitive and specific methods for the quantitative analysis of brain levels of propranolol glycol, N-desisopropylpropranolol and propranolol at times when CNS effects are observed as a result of propranolol administration.

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The present study describes a method for the quantitative determination of propranolol glycol, N-desisopropylpropranolol and propranolol in brain tissue of mice. The method is an extension of a method earlier described for the determination of propranolol in plasma[10]. Propranolol and its metabolites are separated as their trifluoroacetyl derivatives by gas chromatography and detected by electron capture detection (GC–ECD). Conditions for tissue homogenization and extraction are described. Recoveries and precision are measured. Confirmation of chemical structures of derivatives was made by gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

Standards and reagents

Trifluoroacetic anhydride (TFAA) obtained from Pierce (Rockford, Ill., U.S.A.) was used as the derivatization reagent[10]. Trimethylamine (TMA), 1 *M* in benzene, was prepared from gaseous TMA, which was obtained from Eastman-Kodak (St. Louis, Mo., U.S.A.). Nanograde benzene, acetonitrile and perchloric acid were obtained from Fisher Scientific (Fairlawn, N.J., U.S.A.). 1 *N* Sulphuric acid, 5 *N* sodium hydroxide and 0.8 *N* perchloric acid were prepared with glass-distilled water and stored in glass bottles.

Propranolol hydrochloride and N-desisopropylpropranolol hydrochloride were kindly supplied by ICI (Macclesfield, Great Britain). Oxprenolol hydrochloride was a gift from M. S. Merrell Co. (Cincinnati, Ohio, U.S.A.). Propranolol glycol was synthesized in this laboratory as previously described[2,7].

Instruments

GC–ECD. The instrument used was a Varian Model 1440 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column, 180 cm × 2 mm I.D., was made of Pyrex glass and packed with 80–100 mesh re-silanized[11] Chromosorb W, coated with 1% OV-17 and 2% OV-1. The column was conditioned for 36 h at 250° before use. Operating conditions were: column temperature 170° or 195°, injector temperature 265°, detector temperature 280° and carrier gas (nitrogen) flow-rate 30 ml/min.

ECD response expressed as minimum detectable quantity in moles/sec or in picograms, was determined as earlier described[10,11].

GC–MS. The combination instrument used was an LKB 9000S, operated at an accelerating voltage of 3.5 kV, ionization voltage of 20 eV and a trap current of 65 μA. The instrument was equipped with an accelerating voltage alternator. A Pyrex glass column, 120 cm × 2 mm I.D., was packed as described above.

Extraction and derivatization

To each sample of fresh mouse brain tissue (about 500 mg wet weight) were added 1.5 ml acetonitrile, 1.5 ml 0.8 *N* perchloric acid and the internal standard oxprenolol. The sample was homogenized in ice with a Potter-Elvehjem homogenizer, using a PTFE pestle. After centrifugation the supernatant was transferred to a conical centrifuge tube, made alkaline (pH > 12) with 5 *N* sodium hydroxide and extracted with 5 ml benzene.

The benzene was extracted with 2 ml of 1 *N* sulphuric acid, leaving the neutral

metabolite, propranolol glycol, in the benzene phase (neutral fraction). The acidic aqueous phase containing the basic compounds, propranolol, N-desisopropylpropranolol and oxprenolol, was then made alkaline (pH > 12) with 5 *N* sodium hydroxide and extracted with 5 ml benzene (basic fraction).

Both 5 ml benzene extracts were evaporated to 500 μl at 50–60° with a gentle stream of nitrogen. 25 μl of TMA in benzene and 25 μl of TFAA were added to each extract and the reaction mixtures were heated for 5 min at 50–60°. The derivatized samples were washed with 3 ml distilled water and centrifuged before analysis.

All glassware was cleaned and silanized as previously described[10].

Standard curves

To samples of fresh brain tissue (about 500 mg wet weight) from untreated mice were added varying quantities of propranolol glycol (10–250 ng/g), propranolol hydrochloride (10–250 ng/g), N-desisopropylpropranolol hydrochloride (10–250 ng/g) and the internal standard, oxprenolol hydrochloride (400 ng/g). The samples were extracted and derivatized as described above.

Exactly 2.0-μl aliquots of the neutral fraction were analyzed by GC–ECD at a column temperature of 170°. The peak area of propranolol glycol was measured and plotted against propranolol glycol concentrations, expressed in ng/g.

Aliquots (2 μl) of the basic fraction were analyzed by GC–ECD at a column temperature of 195°. The peak area ratios propranolol–oxprenolol and N-desisopropylpropranolol–oxprenolol were calculated. Propranolol and N-desisopropylpropranolol concentrations, expressed as ng/g, *versus* area ratios times oxprenolol concentrations were plotted.

Recovery

The tissue recovery was determined for propranolol glycol, propranolol and N-desisopropylpropranolol at the 100-ng/g brain tissue content and for oxprenolol at 400 ng/g. Individual compounds were added to either brain tissue or distilled water and were carried through the extraction and derivatization procedure described above. GC–ECD analysis of the amount of each compound extracted from brain or from distilled water was used as the measure of recovery. Recoveries from distilled water after single as compared to repeated extractions were determined to be >90% for all compounds.

RESULTS AND DISCUSSION

Trifluoroacetylation of propranolol, N-desisopropylpropranolol, propranolol glycol and the internal standard oxprenolol yielded derivatives containing two trifluoroacetyl groups (Fig. 1) using the derivatization conditions described under Experimental. The structures of these derivatives were confirmed by GC–MS.

The derivatization reaction was complete in 5 min, using TMA as the catalyst (*cf.* ref. 10). Excess of TMA should be avoided for reproducible derivatization of N-desisopropylpropranolol. If the TMA concentrations given in Experimental are exceeded by four times, further but incomplete derivatization of this metabolite takes place at the remaining N–H group, resulting in a tri-TFA derivative (Fig. 2). The peak of this derivative appears at a retention time of 0.45 relative the peak of the di-

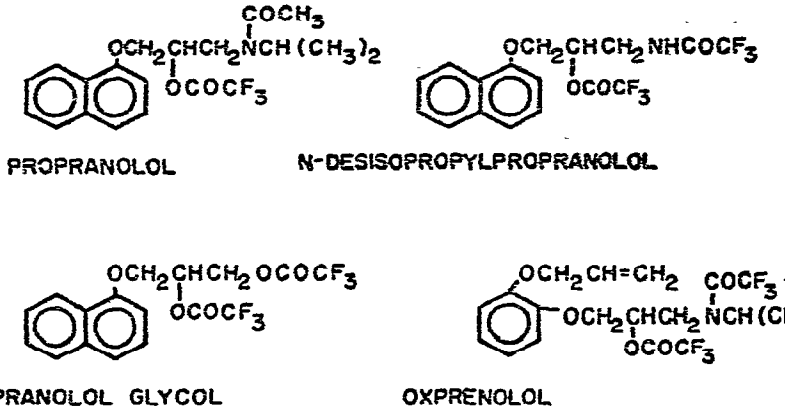

Fig. 1. Di-TFA derivatives of propranolol and oxprenolol and of propranolol metabolites.

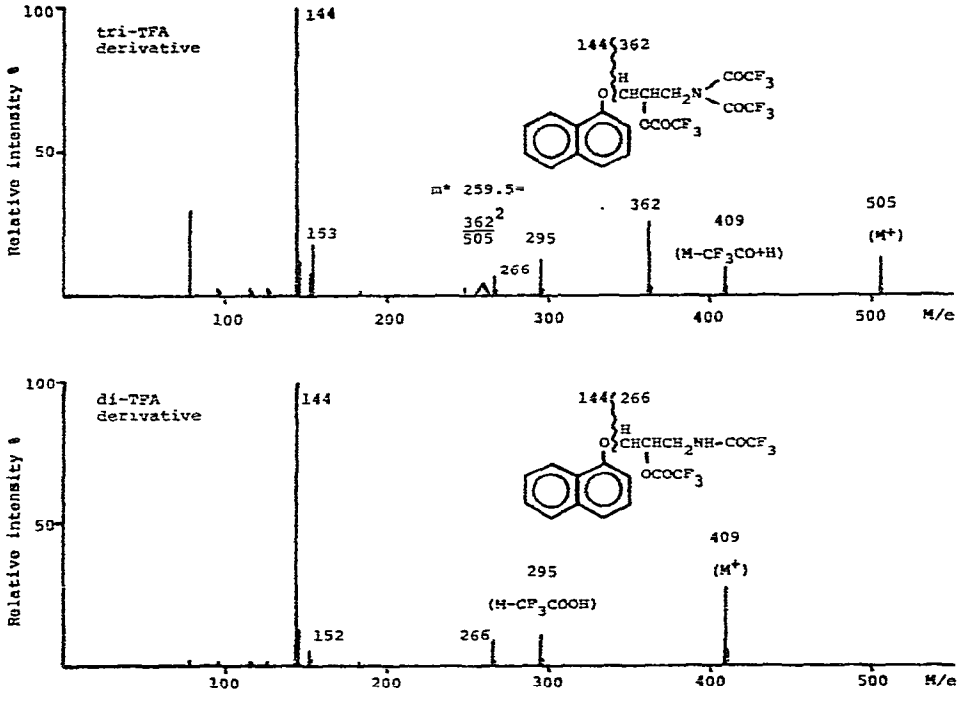

Fig. 2. Mass spectra of tri-TFA and di-TFA derivatives of N-desisopropylpropranolol.

TFA derivative. Ditrifluoroacetylation of simple aliphatic primary amino groups in the presence of high TMA concentrations has earlier been reported[12].

The excess of reagents was removed by shaking the reaction mixture with distilled water. The di-TFA derivatives, remaining in the benzene phase, were stable for at least 24 h. The catalytic action of TMA and the removal of excess of reagents with an aqueous phase has previously been discussed[10–13].

The derivatives have excellent gas chromatographic properties and give a high response on ECD. Retention times and minimum detectable quantities of the derivatives are given in Table I. It is of importance to notice that the two metabolites, the side-chain structures of which are common also for other β-blocking drug metabolites[14–16], are detectable in smaller quantities than the parent compound. The particularly high response of the TFA propranolol glycol is in agreement with earlier reports on TFA ethylene glycols[17].

TABLE I

RELATIVE RETENTION TIMES AND ELECTRON CAPTURE RESPONSES OF TFA DERIVATIVES

Retention time for TFA oxprenolol is 3.9 min. MDQ (minimum detectable quantity) values, expressed in picograms, are valid at a retention time of 3 min for a column with 3800 theoretical plates. Column, see Experimental.

Compound	*Relative retention time*	*MDQ (moles/sec × 10^{-16})*	*MDQ (pg)*
Propranolol glycol	0.64	0.7	0.2
Oxprenolol	1.00	4.3	1.1
N-Desisopropylpropranolol	1.62	1.5	0.4
Propranolol	2.51	2.0	0.5

Isolation of propranolol and its two metabolites from brain tissue through homogenization in perchloric acid–acetonitrile followed by solvent extraction was quite reproducible with a recovery for all three compounds ranging from 58 to 64% (Table II). Oxprenolol also showed the same recovery, indicating its suitability as internal standard. The addition of acetonitrile gave improved and more consistent recoveries of all four compounds and an apparent less interference from lipid material. Interferences from neutral endogenous compounds with retention times similar to oxprenolol, N-desisopropylpropranolol and propranolol shill made analysis after a single extraction difficult at the concentration levels studied. Through back-extraction and separate analysis of the neutral and basic fractions, no interferences were observed. Analysis of the neutral fraction at 170° takes 5 min (Fig. 3A), and of the basic fraction at 195° 8 min (Fig. 3B).

The standard curve for propranolol glycol is shown in Fig. 4. Despite the lack of internal standard for this metabolite, accurate and reproducible injections were

TABLE II

RECOVERIES FROM BRAIN TISSUE

Compound	*Tissue concentration (ng/g)*	*Recovery (%)**
Propranolol	100	58 ± 3 (n = 6)
Propranolol glycol	100	63 ± 4 (n = 5)
N-Desisopropylpropranolol	100	64 ± 3 (n = 10)
Oxprenolol	400	59 ± 6 (n = 4)

* As compared to water from which recoveries are >90%.

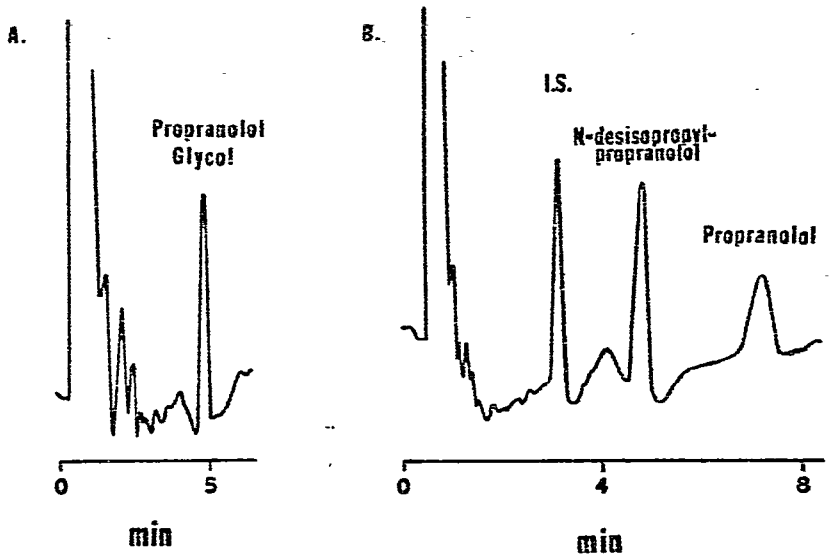

Fig. 3. GC–ECD of brain extracts. A, neutral fraction (column temperature 170°); B, basic fraction (column temperature 195°). The brain content corresponds to 100 ng/g of each of propranolol, N-desisopropylpropranolol and propranolol glycol and to 400 ng/g of oxprenolol.

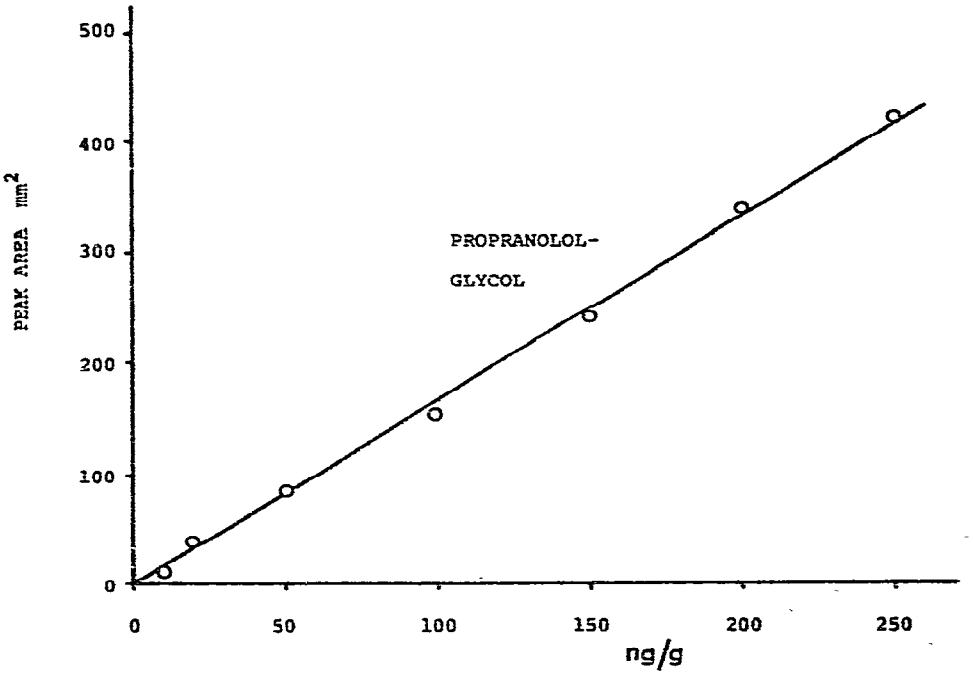

Fig. 4. Standard curve for propranolol glycol based on peak area measurements.

quite satisfactory as a basis for quantitation. The standard curve is linear over the concentration range studied, and goes through the origin. The precision of the method after repeated analysis is about ±5% at the 100-ng/g level. The standard curves for propranolol and N-desisopropylpropranolol shown in Fig. 5 are also quite satisfactory, with a precision at the 100-ng/g level of about ±5% for propranolol and ±10% for N-desisopropylpropranolol.

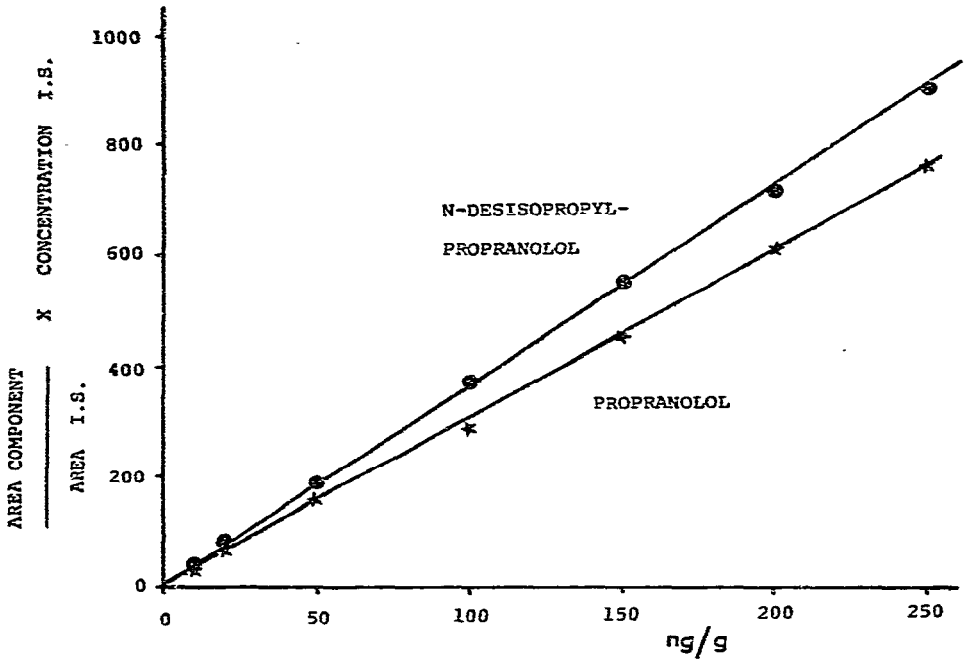

Fig. 5. Standard curves for propranolol and N-desisopropylpropranolol using oxprenolol as internal standard.

The brain levels studied for propranolol and its two metabolites (10–250 ng/g) are at the lower level of what can be expected after propranolol doses, intravenous or oral, that may be used clinically[18]. The sensitivity and specificity of the method should allow for evaluation of the contribution of propranolol glycol and N-desisopropylpropranolol to the effects on the CNS observed after propranolol administration. The method should be applicable for measurements also in other tissues such as the heart where these metabolites may have a pharmacological effect. With slight modifications, the method should be applicable to the determination of similar metabolites of other β-blocking drugs (*cf.* ref. 10).

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