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A NEW METHOD FOR THE DETERMINATION OF METYRAPONE IN PLASMA AND TISSUES

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SUMMARY

A method is described for the determination of metyrapone and its metabolite(s) after extraction from biological samples. The procedure consists of thin-layer or paper chromatographic separation, the KÖNIG reaction and spectrophotometric determination.

INTRODUCTION

Although metyrapone (Metopirone® CIBA, SU-4885, 2-methyl-1,2-bis(3-pyridyl)-1-propanone) has been widely used for diagnostic purposes for 10 years, very little is known about the metabolic fate of this drug because sensitive, accurate and relatively simple methods for its determination in biological samples are still lacking.

The method described here may fulfill this need and has in fact already been utilized for investigating the metabolism of metyrapone *"in vivo"*[1,2].

The principle is based upon the extraction of metyrapone from biological samples with methylene chloride, separation of metyrapone from its metabolites by paper or thin-layer chromatography and spectrophotometric determination of the product obtained after performing the KÖNIG reaction[3].

EXPERIMENTAL

Biological materials and extraction

Plasma samples (1.5 ml) of metyrapone-treated rats or tissue homogenates (prepared with 10 parts of water) were shaken with 4 vol. of methylene chloride for 4 min and the methylene chloride layer was separated by centrifugation for 10 min at 3000 r.p.m.

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A portion (5 ml) of the methylene chloride layer was removed and evaporated to dryness in a stream of nitrogen at 40°.

Thin-layer chromatography

Kieselgel G (Merck, Darmstadt) layers, 250 μ thick, on glass plates[4] were used after activation at 110° for 30 min. The dried residue of the sample extracts, dissolved in 0.4 ml methylene chloride, or the reference standards (1–20 μg) were applied to the thin-layer plates. The chromatograms were developed in a methylene chloride–ethanol (100:4) system in an unsaturated chamber[5] at 22 $\pm$ 0.5° until the front of the solvent had moved about 15 cm.

Paper chromatography

The extracted samples, dissolved in 0.4 ml methylene chloride, were deposited on Whatman No. 1 paper and were equilibrated for 1 h in the tank before adding the mobile phase of a Bush-type solvent system[6]. Petroleum ether–benzene–methanol–water mixtures were used in two different proportions BL_2 (25:25:35:15) or BL_3 (1:1:1:1), respectively, at room temperature.

Detection of spots

This was carried out by iodine vapour (sensitivity is about 3 μg) or by observation in ultraviolet light (at 254 mμ, sensitivity is about 5 μg).

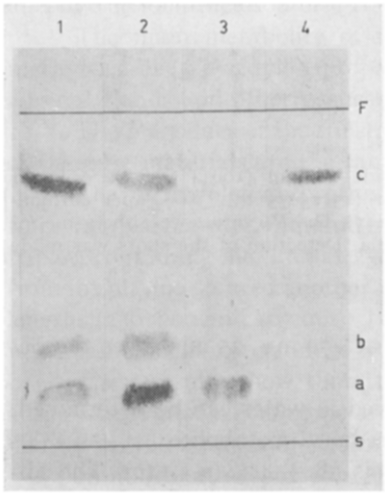
Detection of metyrapone and its metabolite(s) on chromatograms

Semiquantitative estimation. The chromatograms were sprayed with a saturated ethanolic solution of *p*-amino-salicylic acid (PAS) and placed for 15 min in a chamber containing a few crystals of cyanogen bromide (BrCN). The vapour pressure of this substance is high enough to provide a sufficient concentration for the KÖNIG reaction. This reaction is specific for pyridine derivatives with an unsubstituted α-position. The addition of cyanogen bromide is followed by the opening of the pyridine ring with the formation of glutaconic aldehyde, which reacts with the aromatic amine (PAS in this case) forming an intensely coloured imino-derivative of glutaconic aldehyde[7]. By this procedure, metyrapone and its metabolites produce brownish-violet spots. Of the substances 0.5 μg can be easily detected and recorded by a Xerox photocopying machine (Fig. 1).

Quantitative analysis of metyrapone and its reduced derivative. Attempts to elute the coloured spots on thin-layer chromatograms after performing the KÖNIG reaction were unsuccessful. Therefore after the localization of metyrapone and its reduced derivative by iodine vapour or ultraviolet light on thin-layer plates or paper chromatograms, the respective areas were scraped off or cut out and eluted with 3 ml of ethanol. To centrifugated eluates were added: 0.05 ml of 2.5% (w/v) ethanolic solution of *p*-aminosalicylic acid, 0.25 ml of 20% (w/v) ethanolic solution of cyanogen bromide and 0.05 ml of *N* NaOH.

The absorption maximum of the coloured reaction mixture is at 468 mμ and at 472 mμ for metyrapone and its reduced derivative, respectively (Fig. 2).

The colour of the reaction reaches a maximum after around 20 min and remains unchanged for about 10 min (Fig. 3). For serial determinations of metyrapone and



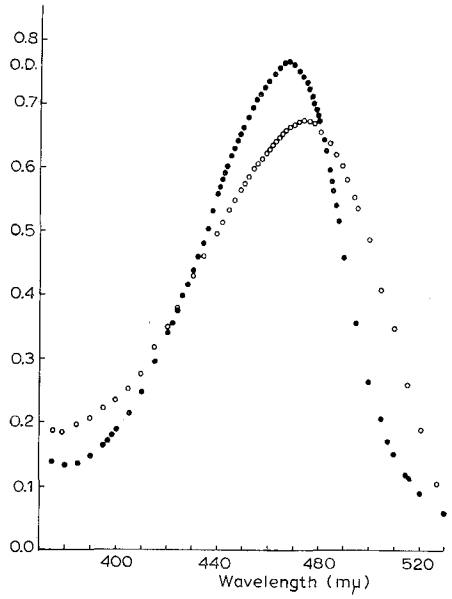
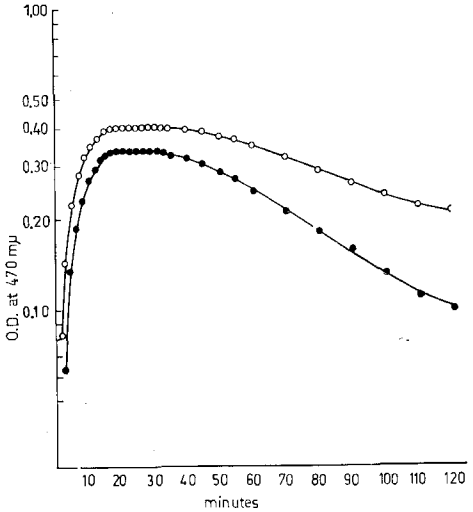


Fig. 1. Thin-layer chromatography carried out according to the method described in the text: (F) front; (a) SU 5236 (2-methyl-1,2-bis(3-pyridyl)-1-propanol); (b) unidentified metabolite; (c) metyrapone; (S) start: 1 and 2; extracts from plasma 20 and 40 min after administration of metyrapone hydrochloride (66 mg/kg i.v.); 3 and 4: pure samples of SU 5236 and metyrapone.

Fig. 2. Optical density of metyrapone (●) or SU 5236 (○) at different wavelengths. Maximum absorption for metyrapone and SU 5236 are respectively 468 and 472 mμ.



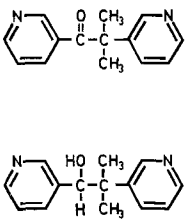


Fig. 3. Development and stability of colour developed after performing KÖNIG reaction on metyrapone (●) or SU 5236 (○). Optical density (O.D.) was measured at 470 mμ.

Fig. 4. Upper formula: 2-methyl-1,2-bis(3-pyridyl)-1-propanone (metyrapone); lower formula: 2-methyl-1,2-bis(3-pyridyl)-1-propanol (SU 5236).

TABLE I

R_F VALUES OF METYRAPONE AND SU 5236 IN DIFFERENT CHROMATOGRAPHIC SYSTEMS

Compound	R_F *in system**		
	1	*2*	*3*
Metyrapone	0.79	0.62	0.81
SU 5236	0.16	0.01	0.05

* 1 = Thin-layer chromatography:methylene chloride–ethanol (100:4 v/v). 2 = Paper chromatography:petroleum ether–benzene–methanol–water (25:25:35:15). 3 = Paper chromatography: petroleum ether–benzene–methanol–water (1:1:1:1). The R_F values were the same for pure substances and for the spots obtained from rat plasma. Detection of the spots was made by the KÖNIG reaction or iodine vapour or U.V.

its metabolite the optical densities were measured at 470 mμ, 25 min after the beginning of the reaction.

By this procedure 1 μg of metyrapone dissolved in water can be determined. The reaction is about 3-fold more sensitive for the reduced metabolite.

The extraction efficiency from rat plasma was 78 $\pm$ 4% (n = 10). The absorption was linear with respect to concentration between 5 and 40 μg for metyrapone and between 2 and 20 μg for the reduced metabolite. The method allows the measurement of a minimum of 5 μg of metyrapone or 2 μg of SU 5236 in 1 ml of rat plasma.

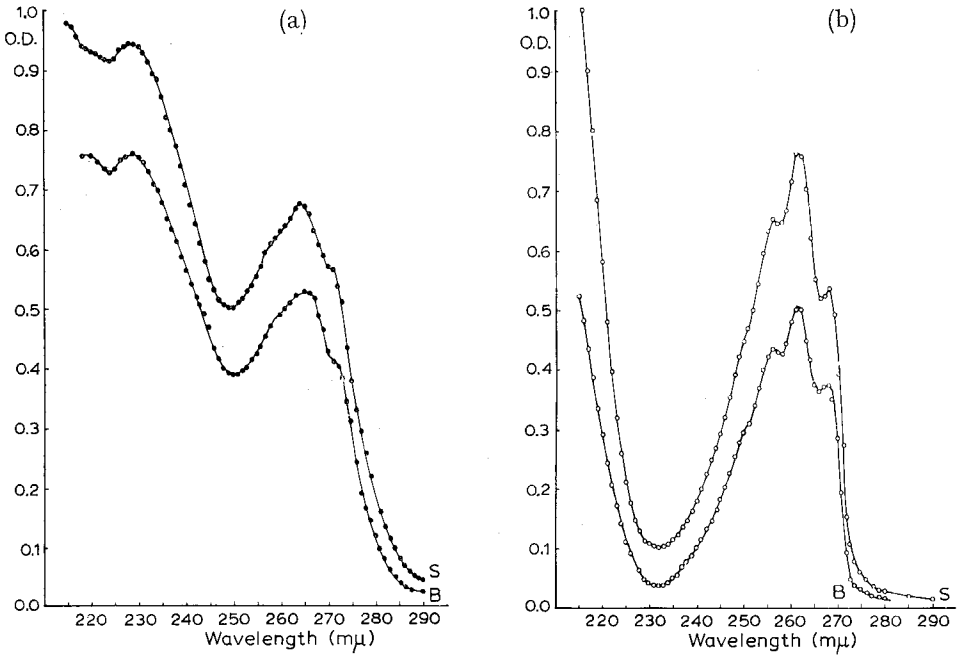


Fig. 5. (a) U.V. absorption spectrum in methanol of metyrapone (S) and of the substance extracted from plasma (B) showing an R_F of 0.79 after thin-layer chromatography. (b) U.V. absorption spectrum in methanol of SU 5236 (S) and of the substance extracted from plasma (B) showing an R_F of 0.16 after thin-layer chromatography.

Identification of the substances extracted from rat plasma and tissues

This was carried out by measuring the R_F values and comparing them with those for pure substances after chromatography in three different solvent systems and by the identification of ultraviolet spectra.

The main metabolite of metyrapone is a reduced derivative (SU-5236: 2-methyl-1,2-bis(3-pyridyl)-1-propanol) found in urine[8] and the incubation fluid of rat adrenal glands and other tissues[9] (Fig. 4).

The R_F values of the substances extracted from rat plasma or tissues and those of the pure materials are the same (Table I).

The ultraviolet absorption spectrum of metyrapone and the spot recovered from rat plasma after administration of metyrapone to rats gave a characteristic triplet, containing maxima at 258, 264 and 270 mμ. SU-5236 and the spot recovered from rat plasma showed another triplet but with the maxima shifted to shorter wavelengths (256, 262 and 267 mμ). The maximum at 229 mμ in the spectrum of SU-4885 was absent in the spectrum of SU-5236 (Fig. 5).

These results show that the substances extracted from rat plasma after the administration of metyrapone are identical with metyrapone and SU-5236, in agreement with the findings of KRAULIS *et al.*[9].

In addition, a spot of a less polar substance giving the KÖNIG reaction was found on the thin-layer plates.

Other spots were also detected sometimes by the KÖNIG reaction which would indicate the presence of trace amounts of similar metabolic products.

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