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Note

Detection of some antihypertensive drugs and their metabolites in urine by thin-layer chromatography.

Five commonly used beta blockers and hydralazine

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Large numbers of patients are currently receiving antihypertensive agents such as the beta adrenoceptor blocking drugs to control their blood pressure. When their action is considered inadequate the dose of drug is increased and/or other antihypertensive agents are added. Though an inability to achieve adequate blood levels or a resistant form of the disease may explain the poor response in some cases, the commonest problem is that the patient fails to take one or more of his drugs. A rapid and easy technique for detecting the presence of the drug in urine which would enable the clinician to detect those patients who were simply not taking their treatment would therefore greatly facilitate patient care. The work described here is an attempt to provide such a method and has been kept deliberately as simple as possible. The structures of the compounds studied are presented in Fig. 1.

MATERIALS AND METHODS

Thin-layer chromatography (TLC)

Silica gel plates with 0.2 mm layer thickness were used without activation (Polygram Sil N-HR, Macherey, Nagel & Co., Düren, G.F.R.). The solvent systems used were ethyl acetate–methanol (40:5) and ethyl acetate–methanol–ammonia (sp.gr. 0.88) (40:5:5). Fluorescent spots were detected using a Chromatolite UV lamp 254 nm (Hanovia Lamps, Slough, Great Britain).

Reagents and materials

Diethyl ether was distilled prior to use, all other organic solvents were analytical reagent grade. A metabolite of metoprolol, H119/66 was supplied by Hässle (Mölndal, Sweden), acebutolol and its metabolite M & B 16942 were supplied by May & Baker (Dagenham, Great Britain), oxprenolol by Ciba (Horsham, Great Britain), propranolol by I.C.I. (Macclesfield, Great Britain), and nadolol by E. R. Squibb & Sons (London, Great Britain). The hydralazine metabolite, methyl triazolophthalazine, was prepared from hydralazine by the action of acetic anhydride[1]. The hydralazine was supplied by Ciba. Visualising reagent: concentrated sulphuric acid or 38% formaldehyde in concentrated sulphuric acid (1:10)[2].

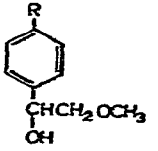
TABLE I

R_F VALUES, CHARACTERISTIC COLOURS AND MINIMUM DETECTABILITIES IN URINE

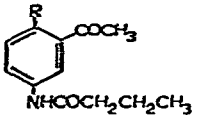
Compound	R_F		UV_{254}	*Colour with conc. sulphuric acid*	*Colour with formaldehyde–conc. sulphuric acid*	*Minimum detectability in urine (mg/l)*
	Solvent system 1	*Solvent system 2*				
H 119/66	0.0	0.65	–	Purple	Yellow	0.5
Acebutolol	0.0	0.73	Yellow-blue	Yellow	Faint yellow	0.05*
M & B 16942	0.0	0.60	Yellow-blue	Yellow	Faint yellow	0.05*
Oxprenolol	0.0	0.81	–	Faint purple	Purple	0.5
Propranolol	0.0	0.81	–	Faint brown	Green	0.5
Nadolol	0.0	0.50	–	–	Orange-red	1.0
Methyltriazolo-phthalazine	0.53	0.81	Pale blue	–	–	0.5*

* Detected by fluorescence at 254 nm.

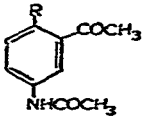
H119/66, a metoprolol metabolite



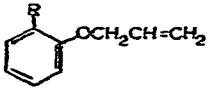
Acebutolol



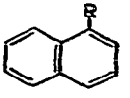
M&B 16942, acebutolol metabolite



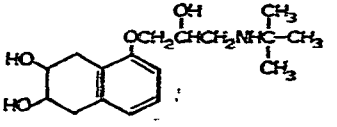
Oxprenolol



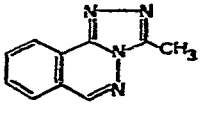
Propranolol



Nadolol



Methyltriazinophthalazine, a hydralazine metabolite



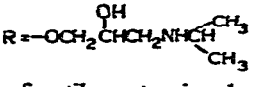


Fig. 1. Structures of antihypertensive drugs and metabolites.

Extraction procedure

A portion of 4 ml of patient urine is made alkaline with 0.05 ml of 7 *N* sodium hydroxide and shaken for 5 sec in a 10-ml test tube with 4 ml of diethyl ether–dichloromethane (4:1). The phases are allowed to separate and the organic phase aspirated and evaporated to dryness under a stream of nitrogen. The residue is dissolved in a drop of methanol, spotted onto a silica gel plate together with the appropriate standards, and allowed to develop for 5 cm in solvent system 1. The plate is then dried and examined under UV light at 254 nm. The appropriate amount of ammonia (sp.gr. 0.88) is added to the tank to produce solvent system 2, the plate replaced and allowed to develop for 8 cm. The plate is removed and oven dried for 10 min. The plate is again examined

under UV light at 254 nm. Finally a large beaker of water is placed below the TLC plate and the appropriate visualizing reagent poured down the plate. The colours developed and the R_F values are compared with the standards on the same plate.

RESULTS AND DISCUSSION

The R_F values of the compounds studied and their colours under UV light and after treating with visualising reagent are given in Table I. The minimum detectability of each drug and/or its metabolite is given in the same table. When both hydralazine and a beta blocker may be present in the urine, solvent system 1 must be used first followed by system 2. If only a beta blocker is anticipated only system 2 is required. In fact, no free hydralazine is present in urine but methyltriazolophthalazine, a major metabolite[3], fluoresces at 254 nm.

Metropolol itself, unlike the other beta blocking drugs studied here, gives no colour with either of the visualizing reagents and does not fluoresce. However, about 10% of an oral dose is excreted in the urine as the 1-hydroxy-2 methoxyethyl metabolite, H119/66 (ref. 4). This metabolite gives an intense purple colour with the sulphuric acid reagent. Acebutolol and its metabolite M & B 16942 show up as yellow spots when treated with concentrated sulphuric acid and much weaker yellow spots with the formaldehyde–sulphuric acid reagent. In contrast, oxprenolol and propranolol only give intense colours with the formaldehyde–sulphuric acid reagent while nadolol gives an orange-red colour with this latter reagent and no colour at all with concentrated sulphuric acid. If acebutolol compliance alone is to be checked, the urine levels of the drug and its metabolite are sufficiently high to make solvent extraction unnecessary. The urine can simply be spotted directly onto a TLC plate, developed in solvent system 2, and examined at 254 nm.

Higher recoveries could, no doubt, be obtained by acid or enzymatic hydrolysis of drug conjugates in urine specimens before solvent extraction. This was considered, however, to be a needless complication and increased greatly the time required to carry out a compliance check. The minimum detectabilities are sufficiently low to allow easy detection of the free drug or metabolite if the patient is taking his medication as directed. No interference from the following drugs, given in normal therapeutic doses, has been observed: methyldopa, guanethidine, prazosin, frusemide, cyclopenthiazide and chlorthalidone. Ths method has now been tested successfully for several months and over one hundred and thirty compliance checks have been carried out.

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