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Note

Fluorodensitometric determination of nadolol in plasma and urine

MONIKA SCHÄFER-KORTING and ERNST MUTSCHLER*

Department of Pharmacology, Faculty of Pharmacy and Biochemistry, University of Frankfurt/Main (G.F.R.)

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Nadolol, *cis*-5-{3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy}-1,2,3,4-tetrahydro-2,3-naphthalenediol, is a long-acting β-adrenoceptor blocking drug [1,2]. Following the administration of therapeutic doses, plasma levels proved to be in the nanogram range [3]. To measure plasma levels of drugs for pharmacokinetic studies or drug monitoring, sensitive and rapid methods are required which allow analysis of many samples per day. Two methods for the analysis of nadolol in biological materials have been described up to now: a fluorimetric method following the oxidation and coupling of the resulting aldehyde with *o*-phenylenediamine [4] and a gas—liquid chromatographic (GLC) determination combined with mass spectrometric quantitation [5]. Both methods are rather laborious. A third assay, using high-performance liquid chromatography, described recently, requires electrochemical detection [6], the latter not being generally available. We describe here a densitometric method with fluorimetric quantitation which proved to be simple and rapid.

EXPERIMENTAL

Apparatus

A chromatogram spectrophotometer KM 3 from Carl Zeiss with a Perkin-Elmer recorder Model 56 was used.

*Address for correspondence and reprint request: Pharmakologisches Institut für Naturwissenschaftler der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt/Main, G.F.R.

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Standard and reagents

Nadolol was obtained from Heyden (Munich, G.F.R.). Standard solutions (10 mg of nadolol dissolved in 100 ml of methanol, and stock solution diluted 1:10) were stored at 4°C for one week. Solvents, chemicals (all analytical grade and used without further purification), and thin-layer plates (10 × 20 cm, coated with silica gel 60, without fluorescence indicator) were purchased from Merck (Darmstadt, G.F.R.).

Glassware

All glassware used in the extraction procedure (100 × 16 mm screw-capped test tubes, and plastic caps with Teflon linings) was washed with detergent solution, rinsed with tap water, then distilled water, and dried. Reagents were added with automatic pipettes.

Method

Extraction procedure for plasma. A 2-ml plasma sample was pipetted into a test tube, and 1.5 g of sodium chloride and 1 ml of 5 *N* sodium hydroxide were added. The mixture was shaken in a horizontal position. Then 5 ml of diethyl ether were added and the tubes shaken again for 10 min. Thereafter the samples were centrifuged at 2000 *g* to separate the layers. The organic phase was removed completely and transferred to another test tube. The solvent was concentrated to dryness at 40°C under an atmosphere of nitrogen. Together with twelve samples of unknown nadolol content, four blank plasma samples (2 ml) spiked with 60, 100, 200, and 600 ng of nadolol were processed.

Extraction procedure for urine. To 0.2 ml (or less) of urine 0.5 ml of 1 *N* hydrochloric acid was added. The acidic solution was pre-extracted with 5 ml of diethyl ether by shaking for 5 min. The layers were separated by centrifuging and the organic layer was discarded. To the aqueous layer 1 g of sodium chloride and 1 ml of 5 *N* sodium hydroxide were added. Extraction of nadolol and evaporation of the solvent were performed as described for plasma. Again, four samples of spiked blank urine (60, 200, 400, and 600 ng per 0.2 ml) were extracted together with twelve patient samples.

Chromatography. The residues were dissolved in 50 µl of methanol by shaking vigorously; 40 µl of the solution were spotted on a thin-layer plate (6 mm wide, Linomat III; Camag, Muttenz, Switzerland). On each plate were spotted twelve extracts of plasma or urine samples of unknown nadolol content and four extracts of spiked plasma. The thin-layer plates spotted with plasma extracts were developed in a saturated glass tank containing the solvent system chloroform—methanol—glacial acetic acid (75:20:5, v/v; R_F = 0.26). Urine extracts were chromatographed in chloroform—methanol—glacial acetic acid (60:35:10, v/v; R_F = 0.47). After developing for 8 cm the plates were air-dried and thereafter dipped into a solution of 4% nujol in cyclohexane. After another drying period of at least 20 min the plates were scanned.

Fluorescence measurement. Fluorimetric measurements were performed using the monochromator—sample mode of the scanner. Fluorescence of nadolol was excited with the 265-nm line of a medium pressure lamp ST 41. A 313-nm monochromatic filter served for the selection of the emitted fluores-

cent light (intensification ten times). The slit was 1 × 8 mm, and the plates were scanned at 100 mm/min. The peaks were registered on an interfaced recorder. The amount of nadolol in plasma and urine was determined by a calibration curve. Peak height was plotted versus nadolol content in spiked samples. The calibration curve was established for each plate.

Nadolol plasma levels and urinary excretion

Nadolol tablets containing 60 and 120 mg of the drug were administered to seven healthy subjects. Blood samples were drawn by venipuncture before the administration of the drug and up to 48 h afterwards. Plasma was separated by centrifugation. Urine was collected in definite intervals for 72 h. Plasma and urine specimens were stored frozen at −20°C until analysis.

RESULTS AND DISCUSSION

Excitation of nadolol on thin-layer chromatographic (TLC) plates with shortwave UV light (265 nm) resulted in the emission of a rather shortwave fluorescent light. A similar behaviour has been reported for atenolol [7,8]. The native fluorescence of nadolol was increased about twofold by spraying the plate with a mixture of 10% citric acid in water—ethylene glycol (1:1), and about fivefold by dipping into a solution of 4% nujol in cyclohexane. Fluorescence enhancement due to nujol is based upon the non-polarity and viscosity of the solvent [9]. Using the latter version the limit of detection of nadolol on TLC plates is in the order of 5 ng per spot. The calibration curve is linear up to 1400 ng per spot. Use of either peak height or peak area for the calibration curve proved to be of the same quality. As the correlation coefficient of both parameters exceeded 0.996, peak height was chosen for analysis as it is obtained more easily. Typical calibration curves are:

pure substance: $Y = 1.160 + 0.187X$, $r = 0.9999$

nadolol extracted from plasma: $Y = 1.386 + 0.088X$, $r = 0.9999$

nadolol extracted from urine: $Y = 2.736 + 0.118X$, $r = 0.9997$.

Extraction of nadolol from urine and plasma was performed by a method similar to that described by Ivashkiv [4], except that plasma samples were extracted by diethyl ether, too. Thus evaporation of butyl acetate, which has a relatively high boiling point leading to a prolonged evaporation time, was avoided. Following the extraction of nadolol from plasma and urine the recovery is incomplete (63% for plasma, 77% for urine samples). Therefore spiked plasma samples were carried through the analysis to avoid major fluctuations in the results due to day-to-day variation. Chromatography of nadolol extracted from plasma in the solvent system described by Dreyfuss et al. [10] leads to chromatograms free of interfering peaks (Fig. 1). Chromatography of urine extracts following the same extraction schedule gives an additional peak interfering with nadolol. This can be avoided by increasing the amounts of methanol and acetic acid in the chromatographic system together with a pre-extraction of the acidified urine specimens with ether.

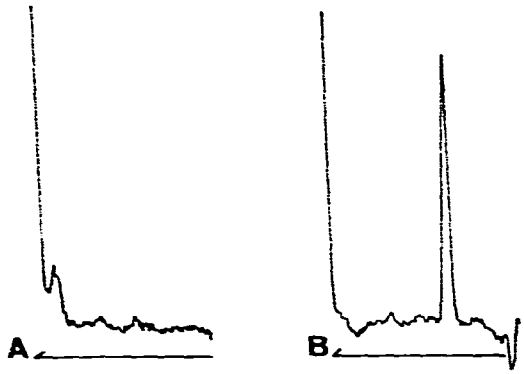

Fig. 1. Chromatograms of blank plasma (A) and of 2 ml of blank plasma spiked with 400 ng of nadolol (B). Analysis performed as described in the text. Direction of development = direction of the scan, as indicated by the arrows.

TABLE I

VARIATION COEFFICIENTS FOR NADOLOL DETERMINATIONS IN PLASMA AND URINE

Seven samples of each concentration were investigated.

Sample	Concentration* of nadolol	Variation coefficient (%)
Plasma	1000	3.9
	400	3.6
	200	3.8
	100	7.1
	50	4.0
Urine	1000	6.5
	50	7.5

*Plasma: ng per 2 ml. Urine: ng per 0.2 ml.

Recovery of nadolol from plasma was 62–64%, recovery from urine samples was 76–78%. Analyses were performed with five and two different concentrations for plasma and urine, respectively, on three different days. Reproducibility of the method proved to be sufficient for the determination of nadolol in biological fluids. The relative standard deviation was calculated from seven replicate analyses of relevant concentrations of nadolol in plasma and urine. The variation coefficient was usually less than 4% for plasma samples and about 7% for urine; for details see Table I. According to the evaluation of other beta-blockers by fluorodensitometry, the variation coefficient exceeds that of the GLC methods slightly [5,8,11].

Following the administration of nadolol to healthy volunteers (60 and 120 mg oral dose) plasma levels could be monitored for at least 12 h. Nadolol could be detected in urine specimens for 72 h. Representative plasma levels and urinary excretion rates ($\Delta U/\Delta t$ versus midpoint time) are shown in Fig. 2. Only unchanged nadolol was excreted in urine and faeces following the administration of [^{14}C]nadolol to hypertensive patients either intravenously or by

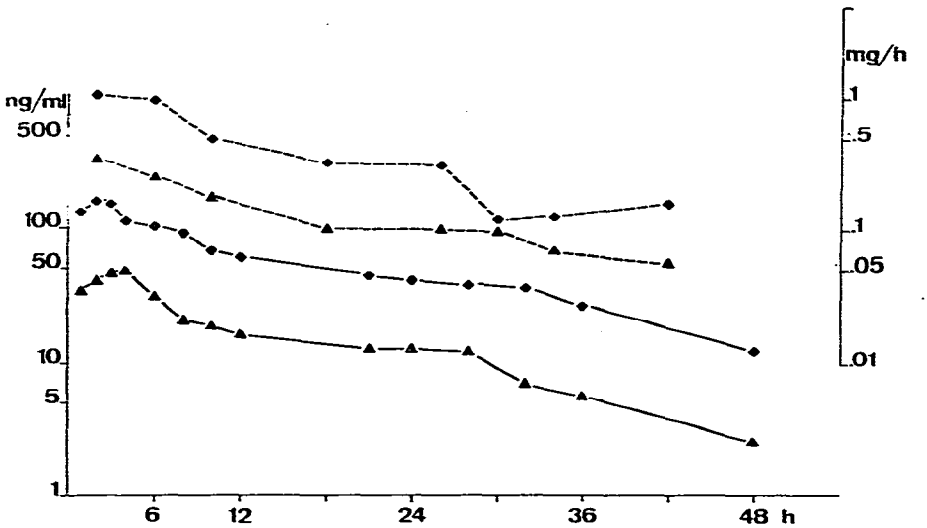

Fig. 2. Nadolol plasma levels (solid line) and urinary excretion rate ($\Delta U/\Delta t$ versus midpoint time; dotted line) of a healthy subject administered 60 mg (▲) and 120 mg (◆) orally.

the oral route [10]. Therefore, the interference of metabolites does not seem to decrease the specificity of the method described.

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