Stereoselective binding of propranolol enantiomers to human α_1 -acid glycoprotein and human plasma

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The binding of propranolol enantiomers to human albumin (ALB), α_1 -acid glycoprotein (α_1 -AGP) and plasma was studied. (–) propranolol is more bound than (+) propranolol to α_1 -AGP ($P < 0.001$) and to plasma ($P < 0.05$). In solutions containing ALB at a constant concentration (580 $\mu\text{mol/l}$) and α_1 -AGP at increasing concentrations, the binding of both isomers increases but the stereo selectivity is evident throughout the α_1 -AGP concentration range examined (25–100 $\mu\text{mol/l}$).

Keywords propranolol stereoselectivity protein binding α_1 -acid glycoprotein

Introduction

($\pm$) Propranolol is a non-selective β -adrenoceptor blocking drug usually employed as a racemic mixture. The two enantiomers, (+)-propranolol and (–)-propranolol share some pharmacological actions whereas for others they show a high degree of stereospecificity (for example, the (–)-propranolol is 60–100 times more potent in blocking the inotropic and chronotropic responses to isoprenaline in dogs) (Barrett & Cullum, 1968). In order to evaluate the relative contribution of the different actions to the overall pharmacological effect, many authors have studied the enantiomers separately, both in experimental (Anlezark *et al.*, 1979; Jaillon *et al.*, 1980) and clinical situations (Tyrer & Lader, 1974; Stensrud & Sjaastad, 1976). Concomitantly the pharmacokinetics (George *et al.*, 1972; Kawashima & Ishikawa, 1980; Tawara *et al.*, 1981; Jackman *et al.*, 1981; Silber *et al.*, 1982) and the metabolism (von Bahr *et al.*, 1982) of the two enantiomers have been studied.

Recently, a paper dealing with the stereo-specific binding to plasma proteins of (+)- and (–)-propranolol in dog has been published (Bai *et al.*, 1983): the authors found a higher binding for (–)-propranolol and speculated that α_1 -acid glycoprotein (α_1 -AGP) might be the stereo-selective binder.

In the present paper, we report data on the binding of propranolol enantiomers to human

albumin (ALB), human α_1 -AGP and human plasma, studied by equilibrium dialysis and liquid chromatography.

Methods

Plasma was obtained from blood collected from five healthy volunteers (males; age 22–40 years), by utilizing glass syringes and polyethylene tubes containing heparin (8–10 i.u./ml of blood). Human albumin (fatty acid free; lot 17; Miles, Milan, Italy) and human α_1 -AGP (Sigma, St. Louis, Mo, USA) were dissolved in Krebs physiological solution. ($\pm$)-, (–)- and (+)-propranolol were kindly supplied by ICI (Macclesfield, Great Britain) as hydrochloride salts. Desipramine (employed as an internal standard in the chromatographic determinations) was a gift from Ciba-Geigy (Basle, Switzerland).

The following procedure was used to add drugs to the ligand solutions: aliquots of a methanolic solution of ($\pm$)-propranolol (or (–)-propranolol, or (+)-propranolol) 40 $\mu\text{mol/l}$ (as free base) were evaporated to dryness in test-tubes, under vacuum; the ligand solutions (plasma; ALB 580 $\mu\text{mol/l}$; α_1 -AGP 25 $\mu\text{mol/l}$) were added to the test-tubes, mixed and allowed

to equilibrate. Final range of concentrations of the drugs determined by liquid chromatography were 350–400 nmol/l. In this way each aliquot of ligand solutions contains only one of the three drugs (($\pm$)-, (+)- or (-)-propranolol).

Binding was also determined in solutions containing ALB 580 μ mol/l plus α_1 -AGP at increasing concentrations (25; 50; 100 μ mol/l).

Equilibrium dialysis was performed in a Dianorm apparatus (Diachema[®], Zurich, Switzerland), using a Spectrapor/2 membrane (Spectrum Medical Ind., Los Angeles, Ca, USA). Ligand solutions (1 ml) were dialyzed against phosphate buffer (1 ml) 0.134M, pH 7.4 for 1.5 h, at 37 °C. These conditions were found to be sufficient for the equilibrium. Concentrations in the ligand and buffer solutions were determined by a liquid chromatographic method (Albani *et al.*, 1982) slightly modified. The fluorometer was set at 285 nm (excitation) and 345 nm (emission), injection volumes were 10–50 μ l; in this condition it is possible to measure concentration of propranolol down to 7 nmol/l with a good reproducibility (C.V. of 6.0%). It should be pointed out that our chromatographic method does not allow for the measurement of (+)- and (-)-propranolol in samples containing both enantiomers.

Free fraction was expressed as the ratio of drug concentrations in buffer and ligand compartments after dialysis. Statistical analysis was performed using Wilcoxon test or Mann-Whitney test, when appropriate.

Results

Drug free fractions in plasma (for (+)- and (-)- propranolol), and in ALB and α_1 -AGP solutions (for (+)-, (-)- and ($\pm$)-propranolol) are reported in Table 1. (-)-propranolol is significantly more bound to α_1 -AGP than (+)-propranolol ($P < 0.001$). Even the differences between ($\pm$)-propranolol and

(+)-propranolol or (-)-propranolol are significant ($P < 0.05$). Data on ALB show a higher mean binding for (+)-propranolol but the difference does not reach statistical significance. In plasma the binding of (-)-propranolol is greater, probably due to a predominance of the stereoselective binding to α_1 -AGP.

Figure 1 shows the free fraction of (+)- and (-)-propranolol in the solutions of ALB plus α_1 -AGP. The difference in free fraction is present throughout the α_1 -AGP concentration range studied.

Figure 1 Binding of (+)- and (-)-propranolol to the solutions containing ALB at constant concentrations (580 μ mol/l), and increasing concentrations of α_1 -AGP (values on abscissa).

Table 1 Free fractions (%) of ($\pm$)-propranolol, (+)-propranolol and (-)-propranolol in the different ligand solutions (mean $\pm$ s.e. mean)

	Plasma (n = 5)	ALB (580 μ mol/l) (n = 7)	α_1 -AGP (25 μ mol/l) (n = 7)
($\pm$)-propranolol	—	50.1 $\pm$ 1.4	26.2 $\pm$ 1.2
(+)-propranolol	12.2 $\pm$ 1.9	48.2 $\pm$ 1.6	30.2 $\pm$ 0.9
(-)-propranolol	10.9 $\pm$ 2.0	51.0 $\pm$ 0.9	23.0 $\pm$ 1.0
	$P < 0.05^*$	NS**	$P < 0.001$ for (-)-propranolol vs ($\pm$)-propranolol** $P < 0.05$ for ($\pm$)-propranolol vs (+)-propranolol vs (-)-propranolol**

* Wilcoxon test, ** Mann-Whitney test

Discussion

Our data show that the binding of (+)- and (-)-propranolol to α_1 -AGP and plasma protein is stereoselective. Evidence of stereoselective binding to human plasma protein of drugs and endogenous substances has been reported for catecholamines (Powis, 1975), warfarin (Sellers & Koch-Weser, 1975) and benzodiazepines (Müller & Wollert, 1975; Simonyi & Fitos, 1983) and, to dog plasma, for propranolol (Bai et al., 1983).

The present data, confirm in man the results of Bai et al. (1983) and identify α_1 -AGP as the main source of this stereoselectivity.

Propranolol enantiomer pharmacokinetics in man treated with the racemic drug are different:

(-)-propranolol shows higher plasma levels, lower intrinsic clearance and longer half-life (George et al., 1972; Tawara et al., 1981; Silber et al., 1982). Accordingly, human liver microsomes were found to metabolize (-)-propranolol more slowly (von Bahr et al., 1982). A different ability of the two isomers to bind to human plasma proteins has now been demonstrated. Unfortunately, as reported under **Methods**, our analytical technique does not allow for the simultaneous determination of the two enantiomers in plasma containing the racemic drug, so plasma of patients treated with ($\pm$)-propranolol cannot be analyzed. However, these differences seem to have little clinical relevance at present.

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(Received October 14, 1983,
accepted March 31, 1984)

Note added in proof:

While this manuscript was under review, a paper on the same topic was published by U.K. Walle et al. in *Clin. Pharmacol. Ther.*, **34**, 718–23.