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The influence of gender and sex steroid hormones on the plasma binding of propranolol enantiomers

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1 Plasma binding of tritium-labelled racemic propranolol (P) was measured by equilibrium dialysis. The unbound enantiomers were separated by h.p.l.c after chiral derivatization. The binding of (−)-P was higher than that of (+)-P.

2 Contrary to previous suggestions, a sex difference in the plasma binding of the P enantiomers (9 young women, 12 young men) was not observed. The unbound percentage of (−)-P was 9.2 ± 1.8 (mean ± s.d.) in women *vs* 9.1 ± 1.7 in men; for (+)-P it was 10.8 ± 1.8 *vs* 10.8 ± 2.1.

3 In the nine women, the binding did not change with fluctuating plasma oestradiol concentrations during the menstrual cycle. Testosterone cypionate doubled the circulating concentrations of testosterone in eight men but had no effect on P binding.

4 Ethinyl oestradiol (50 $\mu g\ day^{-1}$) alone or together with norethindrone (OCD) in eight of the women produced an increase in the unbound percentage of both (−)-P (11.4 ± 2.6 *vs* 9.5 ± 1.6 for control; $P < 0.001$) and (+)-P (13.2 ± 2.5 *vs* 11.2 ± 1.5 for control; $P < 0.001$). This was due to a decrease in the plasma concentrations of α_1-acid glycoprotein from 0.54 ± 0.11 mg ml^{-1} in control to 0.37 ± 0.08 mg ml^{-1} ($P < 0.001$) during ethinyl oestradiol treatment.

5 Enantioselectivity in the unbound fraction of P increased with increasing total binding from a (−)/(+)-ratio of 0.93 at 84% binding to a (−)/(+)-ratio of 0.78 at 94% binding ($P < 0.001$).

Keywords propranolol enantiomers plasma binding ethinyl oestradiol testosterone oestradiol gender menstrual cycle humans

Introduction

Propranolol is one of relatively few drugs with clear sex differences in its human pharmacokinetics [1]. Thus, its oral clearance is lower in women than in men, an effect that is metabolic pathway (enzyme) specific [2]. Although testosterone appears to stimulate these metabolic pathways in men and possibly also in women [3], oestradiol has no effect [3, 4]. In contrast, when the synthetic oestrogen ethinyl oestradiol was administered to young women, both inhibitory and stimulatory effects, dependent on the pathway, on propranolol metabolism were observed [5].

Propranolol is also highly and stereoselectively bound to plasma proteins [6–9]. It has recently been reported that the stereoselectivity in this binding may be sex-dependent [10]. However, further studies on the potential effects of gender and sex steroid hormones on the plasma binding of propranolol are indicated.

The objective of this study was to determine the *in vivo* effect(s) of sex steroid hormones on the enantioselective plasma binding of propranolol in humans. Thus, we measured the binding of the propranolol enantiomers to human plasma in men and women and examined the effects on this binding of fluctuating hormone levels during the menstrual cycle as well as testosterone administration and treatment with ethinyl oestradiol alone or in combination with norethindrone.

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Methods

Materials

Rac-propranolol hydrochloride was purchased from Sigma (St Louis, MO). Rac-[4′-^{3}H]-propranolol hydrochloride (specific activity 14 Ci mmol^{-1}, radiochemical purity 99%) was from Amersham (Arlington Heights, IL). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and (R)-(+)- and (S)-(−)-propranolol were prepared as described previously [11, 12].

Subjects

Nine women (26–41 years old) and 12 men (23–29 years old) participated in the study. They were healthy nonsmokers taking no other drugs. All subjects were studied in a Clinical Research Center and were on an isocaloric diet (15% protein, 50% carbohydrate, 35% fat) for 2 days prior to as well as during the study. The studies were approved by the Institutional Review Boards for Human Research at the Medical University of South Carolina and the University of Arizona. The women were studied on five occasions: three times during a normal menstrual cycle, once after 3 weeks of 50 μg ethinyl oestradiol daily (Estinyl, Schering) and once after 3 weeks of 1 mg of norethindrone acetate + 50 μg ethinyl oestradiol (Norlestrin 1/50, Parke-Davis). In the normal menstrual cycle blood samples were drawn on day 5 of menses, at 16 days prior to expected menses (follicular phase), and at 8 days prior to expected onset of menses (luteal phase). The men were studied before and 7 days after 2 doses of long-acting testosterone cypionate (200 mg i.m., 14 days apart). Blood samples were drawn prior to propranolol administration between 06.00 and 08.00 h on study days by separate venepuncture, using polypropylene syringes. The blood was centrifuged in heparinized polypropylene tubes at 2000 *g* for 15 min to separate plasma, which was kept frozen in glass tubes at −80° C until assay.

Plasma protein binding

Plasma binding was measured by equilibrium dialysis [8], using Lucite chambers and Spectra/Por® dialysis membranes (MWCO 12–14,000) (Spectrum, Houston, TX). Plasma (1.2 ml) spiked with [^{3}H]-propranolol (0.5 μCi ml^{-1} in 25 μl assay buffer, containing 6 ng ml^{-1} unlabelled drug) was dialyzed against 1.2 ml of pH 7.4 phosphate-buffered saline at 37° C for 4 h, i.e. at equilibrium. The binding of racemic propranolol was determined by measuring aliquots of plasma and buffer by liquid scintillation spectrometry after dialysis.

The enantiomeric ratio of unbound propranolol was determined by chiral derivatization and h.p.l.c. Post-dialysis buffer samples (0.9 ml) were extracted with 10 ml of 1% (v/v) *n*-butanol in hexane after addition of 5 μg of unlabelled (±)-propranolol to facilitate u.v. detection. The extracts were taken to dryness and derivatized with GITC [11, 13]. The diastereomers were separated by reversed-phase h.p.l.c. using a Spherisorb ODS-2, 5 μm, 4.6 × 250 mm column (Phenomenex, Torrance, CA) with acetonitrile-water (65:35, v/v) in 0.05 M ammonium acetate, pH 4 as the mobile phase and u.v. detection at 280 nm. The chromatographic peaks corresponding to (−)- and (+)-propranolol, as identified by derivatization of the separate enantiomers, were collected and counted by liquid scintillation spectrometry. The binding of each enantiomer was thus determined in the presence of the other.

Other analyses

Total plasma oestradiol and testosterone concentrations were measured by radioimmunoassays (r.i.a.), using commercial kits from Diagnostics Products Corp. (Los Angeles, CA). In women the reported oestradiol concentrations are the means of study day morning and evening values. In men the testosterone values reported are the means from six plasma samples taken during the study day. All r.i.a. analyses were done in duplicate. Predose (07.00 h) plasma α_1-acid glycoprotein (AGP) levels were measured by radial immunodiffusion with 48 h endpoint area readings (NOR-Partigen, Behring Diagnostics Inc., Somerville, NJ).

Data analysis

Student's *t*-tests with $P < 0.05$ as the significance level were used. Paired *t*-tests were used for all comparisons except for binding in men compared with women, where an unpaired test was used.

Results

The plasma binding of (−)- and (+)-propranolol was similar in both sexes with a smaller unbound fraction of (−)-propranolol (Table 1). The mean (−)/(+)-enantiomer ratio for the unbound fraction of 0.84 was virtually identical to that reported previously [8].

Although intramuscular testosterone cypionate injections doubled the plasma testosterone concentrations in the men, there was no significant change in the plasma binding of the propranolol enantiomers (Table 2).

In women, the phase of the menstrual cycle did not affect plasma binding of the propranolol enantiomers, despite 3- to 4-fold differences in plasma oestradiol concentrations (Table 3).

Both ethinyl oestradiol alone and in combination with norethindrone produced a 20% increase in the free fraction of both (−)- and (+)-propranolol (Table 4). The plasma concentrations of AGP were decreased significantly by 35% after ethinyl oestradiol and by 26% after OCD, 0.35 ± 0.11 mg ml^{-1} and 0.40 ± 0.10 mg ml^{-1} compared with control, 0.54 ± 0.11 mg ml^{-1}. The extents of plasma binding of (−)- and (+)-propranolol in all samples were strongly correlated with AGP levels ($r = 0.80$ and 0.82, respectively; $P < 0.001$).

A linear relationship between the (−)/(+)-enantiomer ratio of unbound propranolol and total binding was found for all samples (i.e. from women and men with and without hormone treatment) (Figure 1). The enantioselectivity in propranolol binding, favouring the (−)-

Table 1 Plasma binding of (−)- and (+)-propranolol in 9 women and 12 men. Results are means ± s.d.

	Women		*Men*	
	Unbound (%)	*Range*	*Unbound (%)*	*Range*
(−)-Propranolol	9.2 ± 1.8	6.0 ± 11.8	9.1 ± 1.7 [−1.8 to 1.7]† NS	6.6–11.7
(+)-Propranolol	10.8 ± 1.8*	7.3 ± 13.3	10.8 ± 2.1* [−1.8 to 1.8]† NS	8.2–13.8
(−)/(+)-Enantiomer ratio	0.84 ± 0.03	0.78 ± 0.90	0.84 ± 0.03 [−0.03 to 0.03]† NS	0.80–0.88

†[95% confidence intervals of the difference between men and women]; NS not significant.
*Significantly different from (−)-propranolol; $P < 0.0001$.

Table 2 The effect of administering testosterone cypionate on the plasma binding of propranolol enantiomers in young, healthy men. Results are means ± s.d. for eight subjects

	Plasma testosterone (ng ml^{-1})	*Percentage unbound*	
		(−)-Propranolol	*(+)-Propranolol*
Control conditions	6.9 ± 1.7	9.5 ± 1.8	11.2 ± 2.2*
After testosterone cypionate	13.6 ± 3.9 [4.1 to 9.3]† $P = 0.0005$	10.4 ± 1.5 [−0.2 to 2.2]† NS	12.1 ± 1.4* [−0.6 to 2.4]† NS

*$P < 0.0001$ compared with (−)-propranolol.
†[95% confidence intervals of the difference between testosterone treatment and control].
NS not significant.

Table 3 The effect of the menstrual cycle on the plasma binding of propranolol enantiomers in young, healthy women. Results are means ± s.d. for nine subjects

	Plasma oestradiol (pg ml^{-1})	*Percentage unbound*	
		(−)-Propranolol	*(+)-Propranolol*
At day 5 of menses (1)	44 ± 17	9.0 ± 1.9	10.7 ± 1.9*
At day 16 prior to expected menses (2)	141 ± 114 [14 to 178]† $P = 0.029$	8.9 ± 2.1 [−1.0 to 0.8]† NS	10.5 ± 2.1* [−1.3 to 0.7]† NS
At day 8 prior to expected menses (3)	116 ± 62 [26 to 118]† $P = 0.009$	9.4 ± 2.0 [−0.5 to 1.1]† NS	11.2 ± 2.1* [−0.6 to 1.4]† NS

*$P < 0.0001$ compared with (−)-propranolol.
†[95% confidence intervals of the difference between menstrual phase 2 or 3 compared with phase 1]; NS not significant.

enantiomer was more pronounced in those samples showing greater binding.

Discussion

The finding of similar plasma binding of each propranolol enantiomer in men and women is in contrast to that of Gilmore *et al.* [10] who reported a higher binding of (−)-propranolol in women than in men but no gender difference in the binding of (+)-propranolol. We can offer no obvious explanation for this discrepancy. However, while we used low concentrations (6 ng ml^{-1}) of radiolabelled racemic propranolol and separated the enantiomers at the end of the experiment, Gilmore *et al.* [10] used individual radiolabelled enantiomers in the presence of 100 ng ml^{-1} of unlabeled racemic propranolol. In the present study neither the administration of testosterone nor the regular course of the menstrual cycle, i.e. when the circulating concentrations of the androgen and oestrogen hormones increased 2- to 3-fold, affected the plasma binding of the propranolol enantiomers. Although Parish & Spivey [14] reported a small effect of the menstrual cycle on plasma AGP concentrations, the main binding protein for basic drugs,

Table 4 The effect of administration of ethinyl oestradiol (EE_2, 50 μg) and an oral contraceptive drug (OCD) (50 μg ethinyl oestradiol +1 mg norethindrone acetate) on the plasma binding of propranolol enantiomers in young, healthy women. Results are means ± s.d., $n = 8$ for control, $n = 7$ for EE_2 and $n = 6$ for OCD

	Percentage unbound		
	Control	*EE_2*	*OCD*
(−)-Propranolol	9.5 ± 1.6	11.4 ± 3.0 [0.12 to 3.4]† $P = 0.039$	11.5 ± 2.3 [1.2 to 3.3]† $P = 0.003$
(+)-Propranolol	11.2 ± 1.5*	13.2 ± 2.9* [0.20 to 3.6]† $P = 0.034$	13.2 ± 2.1* [1.1 to 3.3]† $P = 0.004$

†[95% confidence intervals of the difference between hormone treatment and control].
*$P < 0.0001$ compared with (−)-propranolol.

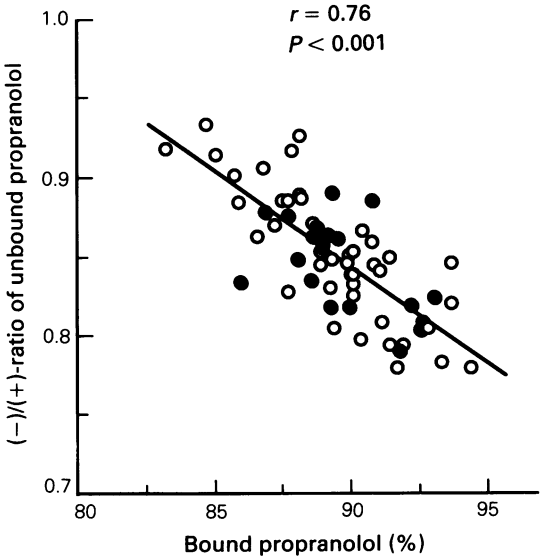

Figure 1 Relationship between the unbound (−)/(+)-enantiomer ratio and total plasma binding of propranolol (43 observations in 9 women (○) and 20 observations in 12 men (●)).

no drug binding experiments were done. To our knowledge there is no example of an effect of normal fluctuating levels of sex steroid hormones on the plasma binding of any drug.

In contrast, there was a significant, albeit non-enantioselective diminution of the binding of propranolol after treatment with the synthetic oestrogen ethinyl oestradiol. The administration of ethinyl oestradiol, with or without a progestin, has been shown to decrease plasma concentrations of AGP in some studies [15–18] but not in others [19]. There are few studies reporting both plasma drug binding and AGP concentrations during oral contraceptive use and the results have not been consistent [20–22]. As propranolol in plasma is bound mainly to AGP [6, 7], a parallel decrease in binding and AGP concentrations would be anticipated. The effects on AGP and binding produced by ethinyl oestradiol are similar to those in pregnant women at 39–41 weeks of gestation when large changes in oestrogen levels occur [21].

The finding that stereoselectivity in propranolol plasma binding is higher at higher total binding (Figure 1) extends a preliminary observation [8] and is consistent with data from Oravcová *et al.* [9]. These investigators demonstrated different binding kinetics of the propranolol enantiomers to AGP, i.e. saturable binding of (−)-propranolol but not of (+)-propranolol.

In summary, in this study we observed a decrease in circulating AGP concentrations during therapy with ethinyl oestradiol or an oral contraceptive drug, resulting in decreased plasma binding of both propranolol enantiomers.

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