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BINDING OF OXPRENOLOL AND PROPRANOLOL TO SERUM, ALBUMIN AND α_1-ACID GLYCOPROTEIN IN MAN AND OTHER SPECIES

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Abstract—Species differences in binding of basic drugs have only occasionally been studied and we have therefore measured the binding of the β-adrenergic blockers oxprenolol and propranolol in (1) serum of healthy humans, dogs, rats and rabbits and of rabbits with experimental arthritis, (2) a solution of albumin of these species and (3) a solution of human α_1-AGP. In humans, dogs, rats and arthritic rabbits, binding of oxprenolol and propranolol was much higher in serum than in albumin solution; in healthy rabbits serum binding was very low and not different from albumin binding. For both drugs, concentration-dependency was seen in serum of dogs, humans and rats and of arthritic rabbits; a similar concentration-dependency was found for human α_1-AGP solution, but not for human albumin and for serum of healthy rabbits.

Tris (2-butoxyethyl)-phosphate (TBEP), a known displacer of drugs from α_1-AGP in humans, decreased binding in serum of all species except the rabbit. For both β-blockers, species differences in capacity constants were found; species differences in affinity constants were present only for propranolol. These results suggest that in humans, dog and rat, but much less in rabbits, oxprenolol and propranolol bind mainly to α_1-AGP and that binding to α_1-AGP is more important for oxprenolol than for propranolol.

Serum binding of some basic drugs is increased in humans with inflammation or stress and it is accepted that this is due to an increase in concentration of the binding protein, serum α_1-acid glycoprotein (α_1-AGP) [1].

Systematic evaluations of species differences in serum binding of basic drugs are scarce. As such differences could be important for our understanding of the kinetics of these drugs in different species, we have studied the serum binding of two basic drugs, the β-blockers oxprenolol and propranolol, in serum of healthy humans, dogs, rats and rabbits and of rabbits with experimental arthritis. In order to interpret the results, the binding of the β-blockers to albumin of the 4 species and to human α_1-AGP was measured. Moreover, all binding experiments were also done in the presence of tris (2-butoxyethyl)-phosphate (TBEP) which is known to displace several basic drugs from serum α_1-AGP [2].

MATERIALS AND METHODS

Chemicals. Human serum albumin was purchased from Behringwerke AG; canine, rabbit and rat serum albumin, and human α_1-AGP from Sigma Chemical Co. ^{14}C-oxprenolol (21.4 μCi/mg) was donated by Ciba–Geigy, Switzerland, and ^{3}H-propranolol (100 μCi/mg) was purchased from Amersham, U.K. Tris (2-butoxyethyl)-phosphate (TBEP) was kindly supplied by Ega-Chemie, Germany. Picofluor-15 was purchased from Packard Instruments.

Serum. Serum was obtained from healthy humans of both sexes and of healthy animals, dogs of both sexes, male Wistar rats and male rabbits; for the rat, serum of several animals was pooled. Serum was also obtained from rabbits in which experimental arthritis had been induced by injection of rabbit fibrin and Freund's complete adjuvant intradermally weekly for 4 weeks and in both knee joints the 5th week, according to the method of Dumonde and Glynn [3] and serum was obtained the 6th week.

Binding experiments. In vitro protein binding of the drugs was measured by equilibrium dialysis. Dialysis was performed in duplicate at 25° for 4 hr in Teflon half-cells separated by a cellophane membrane (Visking). One compartment contained phosphate buffer (0.3 ml; 0.15 M, pH 7.4) in which the drug was dissolved and the other contained either serum or serum albumin (4 g/100 ml) or α_1-AGP (70 mg/100 ml), dissolved in the same buffer (0.3 ml). To obtain the different drug concentrations, a constant amount of labeled substance, and varying amounts of unlabeled drug were added.

After dialysis, 100 μl aliquots from both compartments were counted in a Tri-Carb Liquid Scintillation Spectrometer (3380) after addition of Picofluor 15 (3 ml).

The binding of oxprenolol and propranolol to the different protein solutions was also measured in the presence of TBEP in concentrations ranging from 0 to 100 μg/ml at the beginning of the dialysis.

Calculation of binding. The fraction unbound (f_u) was obtained from the ratio of the number of disintegrations per minute at equilibrium in buffer and protein solution.

The total molar concentration of drug in the protein solution after equilibration, C_t, was determined

by

$$C_t = \frac{C_i}{1 + f_u} \quad (1)$$

where C_i is the initial molar concentration of drug in the buffer compartment.

The molar concentrations of unbound drug, C_u, and bound drug, C_b, after equilibrium were determined by the following equations:

$$C_u = f_u \cdot C_t \quad (2)$$

$$C_b = C_t - C_u \quad (3)$$

The binding data for serum and human α_1-AGP were analysed to obtain the association and capacity constants. The interaction between drugs and protein follows the law of mass action and consequently can be characterized by the equation:

$$C_b = \sum_{i}^{n} \frac{N_i K_i C_u}{1 + K_i C_u} \quad (4)$$

where K_i is the association constant for the ith class of sites, N_i the total binding site concentration (capacity constant) of the ith class, and n the number of classes.

Rosenthal plots were performed, in which bound over free concentration is plotted against bound concentration [4]. To obtain initial estimates of the binding parameters, a multiple linear regression analysis was done after transformation of the experimental data [5]. These preliminary binding parameters were optimized by a non-linear curve fitting of free versus bound concentration based on the law of mass action and using a statistically unbiased method [6]. All calculations were carried out with a BASIC program on a TRS-80 microcomputer. The model and the method of analysis are acceptable as proven by the closeness of the observed points and the Rosenthal plot simulated with the final binding parameters, and by the lack of systematic deviations.

RESULTS

Figure 1 shows the binding of oxprenolol (1.25 μg/ml) and propranolol (10 ng/ml) in serum and to albumin (4 g%) in the different species, and to human α_1-AGP (70 mg%). In the 4 species, the binding of both β-blockers was lower in the albumin solution than in serum; this was more pronounced for oxprenolol. The binding to human α_1-AGP was higher than that to human albumin, but still lower than that in serum. In the rabbit, binding in serum and to albumin was very low; binding in serum of arthritic rabbits was markedly higher.

The binding of both drugs was also measured in function of their pre-equilibrium concentration in serum of the 4 species, and in human albumin and α_1-AGP [for oxprenolol from 0.25 μg to 51.25 μg/ml (0.942 – 193.15 μM) and for propranolol from 0.1 μg to 50 μg/ml (0.338–169.1 μM)]. The binding of both drugs to human α_1-AGP and serum, and to serum of dogs and rats is concentration-dependent. In the rabbit the concentration-dependency was only seen after induction of arthritis; in the arthritic rabbits the binding of both drugs was increased. The results are

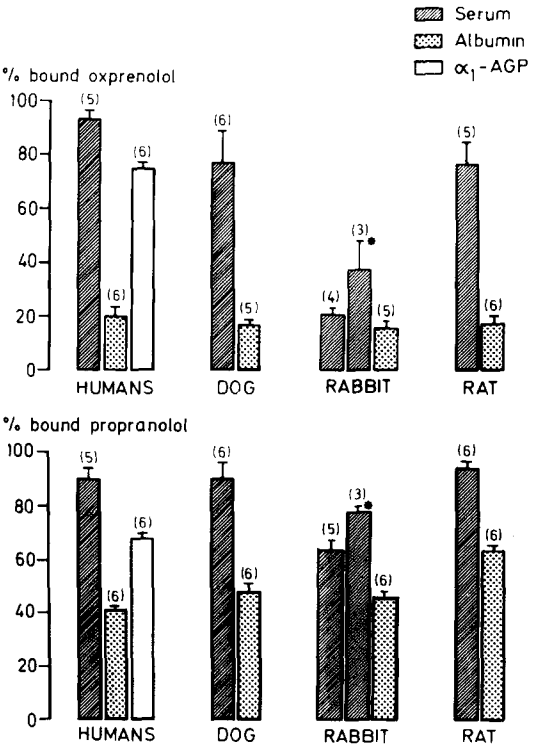

Fig. 1. Binding, in percent, of oxprenolol (1.25 μg/ml) and propranolol (10 ng/ml) to serum and to albumin (4 g%) of different species and to human α_1-AGP (70 mg%). Means (± S.E.M.) are given; the number of experiments is given between brackets. * Serum of arthritic rabbits.

shown in Fig. 2 for oxprenolol; for propranolol the results were similar but the concentration-dependency was less pronounced than for oxprenolol.

Rosenthal plots indicated that in sera of humans, dog and rat, and in human α_1-AGP solution, both drugs are bound to two classes of binding sites; in rabbit serum and in human albumin solution such analysis was impossible in view of the low binding.

Accurate calculation of the association and binding constants was only possible for the first binding site; indeed, within the concentration range that can be used, no saturation occurs for the second binding site. In Table 1 the association and capacity constants for the first binding site are given. For propranolol, large species differences were present for both constants; for oxprenolol the K_i values were comparable in the different species, but the N_i values were different. For both substances, a large intraspecies variability was observed for N_i.

Figure 3 shows the binding of oxprenolol and propranolol in the presence of increasing concentrations of TBEP, in serum and in albumin solutions of the 4 species and in human α_1-AGP. A displacing effect of TBEP was seen in serum of the 4 species, but in rabbits only after induction of arthritis. TBEP had also a clearcut effect on the binding to human α_1-AGP, but not on albumin binding. The effect was more pronounced for oxprenolol than for propranolol.

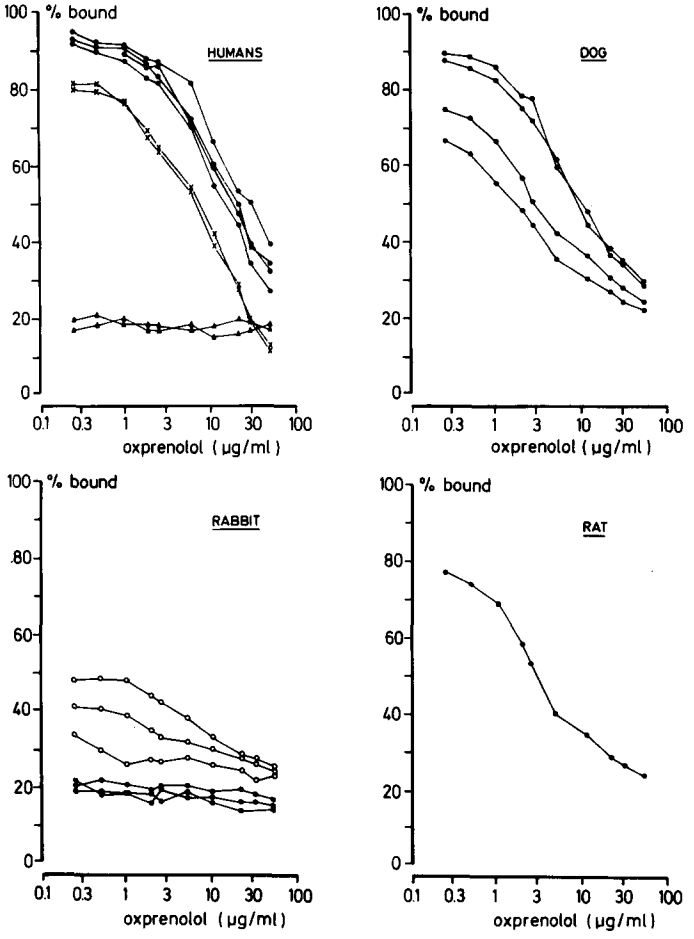

Fig. 2. Binding, in percent, of oxprenolol, in function of drug concentration, to serum of different species (●), to serum of arthritic rabbits (○) and to human albumin (4 g%) (▲) and human α_1-AGP (70 mg%) (×). For serum, each curve denotes one animal or human, except for the rat where serum of different animals was pooled. For both albumin and α_1-AGP, two experiments were performed. Each point is the mean of duplicate determinations.

DISCUSSION

For highly bound drugs, variation in serum binding among individuals or among species can affect pharmacokinetics and pharmacodynamics, leading to differences in tissue levels, effects and toxicity. While species differences in binding of acidic drugs have been adequately studied [7], such studies are scarce for basic drugs.

We have therefore studied in different species the binding of oxprenolol and propranolol. Serum binding of β-blockers has been studied repeatedly in humans. Both oxprenolol and propranolol are, in man, mainly bound to α_1-AGP and to a lesser degree to albumin [8]. For propranolol, binding to lipoproteins was also reported but is probably of minor importance [9, 10].

Our results show that both β-blockers are highly bound in serum of humans, dogs and rats, which corresponds to the data obtained for propranolol by Evans *et al.* [11]. For disopyramide, which is another basic drug mainly bound to α_1-AGP, Lima and Haughey [12] found that binding was lower in dogs than in man and even lower still in rats. In our rabbits, the serum binding of both β-blockers was much lower than in other species, but it increased considerably in rabbits with experimental arthritis. Lima and Haughey [12] found for disopyramide also a very low serum binding in the rabbit.

Albumin binding was in all species lower than serum binding, and was quite similar in all species. The difference in binding between serum and albumin within each species, and the interspecies differences in serum binding are probably related to binding to a protein other than albumin, possibly α_1-AGP. Indeed, it was found that in a solution of human α_1-AGP, binding was more pronounced than in human albumin solution. The importance of α_1-AGP binding for the species differences is also suggested by the fact that serum binding in the rabbit, which is low and hardly higher than binding to albumin, increases markedly after induction of arthritis. Although α_1-AGP concentrations have to our knowledge not been measured in the rabbit, it is to be expected that in inflammatory situations the concentration of this protein increases in this species, as it does in rats [13] and in humans [14]. In healthy

Table 1. Comparison of the constants characterizing the first binding site in different species

		Oxprenolol		Propranolol	
		$K_1(\mu M^{-1})$	$N_1(\mu M)$	$K_1(\mu M^{-1})$	$N_1(\mu M)$
Humans	serum 1	1.40	6.43	0.587	20.4
	2	1.14	10.61	0.522	15.4
	3	1.28	9.36	0.568	15.3
	4	1.32	8.40	0.650	15.1
	α_1-AGP	1.48	2.86	0.244	10.7
		1.05	3.81		
Dog	serum 1	1.05	7.91	1.12	3.67
	2	1.37	5.04	1.25	8.67
	3	1.22	2.65	1.26	7.51
	4	1.07	1.90	1.29	7.01
Rat	pool 1	1.09	3.25	2.04	1.75
	2	1.02	3.12		

rabbits, the concentration of α_1-AGP is probably very low or the protein has a low affinity for the drugs studied.

The suggestion that α_1-AGP binding is responsible for the species differences in binding of the two β-blockers, is further strengthened by the experiments with TBEP. In humans, the plasticizer TBEP decreases markedly the serum binding of some basic drugs and is thought to act by displacing these drugs from their binding sites on α_1-AGP [2, 15]. In man, we found, as expected, an important effect of TBEP on binding of both drugs in serum and α_1-AGP solution, but not in albumin solution. In rats and in dogs too, the displacing effect was seen in serum, but not in albumin, suggesting that in these species, as in man, both drugs bind to an important degree to α_1-AGP. In the rabbit, however, a displacing effect of TBEP on binding in serum was only seen after induction of experimental arthritis. This is in keeping with the idea that, in inflammation, serum α_1-AGP increases and that the increase is responsible for the increased binding of both drugs. In our hands, TBEP had no effect on the binding of the β-blockers to albumin; this is in contrast with the results obtained

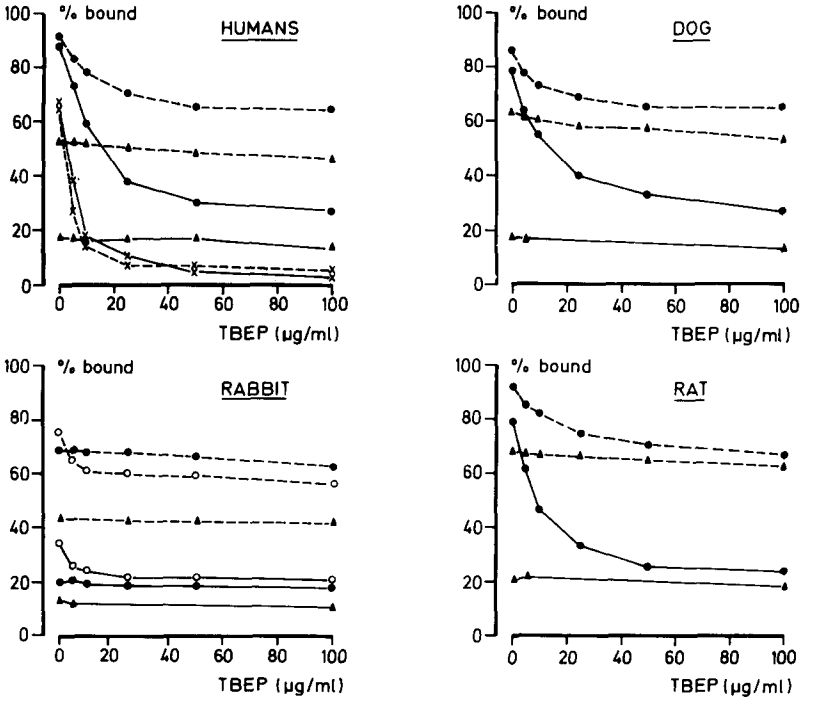

Fig. 3. Binding, in percent, of oxprenolol 1.25 µg/ml (——) and propranolol 10 ng/ml (- - - -) to serum (●) and albumin 4 g% (▲) of different species, to serum of arthritic rabbits (○) and to human α_1-AGP 70 mg% (×), in function of TBEP concentration. Each point is the mean of duplicate determinations.

by Pike *et al.* [15] for quinidine and phenytoin. This can perhaps be explained by differences in the quality of the albumin solution used, with e.g. contamination of commercial albumin with α_1-AGP [16].

The binding of both drugs to serum was in each species, except in healthy rabbits, dependent on the concentration of drug. This corresponds to the data of Evans *et al.* [11] for propranolol and the data of Lima and Haughey [12] for disopyramide in different species. However, within the range of serum concentrations found in humans after therapeutic doses of the two β-blockers, changes in binding are very small.

Plotting of bound over free concentration versus bound concentration according to Rosenthal [4] was performed for serum and for human α_1-AGP. These plots were curvilinear, suggesting the existence of at least two binding sites.

For the calculation of the binding parameters, we decided not to use graphical extrapolation from Rosenthal plots, as this gives only a crude estimation of capacity and affinity constants [17]. Therefore, the initial estimations of the binding parameters were obtained with a computerized method [5]. The initial parameters were optimalized by a non-linear curve fitting of the unmanipulated primary experimental data, i.e. of free versus bound concentration.

The solubility of the products does not permit the use of concentrations high enough to saturate the second binding site. Therefore, the capacity and affinity constants of the second binding site could not be calculated accurately and are not reported. This non-saturable binding site could correspond to an albumin binding site; indeed in human albumin solution too, no saturation occurs, and when high concentrations of TBEP are added to serum, binding of the β-blockers decreases to a value close to that found in the albumin solution.

The saturable binding site which is found for both drugs in each species except the rabbit, is probably located on α_1-AGP. This has already been proven for propranolol in humans by different authors [9, 10, 18]. Saturation was also present in the human α_1-AGP solution.

For both oxprenolol and propranolol, the capacity constants showed a clear-cut species difference, which suggest differences in α_1-AGP serum concentration. For propranolol there were also differences in affinity between the species. Moreover, in humans and dogs for both drugs a large intraspecies variability in capacity was found. The good reproducibility when the same serum was studied 5 times, confirmed that this intraspecies variability is not a methodological artefact (unpublished results). Lima and Haughey [12] found that interspecies variability in disopyramide binding in serum was mostly due to differences in affinity, and not in capacity. However, these authors performed only one experiment per species, and in view of the large intraspecies capacity variation just mentioned, their results should be viewed with caution. The fact that interspecies differences in affinity are present for one β-blocker and not for the other, suggests different binding sites on α_1-AGP; nevertheless, both drugs are displaced by TBEP.

From our results it appears that oxprenolol binds almost exclusively to α_1-AGP; for propranolol, binding to albumin is also important; this explains our observation that the concentration-dependency is less pronounced for propranolol, as the albumin binding is not saturable within the concentration range studied, and also the observation that the effect of TBEP—which is supposed to interact with α_1-AGP binding—is less important for propranolol than for oxprenolol.

Our results suggest that for propranolol and oxprenolol, α_1-AGP is the mean binding protein in serum of humans, dog and rat, while in rabbits binding to α_1-AGP becomes only apparent after induction of inflammation. Saturation of binding on this high affinity site only occurs at supratherapeutic drug concentrations. The species differences in binding can probably be explained by differences in concentration or in affinity of α_1-AGP and could be of importance for the kinetics and dynamics of the substances studied.

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