J. Pharm. Pharmacol. 1988, 40: 459–463
Received September 30, 1987

Buffer and pH Effects on Propranolol Binding by Human Albumin and α_1-Acid Glycoprotein

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Abstract—Propranolol binding to isolated human α_1-acid glycoprotein (AGP) and human albumin (HSA) was studied by equilibrium dialysis at 37°C. With AGP (0·067%) and HSA (4%), total propranolol concentration was varied from 0·7 to 93 000 ng mL^{-1}. Over this concentration range the percentage drug bound to HSA declined from 49 to 39% while that to AGP declined from 68 to 4%. Two classes of sites were identified on AGP with $n_1k_1 = 8{\cdot}50 \times 10^4$ M^{-1} and $n_2k_2 = 3{\cdot}12 \times 10^4$ M^{-1}. With a pH 7·4 phosphate buffer, propranolol binding to AGP was greatest when the protein was initially dissolved in pH 7·4 water compared with pH 7·2 water or the phosphate buffer. Thus, the method of AGP solution preparation affected propranolol binding by this protein. For both AGP and HSA, greater drug binding was noted with phosphate buffers in comparison with a physiological buffer. With phosphate buffers, decreasing pH from 7·4 to 7·0 decreased propranolol binding by AGP, while decreasing pH from 7·7 to 7·4 had little effect. With HSA, the percent propranolol bound consistently decreased on lowering pH from 7·7 to 7·0.

For many basic drugs, binding to human serum albumin (HSA) alone does not completely account for the observed degree of plasma protein binding. Propranolol is known to bind to HSA, lipoproteins, and the acute phase protein α_1-acid glycoprotein (AGP) (Glasson et al 1980). The latter protein plays a major role in the binding of many basic drugs (Lunde et al 1986). Plasma levels of AGP increase in renal failure, rheumatoid arthritis, Crohn's disease, myocardial infarction, burns, and trauma (Abramson et al 1982) and decrease in hepatic disease, pregnancy, nephrotic syndrome, and malnutrition (Gillis et al 1985).

Several basic drugs which bind to AGP display non-restrictive or blood flow-limited elimination. Implications of protein binding on propranolol pharmacokinetics have been reported (Kornhauser et al 1978; Vu et al 1983). Pharmacological effects of propranolol have been best correlated with free drug concentration (McDevitt et al 1976). The purposes of the present studies are to characterize the binding of propranolol to isolated AGP and HSA, and to determine the influence of buffers and pH on this binding. The effect of method of protein solution preparation was also examined for propranolol binding to AGP.

Materials and Methods

The binding of propranolol (Ayerst Labs.) by isolated α_1-acid glycoprotein (Calbiochem-Behring Corp.) and crystallized, essentially globulin-free human albumin (Sigma Chemical Co.) was examined. Propranolol was quantitated through the use of [^{3}H] propranolol (Amersham Corp.). Monobasic and dibasic sodium phosphate were used to prepare freshly the 0·014, 0·028, 0·067 M (Sørensen), 0·1 and 0·134 M phosphate buffer. The 0·014, 0·028, and 0·067 M buffers also contained 0·01925, 0·0385 and 0·0753 M NaCl, respectively. A physiological buffer containing 0·132 M NaCl, 0·0049 M KCl, 0·0012 M $MgSO_4$ and 0·016 M Na_2HPO_4 and trace amounts of HCl was also used. Each buffer had a pH of 7·4.

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Propranolol binding was determined using acrylic equilibrium dialysis cells and membranes with a molecular weight cut off of 6000. Human albumin (HSA), 200 μL, 4%, or 0·067% α_1-acid glycoprotein (AGP) were dialysed against an equal volume of various buffer systems containing tritiated and unlabelled propranolol. Dialysis cells were gently rocked at 37 ± 0·2°C for 24 h. Preliminary studies established that this time was sufficient for obtaining equilibrium and that propranolol did not undergo detectable degradation. Lack of significant weight change in the dialysis system indicated that solution leakage or evaporation did not occur during dialysis. No fluid flux was observed with the use of these protein solutions. Comparative studies were performed with the same batch of protein and ordered to remove bias. All reported percentages bound represent the mean of at least three replications. Comparisons of percentages bound among methods and concentrations were performed by analysis of variance techniques and significant differences ($P < 0{\cdot}05$) were evaluated by the methods of Dunnett (1955).

The radioactivity in 100 μL aliquots of the buffer and protein solutions was determined through liquid scintillation counting. Counts were corrected for background and also for quenching through an external standardization. The fraction of propranolol free was determined as the ratio of the corrected postdialysis counts in the buffer and protein solution.

The effect of buffer composition on HSA binding of propranolol was examined by dissolving HSA in the appropriate buffer and dialysing against the same buffer. The buffers chosen were a pH 7·4, physiological buffer (treatment X), a pH 7·4, 0·067 M Sørensen phosphate buffer (treatment Y), and a pH 7·4, 0·1 M sodium phosphate buffer (treatment Z).

For similar studies with AGP, three methods of preparing the protein solutions were compared. In treatment A, AGP was dissolved in water adjusted to pH 7·4 and dialysed against 0·028 M phosphate buffer. In treatment B, AGP was dissolved in 0·014 M phosphate buffer and dialysed against

0·014 M buffer. The buffer composition at the end of dialysis was identical for treatments A and B. The adjustment of water pH with NaOH had a negligible contribution to sodium concentration. AGP was also dissolved in pH 7·4 water and dialysed against double strength physiological buffer (treatment C).

To investigate further the effect of AGP solution preparation, a study was also conducted with AGP dissolved in pH 7·2 adjusted water with pH 7·4, 0·028 M phosphate buffer as a dialysant (treatment D). The pH of the water was adjusted as previously described with NaOH. All solutions (treatments A–D) had a pH of 7·4 at the end of dialysis and were performed in the same series of studies. Binding parameters were determined for treatments A and D. Values of n_i, the number of binding sites of class i, and k_i, the intrinsic association constant for class i, were obtained by non-linear regression (Metzler et al 1974) of Scatchard plots (Scatchard 1949) using 1/y weighting of data. Binding parameters (n_i, k_i and n_ik_i) were compared as described by Boxenbaum et al (1974).

The effect of pH on HSA binding of propranolol was investigated using 0·067 M isotonic phosphate buffers. For pH 7·0, 7·4, and 7·7 these buffers contained 0·0787, 0·0753 and 0·0736 M NaCl, respectively (Deardorff 1980) and had ionic strengths of 0·226, 0·249, and 0·261, respectively. HSA was dissolved in the appropriate buffer and dialysed against the same buffer. Similar studies with AGP were conducted with 0·028 M phosphate buffers containing 0·0385 M NaCl. At pH values of 7·0, 7·4, and 7·7, the buffers had ionic strengths of 0·100, 0·111, and 0·117, respectively. AGP was dissolved in water adjusted to the appropriate pH and dialysed against the buffer of the same pH.

Results and Discussion

The mean recovery of [^{3}H]propranolol after dialysis was 97·8% ± 2·0 for all HSA studies and 97·0% ± 2·3 for all AGP studies. Thus, loss of propranolol to the dialysis cell and/or membrane was considered negligible. The pH of each solution was verified before dialysis and did not change during dialysis. In general, at pH 7·4 the composition of the standard buffers examined had a small, but significant, effect on propranolol binding by HSA (Table 1). The overall trend was an increase in propranolol binding with increases in phosphate molarity and decreases in the molarity of NaCl and other electrolytes. Thus, buffer components can alter HSA binding of propranolol through possible protein conformational changes and/or competition for binding sites.

Table 2. The influence of pH on propranolol binding by human albumin and α_1-acid glycoprotein.

Propranolol HCl (ng mL^{-1})	Protein	Percent propranolol bound[b] pH 7·0	pH 7·4	pH 7·7
28·0	HSA	38·2 (0·58)*	50·6 (0·59)	53·8 (0·60)*
738·0	HSA	38·1 (0·37)*	48·8 (0·71)	53·3 (1·05)*
4·8	AGP	55·7 (1·96)*	64·8 (0·94)	65·0 (0·96)
73·8	AGP	53·8 (1·71)*	60·5 (2·18)	57·9 (1·56)
512·0	AGP	51·2 (2·33)*	56·3 (3·37)	49·5 (1·09)*
2357·0	AGP	35·9 (5·48)*	41·4 (2·35)	44·5 (2·23)

[a] Approximate, in protein solution postdialysis
[b] Mean (s.d.)
* Significantly different from pH 7·4, $P < 0·05$

In serum at 23°C the percent propranolol free doubled upon decreasing pH from 8·95 to 6·54 (Paxton & Calder 1983). This indicated propranolol binding by HSA and/or AGP was pH-dependent. In the present study, HSA binding of propranolol was found to be pH-dependent (Table 2). The percent propranolol bound consistently decreased on lowering pH from 7·7 to 7·0. Decreasing pH from 7·4 to 7·0 also generally decreased propranolol binding by AGP, while decreasing pH from 7·7 to 7·4 had little effect. These effects are consistent with the effect of pH on AGP binding of disopyramide (Sharps et al 1981). Since the percent propranolol un-ionized only decreases from 1·75% at pH 7·7 to 0·35% at pH 7·0, the observed changes in binding are probably due to pH-dependent protein conformational changes. The small variations in buffer ionic strength may also contribute to these changes.

To characterize propranolol binding further by 4% HSA, studies were conducted over a wider postdialysis drug concentration range (0·75 to 93436 ng mL^{-1}). Over the approximate 125000 fold range of propranolol concentrations, the percent drug bound was constant (49%) up to 5000 ng mL^{-1} (Fig. 1). At the largest drug concentration examined, propranolol was still 39% bound. Thus, HSA has a very high capacity for propranolol. In a similar study over a similar range of propranolol concentrations, the percent drug bound to HSA was slightly higher (55%) and began decreasing at 25 ng mL^{-1} propranolol (Glasson et al 1980). At a concentration of $2·5 \times 10^5$ ng mL^{-1} propranolol was approximately 44% bound. These differences may be, at least partially, due to the different buffer system (0·66 M phosphate

Table 1. The effect of buffer composition on human albumin binding of propranolol.

Propranolol HCl ng mL^{-1}[a]	Percent propranolol bound[b] Physiol. buffer (A)	0·067 M Sørensen Phosphate buffer (B)	0·1 M Sodium Phosphate buffer (C)	Comparisons[c]
3·8	48·6 (0·38)	50·5 (0·28)	51·3 (0·28)	B=C>A, B>A
28·5	48·3 (1·15)	50·6 (0·59)	51·5 (0·18)	B=C>A, B>A
253·0	48·1 (0·66)	49·4 (0·08)	51·3 (0·75)	B<C>A, B>A
751·0	48·1 (0·25)	48·8 (0·71)	51·4 (0·73)	B<C>A, B=A

[a] Approximate concentration in HSA solution postdialysis.
[b] Mean (s.d.)
[c] $P < 0·05$.

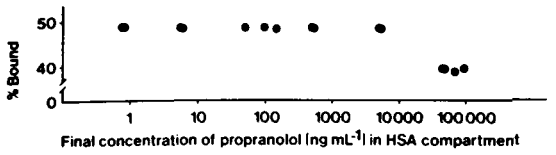

FIG. 1. Percentage of propranolol HCl bound to 4% human albumin in pH 7·4 physiological buffer at 37°C against the final concentration of propranolol in the HSA compartment.

buffer) used in the previous study. The fatty acid free Fraction V used in the previous study often contains AGP as a contaminant (Lunde et al 1986). This contaminant could explain the greater binding of propranolol to HSA and the decrease in HSA binding at propranolol concentrations greater than 25 ng mL^{-1}.

In a study on imipramine and alprenolol binding by AGP the protein was dissolved in water and dialysed against pH 7·4, 0·028 M sodium phosphate buffer (Borga et al 1977). The present study examined whether propranolol binding would be affected by the method of AGP solution preparation. As shown in Fig. 2, at the lower propranolol concentrations examined the percent propranolol bound by AGP was slightly greater for protein dissolved in water (treatment A) compared with protein dissolved in buffer (treatment B). At the end of dialysis the two treatments result in identical solutions in terms of buffer composition. This implies the method of protein dissolution affects the conformation and/or possible aggregation of AGP and the effect is not completely reversed during the time of dialysis. The effect may be primarily associated with higher affinity sites since differences in percent bound were greatest at low propranolol concentrations. At propranolol total concentrations of 4·8 ng mL^{-1}, dissolving AGP in pH 7·4 water (treatment A) yielded percentages bound of $64·8 \pm 0·9$, which was significantly greater than the $57·3 \pm 1·3$ observed for AGP dissolved in buffer (treatment B). With higher drug concentrations of 25000 ng mL^{-1}, treatment A and B showed no significant differences with values of $16·5 \pm 0·7$ and $17·6 \pm 0·9$ percent bound, respectively.

Buffer composition had a much greater effect on percent propranolol bound by AGP than the method of protein solution preparation. Use of the physiological buffer (treatment C) resulted in a significantly lower percent of propranolol bound than the use of the 0·014 M phosphate buffers of treatment A and B (Fig. 2). The percent bound for the physiological buffer system at 4·8 ng mL^{-1} propranolol was $38·8 \pm 2·6$. Since all buffers (A–D) have a similar phosphate concentration, it appears that the electrolytes of the physiological buffer are primarily responsible for the decrease in propranolol binding. The percent propranolol bound was similar only at extremely high drug concentrations.

Propranolol binding by AGP was also examined over a wide range of drug concentrations and comparisons between two methods of protein solution preparation were made. AGP dissolved in either pH 7·4 (treatment A) or pH 7·2 (treatment D) adjusted water was dialysed against 0·028 M, pH 7·4 phosphate buffer. The final pH of all solutions after dialysis was 7·4. Therefore, the only difference between the conditions was the pH at which AGP was dissolved. As observed in a previous study at pH 7·4 (Glasson et al 1980), binding was saturable. The percent drug bound was constant to approximately 500 ng mL^{-1} propranolol, then decreased rapidly. Compared with the previous study (Glasson et al 1980), propranolol binding by AGP dissolved in pH 7·4 water was slightly greater at lower drug concentrations (65% compared with 60%). This could reflect differences in the buffer systems used and/or the method of AGP solution preparation.

The results (Fig. 3) again emphasize that the method of AGP solution preparation significantly affects propranolol binding. With drug concentrations of 4·8 ng mL^{-1}, the percent bound for propranolol initially dissolved in pH 7·2 water was $52·2 \pm 2·1$ which was significantly less than the percent obtained when AGP was dissolved in pH 7·4 water (treatment A) or directly in buffer (treatment B). Except at very large propranolol concentrations, the percent drug bound was lower when AGP was dissolved in pH 7·2 adjusted water (treatment D) when compared with treatments A and B. Decreases in the percent propranolol bound with increasing drug concentration were parallel for the two pH values of preparation conditions. This, again, indicates the method of protein dissolution may affect the conformation and/or possible aggregation of AGP and the effect is not completely reversed during the time of dialysis. Though the postdialysis pH was 7·4 in all of these experiments, the results correlated well with the above data on the effect of pH on

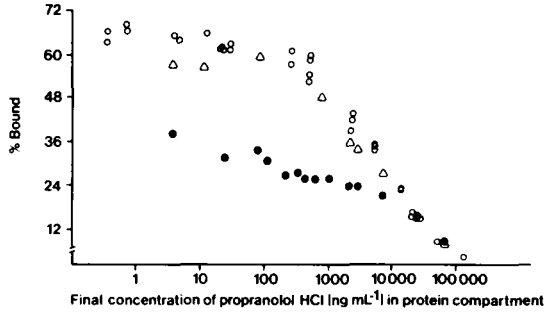

FIG. 2. Buffer and protein solution preparation effects on propranolol binding by α_1-acid glycoprotein at pH 7·4 (○) protein dissolved in pH 7·4 water and dialysed against 0·028 M phosphate buffer (treatment A), (△) protein dissolved in 0·014 M phosphate buffer and dialysed against the same buffers (treatment B), (●) protein dissolved in pH 7·4 water and dialysed against double strength physiological buffer (treatment C).

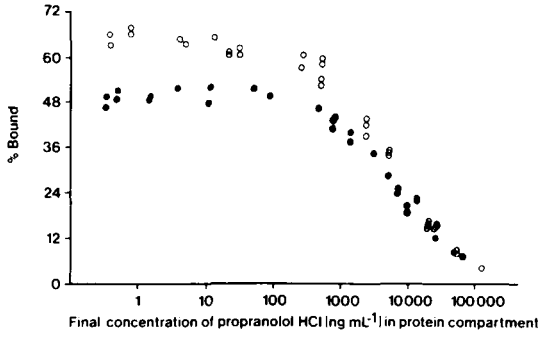

FIG. 3. Protein solution preparation effect on propranolol binding to α_1-acid glycoprotein dialysed against pH 7·4, 0·028 M phosphate buffer. (○) protein dissolved in pH 7·4 water; (●) protein dissolved in pH 7·2 water.

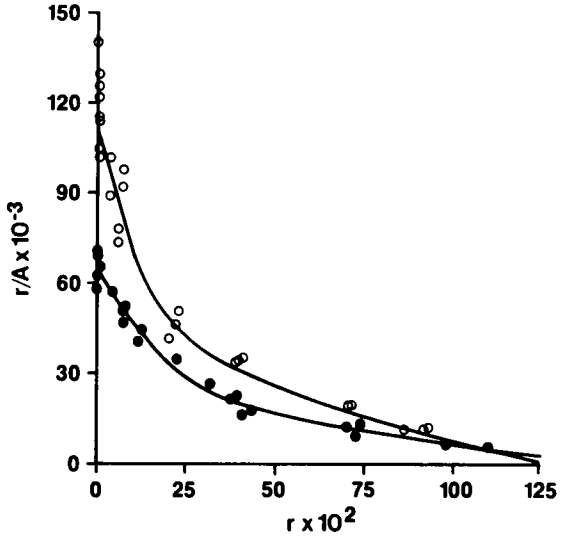

FIG. 4. Scatchard plot of propranolol binding to α_1-acid glycoprotein initially dissolved in pH 7·4 water (○) and in pH 7·2 water (●) and dialysed against 0·028 M phosphate buffer.

propranolol binding by AGP. In these studies, a lowering of pH decreased propranolol binding (Table 2).

Scatchard plots (Fig. 4) of the propranolol-AGP data were constructed to evaluate further the effect of the method of protein solution preparation on binding to AGP dissolved in pH 7·4 water (treatment A) and pH 7·2 water (treatment D). Both plots are curvilinear suggesting each AGP solution contained binding sites with significantly different affinities. Based on standard deviations of estimates and weighted residuals, the best computer fit to the data was obtained when the ordinate values were weighted as the ordinate's reciprocal (1/y) rather than as 1·0. Reciprocal weighting methods are more appropriate for binding data due to the wide range of ordinate values. Binding constants obtained for both initial pH values are presented in Table 3. For both initial pH values (treatments A and D) the values of n_1 are much less than one while the total number of binding sites for AGP is slightly greater than one. Constraining the values of n_2 to 1·0 resulted in only a slight increase in the values of n_1 (Table 3). This implies that all of the AGP molecules have at least one binding site for propranolol with moderate affinity while a small portion of the AGP molecules may also have a second site of much higher affinity. AGP consists of polypeptide variants which may have different binding parameters for the same ligand (Kirley et al 1982). The presence of metal ions and lipids may also affect AGP binding (Westphal 1971). For propranolol, delipidated and non-delipidated AGP yielded stoichiometries of 0·90 and 0·13, respectively (Busby & Ingham 1986). Similar to the present results, methadone is bound by two classes of sites on AGP with $n_1 = 0{\cdot}38$ (Abramson 1982).

The reported binding constants for the interaction of propranolol with AGP at 37°C have been quite varied. Values of n of approximately one with k values ranging from approximately 3×10^4 to 8×10^5 M^{-1} have been reported (Glasson et al 1980; Wong & Hsia 1983; Soltes et al 1985; Morin et al 1986). This range of k values includes the values of both k_1 and k_2 found in the present study (Table 3). Values of $n = 0{\cdot}6$ with $k = 2 \times 10^5$ M^{-1} (Belpaire et al 1984) and $n_1 = 0{\cdot}6$, $k_1 = 4 \times 10^5$ M^{-1} and $n_2 = 1{\cdot}13$, $k_2 = 5 \times 10^3$ M^{-1} (Gillis et al 1985) have also been reported. These variations are quite large even though binding differences are known to occur between different brands and batches of this protein (Morin et al 1986; Lunde et al 1986). Our studies demonstrate that buffer and protein solution preparation effects may explain the large range of binding parameters reported in the literature. Of course, low values of r must be obtained experimentally in order to detect low values of n_1.

In comparing binding parameters obtained for the two methods of AGP solution preparation, it appears that changes in binding were due to changes in the affinity of sites rather than in the number of sites. With a variable n_2, dissolving AGP in pH 7·2 water significantly decreased k_2. In the case where $n_2 = 1$, a significant decrease in k_1 was observed. However, the use of the product $n_i k_i$ is best for comparative purposes since the estimates of k_i are highly correlated with the values of n_i obtained (Parsons, 1980). With n_2 unconstrained, $n_1 k_1$ values of $8{\cdot}50 \times 10^4 \pm 0{\cdot}47 \times 10^4$ M^{-1} and $5{\cdot}13 \times 10^4 \pm 0{\cdot}27 \times 10^4$ M^{-1} were obtained for initial pH values of 7·4 and 7·2 (treatments A and D), respectively. Values of $n_2 k_2$ of $3{\cdot}12 \times 10^4 \pm 0{\cdot}45 \times 10^4$ M^{-1} and $1{\cdot}35 \times 10^4 \pm 0{\cdot}28 \times 10^4$ M^{-1} were obtained for these same preparation methods, respectively. Dissolution of AGP in pH 7·2 water appears to significantly decrease both $n_i k_i$ values. Thus, the use of a lower initial pH for dissolving AGP resulted in a decrease in the overall affinity of both classes of sites for propranolol.

In conclusion, HSA has a very high capacity for propranolol binding. Buffer composition has a small, but significant, effect on propranolol binding to HSA, while decreases in pH result in a decrease in the percent drug bound. Propranolol

Table 3. Comparison of binding constants for propranolol binding to α_1-acid glycoprotein dissolved in pH 7·4 and pH 7·2 water.

Initial pH of water for AGP solution	n_1	$k_1 \times 10^{-5}(M^{-1})$	n_2	$k_2 \times 10^{-4}(M^{-1})$	r^{2a}
7·4	0·111 (0·027)[b]	7·66 (1·67)	1·124 (0·042)	2·78 (0·434)	0·992
7·4, $n_2 = 1{\cdot}0$	0·174 (0·041)	5·02 (0·965)	1·0	2·62 (0·548	0·990
7·2,	0·207 (0·045)	2·48 (0·442)	1·265 (0·119)	1·07 (0·298)*	0·990
7·2, $n_2 = 1{\cdot}0$	0·244 (0·071)	2·08 (0·465)*	1·0	1·32 (0·418)	0·985

[a] Coefficient of determination
[b] Values in parentheses are standard deviations of estimates
* Significantly different from pH 7·4 water, $P < 0{\cdot}05$

binding by AGP was saturable and was dependent upon whether the protein was initially dissolved in pH 7·4 buffer, pH 7·4 water, or pH 7·2 water. Buffer composition had a greater effect on percent propranolol bound by AGP than did the method of protein solution preparation. Decreasing pH from 7·4 to 7·0 generally decreased propranolol binding by AGP, while decreasing pH from 7·7 to 7·4 had little effect.

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