Effects of Human Serum Albumin Concentration on Binding of [^{3}H]Amphetamine, [^{3}H]Atropine, [^{3}H]Epinephrine, and [^{14}C]Histamine

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Abstract □ The binding of [^{3}H]amphetamine, [^{3}H]atropine, [^{3}H]epinephrine, and [^{14}C]histamine to human serum albumin was examined with a constant ligand concentration and varying albumin concentrations. Two concentrations of each ligand were studied. Scatchard plots of the binding data were linear and had positive slopes; the slopes were greater at the higher ligand concentration. The lowest point was for the highest albumin level and increased in sequence to the lowest albumin concentration. The β (fraction bound) value increased with an increasing amount of albumin in the system. The change in β per change in albumin concentration [$d(\beta)/d$(albumin)] when plotted against albumin concentration yielded curves with negative slopes; the slope was greatest at the lower albumin levels and was much less at the higher albumin levels (equivalent to those encountered in human blood). In agreement with literature data for other systems, the nK value (number of binding sites times the association constant) decreased with increasing albumin concentration. In previous studies of the same ligands and protein, when the albumin concentration was kept constant and the ligand concentration was varied, the Scatchard plots had positive slopes. Positive slopes also were found in this study with constant ligand concentrations and varying albumin levels. These results suggest cooperativity in the binding of these ligands to human serum albumin.

Keyphrases □ Amphetamine—effect of human serum albumin concentration on binding □ Atropine—effect of human serum albumin concentration on binding □ Epinephrine—effect of human serum albumin concentration on binding □ Histamine—effect of human serum albumin concentration on binding □ Binding—effect of human serum albumin concentration on binding of amphetamine, atropine, epinephrine, and histamine

Previous studies on the binding of amphetamine, atropine, epinephrine, and histamine to human serum albumin showed that the Scatchard plots for all four ligands had positive slopes (1). Similar Scatchard plots were observed previously in the binding of codeine, morphine, and methadone to human serum albumin (2). In all of these experiments, the protein concentration was kept constant and the ligand concentration was varied. This approach is typical of most binding studies. In several studies of protein binding in which the ligand concentration was kept constant and the protein concentration was varied, Scatchard plots with positive slopes were reported (3–6).

Bowmer and Lindup (7) commented on the need for data on the effects of protein concentration on binding of ligands for more complete characterization of the protein binding of a ligand. In the usual studies, the protein concentration is kept constant and the ligand concentration is varied. With this concept in mind, the previously described studies of the binding of codeine, morphine, and methadone to human serum albumin were extended to provide data on the effects of varying the human serum albumin concentration. Under these conditions (8), the Scatchard plots had positive slopes, as was reported earlier (1, 2) where the protein concentration was kept constant and the ligand concentration was varied.

The present studies represent an extension of the investigations on the binding of amphetamine, atropine, epinephrine, and histamine to human serum albumin (1). In these additional experiments, the ligand concentration was kept constant and the concentration of human serum albumin was varied.

EXPERIMENTAL

Methods—Protein binding was determined using equilibrium dialysis in multiple-cell blocks, each having a volume of 1 ml on each side of the cellulose membrane, as in previous studies (1, 2). Radioactivity was determined in a liquid scintillation system using techniques described previously (1, 2).

Materials—Tritium-labeled and carbon 14-labeled derivatives of the ligands were obtained from the sources reported previously (1). Crystalline human serum albumin was obtained commercially[1]. The unlabeled forms of the ligands were the highest drug quality available, and all other chemicals were reagent grade.

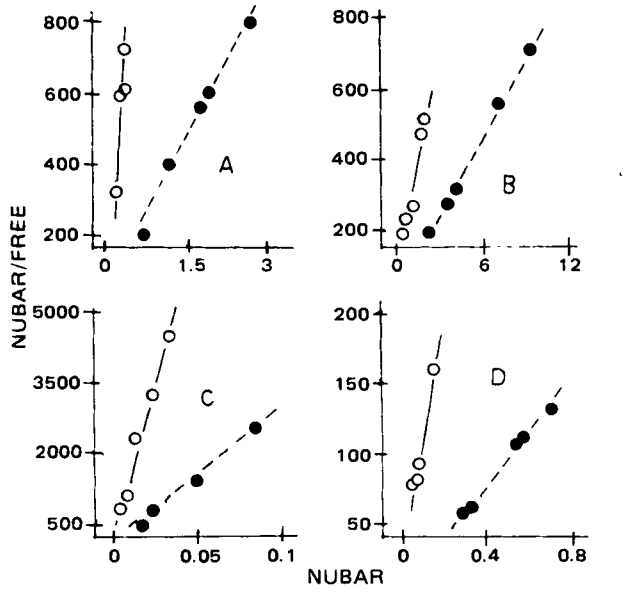

Figure 1—*Scatchard plots of data for binding of [^{3}H]atropine, [^{3}H]amphetamine, [^{3}H]epinephrine, and [^{14}C]histamine to human serum albumin. For each curve, the albumin concentrations are given for each data point starting with the lowest point on the curve. Key: A, [^{3}H]atropine at 4.5×10^{-4} M (○) and 3.43×10^{-3} M (●), with albumin concentrations of 60, 40, 10, and 6 mg/ml for the first ligand concentration and 60, 40, 20, 10, and 6 mg/ml for the second ligand concentration; B, [^{3}H]amphetamine at 3.69×10^{-3} M (○) and 1.48×10^{-2} M (●), with albumin concentrations of 60, 40, 20, 10, and 6 mg/ml for both ligand concentrations; C, [^{3}H]epinephrine at 1.14×10^{-5} M (○) and 4.56×10^{-5} M (●), with albumin concentrations of 60, 40, 20, 10, and 6 mg/ml for the first ligand concentration and 60, 40, 20, and 10 mg/ml for the second ligand concentration; and D, [^{14}C]histamine at 1.15×10^{-3} M (○) and 5.36×10^{-3} M (●), with albumin concentrations of 60, 40, 20, and 10 mg/ml for the first ligand concentration and 60, 40, 20, 10, and 6 mg/ml for the second ligand concentration.*

[1] Nutritional Biochemicals Division, ICN Life Sciences Group.

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Table I—Slopes of Plots of Nubar/Free[a] *versus* Nubar for Binding of [^{3}H]Atropine, [^{3}H]Amphetamine, [^{3}H]Epinephrine, and [^{14}C]Histamine to Human Serum Albumin

Ligand	Total Concentration in System, *M*	Slope	r^b
Atropine	4.5×10^{-4}	1,955.0	0.964
Atropine	3.43×10^{-3}	297.1	0.995
Amphetamine	3.69×10^{-3}	265.4	0.999
Amphetamine	1.48×10^{-2}	69.14	1.000
Epinephrine	1.14×10^{-5}	116,200.0	0.997
Epinephrine	4.56×10^{-5}	28,990.0	1.000
Histamine	1.15×10^{-3}	906.4	0.999
Histamine	5.36×10^{-3}	184.5	1.000

[a] Nubar refers to the molar concentration of ligand bound divided by the molar concentration of human serum albumin in the system. [b] The *r* value refers to the coefficient of correlation of Nubar/free concentration and Nubar.

RESULTS AND DISCUSSION

The human serum albumin concentration was varied from 0.87×10^{-4} to 0.87×10^{-3} *M* (6–60 mg/ml.). Two concentrations of each ligand were used, in the general range of the concentrations used previously (1). The Scatchard plots obtained are shown in Fig. 1. All plots had positive slopes, and the curves were linear (Table I), as can be seen from the coefficients of correlation. In all four cases, a higher slope was obtained with the lower concentration, and higher Nubar (moles of ligand bound per mole of protein in the system) values were seen with the higher concentration.

In the experiments where the protein concentration was kept constant and the ligand concentration was varied, β (fraction bound) values increased with a decrease in ligand concentration (1). In the results obtained in this study, β increased as the albumin concentration increased (Fig. 2). The differences in the plots of β *versus* albumin concentration were not great for the two ligand concentrations. This result is not unexpected when ligand concentrations are not near those required to saturate the protein binding sites. The higher the protein concentration, the more binding sites are available; thus β is expected to increase. For a given ligand, the ratio of the slopes for the two concentrations never exceeded 2.1 (Table II).

Since β varied with albumin concentration for a given ligand, the extent of change of β with a change in albumin concentration was of interest. The rate of change [$d(\beta)/d$(albumin)] was calculated by fitting β values and corresponding albumin concentrations to curves by computer and calculating the first derivative for each albumin concentration for the curve giving the best statistically significant fit (coefficient of correlation of ≥0.95). Plots of these derivatives *versus* albumin concentration are presented in Fig. 3.

The rate of change of β per change in albumin concentration was not constant for all albumin concentrations; it was higher at low albumin concentrations and decreased as the albumin concentration reached that normally found in human blood. Thus, the curves consisted of two major

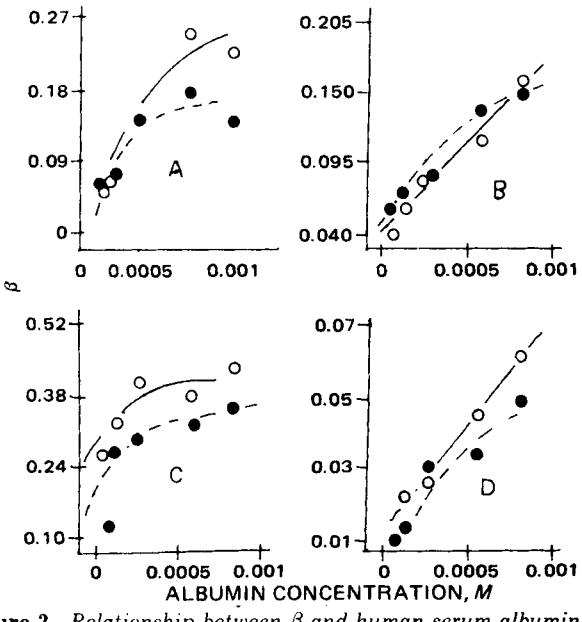

Figure 2—*Relationship between β and human serum albumin concentration for binding of [^{3}H]atropine, [^{3}H]amphetamine, [^{3}H]epinephrine, and [^{14}C]histamine to human serum albumin. Key: A, [^{3}H]atropine at 4.5×10^{-4} M (○) and 3.43×10^{-3} M (●); B, [^{3}H]amphetamine at 3.69×10^{-3} M (○) and 1.48×10^{-2} M (●); C, [^{3}H]epinephrine at 1.14×10^{-5} M (○) and 4.56×10^{-5} M (●); and D, [^{14}C]histamine at 1.15×10^{-3} M (○) and 5.36×10^{-3} M (●).*

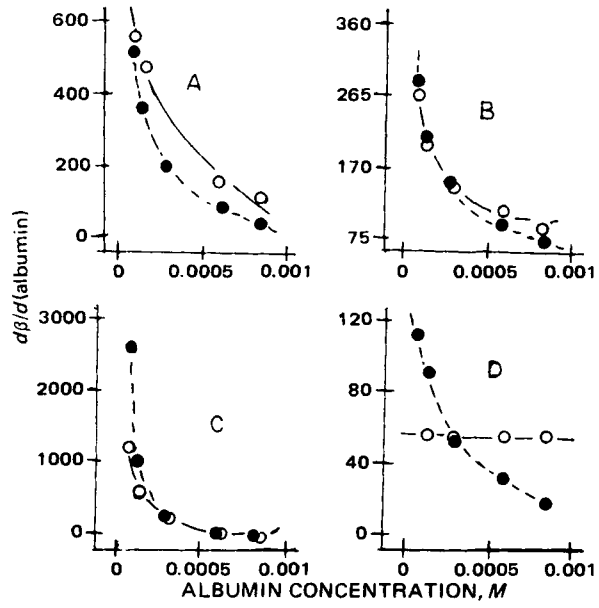

Figure 3—*Change in β per change in human serum albumin concentration* [d(β)/d(*albumin*)] versus *albumin concentration for binding of [^{3}H]atropine, [^{3}H]amphetamine, [^{3}H]epinephrine, and [^{14}C]histamine to human serum albumin. Key: A, [^{3}H]atropine at 4.5×10^{-4} M (○) and 3.43×10^{-3} M (●); B, [^{3}H]amphetamine at 3.69×10^{-3} M (○) and 1.48×10^{-2} M (●); C, [^{3}H]epinephrine at 1.14×10^{-5} M (○) and 4.56×10^{-5} M (●); and D, [^{14}C]histamine at 1.15×10^{-3} M (○) and 5.36×10^{-3} M (●).*

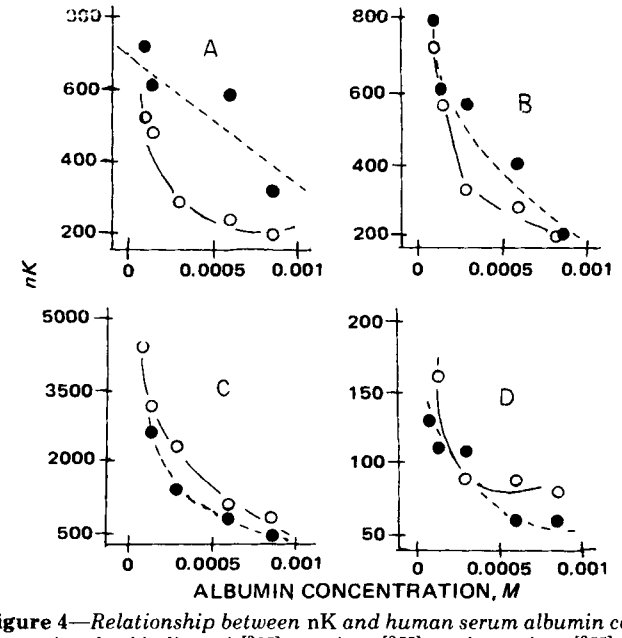

Figure 4—*Relationship between* nK *and human serum albumin concentration for binding of [^{3}H]atropine, [^{3}H]amphetamine, [^{3}H]epinephrine, and [^{14}C]histamine to human serum albumin. Key: A, 3.69×10^{-3} M [^{3}H]amphetamine (○) and 4.5×10^{-4} M [^{3}H]atropine (○); B, 1.48×10^{-2} M [^{3}H]amphetamine (○) and 3.43×10^{-3} M [^{3}H]atropine (●); C, [^{3}H]epinephrine at 1.14×10^{-5} M (○) and 4.56×10^{-5} M (●); and D, [^{14}C]histamine at 1.15×10^{-3} M (○) and 5.36×10^{-3} M (●).*

Table II—Plots of β and nK^a *versus* Human Serum Albumin Concentration (Molar) for Amphetamine, Atropine, Epinephrine, and Histamine

Ligand	Concentration of Ligand, M	β Plot		nK Plot	
		Slope	r^b	Slope	r
Amphetamine	3.69×10^{-3}	142.0	0.995	-3.64×10^5	−0.891
	1.48×10^{-2}	124.7	0.978	-5.58×10^5	−0.870
Atropine	4.5×10^{-4}	241.9	0.906	-3.91×10^5	−0.888
	3.43×10^{-3}	119.0	0.752	-6.44×10^5	−0.964
Epinephrine	1.14×10^{-5}	164.2	0.862	-4.22×10^6	−0.913
	4.56×10^{-5}	98.7	0.922	-2.49×10^6	−0.927
Histamine	1.15×10^{-3}	57.58	0.986	−96,000	−0.748
	5.36×10^{-3}	44.53	0.968	−93,100	−0.946

[a] The n parameter refers to the number of binding sites on the protein molecule, and K is the association constant for the binding system. [b] The r value is the coefficient of correlation for the best fit of the data points to a straight line. The slope given is that calculated for the best-fit straight line.

Table III—Comparison of Slopes of Major Sections of Curves Obtained by Plotting $d(\beta)/d$(Albumin) *versus* Albumin Concentration

Ligand	Concentration of Ligand, M	Slope of Curve Segment[a]		Slope A/Slope B
		A	B	
Amphetamine	3.69×10^{-3}	−1,028,000	−136,100	7.55
	1.48×10^{-2}	−693,000	−114,400	6.06
Atropine	4.5×10^{-4}	−1,780,000	−214,300	8.31
	3.43×10^{-3}	−1,600,000	−249,000	6.43
Epinephrine	1.14×10^{-5}	−4,930,000	−294,000	16.77
	4.56×10^{-5}	−11,960,000	−368,100	32.50
Histamine	1.15×10^{-3}	−253,100	−67,160	3.77
	5.36×10^{-3}	53.54	53.54	1.00[b]

[a] The slope for Segment A was that of the straight line fitted to the three left-hand points of the curve; for Segment B, it was that of the straight line fitted to the three right-hand points. [b] The plot of β *versus* albumin concentration (molar) was a straight line, and $d(\beta)/d$(albumin) was taken as its slope.

segments, one at lower albumin concentrations and the other at higher albumin concentrations. Slopes for the two segments were calculated, and the ratios of the two slopes were computed as a representation of the differences in different albumin concentration ranges (Table III). It appears that β changes the least in the albumin concentration range usually found in human blood. The albumin concentration ranges in which β changes greatly are not encountered clinically.

In several previous studies (7), an increase in protein concentration was accompanied by a decrease in nK[2]. The nK values were calculated using an equation that permits calculation of nK values from β and protein concentrations (9). Plots of nK *versus* albumin concentration are given in Fig. 4. For all four ligands studied, an increase in albumin concentration apparently was accompanied by a decrease in nK. The slopes of these curves are given in Table II; while there were differences in the slopes for the two concentrations of each ligand, these differences were not great (ratios of <2.0), suggesting that the relationship between nK and albumin concentration was essentially independent of ligand concentration for the two ligand concentrations used.

For these four ligands, Scatchard plots with positive slopes were obtained both when the protein concentration was kept constant and the liquid concentration was varied and when the ligand concentration was kept constant and the protein concentration was varied. According to Bowmer and Lindup (7), this result suggests that the positive Scatchard plots may be explained by cooperativity. It also appears that for the compounds examined, the change in β with respect to change in albumin concentration is minor for the albumin concentrations encountered clinically. This finding suggests that β should be similar in normal individuals and those with mild hypoalbuminemia.

[2] The parameter n refers to the number of binding sites or systems on the protein molecule, and the K parameter is the association constant for the protein-ligand system.

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