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Technical Note

The Effect of pH on Gallopamil Protein Binding

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INTRODUCTION

We have previously described pH-dependent binding of gallopamil to human serum proteins (1). Lowering the pH value from 7.4 to 7.0 in healthy volunteer serum "spiked" with gallopamil at a concentration of 10 ng/ml increased free fraction values by 40%. Changes in free fraction have been shown to occur with bupivacaine, where the affinity of the alpha-1 acid glycoprotein solution was affected by the reduction in pH in the same manner as indicated in data obtained with serum (2,3). Since metabolic acidosis is known to increase toxicities of gallopamil, the role of alpha-1 acid glycoprotein and/or albumin in the binding of gallopamil at various physiologic pH values was investigated.

MATERIALS AND METHODS

[^{3}H]Gallopamil, with a specific activity of 87 Ci/mmol (greater than 99% pure), was obtained from New England Nuclear Corp., Boston, Mass. Gallopamil protein binding was determined, in duplicate, *in vitro* over a concentration range of 1.0×10^{-7} (50 ng/ml) to 2.1×10^{-4} *M* (100,000 ng/ml) using equilibrium dialysis techniques (1). Crystallized human serum albumin solution (A-3782, Sigma Chemical Company, St. Louis, Mo.), 4.5 g/dl, alpha-1 acid glycoprotein solution (G-9885 Sigma Chemical Company, St. Louis, Mo.), 60 mg/dl, and serum collected from a fasting, healthy male volunteer were used in binding studies. The pH of the protein and buffer solutions was adjusted immediately prior to binding determinations to 7.0, 7.4, or 8.0 using either concentrated phosphoric acid (14.7 *M*) or sodium hydroxide (4 *M*). Serum for these studies was obtained by drawing blood into a plastic syringe and collection in glass test tubes.

Gallopamil (pK_a = 10.5) and tracer amounts of [^{3}H]gallopamil (0.1 ng) were added to serum or protein solutions (0.35 ml) and dialyzed against an equal volume of isotonic Sorensen's phosphate buffer containing 0.5% (w/v) sodium chloride (4). We corrected all measured free fractions for volume shift (5).

The effect of gallopamil concentration on serum protein binding at each pH value was assessed using the method of Rosenthal (6). Since two types of binding sites were indicated for our data from visual inspection, the Rosenthal equation was fit to the binding data using nonlinear regression analysis (NONLIN) (7). The effect of gallopamil binding at each pH value in the two protein solutions was analyzed by the method of Scatchard (8). From visual inspection, the single class of binding sites for each protein solution permitted the equation to be fit to the binding data using linear least-squares regression analysis.

RESULTS AND DISCUSSION

A reduction in pH from 8.0 to 7.0 did not affect the number of classes of binding sites in serum (Fig. 1). These data suggest the presence of at least two distinct classes of binding sites. Excellent agreement was demonstrated between the observed data and the theoretical curve. Changes in the binding parameters are shown in Table I. At a concentration of 100 ng/ml, the free fraction of gallopamil decreased from 0.1 to 0.05 when the pH was increased from 7 to 8. A reduction in pH from 8.0 to 7.0 decreased the high-affinity binding constant from 1.4×10^6 to 8.8×10^5 M^{-1}. The magnitude of change in the affinity of the second class of binding sites was even larger. The association constant for this class decreased from 3.5×10^4 M^{-1} at a pH of 8.0 to 1.6×10^4 M^{-1} at a pH of 7.0.

Serum albumin solutions were prepared at the concen-

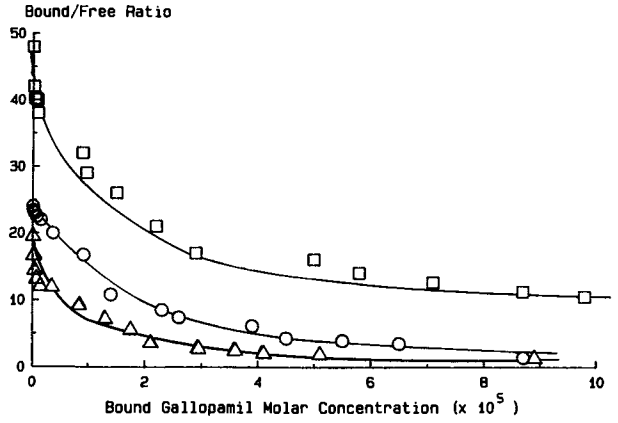

Fig. 1. Protein binding of gallopamil in serum from one subject plotted according to the method of Rosenthal (6). The binding of gallopamil over a broad concentration range was determined at three pH values: (□) 8.0; (○) 7.4; (△) 7.0.

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Table I. Binding Capacities and Affinities for Gallopamil in Human Serum as a Function of pH

pH	N_1P_1[a] (M)	K_1[b] (M^{-1})	N_2P_2[c] (M)	K_2[d] (M^{-1})
7.0	1.5×10^{-5}	8.8×10^{5}	1.2×10^{-4}	1.6×10^{4}
7.4	2.0×10^{-5}	1.0×10^{6}	1.4×10^{-4}	2.3×10^{4}
8.0	2.1×10^{-5}	1.4×10^{6}	2.8×10^{-4}	3.5×10^{4}

[a] Binding capacity or concentration of the first class of binding sites.
[b] Association constant for the first class of binding sites.
[c] Binding capacity or concentration of the second class of binding sites.
[d] Association constant for the second class of binding sites.

Table II. Binding Affinities for Gallopamil in Isolated Human Serum Albumin and Human Alpha-1 Acid Glycoprotein Solutions as a Function of pH

	Albumin		Alpha-1 acid glycoprotein	
pH	K_1[a] (M^{-1})	N[b]	K_1[a] (M^{-1})	N[b]
7.0	9.1×10^{3}	0.15	2.0×10^{5}	0.5
7.4	9.0×10^{3}	0.3	5.1×10^{5}	0.35
8.0	1.4×10^{4}	0.7	1.2×10^{6}	0.3

[a] Association constant.
[b] Number of identical binding sites.

tration of 4.5 g/dl, which was commensurate to the albumin concentration found in the control serum. The binding characteristics of gallopamil in the albumin solution were best described by a one-class binding-site model. Adjustment of the pH from 7.4 to 7.0 (Table II) did not result in a change in affinity (9.0×10^3 to 9.1×10^3 M^{-1}, respectively). However, the association constant at a pH value of 8.0 was higher compared to the association constant at the other pH values. The association constant determined in the albumin study is similar to the low-affinity, high-capacity association constant in the healthy volunteer serum study.

The studies in isolated alpha-1 acid glycoprotein solutions were determined at a concentration of 60 mg/dl, which was equivalent to the concentration in the control serum. The binding characteristics in the alpha-1 acid glycoprotein solution were best described by a one-class binding-site model. The binding affinity decreased from 1.2×10^6 to 2.0×10^5 M^{-1} as the pH was reduced from 8.0 to 7.0, respectively (Table II). Similar high-affinity, low-capacity changes in binding occurred in serum collected from a healthy volunteer. The association constant determined in the alpha-1 acid glycoprotein study is similar to the high-affinity, low-capacity association constant in the healthy volunteer serum study.

Gallopamil systemic toxicities associated with metabolic acidosis may be partially explained by a reduction in the affinity of gallopamil for the high-affinity, low-capacity site on the alpha-1 acid glycoprotein molecule. It appears that this binding environment is sensitive to changes in pH. This pH effect could be of importance in patients with respiratory disease or renal disease, in cardiac arrest, and/or in the early stages of an acute myocardial infarction.

In our previous investigation (1), we demonstrated binding to be constant over the concentration range of 10 to 100 ng/ml. Free fraction values were approximately 0.067 ± 0.0049. In addition, changes in free fraction due to alterations in pH were observed at a concentration of 10 ng/ml. In the present study, our data are consistent with the previous results, although we have used the concentration of 100 ng/ml. The magnitude of change (approximately 40%) in free fraction from a pH of 7.4 to a pH of 7.0 in this study is similar to that from our previous study. Therefore, the results of this study and the implications should be relevant at lower concentrations.

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REFERENCES

1. D. R. Rutledge and J. A. Pieper. *Eur. J. Clin. Pharmacol.* 33:375–380 (1987).
2. D. D. Denson, D. E. Coyle, G. A. Thompson, D. Santos, P. A. Turner, J. A. Myers, and R. Knapp. *Clin. Pharmacol. Ther.* 35:702–799 (1984).
3. D. Denson, D. Coyle, G. Thompson, and J. Myuers. *Clin. Pharmacol. Ther.* 35:409–415 (1984).
4. Geigy Pharmaceuticals. Scientific tables. In K. Diem and C. Lentner (eds.), *Documenta Geigy,* Ardsley, New York, 1962.
5. J. Huang. *J. Pharm. Sci.* 72:1368–1369 (1983).
6. H. E. Rosenthal. *Anal. Biochem.* 20:525–532 (1967).
7. G. M. Metzler, G. L. Elfring, and A. J. McEwen. *A User's Manual for NONLIN and Associated Programs,* Upjohn, Kalamazoo, Mich., 1974.
8. G. Scatchard. *Ann. N.Y. Acad. Sci.* 51:660–672 (1949).
9. P. J. McNamara, R. L. Slaughter, J. A. Pieper, M. G. Wyman, and D. Lalka. *Anesth. Analg.* 60:395–400 (1981).