Steroid Modulation of Human Serum Albumin Binding Properties

A SPIN LABEL STUDY*

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The binding isotherm and unique electron spin resonance spectral characteristics of a monoanionic spin label (1-γ-amino butyrate-5-N-(1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidinyl)-2,4-dinitrobenzene) and a dianionic spin label (1-glutamate-5-N-(1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidinyl)-2,4-dinitrobenzene) are used to prove the steroid modulation of serum albumin binding properties.

Effects of a selected number of steroids (progesterone, testosterone, estradiol, aldosterone, estriol, corticosterone, deoxycorticosterone, hydrocortisone, and cortisol) on the binding isotherm of the monoanionic spin label binding to serum albumin have been determined. At the steroid/albumin ratio of 0.5 to 1, progesterone, testosterone, and estradiol enhance binding of the spin label at all concentrations studied. However, the remaining steroids exert an inhibitory effect at low spin label/albumin ratios and an enhancement effect at high spin label/albumin ratios.

Progestosterone and cortisone effects on the resonance spectra of the spin label bound to serum albumin confirm the enhancement and displacement properties of these ligands. Thus, like fatty acids, steroids may bind to either the primary or secondary bilirubin binding sites and also allosterically perturb the binding properties of serum albumin. The in vivo importance of the steroid-albumin interaction is discussed.

The interaction of hormonal steroids with serum albumin has been a subject of considerable attention (1). Complexing of steroids to albumin appears to induce ligand-stabilized protein conformations (2) as suggested by altered susceptibility to proteolytic hydrolysis (2, 3). Although the physiological importance of steroid-albumin binding has not been established, steroid-albumin binding as suggested by Ryan (3), is an excellent model for the interaction of steroids with high affinity receptors. Furthermore, we have been particularly interested in the study of site-site relations and interactions within the albumin molecule and of the effect of ligand stabilization on albumin binding properties. We report here the steroid effects on the binding of two spin-labeled probes: 1-γ-amino butyrate-5-N-(1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidinyl)-2,4-dinitrobenzene (GABA-DNB-SL)† and 1-glutamate-5-N-(1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidinyl)-2,4-dinitrobenzene (Glu-DNB-SL).

These spin-labeled probes have been shown to bind to the endogenous bilirubin binding sites of albumin (4, 5). It has been found that steroids both enhance spin label binding at certain site(s) and inhibit binding elsewhere. The observed net steroid effect depends on the degree of enhancement versus inhibition behavior characteristics.

MATERIALS AND METHODS

Albumin—Human serum albumin (Fraction V) was purchased from Sigma Chemical Co. The protein was defatted with activated charcoal (6) and monomeric albumin purified as previously described (7). Defatted monomeric albumin at a physiological concentration was determined by ultraviolet light, assuming E1%1cm = 5.30 (8). The buffer system used 0.1 M sodium phosphate buffer, pH 7.4. Albumin solutions were kept at 4°C and used within 1 week. Steroid—Steroids were purchased from Sigma Chemical Co. Because of the limited solubility of steroids, a steroid/albumin molar ratio of 0.5 has been used for all experiments. Steroids were dissolved by drying a stock solution in methanol under nitrogen to form a fine film in a small glass vial. The albumin solution was then added to the vial and gently stirred with a magnetic bar at room temperature for approximately 2 h until optically clear. Stirring has no effect on albumin spin label binding properties. This technique of preparation has been found to be reproducible. Addition of concentrated steroid solution in ethanol typically employed (3) has not been used since as little as 0.1% ethanol is found to have a significant effect on the binding of spin label to albumin.

Spin Label—GABA-DNB-SL synthesis has been described previously (7) and the synthesis of Glu-DNB-SL is previously reported (5). Typically, 50 or 100 μl of the appropriate albumin solution was added to culture tubes containing the dried spin label in the sodium salt form and blended on a Vortex mixer until dissolved. Instrumentation—Electron spin resonance (ESR) spectra were recorded with a Varian E-6 X-band spectrometer equipped with a variable temperature controller. The temperature of the cavity was maintained at 37 ± 0.5°C and monitored with a thermocouple. Samples were contained in glass disposable 25- or 50-μl pipettes. The field was calibrated with Permy’s salt.

Quantitation of Spectral Lines—Free spin label concentrations

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† The abbreviations used are: GABA DNB-SL, 1 γ amino butyrate.
were determined by measurement of the peak-to-peak height of the high field line of the sharp three-line spectrum (9).

RESULTS

We have in the present report investigated the effects of steroid binding on the binding of GABA-DNB-SL to human serum albumin. Study has been limited to a select number of physiological steroids and due to the limited solubility of steroids, a steroid/albumin molar ratio of 0.5 has been used throughout. With the selected steroids studied, it has been observed that steroid binding either enhances or inhibits spin label binding.

Figs. 1 and 2 show representative ESR spectra of the interaction of GABA-DNB-SL and Glu-DNB-SL, respectively, with human serum albumin and the effect of progesterone and cortisone binding. In Fig. 1A and Fig. 2A the changes in the intensity of the sharp three-line spectrum represents free spin label, indicating that cortisone inhibits whereas progesterone enhances the binding of either spin label. The changes in the immobilized spectrum representing protein-bound spin label are better seen in Fig. 1B and Fig. 2B (these changes are discussed below). It is important to note that in the case of Glu-DNB-SL the outer broad line extrema of the immobilized spectrum consists of two components labeled as Lines I and II in Fig. 2B. The lines I and II correspond to Glu-DNB-SL binding to the primary and secondary bilirubin binding sites of albumin (5). The effects of steroid binding on these spectral components have therefore been useful in evaluating the interactions underlying the observed binding phenomena.

The changes in the bound resonance peaks with GABA-DNB-SL and Glu-DNB-SL upon the binding of progesterone and cortisone are more aptly described in reference to Fig. 3. Here, for purposes of presentation, the high field broad line(s) are presented in reversed phase (relative to Figs. 1 and 2) at high instrument sensitivity. In the case of GABA-DNB-SL, Fig. 3A shows line intensity to increase with progesterone and to decrease with cortisone according to the changes observed in the free spin label (Fig. 1). However, no further information is provided as to the nature of these effects (i.e. the effects of GABA-DNB-SL binding sites are both allosteric in nature).

Fig. 3B, in contrast, shows the observed changes with Glu-DNB-SL. It is seen that both steroids show two effects. In the presence of progesterone, Line I intensity decreases while Line II intensity increases. Lines I and II correspond to spin label binding at the primary and secondary bilirubin binding sites, respectively, of albumin. These changes suggest that progesterone may be competing with spin label for binding at the high affinity bilirubin binding site and also that it enhances spin label binding at the secondary site(s). There is also a slight apparent upfield shift of the peak of Line II relative to control and this effect is suggestive of an altered motional freedom, or polarity, or both (10, 11), of the secondary site(s) coincident with an allosteric enhancement of binding. The long chain fatty acids exhibit a similar effect to progesterone as previously described (5). Whether it is the binding at the primary bilirubin binding site which affects the secondary site(s) cannot be said at present. A further interesting point is that the net effect of progesterone binding in the case of Glu-
DNB-SL is that only a slight enhancement of binding is observed (see binding isotherms below). In contrast, Fig. 3B shows that cortisone decreases Line I intensity even further than progesterone. This may indicate that cortisone has a higher affinity relative to progesterone for binding at the primary bilirubin binding site, where it displaces spin label. Furthermore, although the net observable change in the presence of cortisone is an inhibition of binding (see binding isotherms below), cortisone does cause a slight increase in Line II intensity and also shifts the Line II peak further upfield, as compared with progesterone. These changes suggest that cortisone also may allosterically perturb spin label binding at the secondary bilirubin binding site(s).

The binding isotherms for the interaction of GABA-DNB-SL and Glu-DNB-SL in the presence of progesterone or cortisone are presented in Fig. 4. Detailed analysis of these binding isotherms remains outside the scope of the present study and will require adopting computer fitting of the binding data according to the stepwise equilibrium model (12, 13) in order to further characterize steroid binding effects. A few observations, however, will be drawn from these data for present purposes. It is important to note first that both GABA-DNB-SL and Glu-DNB-SL bind to the endogenous bilirubin binding sites of albumin as determined in the previous publications (4, 5). The specificity of GABA-DNB-SL is such that the high affinity bilirubin binding site corresponds to the first two high affinity binding sites of GABA-DNB-SL, while in the case of Glu-DNB-SL this site corresponds to a single high affinity site.

In the case of the GABA-DNB-SL (Fig. 4A) the effects of steroids on the binding isotherm can be accommodated by the binding model advanced from the spectral data with Glu-DNB-SL. Progesterone is shown to enhance spin label binding at all spin label to albumin ratios. Although the net increase in the albumin binding capacity for GABA-DNB-SL, as defined by the ordinate intercept is only a factor of 1.3, the shape of the binding isotherm relative to control supports the idea of binding enhancement at the low affinity site(s) accompanied by competitive binding of steroid with spin label for the primary bilirubin binding site. Cortisone in contrast, displays a net inhibition of binding at low spin label ratios, consistent with increased displacement relative to progesterone at the primary bilirubin binding site. Furthermore, the binding isotherm crosses over the control isotherm at higher spin label ratios (r > 2), consistent with an allosteric effect on the lower affinity site(s). Steroid effects on the GABA-DNB-SL binding isotherm will be further elaborated below.

Fig. 4B shows the comparative changes in the Glu-DNB-SL binding isotherm in the presence of progesterone or corti-
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Fig. 5. Steroid effects on the Scatchard plot of the binding of GABA-DNB-SL to human plasma albumin. The dashed line represents the control curve. The conditions were the same as in Fig. 1.

Alone. Progesterone is seen to exert only a marginal net enhancement of spin label binding, unlike in the case of GABA-DNB-SL. Little comment can be made on this at the present time in view of the complexities underlying the apparent net binding, as revealed by the previous spectral data. Neither can the effect of cortisol be elaborated upon, as cortisol shows a net displacement effect at all spin label ratios.

Fig. 5 presents the effect of a selected number of steroids on the GABA-DNB-SL binding isotherm. The effects observed are peculiar to GABA-DNB-SL. As shown above, cortisol affects the net binding of GABA-DNB-SL in two ways. At low spin label ratios, the binding is inhibited whereas at higher spin label ratios ($r > 2$) there is a net enhancement of binding. A similar effect is exerted by the series of steroids: hydrocortisone, deoxycorticosterone, corticosterone, estradiol, and aldosterone although each succeeding steroid shows less net inhibition at low spin label-albumin ratios and increased enhancement of higher ratios, and also the binding isotherms crossover with the control isotherm at progressively lower spin label ratios. Although these changes await detailed analysis, they clearly show two effects by steroids (i.e., inhibition versus enhancement of spin label binding), and are in accordance with spectral data for Glu-DNB-SL. The effect of the other steroids in Fig. 5 is to produce a net enhancement of binding. The case of estradiol is particularly interesting. Here the total binding capacity of albumin for GABA-DNB-SL as defined by the ordinate intercept, is effectively unchanged in the presence of steroid, although the binding isotherm is markedly different from the control isotherm whereby at $r > 2$ a net enhancement of binding at all spin label ratios is seen. Clearly, the steroid enhancement and inhibition effects have altered the relative apparent affinity constants for GABA-DNB-SL without affecting the total binding capacity. Finally, the effect of testosterone appears similar to that of progesterone. No detailed structure-activity relationships have been attempted until these steroids effects have been further characterized, but, to a first approximation, it appears that the degree of inhibition or enhancement of binding may correlate with the degree of polarity or nonpolarity, respectively, of the steroid molecule.

In conclusion, the present work has shown that steroids apparently can compete with spin label for binding at the single high affinity bilirubin binding site of albumin and also allosterically perturb the interaction of spin label at the secondary bilirubin binding site(s). The net effect on spin label binding appears to be a complex function of these effects by a given steroid.

The present data also provide further support for ligand-stabilized conformations (2) and also advance previous studies from this laboratory as described previously (4, 5) and elsewhere (7) whereby it is here shown that physiological steroids, in addition to long chain fatty acids, can allosterically modulate albumin binding properties.

The in vivo importance of steroid albumin interaction has not been established, as steroids are transported primarily by distinct high affinity binding proteins. However, in the presence of excess steroid levels, (i.e., during pregnancy, steroid treatment etc.) steroids would certainly be expected to interact with albumin, thus modulating its binding properties.

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