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Representation and quantitation of the binding interaction between prednisone, prednisolone and corticosteroid binding globulin

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The interaction between prednisone and prednisolone (0–500 ng ml^{-1}) for binding sites on corticosteroid binding globulin (CBG) in rabbit plasma has been investigated. The fraction unbound of each steroid rose markedly with increasing concentration at low concentrations (250 ng ml^{-1}), tending to a plateau (0·29 prednisone, 0·15 prednisolone) at the higher concentrations (250–500 ng ml^{-1}), indicating both saturation of CBG and the importance of binding to albumin in higher concentrations. A method is proposed of representing graphically the prednisone–prednisolone–protein interaction in three dimensions.

Prednisone and prednisolone are two widely prescribed corticosteroids. In plasma, both bind avidly to corticosteroid binding globulin (CBG), a low capacity protein, but poorly to albumin, a high capacity protein. When either prednisone or prednisolone is administered to man, the other drug is formed as a metabolite (Jenkins & Sampson 1967; Rose et al 1981), hence any displacement interaction between these corticosteroids, for binding sites on CBG, may have important consequences on their disposition in-vivo.

Competition between prednisolone and cortisol (hydrocortisone), the major endogenous corticosteroid in man, has been reported by De Moor et al (1963), who also suggested a binding interaction between prednisone and prednisolone but this has not been evaluated.

We now report on the binding interaction between prednisone and prednisolone, in-vitro, in rabbit plasma pretreated to remove the endogenous corticosteroid, corticosterone. The limitations in the use of linearization procedures, such as the Scatchard and the Rosenthal plot, to represent double ligand–protein interactions is discussed and an alternative method of representing this binding interaction in three dimensions is suggested.

MATERIALS AND METHODS

Plasma (20 ml) from blood removed from five previously unused cross-bred New Zealand white/half-lop rabbits, had the major circulating endogenous corticosteroid, corticosterone (monitored by [^{3}H]corticosterone 80 Ci $mmol^{-1}$, Amersham International) removed by adsorption to charcoal (Heyns et al 1967). All binding studies were conducted using the method of equilibrium dialysis (Dianorm, MSE, UK, 25 µl cells), at 37 °C, run for 3 h, a time that ensured attainment of equilibrium even at the highest binding, i.e. low concentration of ligand.

Eight concentrations of prednisone and prednisolone (0–500 ng ml^{-1}) in isotonic Sørensen's phosphate buffer (pH 7·4, 0·1 M), containing about 0·1 µCi ml^{-1} of either [^{3}H]prednisolone (41 Ci $mmol^{-1}$, >95% pure, Amersham International) or [^{3}H]prednisone (38·3 Ci $mmol^{-1}$, New England Nuclear) were used. [^{3}H]Prednisone was purified (>95%) by hplc before use. An aliquot (120 µl) of each combination of these concentrations, a total of 64, was dialysed twice against the charcoal-treated rabbit plasma (120 µl), once containing [^{3}H]prednisone and once with [^{3}H]prednisolone, to determine the respective percent unbound. At the end of the dialysis period, a 25 µl sample was removed from both the buffer and protein half of the dialysis cells, and counted by scintillation (LKB Wallace, UK), after addition of 3 ml of scintillant (Rialuma, Lumac, Holland). The concentration of albumin was determined by the method of Bartholomew & Delaney (1964).

Data analysis. The fraction unbound of each steroid was determined by calculating the ratio of the radioactivity in the buffer sample (25 µl) to that in the plasma sample (25 µl) at equilibrium.

The model (eqns 1 and 2) proposed to explain the binding data involves competitive nonlinear binding

* Correspondence.

to CBG(T) and linear binding to albumin (A) (Aarons et al 1979):

$$C_{T,PNL} = Cu_{,PNL} + \frac{K_{T,PNL}.Cu_{,PNL}.N_T.C_T}{1+K_{T,PNL}.Cu_{,PNL}+K_{T,PNL}.Cu_{,PN}} + K_{A,PNL}Cu_{,PNL}\,N_A.C_A \quad (1)$$

$$C_{T,PN} = Cu_{,PN} + \frac{K_{T,PN}.Cu_{,PN}.N_T.C_T}{1+K_{T,PN}.Cu_{,PN}+K_{T,PNL}.Cu_{,PNL}} + K_{A,PN}.Cu_{,PN}N_A.C_A \quad (2)$$

where $C_{T,X}$, $Cu_{,X}$ represent the total and unbound concentration of steroid X; $K_{T,X}$ and $K_{A,X}$ represent the association constant of the CBG-X and albumin-X interaction, where X is either prednisone (PN) or prednisolone (PNL); and C_T, C_A, N_T and N_A represent the total concentration and the number of binding sites per molecule on CBG and albumin respectively. The binding interaction between prednisone and prednisolone is considered to take place only for binding sites on CBG.

In fitting the model given by equations 1 and 2 simultaneously to the data, the dependent variables, $Cu_{,PN}$ and $Cu_{,PNL}$ must be regressed against the independent variables $C_{T,PN}$, $C_{T,PNL}$ and C_A. To do this, equations 1 and 2 must be inverted to the form:

$$Cu_{,PNL} = f(C_{T,PNL}, C_{T,PN}, C_A) \quad (3)$$

$$Cu_{,PN} = g(C_{T,PN}, C_{T,PNL}, C_A) \quad (4)$$

where f and g denote that the dependent variables (unbound concentrations) are functions of the respective independent variables in parentheses.

Since there is no analytical expression for these functions, the solutions were obtained numerically using the Newton–Raphson method (Aarons et al 1979). The iteration in the Newton–Raphson algorithm was continued until a present tolerance level was achieved between two successive estimates (0·1%).

In equilibrium dialysis, there is a shift of drug between the two compartments of the dialysis cell. In this present experiment, where there are two cells of equal volume (250 µl) the total drug concentration on the plasma site at equilibrium ($C^E_{T,D}$) is related to the total concentration on the buffer side before dialysis ($C^O_{T,D}$) by the expression:

$$C^E_{T,D} = \frac{C^O_{T,D}}{1 + fu} \quad (5)$$

where fu is the fraction of the unbound drug at equilibrium. Equation 5 is based on the assumption of no uptake of ligand on the dialysis membrane, which was confirmed.

All data were transformed to micromolar concentration. The total drug concentration at equilibrium was calculated using equation 5, before estimating the parameters using a non-linear regression package NONLIN (Metzler 1974). Each datum was weighted by the reciprocal of the square of the estimated unbound concentration, a procedure which resulted in a random distribution of the residuals about the predicted line.

The binding surfaces for the three dimensional plot were generated by substituting the values of the parameter estimates, obtained by non-linear regression, in the inverted forms of equations 1 and 2.

RESULTS AND DISCUSSION

Fig. 1 illustrates the change in the fraction of unbound prednisone with changes in the total concentration of both prednisone and prednisolone. With the addition of either corticosteroid, the fraction of prednisone unbound increased sharply from a starting value of 0·08, at low prednisone concentrations alone, and then more gradually to a limiting value of 0·29 when the corticosteroids were in a combined concentration of approximately 600 ng ml^{-1}. Prednisolone behaved similarly except

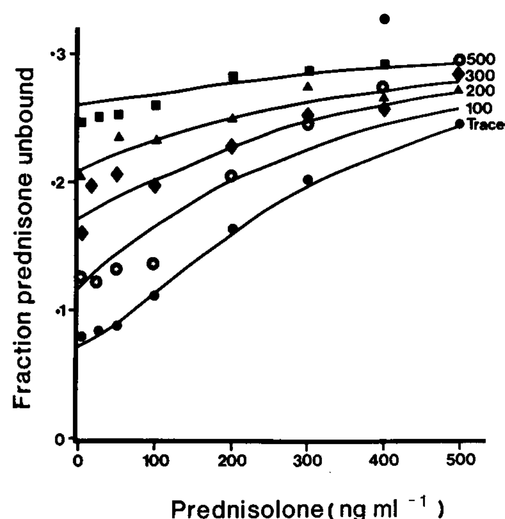

FIG. 1. Fraction of prednisone unbound as a function of the concentrations of both prednisone and prednisolone. The solid lines are the predicted values using eqn 1 and the parameters estimates given in Table 1. The symbols are the experimental points corresponding to various concentrations of prednisolone and tracer (●), 100 (⊛), 200 (♦), 300 (▲) and 500 (■) ng ml^{-1} prednisone.

that the lower and upper limiting values for fraction of unbound drug were 0·06 and 0·15 respectively (Fig. 2).

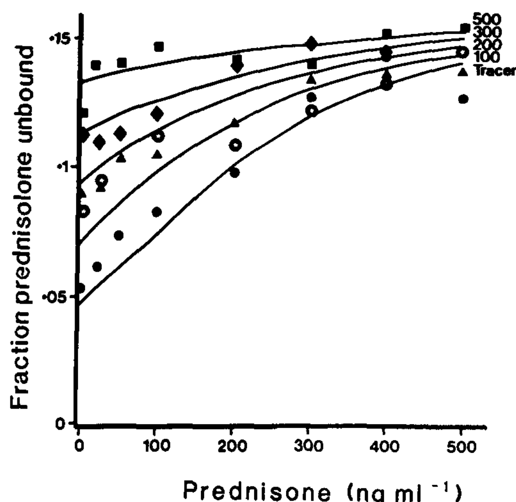

FIG. 2. Fraction of prednisolone unbound as a function of the concentrations of both prednisone and prednisolone. The solid lines are the predicted values using eqn 1 and the parameters estimates given in Table 1. The symbols are the experimental points corresponding to various concentrations of prednisone and tracer (●), 100 (⊕), 200 (⧫), 300 (▲) and 500 (■) ng ml^{-1} prednisolone.

Table 1. Comparison of estimates of parameters describing the binding of prednisone and prednisolone, in rabbit plasma, obtained by us and other workers.

Parameter	Estimate (mean ± s.d.) Our results	From (Rocci & Jusko 1981)
$K_{T,PN}$ (M^{-1})	3·25 (±0·048) × 10^7	—
$K_{T,PNL}$ (M^{-1})	4·44 (±0·068) × 10^7	2·84 (±2·16) × 10^7
N_TC_T (M)	3·42 (±0·354) × 10^{-7}	4·7 (±2·4) × 10^{-7}
$N_AK_{A,PN}$ (M^{-1})	2·59 (±0·184) × 10^3	n.d.
$N_AK_{A,PNL}$ (M^{-1})	6·66 (±0·378) × 10^3	6·24 (±1·5) × 10^3

n.d. = Not determined.

The estimates of the parameters describing the binding of prednisolone to CBG (Table 1) agree with those obtained by Rocci & Jusko (1981). If the number of binding sites per molecule of CBG, (N_T), is assumed to be 1 (as found for the CBG-cortisol interaction, Bernutz et al 1979) the concentration of CBG in rabbit plasma (3·42 ± 0·35 × 10^{-7} M) is of the same magnitude as in human plasma (4·3 × 10^{-7} M), determined by radioimmunoassay. The binding affinity of prednisone for CBG is approximately 40% that of prednisolone, a result which is at variance with that reported by Bernutz et al (1979) for purified human CBG ($K_{T,PNL}$ = 5·4 × 10^7 M^{-1}; $K_{T,PN}$ = 3·4 × 10^6 M^{-1}) determined by displacement of tritiated cortisol. This discrepancy may be explained by the indirect method of analysis used in that work, by a species difference or by both.

In the preparation of isotherms to calculate the binding of corticosteroids to plasma proteins, it is necessary either to remove the competing corticosteroids before the binding study or to include their presence in the binding model. A distinction should be drawn between the in-vitro and in-vivo situation. When control plasma is used for binding studies, the endogenous corticosteroids will be present. However, after the administration of exogenous corticosteroids, the plasma endogenous corticosteroid pool is depleted (Melby 1974). To represent the situation after corticosteroid administration, we chose to remove corticosterone, the major endogenous corticosteroid, from rabbit plasma.

Linearization procedures such as the Scatchard plot are useful when representing a single ligand-protein interaction, where either the protein concentration or the drug ligand concentration is changing.

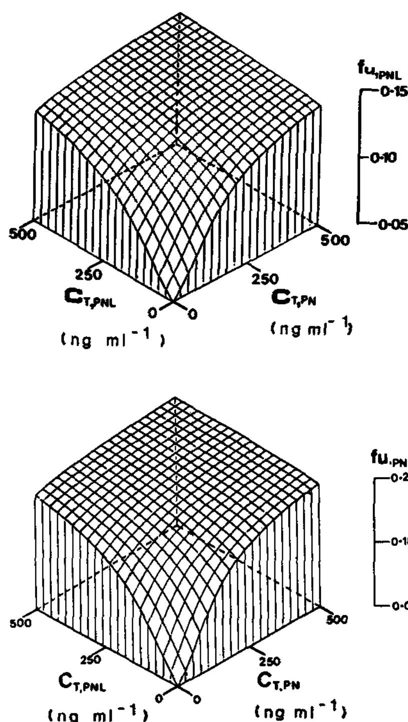

FIG. 3. Binding surfaces of prednisone (PN) and prednisolone (PNL) illustrating the change in fraction unbound of prednisone ($fu_{,PN}$) and prednisolone ($fu_{,PNL}$) in plasma on changing the total plasma concentration of these two steroids, $C_{T,PN}$ and $C_{T,PNL}$ respectively.

These procedures are inadequate when a double ligand–protein interaction is to be displayed, as they implicitly assume that the fraction unbound of one of the two ligands remains constant on changing the total concentration of the other (Aarons et al 1979). To visualize competition of two ligand species for common binding sites, two three-dimensional plots are preferred. In each plot the single ordinate represents the fraction unbound of one of the ligands, while each of the two abscissae represents the total concentration of one of the ligand species.

Fig. 3 illustrates the binding interaction between prednisone and prednisolone for sites on CBG and albumin. The binding surfaces are almost symmetrical due to the similar binding affinity values of both corticosteroids for the proteins. The lower affinity of prednisone is reflected by an almost two-fold greater fraction unbound compared with prednisolone, at equal concentrations. At low concentration of each corticosteroid ($<250\,ng\,ml^{-1}$) the binding values changed markedly when the total concentration of either corticosteroid was changed reflecting the non-linear saturable binding to CBG. At higher concentrations ($>250\,ng\,ml^{-1}$) the fraction unbound tended to reach a plateau (0·29, prednisone; 0·15 prednisolone) suggesting that binding to albumin, which is not saturated, begins to be a major contributor to the total amount of the steroid bound to plasma proteins.

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