

## PLASMA PROTEIN BINDING OF VALPROIC ACID IN HEALTHY SUBJECTS AND IN PATIENTS WITH RENAL DISEASE

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- 1 Based on the Scatchard plot of the binding data of valproic acid (VPA) it is concluded that the drug is bound by two groups of binding sites with the association constants  $K_1=40.0 \times 10^{-3}$  and  $K_2=0.39 \times 10^{-3}$ , and the number of binding sites  $n_1=1.5$  and  $n_2=6.8$ .
- 2 The binding is dependent on dialysis time, on temperature, on the drug concentration, and on the protein concentration in plasma.
- 3 At therapeutic plasma concentrations unbound VPA is  $8.4 \pm 2.5\%$ , but is increased to  $20.3 \pm 4.7\%$  in patients with significant impairment of renal function ( $P < 0.001$ ).
- 4 In patients with renal disease a good correlation is found between unbound VPA and serum creatinine, creatinine clearance, blood nitrogen and uric acid, respectively. A poor correlation is seen between unbound VPA and total protein or albumin concentration in plasma.

### Introduction

Valproic acid (dipropylacetic acid, VPA) is a potent antiepileptic agent, being particularly effective in the treatment of petit mal epilepsy (Simon & Penry, 1975). Since fatty acids are in general extensively bound to plasma proteins in man (Goodman, 1958) it is assumed that VPA as a fatty acid would also be present in plasma in a highly bound form. Preliminary data indicate plasma protein binding of VPA to be in the magnitude of another antiepileptic drug, phenytoin, 90% of which is normally protein bound (Potratz & Schulz, 1976). No information is available, however, on the binding of VPA in disease states such as renal failure or hepatic disease, which have been described to be associated with reduced binding of phenytoin (Reidenberg, Odar-Cederlöf, von Bahr, Borgå & Sjöqvist, 1971; Hooper, Bochner, Eadie & Tyrer, 1974). Alterations in protein binding may be of considerable importance, since they can profoundly affect the pharmacokinetics of drugs (Schoenemann, Yesair, Coffey & Bullock, 1973; Gugler, Shoeman, Huffman, Cohlmiä & Azarnoff, 1975). Furthermore, the interpretation of drug plasma levels can become meaningless, if changes in protein binding are not taken into consideration (Gugler, Azarnoff & Shoeman, 1975).

It was the aim of the present study to characterize the binding of VPA to human plasma protein from healthy subjects and from patients with renal failure.

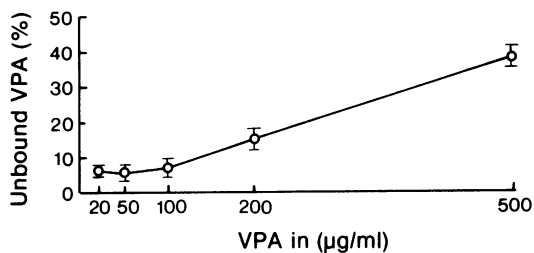
### Methods

#### *Binding sites and association constant*

Human serum albumin (purissimum; Serva Co., Heidelberg, FRG) was used as solution with a concentration of 400 mg/ml ( $5.76 \times 10^{-7}$  mol/l) in 0.05 M phosphate buffer pH 7.4. Scatchard plots were made out of the data calculated from the measurement of unbound and bound VPA at the several concentrations of VPA employed ranging from 20–2000 µg/ml of albumin solution (concentration before dialysis).

#### *Subjects*

Plasma was obtained from sixteen healthy subjects and from twenty-four patients with renal disease randomly selected from the Renal Outpatient Clinic. Sixteen out of the group of renal patients had a reduction in creatinine clearance of at least 25%; patients on hemodialysis were not included. The diagnosis of renal disease was primarily based on biochemical parameters. In fifteen patients diagnosis was confirmed by kidney biopsy. No patients were studied who had evidence of combined renal and hepatic disease. Subjects were judged as being healthy when neither history nor clinical or biochemical evidence for either renal or liver disease or congestive heart failure was found. Laboratory tests included



**Figure 1** % unbound VPA at different total plasma concentrations of VPA (mean  $\pm$  s.d.,  $n=5$ ).

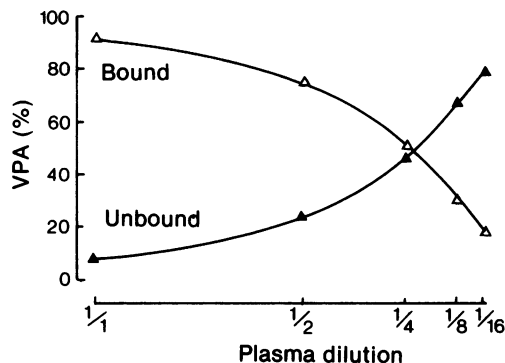
serum electrolytes, blood urea nitrogen, uric acid, creatinine, serum glutamic oxalacetic transaminase (SGOT), total bilirubin, prothrombin time, total protein, and albumin.

#### Estimation of protein binding of VPA

Protein binding determination was carried out at the day of plasma collection or of preparation of the albumin solution. For the determination of the effects of dialysis time, of temperature, of VPA concentration, and of protein concentration, respectively, on the percentage of binding of VPA fresh pooled plasma was obtained from healthy volunteers who had not received any medication over at least 2 weeks prior to the tests. In the standard procedure VPA was added to plasma or albumin in small amounts of an aqueous stock solution to give a final concentration of 50  $\mu\text{g/ml}$ . A dialysis apparatus was used (Dianorm, Diachema AG, Zürich, Switzerland) that allows the simultaneous dialysis of fifteen samples. Each dialysis unit consisted of two 1 ml-chambers separated by a dialysis membrane (Visking 27/35) of 0.0025 mm thickness, pore diameter 15–20 Å, obtained from Serva Co. (Heidelberg, FRG). Plasma (1 ml) containing VPA was pipetted into one chamber, 1 ml of a 0.02 M phosphate buffer pH 7.4 was pipetted into the opposite chamber. Dialysis was performed by rotating the dialysis cells at 15 rev/min in a water bath at 37°C over 16 h. VPA concentrations were measured in the buffer solution (unbound drug) and in the plasma solution (total drug) by a sensitive g.l.c. method (Jensen & Gugler, 1977). Statistical evaluation included regression analysis, *t*-test and multiple regression analysis.

#### Results

Dialysis time affects the achievement of dialysis equilibrium in so far as the percentage of unbound VPA still increases over the first 4 h (5.8% after 1 h, 7% after 4 h), but the system is at equilibrium from 12 h on (8.5%). The binding of VPA relative to the



**Figure 2** The effect of plasma dilution on % of bound ( $\Delta$ ) and unbound ( $\blacktriangle$ ) VPA.

temperature of the water bath used shows the values of unbound drug (mean of five experiments) to be significantly different at 37°C (9.2%) as compared to 22° (5.9%) and 4° (6.0%) indicating higher binding at lower temperature. No difference in binding was observed between 22° and 4°C.

The effect of the concentration of VPA on the degree of plasma protein binding is shown in Figure 1. Binding was relatively constant between 20 and 100  $\mu\text{g/ml}$ , which is in the therapeutic range of VPA, but was considerably reduced at higher concentrations. At 200  $\mu\text{g/ml}$  the percentage of unbound VPA was about twice the value found at 100  $\mu\text{g/ml}$ .

Serial plasma dilutions were performed with a 0.05 M phosphate buffer pH 7.4 to give 1/2, 1/4, 1/8, 1/16 of the original protein concentration. Protein binding of VPA decreased with decreasing protein content, and the unbound fraction of VPA increased accordingly (Figure 2). When plasma was diluted to 1/4 the original concentration unbound VPA was almost 50% of the total VPA concentration (unbound fraction in untreated plasma 8%).

A Scatchard plot of VPA binding data to human serum albumin (400 mg/ml) is shown in Figure 3. The graph indicates that VPA is bound by more than one group of binding sites. From the shape of the curve two groups of binding sites were assumed with the number of binding sites  $n_1$  and  $n_2$  and the association constants  $K_1$  and  $K_2$ . From the asymptotes to the curve as determined by the method of Feldman (1972) the association constant of the primary group of binding sites was calculated to be  $K_1=40.02 \times 10^{-3}$ , the number of binding sites was calculated to be  $n_1=1.5$ . The constant for the secondary group of binding sites was  $K_2=0.39 \times 10^{-3}$ , the number of binding sites was  $n_2=6.8$ .

In normal subjects binding was  $91.6 \pm 2.5\%$  (mean  $\pm$  s.d.) of the total plasma concentration of VPA. Accordingly, the unbound fraction of VPA

**Table 1** Diagnosis, clinical laboratory data and percent unbound VPA of all patients with renal disease and of healthy control subjects (mean  $\pm$  s.d.).

Renal patients number	Diagnosis	Creatinine (mg/100 ml)	Creatinine clearance (ml/min)	Blood urea nitrogen (mg/100 ml)	Uric acid (mg/100 ml)	Total protein (g/100 ml)	Albumin (g/100 ml)	Unbound VPA (%)
1*	Systemic lupus erythematosus	0.8	102	11	5.5	7.8	4.7	9.5
2	Chronic renal failure	3.6	ND	58	9.3	7.6	4.2	13.2
3	Glomerulonephritis	0.8	78	15	8.5	6.7	4.2	12.7
4*	Glomerulonephritis, nephrotic syndrome	0.7	93	12	5.6	7.8	4.8	9.4
5*	Glomerulonephritis	0.7	102	25	2.6	7.5	5.6	6.6
6	Glomerulonephritis	3.4	ND	57	9.4	6.8	4.8	23.0
7*	Chronic renal failure	0.5	99	10	4.3	6.7	4.8	12.0
8	Glomerulonephritis	9.6	12	112	12.0	6.9	4.5	27.7
9*	Hematuria	0.8	90	16	3.6	7.5	4.4	22.0
10	Chronic pyelonephritis	2.9	ND	94	7.3	7.4	5.1	23.4
11	Glomerulosclerosis	6.3	ND	84	9.2	7.1	5.2	18.7
12	Chronic renal failure	2.0	72	26	6.5	6.7	5.2	17.0
13	Chronic renal failure	1.6	88	33	7.2	7.9	4.6	22.0
14	Glomerulonephritis	5.8	24	64	12.0	7.3	5.1	22.0
15	Nephrocalcinosis	1.3	50	13	5.8	7.8	4.6	18.4
16	Glomerulonephritis	1.5	73	19	6.3	7.5	4.2	16.4
17*	Chronic pyelonephritis	0.8	81	15	4.2	7.6	5.3	11.9
18*	Chronic pyelonephritis	1.0	93	18	2.9	7.7	5.0	15.4
19*	Chronic pyelonephritis	3.1	115	11	3.8	8.0	5.0	10.3
20	Chronic renal failure	4.0	ND	39	7.8	7.4	4.1	28.9
21	Chronic renal failure	4.0	ND	51	12.1	7.2	4.1	20.4
22	Glomerulonephritis	5.4	ND	120	10.5	6.7	4.3	24.9
23	Nephrosclerosis	3.1	34	40	6.1	6.9	4.6	17.3
24	Glomerulonephritis	1.7	ND	24	11.0	7.0	5.0	18.0
Mean	—	2.6	76	40	7.2	7.3	4.7	17.5
s.d.	—	2.3	30	33	3.0	0.6	0.5	6.0
Healthy subjects (n = 16)	Mean	0.7	108	15	4.6	7.6	4.9	8.4
	s.d.	0.2	15	4	1.6	0.5	0.2	2.5

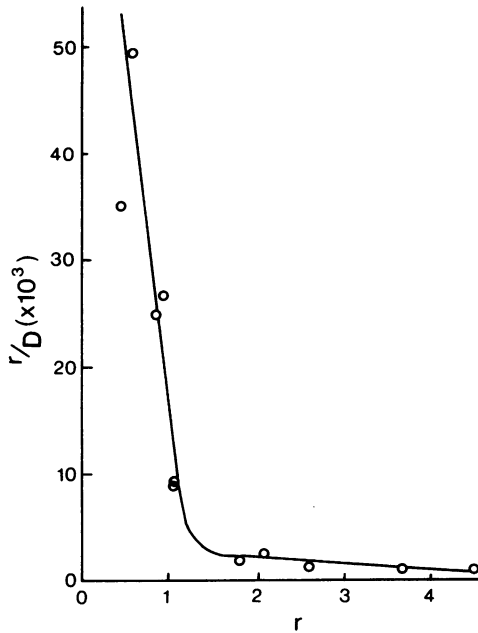
\*Renal patients with relatively normal renal function: normal serum creatinine; GFR reduction less than 25%

ND = not determined

Creatinine mg/100 ml  $\times$  88.4 =  $\mu$ mol/l

Blood urea nitrogen mg/100 ml  $\times$  0.357 = mmol/l

Uric acid mg/100 ml  $\times$  0.05948 = mmol/l



**Figure 3** Scatchard plot of the binding of VPA to human serum albumin.  $r$  = moles of drug bound/mole of albumin;  $D$  = molar concentration of unbound drug.

ranged between 5.2 and 12.3%; mean percentage of unbound VPA was 8.4.

The data for the patients with renal disease are presented in Table 1. From the serum creatinine values as well as from the creatinine clearance values it becomes obvious that most degrees of renal function are covered in this group of patients. However, patients whose creatinine clearance was less than 10 ml/min were not included in this study. Serum albumin concentration in renal patients was not significantly different from the control group. Comparison of results of VPA binding indicates significantly increased unbound VPA in the patients with renal disease ( $17.5 \pm 6.0\%$ ;  $P < 0.001$ ). Unbound VPA was  $20.3 \pm 4.7\%$  when patients with relatively normal renal function were excluded. Table 2

summarizes the correlation of unbound VPA in all renal patients with every biochemical parameter listed in Table 1. VPA binding correlated well to serum creatinine, creatinine clearance, blood urea nitrogen and uric acid, but was independent of total protein and albumin concentration in plasma.

Unbound VPA as a function of creatinine clearance and serum creatinine is shown in Figure 4. For the correlation tests all patients with renal disease ( $n = 24$ ) have been used, those with relatively normal renal function ( $n = 8$ ) included. This seemed to be justified in order to cover the total range between normal and extensively reduced renal function. The healthy subjects were not included in order to achieve a homogenous group with only one major variable, glomerular filtration rate.

**Discussion**

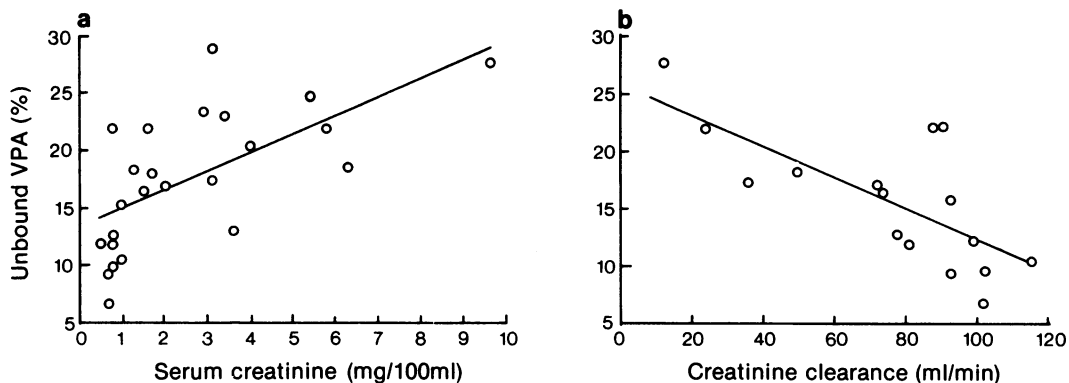
The extent of binding of a drug to plasma proteins can be of considerable importance for its pharmacokinetics. Thus, for some protein bound drugs an increase in the unbound fraction in plasma is associated with an increase in its distribution volume as well as with enhanced elimination by either biotransformation or renal excretion (Gugler *et al.*, 1975; Levi & Yacobi, 1974; Odar-Cederlöf & Borgå, 1974). Information on the degree of binding is also of practical importance, since the knowledge of the total plasma concentration of a normally highly bound drug can be misleading, if the possibility of alterations in binding is not considered.

In the present study the binding of VPA to plasma proteins was studied using an equilibrium dialysis technique. Although VPA is highly bound to plasma proteins, binding varies to a certain degree, ranging from 87.7 to 94.8% in the group of healthy subjects studied here, this variation being in the same magnitude as found with phenytoin (Hooper *et al.*, 1974). Thus, a twofold variation in the unbound fraction in plasma can be observed. Since the degree of binding of VPA is roughly identical to phenytoin (Lunde, Rane, Yaffe, Lund & Sjöqvist, 1970) it is of interest to compare the binding of either antiepileptic

**Table 2** Statistical analyses of the correlation between several biochemical parameters and percent unbound VPA in twenty-four patients with renal disease.

	Creatinine	Creatinine* clearance	Blood urea nitrogen	Uric acid	Total protein	Albumin
Correlation coefficient	0.65	-0.73	0.64	0.60	0.33	0.10
P value	0.002	0.001	0.002	0.005	>0.05	>0.05

\* $n = 16$



**Figure 4** % unbound VPA in plasma of patients with renal disease as related to serum creatinine (a,  $n=24$ ) and creatinine clearance (b,  $n=16$ ). Creatinine mg/100 ml  $\times$  88.4 =  $\mu$ mol/l.

drug under various conditions such as disease states. In an attempt to standardize the experimental conditions for protein binding measurement of VPA to enable a comparison of data obtained in different laboratories, the effect of dialysis time, temperature, VPA concentration, and of plasma dilution on the extent of binding was studied. As it was found that equilibrium is reached after 12 h of dialysis and remains constant over at least 24 h, a dialysis time of 16 h was chosen for all subsequent experiments. The binding of VPA is affected by the temperature of the incubation environment, the binding being less at 37°C. Higher binding at 22° or 4°C may be accompanied with changes in the association constants or in the number of binding sites. It is therefore recommended to measure protein binding of VPA exclusively at 37°C.

Plasma concentrations of VPA between 50 and 120  $\mu$ g/ml are considered therapeutic (Simon & Penry, 1975; Schobben, van der Kleijn & Gabreels, 1975; Windorfer, Vogel & Sauer, 1976). In the upper range of these concentrations the protein binding of VPA is dependent on the plasma concentration. At high VPA concentrations occasionally found with therapeutic doses, but certainly in overdosed patients, the binding is reduced. For standard determination of VPA protein binding a concentration between 50 and 100  $\mu$ g/ml appears appropriate.

Serial plasma dilutions result in a reduced VPA binding. Hypoalbuminemia may be found in newborns (Rane, Lunde, Jalling, Yaffe & Sjöqvist, 1971), in renal failure (Reidenberg *et al.*, 1971; Odar-Cederlöf & Borgå, 1974), in chronic hepatic disease (Affrime & Reidenberg, 1975; Olsen, Bennet & Porter, 1975), but in particular in the nephrotic syndrome (Gugler *et al.*, 1975; Gugler & Azarnoff, 1976). Consequently, VPA binding can be reduced to an unknown extent in these conditions. From the Scatchard plot of the binding data of VPA two groups of binding sites can be

assumed, one being the primary (specific) group with a high association constant and 1.5 binding sites, the secondary (unspecific) group exhibiting a small association constant, but a large number of binding sites. The capacity of the primary group of binding sites appears to be limited to plasma concentrations of about 200  $\mu$ g/ml at a plasma albumin concentration of 400 mg/ml. Therefore, with therapeutic doses of VPA the primary group of binding sites should be occupied predominantly.

The reduction of VPA protein binding in renal impairment appeared to be correlated with serum creatinine, creatinine clearance, blood urea nitrogen and uric acid. Significant difference in albumin and total protein concentrations were not seen between the groups of this study, so that definite conclusions on the effect of a reduced albumin or total protein concentration on the binding of VPA cannot be drawn, although the dilution experiment suggested such relationship. A similar correlation between binding of phenytoin and both, serum creatinine and serum blood urea nitrogen, has been described by Reidenberg and associates (1971) and Hooper *et al.*, (1974), but a correlation between binding and albumin concentration could only be established in one of the studies (Reidenberg *et al.*, 1971). Our findings show that the binding of VPA is altered in renal insufficiency even with normal total plasma protein or plasma albumin concentration. Thus, the reduction of VPA binding in uremia seems to be a result of either a qualitative change in the serum albumin (Shoeman & Azarnoff, 1972) or a displacement from binding sites by endogenous substances accumulating in renal failure (free fatty acids, phenolic acids etc.). It cannot be definitely excluded that some of the decrease in protein binding of VPA in renal failure was a result of displacement by the concurrent medication. However, a reduction in binding was also seen in patients who were on no drugs (No. 3, 9, 15, 18, 22) or who were

on drugs with low protein binding to plasma proteins or small plasma concentrations in the ng range, respectively, such as digoxin, reserpine and clonidine (No. 6, 10, 16, 21). Further studies will be necessary on the possible role of displacement of VPA from protein binding by other highly bound drugs.

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