SERUM PROTEIN BINDING OF VALPROIC ACID AND ITS DISPLACEMENT BY PALMITIC ACID *IN VITRO*

Valproic acid, administered to patients as sodium valproate, has been found to be effective against both major and minor epileptic seizures (Richens & Ahmad, 1975; Gram, Flachs, Wulff & Wurz-Jørgensen, 1977). It is an 8-carbon, branched-chain fatty acid with a molecular weight of 144 and like endogenous fatty acids is highly bound (approximately 90%) to human serum proteins (Jordan, Shillingford & Steed, 1975; Monks, Boobis, Wadsworth & Richens, 1978).

Under physiological conditions the molar ratio of free fatty acid: albumin can vary between 0.5 and 1.5 (Court, Dunlop & Leonard, 1971) and in certain circumstances, such as after vigorous exercise (Rodahl, Miller & Issekutz, 1964), the molar ratio can exceed 4. As free fatty acids are more strongly bound to albumin than any drug so far studied, and physiological concentrations of free fatty acids in human serum fluctuate, the effect of these substances on the binding of several highly bound drugs has previously been examined (Rudman, Baxter & del Rio, 1971; Ozaki & Tejima, 1977). However, with the exception of clofibrate (Spector, Santos, Ashbrook & Fletcher, 1973) and diazepam (Sjödin, 1977), the binding of the majority of drugs tested was not significantly affected until the molar ratio of free fatty acids: albumin exceeded 3. Nevertheless, the similarity of structure between endogenous free fatty acids and valproic acid led us to investigate the effect of palmitic acid (a model free fatty acid) on the serum protein binding of valproic acid.

The binding of valproic acid was measured by the method of Lunde, Rane, Yaffe, Lund & Sjöqvist (1970). Valproic acid (350 μmol/l) was added to two separate pools, one of human serum containing 35g/l of albumin and the other of crystallized human albumin (35g/l) in phosphate buffer at pH 7.4. The binding of valproic acid was measured in the presence of varying concentrations of palmitic acid, from 0 to 1700 μmol/l. In order to examine the nature of the interaction between the two substances, Scatchard plots (Scatchard, 1949) were constructed for the binding of five duplicate concentrations of valproic acid (70, 350, 700, 1400 and 2100 μmol/l) in serum containing 327, 750, 2800 and 4500 μmol/l of palmitic acid. From the Scatchard plots, the values of n and k were calculated, where n is the number of binding sites for the drug on the albumin molecule and k is the apparent association constant of the drug for the n binding sites.

The relationship between the binding of valproic acid in serum and the concentration of palmitic acid is shown in Figure 1. In the absence of palmitic acid, valproic acid was 95.5% bound to serum proteins but with increasing concentrations of palmitic acid, the serum binding of valproic acid decreased in a linear fashion ($r=0.95$) from 95.5 to 65% at a palmitic acid concentration of 1700 μmol/l. Over the physiological range of free fatty acid concentrations (250–750 μmol/l) the bound fraction of valproic acid was reduced from 91 to 82%.

When a solution of crystallized human serum albumin was substituted for serum, there was again a linear decrease in valproic acid binding ($r=0.96$) with increasing palmitic acid concentration (Figure 2). When the binding of valproic acid to serum proteins

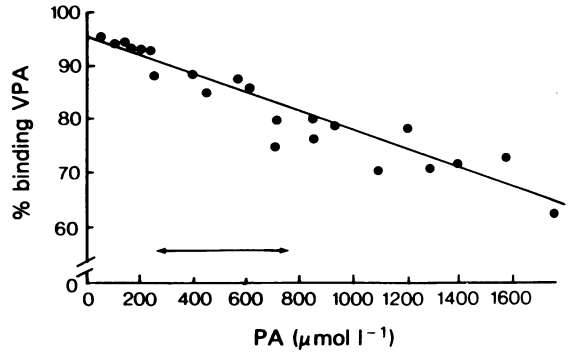

Figure 1 Effect palmitic acid (PA) on the binding of valproic acid (VPA) to human serum *in vitro*. The physiological range of free fatty acid concentrations (250–750 μmol/l) is indicated by the arrows.

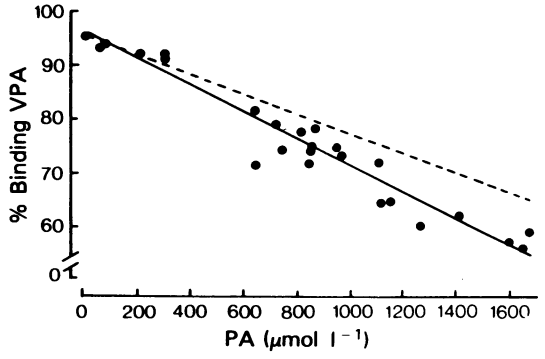

Figure 2 Effect of palmitic acid (PA) on the binding of valproic acid (VPA) in crystallized human serum albumin solution (————). The dashed line (serum) indicates the regression line illustrated in Figure 1.

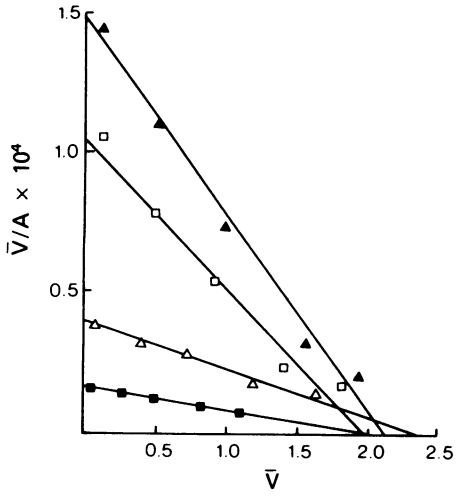

Figure 3 Scatchard plot for the binding of valproic acid in serum at four different concentrations of palmitic acid (327 (▲), 750 (□), 2800 (△) and 4500 (■) μmol/l). $\bar{V}$ = number of moles of drug bound per mole of protein, A = number of unbound moles of drug.

and albumin was compared (Figure 2) no significant difference was found between the intercepts, but the slope of the line for valproic acid binding in albumin solution was significantly greater ($P<0.01$).

Scatchard plots constructed for the binding of valproic acid in serum are illustrated in Figure 3. There was no significant difference in the value of n (mean $\pm$s.e. mean 2.08 ± 0.18) at any of the four concentrations of palmitic acid used but there was a significant fall in k ($P<0.001$) from 0.074 ± 0.04 (s.e. mean) to 0.085 ± 0.01 (s.e. mean) with increasing palmitic acid concentration.

We conclude that valproic acid binding in serum and albumin solution is directly related to the concentration of palmitic acid present. Over the physiological range of free fatty acid concentrations there was a twofold change in the free fraction of valproic acid.

This change may affect the disposition of valproic acid in the body because it has only a small volume of distribution of 8–15 l (Perucca, Gatti, Frigo & Crema, 1978), and because it exhibits restrictive hepatic extraction (Klotz & Antonin, 1977). Displacement of drug will result in a higher concentration of free drug and this will lead to an increase in plasma clearance and a fall in the total serum concentration. It may therefore be difficult to interpret the result of a serum valproic acid measurement unless the free fatty acid concentration is known.

The difference in slopes of the regression lines for valproic acid binding on palmitic acid concentration in serum and in crystallized albumin solution indicates that, in the absence of competing ligands, valproic acid is bound almost exclusively to albumin binding sites, but as valproic acid is displaced it begins to bind to protein sites other than those on the albumin molecules. Finally, Scatchard plots indicate that valproic acid and palmitic acid compete for the same albumin binding sites because the number of binding sites remained unchanged while the affinity of valproic acid for these sites was reduced.

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LACK OF HEPATIC ENZYME INDUCING EFFECT OF SODIUM VALPROATE

Many of the drugs prescribed for the major epilepsies, e.g. phenytoin and phenobarbitone, are potent inducers of hepatic microsomal enzymes. This property is responsible for a number of drug interactions (Richens, 1977) and for altering the metabolism of various endogenous substances. The latter may account for certain chronic adverse effects such as anticonvulsant osteomalacia and folate deficiency (Richens & Rowe, 1970; Maxwell, Hunter, Stewart, Ardeman & Williams, 1972). The most recently introduced antiepileptic drug, sodium valproate, has been shown not to induce liver enzymes in rats (Jordan, Shillingford & Steed, 1976). The present study was designed to evaluate this property in humans.

Eight adult subjects who were considered to require antiepileptic drug therapy because of recurrent fits of recent onset were studied on two occasions, one immediately before starting sodium valproate therapy and the second after at least 3 months (mean 4 months) of regular treatment with this drug. None of the subjects had been previously treated with antiepileptic drugs, and none had taken any other drugs known to induce liver enzymes for at least 6 months prior to the study. Sodium valproate was administered as sole drug therapy during the period of the study. The daily dose was 600–800 mg in divided doses. The subjects were admitted to hospital for 3 days on both occasions and the following indirect indices of liver enzyme induction were measured: (i) serum antipyrine half-life, using the analytical method described by van Boxtel, Wilson, Lindgren & Sjöqvist (1976), and calculating the half-life by the method of least squares regression; (ii)

Table 1 Plasma antipyrine half-life and urinary D-glucaric acid excretion in eight patients before and during sodium valproate therapy

Subject	*Age (years)*	*Sex*	*Daily dose of sodium valproate (mg)*	*Serum valproic acid concentration* (μmol/l)*	*[1]Antipyrine half-life (h)*		*[2]D-glucaric acid excretion (μmol/24 h)*	
					Before	*During*	*Before*	*During*
1	19	F	600	515	11.4	10.4	10.3	10.6
2	51	F	600	316	5.7	5.9	+	10.5
3	45	F	800	689	12.4	14.1	19.3	11.6
4	22	F	800	476	13.9	11.0	4.7	9.1
5	37	M	600	364	14.1	15.1	3.0	4.5
6	33	M	600	424	7.3	7.4	11.4	7.4
7	22	M	800	441	18.1	14.7	1.1	11.9
8	32	M	800	511	14.7	13.4	16.5	19.0
Mean	35.3			491	12.2	11.5	9.5	10.6
s.d.	11.7			112	4.0	3.5	6.5	4.2
					NS		NS	

*mean of two samples, + no sample
*7 μmol/l = 1 μg/ml
[1]Normal range 7–19 h; [2]Normal range < 20 μmol/24 h