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

Diazepam is frequently used in the epileptic patient, either for its anticonvulsant effect or in the treatment of coexisting anxiety or spasticity. The possibility of interaction with other antiepileptic drugs has been little studied. As diazepam is approximately 98% bound to plasma proteins (Van Der Kleijn *et al.*, 1971; Thiessen *et al.*, 1976; Klotz, Antonin & Bieck, 1976) the possibility of binding interactions with other drugs arises. Free fatty acids are known to displace diazepam *in vitro* (Tsutsumi *et al.*, 1975; Sjödin, 1977) and *in vivo* (Colburn & Gibaldi, 1978; Sellers *et al.*, 1980). The antiepileptic drug valproic acid is a two chain fatty acid, with similar displacing properties to endogeneous free fatty acids, including the ability to bind to fatty acid binding sites on albumin (Monks & Richens, 1979), and so we decided to investigate the effect of this drug on the binding of diazepam in serum. We were stimulated to do so by the observation that the apparent volume of distribution of diazepam tended to be higher in epileptic patients on sodium valproate (Dhillon & Richens, 1981).

Binding was measured by the method of Lunde *et al.* (1970) in pooled serum collected from normal volunteers. 5-[C^{14}] diazepam (specific activity 206 μCi/mg) (Roche Products Ltd) was added to serum *in vitro* in a concentration of 0.52 μmol/l. The binding of diazepam was measured over a 'therapeutic' range of concentrations, namely 0.53–4.04 μmol/l. The effect of varying valproic acid concentrations (175–700 μmol/l) was determined at a diazepam concentration of 1.58 μmol/l.

In order to examine the nature of the interaction between the two substances a Scatchard plot (Scatchard, 1949) was constructed over the relatively large range of diazepam concentrations of 3.5×10^{-6} mol/l to 2.11×10^{-4} mol/l.

Diazepam binding to human serum albumin was found to be 98.1 ± 0.08% at a serum diazepam concentration 1.58 μmol/l. This is in agreement with values reported earlier (Van der Kleijn *et al.*, 1971; Klotz *et al.*, 1976). (The coefficient of variation in the pool, within batch for the unbound fraction was 3.8% (n = 10)). Binding was found to be independent of the drug concentration over a range of 0.53–4.03 μmol/l but was shown to be concentration dependent at high serum concentrations, above those normally encountered with the therapeutic use of diazepam (3.51–2.10 μmol/l).

The effect of valproic acid on the binding of diazepam (serum diazepam concentration 1.58 μmol/l) is shown in Figure 1. In the absence of valproic acid, diazepam binding was 98.1 ± 0.08% but with increasing concentrations of valproic acid the binding was reduced from 98.1 ± 0.08 to 96.1 ± 0.21% at a valproic acid concentration of 700 μmol/l. Over the therapeutic range of valproic acid concentrations (350–700 μmol/l) unbound fraction of diazepam was increased two fold from 1.8 to 3.9%. Figure 2 shows the Scatchard plot for diazepam in the absence and presence of 700 μmol/l of valproic acid. The intercepts on the y axis were close to unity (n = 0.93; n = 0.95 respectively, Figure 1). There was however a

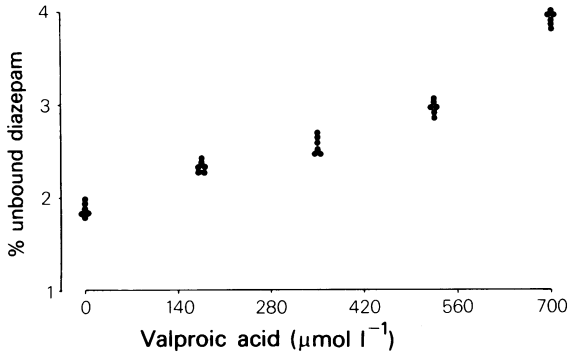

Figure 1 Effect of valproic acid on the free fraction of diazepam in human serum *in vitro*.

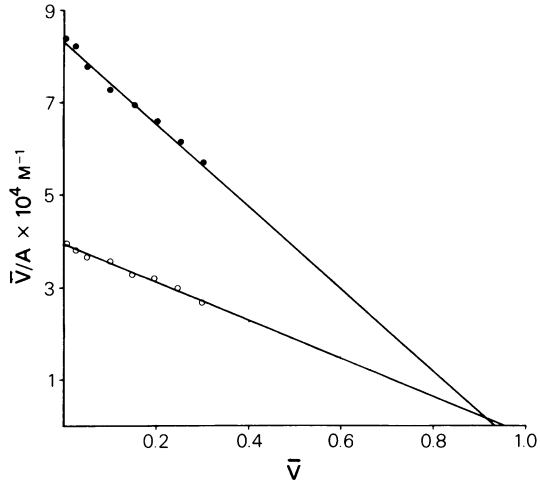

Figure 2 Scatchard plot for the binding of diazepam in serum in the absence (●) and presence (○) of valproic acid. V = number of moles of drug bound/mol of protein. A = number of moles of unbound drug.

Diazepam only Ka 8.9×10^4 M^{-1}, n = 0.93, diazepam plus valproic acid Ka 4.1×10^4 M^{-1}, n = 0.95.

marked fall in the association constant for diazepam in the presence of valproic acid: Ka = 8.9×10^4 M^{-1} (diazepam only); Ka = 4.1×10^4 M^{-1} (diazepam and valproic acid).

The results suggest that diazepam, under the conditions used, has one primary binding site on human serum albumin. This is in agreement with the findings of previous authors (Müller & Wollert, 1973; Kober *et al.*, 1979). The inhibition of diazepam binding by valproic acid has been shown to be competitive since the number of binding sites for diazepam remained unchanged while the affinity of diazepam for these sites was reduced.

The disposition kinetics of many drugs can be influenced by changes in plasma protein binding. Diazepam is a drug subject to restrictive elimination by hepatic metabolism and it has been shown that for such drugs the total clearance is directly proportional to the free fraction of the drug (Shand, Mitchell & Oates, 1975). Changes in diazepam free fraction may therefore cause an important alteration in disposition.

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EFFECT OF LOW-DOSE PHENOBARBITONE ON FIVE INDIRECT INDICES OF HEPATIC MICROSOMAL ENZYME INDUCTION AND PLASMA LIPOPROTEINS IN NORMAL SUBJECTS

Although the concept of dose-dependent enzyme induction with hypnotic doses of barbiturates is well established (Breckenridge *et al.*, 1973), little information is available on the minimum dosage of these drugs that is necessary to produce enzyme-induction in man. We have therefore decided to evaluate the enzyme-inducing properties of low doses of phenobarbitone (15 and 30 mg daily respectively) following repeated administration in normal volunteers. Five indirect indices of hepatic microsomal enzyme-