LACK OF EFFECT OF PHENYTOIN ON FUNCTIONS RELATED TO Na^+ , K^+ -ATPASE ACTIVITY OF THE INTACT ERYTHROCYTES OF EPILEPTIC PATIENTS

In a recent letter to this journal (Scott, Gilbert & Webster, 1980) it was reported that there was reduced activity of the Na^+ , K^+ -ATPase of ghosts prepared from the erythrocytes of patients taking phenytoin or phenobarbitone for grand mal epilepsy. The mean Na^+ , K^+ -ATPase activity was 12% of that in ghosts prepared from the erythrocytes of matched control subjects.

In view of the extent of the reported reduction of Na^+ , K^+ -ATPase activity, we thought it important to assess the effects of anticonvulsant treatment on functions related to Na^+ , K^+ -ATPase activity in *intact* human erythrocytes. We have therefore made the following measurements in the erythrocytes of epileptic patients taking anticonvulsants and in age-matched controls:

1. The maximum ability of the membranes of intact erythrocytes to bind 12- α - ^3H -digoxin specifically *in vitro* (^3H -digoxin binding).
2. The ability of those erythrocytes to transport ^{86}Rb (as a substitute for potassium) across the cell membrane *in vitro* (^{86}Rb uptake).
3. The intraerythrocytic sodium and potassium concentrations.

We have previously shown (Aronson *et al.*, 1977; Ford *et al.*, 1979b) that during therapy with digoxin, which binds to and inhibits membrane-bound Na^+ , K^+ -ATPase, changes occur which are consistent with *in vivo* occupation of erythrocytic Na^+ , K^+ -ATPase receptor sites by digoxin and consequent inhibition of

Na^+ , K^+ -ATPase, i.e. *in vitro* ^3H -digoxin binding is reduced, *in vitro* ^{86}Rb uptake is inhibited and intraerythrocytic Na^+ concentrations rise.

We have studied thirteen patients taking phenytoin for epileptic seizures—seven generalised tonic-clonic (grand mal), five complex partial (temporal lobe), and one simple partial (Jacksonian). The median duration of treatment was 2 years (range 3 weeks to 28 years). The median (range) numbers of seizures in the year before the study were: generalised tonic-clonic 6 (0–12), complex partial 12 (0–100), simple partial 0. Of these patients two were taking another anti-convulsant (diazepam). Thirteen age-matched, non-epileptic control subjects who were attending the hospital for various reasons were also studied. In addition we have studied three patients taking sodium valproate alone, two for generalised tonic-clonic and one for complex partial (temporal lobe) seizures, and one patient taking carbamazepine alone for generalised tonic-clonic seizures. All the subjects had normal plasma urea and electrolyte concentrations.

We have described our erythrocyte measurement techniques in detail elsewhere (Aronson *et al.*, 1977; Ford *et al.*, 1979a, 1979b). Plasma anticonvulsant concentrations were measured by enzyme-immunoassay using EMIT® kits (Syva).

The results of ^3H -digoxin binding, ^{86}Rb uptake and intraerythrocytic Na^+ and K^+ concentrations in control and phenytoin-treated subjects are illustrated

Figure 1 Values of erythrocytic ^3H -digoxin binding and ^{86}Rb uptake, and intraerythrocytic sodium and potassium concentrations in controls and treated epileptic patients.

○ controls, others—epileptic patients on: □ phenytoin > 6 months, ■ phenytoin ≤ 6 months, △ valproate and ▲ carbamazepine.

Table 1 Comparison of results from control and phenytoin-treated subjects

Measurement	Group	Mean	s.d.	n	Mean p – Mean C (95% limits)
³ H]-digoxin binding (pg per 0.5 ml cells)	C	2236	440	13	189 (–184 to 562)
	P	2425	434	13	
⁸⁶ Rb uptake (%)	C	28.7	4.0	13	2.3 (–1.6 to 6.2)
	P	31.0	5.1	13	
Intraerythrocytic [Na ⁺] (mmol l ^{–1})	C	7.1	1.8	13	–0.6 (–1.8 to 0.6)
	P	6.5	1.0	13	
Intraerythrocytic [K ⁺] (mmol l ^{–1})	C	89	5	13	–1 (–7 to 5)
	P	88	8	12	

C – control subjects; P – phenytoin-treated subjects.

in Figure 1 and listed in Table 1. None of the differences between patients and controls was statistically significant.

Plasma phenytoin concentrations ranged between 8 $\mu\text{mol l}^{-1}$ and 140 $\mu\text{mol l}^{-1}$ (median 47 $\mu\text{mol l}^{-1}$). There was no correlation between plasma phenytoin concentrations and any of the erythrocyte measurements related to Na⁺,K⁺-ATPase activity.

The results of the erythrocyte studies for the epileptic patients not taking phenytoin but taking valproate (plasma concentrations in the range 200–700 $\mu\text{mol l}^{-1}$) or carbamazepine (plasma concentration 35 $\mu\text{mol l}^{-1}$) are shown in Figure 1 for comparison with the other data.

These results suggest that phenytoin does not inhibit Na⁺,K⁺-ATPase activity in the membranes of the intact erythrocytes of patients taking phenytoin orally for epilepsy. These data are at variance with the results of Scott *et al.* (1980) who observed 88% inhibition of the Na⁺,K⁺-ATPase present in ghosts prepared from the erythrocytes of phenytoin-treated patients. We do not think that the residual 12% activity would be capable of maintaining the integrity of the erythrocyte functions that we have measured. It has been shown, for example, that 20% inhibition of Na⁺,K⁺-ATPase by digoxin *in vivo* is associated with a 32% reduction in sodium efflux rate constant and a 24% increase in intraerythrocytic sodium concentration (Cumberbatch *et al.*, 1981).

We think that this discrepancy cannot be explained on the basis of differences in either duration of treatment or plasma phenytoin concentrations between the patients in the two studies. The patients of Scott *et al.* (1980) had been treated for more than 3 months but exact durations of treatment were not stated. Our patients had been treated for between 3 weeks and 28 years (median 2 years) and may on average have been taking phenytoin for longer than the patients studied by Scott *et al.* (1980). However, the distribution of values of the erythrocyte measurements in the three patients who had been taking phenytoin for 6 months or less (closed squares in the figure) suggests that

even relatively short-term treatment with phenytoin is not associated with Na⁺,K⁺-ATPase inhibition. The 95% confidence limits of the range of plasma phenytoin concentrations in the patients of Scott *et al.* (1980) (calculated from the data given in their paper) were 0 and 32 $\mu\text{mol l}^{-1}$ (mean 16). Five of our patients had plasma phenytoin concentrations less than 40 $\mu\text{mol l}^{-1}$ and comparison of the values of their erythrocyte measurements with those of the control subjects reveals no differences.

We think that the difference in results may have arisen from some effect of phenytoin on the activity of membrane-bound Na⁺,K⁺-ATPase during preparation of the ghosts. Scott *et al.* (1980) showed that Na⁺,K⁺-ATPase activity could be inhibited in ghosts prepared from the erythrocytes of untreated subjects by incubation *in vitro* with phenytoin. The extent of inhibition was 60% at a phenytoin concentration of 0.8 $\mu\text{mol l}^{-1}$ and 95% at a phenytoin concentration of 8 $\mu\text{mol l}^{-1}$. Concentrations of phenytoin in human erythrocytes are about one-fifth of those in plasma (Kurata & Wilkinson, 1974) and thus the ghosts prepared from the erythrocytes of patients with plasma concentrations of 16 $\mu\text{mol l}^{-1}$ (as observed by Scott *et al.* (1980)) could have contained sufficient phenytoin to have caused *in vitro* inhibition of Na⁺,K⁺-ATPase to the extent they observed during preparation of the ghosts, depending on the amount of phenytoin which might have been lost during ghost preparation. The effects of phenytoin on the Na⁺,K⁺-ATPase of other, normal, excitable tissues has been widely studied in animals with inconclusive results. In some cases stimulation has been observed, in others inhibition and in others no effect (for a review see Deupree, 1977). Previous work in this laboratory has shown that incubation of phenytoin with intact human erythrocytes *in vitro* has no effect on ⁸⁶Rb uptake by the cells and does not alter the effects of cardiac glycosides on ⁸⁶Rb uptake (Hibbelle & Grahame-Smith, unpublished observations).

It is known that several constituents of erythrocyte membranes are lost during ghost preparation, that

membrane-bound enzyme activities may change as a consequence and that the cation transport mechanisms of the resulting ghosts may be different from those of the original cells (for a review see Schwoch & Passow, 1973). It may be that such changes during the preparation of erythrocyte ghosts render the Na^+, K^+ -ATPase more susceptible to the effects of phenytoin *in vitro* resulting in the effects observed by Scott *et al.* (1980).

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VALPROIC ACID FAILS TO DISPLACE PHENOBARBITONE AND CARBAMAZEPINE FROM PLASMA PROTEIN BINDING SITES IN EPILEPTIC PATIENTS

Valproic acid is extensively (90% approximately) bound to plasma proteins (Gugler & Mueller, 1978) and therefore the possibility that it may displace other drugs from binding sites needs to be considered. In a recent study (Pisani & Di Perri, 1981), we have shown that a single dose of sodium valproate given intravenously to epileptic patients stabilized on phenytoin therapy results in a significant increase in the saliva: plasma ratio of the latter drug. Since this reflects the unbound fraction in plasma (Troupin & Friel, 1975; McAuliff *et al.*, 1977), these data indicate that valproic acid displaces phenytoin from plasma proteins in agreement with previous reports (Mattson *et al.*, 1978; Monks & Richens, 1980; Perucca *et al.*, 1980). Whether valproic acid could also affect the plasma protein binding of other antiepileptic drugs

has been little investigated. Jordan, Shillingford & Steed (1976) observed *in vitro* a significant displacement of phenobarbitone by valproic acid. In order to examine this possibility we have studied the effect of a single dose of sodium valproate on the steady-state concentration of phenobarbitone and carbamazepine in the plasma and saliva of patients with epilepsy.

Ten patients (six males, four females) aged 14–37 years and weighing 49–78 kg, were studied. Five patients were treated with phenobarbitone alone (100–250 mg/day) and five with carbamazepine alone (1000–1200 mg/day). Plasma and mixed saliva samples were collected for each patient immediately before and 15 and 45 min after intravenous administration of single dose (1200 mg) of sodium valproate (Sigma-Tau, Italy). The plasma and saliva levels of