Differential transplacental binding of valproic acid: influence of free fatty acids

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The unbound fraction of valproic acid (VPA) was found to be significantly lower in cord serum ($6.0 \pm 0.8\%$) than in maternal serum collected before oxytocin ($12.2 \pm 2.7\%$) or after delivery ($9.9 \pm 2.3\%$). The difference was probably due to the concentration of free fatty acids (acting as displacing agents) being higher in maternal serum. The transplacental binding gradient explains the clinical observation that total VPA levels at delivery are higher in the newborn than in the mother.

Keywords valproic acid free fatty acids transplacental binding

Introduction

A number of investigators have reported that valproic acid (VPA) levels are higher in umbilical cord or neonatal serum than in maternal serum (Dickinson *et al.*, 1979; Fröscher *et al.*, 1981; Ishizaki *et al.*, 1981; Nau *et al.*, 1981; Kaneko *et al.*, 1983). Possible mechanisms proposed to explain this finding include (1) the possibility of the foetus acting as a deep compartment (Dickinson *et al.*, 1979; Kaneko *et al.*, 1983), (2) the presence of an active transport of VPA across the placenta (Nau *et al.*, 1981; Kaneko *et al.*, 1983) and/or (3) more extensive protein binding of the drug in foetal as compared with maternal blood (Nau *et al.*, 1981). Since only the free fraction is pharmacologically active, the existence of a transplacental binding gradient, such as suggested in preliminary observations by Nau *et al.* (1981), will have important implications in the interpretation of the observed differences in total serum VPA levels between mother and newborn.

The present study was designed to compare the plasma protein binding of VPA in maternal and cord blood *in vitro*. Since the free fraction of VPA is highly dependent on the level of albumin and free fatty acids (FFA) (Perucca *et al.*, 1981; Riva *et al.*, 1982, 1984; Albani *et al.*, 1983) we also considered it of interest to examine the

transplacental gradient of these compounds and their possible contribution to the drug binding differences between mother and newborn.

Methods

As oxytocin has been reported to alter the plasma protein binding of certain drugs (Hamar & Levy, 1980), blood was collected from each of five healthy women on two occasions: (a) immediately before administration of oxytocin (i.e. 20–30 min before delivery) (Syntocinon®, Sandoz) and (b) immediately after delivery. All women had a normal gestation and an uneventful delivery and were free of medications (other than oxytocin) at the time the blood was collected. Mixed (arterial-venous) umbilical cord blood was obtained immediately after birth. In order to prevent spurious binding results caused by *in vitro* release of FFA (Albani *et al.*, 1983) the serum was separated within 1 h and stored at -20°C until analysis. Drug spiking was performed by evaporating to dryness a solution of sodium valproate in methanol (50 mmol/l) in glass stoppered tubes prior to addition of the serum. The protein binding of VPA was assessed by using a rapid equilibrium dialysis technique

free from *in vitro* alterations of FFA at a temperature of 37°C (Riva *et al.*, 1982). Values of free fraction refer to the ratio between the drug concentration in the buffer and serum dialysates at equilibrium.

Valproic acid was measured in buffer and serum dialysates by g.l.c. (Riva *et al.*, 1982). Serum albumin and FFA in drug-free serum aliquots were determined according to Laurell (1966) and Duncombe (1964) respectively.

Statistical analysis was made by using the Student's *t*-test for paired data. Correlations were examined by regression analysis.

Results

The concentration of VPA (mean $\pm$ s.d.) in the serum compartment of the dialysis cell at equilibrium was 527 ± 62 $\mu\text{mol/l}$ (pre-oxytocin maternal samples), 493 ± 55 $\mu\text{mol/l}$ (post-delivery maternal samples) and 500 ± 55 $\mu\text{mol/l}$ (cord serum). Therefore estimated values of free fraction refer to a very similar total concentration in all samples, thus avoiding the interpretative problems related to the concentration dependent nature of VPA binding (Riva *et al.*, 1982).

As summarized in Table 1, the free VPA fraction was much greater in maternal than in cord serum ($P < 0.02$). There was also an appreciable difference between the two maternal samples, with a mean 23% decrease in free fraction in the sera collected after delivery ($P < 0.05$).

Serum albumin levels tended to be greater in umbilical serum but the magnitude of the difference was small, with a wide overlapping between maternal and cord values (Figure 1a). The maternal samples with the lowest serum albumin were those collected after delivery, in spite of the fact that these samples showed a VPA binding greater than that observed before oxytocin. As shown in Table 1, FFA were markedly more elevated in maternal (especially in the samples collected before oxytocin) than in umbilical blood.

Figure 1 relationship between free VPA fraction and: (a) albumin concentration; (b) FFA concentration in mixed cord serum ($\square$); maternal serum, before oxytocin ($\blacktriangle$) and after delivery (∇).

The relationship between free drug fraction and serum albumin was relatively weak ($r = 0.55$, $P < 0.05$). As clearly shown in Figure 1a, at equivalent values of serum albumin the free fraction was much smaller in cord than in maternal blood. By contrast, a strong positive correlation could be found between free VPA fraction and FFA ($r = 0.86$, $P < 0.001$, Figure 1b).

Table 1 Free VPA fraction, albumin and FFA concentration (means $\pm$ s.d.) in the samples examined ($n = 5$ for each set).

	(%)	Albumin ($\mu\text{mol/l}$)	FFA ($\mu\text{Eq/l}$)
Maternal serum (pre-oxytocin)	12.2 ± 2.7	505 ± 51	1099 ± 199
Maternal serum (after delivery)	$9.9 \pm 2.3^*$	484 ± 30	$746 \pm 338^*$
Cord serum	$6.0 \pm 0.8^*$	545 ± 42	$398 \pm 238^*$

* significantly different from the values observed in maternal serum pre-oxytocin ($P < 0.05$).

† significantly different from the value observed in maternal serum after delivery ($P < 0.05$).

Discussion

These experiments clearly demonstrate that VPA binds to cord serum much more extensively than to maternal serum. These data confirm preliminary *in vivo* observations made by Nau *et al.* (1981), who studied four newborns of epileptic mothers and quoted a mean neonatal/maternal free VPA fraction ratio of 0.72. The differential binding between mother and foetus (newborn) is consistent with and explains, at least in part, clinical observations that total VPA levels at delivery are higher in neonatal than in maternal blood (Dickinson *et al.*, 1979; Fröscher *et al.*, 1981; Ishizaki *et al.*, 1981; Nau *et al.*, 1981; Kaneko *et al.*, 1983). The presence of additional mechanisms, however, cannot be excluded since the 3-keto-metabolite of VPA, which is not highly protein-bound, has also been found in higher concentrations in cord than in maternal serum (Nau *et al.*, 1981).

In serum, VPA is bound virtually entirely to albumin (Monks & Richens, 1979) and the impairment in binding observed in pregnancy is known to correlate with a decrease in albumin concentration (Perucca *et al.*, 1981; Riva *et al.*, 1984). Albumin, however, did not appear to be important in determining the transplacental gradient in VPA binding. In fact the level of this protein in cord blood was only marginally greater than that in the maternal blood. Furthermore, the free drug fraction at equivalent values of albumin concentration was clearly much higher in the maternal samples indicating that other factors were mainly responsible for the binding difference.

The striking elevation of FFA in maternal samples and their correlation with free VPA strongly suggest that these factors can be identified at least in part as FFA. FFA have previously been shown to displace VPA from binding sites in a concentration-dependent manner (Monks & Richens, 1979; Riva *et al.*, 1982, 1984; Albani *et al.*, 1983, and to be a dominant factor in governing the maternal swings in free fraction and the transplacental binding gradient of another highly albumin-bound drug, diazepam, which is also found in greater concentration in cord as compared with maternal serum (Ridd *et al.*, 1982, 1983). Since the dramatic maternal elevation of FFA is confined to the stress of the perinatal period, it is unlikely that the transplacental binding gradient of these drugs is maintained throughout pregnancy.

A final comment is required on the decrease in free VPA fraction observed in the maternal serum after delivery. In studies with diazepam and salicylic acid, Hamar & Levy (1980) reported that administration of oxytocin *in vivo* (but not, at least for salicylate, its addition *in vitro*) caused a decrease in serum protein binding. In our own studies we observed an increase in VPA binding after administration of oxytocin and subsequent delivery. The effect, however, was associated with a 30% fall in serum FFA which could itself easily account for the observed change in binding capacity. The assessment of any effect of oxytocin *per se* will require more detailed investigations, possibly including comparison with a group of women not treated with this drug.

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