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Effect of sodium valproate on midazolam distribution

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Abstract—The displacement of midazolam, a new water-soluble, short-acting benzodiazepine, from its plasma binding sites by sodium valproate, has been studied in man. An increase of its free fraction (ranging from 2·71 to 5·35%) in plasma from epileptic patients receiving sodium valproate was observed. A similar situation was created in rabbits by pretreatment with sodium valproate (600 mg kg^{-1} day^{-1}) and posterior hypnosis with midazolam. Due to the interaction, sodium valproate-pretreated rabbits showed an increase in midazolam brain levels (130·91 μg g^{-1} in cortex vs 84·55 μg g^{-1} in control animals). Therefore, it seems likely that displacement of midazolam by sodium valproate in epileptic patients could lead to an increase of the midazolam response.

It is generally accepted that drug effects are a function of free drug concentration rather than total drug concentration in plasma. There is an increasing awareness of the importance of pharmacologically active unbound serum fraction of drugs, particularly in relation to central depressant agents (Greenblatt & Koch-Wesser 1974; Shoeman & Azarnoff 1975; Ochs et al 1980; Calvo et al 1986).

Since plasma protein binding is a limiting factor in the access of drugs to the central nervous system, displacement-induced modifications of the degree of binding could influence the effect of drugs used as intravenous anaesthetic agents (Brodie et al 1960; McElnay & D'Arcy 1983).

The effect of sodium valproate, a highly bound antiepileptic drug, on the binding to plasma proteins of diazepam and thiopentone has been examined and found to result in an increase in their free fraction and response (Dhillon & Richens 1981, 1982; Calvo et al 1986; Aguilera et al 1986). However, as both of those drugs have an intrinsic sedative action, it must be demonstrated that they produce their increased effect by means of a pharmacokinetic interaction rather than a pharmacodynamic potentiation.

Midazolam is a new water-soluble, short-acting benzodiazepine, mainly used in anaesthetic procedures (Dundee et al 1984). It is also extensively bound to plasma proteins (96%), most probably to site II on albumin molecule (Dundee et al 1986). No data have been presented about any interaction between midazolam and sodium valproate, so we have set out to determine the magnitude of the protein binding displacement of midazolam by sodium valproate in epileptic patients, and, using an animal model, to assess the relationship between these changes and midazolam distribution into different brain areas.

Although the extent of drug protein binding (despite similar protein concentrations) can differ between species, many authors (Shoeman & Azarnoff 1975; Ghoneim et al 1976; Belpaire et al 1977; Chou & Levy 1981) have used animals as suitable models to investigate the pharmacokinetic and pharmacodynamic consequences of drug plasma binding displacements.

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Material and methods

Drugs. [^{3}H]Midazolam maleate, water solution (specific activity, 25 Ci $mmol^{-1}$) was obtained from Nuclear Chicago. The radiochemical purity was over 97%. The midazolam for in-vivo studies was used as the commercially available pharmaceutical speciality (Dormicum, Roche, Basle, Switzerland) mixed with the labelled midazolam solution. Sodium valproate was administered as aqueous solution of the pure product obtained from Labaz (Barcelona, Spain).

Human subjects. Serum samples were obtained from 30 healthy drug-free adults (18–45 years) and from 30 adult epileptic patients (20–40 years), chronically receiving sodium valproate alone. Blood samples were drawn by venepuncture of the left arm using Vacutainer tubes without anticoagulant. They were not shaken and therefore there was no contact with the caps. Serum was obtained by centrifugation at 20 °C and stored at −20 °C until analysis.

Animals. Two groups of five female rabbits (New Zealand White, 1·5–2·5 kg), were used. They had free acces to water and food before the experiments. One group was treated with sodium valproate and other with saline (0·9% NaCl).

Valproate-pretreated rabbits received an intraperitoneal injection of 300 mg kg^{-1} twice a day for ten days (therapeutical dose in rabbits) (Pei et al 1983). Thirty minutes after the last administration of sodium valproate, 2 mL of blood were drawn and immediately centrifuged, and the sera were frozen until valproate determination. All rabbits received [^{3}H]midazolam as intravenous injection of 24 mg kg^{-1} (hypnotic dose in rabbits) with an administration time of 30 s. This hypnotic dose was previously calculated as the average of a group including ten animals with the same age, sex and weight. Animals were killed 45 s after the end of the midazolam administration.

Blood samples were collected and immediately centrifuged, and the sera were separated to measure total and free midazolam concentration.

Brains were rapidly removed and brain areas (cortex, hippocampus and pons-medulla), were dissected according to the procedure of Glowinski & Iversen (1966). Samples were accurately weighed and stored until [^{3}H]midazolam determination.

Drug analysis

In-vitro studies. In serum from human subjects, [^{3}H]midazolam binding was determined after adding 10 μL of an aqueous solution of [^{3}H]midazolam to 1 mL serum samples (final concn of 0·32 μg mL^{-1}, therapeutic concn in man) by ultrafiltration at 37 °C, using a micropartition system (Amicon MPS-1). [^{3}H]Midazolam free concentration was measured by scintillation counting using a Packard Model 300-Tri-Carb Spectrometer.

Sodium valproate concentration in serum was measured by enzyme-immunoassay (Emit) (Elyas 1980). Serum albumin was determined by a radialimmuno diffusion technique (Behring Inst.).

In-vivo studies. Measured samples of rabbit brain and serum were placed in scintillation counting vials, treated with 2 mL of Protosol solubilizer and heated at a temperature not exceeding 50 °C. After they were completely solubilized, 10 mL of Econofluor counting solution were added to each vial and radioactivity was measured as described above. Also, rabbit serum samples (0·5 mL) were used to determine midazolam free fraction by ultrafiltration and serum albumin was measured by electrophoresis. Sodium valproate in pretreated rabbits was measured as under in-vitro studies.

Statistical analyses. All data are presented as the mean ± s.e.m. Student's *t*-test was used. The criterion for significance was $P < 0·05$.

Results

In-vitro studies. The percentages of unbound midazolam in sera of normal subjects and epileptic patients, and sodium valproate concentrations are shown in Table 1. The free fraction of midazolam in control samples was 2·71 ± 0·07% and the percentage increased to 5·35 ± 0·37 in epileptic patients ($P < 0·001$). No changes were detected in albumin levels.

Table 1. Percentage of unbound midazolam and sodium valproate concentration in sera from epileptic patients on valproate (mean ± s.e.m.).

	% Free midazolam	Serum albumin concn (g L^{-1})	Sodium valproate concn (μg mL^{-1})
Control (n = 30)	2·71 ± 0·07	45·8 ± 0·15	0
Epileptic patients (n = 30)	5·35 ± 0·27*	44·1 ± 3·1	67·29 ± 4·82

*$P < 0·001$.

In-vivo studies. The free fraction of midazolam in control animals was 17·78 ± 1·01% and in sodium valproate-pretreated rabbits was 29·86 ± 2·96 ($P < 0·005$). Besides, total concentrations in serum were significantly less in pretreated rabbits than in control animals, 28·2 ± 1·7 μg mL^{-1} vs 46·2 ± 3·6 μg mL^{-1}. Sodium valproate levels were 286 ± 16 μg mL. No changes were detected in albumin levels (Table 2).

Table 2. Comparison of free fraction and total serum levels of midazolam after an hypnotic dose of 24 mg kg^{-1} in control and sodium valproate-treated animals (600 mg kg^{-1} day^{-1}).

	% Free midazolam	Total serum midazolam (μg mL^{-1})	Sodium valproate concn (μg mL^{-1})	Albumin concn (g L^{-1})
Control (n = 5)	17·78 ± 1·01	46·2 ± 3·6	0	42 ± 0·13
Sodium valproate-pretreated (n = 5)	29·86 ± 2·96*	28·2 ± 1·7*	286 ± 16	40 ± 0·17

*$P < 0·005$.

There was an increment in brain midazolam levels (μg g^{-1} tissue) in all areas in pretreated animals, and this was significantly greater in cortex ($P < 0·01$) (Table 3). Significantly ($P < 0·01$) a positive correlation existed between brain/total serum midazolam ratio and percentage of free midazolam (Fig. 1).

Table 3. Comparison of brain midazolam levels (μg g^{-1} tissue) after a hypnotic dose of 24 mg kg^{-1} in control and sodium valproate-treated animals (600 mg kg^{-1} day^{-1}).

	Cortex (μg g^{-1})	Hippocampus (μg g^{-1})	Pons-medulla (μg g^{-1})
Control (n = 5)	84·55 ± 9·91	102·99 ± 13·73	86·88 ± 13·32
Sodium valproate (n = 5)	130·91 ± 8·13*	119·93 ± 7·27**	100·52 ± 13·17**

*$P < 0·01$.
**N.S.

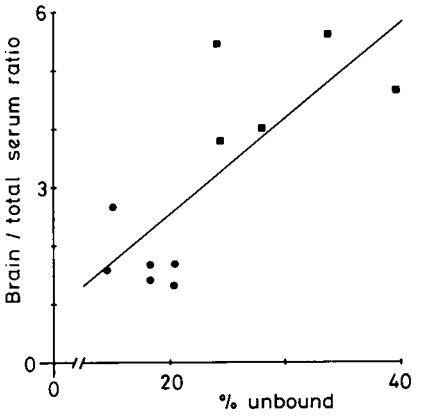

FIG. 1. Correlation between brain (μg g^{-1})/total serum (μg mL^{-1}) midazolam ratio and plasma free fraction in control (●) and valproate-pretreated (■) rabbits (r = 0·7656; $P < 0·01$).

Discussion

It is difficult to evaluate whether an in-vivo pharmacokinetic interaction can lead to an increased or decreased drug effect. Frequently, a displacement in the binding of a drug to plasma proteins does not greatly affect the access of displaced drug to receptors and there is little change in the drug's effect. However, the importance of plasma protein binding on the effects of highly bound drugs is more significant, especially if the drug acts on the central nervous system, and is intravenously injected. In this case, small changes in plasma binding will lead to large alterations in the pharmacologically active free fraction of the drug thereby producing a more intense effect (D'Arcy & McElnay 1986).

The amount of information available on the effect of displacement on the plasma protein binding of intravenous anaesthetic drugs is small (Pagnini et al 1971; Ghoneim et al 1976) and the relationships between an increase in unbound concentrations in plasma and pharmacological, or even adverse effects have been verified in only a few studies. Kaukinen et al (1980), observed a prolongation of the action of thiopentone following probenecid treatment, and suggested that this potentiation might be due to greater dispersal of the barbiturate through the brain to its sites of action. With other acidic drugs, like sulphonamides or salicylates, smaller doses

of thiopentone are required to obtain an anaesthetic effect (Chaplin et al 1973). Like thiopentone, midazolam is a drug currently used to induce sleep or as premedication in anaesthesia, and is also highly protein-bound. Dundee et al (1986) reported a potentiation of the depressant action of midazolam caused by aspirin or probenecid, that could be due to competition with the benzodiazepine for its albumin binding sites. The hypothesis is also supported by the correlation found between serum albumin and induction time in man (Halliday et al 1985), but the confirmation of the greater levels of midazolam in central nervous system is not available.

Sodium valproate is an antiepileptic drug, strongly bound to plasma albumin, that displaces other highly bound drugs in-vitro and in-vivo (Dhillon & Richens 1981, 1982). In rabbits, Aguilera et al (1986) noted that the recovery time from thiopentone was markedly longer in the presence of this anticonvulsant.

We have shown here that for valproate-pretreated epileptic patients, the in-vitro free fraction of midazolam is almost doubled when compared with normal subjects, but the direct evidence that a decrease in plasma protein binding of midazolam increases its distribution into CNS in man has not been confirmed.

So, we conducted experiments in rabbits chronically treated with sodium valproate and found that valproate also produced a two-fold increment in the midazolam-free fraction. This increase also led to potentiation of brain uptake of midazolam because a highly significant positive correlation between brain/total serum concentration ratio and percentage of free serum midazolam was observed.

Therefore our results offer evidence that sodium valproate exerts an action on the kinetics of midazolam in the CNS in rabbits and we suspect that the displacement observed in-vitro, could produce a similar increase of midazolam brain concentrations. It suggests that when midazolam is used as an intravenous anaesthetic in epileptic patients on valproate, care should be taken in choosing dosage.

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