Assessment of Valproic Acid Serum–Cerebrospinal Fluid Transport by Microdialysis[1]

Pamela L. Golden,[2] Kenneth R. Brouwer,[3] and Gary M. Pollack[2,4]

Received February 11, 1993; accepted June 8, 1993

The systemic disposition and serum–cerebrospinal fluid (CSF) translocation of valproic acid (VPA) were examined in rats after administration of VPA as a bolus, as a continuous infusion, or with probenecid. VPA in CSF was monitored continuously by *in vivo* microdialysis. Both prolonged VPA infusion and probenecid pretreatment increased the K_m for saturable VPA elimination and decreased intrinsic hepatic clearance, perhaps due to competition of probenecid or accumulated VPA metabolites for glucuronidation or depletion of hepatic UDP-glucuronic acid. The CSF/serum VPA ratio increased rapidly initially, then decreased with time throughout the remainder of the experiment in all three groups. This time- and/or concentration-dependent behavior suggested that the rate of CSF penetration increased disproportionately with increasing serum VPA and could be described by a kinetic model incorporating a concentration-dependent first-order rate constant for VPA influx into CSF. Under all experimental conditions, the VPA efflux from CSF appeared to be saturable; an increase in the Michaelis constant for efflux was observed following probenecid pretreatment and during VPA infusion, suggesting competitive inhibition of transport by probenecid and derived metabolites of VPA, respectively. The mechanisms responsible for asymmetric VPA transport between serum and CSF, in particular the apparent concentration-dependent change in the rate constant governing CSF penetration, remain to be elucidated.

KEY WORDS: valproic acid; CNS transport; microdialysis; nonlinear disposition; nonstationary disposition.

INTRODUCTION

Previous studies have suggested that the serum–CNS translocation of valproic acid (VPA) is complex. An active system for monocarboxylic acid transport into and out of the CNS has been identified (1), and several lines of evidence suggest that serum–CNS VPA transfer is mediated in part by this system. Net VPA efflux from CSF was decreased by probenecid (2), a competitive inhibitor of many organic anion transporters. VPA uptake into the CNS appears to be saturable, as suggested by a less-than-proportionate increase in brain tissue VPA concentration with increasing serum VPA exceeding 300 μg/mL (3). A potential contribution of protein-bound VPA to serum–CNS transport also has been suggested but has yet to be elucidated (4).

In addition to active transport, passive diffusion of VPA may contribute to serum–CNS translocation. Although the undissociated fraction of VPA is less than 0.005 at physiologic pH, the CSF/unbound serum VPA ratio in dogs increased by approximately 50% as the serum pH decreased from 7.4 to 6.9 (2). The mechanism underlying this pH-dependent increase in CNS penetration (i.e., diffusion of nonionized VPA across the blood–CSF barrier versus pH-dependent changes in barrier integrity or the activity of transporters) was not determined. The quantitative contribution of passive diffusion to the overall serum–CSF VPA translocation also is unknown.

One consistent observation regarding the kinetics of VPA in serum and brain tissue is that the brain/serum concentration ratio appears to be time-dependent. Instead of increasing to a plateau after VPA administration, as would be anticipated for distribution via passive diffusion, or continuing to increase with time, as would be observed for slow efflux from the organ, the brain/serum concentration ratio for VPA increases rapidly postadministration and then *decreases* with time (5,6). This situation is suggestive of a time-dependent change in the kinetics of VPA uptake into and/or efflux from brain tissue. At present, the mechanism controlling this unusual behavior has not been examined. In addition, it is not clear whether the VPA CSF/serum concentration ratio would evidence a similar time dependency.

The present study was undertaken to examine the translocation kinetics of VPA between serum and CSF in the rat. Typically, studies of CNS kinetics in rodents involve sacrifice of groups of animals at specified time points, allowing collection of only one sample per animal. The experiments described herein utilized *in vivo* microdialysis to estimate VPA concentrations in CSF, allowing the collection of complete serum and CSF concentration–time profiles in individual rats. Utilizing this approach, the translocation kinetics of VPA were assessed under three experimental conditions (after an iv bolus dose, during continuous infusion, and in the presence of probenecid) in an effort to isolate the various factors governing transfer of the drug between serum and CSF.

METHODS

VPA Disposition in Serum and CSF. Male Sprague–Dawley rats (270–310 g) were anesthetized with urethane, 1 g/kq ip, and body temperature was maintained at 37°C. The right jugular (for blood sampling) and femoral (for drug administration) veins were cannulated, and a microdialysis probe (CMA/10, Carnegie Medicin, 3 × 0.5-mm tip) was implanted in the fourth ventricle (11.6 mm posterior to bregma, 7.8 mm subdurally). The probe equilibrated for 30 min prior to the experiment to allow stabilization of the blood–brain barrier, probe environment, and probe recovery (7,8). Probes were perfused with artificial CSF (pH = 7.5, 2 μL/min) prior to implantation and throughout the ex-

[1] This work was presented in part at the American Association of Pharmaceutical Scientists Annual Meeting, Washington, DC, November 1991.

[2] Division of Pharmaceutics, School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7360.

[3] Drug Metabolism Department, Glaxo Inc. Research Institute, Five Moore Drive, Research Triangle Park, North Carolina 27709.

[4] To whom correspondence should be addressed.

0724-8741/93/1200-1765$07.00/0

periment via a microsyringe pump (BAS, West Lafayette, IN). Fractional VPA recovery by each probe was determined *in vitro* after the experiment by placing the probe sequentially in vials of artificial CSF (1 mL) containing 10, 50, and 100 μg/mL VPA. Fractional recovery was calculated as the ratio of microdialysate concentration to VPA concentration in each vial; results were averaged across vials for each probe. Probe recovery (average of 33%) was independent of concentration. VPA concentrations in microdialysate from the *in vivo* experiment were corrected for recovery to estimate VPA concentrations in CSF (9).

Treatment Groups. Rats in Group 1 ($n = 4$) received a VPA bolus (100 mg/kg) and served as controls for the other groups. Rats in Group 2 ($n = 5$) received a VPA bolus (100 mg/kg) plus infusion (17 mg/kg/hr) to examine serum/CSF VPA transfer in the presence of sustained VPA concentrations; doses were selected to produce serum VPA similar to that in the third group. Rats in Group 3 ($n = 5$) received probenecid (50 mg/kg bolus plus 17.5 mg/kg/hr infusion for the duration of the experiment) 30 min prior to a VPA bolus (100 mg/kg) to assess probenecid inhibition of VPA serum/CSF translocation, as has been shown for other organic acids (10–12).

Sample Collection. CSF microdialysate (10 μL/sample) was collected over 5-min intervals, capped, and frozen (−20°C) until analysis. Blood (300 μL/sample) was collected via the jugular vein cannula at timed intervals, and the serum harvested and frozen (−20°C) until analysis.

VPA Assay. VPA in microdialysate (10 μL) and serum (100 μL) was determined by GLC with flame ionization detection. Samples were acidified with 0.5 *N* HCl and extracted with 2 volumes of ethyl acetate containing cyclohexanecarboxylic acid (100 μg/mL) as an internal standard. Aliquots (1–3 μL) of the organic layer were analyzed on a wide-bore (0.53-mm id) fused silica FFAP capillary column (15 m) at 120°C (microdialysate) or 70°C (1 min) to 120°C at 40°C/min (serum). Carrier gas (helium) was delivered at 10 mL/min. Standard curves were linear throughout the concentration range, with a concentration-independent CV <5%.

Probenecid Assay. Proteins in serum (50 μL) were precipitated by cold acetonitrile with acetaminophen as an internal standard. After centrifugation, 50-μL aliquots of supernatant were diluted with 50 μL water. Microdialysate (10 μL) was mixed with 20 μL acetonitrile and 30 μL water. Fifty-microliter aliquots of the final preparation were analyzed on a 30-cm 5-μm C_{18} column at 28°C, with a mobile phase of 25% acetonitrile in 0.01 *M* sodium acetate (pH 7.4) delivered at 1 mL/min. The absorbance of column eluent was monitored continuously at 254 nm.

Pharmacokinetic Analysis. VPA disposition in serum and CSF was analyzed by nonlinear least-squares regression (PCNONLIN, SCI, Lexington, KY). Prior to analysis, total serum VPA concentrations were converted to estimated unbound concentrations with *ex vivo* binding parameters determined in separate groups of animals. To assess protein binding, rats ($n = 5$) were anesthetized and cannulated as described above, and VPA (100 mg/kg) was administered as a bolus. Blood samples (300 μL) were collected at timed intervals for 4 hr; serum was harvested and pooled for determination of VPA binding by ultrafiltration. A one-site saturable binding model was fit to the unbound (ultrafiltrate) versus bound VPA concentration data to recover the following parameters: binding capacity, 448 μM; binding affinity, 4.60×10^{-3} mM^{-1}.

Three models based on the scheme in Fig. 1 were developed; each was fit simultaneously to the unbound serum and CSF data. Each model incorporated saturable clearance from serum (V_{max}, K_m) and differed only in VPA serum–CSF transfer. In all cases, X_0 refers to the VPA bolus (or loading) dose, V is the central volume, and C_S and C_{CSF} are VPA in serum and CSF.

Model 1. This model was based on passive diffusion into and out of CSF governed by first-order rate constants (K_{IN}, K_{OUT}, respectively):

$$\frac{dC_S}{dt} = K_{OUT} \cdot C_{CSF} - K_{IN} \cdot C_S - \frac{V_{max} \cdot C_S}{K_m + C_S}, \qquad C_{S,0} = \frac{X_0}{V}$$

$$\frac{dC_{CSF}}{dt} = K_{IN} \cdot C_S - K_{OUT} \cdot C_{CSF}, \qquad C_{CSF,0} = 0$$

Model 2. Model 2 was based on passive diffusion of VPA into the CSF according to a C_S-dependent rate constant (K^*_{IN}) determined by a maximal rate constant for uptake (K_{max}) and a serum concentration (C_{50}) associated with an uptake rate constant equal to one-half of K_{max}.

$$K^*_{IN} = \frac{K_{max} \cdot C_S}{C_{50} + C_S}$$

CSF efflux was governed by a transport maximum (T_{max}) and a Michaelis constant ($K_{m,CSF}$):

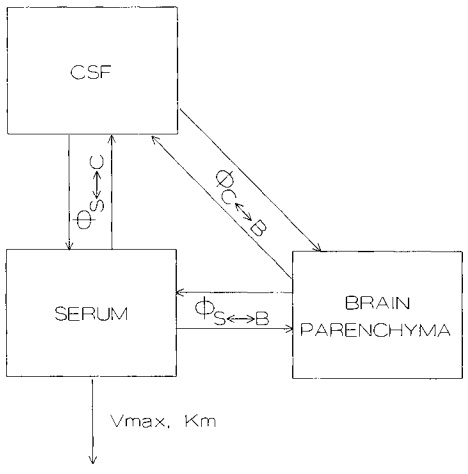

Fig. 1. Scheme for serum–CNS transfer of VPA. Arrows indicate transfer mechanisms with associated rates of flux ϕ_i. In the present study, only transfer between serum and CSF was examined; transfer between CSF and brain parenchyma was assumed not to influence the CSF concentration–time profile. Three models based upon this scheme were examined, all of which assumed saturable elimination from serum. Model 1: $\phi_{S\text{-}C}$, passive diffusion; $\phi_{C\text{-}S}$, passive diffusion. Model 2: $\phi_{S\text{-}C}$, concentration-dependent influx; $\phi_{C\text{-}S}$, active transport. Model 3: $\phi_{S\text{-}C}$, passive diffusion; $\phi_{C\text{-}S}$, active transport.

$$\frac{dC_S}{dt} = \frac{T_{max} \cdot C_{CSF}}{K_{m,CSF} + C_{CSF}} - K^*_{IN} \cdot C_S - \frac{V_{max} \cdot C_S}{K_m + C_S}, \qquad C_{S,0} = \frac{X_0}{V}$$

$$\frac{dC_{CSF}}{dt} = K^*_{IN} \cdot C_S - \frac{T_{max} \cdot C_{CSF}}{K_{m,CSF} + C_{CSF}}, \qquad C_{CSF,0} = 0$$

Model 3. This model assumed passive uptake (K_{IN}) and active efflux (T_{max} and $K_{m,CSF}$) of VPA; equations for Model 3 were identical to those for Model 2, with K_{IN} replacing K^*_{IN}.

Model selection was based on Akaike's information criterion (AIC) (13). Models incorporating other CNS uptake processes (e.g., active transport into CSF) were not examined since these were unable to describe VPA flux between serum and brain tissue (6). It is likely that the inability to describe the active component of VPA uptake was due to the fact that VPA concentrations were within the linear kinetic range (<1 m*M*) observed for monocarboxylic acid uptake (1,14).

Statistical Analysis. Where appropriate, the significance of differences in kinetic parameters between groups was determined by the unpaired Student's *t* test. Since the iv bolus dose group served as a reference for both the iv infusion and the probenecid-treated groups, Bonferroni's correction for multiple comparisons was employed. The influence of treatment group and time after VPA administration on the CSF/serum concentration ratio was assessed by two-way analysis of variance. In all cases, $P < 0.05$ was considered statistically significant.

RESULTS

The time course of VPA CSF/serum concentration ratios is presented in Fig. 2. In all groups, the CSF/serum ratio increased rapidly immediately after VPA administration, achieving a maximum at 15 min, followed by a progressive decline. Based on two-way ANOVA, the time-dependent decrease in the CSF/serum ratio was statistically significant ($P < 0.005$). The only treatment-related differences in mean CSF/serum ratios were at 5 min (VPA infusion and probenecid groups were statistically lower than the VPA bolus group) and 15 min (the probenecid group was statistically lower than the VPA bolus group) after VPA administration.

Mean (±SD) VPA profiles in serum and CSF are shown in Figs. 3–5. Each model provided identical fits to the serum data, suggesting that VPA serum–CSF flux does not influence serum VPA. In every case, Model 1 (bidirectional passive diffusion) provided the worst fit to the data based upon AIC. For the bolus-dose group, Model 3 (passive influx and active efflux) provided the best fit to the data (Fig. 3C). In contrast, Model 2 provided a superior fit to the data obtained from both the VPA infusion and the probenecid groups (Figs. 4B and 5B, respectively). In these groups, Model 3 overestimated the CSF concentration of VPA after 120 min (Figs. 4C and 5C).

Parameters governing systemic VPA kinetics, generated with the optimal model identified for each group, are compiled in Table I. These parameters represent the mean ± SD of estimates obtained from individual rats. The distribution volume for unbound VPA (~300 mL/kg) was unaffected by treatment. Increases in both V_{max} (5- to 10-fold) and K_m (30- to 40-fold) were observed in both the infusion and the probenecid groups. The observed variability in Michaelis–Menten parameters was low in the bolus-dose group but high in the other two groups. This observation is not surprising, since the apparent nonlinear disposition of VPA was masked during VPA infusion (as serum concentrations approached steady-state conditions) or in the presence of probenecid (due to a decrease in VPA clearance). Thus, the model was overparameterized for the infusion and probenecid groups. To assess the influence of each treatment on VPA elimination, the unbound intrinsic clearance (Cl′) of VPA was estimated for each animal as $(V_{max}/K_m) \cdot V$. Cl′ was decreased significantly ($P < 0.01$) during constant infusion of VPA (approximate 6-fold decrease) and in the presence of probenecid (approximate 10-fold decrease).

The kinetic parameters controlling VPA flux between

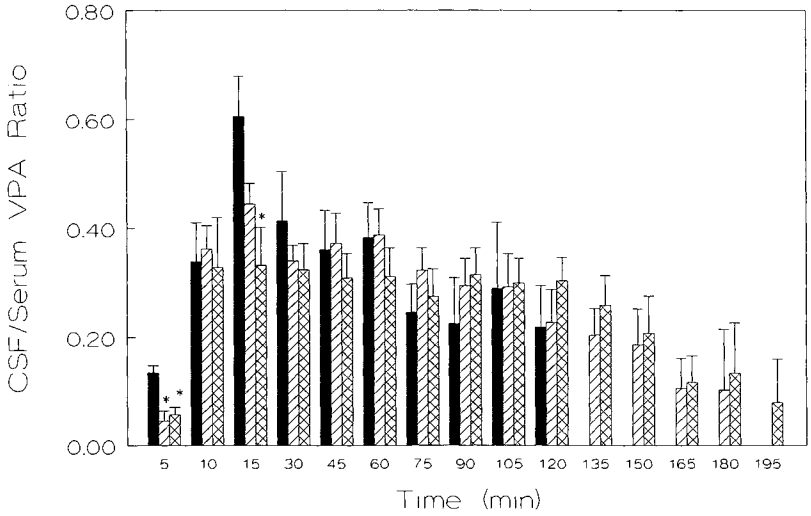

Fig. 2. Time course of CSF/serum VPA concentration ratio in rats receiving a VPA bolus (solid), a VPA infusion (hatched), or probenecid coadministration (cross-hatched). Bars indicate mean ± SD of four (bolus-dose group) or five observations.

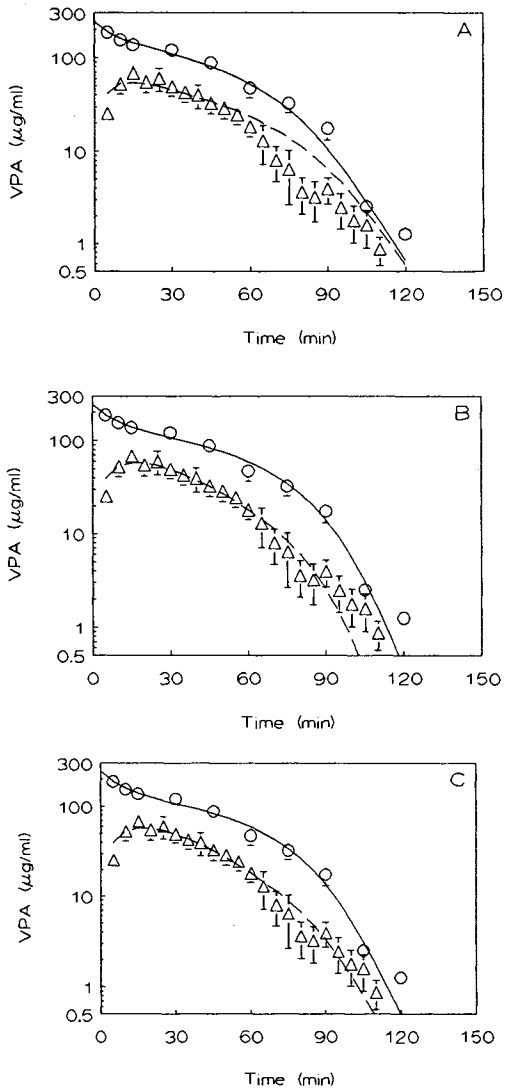

Fig. 3. Concentration–time profiles for VPA in serum (circles) and CSF (triangles) after a 100 mg/kg iv bolus of VPA. Error bars indicate the mean ± SD; lines indicate the fit of (A) Model 1, (B) Model 2, and (C) Model 3 to the mean data. See Fig. 1 and Methods for model identification.

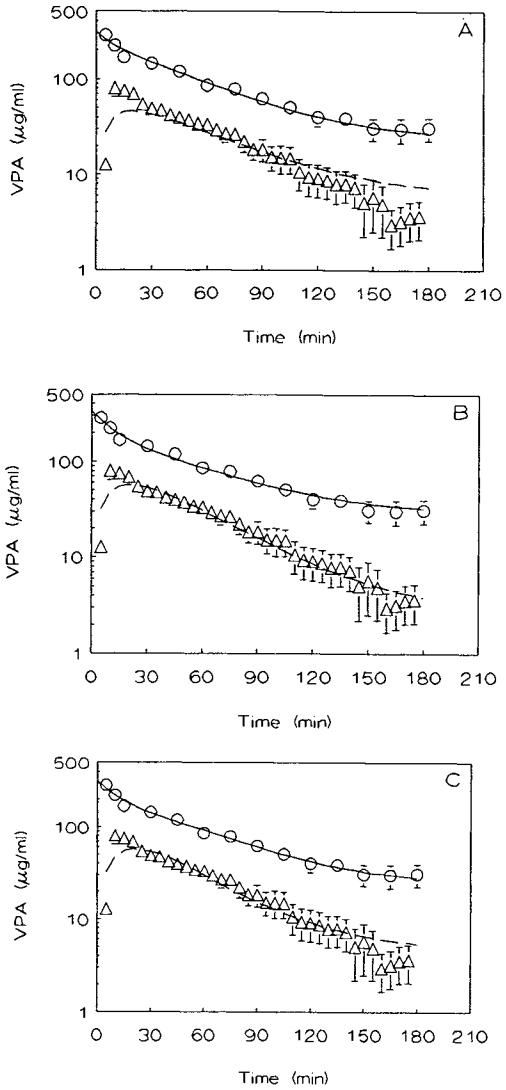

Fig. 4. Concentration–time profiles for VPA in serum (circles) and CSF (triangles) during constant iv infusion of VPA. Error bars indicate the mean ± SD; lines indicate the fit of (A) Model 1, (B) Model 2, and (C) Model 3 to the mean data. See Fig. 1 and Methods for model identification.

serum and CSF are presented in Table II. The maximum rate constant for VPA influx (or, in the case of the bolus-dose group, the concentration-independent rate constant for influx) was approximately 0.05 min^{-1}, and did not differ statistically between treatment groups. Similarly, the serum concentration at which the influx rate constant was 50% of maximum (C_{50}) was consistent between the VPA infusion and the probenecid-treated groups, and tended to be 10–30 μg/mL. T_{max} for VPA efflux from CSF did not differ between treatment groups. However, $K_{m,CSF}$ was significantly higher in both the infusion and the probenecid-treated groups compared to the bolus-dose group ($P < 0.05$). Peak VPA concentrations in CSF, normalized for VPA in serum, were significantly lower in rats receiving probenecid coadministration compared to animals receiving only a VPA bolus ($P < 0.05$). In all groups, the peak CSF/serum concentration ratio was observed at 15 min.

Mean (±SD) probenecid concentrations were 174 ± 14 μg/mL in serum and 28.5 ± 18.3 μg/mL in CSF. Correlations of VPA kinetic parameters with probenecid concentrations were not significant due to the small number of rats and narrow range of probenecid concentrations.

To demonstrate differences in model performance, the concentration–time data for the infusion group were converted to CSF/serum concentration ratios versus time. The observed ratios were compared to predicted ratios based upon parameter estimates obtained from application of Model 2 and Model 3 to the data obtained from this group. The results of this comparison are displayed in Fig. 6. A traditional pharmacokinetic model (i.e., Model 3, based upon assumptions of passive diffusional influx and active efflux) predicted that the CSF/serum ratio would increase rapidly after the VPA loading dose and would become essentially constant by 1 hr. Clearly the CSF/serum ratio did

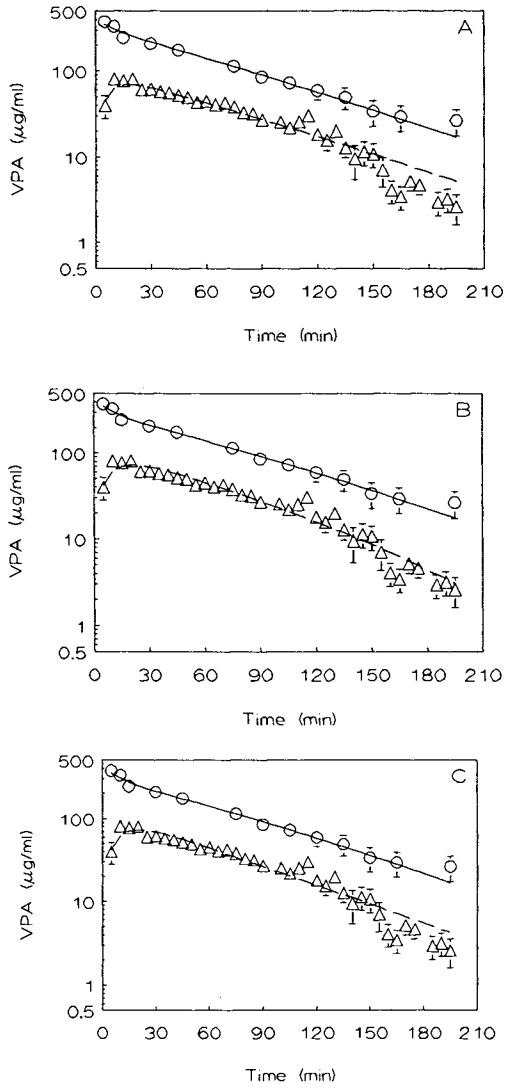

Fig. 5. Concentration–time profiles for VPA in serum (circles) and CSF (triangles) after a 100 mg/kg iv bolus of VPA to rats pretreated with probenecid. Error bars indicate the mean ± SD; lines indicate the fit of (A) Model 1, (B) Model 2, and (C) Model 3 to the mean data. See Fig. 1 and Methods for model identification.

not behave in this manner. The observed CSF/serum ratio increased rapidly, reaching a maximum value at 15 min and declining thereafter. Model 2, which assumed that the rate constant for VPA influx into CSF decreased as serum VPA decreased, was able to account for this unusual time-dependent concentration ratio.

DISCUSSION

The present study elucidated several aspects of the kinetics of VPA flux between serum and CSF. As has been observed for VPA partitioning between serum and brain tissue (5,6), the CSF/serum VPA ratio did not achieve a distributional steady state. The initial rate of influx into the CSF was rapid, with maximal CSF/serum ratios observed within 15 min; however, the CSF/serum ratio decreased significantly with time throughout the remainder of the experiment.

VPA serum-to-CSF translocation did not appear to be saturable. However, the serum concentrations observed in this study may have been below the K_m for active VPA uptake, resulting in an apparent first-order process. Uptake of monocarboxylic acids tends to be linear at concentrations <1 m*M* (i.e., VPA concentrations of 150 μg/mL) (1). The K_m for this transport system is 1.8 ± 0.6 m*M* (15), corresponding to VPA concentrations of 260 μg/mL. Nonlinear distribution of VPA between brain and serum appears to occur at concentrations of 300 μg/mL (3), which is consistent with the aforementioned K_m. Since serum concentrations were above this value only briefly in the bolus-dose and infusion groups, it is not surprising that VPA influx did not appear to be saturable. Although probenecid increased VPA serum concentrations, the influx kinetics in this group also appeared to be nonsaturable. If probenecid inhibited active VPA uptake competitively (therefore elevating the K_m), VPA serum concentrations may have been too low to achieve significant saturation. Indeed, the observed data were consistent with a slight inhibition of serum-to-CSF translocation of VPA by probenecid: the maximum CSF/serum VPA ratio was significantly lower in rats receiving probenecid, and the apparent maximal rate constant for VPA uptake into CSF tended to be lower in these animals.

The apparent inhibition of VPA uptake by probenecid is consistent with the influence on CNS uptake of other organic acids (16). These results contrast with the observation of Frey and Loscher (2) that probenecid increased the initial rate of net VPA influx into the CSF. These authors suggested that probenecid inhibited carrier-mediated efflux of VPA from CSF and that VPA uptake into CSF was unaffected. This discrepancy between studies may be due to differences in VPA concentration (>200 μg/mL in the present study versus <80 μg/mL).

Table I. Influence of VPA Infusion or Probenecid Coadministration on the Systemic Disposition of VPA[a]

Parameter	Bolus dose (n = 4)	Infusion (n = 5)	Probenecid (n = 5)
V (mL/kg)	314 ± 73	288 ± 62	257 ± 68
V_{max} (μg/mL/min)	3.32 ± 0.47	31.2 ± 24.4	17.6 ± 9.3
K_m (μg/mL)	20.2 ± 11.7	667 ± 456	814 ± 406
Cl′ (mL/min/kg)	77.4 ± 23.9	13.0 ± 3.2*	7.18 ± 5.16*

[a] Data expressed as mean ± SD.
* Significantly different from bolus dose, $P < 0.01$.

Table II. Influence of VPA Infusion or Probenecid Coadministration on the Serum–CSF Transfer Kinetics of VPA[a]

Parameter	Bolus dose (n = 4)	Infusion (n = 5)	Probenecid (n = 5)
K_{IN} (K_{max}) (min^{-1})	0.0634 ± 0.0148	0.0475 ± 0.0286	0.0364 ± 0.0177
C_{50} (μg/mL)	NA[b]	43.7 ± 26.7	19.3 ± 17.3
T_{max} (μg/mL/min)	15.5 ± 5.0	27.7 ± 11.3	10.6 ± 2.9
$K_{m,CSF}$ (μg/mL)	39.5 ± 14.9	133 ± 33*	78.4 ± 29.4*
Peak CSF/serum ratio	0.606 ± 0.147	0.514 ± 0.074	0.332 ± 0.140*

[a] Data expressed as mean ± SD.
[b] Not applicable to the kinetic model used.
* Significantly different from bolus dose, $P < 0.05$.

In contrast to influx, VPA efflux from CSF clearly was saturable. The existence of saturable VPA efflux from CSF has been proposed to account for the fact that VPA concentrations in CSF are lower than unbound serum VPA at steady state (17). In rats receiving a VPA bolus, $K_{m,CSF}$ was approximately 40 μg/mL. $K_{m,CSF}$ was elevated during VPA infusion and by probenecid; neither treatment influenced T_{max}. This observation is consistent with competitive inhibition of VPA efflux. For VPA infusion, inhibition may be due to accumulation of VPA metabolites in the CSF. Probenecid may have inhibited VPA efflux by one of several mechanisms, including inhibition by probenecid or a probenecid metabolite or inhibition by a VPA metabolite that accumulated in the presence of probenecid. Since probenecid inhibits the serum–CNS transport of several compounds including VPA, the former mechanism is most likely. Indeed, both VPA and probenecid increase CSF concentrations of various organic acids in the rat, presumably via competitive inhibition of the monocarboxylic acid transport system (9,12,18).

The kinetic models applied to the present data are worthy of note. In most cases, the tissue-to-blood partitioning of a compound administered systemically will increase with time and eventually approach a constant value. The distribution of VPA between serum and CSF is unusual in that it evidences an "overshoot" (i.e., the CSF/serum ratio displays an initial rapid increase followed by a decrease). It has been proposed that the serum–CNS transport of VPA is asymmetric, with the CNS efflux rate exceeding the influx rate (4). Models based on asymmetric transport (i.e., diffusional input coupled with saturable efflux) produce an overshoot in the CSF/serum concentration ratio. However, the observed data deviate from model predictions based upon simple asymmetric transport in two important respects. The parameters generated with Model 3 predict that maximal CSF/serum ratios will occur at 40–60 min, depending upon the experimental group. In every rat examined, the maximal CSF/serum ratio was observed at 10–15 min. Thus, the simple asymmetric model cannot account for rapid initial influx into CSF. More importantly, the asymmetric model predicts that at some point the CSF/serum concentration ratio will become constant. With the parameter estimates generated in this experiment, the CSF/serum ratio should remain constant after approximately 2 hr. In the present study, rats receiving a bolus dose of VPA appeared to evidence such a plateau in distribution ratio (Fig. 2). This is somewhat misleading, however, since CSF VPA was undetectable in this group after 2 hr. In the presence of sustained VPA concentrations, where assay limitations are not a consideration, the CSF/serum ratio continued to decrease throughout the experiment. This prolonged decrease cannot be accounted for with a simple asymmetric distribution model.

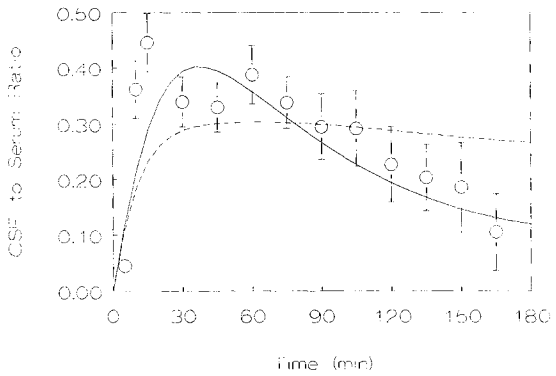

Fig. 6. Observed (mean ± SD) and predicted CSF/serum concentration ratio during VPA infusion. Lines indicate predictions based upon Model 2 (solid line) and Model 3 (dashed line).

The present data, as well as previously published reports (5,6), suggest that the rate of VPA penetration into the CNS increases disproportionately with increases in unbound serum VPA. There are several mechanisms by which a drug could enhance its own CNS uptake. It is possible that VPA transport is cooperative (i.e., binding of one VPA molecule to the transport site increases the likelihood that a second molecule will be transported). Such a cooperative system, while possible in theory, has not been documented. Alternatively, VPA may decrease cerebral blood flow, thereby increasing both the cerebral capillary transit time and the opportunity for diffusion across capillary endothelia. Whether VPA does alter cerebral blood flow is unknown.

An alternative hypothesis is that VPA alters serum–CNS transport in a nonspecific fashion via a transient, concentration-dependent perturbation of the blood–brain or blood–CSF barrier. Several pieces of evidence support the latter hypothesis. VPA has been shown to increase the brain uptake index of both diazepam (19) and chlordiazepoxide (20) in rats. For both drugs, the increase in brain uptake could not be accounted for on the basis of alterations in protein binding. Similar increases in blood–brain barrier permeability have been induced by administration of ali-

phatic alcohols to dogs (21). Furthermore, VPA and ethanol both are capable of disordering neuronal cellular membranes, although VPA is nearly an order of magnitude more potent than ethanol in this regard (22); 50% of maximum change in membrane fluidity was achieved with 9 m*M* VPA versus 72 m*M* ethanol. It is possible that the unusual partitioning kinetics of VPA, and the VPA-enhanced brain uptake of benzodiazepines, is due to disordering of the endothelial membranes comprising the blood–brain and blood–CSF barriers. Experimentation is under way currently to test this hypothesis.

REFERENCES

1. W. H. Oldendorf. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am. J. Physiol.* 224:1450–1453 (1973).
2. H. H. Frey and W. Loscher. Distribution of valproate across the interface between blood and cerebrospinal fluid. *Pharmacology* 17:637–642 (1978).
3. G. M. Pollack and D. D. Shen. A timed intravenous pentylenetetrazol infusion seizure model for quantitating the anticonvulsant effect of valproic acid in the rat. *J. Pharmacol. Meth.* 13:135–146 (1985).
4. E. M. Cornford, C. P. Diep, and W. M. Pardridge. Blood-brain barrier transport of valproic acid. *J. Neurochem.* 44:1541–1550 (1985).
5. E. J. Hammond, R. J. Perchalski, H. J. Villarreal, and B. J. Wilder. In vivo uptake of valproic acid into brain. *Brain Res.* 240:195–198 (1982).
6. L. J. Haberer and G. M. Pollack. Blood-brain transport kinetics of valproic acid (VPA) in the developing rat. *Pharm. Res.* 8:S-322 (1991).
7. H. Benveniste, J. Drejer, A. Schousboe, and N. H. Diemer. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.* 43:1369–1374 (1984).
8. H. Benveniste, A. J. Hansen, and N. S. Ottosen. Determination of brain interstitial concentrations by microdialysis. *J. Neurochem.* 52:1741–1750 (1989).
9. H. Benveniste and P. C. Huttemeier. Microdialysis—theory and application. *Prog. Neurobiol.* 35:195–215 (1990).
10. N. H. Bass and P. Lundborg. Postnatal development of mechanisms for the elimination of organic acids from the brain and cerebrospinal fluid system of the rat: Rapid efflux of [^{3}H]para-aminohippuric acid following intrathecal infusion. *Brain Res.* 56:285–298 (1973).
11. F. R. Domer. Drug inhibition of PAH transport from cerebrospinal fluid in rabbits. *Exp. Neurol.* 40:414–423 (1973).
12. V. MacMillan, J. Leake, T. Chung, and M. Bovell. The effect of valproic acid on the 5-hydroxyindoleacetic, homovanillic and lactic acid levels of cerebrospinal fluid. *Brain Res.* 420:268–276 (1987).
13. A. Akaike. Posterior probabilities for choosing a regression model. *Ann. Inst. Math. Stat.* 30:A9–14 (1978).
14. J. E. Cremer, H. M. Teal, and V. J. Cunningham. Inhibition, by 2-oxo acids that accumulate in maple-syrup-urine disease, of lactate, pyruvate, and 3-hydroxybutyrate transport across the blood-brain barrier. *J. Neurochem.* 39:674–677 (1982).
15. W. M. Pardridge. Recent advances in blood-brain barrier transport. *Annu. Rev. Pharmacol. Toxicol.* 28:25–39 (1988).
16. R. A. Fishman. Blood-brain and CSF barriers to penicillin and related organic acids. *Arch. Neurol.* 15:113–124 (1966).
17. J. S. Lockard, C. T. Viswanathan, and R. H. Levy. Diurnal oscillations of CSF valproate in monkey. *Life Sci.* 36:1281–1285 (1985).
18. T. B. Miller and C. R. Ross. Transport of organic cations and anions by choroid plexus. *J. Pharmacol. Exp. Ther.* 196:771–777 (1976).
19. C. Hariton, G. Jadot, E. Mesdjian, J.-P. Cano, and P. Mandel. Valproate increases diazepam impregnation selectively in CNS of the rat after subchronic administration. *Life Sci.* 37:1343–1349 (1985).
20. P. Riant, F. Bree, S. Urien, C. Hamberger, E. Albengres, and J. P. Tillement. How chronically administered valproate increases chlordiazepoxide transfer through the blood-brain barrier. *Fundam. Clin. Pharmacol.* 4:105–114 (1990).
21. A. Gulati, C. Nath, K. Shanker, and R. C. Srimal. Effect of alcohols on the permeability of blood-brain barrier. *Pharmacol. Res. Comm.* 17:85–93 (1985).
22. B. J. Perlman and D. B. Goldstein. Genetic influences on the central nervous system depressant and membrane-disordering actions of ethanol and sodium valproate. *Mol. Pharmacol.* 26:547–552 (1984).