Pharmacokinetic Analysis and Antiepileptic Activity of N-Valproyl Derivatives of GABA and Glycine

Salim Hadad[1] and Meir Bialer[1,2]

Received November 15, 1994; accepted January 9, 1995

Purpose. To explore the possibility of utilizing valproyl derivatives of GABA and glycine as new antiepileptics by using the structure pharmacokinetic-pharmacodynamic relationship (SPPR) approach. ***Methods.*** The pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of the following four conjugation products of valproic acid (VPA), glycine and GABA were investigated: valproyl glycine, valproyl glycinamide, valproyl GABA and valproyl gabamide. ***Results.*** Only valproyl glycinamide showed a good anticonvulsant profile in both mice and rats due to its better pharmacokinetic profile. Valproyl glycinamide was more potent than one of the major antiepileptic agents - VPA and showed a better margin between activity and neurotoxicity. Valproyl glycine and valproyl GABA were partially excreted unchanged in the urine (fe = 50% and 34%, respectively), while the urinary metabolites of the amide derivatives were valproyl glycine and valproyl GABA. ***Conclusions.*** The four investigated valproyl derivatives did not operate as chemical drug delivery systems (CDDS) of glycine or GABA, but acted rather as drugs on their own. The current study demonstrates the benefit of the SPPR approach in developing and selecting a potent antiepileptic compound in intact animals based not only on its intrinsic pharmacodynamic activity, but also on its better pharmacokinetic profile.

KEY WORDS: valproyl glycine; valproyl glycinamide; anticonvulsant activity; structure pharmacokinetic pharmacodynamic relationships (SPPR); valproyl GABA; valproyl gabamide.

INTRODUCTION

GABA is an inhibitory neurotransmitter which plays an important role in the control of neuronal activity in the mammalian central nervous system (CNS). A deficiency in brain GABA levels has been shown to cause convulsions or epilepsy (1,2). Consequently, drugs which increase the amount of GABA available in the brain for neurotransmission have the potential of becoming antiepileptic agents. GABA derivatives, such as gamma-vinyl-GABA (GVG) (3) and gabapentin (4) are two of the newest antiepileptics which have reached the clinic in recent years. The antiepileptic activity of GVG in epileptic patients shows that a chemical addition of vinyl to GABA enhances brain penetration (in comparison to GABA) and contributes to its physiological stability (5). Gabapentin contains a GABA molecule symmetrically integrated into a lipophilic cyclohexane system, which unlike GABA has the ability of crossing the blood brain barrier (BBB). Apart from GABA, glycine one of the most important inhibitory neurotransmitters has also been incorporated into a new antiepileptic agent - milacemide (6) and into an active derivative - N-benzyloxycarbonylglycine (7). Recent reports have shown that co-administration of glycine and other antiepileptics, such as phenytoin, phenobarbital and GVG, potentiate the anticonvulsant activity in several rats models, due to synergism (8-11). However, neither GABA nor glycine are effective upon oral or systemic administration and therefore their delivery into the brain can be accomplished by designing derivatives or chemical drug delivery system (CDDS) which will be orally available and will serve as BBB penetrable carriers.

Valproic acid (VPA-I) is one of the four major antiepileptic drugs which has a wide antiepileptic spectrum of activity (12-14). In this study the following four valproyl derivatives were synthesized and evaluated (Fig 1): valproyl glycine (II), valproyl glycinamide (III), valproyl GABA (IV) and valproyl gabamide (V). Valproyl glycine, a minor metabolite of VPA in rats (15) which has some anticonvulsant activity although less than that of VPA (16). Valproyl GABA has also been reported to possess some anticonvulsant activity in mice (17). VPA glycinamide and VPA gabamide are novel compounds.

One of the major side effects of VPA is teratogenicity. Structure- teratogenicity relationship studies (in rodents) showed that the presence of a free carboxylic moiety in the VPA molecule is essential for teratogenicity and therefore, unlike VPA, its primary amide - valpromide (VPD-VI) is not teratogenic (18,19). The amidation of the VPA carboxylic moiety forming compounds II to V might lead to less teratogenic compounds than VPA.

The current pharmacokinetic study was designed in order to investigate the *in vivo* performance of compounds II-V, and to assess whether these compounds undergo a metabolic cleavage to VPA and to the neuroinhibitory transmitters GABA and glycine, and if so, to what extent. In addition, this study was designed to evaluate the structure pharmacokinetic-pharmacodynamic (anticonvulsant activity and neurotoxicity) relationships (SPPR) of the above mentioned compounds. The pharmacodynamic evaluation was carried out in collaboration with the anticonvulsant screening project of the NIH Epilepsy Branch (20).

MATERIALS AND METHODS

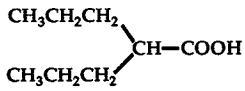
Materials

VPA was supplied to us by Teva Pharmaceutical Industries, Israel. Compounds II-V were prepared by reacting valproyl chloride with glycine, glycinamide and GABA in the presence of 10% aqueous sodium hydroxide. Valproyl gabamide was prepared by reacting valproyl gabaoyl chloride in dry chloroform with gaseous ammonia. The reaction was performed in an ice-cooled flask and the valproyl gabaoyl chloride was added dropwise with stirring. Valproyl gabaoyl chloride was prepared by reacting valproyl GABA in dry chloroform at 0°C in the presence of dimethylaminopyridine with thionyl chloride. The chemical structures of compounds II-V and their purity were confirmed by nuclear magnetic resonance (NMR) and elemental microanalysis.

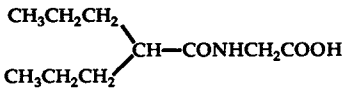
[1] Department of Pharmacy, School of Pharmacy, Faculty of Medicine, The Hebrew University, P.O. Box 12065, Jerusalem 91120, Israel.

[2] To whom correspondence should be addressed.

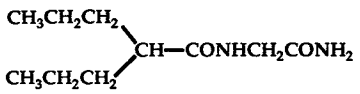
0724-8741/95/0600-0905$07.50/0



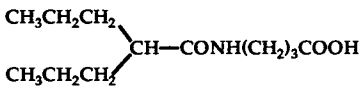
Valproic acid - VPA (I)



Valproyl glycine (II)

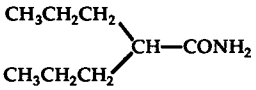


Valproyl glycinamide (III)



Valproyl GABA (IV)

Valproyl gabamide (V)



Valpromide (VI)

Fig. 1. The chemical structures of valproic acid (VPA) (I), valproyl glycine (II), valproyl glycinamide (III) valproyl GABA (IV), valproyl gabamide (V) and valpromide (VI).

Animals

The experiments were carried out on seven dogs (mongrels), four males and three females, ranging in weight between 16 and 21 kg. (The experiment with compounds IV and V were carried out on six dogs.) In a randomized cross-over design, each dog was injected intravenously (in 1.5 ml 70% alcohol) with a dose equivalent to 400 mg of VPA, of compounds II-IV (valproyl glycine 558 mg; valproyl glycinamide 556 mg, valproyl GABA 665 mg, valproyl gabamide 633 mg). In four (male) dogs, urine was collected systematically for 18 hours after dosing, by means of an indwelling catheter. The pharmacokinetics of valproyl glycinamide (III) and valproyl glycine (II) was also studied following oral administration to six and four dogs, respectively.

Protocol

Venous blood samples (5 ml) were collected via an indwelling catheter (from the cephalic vein) at specified intervals following injection (0, 5, 10, 15, 20, 30, 40 and 50 min and 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hr, respectively). Following oral dosing the sampling times were the same except for the first hour, during which blood was withdrawn every 15 minutes. The plasma was then immediately separated by centrifugation at 7000 rpm for 15 min and stored at −20°C. Before each assay, the plasma was allowed to reach room temperature, vortexed, centrifuged and the residual clot removed. Plasma and urine levels of compounds II-V were then assayed by a new HPLC assay. VPA plasma levels were assayed by a GLC assay previously reported by us (21).

HPLC Assay

To 0.5 ml of plasma, 20 μl of internal standard solution (N-valproylnipecotamide 1 mg/ml in acetonitrile), 250 μl of phthalate buffer pH = 5.4 (50 ml of 0.1M potassium hydrogen phthalate and 34 ml of NaOH 0.1M) and 2 ml of acetonitrile were added. The mixture was centrifuged for 10 minutes at 3000 g and the organic phase was separated and evaporated (under vacuum at ambient temperature using a vortex evaporator apparatus) to a total volume of about 0.5 ml of aqueous residue. To the aqueous residue, 4 ml of tert. butyl methyl ether was added followed by vigorous vortex for 30 seconds. The mixture was centrifuged for 10 minutes at 3000 g and the organic phase was separated, and evaporated (using a vortex evaporator) to dryness. To the dry residue 120 μl of acetonitrile were added, the mixture was vortexed and 20 μl was injected into the HPLC apparatus. HPLC Conditions: Column - RP-18 reverse phase column equipped with a precolumn. Mobile phase: acetonitrile 45%, bidistilled water 55%; and trifluoroacetic acid (TFA) 0.1%.

UV Wave length - 220 nm
Flow rate - 1-1.2 ml/min

A linear response was observed for compounds II-V at a concentration range of 3 to 40 mg/L. The inter-day percentage coefficient of variation (CV) among replicates ranged between 3.7 to 11.3% for valproyl glycinamide, and 5.6 to 11.7% for valproyl glycine with 24% CV at the lower limit of quantification (LOQ) of 3 mg/L.

Anticonvulsant Activity

The following compounds VPA (I), compounds II-V and VPD (VI) have been screened in Carworth Farm #1 mice (ip - in a volume of 0.01 ml/g of body weight) and Sprague-Dawley rats (po - in a volume of 0.004 ml/g of body weight) for their anticonvulsant activity and neurotoxicity by the NIH Epilepsy Branch (20). The screening procedure involved the following: 1) the maximal electroshock (MES) test, which measures seizure spread; 2) the subcutaneous pentylenetetrazol test (sc Met test), which measures seizure threshold; and 3) the rotorod ataxia test which assesses neurotoxicity (20).

Pharmacokinetic Analysis

The linear terminal slope (β) of log C (drug plasma concentration) versus t (time) was calculated by the method of least squares. The terminal half-life of the compound ($t_{1/2}\beta$) was calculated from the quotient 0.69/terminal slope. The AUC (area under the C versus t curve) was calculated by using the trapezoidal rule with extrapolation to infinity (22). The total body clearance (CL) of compounds II-V was calculated by using the quotient of the i.v. dose (D) and the AUC. The volume of distribution (Vβ) was calculated using the quotient of the clearance and the linear terminal slope. The volume of distribution at steady state (V_{ss}) and the mean residence time (MRT) were calculated by classical methods (22-25).

The fraction excreted unchanged (fe) of valproyl glycine and valproyl GABA was calculated from the ratio of the cumulative amount excreted in the urine to the dose. The fraction metabolised (fm) of valproyl glycinamide to valproyl glycine and valproyl gabamide to valproyl GABA was calculated from the ratio of the fe obtained after iv administration of the amide and its corresponding acid (metabolite).

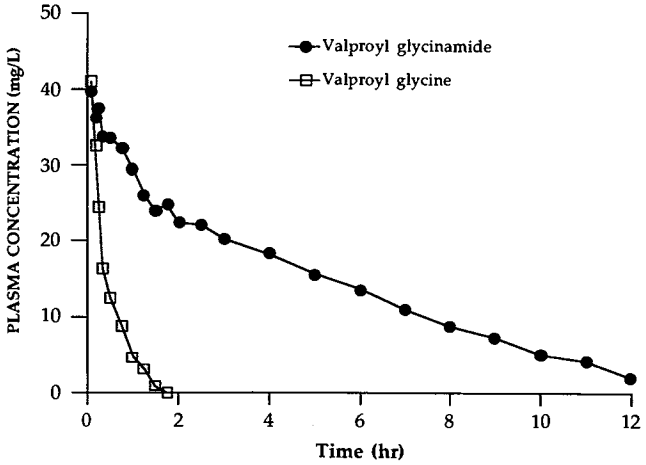


Fig. 2. Mean plasma levels of valproyl glycinamide and valproyl glycine following their iv administration (556 mg and 558, respectively) to seven dogs.

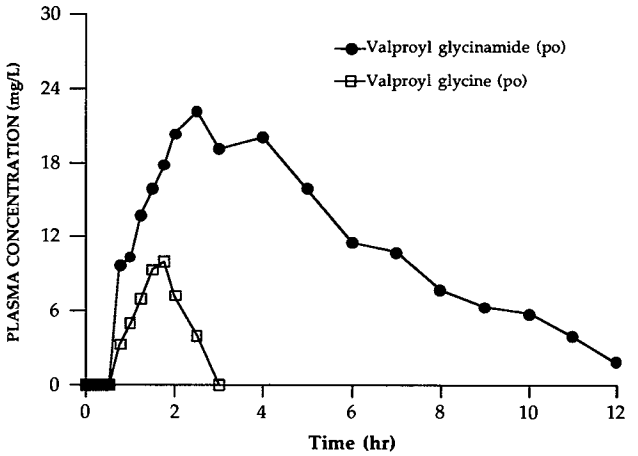


Fig. 3. Mean plasma levels of valproyl glycinamide and valproyl glycine following their oral administration (556 mg and 558, respectively) to six and four dogs, respectively.

Partition, Stability, Water Solubility and Protein Binding Studies

The blood-plasma concentration ratio (26,27) of compound III (partition study) was carried out at room temperature (25°C) by spiking known amounts of the compound in three samples of fresh blood taken from a dog prior to drug administration. Valproyl glycinamide concentrations were 5, 10 and 20 mg/L. Each blood sample was centrifuged immediately after spiking and the separation of the plasma was carried out according to the procedure mentioned above. Plasma levels of valproyl glycinamide were determined by HPLC.

A blood stability study of compounds II-V was carried out by incubating 400 μg of each compound in 30 ml of dog blood (placed in heparinized test tubes) at 37°C with continuous shaking. Blood samples (2 ml) were then collected at

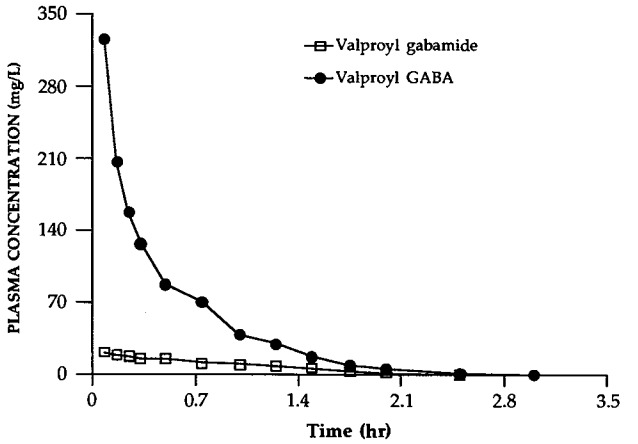


Fig. 4. Mean plasma levels of valproyl GABA and valproyl gabamide following their iv administration (636 mg and 633 mg, respectively) to six dogs.

Table I. Mean (±SD) Pharmacokinetic Parameters of Valproyl Glycine (II), Valproyl Glycinamide (III), Valproyl GABA (IV) and Valproyl Gabamide (V) Obtained Following iv Administration (of a Dose Equivalent to 400 mg VPA) to Dogs

Dogs	Valproyl glycine	Valproyl glycinamide	Valproyl GABA	Valproyl gabamide
t1/2β (hr)	0.35 ± 0.07	2.7 ± 0.5	0.32 ± 0.08	0.44 ± 0.12
AUC (mg/hr)	18 ± 6	186 ± 71	136 ± 62	21 ± 11
CL (L/hr)	25 ± 6	2.9 ± 0.8	5.3 ± 1.7	33 ± 10
V_{ss} (L)	12 ± 3	12 ± 3	3.0 ± 1.3	28 ± 8
Vβ (L)	11 ± 2	11 ± 3	2.4 ± 0.8	21 ± 8
MRT (hr)	0.51 ± 0.1	4.6 ± 0.7	0.55 ± 0.08	0.84 ± 0.11
fe (%)[a]	49 ± 5	—	35 ± 5	—
M/D (%)[b]	—	34 ± 12	—	26 ± 3
fm (%)	—	70 ± 17	—	77 ± 19
t1/2 urine (hr)[c]	—	2.5 ± 0.6	0.61 ± 0.35	1.2 ± 0.3

[a] All pharmacokinetic parameters calculated from urine data are mean of four dogs.
[b] The fraction (percent) of valproyl glycinamide or valproyl gabamide (or the ratio of the cumulative amount of urinary metabolite and the dose) excreted in the urine as valproyl glycine or valproyl GABA respectively.
[c] Half life calculated from a sigma minus plot of the urinary data of valproyl glycine or valproyl GABA.

the following times: 0, 0.5, 1, 2, 3, 4, 5, 6 and 7 hr. Plasma was immediately separated and the amide concentration in the plasma assayed by HPLC.

Protein binding of compounds II-V was evaluated using the ultrafiltration method. This was carried out in four plasma samples of compounds II-V. The compound levels in the filtrate (plasma water) were assayed by HPLC. The free fraction (fu) of the amides was calculated from the quotient of the drug concentration in the filtrate to the initial drug concentration in the plasma.

The water solubility of compounds II-V was determined by stirring 40 mg of the appropriate compound into 3 ml of distilled water for 2 hr. At the end of the 2 hr period, the sample was centrifuged and 3-μl aliquots were taken for HPLC assay.

RESULTS

Stability studies showed that compounds II-V were stable in dog blood for 8 hr at physiological conditions. The protein binding and water solubility data of compounds II-V were as follows: valproyl glycine: fu 46±9%, solubility 6.8 mg/ml; valproyl glycinamide: fu = 39±2%; solubility 3.8 mg/ml; valproyl GABA: fu 27±5%, solubility 7.3 mg/ml; valproyl gabamide: 68±4%, solubility 6.5 mg/ml. Valproyl glycinamide was evenly distributed between blood and plasma with a blood to plasma ratio of 0.83±0.03.

The mean plasma levels of compounds II-V are presented in Figs 2-4, respectively. Following the administration of the four investigated compounds, no VPA was found either in the plasma or in the urine. The only metabolites found and quantified in the urine were valproyl glycine and valproyl GABA following the administration of compounds III and V, respectively. Tables I and II summarize the mean pharmacokinetic parameters of compounds II-V obtained following their iv and oral administration to dogs.

In phase I of the anticonvulsant screening project of the NIH Epilepsy Branch, out of the four investigated compounds only valproyl glycinamide (III) demonstrated qualitative anticonvulsant activity in mice. Subsequently, we decided to test valproyl glycinamide (III) in phases II (mice) and VI (rats) of the NIH-anticonvulsant screening project, in order to determine its ED_{50} and TD_{50} values, as well as its protective indices - PI (the ratio between the TD_{50} and ED_{50} values). The pharmacodynamic (anticonvulsant activity and neurotoxicity) results of valproyl glycinamide in comparison to VPA (I) and VPD (VI) are shown in Table III.

DISCUSSION

Pharmacokinetic analysis showed that of the four investigated compounds valproyl glycinamide (III) had the lowest clearance value. Its clearance was one tenth of that of its corresponding acid, valproyl glycine (II) and of its analogous compound valproyl gabamide (V). This low clearance value leads to the fact that valproyl glycinamide had a mean half life of 3 hours, which was about ten times longer than that of the other three investigated compounds. Valproyl GABA had a clearance value, similar to that of valproyl glycinamide, however it has a short half life of 0.32 hr, due to its small volume of distribution. A comparison between valproyl glycinamide and valproyl glycine showed that these two compounds had an identical volume of distribution and therefore the ten fold difference in clearance is reflected in a similar difference in the half life of these compounds. A comparison between valproyl GABA and valproyl gabamide showed a different pattern than that of the two glycine de-

Table II. Mean (±SD) Pharmacokinetic Parameters of Valproyl Glycinamide and Valproyl Glycine Obtained Following Oral Administration (556 mg—equivalent to 400 mg of VPA) to Six Dogs and Four Dogs, Respectively

Pharmacokinetic parameter	Valproyl glycinamide	Valproyl glycine
t 1/2β (hr)	2.9 ± 0.9	0.7 ± 0.35
AUC (mg/L hr)	147 ± 23	19 ± 4
Cmax (mg/L)	25 ± 3	10 ± 3
tmax (hr)	2.8 ± 1.1	1.7 ± 0.2
MRT (hr)	5.8 ± 0.9	2.5 ± 0.3
F (%)	95 ± 34	107 ± 41
CL/F (L/hr)	3.4 ± 0.9	31 ± 7

Table III. Anticonvulsant Activity Data of Valproyl Glycinamide (III), Valproic Acid (VPA), and Valpromide (VPD) Obtained Following ip Administration to Mice and Oral Administration to Rats and Mice[a]

	Mice			Rats		
	VPA	VPD	III	VPA	VPD	III
MES, ED_{50} (mg/kg)	200	56	152	490	32	73
sc Met, ED_{50} (mg/kg)	146	55	127	180	59	>250
Neurotoxicity, TD_{50} (mg/kg)	283	81	369	200	87	>1000
PI, MES	1.4	1.4	2.4	0.6	2.7	13.7
PI, sc Met	1.9	1.5	2.9	1.6	1.5	—

[a] MES = Maximal electroshock, sc Met = Chemically induced shock obtained following subcutaneous injection of metrazol, ED_{50} = Effective dose in 50% of the animals, TD_{50} = Neurotoxic dose in 50% of the tested animals, PI = Protective index—The ratio of the TD_{50} to the ED_{50}.

rivatives. Valproyl gabamide had larger clearance and volume of distribution values than valproyl GABA. Consequently the difference in half life of these two compounds was minor with both compounds having a short half life of less than half an hour. Urine analysis showed that most of the amide (valproyl glycinamide or valproyl gabamide) was biotransformed to its corresponding acid (fm = 70% for compound III or fm = 77% for compound V), while 35% and 49% of acid was excreted intact. No metabolic cleavage of each of the four investigated compounds, to the two components of the conjugation products (VPA and the neuroinhibitory transmitters, GABA or glycine), were observed in this study. Thus, it can be concluded that in dogs, none of the four investigated compounds serve as a prodrug or a chemical drug delivery system for VPA and glycine or GABA.

Anticonvulsant testing showed that of the four investigated compounds only valproyl glycinamide (III) demonstrated anticonvulsant activity. This compound was more potent than VPA in mice and rats (at the MES test) and showed a better margin between activity and neurotoxicity. Thus, the anticonvulsant profile of valproyl glycinamide was different than that of VPA, supporting the pharmacokinetics results, that in this study, valproyl glycinamide did not appear to be a chemical delivery system of glycine.

In the literature there are several reports of GABA and glycine derivatives which possess anticonvulsant activity. In contrast to our data Blowmick et al., showed that valproyl GABA (IV) was as effective as VPA (I) in mice, with no sedative side effects (17). Silverman et al. developed a series of 3-alkyl GABA analogues of which S(+)3-isobutyl GABA was found to be the most potent anticonvulsant compound (28,29). Vamvakides et al. developed conjugation products between linolenic acid and GABA or glycine which demonstrated some anticonvulsant activity in rats in the sc Met test (16,30,31). Recently O'Brien et al. found that α-methyl milacemide demonstrated anticonvulsant properties despite the fact that it was not metabolized to glycinamide (32). These findings cast doubt on the importance of the oxidative cleavage of milacemide to glycinamide, as a major factor in the anticonvulsant activity of the compound. Our study also showed that valproyl glycinamide is active without serving as a chemical delivery system to glycine or glycinamide.

In spite of the different animal species used for pharmacokinetic-pharmacodynamic analysis, this study showed a good pharmacokinetic-pharmacodynamic (anticonvulsant activity) correlation. The better pharmacokinetic profile of valproyl glycinamide (low CL, V_{ss} - 0.6 L/Kg and long $t_{1/2}$- 3.0 hours) over the other three investigated compounds may explain its anticonvulsant activity. The current study showed four conjugation products between VPA and the neuroinhibitory transmitters GABA and glycine, which did not operate as chemical delivery systems for VPA and glycine or GABA. Out of the four investigated compounds only VPA glycinamide showed a good and promising anticonvulsant profile in the classical animal models for antiepileptic screening, due to its better pharmacokinetic profile.

ACKNOWLEDGMENTS

The research was supported by the Teva Yissum Fund. This work is abstracted from the Ph.D. thesis of Dr. Salim Hadad, in partial fulfillment of the Ph.D. degree requirement of the Hebrew University of Jerusalem. The authors thank Dr. Harvey J. Kupferberg and Mr. James Stables of the NIH Epilepsy Branch for screening the compounds in their anticonvulsant screening project. Our thanks also to Mr. Kadry Basheir for his skillful technical assistance.

REFERENCES

1. F.E. Dreifus. New anticonvulsant drugs in "Epilepsy, Progress in Treatment". M Dam, S.I. Johannessen, B. Nilsson and M. Sillapaa (eds.), Wiley & Sons, NY (1987) pp. 247-256.
2. E. Roberts, T.N. Chase, D.B. Tower (eds.). "GABA in Nervous System Function", Raven Press, NY (1976).
3. E.J. Hammond and B. Wilder. A gamma-vinyl GABA: as new antiepileptic drug. Clin. Neuropharmacol., *8*, 1-12 (1985).
4. D. Chadwick (ed). New Trends in Epilepsy Management: The Role of Gabapentin. Royal Society of Medicine Services Ltd., London, 1993.
5. S.M. Grant and R.C. Heel. Vigabatrin - A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in epilepsy and disorders of motor control. Drugs, *41*, 889-926 (1991).
6. J. Roba, R. Cavalier, A. Cordi, H. Gorissen, M. Herin, P. Janssens de Varebeke, C. Onkelinx, M. Remacle and W. van Dorsser. Milacemide in "New Anticonvulsant Drug", B.S. Meldrum and R.J. Porter (eds.), John Libby, London (1986) pp. 179-190.
7. D.M. Lambert, J.H. Poupaert, J-M. Maloteaux and P. Dumont. Anticonvulsant activities of N-benzloxycarbonylglycine after parenteral administration. Neuropharmacol. Neurotoxicol. *5*, 777-780 (1994).
8. J. Liu, N. Seiler, C. Marescaux, A. Depaulis and M. Vergns.

Potentiation of γ-vinyl GABA (vigabatrin) effects by glycine. Eur. J. Pharmacol. *182,* 109-115 (1990).
9. E. Toth and A. Lajtha. Glycine potentiates the action of some anticonvulsant drugs in some seizure models. Neurochem. Res. *8,* 1711-1718 (1984).
10. J.D. Wood, P. Krogsgaard-Larsen and A. Schousboe. Amplification by glycine of the effect of the GABA transport inhibitor THPO on synaptosomal GABA level. Neurochem. Res. *13,* 917-921 (1988).
11. N. Seiler and S. Sarhan. Synergistic anticonvulsant effects of a GABA agonist and glycine. Gen Pharmacol. *15,* 367-369 (1984).
12. R.H. Levy. Valproate: Modern perspectives. Epilepsia (Suppl. 1) S1-S77 (1984).
13. R.H. Levy and D.D. Shen. Valproate absorption, distribution and excretion. In: R.H. Levy, R.H. Mattson and B.S. Meldrum (Eds) Antiepileptic Drugs, 4th ed. Raven Press, New York, in press, 1995.
14. G. Zaccara, A. Messori and F. Moroni. Clinical pharmacokinetics of valproic acid-1988. Clin Pharmacokinet. *15,* 367-389 (1988).
15. R.G. Granneman, S-I. Wang, J.M. Machinist and J.W. Kesterson. Aspects of metabolism of valproic acid. Xinobiotica *14,* 375-387 (1984).
16. A. Vamvakides and N. Kolokouris. Effect synergique du GABA et de la glycine dans l'antagonisme des convulsions du pentetrazole chez le rat. Etude du valpromide de glycine. Ann. Pharm. Fr. *44,* 501-508 (1986).
17. S. Blowmick, M. Pal and S.P. Pal. N-valproyl GABA as a potential anticonvulsant agent. Med. Sci. Res. *17,* 491-492 (1989).
18. H. Nau, R-S. Siegbert and K. Ebley. Valproic acid - induced neural table defects in mouse and humans: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanism. Pharmacol. Toxicol. *69,* 310-321 (1991).
19. H. Nau and G. Hendrickx. Valproic acid teratogenesis. Atlas Sci. Pharmacol. 52-56 (1987).
20. R.J. Porter, J.J. Cereghino, G.D. Gladding, B.J. Hessie, H.J. Kupferberg, B. Scoville and B.G. White. Antiepileptic Drug Development Program. Cliv. Cin. Quat. *51,* 293-305 (1984).
21. M. Bialer, M. Friedman and J. Dubrovsky. A rapid GLC assay for monitoring valproic acid and valpromide in plasma. J. Pharm. Sci. *73,* 991-993 (1984).
22. M. Gibaldi and D. Perrier. Pharmacokinetics, Ed. 2, Marcel Dekker, New York 1982, pp. 445-449.
23. L.Z. Benet and R.L. Galeazzi. Non-compartmental determination of steady-state volume of distribution. J. Pharm. Sci. *68,* 1071-1074 (1979).
24. K. Yamaoka, T. Nakagawa and T. Uno. Statistical moments in pharmacokinetics. J. Pharmacokinet. Biopharm. *6,* 547-558, 1978.
25. K. Yamaoka. Methods for Pharmacokinetic Analysis for Personal Computers, Ed. 2, Nanko-D Ltd., Tokyo, 1986, pp. 145-175.
26. M. Rowland and T. Tozer. Clin. Pharmacokinet. Ed. 2, Lea and Febiger, Philadelphia, 1989, pp. 151-152.
27. M. Gibaldi and D. Perrier. Pharmacokinetics, Ed. 2, Marcel Dekker, New York, 1982, pp. 327-330.
28. R.B. Silverman, R. Andruszkiewicz, S.M. Nanavati, C.P. Taylor and M.G. Vartanian. 3-Alkyl-4-aminobutyric acids: The first class of anticonvulsant agents that activates L-glutamic acid decarboxylase. J. Med. Chem. *34,* 2295-2298 (1991).
29. C.P. Taylor, M.G. Vartanian, P-W. Yuen, C. Bigge, N. Suman-Chauban and D.R. Hill. Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vivo binding at a novel site labeled by tritiated gabapentin. Epileps. Res. *14,* 11-15 (1993).
30. A. Vamvakides, Z. Papadopoulou-Daifotis and J. Noras. Etudes in vivo et in vitro avec une nouvelle drogue GABA-ergique (Gabalid) et son impact sur la liberation de la dopamine cerebale. J. Pharmacol. (Paris) *14,* 248-249 (1983).
31. A. Vamvakides. Synthese et etude pharmacologique du linoleamide de glycine. Ann. Pharm. Fr. *44,* 145-155 (1986).
32. E.M. O'Brien, K.T. Tipton, M.S. Benedetti, A. Bonsignori, P. Marrari and P. Dostert. Is the oxidation of milacemide by monoamine oxidase a major factor in its anticonvulsant action? Biochem. Pharmacol. *41,* 1731-1737 (1991).