ELSEVIER

European Journal of Pharmacology 294 (1995) 541–546

ejp

# Differential effects of phenytoin and sodium valproate on seizure-induced changes in γ-aminobutyric acid and glutamate release in vivo

Helen L. Rowley [a,*], Charles A. Marsden [a], Keith F. Martin [b]

[a] *Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK*
[b] *Knoll Pharmaceuticals Research Department, Nottingham, NG2 3AA, UK*

Received 2 May 1995; revised 15 August 1995; accepted 12 September 1995

**Abstract**

The effects of intraperitoneal administration of the anticonvulsants phenytoin and sodium valproate were compared with ethosuximide on maximal electroshock seizure-related changes in rat hippocampal γ-aminobutyric acid (GABA) and glutamate release in vivo as measured by microdialysis. There were immediate increases in GABA and glutamate in the 5 min post-ictal period, followed by a sustained reduction in GABA levels. Glutamate levels, however, were subsequently reduced until 20 min post-ictal before gradually increasing above basal. All animals displayed tonic hind-limb extension that was blocked by phenytoin (20 mg/kg) and sodium valproate (400 mg/kg) but not ethosuximide (150 mg/kg). Phenytoin attenuated the immediate post-ictal increase observed in glutamate whilst sodium valproate enhanced GABA release and prevented its secondary post-ictal inhibition. Ethosuximide was without effect on the post-ictal changes. These are the first data to show detailed seizure-induced amino acid changes and the in vivo effects of anticonvulsants on them in the seizure model.

*Keywords:* Anticonvulsant; Generalised seizure; Amino acid; Microdialysis

## 1. Introduction

Phenytoin and sodium valproate are widely used anticonvulsants to treat a range of epileptic seizure types. In animal models they are particularly effective against the maximal electroshock seizure model of generalised tonic-clonic seizures (Löscher and Schmidt, 1988; Swinyard et al., 1989). Research has led to the belief that phenytoin is effective through an action on voltage-dependent $Na^+$ and $Ca^{2+}$ channels, as reviewed recently by Rogawski and Porter (1990). There is also evidence that phenytoin blocks neuronal excitatory transmitter release in vitro (Crowder and Bradford, 1987), as well as conflicting reports that it can enhance γ-aminobutyric acid (GABA)-mediated synaptic inhibition or responses to exogenously applied GABA (Hershkowitz and Ayala, 1981; McLean and Macdonald, 1983).

Similar mechanisms of action are proposed for sodium valproate, although there is much stronger evidence for a GABA-mediated mechanism. Administration of sodium valproate causes an increase in GABA concentrations in whole brain (Godin et al., 1969), synaptosomes (Löscher, 1981) and hippocampal release in vivo (Biggs et al., 1992). Significantly, patients being treated with clinically effective doses of sodium valproate have shown an increase in cerebrospinal fluid and plasma levels of GABA (Löscher and Schmidt, 1980, 1981). Since sodium valproate appears to preferentially enhance GABA turnover in the neuronal compartment (Iadarola and Gale, 1981), its effect on GABA release may be an important part of its anticonvulsant action. However, as with phenytoin, sodium valproate limits the firing of voltage-dependent action potentials at high frequency in cultured central nervous system neurones (McLean and Macdonald, 1986), at concentrations that are clinically effective in

* Corresponding author. Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH, UK. Tel.: 0115 970 9480; fax: 0115 970 9259.

0014-2999/95/$09.50 
*SSDI* 0014-2999(95)00589-7

humans, suggesting that this may also account for its anticonvulsant action.

We have previously shown the effects of tonic-clonic seizures on hippocampal GABA and glutamate release, in vivo (Rowley et al., 1995a). However, due to the relatively long sampling time employed (20 min), it was not possible to determine the immediate post-ictal changes in the release of these amino acid neurotransmitters. Therefore, the present study was designed to investigate, with improved temporal resolution, the generalised seizure-induced amino acid changes observed in the post-ictal period by using a shorter sampling time (5 min) and to compare the effects of the anticonvulsants, phenytoin and valproate, with ethosuximide on these changes, using the maximal electroshock seizure model.

## 2. Materials and methods

### 2.1. Surgery and microdialysis

Male, Lister hooded rats (250–350 g; Olac) were anaesthetised with Equithesin (400 mg/kg i.p.) and a concentric microdialysis probe with 4 mm membrane, constructed as previously described (Wright et al., 1992), was stereotaxically implanted into the ventral hippocampus (coordinates: P: −4.8 mm; L: −5.0 mm relative to bregma; V: −7.7 mm relative to the dural surface, according to Paxinos and Watson, 1986). Following surgery, animals were returned to a home cage and allowed a recovery period of at least 20 h with food and water available ad libitum. Probes were continuously perfused with artificial cerebrospinal fluid with the following composition: 140 mM NaCl; 3.0 mM KCl; 1.2 mM $Na_2HPO_4$; 0.27 mM $NaH_2PO_4$; 1.0 mM $MgCl_2$; 1.2 mM $CaCl_2$; 7.2 mM glucose; pH 7.4. A flow rate of 0.8 μl/min was used overnight and this was increased to the working rate of 2.0 μl/min, 1.5 h before the onset of sample collection. Samples were collected at 5 min intervals and collection began 20 min prior to administration of maximal electroshock seizures. Dialysates were analysed for GABA and glutamate content using high-performance liquid chromatography with electrochemical detection, as previously described (Rowley et al., 1995b).

### 2.2. Induction of generalised seizures

Maximal electroshock seizures were administered to conscious animals via ear-clip electrodes. Stimulus parameters were 200 V for 2 s which induced tonic hind-limb extension followed by clonic seizures in all untreated animals and saline-treated controls. Sham controls were ear-clipped only. Dialysate samples were collected for 40 min following the seizure.

### 2.3. Drug treatment

Injections of either saline, phenytoin (20 mg/kg; Parke Davis), sodium valproate (400 mg/kg; Knoll Pharmaceuticals) or ethosuximide (150 mg/kg; Sigma), were administered intraperitoneally in a volume of 1 ml/kg of body weight, 60 min prior to maximal electroshock seizures. Doses of phenytoin and sodium valproate were chosen following preliminary studies, as the minimum dose required to block tonic hind-limb extension in all animals. At these doses clonic seizures were still present.

### 2.4. Statistical analysis

In each experiment, the levels of GABA and glutamate in the four dialysis samples collected prior to the maximal electroshock seizure, were averaged and used as a measure of the basal levels. All levels are corrected for in vitro recovery of 17.8 ± 2.2% for GABA and 13.8 ± 0.9% for glutamate. All values are means ± S.E.M. for $n = 6$ animals. Statistical analysis of GABA and glutamate levels between maximal electroshock seizure and sham groups was carried out using one- and two-way analysis of variance (ANOVA), with post-hoc Dunnett's $t$-test for multiple comparisons. Statistical comparisons between drug treatments and saline, in animals receiving maximal electroshock seizures, were made using a Mann-Whitney $U$-test. A $P$ value of $< 0.05$ was considered significant.

## 3. Results

### 3.1. Effect of maximal electroshock in untreated animals

A single maximal electroshock seizure produced an immediate tonic hind-limb extension of 5–10 s duration followed by clonic seizures, lasting up to 20 s. Following a maximal electroshock seizure, there was a significant ($P < 0.01$; ANOVA with post-hoc Dunnett's $t$-test) increase of 102 ± 16% in basal GABA levels (0.21 ± 0.01 pmol/20 μl), in the post-ictal 5 min period, compared to sham controls (Fig. 1A). This was followed by a secondary, sustained reduction in levels to 0.11 ± 0.01 pmol/20 μl, 10 min post-ictally, compared to sham controls and levels remained at this depressed level until the end of the experiment, 40 min after the seizure.

Basal glutamate levels (0.61 ± 0.01 pmol/20 μl) were also significantly ($P < 0.01$; ANOVA with post-hoc Dunnett's $t$-test) increased by 26 ± 6%, in the first 5 min post-ictal sample, compared to sham controls (Fig. 1B). Levels then decreased over the next 15 min to 0.52 ± 0.02 pmol/20 μl, before a gradual, secondary increase of 53 ± 8% to 0.93 ± 0.03 pmol/20 μl, 40 min following the generalised seizure.

### 3.2. Modulation by anticonvulsant treatment

Pretreatment with phenytoin did not alter basal GABA ($0.21 \pm 0.01$ pmol/20 $\mu$l) or glutamate ($0.58 \pm 0.02$ pmol/20 $\mu$l) levels and there were no significant effects on GABA release following a maximal electroshock seizure (Fig. 2A). In contrast, the increase ($30 \pm 8\%$) in glutamate levels in saline controls in the first 5 min post-ictal sample was significantly ($P < 0.05$; Mann-Whitney $U$-test) attenuated by $46 \pm 9\%$ in phenytoin-treated animals (Fig. 2B). There were no differences in the secondary post-ictal changes in glutamate.

Following treatment with sodium valproate, basal GABA levels ($0.21 \pm 0.01$ pmol/20 $\mu$l) were significantly ($P < 0.001$; ANOVA with repeated measures) increased ($50 \pm 9\%$) to $0.31 \pm 0.01$ pmol/20 $\mu$l (Fig. 3A). Following a maximal electroshock seizure, the immediate post-ictal rise in GABA was unaltered but the secondary, sustained reduction was prevented compared to saline controls (Fig. 3A). Neither basal glutamate levels ($0.60 \pm 0.02$ pmol/20 $\mu$l) nor the seizure-induced changes up to 25 min following the seizure were altered (Fig. 3B). However, the secondary increase in glutamate release observed from 25 min onwards was prevented in sodium valproate-treated animals (Fig. 3B).

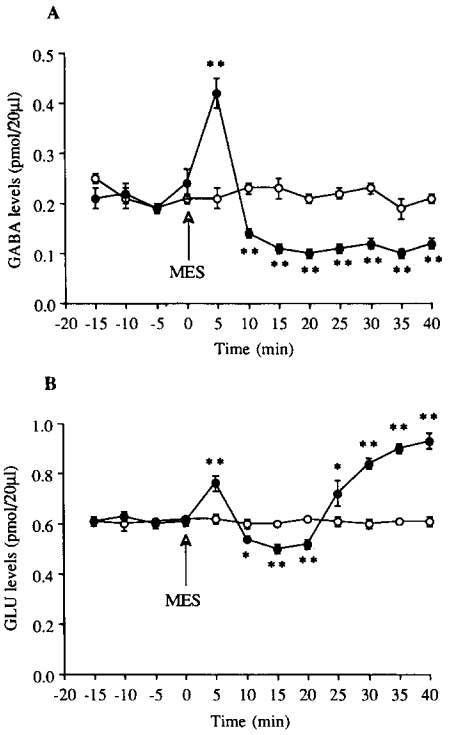


Fig. 1. Effects of a single maximal electroshock seizure on basal GABA (A) and glutamate (B) dialysate levels in the ventral hippocampus of a freely moving rat. Administration of maximal electroshock seizure (filled circles) or sham (open circles) is indicated by the vertical arrow. Each data point represents the mean ± S.E.M. for six animals. * $P < 0.05$; ** $P < 0.01$ compared to sham control according to ANOVA with post-hoc Dunnett's $t$-test for multiple comparisons.

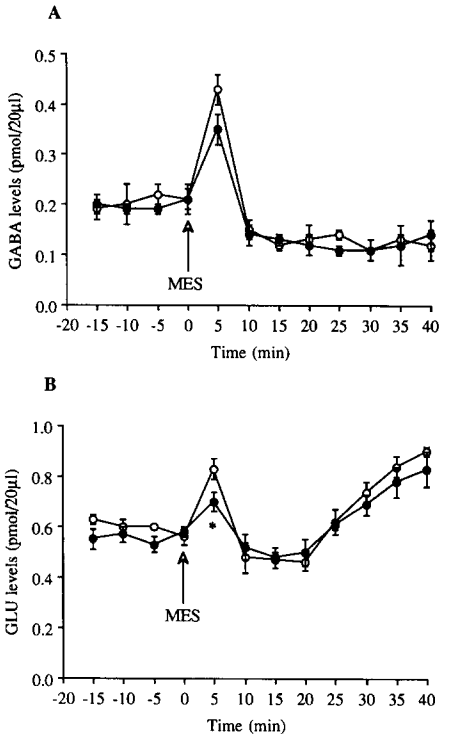


Fig. 2. Effects of phenytoin (20 mg/kg i.p.) on maximal electroshock seizure-induced changes in extracellular GABA (A) and glutamate (B) levels. Each data point represents mean ± S.E.M. ($n = 6$). Maximal electroshock seizure is indicated by the vertical arrow and for clarity sham controls are not shown. Phenytoin (filled circles) or saline (open circles) were administered 60 min prior to maximal electroshock seizure. * $P < 0.05$ compared with saline controls (Mann-Whitney $U$-test).

Maximal electroshock seizure-induced changes in both GABA (Fig. 4A) and glutamate (Fig. 4B) levels were not affected by pretreatment with ethosuximide when compared to saline controls. All animals receiving ethosuximide followed by maximal electroshock seizure exhibited tonic hind-limb extension followed by clonic activity.

## 4. Discussion

These data confirm our previous observations that in the 20–40 min period following a tonic-clonic seizure, neuronal release of GABA is decreased and glutamate increased (Rowley et al., 1995a). The present data extend these findings by demonstrating that shorter sampling times allow a more detailed view of the seizure-induced changes in amino acid neurotransmitter release since in the first 5 min post-ictal sample,

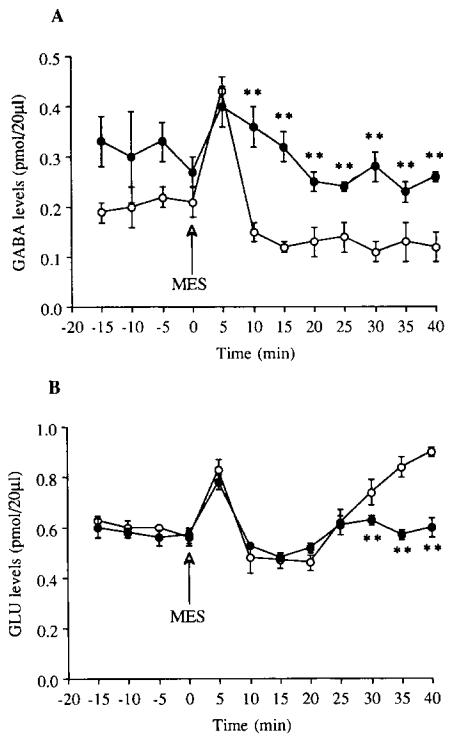


Fig. 3. Effects of sodium valproate (400 mg/kg i.p.) on extracellular GABA (*A*) and glutamate (B) levels following maximal electroshock seizure. Each data point represents mean ± S.E.M. ($n = 6$). Sodium valproate (filled circles) or saline (open circles) were administered 60 min prior to maximal electroshock seizure, which is indicated by the vertical arrow. Sham controls are not shown. ** $P < 0.01$ compared with saline controls (Mann-Whitney *U*-test).

a marked increase in both GABA and glutamate levels was observed. This was masked in our earlier study by the secondary changes occurring in the following 15 min.

There appear to be three phases to the functional post-ictal changes. Firstly, an immediate increase in both GABA and glutamate which is followed by the secondary, inhibition of GABA and glutamate release. Finally, the decrease in GABA is sustained and this is accompanied by an increase in glutamate release. Previous studies have reported immediate maximal electroshock seizure-induced increases in monoamine neurotransmitters in vivo over the 20–30 min post-ictal period (Glue et al., 1990; Nomikos et al., 1991; Zis et al., 1992) but this is the first study to report post-ictal changes in extracellular levels of amino acids in vivo, using the maximal electroshock seizure model. The initial rise in GABA release is in agreement with the results of Bowdler and Green (1982), who showed that ex vivo tissue levels of GABA were rapidly increased following a maximal electroshock seizure. However, these authors also found that tissue GABA levels remained elevated for 120 min following the seizure. Although our in vivo release data, in showing a subsequent secondary, sustained decrease in GABA, appear to conflict with this observation, they are in agreement with the in vitro release data subsequently reported by Green et al. (1987). These authors reported that GABA release from rat cortical, hippocampal and striatal slices was inhibited for up to 30 min following a maximal electroshock seizure. These data demonstrate the importance of measuring extracellular levels in vivo to determine the functional effects of maximal electroshock seizures on neurotransmission. Interestingly, the immediate post-ictal increase in glutamate levels agrees with the microdialysis data obtained from human patients with complex partial epilepsy (During and Spencer, 1993). An increase in glutamate levels was observed 1.5 min prior to seizure-onset and levels remained elevated for up to 16 min after the seizure.

Treatment with either phenytoin or sodium valproate both prevented tonic hind-limb extension but had no effect on the clonic phase. Ethosuximide had no effect on either the tonic or clonic phases. These findings are in agreement with previous studies (Swinyard et al., 1989). The effects of phenytoin and sodium valproate on GABA and glutamate release, however, were markedly different. Phenytoin exerts its

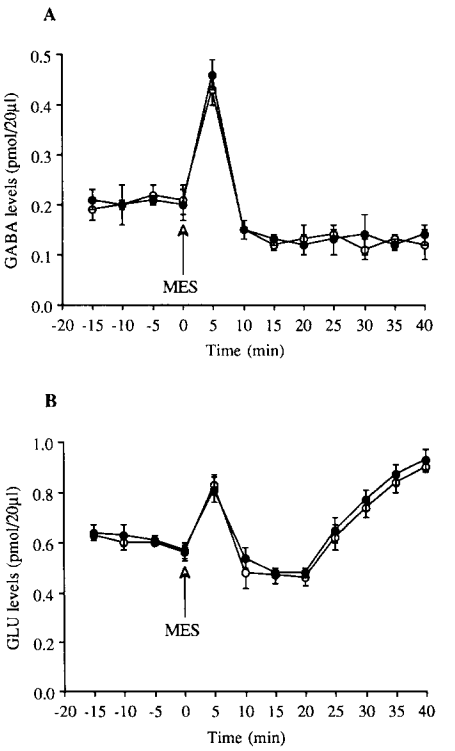


Fig. 4. Effects of ethosuximide (150 mg/kg i.p.) on maximal electroshock seizure-induced changes in GABA (A) and glutamate (B) levels. Each point represents mean ± S.E.M. ($n = 6$). Maximal electroshock seizure is indicated by the vertical arrow and was administered 60 min post-treatment with either ethosuximide (filled circles) or saline (open circles).

anticonvulsant action against generalised tonic seizures by preventing seizure spread. Whilst the temporal resolution of the present experiments does not allow correlation of glutamate release directly with the initiation of the tonic hind-limb extension phase of maximal electroshock seizure, it is tempting to speculate that at least part of phenytoin's efficacy against this type of seizure is mediated through its suppression of the immediate post-ictal increase in glutamate levels. Phenytoin is known to block voltage-dependent $Na^+$ channels in a frequency-dependent manner. Since these channels are responsible for action potential upstroke, this may explain phenytoin's ability to prevent the propagation of high-frequency firing during epileptic activity but not cause general neuronal depression. As the post-ictal increase in GABA was not attenuated by phenytoin, it appears that this mechanism of action favours $Na^+$ channels on excitatory neurones. It has previously been shown that phenytoin depresses excitatory amino acid neurotransmitter release (Gage et al., 1980), however this was believed to be due to presynaptic inhibition of $Ca^{2+}$ entry into nerve terminals (Crowder and Bradford, 1987), as opposed to an action on $Na^+$ channels.

Sodium valproate, in contrast, enhanced GABA levels which is in agreement with previous in vivo observations (Biggs et al., 1992). The mechanism by which this was achieved is not completely understood although other studies have suggested that GABA synthesis is increased. Thus, the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis, was found to increase following sodium valproate administration (Phillips and Fowler, 1982) and the increase in brain GABA levels paralleled the increase in enzyme activity (Nau and Löscher, 1982). Also, sodium valproate has been shown to inhibit enzymes involved in GABA degeneration (Godin et al., 1969; Van der Laan et al., 1979). It would appear, therefore, that enhanced GABA release observed in our experiments may be due to these mechanisms.

We have proposed that the secondary increase in glutamate levels observed from 25 min post-seizure, is due to the inhibition of GABA release and that, in the hippocampus, GABA exerts a tonic inhibition on glutamate release (Rowley et al., 1995a). In previous experiments we have shown that by preventing the sustained decrease in GABA levels by local perfusion with the GABA uptake inhibitor 1-(2-(((diphenylmethylene)-amino)oxy)-ethyl)-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid hydrochloride (NNC-711), the secondary increase in glutamate levels is also prevented (Rowley et al., 1995a). Thus, in the present study, abolition of the secondary increase in glutamate release is most likely due to the prevention of the reduction in GABA levels by sodium valproate. Since increases in glutamate release in epilepsy are known to result in neuronal damage (Meldrum, 1993), prevention of the secondary, prolonged increase in glutamate levels by sodium valproate may also protect against seizure-induced neuronal damage.

Ethosuximide is ineffective in the maximal electroshock seizure model but highly efficacious in the treatment of absence seizures. In the present study, ethosuximide failed to prevent tonic hind-limb extension or clonic seizure activity and did not alter the post-ictal changes in either GABA or glutamate. Presumably, since it does not limit the high-frequency repetitive firing of neurones at clinically relevant concentrations (McLean and Macdonald, 1986), ethosuximide lacks a phenytoin-like effect on voltage-dependent $Na^+$ channels. This, along with its lack of effect on the GABAergic system, may account for its ineffectiveness in the maximal electroshock seizure model, based on the present data obtained with phenytoin and sodium valproate.

To conclude, the data obtained from these experiments demonstrate that following a generalised tonic-clonic seizure of 15–20 s duration, there were prolonged changes in the release of both GABA and glutamate, which were observed up to 40 min after the seizure. The duration of the ictal event was a maximum of 30 s duration but the microdialysis sampling time was 5 min. Therefore, in this study, it was not possible to distinguish between ictal and post-ictal changes in amino acid neurotransmitter release. In order to investigate the effects of anticonvulsants on pre-ictal, ictal and post-ictal release it will be necessary to significantly improve the temporal resolution of the microdialysis technique. Nevertheless, the present data provide valuable information on the prolonged changes in GABA and glutamate that occur following generalised seizures.

This study demonstrated that pretreatment with the anticonvulsants phenytoin and sodium valproate both prevented tonic hind-limb extension. The in vivo neurochemical evidence supports the view that they differentially affected the post seizure-induced changes in GABA and glutamate release. Phenytoin prevented generalised tonic seizures and also attenuated the post-ictal increase in glutamate, whilst sodium valproate's anticonvulsant actions were associated with enhanced GABA-mediated inhibition. Since neither drug modified clonic seizure activity, it is unlikely that these differences in neurochemical response were due to altered clonic activity. Therefore, future anticonvulsant development concerned with amino acid neurotransmitters, should aim to target multiple transmitter systems and also to prevent long term post-ictal changes, as described by these data and our previous study (Rowley et al., 1995a).

## Acknowledgements

Financial support from the Biotechnology and Biological Sciences Research Council and Knoll Pharmaceuticals is acknowledged, with whom H.L.R. holds a CASE Award.

## References

Biggs, C.S., B.R. Pearce, C.J. Fowler and P.S. Whitton, 1992, The effect of sodium valproate on extracellular GABA and other amino acids in the rat ventral hippocampus: an in vivo microdialysis study, Brain Res. 594, 138.

Bowdler, J.M. and A.R. Green, 1982, Regional rat brain benzodiazepine receptor number and γ-aminobutyric acid concentration following a convulsion, Br. J. Pharmacol. 76, 291.

Crowder, J.M. and H.F. Bradford, 1987, Common anticonvulsants inhibit $Ca^{2+}$ uptake and amino acid neurotransmitter release in vitro, Epilepsia 28, 378.

During, M.J. and D.D. Spencer, 1993, Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain, The Lancet 341, 1607.

Gage, P.W., M. Lonergan and T.A. Torda, 1980, Presynaptic and postsynaptic depressant effects of phenytoin sodium at the neuromuscular junction, Br. J. Pharmacol. 69, 119.

Glue, P.M.J. Costello, A. Pert, A. Mele and D.J. Nutt, 1990, Regional neurotransmitter responses after acute and chronic electroconvulsive shock, Psychopharmacology 100, 60.

Godin, Y., L. Heiner, J. Mark and P. Mandel, 1969, Effects of di-*n*-propylacetate, an anticonvulsive compound, on GABA metabolism, J. Neurochem. 16, 869.

Green, A.R., M.C.W. Minchin and N.D. Vincent, 1987, Inhibition of GABA release from slices prepared from several brain regions of rats at various times following a convulsion, Br. J. Pharmacol. 92, 13.

Hershkowitz, N. and G.F. Ayala, 1981, Effects of phenytoin on pyramidal neurones of the rat hippocampus, Brain Res. 208, 487.

Iadarola, M.J. and K. Gale, 1981, Cellular compartments of GABA in brain and their relationship to anticonvulsant activity, Mol. Cell. Biochem. 39, 305.

Löscher, W., 1981, Relationship between drug-induced changes in seizure thresholds and the GABA content of brain and brain nerve endings, Naunyn-Schmied. Arch. Pharmacol. 317, 131.

Löscher, W. and D. Schmidt, 1980, Increase in human plasma GABA by sodium valproate, Epilepsia 21, 611.

Löscher, W. and D. Schmidt, 1981, Plasma GABA levels in neurological patients under treatment with valproic acid, Life Sci. 28, 2383.

Löscher, W. and D. Schmidt, 1988, Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical observations, Epilepsy Res. 2, 145.

McLean, M.J. and R.L. Macdonald, 1983, Multiple actions of phenytoin on mouse spinal cord neurons in cell culture, J. Pharmacol. Exp. Ther. 227, 779.

McLean, M.J. and R.L. Macdonald, 1986, Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture, J. Pharmacol. Exp. Ther. 237, 1001.

Meldrum, B.S., 1993, Excitotoxicity and selective neuronal loss in epilepsy, Brain Pathol. 3, 405.

Nau, H. and W. Löscher, 1982, Valproic acid: brain and plasma levels of the drug and its metabolites, anticonvulsant effects and γ-aminobutyric acid (GABA) metabolism in the mouse, J. Pharmacol. Exp. Ther. 220, 654.

Nomikos, G.G., A.P. Zis, G. Damsma and H.C. Fibiger, 1991, Electroconvulsive shock produces large increases in interstitial concentrations of dopamine in the rat striatum: an in vivo microdialysis study, Neuropsychopharmacology 4, 65.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, London).

Phillips, N.I. and L.J. Fowler, 1982, The effects of sodium valproate on gamma-aminobutyrate metabolism and behaviour in naive and ethanolamine-*o*-sulphate pretreated rats and mice, Biochem. Pharmacol. 31, 2257.

Rogawski, M.A. and R.J. Porter, 1990, Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds, Pharmacol. Rev. 42, 223.

Rowley, H.L., K.F. Martin and C.A. Marsden, 1995a, Decreased GABA release following tonic-clonic seizures is associated with an increase in extracellular glutamate in rat hippocampus in vivo, Neuroscience 68, 415.

Rowley, H.L., K.F. Martin and C.A. Marsden, 1995b, Determination of in vivo amino acid neurotransmitters by high-performance liquid chromatography with *o*-phthalaldehyde-sulphite derivatisation, J. Neurosci. Meth. 57, 93.

Swinyard, E.A., J.H. Woodhead, H.S. White and M.R. Franklin, 1989, General principles. Experimental selection, quantification and evaluation of anticonvulsants, in: Antiepileptic Drugs, eds. R. Levy, R. Mattson, B. Meldrum, J. K. Penry and F. E. Dreifuss (Raven Press, New York) p. 85.

Van der Laan, J.W., T. De Boer and J. Bruinvels, 1979, Di-*n*-propylacetate and GABA degradation. Preferential inhibition of succinic semialdehyde dehydrogenase and indirect inhibition of GABA transaminase, J. Neurochem. 32, 1769.

Wright, I.K., M.J. Bickerdike and C.A. Marsden, 1992, Preparation of low cost microdialysis probes, Br. J. Pharmacol. 107, 233P.

Zis, A.P., G.G. Nomikos, E.E. Brown, G. Damsma and H.C. Fibiger, 1992, Neurochemical effects of electrically and chemically-induced seizures: an in vivo microdialysis study in the rat hippocampus, Neuropsychopharmacology 7, 189.