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Vigabatrin reduces epileptiform activity in brain slices from pharmacoresistant epilepsy patients

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Abstract

Human neocortical temporal lobe tissue resected for treatment of pharmacoresistant epilepsy was investigated. In slices prepared from this tissue, epileptiform field potentials (EFP) were induced by omission of magnesium from the artificial cerebrospinal fluid (ACSF). The effects of the gamma-aminobutyric acid transaminase inhibitor vigabatrin on EFP were tested. Vigabatrin exerted a dose-dependent reduction of the repetition rate of EFP: after 3 h of administration of vigabatrin in concentrations of 100 and 200 μmol/l, the repetition rate of EFP was reduced to 35% and 18% of the initial values, respectively. This effect was not reversible. In control experiments with neocortical slices from rats, vigabatrin reduced EFP in a comparable range. The results demonstrate a strong antiepileptic effect of vigabatrin on EFP in tissues from pharmacoresistant epilepsy patients. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Antiepileptic drug; Vigabatrin; Epileptic activity; Temporal lobe epilepsy; Pharmacoresistant; Brain slice

1. Introduction

Physiological investigations of human brain tissue that has been removed during surgical treatment of epilepsy patients resistant to medical treatment, have been of increasing interest in recent years. Experiments revealed that in many aspects, the chronic epileptic human tissue had similar neuronal characteristics to those seen in tissue from experimental animals. Only a few studies found differences in electrophysiological characteristics such as membrane and repetitive firing properties or postsynaptic potentials (Avoli et al., 1994; Avoli and Olivier, 1989; Beck et al., 1997; Isokawa, 1996; McCormick, 1989; McCormick and Williamson, 1989; Prince and Wong, 1981; Schwartzkroin and Knowles, 1984; Schwartzkroin and Prince, 1976; Tasker et al., 1992; Williamson et al., 1993; Williamson et al., 1995).

These differences, however, could not explain the refractoriness of patients to medical therapy. Up to now, no information is available on whether drugs, which are proven to be ineffective in the medical treatment of patients can still be antiepileptically effective in human epileptic tissue in vitro. We analyzed the effects of the γ-aminobutyric acid (GABA) transaminase inhibitor vigabatrin on epileptiform activity induced by perfusing the slices of surgically resected human neocortex maintained in vitro with Mg^{2+}-free artificial cerebrospinal fluid (ACSF). We prefer this epilepsy model, since the recurrent short epileptiform discharges induced by omission of Mg^{2+} from the bath solution are insensitive to a variety of antiepileptic drugs (Heinemann et al., 1994; Lücke et al., 1998). Therefore, this model, which is frequently used in experimental epilepsy research, can be regarded as a model for pharmacoresistant epilepsy (Heinemann et al., 1994). This model is also in line with the pharmacoresistancy of the patients.

2. Materials and methods

2.1. Epilepsy patients

Human neocortical tissue (anterior portion of left and right gyrus temporalis inferior) was obtained from 10

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Table 1
Clinical data of epilepsy patients whose tissue was investigated in this study

Case	Gender	Age	Seizure type	Seizure frequency (month)	Seizures for *n* years	AED	Pathology
1	M	46	CPS, SGTCS		15		astrocytosis, gliosis (fiber)
2	M	40	CPS, SGTCS	8	14	VGB, CBZ, DPH	astrocytosis, gliosis (fiber)
3	M	25	CPS, aura	12	11	PHB, CBZ	astrocytosis, gliosis (fiber), AHS
4	F	13	CPS, SGTCS, aura	45	8	CBZ	gliosis, AHS
5	F	18	CPS, SGTCS, aura	10	13	CBZ, VPA, PHB, LTG, VGB, STM	astrocytosis, gliosis, AHS
6	F	23	CPS, SGTCS	9	5	CBZ, PHB	astrocytosis, AHS
7	M	15	CPS	75	10	CBZ	gliosis, AHS
8	F	39	CPS, SGTCS	1.5	25	PHT, GPT	astrocytosis, gliosis (fiber), AHS
9	F	40	CPS, aura	7	26	CBZ, PHT, PHB, PBP, LTG, VGB, VPA	astrocytosis, gliosis (fiber), AHS
10	M	36	CPS, SGTCS, aura	6	32	CBZ, VGB, ES	astrocytosis, AHS

AED = antiepileptic drug, AHS = Ammon's horn sclerosis, CBZ = carbamazepine, CPS = complex partial seizure, ES = ethosuximide, F = female, GBT = gabapentin, LTG = lamaotrigine, M = male, PHB = phenobarbital, PHT = phenytoin, (S)GTCS = (secondary) generalized tonic–clonic seizure, STM = sultiam, VGB = vigabatrin, VPA = valproate.

patients who underwent resection of the temporal lobe for surgical treatment of pharmacoresistant focal epilepsy (Table 1). The patients were of both sexes (five females, five males), aged 13 to 46 years. All patients had been treated unsuccessfully with a variety of medications, regularly including carbamazepine, and one or more of phenytoin, valproate, gabapentin, vigabatrin, sultiam, lamotrigine, ethosuximide and phenobarbital. Drugs were given until surgery. The neuropathological diagnosis included hippocampal sclerosis ($n = 8$), astrocytosis ($n = 8$), and gliosis of neocortical tissue ($n = 8$). Informed consent was obtained from all patients. The experiments were approved by the local ethics committee.

2.2. Preparation of brain slices

Since the hippocampal tissue obtained from the neurosurgical resection did not allow slice preparation, the investigations were restricted to neocortical tissue. The technique for slice preparation of human tissue has been described in detail elsewhere (Köhling et al., 1998). Briefly, neocortical specimens were immediately after resection immersed in ice-cold ACSF. The ACSF contained (in mmol/l) 124 NaCl, 4 KCl, 1 $CaCl_2$, 1.24 NaH_2PO_4, 26 $NaHCO_3$, 1.3 $MgCl_2$ and 10 glucose, and was constantly bubbled with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. Slices (500 μm) were sectioned perpendicularly to the pial surface using a vibroslice and transferred into a portable incubation bath containing ACSF at a temperature of 28°C (Köhling et al., 1996). After 30 min, the $CaCl_2$ concentration was raised to 2 mmol/l. The total pre-incubation time was of at least 2 h. All human neocortical slices investigated electrophysiologically were examined and none had pathological abnormalities. Only in a few cases were mild dysplastic changes and mild gliosis found.

The technique for slice preparation of animal tissue have been described in detail elsewhere (Straub et al., 2000). Briefly, rats of both sexes, weighting 300–400 g, were used. The brain was removed under ether anaesthesia. The neocortex were dissected and transverse slices (500 μm thick) were cut by means of a McIlwain tissue chopper. Slices were pre-incubated in a submersion chamber for at least 1 h in ACSF at 28°C.

2.3. Electrophysiology and drug administrations

For electrophysiological investigations, slices were maintained in a submerged type recording chamber. Temperature (32°C), pH (7.4), and flow rate (4 ml/min, bath volume 1 ml) were continuously monitored. Extracellular field potentials were recorded from layers II to V with glass microelectrodes (0.5–1.5 MΩ) filled with 150 mmol/l NaCl. Epileptiform field potentials (EFP) were elicited by omission of $MgCl_2$ from the ACSF. Vigabatrin (kind gift from Hoechst Marion Roussel) was dissolved in Mg^{2+}-free ACSF. To achieve effective concentrations of vigabatrin in the slices within the relatively short period of administration, we used concentrations of 100, 200, and 500 μmol/l. Vigabatrin was applied 60 to 90 min after superfusion with Mg^{2+}-free ACSF, when EFP were nearly stable in frequency, amplitude, and duration. After 3 h of drug application, a wash-out period of 1 h in Mg^{2+}-free ACSF followed (Fig. 1A).

The EFP were evaluated with respect to frequency, amplitude, and duration over a period of 5 min. The effects of vigabatrin on epileptiform activity are given as percent

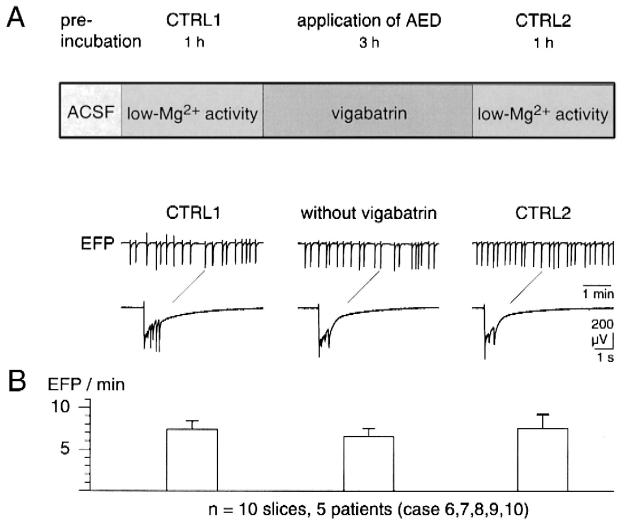

Fig. 1. EFP induced by omission of Mg^{2+} from the bath solution recorded from human neocortical slice preparations obtained during epilepsy surgery from the inferior temporal gyrus. (A) Experimental protocol for the application of vigabatrin. (B) Control experiment: recordings of EFP for 5 h without administration of vigabatrin. ACSF: artificial cerebrospinal fluid.

of control. Data were analyzed statistically with Wilcoxon rank test and Kruskal–Wallis test. All values represent mean ± S.E.M. (significance level $P < 0.05$).

3. Results

Extracellular field potentials were recorded from a total of 28 neocortical slices from 10 patients. The omission of extracellular Mg^{2+} from the bath solution induced epileptiform activity after 30 to 90 min of incubation. Only slices with a regular discharge frequency were selected for further investigations. The EFP occurred at a frequency of 7.5 ± 1/min ($n = 10$ slices; 5 patients) and remained nearly stable in frequency, duration, and amplitude throughout 5 h of investigation (Fig. 1B).

The antiepileptic effect of vigabatrin on EFP was tested on 18 neocortical slices from eight patients. Vigabatrin reduced the frequency of EFP in a concentration-dependent manner. The discharge frequency, at a concentration of 100 µmol/l vigabatrin, was reduced after 1 h of application to 72 ± 7%, after 2 h to 48 ± 7% and after 3 h to 35 ± 6% of control (3 h: $P = 0.001$; $n = 9$ slices, 4 patients; Fig. 2). After wash-out, the discharge frequency increased slightly to 39 ± 6%. At a concentration of 200 µmol/l, the antiepileptic effect of vigabatrin increased. The discharge activity was reduced after 1, 2, and 3 h to 60 ± 6%, 34 ± 6%, and 18 ± 3%, respectively (3 h: $P = 0.001$; $n = 7$ slices, 2 patients; Fig. 2). One hour of wash-out led to a small re-increase in the discharge frequency to 29 ± 4%. With application of 500 µmol/l vigabatrin, the epileptiform activity was completely

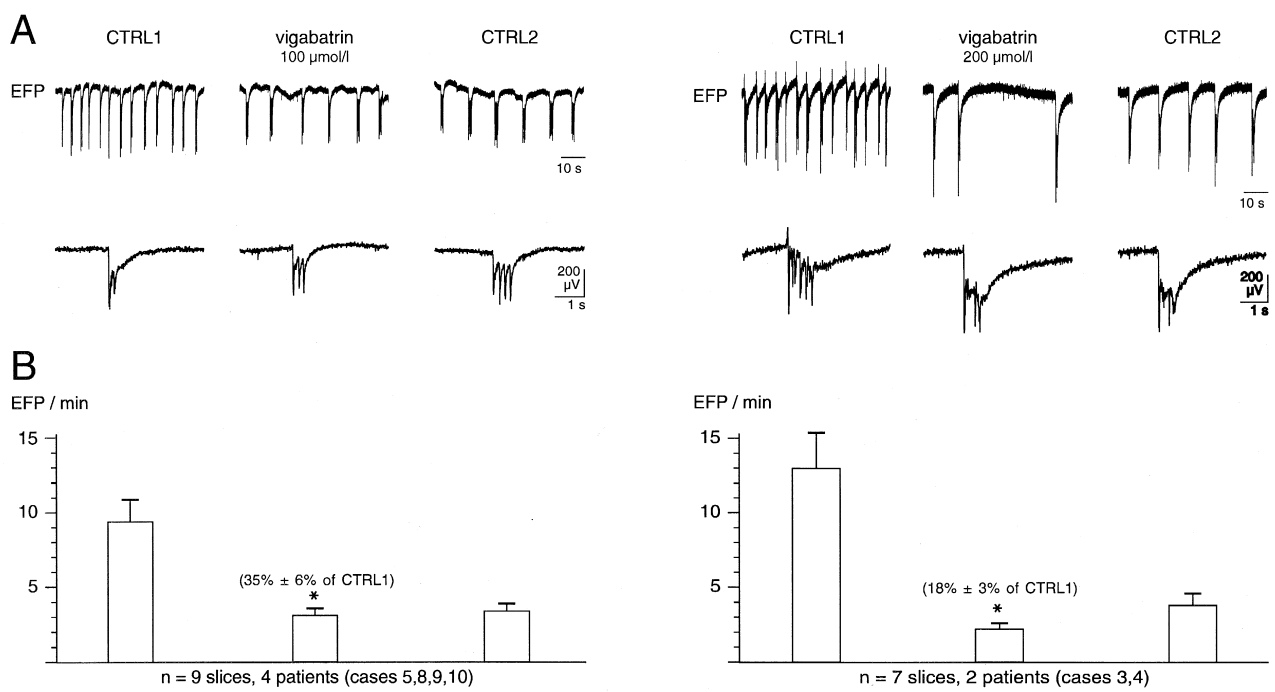

Fig. 2. Antiepileptic effects of vigabatrin on epileptiform activity recorded from human neocortical slice preparations obtained during epilepsy surgery from the inferior temporal gyrus. (A) Dose-dependent effects of vigabatrin (100 and 200 µmol/l for 3 h) on EFP induced by omission of Mg^{2+} from the bath solution. (B) Statistical evaluation of the antiepileptic effects of vigabatrin. CTRL1 and CTRL2 represent activity recorded before and 1 h after administration of vigabatrin, respectively.

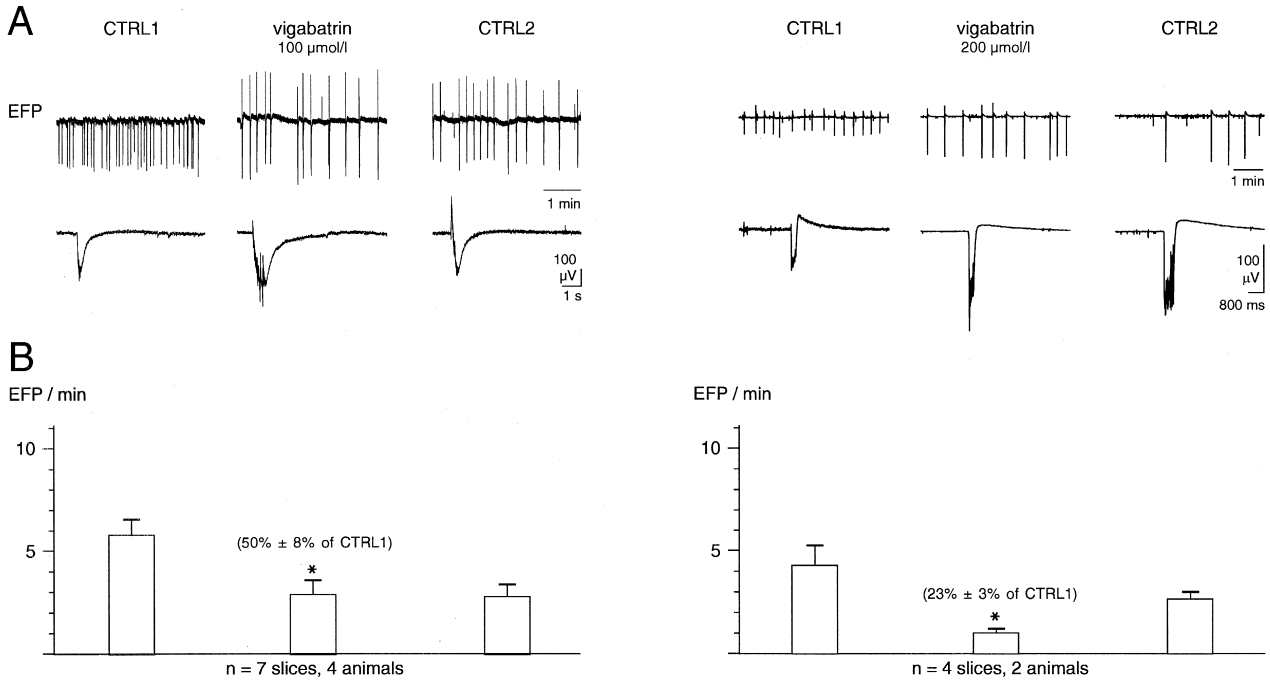

Fig. 3. Antiepileptic effects of vigabatrin on epileptiform activity recorded from rat neocortical slice preparations. (A) Dose-dependent effects of vigabatrin (100 and 200 μmol/l for 3 h) on EFP induced by omission of Mg^{2+} from the bath solution. (B) Statistical evaluation of the antiepileptic effects of vigabatrin. CTRL1 and CTRL2 represent activity recorded before and 1 h after administration of vigabatrin, respectively.

blocked after 12 ± 2 min ($n = 2$ slices, 2 patients; data not shown). At this concentration, the recovery was rather slow and upon wash-out of vigabatrin, the first EFP re-appeared after 60–80 min. Neither amplitude ($P = 0.059$) nor duration of EFP ($P = 0.694$) was significantly changed during application and after wash-out of vigabatrin.

The antiepileptic effect of vigabatrin on EFP was furthermore tested on 11 neocortical slices from six control rats. Again, vigabatrin reduced the frequency of EFP in a concentration-dependent manner. The discharge frequency, at a concentration of 100 μmol/l vigabatrin, was reduced after 3 h to $50 \pm 8\%$ of controls ($P = 0.001$; $n = 7$ slices, 4 rats; Fig. 3). At a concentration of 200 μmol/l, the antiepileptic effect of vigabatrin increased. The discharge activity was reduced after 3 h to $23 \pm 4\%$ ($P = 0.001$; $n = 4$ slices, 2 rats; Fig. 3).

4. Discussion

Vigabatrin exerted an antiepileptic effect on epileptiform discharges in neocortical slice preparations from pharmacoresistant human epileptogenic tissue. The EFP were significantly decreased in frequency by vigabatrin in bath concentrations of 100 and 200 μmol/l. Although vigabatrin is an irreversible blocker of the GABA transaminase and therefore exerts its effects independent of its concentration, we found a dose-dependent effect of the drug in our experiments. This is probably caused by the time course necessary to achieve the effective concentrations in the slice. These concentrations of the drug in the bath solution correspond quite well to serum levels found in patients receiving chronic treatment with vigabatrin. In these patients, vigabatrin was found in plasma concentrations up to 300 μmol/l, with mean values of 42–100 μmol/l (Foletti et al., 1995). However, the concentration of vigabatrin in the cerebrospinal fluid (CSF) is markedly lower since the concentration ratio between CSF and serum is approximately 0.1 (Rey et al., 1992). Nevertheless, it is possible that changes in the extracellular micromilieu, for reasons of cellular activity or location, may drastically increase the concentration of vigabatrin in the immediate vicinity of the cells. Furthermore, one may have to take into account the retardation in the equilibration time course of bath applied vigabatrin in the neocortical slices in our experiments. Heinemann et al. (1994) have reported that even after 40 min of equilibration, the concentration of valproate measured at the depth of 100 μm below the cut surface was only 25% of that in the bath. In contrast to vigabatrin, other drugs did not exert a clear antiepileptic effect in human neocortical slices. Carbamazepine, applied in a concentration of 50 μmol/l, reversibly increased the EFP frequency induced by omission of Mg^{2+} to 174%, while the duration of EFP was reduced to 30% of the initial value (Musshoff et al., 1997). Furthermore, in human neocortical slices generating spontaneous epileptiform activity, carbamazepine and phenytoin, both applied in a concentration of 25 μmol/l, failed to suppress this activity (Köhling et al., 1998).

Epileptiform activity, induced by omission of Mg^{2+}, is characterized by an increase in the activity of NMDA receptors and a reduction of inhibitory responses (Traub et al., 1994; Whittington et al., 1995). Concerning the mechanism of action of vigabatrin, it is unlikely that the antiepileptic effect is mediated by a direct blockade of NMDA receptors since the maximal effects of the drug occurred after several hours. Furthermore, in the oocyte expression system vigabatrin exerted no effects on membrane currents, induced by NMDA receptors from rat brain (Wirth et al., 1998). It is more likely that vigabatrin increases in one way or another, the GABA-mediated potentials mainly by the irreversible inhibition of GABA-transaminase, by an inhibition of GABA uptake or by an enhancement of GABA release (Jung and Paltreyman, 1995). This is also supported by the fact that the antiepileptic effects of vigabatrin are blocked by simultaneous administration of bicuculline (Lücke et al., 1998) and that vigabatrin induces an increase in the GABA concentration of the whole slice (Neal and Shah, 1990; Wadman and Nunes Felipe, 1992). The time course of the antiepileptic effect and the small increase in frequency of the epileptic activity after wash-out of vigabatrin is in line with an irreversible inhibition of the GABA-transaminase, whereas the effects on GABA uptake and release are completely reversible (cf. Lücke et al., 1998). Furthermore, a pharmacological interaction of vigabatrin with other antiepileptic drugs, administered to the patients up to the operation, can be excluded since these drugs were washed out completely during incubation of the slices. No quantifiable concentrations of carbamazepine and phenytoin could be detected in high-pressure liquid chromatography analyses (Köhling et al., 1998). Since there was no difference in the antiepileptic effect of vigabatrin between the pharmacoresistant human tissue and the animal control tissue, one may assume that the mechanism responsible for the antiepileptic effect of vigabatrin in the slices, probably an increase of the GABA-mediated potentials induced by the irreversible inhibition of GABA-transaminase, is not altered in the human epileptic tissue in vitro.

The antiepileptic effect of vigabatrin occurred in slice preparations obtained from all patients. Therefore, the effect of the drug was not correlated with sex, age, seizure history or antiepileptic medication of the patients nor with histopathological findings (e.g. hippocampal sclerosis). Surprisingly, three patients (case nos. 5, 9, and 10) received vigabatrin during their medical treatment for a short period but did not develop a significant reduction in seizure frequency. This corresponds with clinical data demonstrating that a reduction of seizure frequency by more than 50% can be expected in only 50% of patients with refractory partial seizures treated with vigabatrin (Jung and Palfreyman, 1995). However, the ineffectiveness of vigabatrin in these patients in vivo is in contrast to the strong antiepileptic effects in vitro. Although this question remains completely unanswered, one could speculate that this result may be due to differences in the mechanisms leading to epileptiform activity in vitro and mechanisms leading to seizures in vivo.

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References

Avoli, M., Olivier, A., 1989. Electrophysiological properties and synaptic responses in the deep layers of the human epileptogenic neocortex in vitro. J. Neurophysiol. 61, 589–606.

Avoli, M., Hwa, G.G.C., Lacaille, J.-C., Olivier, A., Villemure, J.G., 1994. Electrophysiological and repetitive firing properties of neurons in the superficial/middle layers of the human neocortex maintained in vitro. Exp. Brain Res. 98, 135–144.

Beck, H., Clusmann, H., Kral, T., Schramm, J., Heinemann, U., Elger, C.E., 1997. Potassium currents in acutely isolated human hippocampal dentate granule cells. J. Physiol. 498, 73–85.

Foletti, G.B., Delisle, M.-C., Bachmann, C., 1995. Reduction of plasma alanine aminotransferase during vigabatrin treatment. Epilepsia 36, 804–809.

Heinemann, U., Dreier, J., Leschinger, A., Stabel, J., Draguhn, A., Zhang, C., 1994. Effects of anticonvulsant drugs on hippocampal neurons. Hippocampus 4, 291–296.

Isokawa, M.J., 1996. Decrement of $GABA_A$ receptor-mediated inhibitory postsynaptic currents in dentate granule cells in epileptic hippocampus. J. Neurophysiol. 75, 1901–1908.

Jung, M.J., Palfreyman, M., 1995. Vigabatrin: mechanisms of action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs. Raven Press, New York, pp. 903–939.

Köhling, R., Lücke, A., Straub, H., Speckmann, E.-J., 1996. A portable chamber for long-distance transport of survival human brain slice preparations. J. Neurosci. Methods 67, 233–236.

Köhling, R., Lücke, A., Straub, H., Speckmann, E.-J., Tuxhorn, I., Wolf, P., Pannek, H., Oppel, F., 1998. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain 121, 1073–1087.

Lücke, A., Musshoff, U., Köhling, R., Osterfeld, M., Mayer, T., Wolf, P., Schütte, W., Speckmann, E.-J., 1998. Gabapentin potentiation of the antiepileptic efficacy of vigabatrin in an in vitro model of epilepsy. Br. J. Pharmacol. 124, 370–376.

McCormick, D.A., 1989. GABA as an inhibitory neurotransmitter in human cerebral cortex. J. Neurophysiol. 62, 1018–1027.

McCormick, D.A., Williamson, A., 1989. Convergence and divergence of neurotransmitter action in human cerebral cortex. Proc. Natl. Acad. Sci. U. S. A. 86, 8098–8102.

Musshoff, U., Lücke, A., Köhling, R., Speckmann, E.-J., Tuxhorn, I., Wolf, P., Pannek, H., Oppel, F., 1997. Efficacy of conventional and new antiepileptic drugs in human neocortical slices. Eur. J. Physiol., R181, Suppl. 433.

Neal, M.J., Shah, M.A., 1990. Development of tolerance to the effects of vigabatrin (gamma-vinyl-GABA) on GABA release from rat cerebral cortex, spinal chord and retina. Br. J. Pharmacol. 100, 324–328.

Prince, D.A., Wong, R.K., 1981. Human epileptic neurons studied in vitro. Brain Res. 210, 323–333.

Rey, E., Pons, G., Olive, G., 1992. Vigabatrin-clinical pharmacokinetics. Clin. Pharmacokinet. 23, 267–278.

Schwartzkroin, P.A., Knowles, W.D., 1984. Intracellular study of human epileptic cortex: in vitro maintenance of epileptiform activity?. Science 223, 709–712.

Schwartzkroin, P.A., Prince, D.A., 1976. Microphysiology of human cerebral cortex studied in vitro. Brain Res. 115, 497–500.

Straub, H., Köhling, R., Frieler, A., Grigat, M., Speckmann, E.-J., 2000. Contribution of L-type calcium channels to epileptiform activity in hippocampal and neocortical slices of guinea-pigs. Neuroscience 95, 63–72.

Tasker, J.G., Peackock, W.D., Dudeck, F.E., 1992. Local synaptic circuits and epileptiform activity in slices of neocortex from children with intractable epilepsy. J. Neurophysiol. 67, 496–507.

Traub, R.D., Jeffreys, J.G.R., Whittington, M.A., 1994. Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. J. Physiol. 478, 379–393.

Wadman, W.J., Nunes Filipe, C.M., 1992. Gamma-vinyl-GABA induced changes in excitability in rat hippocampal slices. In: Speckmann, E.-J., Gutnick, M.J. (Eds.), Epilepsy and Inhibition. Urban and Schwarzenberg, München-Wien-Baltimore, pp. 107–121.

Whittington, M.A., Traub, R.D., Jeffreys, J.G.R., 1995. Erosion of inhibition contributes to the progression of low magnesium bursts in rat hippocampal slices. J. Physiol. 486, 723–734.

Williamson, A., Spencer, D.D., Shepherd, G.M., 1993. Comparison between the membrane and synaptic properties of human and rodent dentate granule cells. Brain Res. 622, 194–202.

Williamson, A., Telfeian, A.E., Spencer, D.D., 1995. Prolonged GABA responses in dentate granule cells in slices isolated from patients with temporal lobe sclerosis. J. Neurophysiol. 74, 378–387.

Wirth, B., Golla, H., Mußhoff, U., Madeja, M., Speckmann, E.-J., 1998. Effects of new antiepileptic drugs on GABA and glutamate receptors. Eur. J. Neurosci., R49, Suppl. 10.