Effectiveness of Vigabatrin against Focally Evoked Pilocarpine-Induced Seizures and Concomitant Changes in Extracellular Hippocampal and Cerebellar Glutamate, γ-Aminobutyric Acid and Dopamine Levels, a Microdialysis-Electrocorticography Study in Freely Moving Rats

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ABSTRACT

Limbic seizures were evoked in freely moving rats by intrahippocampal administration of the muscarinic agonist pilocarpine via the microdialysis probe (10 mM for 40 min at 2 μl/min). This study monitored changes in extracellular hippocampal γ-aminobutyric acid (GABA), glutamate and dopamine levels after systemic (30 mg/kg/day) or local (intrahippocampal or intranigral, 5 mM or 600 μM for 180 min at 2 μl/min) vigabatrin administration, and evaluated the effectiveness of this antiepileptic drug against pilocarpine-induced seizure activity. Extracellular GABA and glutamate overflow in the ipsilateral cerebellum was studied simultaneously. Microdialysis was used as an in vivo sampling technique and as a drug-delivery tool. Electrophysiological evidence for the presence or absence of seizures was recorded with electrocorticography. The observed alterations in extracellular hippocampal amino acid levels support the hypothesis that muscarinic receptor stimulation by the intrahippocampal administration of 10 mM pilocarpine is responsible for the seizure onset, and that the amino acids maintain the sustained seizure activity. The focally evoked pilocarpine-induced seizures were completely prevented by intraperitoneal vigabatrin premedication for 7 days or by a single intraperitoneal injection. Effective protection was reflected in a lack of sustained elevations of hippocampal glutamate levels. Rats receiving vigabatrin intrahippocampally or intranigrally still developed seizures, although there appeared to be a partial protective effect. During the intrahippocampal perfusion with 5 mM vigabatrin, extracellular hippocampal GABA levels increased, whereas the extracellular glutamate and dopamine overflow decreased. The lack of a complete neuroprotection after local vigabatrin treatment is discussed.

Vigabatrin (γ-vinyl-GABA), which is the first rationally designed antiepileptic drug, acts as an irreversible inhibitor of the GABA-metabolizing enzyme, GABA-transaminase, which results in an augmentation of GABA concentrations in the brain. Vigabatrin causes a selective increase in the GABA transmitter pool as well as an increased GABA release after synaptic stimulation, which indicates that this drug acts through GABA-mediated inhibition (Gram et al., 1988). Indeed, pharmacological actions are presumed to be the result of extracellular transmitters interacting with receptors located on the outer surface of cell membranes. Controlled clinical trials have demonstrated the excellent antiepileptic effect of vigabatrin, especially in the treatment of complex partial seizures (Mumford and Dam, 1989). A dose-dependent decrease in the whole tissue concentration of glutamate in the hippocampal region after intraperitoneal vigabatrin administration has been described (Halonen et al., 1991), whereas no influences on cholinergic, dopaminergic, serotonergic and peptidergic systems have yet been reported (Sabers and Gram, 1992). Microdialysis provides information about extracellular transmitter levels after systemic or local vigabatrin application: a few microdialysis studies have reported increases in extracellular GABA concentrations (Jolkkonen et al., 1992; Qume et al., 1995; Sayin et al., 1995), but no data

ABBREVIATIONS: ANOVA, analysis of variance; ECoG, electrocorticography; Fisher’s PLSD, Fisher’s protected least significant difference; GABA, γ-aminobutyric acid; LC, liquid chromatography; MK-801, dizocilpine maleate; NMDA, N-methyl-D-aspartate; S.E.M., standard error on the mean.
on changes in other extracellular neurotransmitter levels are available.

Systemic administration of pilocarpine, a nonselective muscarinic agonist, is used as an animal model of intractable epilepsy (Turski et al., 1989). Histological studies have shown important similarities between this model and temporal lobe epilepsy in humans (Isokawa and Mello, 1991). “Focally evoked” pilocarpine-induced seizures provide an even better model for the generation of complex partial seizures. Millan et al. (1993) were the first to evoke seizures by administering pilocarpine via a microdialysis probe in the hippocampus of anesthetized rats. We have used this experimental approach in freely moving rats, and further characterized this model with experiments using the muscarinic receptor antagonist atropine, the voltage-dependent sodium channel blocker tetrodotoxin and the NMDA receptor antagonist dizocilpine maleate (MK-801). Muscarinic receptor blockade with atropine prevented the changes in extracellular hippocampal dopamine, glutamate and GABA levels normally observed during and after local pilocarpine administration. Tetrodotoxin abolished the increases in extracellular dopamine, glutamate and GABA levels in the hippocampus after pilocarpine perfusion, thereby indicating the voltage-dependent release of these transmitters. Intrahippocampal MK-801 administration was able to prevent the pilocarpine-induced seizures, which suggested an NMDA receptor-mediated mechanism (Smolders et al., 1997).

In this combined microdialysis-ECG study, epilepsy was evoked in a control group of freely moving rats by intrahippocampal pilocarpine perfusion. Changes in hippocampal and cerebellar GABA, glutamate and dopamine efflux were monitored via microdialysis sampling. The effectiveness of systemically or locally applied vigabatrin against these pilocarpine-induced seizures was evaluated in treatment groups, and concomitant alterations in transmitter levels were monitored. Local administration of the antiepileptic drug may provide more information about its biochemical effects or unrevealed mechanisms of action.

Materials and Methods

Chemicals and Reagents

GABA, l-glutamate, dopamine and pilocarpine were supplied by Sigma (St. Louis, MO). Vigabatrin (γ-vinyl-GABA) was obtained from Marion Merrell Dow Research Institute (Strasbourg, France). All other chemicals were analytical reagent grade or better and were supplied by Merck (Darmstadt, Germany). Aqueous solutions were made with purified water (Seralpur pro 90 CN, Belgolabo, Overijse, Belgium) and filtered through a 0.2-μm membrane filter. The aqueous perfusion medium for the microdialysis experiments, the so-called microdialysis solution, contained 147 mM NaCl, 2.3 mM CaCl₂ and 4 mM KCl. An antioxidant solution containing 0.02 M HCl, 0.2% sodium metabisulfite and 0.02% Na₂EDTA was added to all dialysate samples to stabilize collected dopamine. Vigabatrin was dissolved in physiological saline when administered intraperitonely. Vigabatrin and pilocarpine were dissolved in the microdialysis solution when applied via the microdialysis probe.

Microdialysis

The protocols for the animal experiments described in this study were performed according to national rules on animal experiments and guidelines from the Faculty of Medicine, Free University of Brussels. Male albino Wistar rats, weighing 270 to 300 g, were anesthetized with a mixture of ketamine/diazepam (50:55 mg/kg) and mounted in a stereotaxic frame. Intracranial guides (CMA Microdialysis, Stockholm, Sweden) were implanted in the hippocampus and ipsilateral cerebellum (groups 1, 2, 3, 4, 5, or in hippocampus and ipsilateral substantia nigra (group 6). Coordinates toward bregma were L = +4.6, A −5.6 and V +4.6 for hippocampus, L +3.0, A −13.0 and V +4.0 for cerebellum and L +2.0, A −5.2 and V +7.0 for substantia nigra (Paxinos and Watson, 1986). Immediately after surgery, the guide cannula obturators were replaced with CMA 10 microdialysis probes (CMA Microdialysis, Stockholm, Sweden). The probes had a 3-mm membrane length for use in hippocampus and cerebellum, and a 2-mm membrane length for use in substantia nigra. After implantation, probes were continuously perfused with microdialysis solution at a flow rate of 2 μl/min (CMA 100 microdialysis pump, CMA Microdialysis, Stockholm, Sweden). The rats were placed in the experimental cages, and allowed to recover from surgery overnight. During the experiment, dialysates were collected every 20 min from the freely moving animals (from six treatment groups). Each collection vial contained 10 μl of the aforementioned antioxidant mixture.

Group 1 (n = 6): Control group. Eight hippocampal dialysate samples were collected under basal conditions. Epilepsy was then evoked by administration of 10 mM pilocarpine for 40 min (two dialysates) via the microdialysis probe in the hippocampus. After pilocarpine treatment, the perfusion media was switched back to the original microdialysis solution for another 10 sampling intervals. Simultaneously, the ipsilateral cerebellum was perfused with microdialysis solution and 20 dialysate samples were collected.

Group 2 (n = 6): Animals intraperitonely pretreated with 30 mg/kg/day vigabatrin for 7 days. During 7 days of pretreatment, rats received one i.p. injection of 30 mg/kg vigabatrin every day. On the day of the experiment, the same protocol was followed as for the control group.

Group 3 (n = 2): Animals acutely intraperitonely pretreated with 30 mg/kg vigabatrin. On the day of the experiment, rats received a single i.p. injection of 30 mg/kg vigabatrin after which the same sampling protocol as used for the control group was followed.

Group 4 (n = 6): Animals receiving an intrahippocampal perfusion with 5 mM vigabatrin. After six basal dialysate samples, 5 mM vigabatrin dissolved in the microdialysis solution was administered intrahippocampally via the probe for 180 min. The perfusion fluid was then switched to a solution containing 5 mM vigabatrin and 10 mM pilocarpine for two collection intervals. The perfusion fluid was then switched back to the initial vigabatrin solution. Simultaneously, the ipsilateral cerebellum was perfused with microdialysis solution and 23 dialysate samples were collected.

Group 5 (n = 6): Animals receiving an intrahippocampal perfusion with 600 μM vigabatrin. The same protocol as described for group 4 was followed, but with 600 μM vigabatrin instead of 5 mM vigabatrin.

Group 6 (n = 3): Animals receiving an intranigral perfusion with 600 μM vigabatrin. After six basal dialysate samples, 600 μM vigabatrin dissolved in microdialysis solution was administered via the probe in substantia nigra throughout the course of the experiment (collection periods 7 to 23). The hippocampus was perfused with microdialysis solution during basal conditions and during collection intervals 7 to 15. This was followed by an intrahippocampal administration of pilocarpine (10 mM) for two sampling intervals. The perfusion fluid was then switched back to the microdialysis solution for another six sampling periods.

Histological Control

Histology was performed regularly to verify the placement of the microdialysis probes. This was especially critical for small brain nuclei such as the substantia nigra. At the end of the experiments, animals were sacrificed via an overdose of pentobarbital. Brains were surgically excised and fixed in 10% formalin. Paraffin sections...
of 5-μm thickness were stained by hematoxylin-eosin and examined by microscopic evaluation.

**ECoG**

Combined microdialysis-ECoG was performed in two animals of each experimental group. Two parasagittal grooves were drilled in the skull of anesthetized animals (ketamine/diazepam, 50:5 mg/kg). Electrodes for ECoG recordings for groups 1, 2, 4 and 5 were implanted as shown in figure 1, and fixed with dental cement so the electrode tips touched the dura mater. For group 6, electrodes could only be implanted at the contralateral side. The microdialysis probe in substantia nigra prevented ipsilateral implantation of ECoG electrodes. Monopolar ECoG toward a prefrontal reference electrode was polygraphically amplified and recorded with a time constant of 0.15 s, high cut-off filter of 70 Hz, and sensitivity of 500 μV/cm. Signals were further sampled at a frequency of 256 Hz with the Nicolet Brainlab System.

**Chromatographic Assays**

Chromatographic conditions and precolumn derivatization procedures for the amino acids have been described previously (Smolders et al., 1996). For GABA determinations isocratic, reversed-phase, microbore LC with electrochemical detection was used. Precolumn derivatization was performed with o-phthalaldehyde/β-mercaptoethanol and iodoacetamide. Glutamate derivatizations were carried out after precolumn derivatization with o-phthalaldehyde/β-mercaptoethanol, by gradient, reversed-phase, microbore LC with fluorescence detection. The chromatographic assay for dopamine used isocratic, reversed-phase, ion-pair, microbore LC with electrochemical detection (Smolders et al., 1996).

**Statistical Analysis**

All results presented in the figures are expressed as the mean amino acid or dopamine concentrations in μM or nM, respectively, with S.E.M. These dialysis sample concentrations were not corrected for recovery across the dialysis membrane. Basal values represent the mean transmitter concentration as obtained under basal conditions, i.e., before administration of pilocarpine or vigabatrin via the microdialysis probe. Statistical analysis of alterations of neurotransmitter concentrations in time was performed by ANOVA for repeated measurements and Fisher's PLSD post hoc test (α = .05). The significance of difference between peak sample concentrations was determined by Mann-Whitney's test (α = .05).

**Results**

**Basal GABA, glutamate and dopamine concentrations in hippocampus and cerebellum of drug-free animals.** Basal hippocampal dialysis sample concentrations (mean ± S.E.M.) (n = 20) were 0.043 ± 0.012 μM for GABA, 0.47 ± 0.16 μM for glutamate and 0.34 ± 0.10 nM for dopamine. Basal cerebellar sample concentrations (mean ± S.E.M.) (n = 20) were 0.054 ± 0.015 μM for GABA and 0.86 ± 0.19 μM for glutamate. No dopamine was measured in the cerebellum, because this brain nucleus lacks dopaminergic innervation. Extracellular basal hippocampal and cerebellar neurotransmitter levels in the control rats were not significantly different from the levels obtained in the animals premedicated with vigabatrin.

**Group 1: Control group.** All rats demonstrated limbic seizures characterized by tremor, scratching and wet dog shakes starting approximately 1 h after initiation of pilocarpine infusion. This behavior was followed by tonic-clonic movements of the limbs, salivation, intense masticatory jaw movements, rearing and occasional loss of balance. During pilocarpine administration, ECoG recordings showed a slowing of rhythmic activity resulting in theta and delta waves. About 1 h after the start of pilocarpine perfusion, ECoG recordings showed clear patterns of tonic and tonic-clonic epileptic seizure activity which was sustained until the end of the experiment (fig. 2). The focally evoked seizures immediately became secondarily generalized, indicated by seizure activity at both the ipsi- and contralateral side of focus. Intrahippocampal administration of pilocarpine (fig. 3a) significantly decreased basal hippocampal overflow of glutamate (significant decrease during collection PILO1, PILO2 and H11; P = .0003) and GABA (significant decrease during collection PILO1 and PILO2; P = .0082). Cessation of pilocarpine administration resulted in a significant increase in glutamate and GABA concentrations, 225% and 183%, respectively (P = .0001) (fig. 3a). This significant elevation of both amino acid transmitters remained until the end of the experiment. During the pilocarpine-induced seizures, simultaneous ipsilateral elevation of glutamate (378%, P = .0007, fig. 3b) and GABA (171%, P = .0002, fig. 3b) in cerebellum was observed. Extracellular cerebellar levels of glutamate remained elevated until the end of the experiment. Extracellular cerebellar GABA concentrations returned to baseline from collection C15. Intrahippocampal administration of pilocarpine resulted in a significant increase of basal hippocampal dopamine release (fig. 4, 478%, P = .0001). Extracellular dopamine levels were significantly elevated from collection PILO2 until collection H16.

**Groups 2 and 3: Animals pretreated intraperitoneally with 30 mg/kg/day vigabatrin.** Rats premedicated intraperitoneally with vigabatrin did not develop limbic seizures as described for control animals. The animals were protected against the convulsions with both the 7-day treatment and an acute injection of vigabatrin. ECoG recordings showed no patterns of epileptic seizure activity (data not shown). During hippocampal perfusion with pilocarpine in the rats premedicated for 7 days (fig. 5a), a decrease in extracellular hippocampal levels of glutamate (to 2%, P = .0001) and GABA (to 29%, P = .0016) was observed, a response similar to that observed in the control group. The decrease in extracellular hippocampal glutamate overflow was not followed by an increase (fig. 5a), whereas the decrease in extracellular hippocampal GABA levels was again followed by a significant elevation that was maintained until the end of the experiment (fig. 5a, P = .0001, maximum increase 213%). In ipsilateral cerebellum, extracellular glutamate and GABA concentrations remained unaltered (fig. 5b). Intrahippocampal administration of pilocarpine in rats premedicated with vigabatrin for 7 days resulted in a significant increase of basal hippocampal dopamine release (fig. 4, to 261%, P = .0083). Extracellular dopamine levels were significantly elevated during collection PILO2 and H11. The dopamine increase in these premedicated animals was sig-

![View of rat skull and implantation sites for microdialysis probes (a, b) and electrodes for ECoG recordings (c–h).](Image)
nificantly lower (P = 0.028) than the dopamine increase observed in control rats. Animals given a single intraperitoneal injection of vigabatrin on the day of the experiment showed changes in transmitter levels similar to animals pretreated for 7 days (data not shown).

**Group 4: Animals receiving an intrahippocampal perfusion with 5 mM vigabatrin.** Intra-hippocampal administration of 5 mM vigabatrin did not protect animals from pilocarpine-induced seizures. ECoG recordings showed patterns of tonic seizures (fig. 6a). Local administration of 5 mM vigabatrin via the microdialysis probe resulted in elevated GABA levels (to 1046%, P = 0.0001) from collection H7 to H15 (fig. 7a), decreased glutamate levels (to 42%, P = 0.0001) from collection H7 to H15 (fig. 8a) and decreased dopamine levels (to 60%, P = 0.0001) from collection H9 to H15 (fig. 9). During simultaneous pilocarpine perfusion (collection H16 and H17), a decrease in the elevated GABA levels (to 385%, P = 0.0001, fig. 7a), an even more dramatic decrease in glutamate overflow (to 3%, P = 0.0001, fig. 8a) and an increase in dopamine release (to 295%, P = 0.0001, fig. 9) was observed. After administration of pilocarpine, a significant increase in extracellular hippocampal GABA levels compared with GABA concentrations obtained during collection H7 to H15 (fig. 7a, P = 0.02)
Vigabatrin and Pilocarpine Seizures

1243

1997

perfusion with 600 mM pilocarpine (PILO) via the microdialysis probe. Each bar represents a 20-min collection period. Asterisks denote only the first values significantly different from corresponding base-line values [P < .01 (**)].

Alterations in extracellular levels of glutamate, GABA and dopamine before and during pilocarpine-induced seizures. During intrahippocampal pilocarpine perfusion, a slowing of the rhythmic activity was recorded on the ECoG, indicated by the appearance of theta and delta waves. Involvement of cholinergic receptors in hippocampal theta rhythm in vitro has been reported (Konopacki et al., 1988). In this study, simultaneous inhibition of hippocampal glutamate release was observed, confirming previous in vitro studies which suggested that the activation of presynaptic muscarinic M_2 receptors, present on hippocampal glutamatergic nerve terminals, might directly inhibit glutamate release (Marchi and Raiteri, 1989; Segal, 1989). Muscarinic receptor-mediated fast onset depression of excitatory postsynaptic potentials was also observed in rat hippocampal brain slices, but was antagonized by a muscarinic M_3 receptor blocker (Auerbach and Segal, 1996). Current- and voltage-clamp experiments demonstrated that acetylcholine, acting on muscarinic receptors, produced a long-lasting facilitation of NMDA receptor-mediated responses after initial suppression of excitatory postsynaptic potentials (Markram and Segal, 1990). After cessation of intrahippocampal administration of pilocarpine in the control rats of the present study, the decrease in glutamate release was not been reported previously. Extracellular GABA and glutamate levels after treatment with vigabatrin, have alteration in extracellular hippocampal glutamate and dopamine concentrations compared with levels collected from dialysates H7 to H15 (figs. 7a, 8a and 9). There were no changes in cerebellar GABA concentrations (fig. 7b), although an increase in cerebellar glutamate levels (to 288%, P < .0001, fig. 7a) and an increase in dopamine release (to 1899%) was observed. However, after pilocarpine, we observed no further changes in extracellular hippocampal glutamate and dopamine concentrations compared with levels collected from dialysates H7 to H15 (figs. 7a, 8a and 9). After pilocarpine, no further changes in extracellular hippocampal GABA, glutamate and dopamine levels, as compared with levels obtained from collection H7 to H15, were noticed (figs. 7a, 8a and 9). After pilocarpine perfusion, there were no alterations in cerebellar GABA concentrations (fig. 7b). During the same period, an increase in cerebellar glutamate levels (to 162%, P < .0008, from collection C17 to C20) was observed (fig. 8b).

Group 6: Animals receiving an intranigral perfusion with 600 μM vigabatrin. Local administration of 600 μM vigabatrin in substantia nigra could not protect the rats from developing seizures after intrahippocampal administration of pilocarpine. ECoG recordings clearly showed epileptic seizure patterns and changes in extracellular hippocampal transmitter concentrations were similar to extracellular transmitter changes observed in the control group (data not shown).

Discussion

This study provides in vivo data concerning changes in extracellular hippocampal GABA, glutamate and dopamine concentrations in freely moving rats after systemic or local administration of vigabatrin and focally evoked pilocarpine-induced seizure activity. To our knowledge, data regarding alterations in extracellular hippocampal dopamine and GABA concentrations in animals developing pilocarpine-induced seizures, and on changes in extracellular dopamine and glutamate levels after treatment with vigabatrin, have not been reported previously. Extracellular GABA and glutamate overflow in ipsilateral cerebellum was characterized simultaneously, and electrophysiological evidence for the presence or absence of seizures was recorded. Muscarinic receptor stimulation is presumed to be responsible for the onset of pilocarpine-induced seizures, whereas amino acid mechanisms are presumed to maintain sustained seizure activity and to lead to neuronal damage (Turski et al., 1989; Smolders et al., 1997).

Fig. 5. Extracellular hippocampal (H) (a) and cerebellar (C) (b) concentrations (% of the baseline level) (mean ± S.E.M.) (n = 6) of glutamate and GABA in group pretreated with 30 mg/kg/day vigabatrin i.p., before [basal], during [PILO1, PILO2 or C9, C10] and after [H/C11–H/C20] intrahippocampal administration of 10 mM pilocarpine (PILO) from pilocarpine-induced seizures. ECoG recordings showed epileptic seizure patterns and changes in extracellular hippocampal transmitter concentrations were similar to extracellular transmitter changes observed in the control group (data not shown).

Group 5: Animals receiving an intrahippocampal perfusion with 600 μM vigabatrin. Intrahippocampal perfusion with 600 μM vigabatrin did not protect animals from pilocarpine-induced seizures. ECoG recordings showed patterns of tonic-clonic seizures (fig. 6b). Local administration of 600 μM vigabatrin resulted in elevated GABA levels (to 431%, P = .0001) from collection H8 to H15 (fig. 7a), but did not alter glutamate (fig. 8a) nor dopamine levels (fig. 9). During simultaneous pilocarpine perfusion (collection H16 and H17), a decrease in the elevated GABA levels (to 183%, P = .0001, fig. 7a), a decrease in glutamate overflow (to 9%, P = .0001, fig. 8a) and an increase in dopamine release (to 274%, P = .0001, fig. 9) was observed. After intrahippocam-
followed by a significant elevation of extracellular glutamate levels. Simultaneous ECoG recordings showed clear patterns of epileptic seizure activity. This suggests that the sustained increase in glutamate concentrations is related to the pilocarpine-induced convulsions, and therefore may play a key role in maintenance and spread of seizure activity. These results support the critical role glutamate plays in epileptogenesis as suggested by Meldrum (1994). Elevated glutamate levels are presumed to elicit morphological changes of neurons, to be involved in NMDA receptor-mediated excitotoxicity (Isokawa and Mello, 1991; Smolders et al., 1997) and may be partly responsible for the recurrence of seizure activity.

An inhibition of hippocampal GABA efflux during administration of pilocarpine was observed. Whether the decrease in extracellular levels of GABA contributed to seizure onset in this animal model could be hypothesized. However, this contribution is unlikely because the same decrease in extracellular GABA levels was noticed in animals protected from seizures by vigabatrin premedication. In the control group, initial GABA decrease was followed by a sustained increase of the extracellular levels of this inhibitory transmitter, and thus showed a similar profile to glutamate levels. This increase in GABA release may act as a compensatory mechanism to suppress the firing of glutamatergic neurons and to maintain the balance between excitation and inhibition. Indeed, it has been shown that GABA tonically inhibits hippocampal glutamate release (Rowley et al., 1995). However, prolonged stimulation of GABA receptors may lead to GABA desensitization and fading of its inhibitory effect (Krnjevic, 1990), resulting in an imbalance between excitation and inhibition, and deterioration of seizure activity. These observations support the hypothesis that, in the pilocarpine rat model, the cholinergic system is responsible for seizure onset and the amino acid system for sustaining seizure activity.

Extracellular hippocampal dopamine concentrations increased during and after intrahippocampal pilocarpine perfusion. The elevation in the control group, in which all rats developed epileptic convulsions, was higher and longer lasting when compared with the increase in dopamine release observed in vigabatrin-pretreated and seizure-free animals.
Behavioral studies showed that dopamine controls hippocampal excitability via opposing actions at D₁ and D₂ dopamine receptors (Alam and Starr, 1992, 1993). Electrophysiological results indicated that the predominant action of dopamine on hippocampal excitability is inhibition (Benardo and Prince, 1982), and that dopamine tends to decrease the incidence of epileptogenic events (Suppes et al., 1985). The results of the present study suggest that the increase in hippocampal dopamine release during administration of pilocarpine could result from muscarinic receptor stimulation, and that the increase in dopamine release during the convulsions could provide an anticonvulsant and protective effect curtailing epileptic seizure activity.

Cerebellar amino acid changes during pilocarpine-induced seizure activity. In the cerebellum of the control group, the extracellular GABA and glutamate overflow was elevated significantly during the pilocarpine-induced convulsions. Seizure-related stimulation of the hypothalamocerebellar GABA-ergic projection (Dietrichs et al., 1992) may be responsible for the increased cerebellar GABA release, but does not directly explain simultaneous and longer lasting glutamate increases. During limbic seizures and during the interictal period, changes in metabolic activity and cerebral blood flow occur (Park et al., 1992). Hyperperfusion at the ipsilateral side of epileptic focus, as demonstrated in a case report (Overbeck et al., 1990), may partly explain the increased amino acid concentrations in ipsilateral cerebellum observed in this study. The increases in cerebellar amino acid levels may be related to the evoked limbic convulsions rather than to the administration of pilocarpine. These increases were abolished when pilocarpine-induced seizures were prevented by antiepileptic drug treatment, which further suggested limbic involvement.

Effective protection by systemic administration of vigabatrin is reflected by a lack of elevated glutamate levels. Rats pretreated intraperitoneally with vigabatrin were protected from pilocarpine-induced seizures. The decrease in extracellular hippocampal glutamate overflow caused by pilocarpine perfusion was not followed by a persistent increase as demonstrated in the control group. After administration of pilocarpine, the extracellular hippocampal glutamate levels did not raise above basal levels. This impor-
Fig. 9. Extracellular hippocampal (H) concentrations (in % of the baseline level) (mean ± S.E.M.) (n = 6) of dopamine in animals receiving 5 mM or 600 μM vigabatrin via the microdialysis probe in the hippocampus from collection H7 to H23, before [H7–H15], during simultaneous [H16, H17] and after [H18–H23] intrahippocampal administration of 10 mM pilocarpine (PILO) via the microdialysis probe. Each bar represents a 20-min collection period. Asterisks denote only the first values significantly different from corresponding baseline values [P = .01 (**)].

The significant result supports the hypothesis that a sustained release of glutamate is related to the seizures and may point to the absence of NMDA receptor-mediated excitotoxicity. Furthermore, no alterations in rhythmic activity were observed via ECoG after pilocarpine perfusion in these pretreated rats, and no variations in extracellular cerebellar glutamate and GABA levels were observed.

**Vigabatrin influences GABA transmission only after seizure initiation.** Extracellular hippocampal GABA levels in animals premedicated intraperitoneally with vigabatrin increased after administration of pilocarpine. This increase can be explained by the mechanism of action of vigabatrin. Vigabatrin inhibits the mitochondrial enzyme GABA-transaminase, which results in an intracellular augmentation of the presynaptic pool of releasable GABA (Qume et al., 1995). In seizure-free periods, vigabatrin had little effect on ECoG after pilocarpine perfusion in these pretreated rats, and no variations in extracellular cerebellar glutamate and GABA levels were observed.

**Intrahippocampal vigabatrin administration: Role of GABA uptake inhibition and suppression of glutamate release.** Intrahippocampal administration of both 5 mM and 600 μM vigabatrin resulted in significant increases of the extracellular GABA concentrations. These results are in accordance with the acute effects of vigabatrin application observed by Jolkkonen et al. (1992), and suggest that the immediate increase of GABA overflow after local administration of vigabatrin (1.6 and 8 mM) may result from a direct blockade of GABA uptake sites. Thus, the antiepileptic action of GABA-transaminase inhibitors may be partly mediated through inhibition of GABA uptake. In the present study, a significant decrease of extracellular glutamate levels during local perfusion with 5 mM vigabatrin was observed. This dose-dependent decrease was abolished when perfusing with 600 μM vigabatrin. In a postmortem analysis study, Halonen et al. (1991) reported a dose-dependent decrease in the hippocampal tissue content of glutamate after intraperitoneal administration of vigabatrin. A clinical study showed that vigabatrin attenuated elevated glutamate levels in cerebrospinal fluid of newly diagnosed epileptic patients who responded to vigabatrin treatment (Kalviainen et al., 1993). Thus, GABA elevation did not seem to be the only means for achieving seizure control after administration of vigabatrin. Decreased extracellular concentrations of glutamate, whether of neuronal or metabolic origin (Halonen et al., 1995), might exert less stimulation of excitatory amino acid receptors on both neuronal and non-neuronal elements which results in a reinforcement of the protective effect of vigabatrin.

**Partial protection by local administration of vigabatrin?** Rats receiving intrahippocampal administration of vigabatrin were not protected from the focally evoked pilocarpine-induced seizures, although the results of the present study suggest a partial protection. After pilocarpine perfusion, no increases in extracellular hippocampal glutamate concentrations nor in extracellular cerebellar GABA levels were observed. An increase in extracellular cerebellar glutamate overflow of shorter duration was observed in comparison with the control group response. This lack of elevated hippocampal glutamate levels, and the attenuation of the increases in cerebellar GABA and glutamate overflow support the partial protection hypothesis because increases in hippocampal glutamate and in cerebellar amino acids in control animals were related to the epileptic convulsions. A partial neuroprotective effect of vigabatrin has been reported in the kainic acid model for status epilepticus: vigabatrin protected against the induced neuronal damage, but was unable to suppress the convulsions completely (Halonen et al., 1995). Several suggestions can be made concerning the lack of complete neuroprotection after local administration of vigabatrin. First, 3 h of vigabatrin administration may be insufficient to induce full protection and longer term effects were necessary. Therefore, an experiment involving a single systemic injection of vigabatrin was performed. As described above, one injection of vigabatrin before administration of pilocarpine was sufficient to protect the rats from the limbic seizures. Thus, this first hypothesis can be rejected. Second, intrahippocampal vigabatrin application may lower the threshold for convulsions or the applied doses were too high. This brain area is very susceptible to seizure generation. Chronic high-dose vigabatrin treatment of rats has been reported to induce convulsions, possibly related to negative feed back inhibition affecting the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) (Sabers and Gram, 1992). With 5 mM vigabatrin application tonic seizures were observed on the ECoG recordings, while with 600 μM tonic-
clonic convulsions were recorded. Therefore, the latter dose may be more appropriate. Indeed, tonic seizures lack a normal GABA-ergic inhibition (i.e., disinhibition) and in addition to disinhibition anomalous excitatory transmission can occur (Champagnat et al., 1990).

**Role of substantia nigra in seizures**

It is now generally accepted that the substantia nigra plays a critical role in curtailing the spread of seizures and in terminating paroxysmal activity (Moshe and Sperber, 1990). Pharmacological treatments that increase GABA transmission of the substantia nigra were able to suppress experimentally induced seizures (Iadarola and Gale, 1982). The profile of action of antiepileptic drugs on systemically evoked pilocarpine seizures (Iadarola and Gale, 1982). The profile of action of valproate, vigabatrin and aminooxyacetic acid on release of endogenous GABA from cultured neurons. Epilepsy Res. 2: 87–95, 1998. Halonen, T., Pitkänen, A., Saanu, V. and Riekkinen, P. J.: Effects of vigabatrin on neurotransmitter in epilepsy of preclinical research. Epilepsy 33: suppl. 5, S3–S12, 1992.

**Vigabatrin and Pilocarpine Seizures**


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