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Hippocampal and cerebellar extracellular amino acids during pilocarpine-induced seizures in freely moving rats

Ilse Smolders [a], Kristel Van Belle [a], Guy Ebinger [b], Yvette Michotte [a,*]

[a] *Department of Pharmaceutical Chemistry and Drug Analysis, Pharmaceutical Institute, Vrije Universteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium*

[b] *Department of Neurology, University Hospital, Vrije Universteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium*

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Abstract

Limbic seizures were provoked in freely moving rats by intrahippocampal administration of the muscarinic receptor agonist pilocarpine via a microdialysis probe (10 mM for 40 min at 2 μl/min). Changes in extracellular hippocampal and cerebellar glutamate, aspartate and γ-aminobutyric acid (GABA) levels were monitored during and after pilocarpine administration. Effects of systemic or local administration of anticonvulsants on the seizures and concomitant changes in amino-acid concentrations, were investigated. Pilocarpine-induced seizures were completely abolished after intraperitoneal premedication for 7 days with phenobarbital (15 mg/kg per day) and after intrahippocampal administration of 10 mM phenobarbital and 1 mM carbamazepine (180 min at 2 μl/min). Rats premedicated with carbamazepine (5 mg/kg per day) still developed seizures. The changes in extracellular hippocampal amino-acid levels suggest that glutamate, aspartate and GABA are not involved in seizure onset, but may play a role in seizure maintenance and/or spread in the pilocarpine animal model of epilepsy. The increases in extracellular amino acids in ipsi- and contralateral cerebellum following limbic seizures provoked in the hippocampus, probably play a role in the 'reversed' diaschisis phenomenon.

Keywords: Microdialysis; Hippocampus; Cerebellum; Pilocarpine; Epilepsy; Amino acid

1. Introduction

Local application of acetylcholine in certain brain regions, such as the hippocampus, can produce focal epilepsy. The presumed cellular mechanisms of action involve changes in ionic conductances and second messengers by stimulating muscarinic M_1 and M_2 receptors (Dichter and Ayala, 1987), both receptor types occurring in hippocampus. Electrophysiological studies have revealed the main effects of muscarinic receptor activation on Ca^{2+} currents, excitatory postsynaptic potentials and K^+ conductances (Segal, 1988). The result of these complex actions is increased neuronal excitability in the brain. Cruickshank et al. (1994) demonstrated that muscarinic M_1 receptors are preferentially involved in the initiation of seizures produced by the acetylcholine receptor agonist carbachol. Activation of muscarinic M_1 receptors results in the formation of inositol triphosphate and diacylglycerol, whereas muscarinic M_2-mediated activation inhibits adenylate cyclase (Millan et al., 1993; Minisclou et al., 1994). In addition, activation of presynaptic muscarinic M_2 receptors is expected to reduce acetylcholine release and thus inhibit neuronal membrane excitation.

Pilocarpine, a partial agonist at both muscarinic M_1 and M_2 receptors (Hoss et al., 1990), is used after systemic (Turski et al., 1989) and intracerebral administration (Millan et al., 1993) to induce limbic seizures in rodents. The seizures induced by pilocarpine in rats are prevented by systemically administered phenobarbital, diazepam, trimethadione and valproate. Surprisingly, systemic injection of carbamazepine and phenytoin, both drugs of choice for the treatment of 'limbic' seizures in human, are ineffective against pilocarpine-induced seizures (Turski et al., 1989). At the electron microscopic level, the pilocarpine-induced seizures cause massive swelling of dendrites and neuronal cell bodies with the axons remaining relatively spared (Clifford et al., 1987). This cytopathology is similar to excitotoxic tissue damage caused by glutamate, in con-

* Corresponding author. Tel.: (32-2) 477-4744; Fax: (32-2) 477-4113.

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trast to tissue damage caused by acetylcholine, which after synaptic release keeps neurons firing without breaking down the membrane (Olney et al., 1986). It is hypothesized that the cholinergic system has a function in the maintenance of seizures driving amino-acid mechanisms responsible for neuronal damage. However, no consistent changes in excitatory brain amino-acid content after postmortem analysis have been reported in animal models after systemic administration of pilocarpine (Walton et al., 1990; Cavalheiro et al., 1994). Furthermore, it has been suggested that prompt reuptake of glutamate is responsible for not detecting the presumed increase in its extracellular concentrations, since pretreatment with a glutamate uptake inhibitor before the systemic pilocarpine injection resulted in large and sustained increases in extracellular hippocampal glutamate and aspartate (Millan et al., 1993). Contradictory data on changes in extracellular γ-aminobutyric acid (GABA) concentrations prevented the elucidation of the role of this inhibitory transmitter in limbic seizures (Lehmann et al., 1985; Walton et al., 1990; Bruhn et al., 1992).

Both hippocampus and cerebellum are areas of interest to investigate pilocarpine-induced limbic seizures. The hippocampus is one of the most sensitive regions to epilepsy-related damage following convulsions produced by pilocarpine and plays a major role in the development and maintenance of limbic seizures (Turski et al., 1989). It receives a major cholinergic innervation from the medial septal area (Moor et al., 1994) and contains glutamatergic and GABA-ergic input and output neurons (Headley and Grillner, 1990). Little is known about changes in neurotransmitters in the cerebellum during limbic seizures. The dual microdialysis probe technique allows to monitor changes in extracellular cerebellar amino-acid levels following focal seizures evoked in the hippocampus.

In this study, limbic seizures were provoked by administration of pilocarpine via the microdialysis probe in the hippocampus of freely moving rats. Changes in extracellular concentrations of glutamate, aspartate and GABA before, during and following seizure onset were monitored simultaneously in hippocampus, ipsi- and contralateral cerebellum. Additionally, we investigated the effect of a systemic premedication and an acute intrahippocampal administration of the anticonvulsant drugs phenobarbital and carbamazepine on the seizures and the accompanying changes in extracellular amino-acid levels in hippocampus and cerebellum.

2. Materials and methods

2.1. Chemicals and reagents

L-Glutamate, L-aspartate, GABA and pilocarpine were supplied by Sigma (St. Louis, MO, USA). Sodium phenobarbital was purchased from Bios (Brussels, Belgium) and carbamazepine was a gift from Ciba-Geigy (Basle, Switzerland). All other chemicals were analytical reagent grade or better and supplied by Merck (Darmstadt, Germany). Aqueous solutions were made using fresh water purified by a Seralpur pro 90 CN (Belgolabo, Overijse, Belgium) and filtered through a 0.2 μm membrane filter.

As perfusion solution for the microdialysis experiments, a modified Ringer's solution was used containing 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl, pH 7.3. All drugs administered via the microdialysis probe were dissolved in this modified Ringer's solution. When administered intraperitoneally, phenobarbital was dissolved in physiological saline and carbamazepine in a saline/propyleneglycol (50:50) mixture.

2.2. Chromatographic conditions

The microbore liquid chromatography (LC) systems for analysis of glutamate, aspartate and GABA have been previously described (Smolders et al., 1995). Briefly, measurement of glutamate and aspartate was performed, after precolumn derivatization with *o*-phtalaldehyde/β-mercaptoethanol, by gradient elution and fluorescence detection. Analysis of GABA was carried out after precolumn derivatization with *o*-phtalaldehyde/*tert*-butylthiol and iodoacetamide, by isocratic elution and electrochemical detection. The limits of detection obtained with these microbore LC systems were 0.001 μM for GABA and 0.008 μM for glutamate and aspartate. The collected dialysates (40 μl) were splitted so 10 μl injections could be made on both LC systems to determine the three amino acids in each dialysate.

2.3. Microdialysis experiments

2.3.1. Surgery

Male albino Wistar rats, weighing 270–300 g, were anaesthetized with a ketamine/diazepam (50:5 mg/kg) mixture. Intracranial guides (CMA Microdialysis, Stockholm, Sweden) were implanted in hippocampus and ipsilateral cerebellum (groups 1, 3, 4, 5, 6) or in hippocampus and contralateral cerebellum (group 2) according to the following coordinates towards bregma: L +4.6, A −5.6 and V +4.6 for hippocampus, and L +3.0 (ipsilateral) or −3.0 (contralateral), A −13.0 and V +4.0 for the cerebellum (Paxinos and Watson, 1986). Immediately after surgery the inner guides were replaced by CMA/10 microdialysis probes (CMA Microdialysis, Stockholm, Sweden) with a 3 mm membrane length and were continuously perfused with modified Ringer's solution at a flow rate of 2 μl/min (CMA/100 microdialysis pump, CMA Microdialysis). The animals were allowed to recover from surgery overnight. During the experiment, dialysates were collected every 20 min, yielding 40 μl samples, from freely moving animals which are divided into six groups.

2.3.2. Group 1 (n = 6): changes in hippocampal and in ipsilateral cerebellar extracellular amino-acid concentrations determined during pilocarpine-induced seizures in the hippocampus

The hippocampus was perfused with modified Ringer's solution and six dialysates were collected in basal conditions. Then, pilocarpine (10 mM) was administered via the microdialysis probe for 40 min (two dialysates), followed by perfusion with modified Ringer's solution for another seven dialysates. Simultaneously, the ipsilateral cerebellum was perfused with modified Ringer's solution and 15 dialysates were collected throughout the experiment.

2.3.3. Group 2 (n = 6): changes in contralateral cerebellar extracellular amino-acid concentrations determined during pilocarpine-induced seizures in the hippocampus

For the hippocampus the same protocol was followed as described for group 1, but now the contralateral cerebellum was perfused with modified Ringer's solution and dialysates were collected simultaneously throughout the experiment.

2.3.4. Groups 3 (n = 4) and 4 (n = 4): changes in hippocampal and ipsilateral cerebellar extracellular amino-acid concentrations determined during pilocarpine-induced seizures in the hippocampus of rats premedicated intraperitoneally with phenobarbital or carbamazepine

The animals were premedicated for 7 days prior to the experiment with phenobarbital (15 mg/kg per day intraperitoneally) (group 3) or carbamazepine (5 mg/kg per day intraperitoneally) (group 4). Then, the same perfusion scheme as described for group 1 was performed.

2.3.5. Groups 5 (n = 4) and 6 (n = 4): changes in hippocampal and ipsilateral cerebellar extracellular amino-acid concentrations determined during pilocarpine-induced seizures in the hippocampus of rats while administrating phenobarbital or carbamazepine via the microdialysis probe

The hippocampus was perfused with modified Ringer's solution and four dialysates were collected in basal conditions. Then, during nine dialysates phenobarbital (10 mM) (group 5) or carbamazepine (1 mM) (group 6) was administered via the microdialysis probe, followed by a simultaneous perfusion of pilocarpine (10 mM) and phenobarbital (10 mM) or pilocarpine (10 mM) and carbamazepine (1 mM) for 40 min. Continuous perfusion with phenobarbital (10 mM) or carbamazepine (1 mM) was performed for another seven dialysates. Simultaneously, the ipsilateral cerebellum was perfused with modified Ringer's solution and dialysates were collected throughout the experiment.

2.4. Statistical analysis

All results in tables and figures are expressed as the mean amino-acid dialysate concentrations in μM with standard deviations (S.D.). The basal values in the figures are the mean of the three last stable amino-acid dialysate concentrations obtained in basal conditions, prior to pilocarpine or drug administration via the microdialysis probe. The dialysate concentrations were not corrected for the recovery across the dialysis membrane.

Statistical analysis of the changes of amino-acid dialysate concentrations in time was performed using one-way analysis of variance (ANOVA) for repeated measures and Fisher's protected least significance difference (Fisher's PLSD) post hoc test ($\alpha = 0.05$). The significance of differences between basal or peak amino-acid dialysate concentrations was determined by Mann Whitney's test ($\alpha = 0.05$).

3. Results

3.1. Groups 1 and 2: changes in hippocampal and cerebellar extracellular amino-acid levels during pilocarpine-induced seizures evoked in hippocampus

In all rats we observed limbic seizures, characterized by tremor, scratching and wet dog shakes starting between 30 min to 1 h after the start of perfusion with pilocarpine, followed by myoclonic movements of the limbs, salivation and occasionally falling at the end of the experiment.

Basal dialysate concentrations of glutamate, aspartate and GABA obtained from hippocampus, ipsi- and contralateral cerebellum are reported in Table 1. Pilocarpine administration (40 min) in the hippocampus, resulted in significant decreases of the basal hippocampal overflow of glutamate to 12% ($P = 0.0001$) (Fig. 1a), aspartate to 36% ($P = 0.0006$) and GABA to 20% ($P = 0.0001$) (Fig. 1b). For both glutamate and GABA, ceasing the administration

Table 1
Basal glutamate, aspartate and GABA dialysate concentrations in control rats (CON) and in rats premedicated with phenobarbital (PB) (15 mg/kg per day intraperitoneally) or carbamazepine (CBZ) (5 mg/kg per day intraperitoneally)

	Glutamate	Aspartate	GABA
CON			
hippocampus	1.47 ± 0.59	0.11 ± 0.04	0.04 ± 0.01
ipsilateral cerebellum	2.47 ± 0.49	0.23 ± 0.06	0.09 ± 0.04
contralateral cerebellum	2.19 ± 0.51	0.29 ± 0.12	0.10 ± 0.04
PB			
hippocampus	1.28 ± 0.29	0.07 ± 0.03	0.04 ± 0.02
ipsilateral cerebellum	2.45 ± 0.17	0.12 ± 0.05	0.05 ± 0.02
CBZ			
hippocampus	1.47 ± 0.94	0.14 ± 0.07	0.03 ± 0.01
ipsilateral cerebellum	1.87 ± 0.55	0.18 ± 0.10	0.02 ± 0.01 [a]

Values are expressed in μM (mean ± S.D.) without correction for the recovery across the dialysis membrane, $n = 6$ for the control group, $n = 4$ for the premedicated animals.

[a] denotes significantly different value compared to the corresponding basal value obtained in the control group ($P < 0.05$).

of pilocarpine resulted in a significant increase to 230% and 240% respectively ($P = 0.0001$), with a significant elevation remaining until the end of the experiment. Because of its similar behaviour as glutamate throughout all the experiments, no figures of aspartate will be shown.

During the pilocarpine-induced seizures, simultaneous elevations of glutamate to 176% ($P = 0.0009$) (Fig. 2a), aspartate to 172% ($P = 0.0002$) and GABA to 242% ($P = 0.0001$) (Fig. 2b) in ipsilateral cerebellum; and of glutamate to 215% ($P = 0.008$) (Fig. 2a), aspartate to 263% ($P = 0.004$) and GABA to 164% ($P = 0.001$) (Fig. 2b) in contralateral cerebellum, were observed. Ipsilaterally, the GABA changes are earlier and more marked: the GABA increase in ipsilateral cerebellum was significantly higher ($P = 0.02$) than the increase at the contralateral side. Contralaterally, it seems that the glutamate changes are more prominent. At the end of the experiments, glutamate, aspartate and GABA dialysate concentrations obtained from ipsi- and contralateral cerebellum returned to basal values.

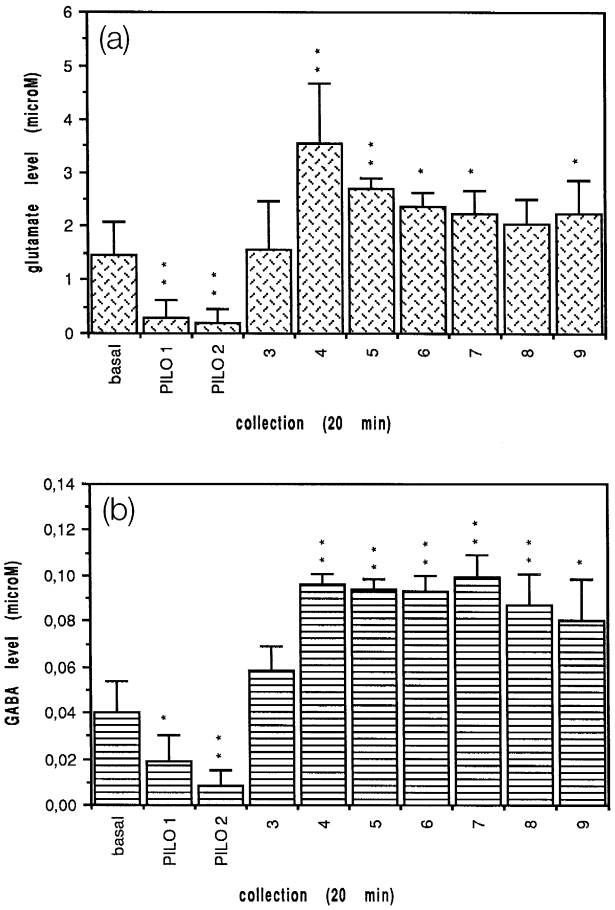

Fig. 1. Hippocampal dialysate concentrations (in μM) (mean ± S.D.) ($n = 6$) of glutamate (*a*) and GABA (*b*) before (basal), during (PILO1, PILO2) and after (3–9) administration of pilocarpine (PILO) (10 mM) via the microdialysis probe. Each bar represents a 20 min collection period. Asterisks denote values significantly different from corresponding baseline values ($P < 0.05$ * or 0.01 * *).

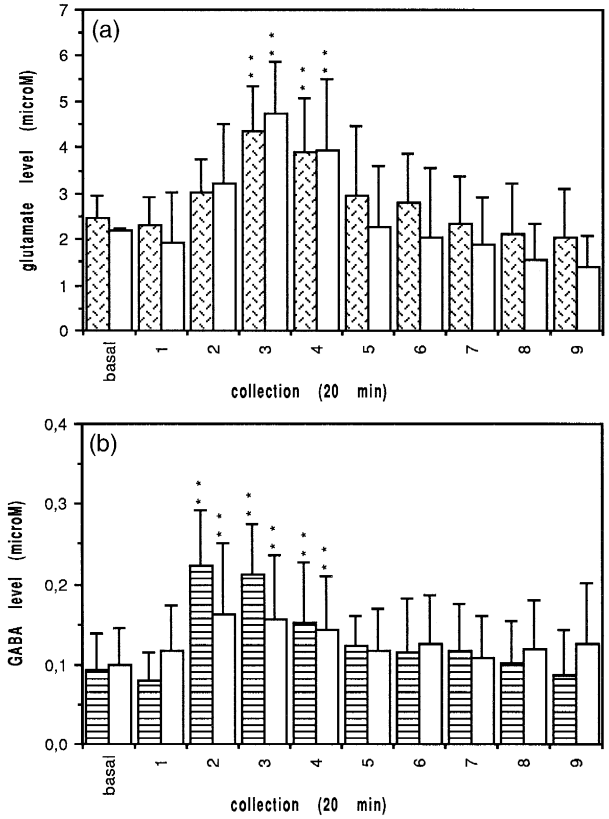

Fig. 2. Glutamate (*a*) and GABA (*b*) dialysate concentrations (in μM) (mean ± S.D.) ($n = 6$) in ipsilateral (striped bars) and contralateral cerebellum (open bars) before (basal), during (1–2) and after (3–9) administration of pilocarpine (PILO) (10 mM) in the hippocampus via the microdialysis probe. Asterisks denote values significantly different from corresponding baseline values ($P < 0.01$ * *).

3.2. Group 3: changes in hippocampal and ipsilateral cerebellar extracellular amino-acid levels determined during pilocarpine-induced seizures evoked in the hippocampus of rats premedicated intraperitoneally with phenobarbital

None of the animals premedicated intraperitoneally with phenobarbital developed limbic seizures similar to those described above (groups 1 and 2). The basal amino-acid dialysate concentrations for hippocampus and ipsilateral cerebellum of the with phenobarbital premedicated animals are given in Table 1. These extracellular hippocampal and cerebellar amino-acid levels were not significantly different from the levels obtained in non-premedicated animals (group 1, see also Table 1).

During the local perfusion of the hippocampus with pilocarpine in these premedicated rats, a decrease in extracellular hippocampal dialysate levels of glutamate to 9% ($P = 0.0001$) (Fig. 3a), aspartate to 19% ($P = 0.0006$) and GABA to 26% ($P = 0.01$) (Fig. 3b) was observed, similar as in the animals of group 1. However, these decreases in the hippocampus were not followed by an elevation of the

glutamate, aspartate and GABA levels, as reported for the animals of group 1. In the ipsilateral cerebellum, extracellular glutamate (Fig. 3a), aspartate and GABA (Fig. 3b) concentrations were unaltered.

3.3. Group 4: changes in hippocampal and ipsilateral cerebellar extracellular amino-acid levels determined during pilocarpine-induced seizures evoked in the hippocampus of rats premedicated intraperitoneally with carbamazepine

Basal amino-acid dialysate concentrations obtained from hippocampus and ipsilateral cerebellum of rats premedicated intraperitoneally with carbamazepine are given in Table 1. The basal cerebellar GABA levels were significantly lower ($P = 0.028$) than those in the non-premedicated animals (group 1). All rats premedicated intraperitoneally with carbamazepine developed similar limbic seizures as the rats of group 1 after intrahippocampal administration of pilocarpine. Similar changes in extracellular amino-acid levels were noticed in hippocampus and ipsilateral cerebellum. A significant decrease of the basal hippocampal overflow of glutamate to 7% ($P = 0.0001$) (Fig. 4a), aspartate to 7% ($P = 0.01$) and GABA to 27% ($P = 0.0001$) (Fig. 4b), was followed by a significant elevation of the extracellular glutamate levels to 211% ($P = 0.0001$) (Fig. 4a). However, no increase in GABA overflow was observed (Fig. 4b). During the pilocarpine-induced seizures evoked in the hippocampus, simultaneous elevations of glutamate to 178% ($P = 0.0008$) (Fig. 4a), aspartate to 206% ($P = 0.01$) and GABA to 281% ($P = 0.04$) (Fig. 4b) in ipsilateral cerebellum occurred. At the end of the experiments, extracellular glutamate, aspartate and GABA dialysate concentrations in hippocampus and ipsilateral cerebellum returned to basal values.

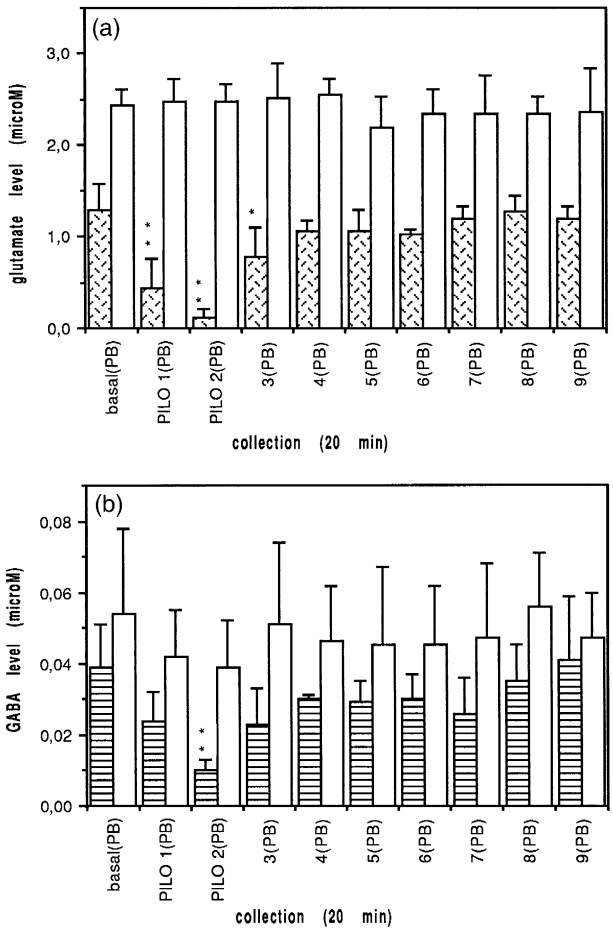

Fig. 3. Hippocampal (striped bars) and cerebellar (open bars) dialysate concentrations (in μM) (mean ± S.D.) ($n = 4$) of glutamate (*a*) and GABA (*b*) before (basal(PB)), during (PILO1(PB), PILO2(PB)) and after (3(PB)-9(PB)) administration of pilocarpine (PILO) (10 mM) in the hippocampus via the microdialysis probe in rats premedicated with phenobarbital (PB) (15 mg/kg per day intraperitoneally) 7 days prior to the experiment. Asterisks denote values significantly different from corresponding baseline values ($P < 0.05$ * or 0.01 * *).

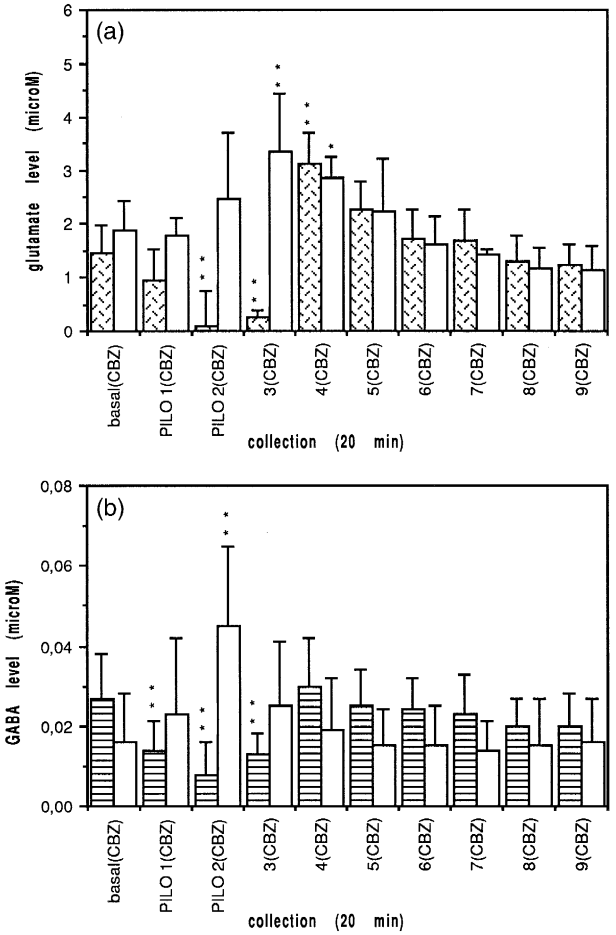

Fig. 4. Hippocampal (striped bars) and cerebellar (open bars) dialysate concentrations (in μM) (mean ± S.D.) ($n = 4$) of glutamate (*a*) and GABA (*b*) before (basal(CBZ)), during (PILO1(CBZ), PILO2(CBZ)) and after (3(CBZ)-9(CBZ)) administration of pilocarpine (PILO) (10 mM) in the hippocampus via the microdialysis probe in rats premedicated with carbamazepine (CBZ) (5 mg/kg per day intraperitoneally) 7 days prior to the experiment. Asterisks denote values significantly different from corresponding baseline values ($P < 0.05$ * or 0.01 * *).

3.4. Group 5: changes in hippocampal and ipsilateral cerebellar extracellular amino-acid levels determined during pilocarpine-induced seizures evoked in hippocampus while administrating phenobarbital via the microdialysis probe

Intrahippocampal administration of phenobarbital via the microdialysis probe resulted in elevated glutamate (to 453%) ($P = 0.0001$) (Fig. 5) and aspartate (to 294%) ($P = 0.02$) levels in the hippocampus. No changes in extracellular GABA concentrations were noticed. During and after the intrahippocampal pilocarpine administration, no further changes in extracellular hippocampal glutamate levels compared to basal values were observed, but a significant decrease compared to the under phenobarbital elevated levels was noticed. There were no changes in cerebellar amino-acid dialysate levels and no limbic seizures were developed during the entire experiment.

3.5. Group 6: changes in hippocampal and ipsilateral cerebellar extracellular amino-acid levels determined during pilocarpine-induced seizures evoked in hippocampus while administrating carbamazepine via the microdialysis probe

Intrahippocampal administration of carbamazepine had no effect on glutamate (Fig. 6), aspartate and GABA levels in hippocampus nor in cerebellum. During pilocarpine co-administration in the hippocampus, a significant decrease in the extracellular concentration was observed for glutamate (to 15%) ($P = 0.0001$) (Fig. 6) and aspartate (to 10%) ($P = 0.0006$), but not for GABA. In contrast to the data obtained for rats premedicated intraperitoneally with carbamazepine, the intrahippocampal pilocarpine perfusion in rats of group 6 provoked no seizures and after ceasing the pilocarpine administration no further changes in amino-acid levels in hippocampus or cerebellum appeared.

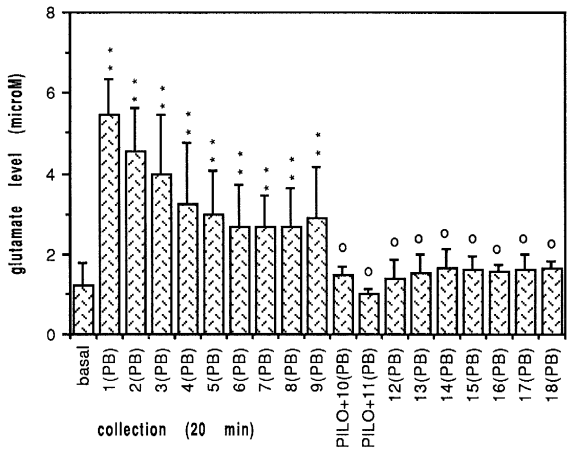

Fig. 5. Hippocampal dialysate concentrations (in μM) (mean ± S.D.) (n = 4) of glutamate in basal conditions and during intrahippocampal administration of phenobarbital (PB) (10 mM) alone (1(PB)–9(PB9); 12(PB)–18(PB)) or during co-administration of pilocarpine (PILO) (10 mM) (PILO + 10(PB), PILO + 11(PB)) via the microdialysis probe. Asterisks denote values significantly different from corresponding baseline values ($P < 0.05$ * or 0.01 * *). ° denotes values significantly different from the under phenobarbital elevated glutamate levels ($P < 0.05$°).

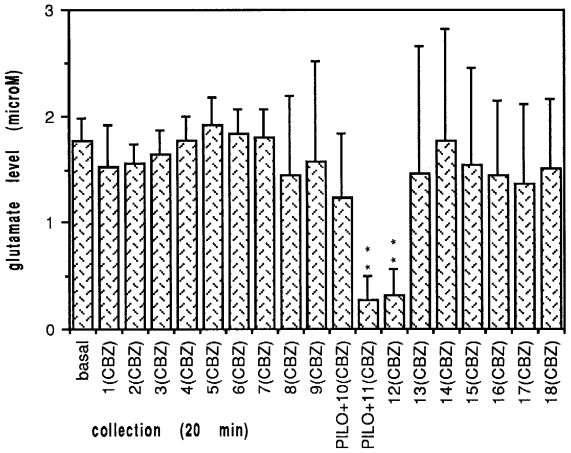

Fig. 6. Hippocampal dialysate concentrations (in μM) (mean ± S.D.) ($n = 4$) of glutamate in basal conditions and during intrahippocampal administration of carbamazepine (CBZ) (1 mM) alone (1(CBZ)–9(CBZ); 12(CBZ)–18(CBZ)) or during co-administration of pilocarpine (PILO) (10 mM) (PILO + 10(CBZ), PILO + 11(CBZ)) via the microdialysis probe. Asterisks denote values significantly different from corresponding baseline values ($P < 0.01$ * *).

4. Discussion

Brain microdialysis is an interesting technique to study changes in extracellular amino-acid levels during seizures and several studies have been performed in different animal models (Lehmann et al., 1985; Vezzani et al., 1985; Zhang et al., 1991; Bruhn et al., 1992; Millan et al., 1993) and also in human epileptic foci (Carlson et al., 1992; Ronne-Engström et al., 1992; During and Spencer, 1993). We studied the effect of pilocarpine-induced hippocampal seizure activity on the extracellular levels of excitatory and inhibitory amino acids involved in hippocampal and cerebellar neurotransmission.

Our results show an initial inhibition of the glutamate, aspartate and GABA release during the local application of pilocarpine in the hippocampus of freely moving rats. Activation of presynaptic muscarinic M_2 receptors on hippocampal glutamatergic nerve terminals can inhibit directly the glutamate release (Marchi et al., 1989). Furthermore, a current- and voltage-clamp study showed that acetylcholine – acting on muscarinic receptors – produces first an initial suppression of the excitatory postsynaptic potentials, followed by a long-lasting facilitation of NMDA receptor-mediated responses (Markam and Segal, 1990). The latter effect of acetylcholine on excitatory postsynaptic potentials appears easily to reconcile with the fact that acetylcholine is thought to be important in the excita-

tory processes underlying epilepsy. The decrease in extracellular glutamate/aspartate levels seen in our experiments confirms that in the pilocarpine animal model of limbic seizures, an increase in excitatory amino acids is not responsible for the onset of seizures. In contrast, in the human hippocampus extracellular levels of glutamate rise prior to the seizures (During and Spencer, 1993; Massieu et al., 1995). Millan et al. (1993) observed an immediate increase in extracellular glutamate/aspartate during intrahippocampal pilocarpine application in anaesthetized rats. The reasons for these discrepancies between freely moving and anaesthetized animals are unclear. For GABA, presynaptic muscarinic M_1 receptors were identified in the septal nuclei with activation leading to disinhibition (Hasuo et al., 1988). In striatum, acetylcholine inhibits GABA release via presynaptic muscarinic non-M_1 receptors (Marchi et al., 1990). We observed in this study a similar inhibition of hippocampal GABA efflux during pilocarpine administration. Whether the decreased extracellular GABA concentrations contribute to seizure onset in this pilocarpine animal model could be hypothesized, but is unlikely since the same decrease in extracellular GABA concentrations was noticed in the animals protected from seizures by phenobarbital premedication.

The extracellular levels of glutamate/aspartate have been reported to be tightly regulated, even during severe seizures (Millan et al., 1993; Wasterlain et al., 1993; Cavalheiro et al., 1994). In this study, after ceasing the intrahippocampal pilocarpine administration the decrease in glutamate/aspartate release was followed by a significant elevation of the extracellular levels of both excitatory amino acids. It is obvious that these increases are related to the pilocarpine-induced convulsions. The sustained increase in glutamate levels may therefore play a key role in the maintenance and spread of the seizures which continue until the end of the experiment. This supports the hypothesis of the cholinergic system being responsible for seizure onset and maintenance, and of driving amino-acid mechanisms to support sustained seizure activity with neuronal damage (Turski et al., 1989), probably mediated via NMDA receptors (Schwarcz and Meldrum, 1985; Isokawa and Mello, 1991). The initial GABA decrease during pilocarpine administration was followed by an increase of the extracellular levels of this inhibitory transmitter, which also sustained during the whole experiment. This increased GABA release may act as a compensatory mechanism and suppress the firing of the glutamatergic neurons and minimize neuronal injury. Indeed, it has been suggested that the balance between excitatory and inhibitory transmitters may be more relevant in reflecting the contribution to brain damage than the absolute extracellular excitatory amino-acid concentrations (During and Spencer, 1993).

Further, our findings confirm that pilocarpine-induced seizures are prevented in rats premedicated intraperitoneally with phenobarbital (Turski et al., 1989). Phenobarbital did not abolish the diminished amino-acid release during pilocarpine administration, since barbiturates themselves are able to reduce presynaptic release of GABA and glutamate (Tauboll et al., 1993). It did prevent the persistent enhanced extracellular glutamate and GABA levels after pilocarpine perfusion. These results agree with the hypothesis that decreased GABA levels are not directly involved in the onset of pilocarpine-induced seizures, and that the enhanced release of glutamate is related to the seizures. During intrahippocampal phenobarbital administration, significant elevations of extracellular glutamate/aspartate levels occurred. Locally applied barbiturates induce various changes in glutamate neurotransmission (Cai and McCaslin, 1993). Besides inhibiting presynaptic glutamate release, phenobarbital also antagonizes glutamate-mediated postsynaptic excitation (McDonald and Barker, 1979). The increase in glutamate release induced by locally applied phenobarbital could be the consequence of postsynaptic blockade of excitatory amino-acid receptors, leading to a feedback enhancement of glutamate release. The excitatory amino-acid receptor subtype involved, is probably the kainate receptor. Phenobarbital directly interacts with kainate receptors or with processes selectively coupled to activation of kainate receptors, but not with other excitatory amino-acid receptors (Frandsen et al., 1990). It must be mentioned that this enhancement of extracellular hippocampal glutamate did not result in seizure activity. Nevertheless, the reported extracellular glutamate levels may be sufficiently high to produce neurotoxicity. 10–100 μM glutamate is toxic to neurons grown in culture (Choi, 1988), but neurons in vivo are less vulnerable due to the presence of highly effective uptake carriers (Bruhn et al., 1992). Elevation of extracellular levels of endogenous glutamate is however not sufficient for induction of neuronal degradation. Other factors that facilitate overactivation of excitatory amino-acid receptors, for instance hyperexcitation present during seizures, seemed to be necessary to induce neuronal damage. The phenobarbital-induced elevated glutamate levels significantly decreased during and after pilocarpine perfusion. No epileptic seizures were observed, indicating protective effects of locally administered phenobarbital in this animal model.

Animals premedicated intraperitoneally with carbamazepine were not protected against pilocarpine-induced seizures (Turski et al., 1989) and our results show similar changes in extracellular glutamate levels as described for control rats. However, after the increase basal glutamate levels were rapidly reached. In addition, basal cerebellar GABA concentrations were significant lower in rats premedicated with carbamazepine than in non-premedicated ones and in hippocampus no increases in extracellular GABA levels were noticed after the decrease due to pilocarpine administration. These attenuations of glutamate and GABA release are probably due to blockade of voltage-dependent Na^+ channels – main mechanism of action of carbamazepine – on glutamatergic and GABAergic

(Wolf et al., 1993) nerve terminals. Furthermore, this may indicate a partial protective effect of systemic pretreatment with carbamazepine. Intrahippocampal carbamazepine administration did not abolish the inhibition of glutamate release during pilocarpine perfusion. In contrast to the intraperitoneal premedication, it did suppress the pilocarpine-induced seizures and all the accompanying changes in amino-acid levels observed in control rats after ceasing pilocarpine administration. These contradictory results, obtained by using different administration routes of an anticonvulsant drug, confirm that it is important to recognize the limits of the existing experimental models of epilepsy.

Limbic epilepsy is a complex phenomenon showing changes in cerebral blood flow not limited to the epileptogenic focus but involving multiple cerebral areas. During the interictal period, depression of perfusion, metabolism and neuronal function in a remote area is referred to as diaschisis phenomenon (Meyer et al., 1993). A parallel reduction in blood flow and oxygen use in the contralateral cerebellum is called crossed cerebellar diaschisis. Reversed crossed diaschisis is characterized by hyperperfusion in hippocampus, amygdala and contralateral cerebellum during the ictal phase (Park et al., 1992). A few case reports using magnetic resonance imaging indicated reversed crossed cerebellar diaschisis in patients with focal epilepsy (Duncan et al., 1987; Park et al., 1992; Stübgren, 1995). We demonstrate in this study increases in extracellular GABA, glutamate and aspartate levels in ipsi- and contralateral cerebellum during limbic seizures evoked by intrahippocampal pilocarpine perfusion. These changes in cerebellar amino acids are related to the seizures rather than to pilocarpine administration, because they are completely abolished when pilocarpine-induced seizures are prevented by anticonvulsant drugs. We suggest a possible role of these amino acids in the reversed cerebellar diaschisis phenomenon during limbic seizures. Interestingly, the extracellular GABA changes are earlier and more marked ipsilaterally, whereas contralaterally the extracellular glutamate changes are more prominent. These data link in strongly with the increased cerebellar metabolism and perfusion during focal seizures. The hippocampus is connected with the hypothalamus through the fibers of the Papez circuit (Niewenhuys et al., 1988), and via hypothalamocerebellar GABA-ergic and histaminergic projections with the cerebellum (Dietrichs et al., 1992). Seizure related stimulation of this pathway may be responsible for the marked GABA release in the ipsilateral cerebellum, but does not explain simultaneous but less pronounced changes on the contralateral side. Excessive neuronal firing in the cortex as a result of seizure spread, can stimulate the excitatory crossed corticopontocerebellar pathway leading to a marked glutamate release and probably contributing to excitotoxic cerebellar atrophy (Stübgren, 1995). Another case report showed also hyperperfusion at the ipsilateral side of the focus (Overbeck et al., 1990), possibly explaining the increase in extracellular glutamate levels in ipsilateral cerebellum.

In conclusion, brain microdialysis combined with microbore liquid chromatography was used to determine seizure-related hippocampal changes of extracellular amino-acid levels in a pilocarpine model of limbic epilepsy in rats. Simultaneously, seizure-related enhanced extracellular amino-acid levels were detected in ipsi- and contralateral cerebellum. Further exploration of the use of microdialysis may provide more information for assessing the usefulness of chemical models in selecting antiepileptic drugs and modeling the human conditions.

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