Effects of Three Anti-Seizure Drugs on Cholinergic and Metabolic Activity in Experimental Status Epilepticus

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Received, February 22, 2019; Revised, June 15, 2019; Accepted, July 9, 2019; Published, July 19, 2019.

Abstract - Purpose. Status epilepticus (SE) is characterized by recurrent seizure activity and can be drug-resistant. Knowledge of neuronal and metabolic activity of the brain during SE may be helpful to improve medical care. We here report the effects of three anti-seizure drugs on changes of acetylcholine energy metabolites and oxidative stress during SE. **Methods.** We used the lithium-pilocarpine model in rats to induce SE and in vivo-microdialysis to monitor cholinergic and metabolic activity in the hippocampus. We measured extracellular concentrations of acetylcholine, glucose, lactate, pyruvate, glycerol and isoprostanes before and during SE, and after acute treatment with pregabalin, valproic acid, and levetiracteam. **Results.** Upon onset of SE, acetylcholine (ACh) release increased six- to eightfold. Glucose was increased only transiently by 30% but lactate levels rose four-fold, and extracellular concentrations of glycerol ten-fold. Isoprostanes are markers of oxidative stress and increased more than 20-fold. Two hours after pilocarpine adminstration, rats were treated with pregabalin (100 mg/kg), levetiracetam (200 mg/kg) or valproic acid (400 mg/kg) by i.p. injection. All three drugs stopped seizure activity in a delayed fashion, but at the doses indicated, only animals that received levetiracetam reached consciousness. All drugs reduced ACh release within 60-120 minutes. Lactate/pyruvate ratios, glycerol and isoprostanne levels were also reduced significantly after drug administration. **Conclusions.** Hippocampal ACh release closely follows seizure activity in SE and is attenuated when SE subsides. Pregabalin, valproic acid and levetiracetam all terminate seizures in the rat SE model and attenuate cholinergic and metabolic changes within two hours.

INTRODUCTION

Status epilepticus (SE) is defined as continuous seizure activity that does not resolve spontaneously (1). SE is a medical emergency and is frequently lethal. If SE is survived, temporal lobe epilepsy is a common consequence. Treatment of SE is usually by intravenous administration of a benzodiazepine such as lorazepam (2). In refractory cases, several anticonvulsant drugs have been considered as 2nd line treatments, including valproic acid, fosphenytoin and more recently levetiracetam (3). Valproic acid is an old broad-spectrum anti-seizure drug (ASD) for which numerous activities have been suggested including GABAergic enhancement and blockade of sodium channels (4); its activity in SE is well documented (5). Levetiracetam is a 2nd generation ASD with a good clinical record; it evidently acts by interaction with synaptic vesicle protein 2a (6,7). In the present study, we have also included pregabalin, an ASD with a broad range of indications that acts on a subunit of voltage-operated calcium channels (8,9).

Drug development for epilepsy depends on experimental models (10,11). For status epilepticus, various chemically induced seizure models have been described, with the lithium-pilocarpine model in rats being one of the most popular (12,13). In this model, seizures develop in animals that were pretreated with lithium chloride upon administration of pilocarpine, a muscarinic agonist (14). Various parameters were monitored during seizures to understand the underlying pathophysiology, including neurotransmitter release, glycolytic metabolism and oxidative stress (15,16). Overactivation of glutamatergic systems and loss of GABAergic inhibition were suggested as cause of continuous seizures (17,18).

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Very recently, studies with microdialysis and studies using drug cocktails to terminate SE have pointed to a role of cholinergic system in seizure activity (19,20). In our work in rat hippocampus, extracellular acetylcholine (ACh) was found to increase several-fold when seizures developed (19) while neuronal hyperexcitation was reflected in an increase of aerobic glycolysis and ATP release (21,22). In parallel, membrane damage and oxidative stress were prominent (21). Moreover, recent work demonstrated the effectiveness of scopolamine, a muscarinic antagonist, to terminate drug-resistant seizures (20,23), and scopolamine also effectively reduced seizure activity induced by electrical stimulation of the basal amygdala (24). These findings point to a more general role of cholinergic activation in SE.

In the present work, we have followed up on our previous findings and compared three anti-seizure drugs (pregabalin, valproic acid and levetiracetam) with respect to their acute actions in rats undergoing SE. We monitored ACh release, energy metabolites and indicators of cell damage. Our results show that these parameters are closely coupled to seizure activity, and that seizure-induced changes in these parameters are significantly attenuated when seizures are terminated by ASD administration.

METHODS

Animals

Male Sprague–Dawley rats were obtained from Janvier laboratories (Le Genest-Saint-Isle, France) and housed in standard rodent cages (3 rats per cage) in the departmental animal house facility at 22°C, 50-70% humidity and a day/night cycle of 12/12 h. They had free access to food (Altromin 1320, Lage, Germany) and water. After at least 1 week of adaptation in the animal room, 6-8 week-old rats (220–300 g) were randomized to study groups. A total of 45 rats were used; at 4% mortality (2 animals) and 3 further experiments that failed (leaking or blocked microdialysis probes etc.), a total of 40 successful experiments was performed for the present results. All experiments were carried out in accordance with the guidelines as set and approved by the responsible government agency (Regierungspräsidium Darmstadt, Germany).

Microdialysis procedure

Rats were transferred to microdialysis cages at least 24 hours before probe implantation and had free access to food and water. Rats were weighed and anesthetized with isoflurane (induction dose 4%, maintenance dose 1.5–2% v/v) in synthetic air (Praxair, Düsseldorf, Germany), and placed in a stereotaxic frame (Stoelting, Chicago, USA). Self-constructed, Y-shaped, concentric dialysis probes with an outer diameter of 280 µm, an exchange length of 3.5 mm and a molecular cut-off of 10,000 Da (25) were implanted in the right ventral hippocampus using the following coordinates (from bregma): AP -5.2 mm; L –5.2 mm; DV –7.0 mm (26). After surgical implantation of probes, all rats received an injection of lithium chloride (3 mmol/kg i.p., equivalent to 127 mg/kg). Also, all rats received 2 ml of Ringer-lactate solution i.p. to prevent dehydration, and a treatment with local anesthetic to suppress wound pain; then they were allowed to recover over night. On the following day, the microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$ and 1.2 mM $MgCl_2$; all Merck VWR, Darmstadt, Germany) containing 0.1 µM neostigmine. The perfusion rate was 2 µl/min, and efflux from the microdialysis probe was collected in 15 min intervals (30 minutes for isoprostanes). After equilibration, samples were collected for 90 min (six samples), which were used to determine baseline levels of the analytes of interest. Then, pilocarpine (30 mg/kg s.c. in saline) was given to induce SE which developed within 25-30 min. 2 h after pilocarpine (i.e. after ca. 90 min of ongoing seizures), the animals received either pregabalin (100 mg/kg), levetiracetam (200 mg/kg) or valproic acid (400 mg/kg) by i.p. injection. Microdialysis was continued for 2 hours, then rats were anethetized with isoflurane and sacrificed. Brain slices were prepared to verify the correct position of the microdialysis probes.

Behavioral scoring

Rat behavior was scored every 5 min after pilocarpine injection according to a modified Racine scale (27). The following grades of seizure development were distinguished: Stage 0, no phenotype, no tremor or seizures. Stage 1, tremor and signs of autonomic stimulation such as piloerection, salivation, chromodacryorrhea, diarrhea. Stage 2, stereotypical behavior, continuous sniffing or chewing, facial twisting, stereotypical movements (e.g., head bobbing), rats may be calm

and stare into space. Stage 3, motor seizures in limbs, e.g., during walking, fore-limb clonus, rearing and lowering of body, wet dog shakes, animals conscious and respond to tactile stimuli. Stage 4, status epilepticus, typically with alternating rearing and lowering of the body every 30-60 s, and unconsciousness (animal does not respond to tactile stimuli). Stage 5 (tonic-clonic seizures) were not observed in our experiments.

HPLC analysis of acetylcholine

Acetylcholine (ACh) and choline in dialysates were determined by microbore HPLC-ECD using the Eicom HTEC-500 system (Kyoto, Japan) that included degasser, low-speed pump, pre- and separation column, enzyme reactor carrying immobilised AChE and choline oxidase, and electrochemical detector with a platinum electrode operating at 500 mV relative to the Ag/AgCl reference electrode (19,22). The system is contained in a temperature controlled cabinet. ACh was retained on the separation column and cleaved to choline and acetate by acetylcholinesterase (AChE); choline was then oxidised to hydrogen peroxide by choline oxidase. Hydrogen peroxide was detected electrochemically. The mobile phase consisted of KHCO3 50 mmol/L (Merck, Darmstadt, Germany), EDTA-2Na 134.3 µmol/L (BDH, Poole, UK) and sodium decane-1-sulfonate 1.64 mmol/L (Alfa Aesar, Karlsruhe, Germany) in RotisolV® HPLC gradient grade water (Sigma-Aldrich, Munich, Germany), brought to pH 8.4. The flow rate was 150 µL/min. At an injection volume of 5 µL, the detection limit of this system was 1-2 fmol/injection. Data acquisition was performed using EPC-500 PowerChrom software.

Chemical analysis of microdialysates

Glucose, lactate, pyruvate and glycerol concentrations in the dialysate samples were quantified by a colorimetric method (530 nm) using a CMA-600 microanalyzer (CMA Microdialysis, Solna, Sweden).

Quantification of oxidative stress

Isoprostanes were determined by the 8-isoprostane express EIA kit manufactured by Cayman Chemicals (Biomol, Hamburg, Germany). The kit was developed according to the manufacturer´s protocol. The limit of detection was approximately 10 pg/ml.

STATISTICAL ANALYSIS

Data in Figs. 1-3 were calculated as percentages of baseline levels (100%) of respective analytes from individual animals, averaged from 6 consecutive 15 min-sampling periods for each rat (-90 min to 0 min). Lactate und pyruvate data are given as L/P ratios (Fig. 4) while isoprostane data are given in absolute values (Fig. 5). All data are given as means ± S.D. of N experiments. Time courses of ACh and L/P ratios were analyzed using two-way ANOVA for repeated measurements (GraphPadR Prism 5.03) with Bonferroni's post-test (Figs. 1-4). One-way ANOVA followed by Dunnett's multiple comparison test was used for the calculation of isoprostane data (Fig. 5). P-values <0.05 were considered to be statistically significant.

RESULTS

Cholinergic activation during status epilepticus

We induced status epilepticus (SE) in rats using the established lithium-pilocarpine method (Figs. 1-5). After pretreatment with lithium chloride one day earlier, the administration of pilocarpine, within a few minutes, evoked cholinergic signs and tremor ("stage 1") followed by stereotypical behavior ("stage 2", corresponding to limbic serizures) and motor seizures ("stage 3"). SE ("stage 4") set in after 25-30 minutes and was characterized by alternating rearing and lowering of the body and unresponsiveness of the rats to external stimuli. Tonic-clonic seizures ("stage 5") were not observed in our experiments. Due to the regularity of the movements in stage 4, microdialysis could be performed during active SE in unconscious, but unanesthetized animals. The most striking finding was a several-fold increase of acetylcholine (ACh) concentrations in the extracellular space of rat hippocampus reflecting a strong stimulation of the septo-hippocampal pathway. ACh levels were increased 6-8-fold within 30-60 minutes after pilocarpine administration and, therefore, closely followed the development of SE (Figs. 1-3). In untreated animals, ACh levels remained high for up to four hours (Figs. 1-3).

We tested three antiseizure drugs for their ability to terminate SE and followed their actions on cholinergic and metabolic activity, at doses that were previously described as active in seizure models. All three drugs (pregabalin, Fig. 1; valproic acid, Fig. 2; levetiracetam, Fig. 3) were able to terminate SE in a delayed fashion. It is remarkable that the onset of action of all three drugs was rapid in terms of ACh

release which immediately dropped after drug administration. However, the behavioural response was delayed by 30-60 minutes when compared to earlier data with diazepam and ketamine (19), and rats stopped convulsing only when maximum ACh levels were reduced by more than 50% (Figs. 1-3). Pregabalin and valproic acid stopped the convulsions but the animals remained unconscious and immobile and did not regain consciousness. Levetiracetam, in contrast, not only stopped the convulsions but the rats regained consciousness and only suffered from tremors. Interestingly, this difference was not reflected in the microdialysis data since all three anticonvulsants brought ACh levels down to baseline, with levetiracetam actually acting somewhat less rapidly than pregabalin and (especially) valproic acid.

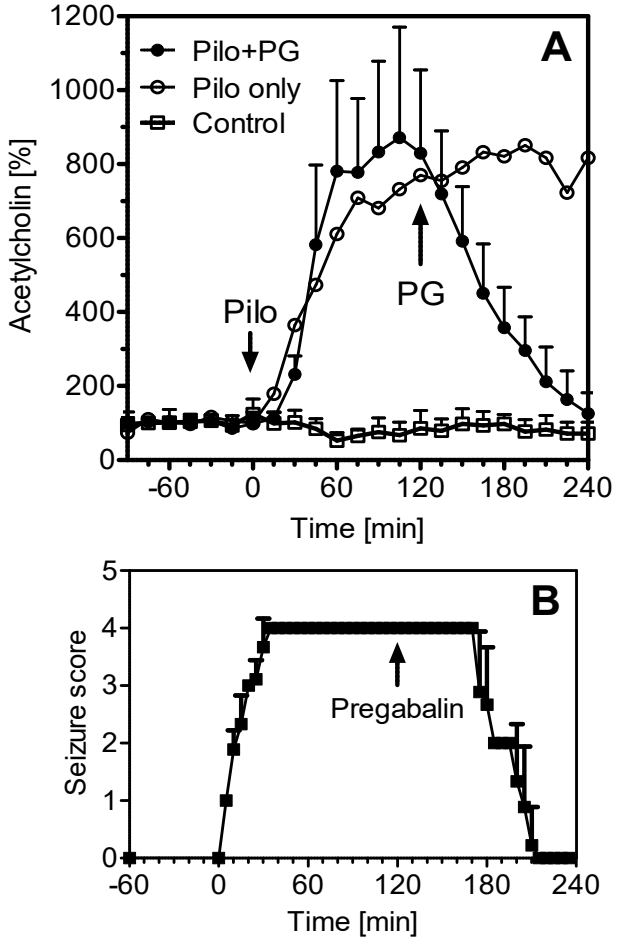

Figure 1. (A) Acetylcholine (ACh) concentrations in rat hippocampal microdialysates during status epilepticus (SE). Rats were pretreated with lithium chloride (127 mg/kg i.p.), and pilocarpine (30 mg/kg s.c.) was given 24 hours later, at time zero ("Pilo"). Pregabalin ("PG", 100 mg/kg; N=10) was given two hours later. The control group (N=5) did not receive pilocarpine. Data are presented as percentages (means ± S.D.) of basal extracellular concentrations of acetylcholine which were determined as averages from six samples (-90 to 0 min) obtained prior to pilocarpine treatment. The time course for ACh in rats that were only treated with pilocarpine has been added for comparion (N=6; error bars omitted). Statistics: (two-way ANOVA with Bonferroni post test, differences between Pilo+PG vs. controls): $F_{1,13}$=65.9; p<0.001. (B) Seizure scores ranging from 0-5 as measured by the modified Racine scale in 5 min intervals. Seizures were scored in parallel to the experiments shown in (A). In fully developed status epilepticus, all rats scored "4" and remained there until interrupted by pregabalin administration.

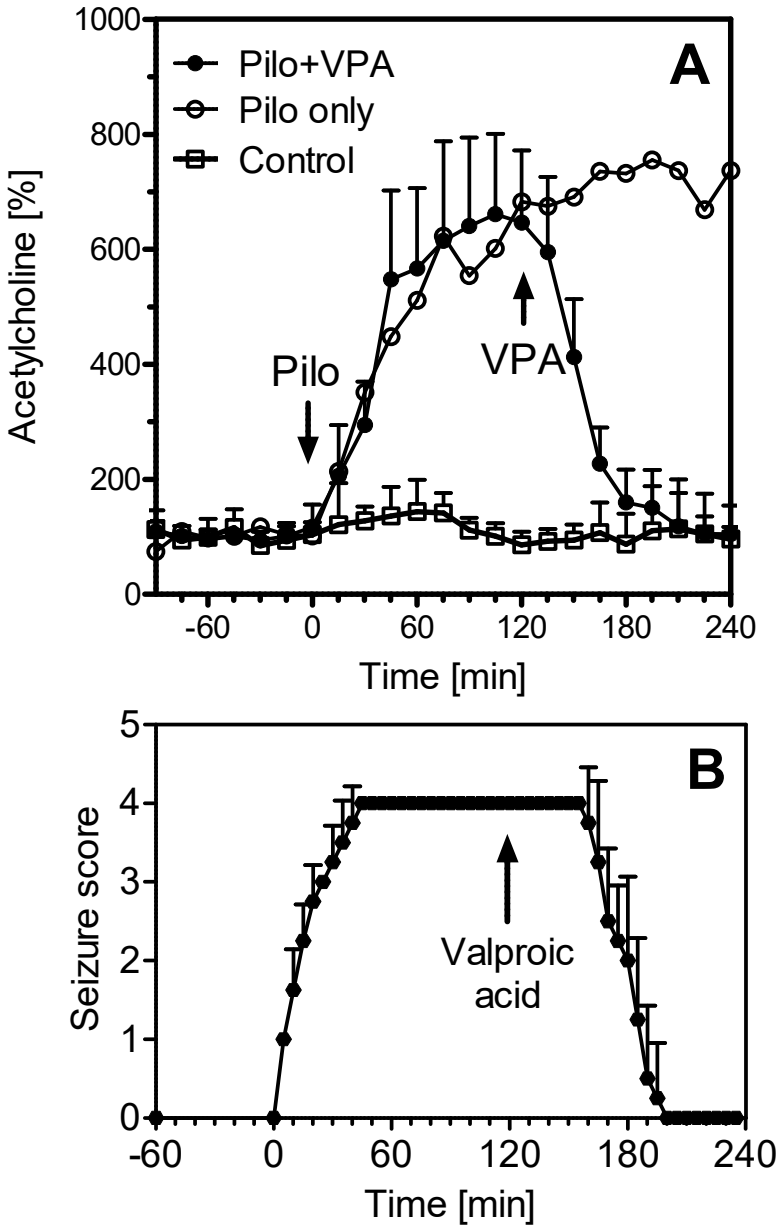

Figure 2. (A) Acetylcholine (ACh) concentrations in rat hippocampal microdialysates during status epilepticus (SE). Rats were pretreated with lithium chloride (127 mg/kg i.p.), and pilocarpine (30 mg/kg s.c.) was given 24 hours later, at time zero ("Pilo"). Valproic acid ("VPA", 400 mg/kg; N=9) was given two hours later. The control group (N=5) did not receive pilocarpine. Data are presented as percentages (means ± S.D.) of basal extracellular concentrations of acetylcholine which were determined as averages from six samples (-90 to 0 min) obtained prior to pilocarpine treatment. The time course for ACh in rats that were only treated with pilocarpine has been added for comparion (N=6; error bars omitted). Statistics: (two-way ANOVA with Bonferroni post test, differences between Pilo+VPA and controls): $F_{1,13}=76.8$; $p<0.001$. (B) Seizure scores ranging from 0-5 as measured by the modified Racine scale in 5 min intervals. Seizures were scored in parallel to the experiments shown in (A). In fully developed status epilepticus, all rats scored "4" and remained there until interrupted by valproic acid.

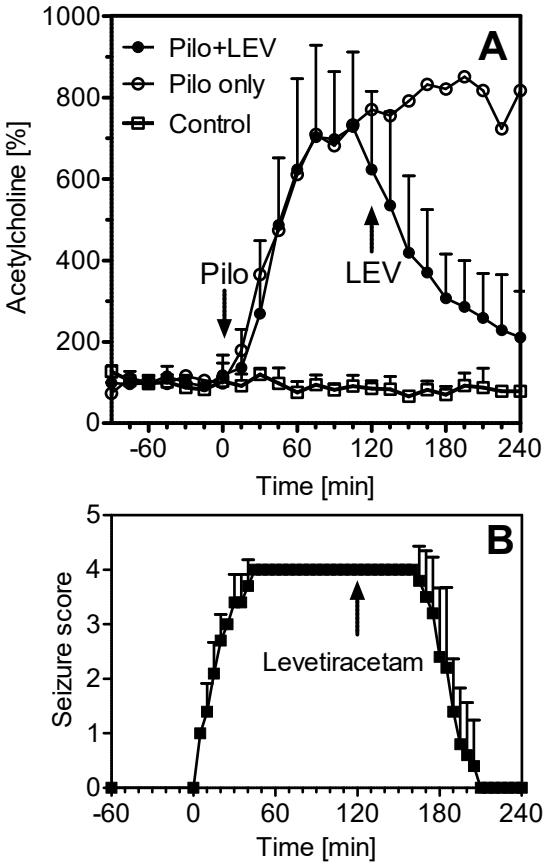

Figure. 3. (A) Acetylcholine (ACh) concentrations in rat hippocampal microdialysates during status epilepticus (SE). Rats were pretreated with lithium chloride (127 mg/kg i.p.), and pilocarpine (30 mg/kg s.c.) was given 24 hours later, at time zero ("Pilo"). Levetiracetam ("LEV", 200 mg/kg; N=10) was given two hours later. The control group (N=5) did not receive pilocarpine. Data are presented as percentages (means ± S.D.) of basal extracellular concentrations of acetylcholine which were determined as averages from six samples (-90 to 0 min) obtained prior to pilocarpine treatment. The time course for ACh in rats that were only treated with pilocarpine has been added for comparion (N=6; error bars omitted). Statistics: (two-way ANOVA with Bonferroni post test, differences between curves): $F_{1,13}$=65.8; $p<0.001$. (B) Seizure scores ranging from 0-5 as measured by the modified Racine scale in 5 min intervals. Seizures were scored in parallel to the experiments shown in (A). In fully developed status epilepticus, all rats scored "4" and remained there until interrupted by levetiracetam.

Metabolic parameters during status epilepticus

In addition to ACh, we monitored parameters of energy metabolism and of cell damage by microdialysis. The basal glucose concentration in hippocampal microdialysates was 338 ± 133 µM (N=40). As described before (21), glucose levels rose after pilocarpine administration to 444 ± 198 µM (+31%) but returned to baseline within 60-90 min after seizures were terminated. Basal lactate and pyruvate levels in microdialysates were 319 ± 126 µM (N=40) and 21.5 ± 7.7 µM, yielding an L/P ratio of 14.8 ± 2.4 under basal conditions. When SE developed, lactate concentrations increased 4-5 fold, while pyruvate levels did not change appreciably; hence, the lactate/pyruvate (L/P) ratio increased to values between 40 and 50 (Fig. 4). In untreated animals, lactate levels remained at or above 400% for up to four hours (21). When anticonvulsant drugs were given, the L/P ratio was lowered to baseline levels while convulsions subsided (Fig. 4).

We also measured ß-hydroxybutyrate (BHB), a ketone body that can be used as a fuel for brain metabolism under special circumstances (28). BHB levels were 4.63 ± 1.05 µM (N=22), increased to 7.46 ± 2.00 µM during SE (+61%; $p<0.01$) and

remained at that level even after administration of anticonvulsants (data not shown).

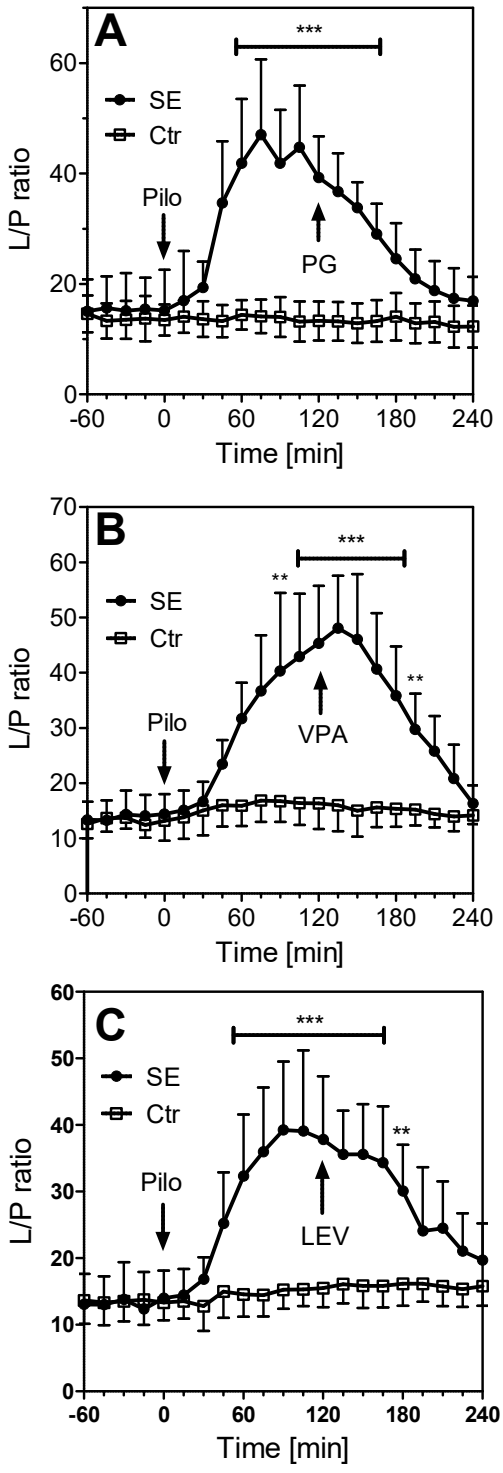

Figure. 4. Lactate/pyruvate ratios (L/P ratio) as calculated from extracellular lactate and pyruvate levels in rat hippocampal microdialysates obtained before and during status epilepticus (SE). Rats were pretreated with lithium chloride (127 mg/kg i.p.), and pilocarpine (30 mg/kg s.c.) was given 24 hours later, at time zero ("Pilo"). The control groups (N=5) did not receive pilocarpine. Data are presented as ratios (means ± S.D.). (A) Pregabalin ("PG", 100 mg/kg; N=10) was given two hours later. Statistics: (two-way ANOVA with Bonferroni post test, differences between curves): $F_{1,11}$=23.5; p<0.001. (B) Valproic acid ("VPA", 400 mg/kg; N=9) was given two hours later. Statistics: (two-way ANOVA with Bonferroni post test, differences between curves): $F_{1,12}$=18.7; p=0.001. (C) Levetiracetam ("LEV", 200 mg/kg; N=10) was given two hours later. Statistics: (two-way ANOVA with Bonferroni post test, differences between curves): $F_{1,13}$=16.2; p=0.001. The bar indicates individual data points that were significantly different from control data at p<0.001. ** indicates p<0.01.

Parameters of cellular damage during status epilepticus

We also followed the formation of F2-isoprostanes during SE. Isoprostanes are reliable markers of oxidative stress in situ and remained stable at 0.1-0.2 nM in untreated rats. Upon induction of SE, hippocampal levels of isoprostanes increased more than twentyfold (Fig. 5). Again, treatment with the three antiseizure drugs caused a reduction of isoprostane levels although the levels remained elevated for more than sixty minutes past treatment (Fig. 5).

Finally, we monitored hippocampal levels of glycerol by microdialysis. In agreement with our previous study, glycerol levels (basal level: 7.13 ± 3.58 µM, N=40) increased approx. tenfold during SE and remained high for several hours (21). Treatment with the three anticonvulsant drugs caused a slow but steady decline of glycerol levels (data not shown).

DISCUSSION

Experimental status epilepticus (SE) can be induced by cholinergic hyperexcitation in the lithium-pilocarpine model in rats and the pilocarpine model in mice. Previous data have shown that increases of acetylcholine (ACh) are observed during SE (29), and our studies using microdialysis have demonstrated a several-fold increase of ACh concentrations in rat hippocampus as well as in striatum, even after kainate-induced SE (19). A role of the cholinergic system in the development and maintenance of SE was also demonstrated by recent work from the Löscher group (20,23) who found that SE that was resistant to diazepam and phenobarbital could be terminated by the additional administration

of scopolamine, a muscarinic antagonist. Further work demonstrated that cholinergic blockade reduces severity and duration of SE in a model of basolateral amygdala stimulation (24). These findings indicate that central cholinergic pathways contribute to SE and may even be potential targets for drugs.

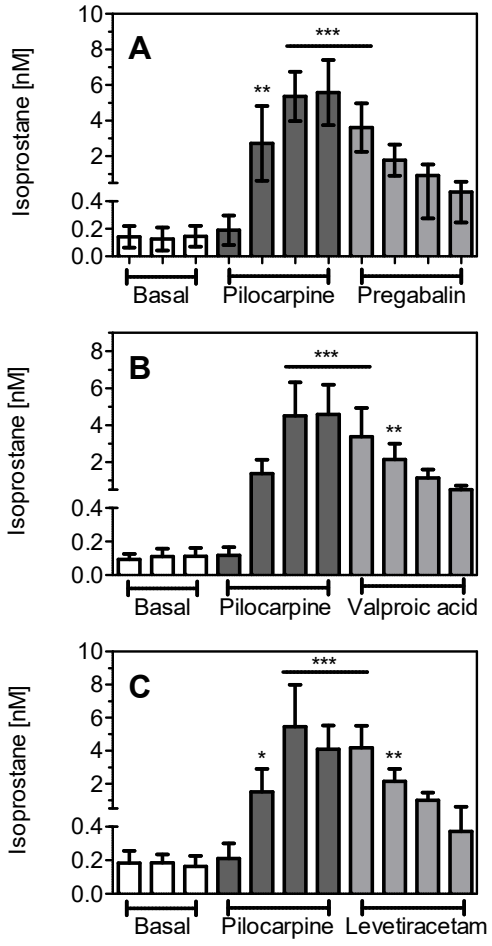

Figure 5. Extracellular concentrations of F2-isoprostanes in rat hippocampal microdialysates during status epilepticus (SE) and after treatment with (A) pregabalin (100 mg/kg); (B) valproic acid (400 mg/kg); and (C) levetiracetam (200 mg/kg, N=9-10). Rats were pretreated with lithium chloride (127 mg/kg i.p.), and basal isoprostane levels were monitored on the next day for 90 min (in 30 min intervals). Four samples (30 min each) were sampled after administration of pilocarpine (30 mg/kg s.c.) during SE, and four samples after administration of anticonvulsant drugs which were given 2 hours after pilocarpine. The isoprostane values are presented in absolute terms (nM) as means ± S.D. Statistical analysis (one-way ANOVA followed by Dunnett's multiple comparison test considering the last basal value as a reference): *, P<0.05; **, P<0.01; *** P<0.001 (N=11). The bar indicates individual data points that were significantly different from control data at p<0.001.

Effects of anticonvulsant treatment on cholinergic activation

In the present study, we first confirmed our earlier report of a strong activation of the septo-hippocampal cholinergic pathway by lithium-pilocarpine. The increase of hippocampal ACh closely followed the development of SE and ACh levels remained high for 90 min of unabated seizures. In our previous work, we had observed rapid termination of seizures and reductions of ACh after diazepam and, with an even more rapid action, after ketamine (19). Here, we tested three antiseizure drugs with different mechanisms of action. Pregabalin, an inhibitor of presynaptic calcium channels, was included because it was active as a pretreatment in a previous study on metabolic changes of SE (21) and in a study using long-term treatment (30). Valproic acid (VPA) is a broadly acting antiepileptic drug that is a regular 2nd line drug for SE in humans (2,4); among other activities, VPA inhibits sodium channels. Levetiracetam has recently become a 2nd line drug for drug-resistant SE in humans; it binds to a presynaptic protein, synaptic vesicle protein 2a and was also described to be active in the lithium-pilocarpine model (31,32). In our hands, all three drugs terminated SE, but with a delay of 30-60 min. This is in contrast with the observation that diazepam and ketamine stop seizures almost immediately after administration (19). Moreover, after pregabalin and VPA, the animals stopped convulsing but remained unconscious for a considerable time after treatment (more than 60 minutes on average). Only levetiracetam treatment caused the rats to become conscious soon after the termination of seizures.

It is speculative to extrapolate the present data to the clinical situation. As we did not have the resources to do full dose-response curves, we used only one dose of each anticonvulsant; the doses were selected based on previous work using these anticonvulsants in seizure studies (30,33,34). In most clinical guidelines, first-line therapy of status epilepticus involves benzodiazepines and barbiturates. As second-line therapy (if convulsions persist), valproic acid and levetiracetam are often recommended (1,2). Pregabalin is rarely used for status epilepticus but has been used as add-on treatment and for non-convulsive status epilepticus

(35,36). Judging from the present obervations in rats, levetiracetam may be the best choice for drug-resistant seizures because gain of consciousness was obtained in most rats with levetiracetam. A unique profile for levetiracetam in rodent models of status epilepticus was previously reported (37,38), but at this time, there are no clinical data to support this concept. Nevertheless, at the doses used here, levetiracetam seems to be the most useful as 2nd line treatment in drug-resistant SE.

The molecular mechanisms of antiseizure drug actions in SE remain obscure. It was suggested that hypersynchronization during SE results from an imbalance of (increased) glutamatergic and (decreased) GABAergic tone, with $GABA_A$ receptors showing a progressive down-regulation during SE (17,18). Our data show, however, that diazepam, a GABAergic enhancer, is an effective anticonvulsant even after 90 min of ongoing seizures (19), and the drugs active in the present study are not primarily targeted at GABAergic pathways (although they may affect GABAergic neurons indirectly). More likely, the presynaptically acting drugs pregabalin and levetiracetam may reduce hyperexcitation by impairing glutamate and acetylcholine release which are both predominantly excitatory neurotransmitters acting through AMPA/NMDA and M1 muscarinic receptors, respectively. This speculation is in agreement with the rapid and effective action of ketamine, an antagonist at glutamatergic NMDA receptors (19). The important role of glutamatergic hyperexcitation was also observed in studies with nerve agents such as soman which are effective inhibibitors of acetylcholinesterase (AChE) and therefore, like pilocarpine, procholinergic agents (39,40); here, increases of ACh levels are followed by delayed increases of glutamate-activated NMDA receptors. Impairment of glutamate or ACh release, or both, may be involved in the antiseizure activity of the drugs used here. In summary, glutamatergic and cholinergic drive seem to work together during SE, and effective treatment of diazepam-resistant seizures may require the reduction of glutamate or ACh release (e.g., by pregabalin and levetiracetam) and the blockade of glutamatergic and cholinergic receptors (e.g., by ketamine and scopolamine).

Effects of anticonvulsant treatment on parameters of metabolism and cellular damage

SE is accompanied by hypermetabolism (41) which is reflected in enhanced formation of lactate, likely formed by glial cells to support neuronal energy metabolism (42). Lactate release has long been known to correlate with neuronal activity and transmitter action (43,44) and was found to be increased during SE evoked by lithium-pilocarpine (21,45). Here we document changes of the lactate/pyruvate (L/P) ratio in rat hippocampus during seizures. Strong increases of the L/P ratio to values up to 50 indicate an increase of aerobic glycolysis which was attenuated when anticonvulsant drugs were given. In comparison, changes of glucose (transient increase) and the ketone body BHB (small increase) were minor. It should be noted that our previous study (21) reported a strong decrease of lactate after atropine administration, pointing to a prominent role of cholinergic inputs for hypermetabolism.

Enhanced release of excitatory transmitters such as glutamate and ACh likely causes excitotoxicity which we observed as a strong increase of glycerol and isoprostanes. Glycerol is a breakdown product of cellular phospholipids and indicates membrane degradation (46,47). F2-isoprostanes are reliable indicators of oxidative stress (48). Our observation of oxidative stress during SE confirms earlier studies (21,49); also, an effect of levetiracetam on oxidative stress parameters in mice has previously been reported (34). In the present study, we found that anticonvulsant treatment rapidly lowers isoprostane levels that were 20-fold increased during SE. Again, all three anticonvulsant drugs were effective in this respect.

CONCLUSION

In summary, the present study gives evidence for a strong cholinergic component in SE induced by lithium-pilocarpine administration. In parallel to seizure development, increases of ACh are accompanied by increases in lactate (hypermetabolism) and isoprostanes (oxidative stress). All parameters are attenuated by the administration of three anticonvulsant drugs, pregabalin, valproic acid and levetiracetam, although only levetiracetam caused the rats to gain consciousness rapidly. The mechanism of action of these drugs for SE termination remains elusive, but reductions of ACh and glutamate release may be involved. A role for levetiracetam in the treatment of SE is suggested by the present data, but more importantly, the study shows that rapid termination

of seizures not only stops hypermetabolism but also cellular (long-term) damage in SE.

NOVELTY OF THE WORK

This is the first study of pregabalin, valproic acid and levetiracetam in a rat model of status epilepticus combined with microdialysis. We show that the three drugs act similarly, suppressing seizure activity in a delayed manner and slower than previously described for diazepam or ketamine. At the doses tested, only levetiracetam helps rats to regain consciousness. However, all three drugs terminate cholinergic hyperactivity and cellular damage as measured by glycerol release and oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

The study was supported by a stipend from the German Academic Exchange Service (DAAD) to I. Imran and by internal funds from Goethe University to J.K.

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