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Effects of γ-aminobutyric acid (GABA) agonists and GABA uptake inhibitors on pharmacosensitive and pharmacoresistant epileptiform activity *in vitro*

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1 Lowering of the extracellular Mg^{2+}-concentration induces various patterns of epileptiform activity in combined rat entorhinal cortex-hippocampal brain slices. After a prolonged period of exposure to Mg^{2+}-free medium seizure-like events in the entorhinal cortex change to a state of late recurrent discharges which cannot be blocked by clinically available antiepileptic drugs. This late epileptiform activity thus represents a useful model to test the effects of new anticonvulsant substances.

2 A mechanism possibly underlying the development of sustained seizure-like activity is the loss of synaptically released γ-aminobutyric acid (GABA). Drugs which increase the amount of GABA available in presynaptic endings might thus be useful in the treatment of these therapeutically complicated forms of epilepsy.

3 Therefore, we studied the effects of various substances increasing GABA-mediated inhibition on early and late forms of epileptiform activity. GABA and the $GABA_A$ receptor agonist muscimol blocked both the pharmacosensitive discharges in the hippocampus and entorhinal cortex as well as the late recurrent discharges in the medial entorhinal cortex. The $GABA_B$ receptor agonist baclofen blocked the recurrent short discharges very potently, but did not consistently block seizure-like events and late recurrent discharges in the entorhinal cortex.

4 GABA uptake blockers showed a differential potency to block the various discharge patterns. Whereas nipecotic acid and β-alanine suppressed all forms of epileptiform activity albeit at high concentrations (1–5 mM), tiagabine was much more potent in blocking the hippocampal recurrent short discharges and the seizure-like events in the medial entorhinal cortex, but could not block the late recurrent discharges.

5 Our data support the idea that prolonged neuronal overactivity might result in a loss of synaptically available GABA. Selective block of uptake into glia cells or substitution of the transmitter may therefore be an efficient strategy for the treatment of severe prolonged epileptic discharges whereas block of neuronal GABA uptake fails to counteract synchronized discharges in this situation.

Keywords: Epilepsy; seizure-like events; γ-aminobutyric acid (GABA) uptake; tiagabine; nipecotic acid; β-alanine; entorhinal cortex; hippocampus

Introduction

Many anticonvulsant drugs act by potentiating γ-aminobutyric acid (GABA)-mediated inhibition in the nervous system (Macdonald & McLean, 1986). However, some of the substances increasing $GABA_A$ receptor-mediated responses have severe sedative side-effects (barbiturates) or lead to tolerance upon prolonged treatment (benzodiazepines). Therefore, alternative strategies have been chosen to strengthen GABA-mediated inhibition.

Special interest has been devoted to GABA uptake blockers which exert their anticonvulsant effects by prolonging the duration of inhibitory postsynaptic potentials (Rekling *et al.*, 1990; Fink Jensen *et al.*, 1992; Gram, 1994; Suzdak & Jansen, 1995; Draguhn & Heinemann, unpublished). They are highly efficient anticonvulsants in several animal models of chronic epilepsy (Heit & Schwark, 1988; Suzdak, 1994; Walton *et al.*, 1994; Suzdak & Jansen, 1995; Smith *et al.*, 1995). Tiagabine, a lipophilic derivative of nipecotic acid (NPA) is presently tested as add-on therapy against complex partial seizures in man (Gram, 1994). Alterations of the GABA uptake system in chronic epileptic syndromes have been found both in animal models and in man. Recent evidence suggests that the GABA uptake is reduced in tissue from animals and humans with chronic temporal lobe epilepsy (Janjua *et al.*, 1991; During *et al.*, 1995). This should lead to prolonged synaptic inhibition and might reflect an adaptation to hyperexcitability. GABA uptake molecules can release the transmitter from cells by reversed function under certain ionic and electrical conditions (Attwell *et al.*, 1993; Cammack *et al.*, 1994). It has therefore been suggested that the loss of reverse transport capacity might reduce the inhibitory tone in hyperactive tissue and thus aggravate seizures (During *et al.*, 1995).

Acute neuronal hyperactivity is characterized by enhanced synaptic transmission and leads to an augmented release of excitatory as well as inhibitory neurotransmitters (Sayin *et al.*, 1995). Chronic epilepsy can also be accompanied by enhanced inhibitory transmission (Whittington & Jefferys, 1994). Following synaptic release, GABA is transported into presynaptic endings as well as into glia cells where some of the transmitter is metabolized by the GABA transaminase (Schousboe, 1982). The relative amount of neuronal vs. glial GABA uptake is not known. This metabolic shunt, however, might lead to a progressive loss of synaptically available GABA during prolonged epileptic hyperactivity. This may result in transition to prolonged and therapeutically complicated forms of status epilepticus. Experimental and clinical evidence for this concept comes from the effect of γ-vinyl-GABA (vigabatrin). This substance is an irreversible blocker of the GABA transaminase and

(Ben-Menachem, 1995; Halonen *et al.*, 1995; Buchanan, 1993). Conventional GABAergic drugs acting on $GABA_A$ receptors will become progressively less potent if the presynaptic GABA content and consequently the amount of synaptically released GABA decreases. Indeed, severe forms of epilepsy like status epilepticus are frequently resistant towards these agents.

In such cases, it might be therapeutically useful to enhance the amount of transmitter in the neuronal GABA pool by selectively blocking the glial uptake or by adding GABA receptor agonists. We therefore tested various GABAergic drugs on acute and prolonged epileptiform neuronal discharges. We employed the low-Mg^{2+} model of temporal lobe epilepsy in combined entorhinal cortex-hippocampal brain slices of rats. Whereas early stages of epileptiform activity in this model can be blocked by clinically available antiepileptic drugs (AEDs), the later form of discharges in the entorhinal cortex is resistant to presently employed AEDs (Walther *et al.*, 1986; Zhang *et al.*, 1995). Based on the assumption that prolonged hyperactivity might result in reduced availability of GABA we analyzed the effect of GABA receptor agonists as well as various GABA uptake blockers on the early and late patterns of epileptiform field potentials.

Methods

Slice preparation

The experiments were performed as described previously (Dreier & Heinemann, 1990). In brief, combined entorhinal cortex-hippocampal slices (400 μm) containing the temporal cortex area 3, the perirhinal cortex, the entorhinal cortex, the subiculum, the dentate gyrus and the ventral hippocampus were prepared in a nearly horizontal plane from male or female adult Wistar rats (150–250 g) decapitated under deep ether anaesthesia. The slices were placed in an interface chamber and were continuously perfused (1.5–2 ml min^{-1}) with prewarmed (35°C) artificial cerebrospinal fluid (ACSF) which contained (in mM): NaCl 124, KCl 3, $MgSO_4$ 1.8, $CaCl_2$ 1.6, NaH_2PO_4 1.25, $NaHCO_3$ 26, and glucose 10 (pH 7.4). The ASCF was saturated with carbogen (95% O_2, 5% CO_2). $MgSO_4$ was omitted in the nominally Mg^{2+}-free ACSF. Drugs were added to the Mg^{2+}-free ACSF after onset of epileptiform activity. Before the experiment was started the slices were allowed to recover from their preparation for at least one hour. The viability of the slices was tested through stimulation of the Schaffer collaterals and recording of the field response in the hippocampal area CA1 before washout of Mg^{2+}. Only slices in which a population spike of at least 5 mV amplitude could be obtained were used in the experiments.

Recording system

Extracellular field potentials were recorded with glass microelectrodes filled with 150 mM NaCl which were placed in the pyramidal layer of area CA1 and in the medial entorhinal cortex, layer 5 to 6. Data were recorded on chart recorder (Linearcorder MkVII WR3101, Graphtech Corp., Japan or DASH IV, Astro-med, Inc., West Warwick, RI, U.S.A.). Some data were digitized with a CRC VR-100 A interface (Instrutech Corp., Mineola, U.S.A.), recorded on video tape and subsequently analyzed off-line.

Data analysis

Data were analyzed by counting the number of epileptiform events during a period of 4 min in the case of recurrent short discharges (RSDs) and late recurrent discharges (LRDs), and for 12 min in the case of seizure-like events (SLEs). The effects of the different substances tested are given as % of control. All results are expressed as mean value $\pm$ s.e.mean. Statistical significance was determined by Wilcoxon matched-pairs signed-

Drugs

Bicuculline methiodide and muscimol were obtained from Research Biochemicals International, Natick, MA, U.S.A. γ-Aminobutyric acid (GABA), (−)-baclofen, β-alanine and (±)-nipecotic acid (3-piperidine carboxylic acid, NPA) were obtained from Sigma, Deisenhofen, Germany. Tiagabine [(R)-N-(4,4-bis(3-methyl-2-thienyl) but 3-en-1-yl nipecotic acid] was a kind gift from Novo Nordisk, Maloev, Denmark. CGP 55845A was kindly provided by Ciba-Geigy Ltd., Basel, Switzerland.

Results

Spontaneous extracellular field potentials were recorded from area CA1 and the medial entorhinal cortex (mEC) of 221 brain slices from 123 animals. Lowering of the extracellular Mg^{2+}-concentration induced different patterns of epileptiform activity. All discharges were completely reversible within 6 to 30 min after re-perfusion with normal extracellular solution. Field potential recordings in the pyramidal cell layer of area CA1 revealed recurrent short discharges (RSDs; Figure 1a) after 20 to 90 min of incubation with magnesium-free solution. This activity remained stable during the experiment even upon prolonged treatment with Mg^{2+}-free ACSF. In 40% of the experiments we also observed some (1 to 20) initial seizure-like events (SLEs) in CA1 characterized by a marked negative potential shift of 10 to 70 s duration with a tonic and a clonic phase before the regular activity of RSDs developed. In the medial entorhinal cortex (mEC), spontaneous epileptiform activity regularly started with SLEs (Figure 1b). However, upon prolonged exposure to Mg^{2+}-free ACSF the SLEs changed to a characteristic pattern of short-lasting high-frequency negative potential shifts termed late recurrent discharges (LRDs; Figure 1c, d). The transition from SLEs to LRDs occurred at 46 $\pm$ 9 min after the first SLE. The LRDs remained stable for several hours, although the duration of the events decreased time while the frequency of the discharges increased.

GABA receptor agonists

The natural agonist GABA reduced the frequency of the recurrent short discharges in area CA1 in a concentration-dependent manner. Whereas 1.0 mM showed only little effect, 2.5 mM GABA blocked the RSDs in two thirds of the experiments. With 5 mM, in almost all slices tested RSDs were blocked (Figures 1a and 2a). The seizure-like events in the mEC were more sensitive to GABA-application than the RSDs in the hippocampus. Here, the antiepileptic effect started at 0.5 mM GABA and the discharges were completely blocked at 2.5 mM (Figures 1b and 2a). In two experiments, however, 0.5 mM GABA could not prevent the transition from SLEs to late recurrent discharges. This late activity was antagonized by higher concentrations of GABA beginning at 1 mM with a complete block at 5 mM (Figures 1c and 2a). In 4 out of 48 experiments LRDs were reversed to SLEs when GABA was applied shortly after the transition from SLEs to LRDs.

The $GABA_A$ receptor agonist muscimol suppressed all patterns of epileptiform activity much more potently than GABA. Effects on RSDs in the hippocampal area CA1 were seen at concentrations as low as 5 μM and at 25 μM the discharges were almost completely blocked. The suppression of SLEs in the mEC had a similar dose-dependence (Figure 2b). Interestingly, muscimol consistently reversed LRDs into SLEs (Figure 1d). This effect was seen in all 16 experiments at concentrations between 5 and 25 μM. In 4 of 9 slices the SLEs were subsequently blocked by further application of 5 μM muscimol and at 25 μM the SLEs were always blocked.

The $GABA_A$ receptor agonist baclofen suppressed the hippocampal RSDs more potently than both muscimol or GABA.

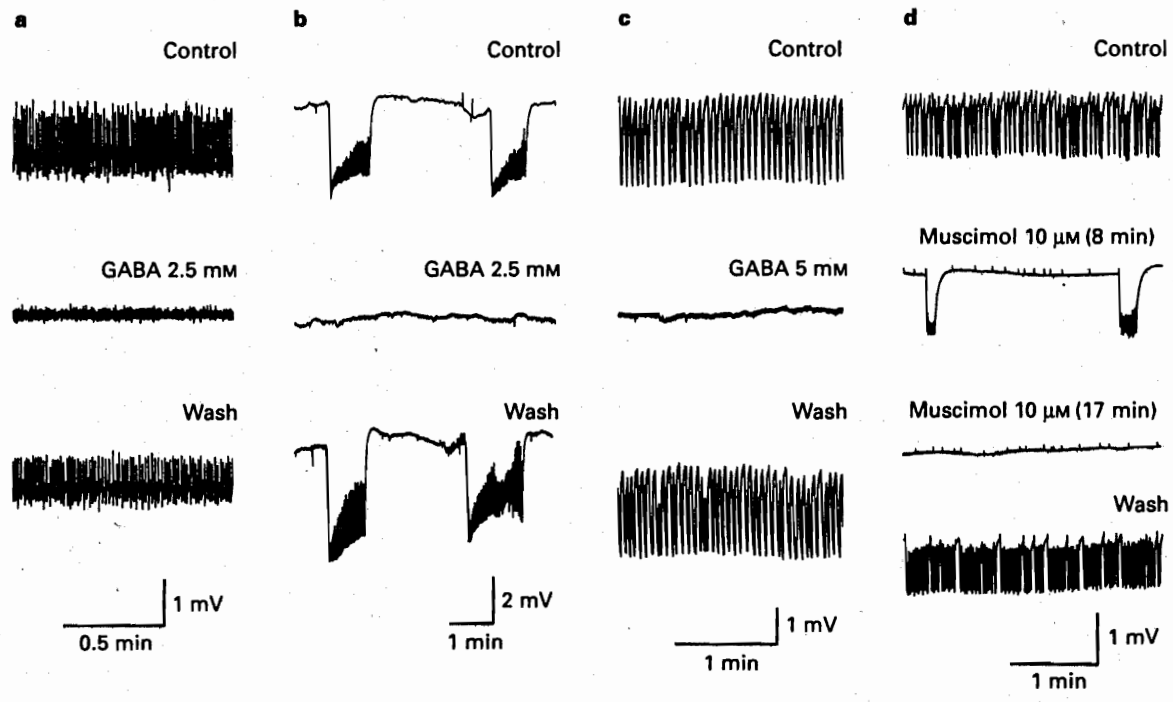

Figure 1 Effects of GABA and muscimol on the different forms of epileptiform discharges. (a) GABA (2.5 mM, 19 min) suppressed the recurrent short discharges in the hippocampal area CA1. (b) GABA (2.5 mM for 13 min) blocked the seizure-like events (SLEs) in the entorhinal cortex (EC). (c) GABA (5 mM, 10 min) reversibly suppressed the pharmacoresistant late activity in the EC. (d) Muscimol (10 μM) reversed the entorhinal late recurrent discharges (LRDs) to SLEs (8 min) and subsequently blocked these SLEs (17 min). Washout of the substance resulted in the reappearance of seizure-like events after 22 min and of LRDs after 42 min.

reversible block of the activity in area CA1. The frequency of SLEs in the EC was clearly reduced beginning at 5 μM baclofen. However, increasing the concentration up to 100 μM did not consistently enhance the antiepileptic effect (Figure 2c). Similar to muscimol, baclofen could reverse LRDs to SLEs in all 19 experiments, but the resulting SLEs were slightly atypical lacking a marked clonic-like discharge pattern in the field potentials (not shown). Like the initial SLEs these discharges were also resistant towards baclofen. A comparison of the main effects of the GABA receptor agonists is shown in Figure 2.

GABA uptake inhibitors

The GABA uptake blocker, (±)-nipecotic acid (NPA) reduced the frequency of the recurrent short discharges in the hippocampus concentration-dependently. The antiepileptic effect started at 1 mM and at 5 mM NPA the RSDs were completely blocked (Figure 4a). When NPA (1–2.5 mM) was applied after the appearance of SLEs in the mEC it also suppressed this activity (Figure 4a). Nipecotic acid (1–5 mM) reduced the frequency of LRDs in the mEC in a concentration-dependent manner. At the same time, the amplitude and duration of single LRDs were increased. A complete block was reached at 5 mM (±)-nipecotic acid (Figures 3d and 4a). In contrast to the effect of the GABA receptor agonists, we never observed a transition from LRDs back to the pattern of seizure-like events during the application of nipecotic acid.

The GABA uptake blocker β-alanine was slightly less potent than (±)-nipecotic acid in suppressing the different forms of epileptiform activity (Figure 4b). The antiepileptic effect on the hippocampal recurrent short discharges started at a concentration of 1 mM and at 5 mM the RSDs were always blocked. SLEs were suppressed by β-alanine with lower potency than by NPA, but at 5 mM a complete block was achieved. The late recurrent discharges in the mEC required even higher concentrations for suppression starting at 2.5 mM. In 4 out of 10 slices there was no complete block of this late

Tiagabine was studied at concentrations between 10 and 250 μM. Hippocampal recurrent short discharges were blocked or reduced in frequency by 10 μM tiagabine in 17 of 24 experiments (Figures 3a and 4c). Surprisingly, the substance lost its efficacy at higher concentrations and developed a proconvulsive effect at 250 μM (Figures 3a and 4c). In 4 slices which had shown a reduction of RSD frequency by 10 μM tiagabine, the subsequent application of 50 μM had no effect (RSD frequency 107.9 ± 17.3% of control) and 250 μM tiagabine even increased the frequency of the discharges to 153.1 ± 30.3% (Figures 3a and 4c). In 4 experiments where 10 μM tiagabine had no effect, increasing the concentration to 250 μM also had no influence on the activity. In contrast, SLEs in the entorhinal cortex were always blocked by 10 μM tiagabine (Figures 3b and 4c). The recovery was rather slow and upon washout of the substance the first SLE reappeared after 40 to 120 min. The uptake blocker was also applied to slices showing LRDs in the mEC. We tested concentrations between 10 and 250 μM. At none of the concentrations tested (10, 50, and 250 μM) was tiagabine able to influence the duration, amplitude or frequency of entorhinal LRDs (Figures 3c and 4c). The effects of the various GABA uptake blockers are summarized in Figure 4.

GABA receptor antagonists

Application of the $GABA_A$ receptor antagonist bicuculline (10 and 25 μM) in normal ACSF caused interictal discharges in the hippocampus (Figure 5a) and in the mEC (Figure 5b), but did not elicit SLEs ($n=7$ for each region). When bicuculline was applied together with the Mg^{2+}-free medium, all forms of epileptiform activity were severely aggravated. The frequency of RSDs in CA1 was increased by 80% ($n=4$; Figure 5c). SLEs in the mEC quickly transformed to LRDs upon addition of bicuculline within 6–12 min ($n=7$; Figure 5d). Application of bicuculline to slices already displaying LRDs increased the frequency of these events by 118% ($n=4$; Figure 5e).

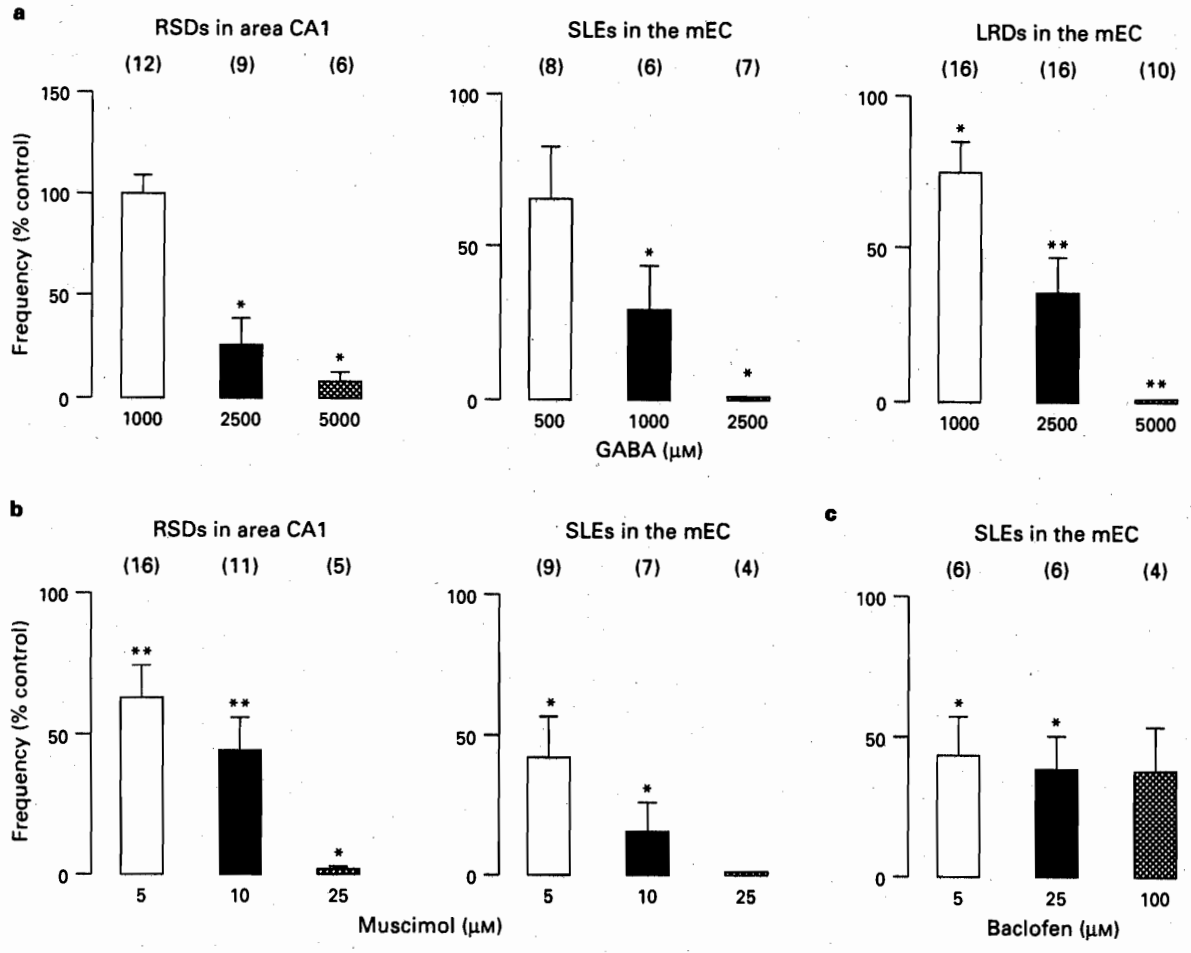

Figure 2 Effects of different GABA receptor agonists on the various epileptiform discharges. Ordinates indicate discharge frequency as % of the frequency under low-magnesium medium without drugs. (a) The natural agonist GABA suppressed all forms of epileptiform activity in a concentration-dependent manner. LRDs, late recurrent discharges; mEC, medial entorhinal cortex. (b) The $GABA_A$ receptor agonist muscimol reduced the frequency of recurrent short discharges (RSDs) and of seizure-like events (SLEs). (c) (−)-Baclofen reduced the frequency of the SLEs at all concentrations tested, but did not consistently block the events even at high concentrations. Asterisks indicate the significance of the drug effect vs. control (Wilcoxon matched-pairs signed rank test: $*P<0.05$; $**P<0.01$).

ther by addition of the $GABA_B$ receptor antagonist CGP 55845A to Mg^{2+}-free medium. At 2 μM, CGP 55845A increased the frequency of RSDs in CA1 by 84±45% ($n=7$; $P<0.03$). The transition from SLEs to LRDs in the entorhinal cortex was not accelerated by addition of 2 μM CGP 55845A (transition time 32±11 min $n=8$; $P>0.33$). Addition of the $GABA_B$ receptor antagonist after the occurrence of LRDs in the entorhinal cortex also did not increase the frequency of these events ($n=8$; $P>0.25$).

Discussion

The present data indicate that GABAergic inhibition is differentially involved in different forms of epileptiform activity in brain slices. GABA receptor agonists and various GABA uptake blockers showed characteristic differences in potency and efficacy both between the drugs tested as well as between the different patterns of epileptiform activity.

Different patterns of activity

Lowering extracellular Mg^{2+} induces various forms of epileptiform activity. The synchronized discharges depend on activation of N-methyl-D-aspartate (NMDA) receptors for glutamate and are therefore sensitive to NMDA receptor antagonists (Herron *et al.*, 1985; Zhang *et al.*, 1994; Stanton *et* in area CA1 (recurrent short discharges, RSDs) are sensitive to valproic acid, but do not respond to benzodiazepines and barbiturates at clinically relevant concentrations (Heinemann *et al.*, 1994). The seizure-like events in the entorhinal cortex are sensitive to phenytoin and carbamazepine, to $GABA_A$ receptor modulators like benzodiazepines and barbiturates, and to $GABA_A$- and $GABA_B$-receptor agonists (Jones, 1989; Heinemann *et al.*, 1994).

The most interesting discharge patterns are the short negative-going field potentials in the entorhinal cortex (late recurrent discharges, LRDs) which occur upon prolonged washout of Mg^{2+} (Dreier & Heinemann, 1990). These events are insensitive to clinically employed anticonvulsants and can therefore serve as a test system for drugs developed against pharmacoresistant forms of epilepsy (Zhang *et al.*, 1995).

GABAergic inhibition plays an important role in the maintenance of neuronal function even during epileptiform activity. Block of the $GABA_A$ receptor aggravated the epileptiform activity in our experiments as previously described (Jones, 1988; 1994; Jones & Lambert, 1990). The highly potent $GABA_B$ receptor antagonist CGP 55845A (Jarolimek *et al.*, 1993) had no proconvulsant effect in the EC but increased the frequency of RSDs in CA1. Thus, $GABA_B$-mediated inhibition is more important in CA1 than in the EC in the low-Mg^{2+}-model; this has also been found in 4-aminopyridine-induced seizures (Jones, 1994). The aim of the present study was to examine further the antiepileptic potency of different

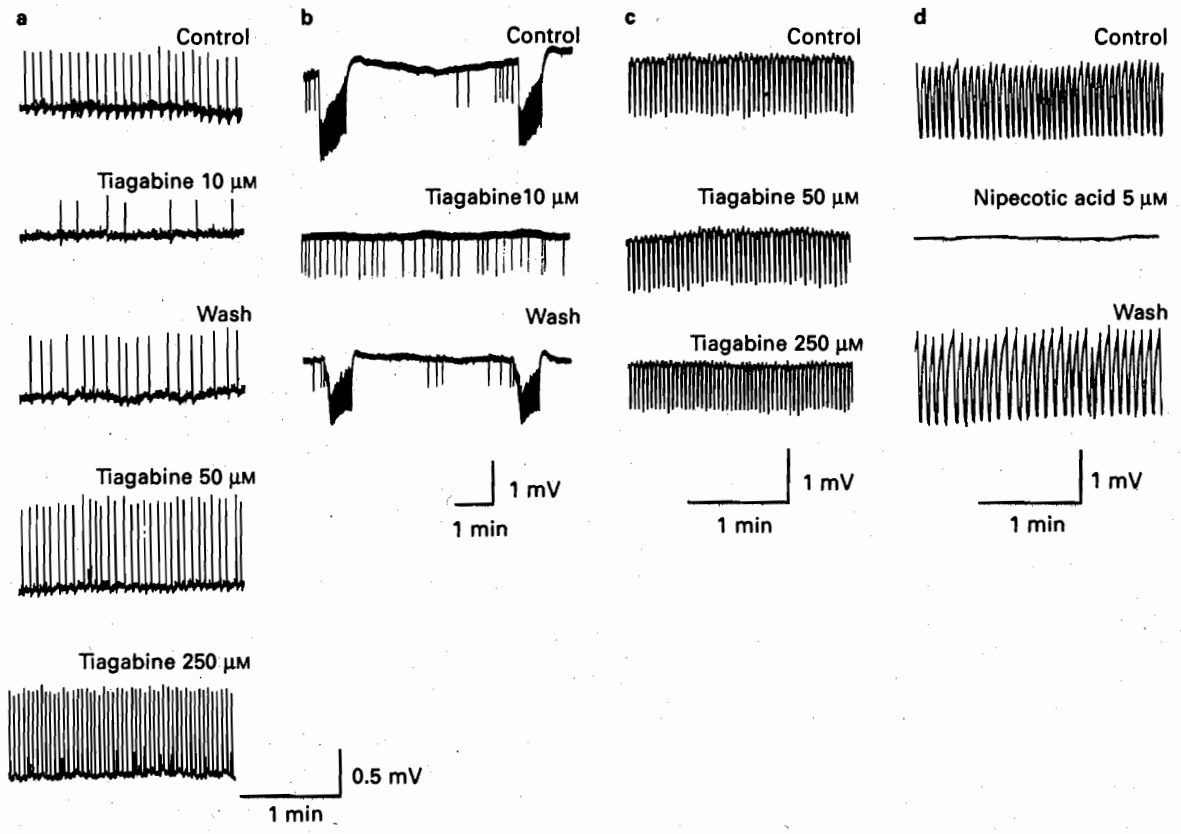

Figure 3 Effects of the GABA uptake blocker tiagabine on the various patterns of epileptiform activity. (a) At 10 μM, tiagabine (22 min) clearly reduced the frequency of the hippocampal recurrent short discharges. After washout of the drug, recovery of the epileptiform discharges is visible. At 50 μM (23 min) tiagabine showed no effect on the RSDs and at 250 μM (26 min) the frequency of the discharges was significantly increased. (b) At 10 μM, tiagabine suppressed the seizure-like events, which reappeared after washout of the substance. Note that tiagabine did not block the interictal-like activity in the entorhinal cortex. (c) Tiagabine showed no effect on the late epileptiform activity. In this experiment, tiagabine was applied at 50 μM for 23 min and subsequently at 250 μM for 26 min. (d) Nipecotic acid (5 mM) reversibly suppressed late recurrent discharges (LRDs) in the entorhinal cortex.

Effects of GABAergic drugs on LRDs

In contrast to clinically used antiepileptics, GABA suppressed the LRDs concentration-dependently. Both the $GABA_A$ and the $GABA_B$ receptor agonists, muscimol and baclofen, respectively consistently reversed LRDs to SLEs in all experiments. Thus, supplementing the tissue with GABA receptor agonists is an efficient antiepileptic mechanism for LRDs.

The second approach followed in our experiments was to strengthen inhibitory transmission by GABA uptake blockers. These drugs prolong the postsynaptic effects of GABA (Dingledine & Korn, 1985; Thompson & Gähwiler, 1992; Oh & Dichter, 1994; Roepstorff & Lambert, 1994; Draguhn & Heinemann, unpublished). The three GABA uptake blockers tested in our experiments displayed diverse effects. Whereas nipecotic acid (NPA) and β-alanine were both able to suppress the LRDs, tiagabine failed to influence this activity even at very high concentrations (250 μM). Both β-alanine and NPA had effective concentrations clearly above their apparent K_D for uptake inhibition (Guastella *et al.*, 1990; Borden *et al.*, 1992; Liu *et al.*, 1993). However, Dingledine & Korn (1985) used similar high concentrations of NPA to prolong inhibitory postsynaptic potentials in CA1 neurones. This discrepancy is possibly due to uptake of the substances and the slow equilibration time in brain slices (Müller *et al.*, 1988).

The recent cloning of cDNAs coding for GABA uptake molecules (GAT-1 to GAT-4) has revealed that tiagabine is a highly potent and selective inhibitor of the neuronally expressed GAT-1 (Clark *et al.*, 1992; Ikegaki *et al.*, 1994; Borden *et al.*, 1994), whereas β-alanine and NPA act on a variety of transporters which might also be expressed in glia (Guastella *et al.*, 1990; Clark *et al.*, 1992; Liu *et al.*, 1993; Borden *et al.*, GABA uptake and thereby enhance the extracellular amount of free GABA by a non-synaptic mechanism (Solis & Nicoll, 1992; Honmou *et al.*, 1995). Reversed uptake has been suggested to be a major inhibitory mechanism under pathophysiological conditions (Attwell *et al.*, 1993; Cammack *et al.*, 1994; During *et al.*, 1995) and therefore NPA might be particularly effective against epileptiform activity.

Possible mechanism of action

In contrast to clinically established antiepileptic drugs, $GABA_A$- and $GABA_B$ receptor agonists and non-neuronal GABA uptake blockers could block or reverse the LRDs in the entorhinal cortex. How can these effects be interpreted?

Upon prolonged seizure activity, inhibition becomes progressively weaker due to loss of synaptically available GABA (Schousboe *et al.*, 1983; Gonsalves *et al.*, 1989). LRDs do not react to potentiating modulators of $GABA_A$ receptors (Heinemann *et al.*, 1994) which might indicate that the amount of synaptically released transmitter is already diminished during this late activity pattern (Mody *et al.*, 1994). Supplementing GABA receptor agonists should then be effective in restoring the inhibitory transmission which was indeed the case in our experiments. Secondly, a selective block of glial uptake would redirect extracellular GABA to the axonal endings of interneurones thereby preventing the metabolism of GABA by the GABA-transaminase in glia cells (Schousboe *et al.*, 1983). In contrast, a selective blockade of neuronal GABA uptake would aggravate the failure of inhibition. Our observations on the differential effects of NPA and β-alanine versus tiagabine coincide with these predictions. We therefore support the hypothesis that depletion of synaptically available GABA

similar concept underlies the treatment of epilepsies with γ-vinyl-GABA, an irreversible blocker of the GABA transaminase which enhances the synaptic pool of GABA by closing the metabolic shunt (Ylinen *et al.*, 1991; Buchanan, 1993; Petroff *et al.*, 1995; Ben-Menachem, 1995).

Antiepileptic effects on SLEs and RSDs

The SLEs in the EC are sensitive to most clinically employed antiepileptic drugs, whereas the RSDs in CA1 react only to GABA agonists and valproic acid (Heinemann *et al.*, 1994; Zhang *et al.*, 1995). In accordance with previous findings (Jones, 1989) we found that GABA receptor agonists (GABA, muscimol and (−)-baclofen) were effective against RSDs and SLEs. Baclofen potently suppressed the entorhinal SLEs, but in contrast to previous findings (Jones, 1989) some SLEs remained even at high concentrations. This discrepancy is difficult to explain, especially since GABA had a similar low potent effect against SLEs in this study and our experiments. The difference might appear pronounced due to the long period (12 min) used for counting the frequency of SLEs in our study. Alternatively, minor strain differences cannot be excluded.

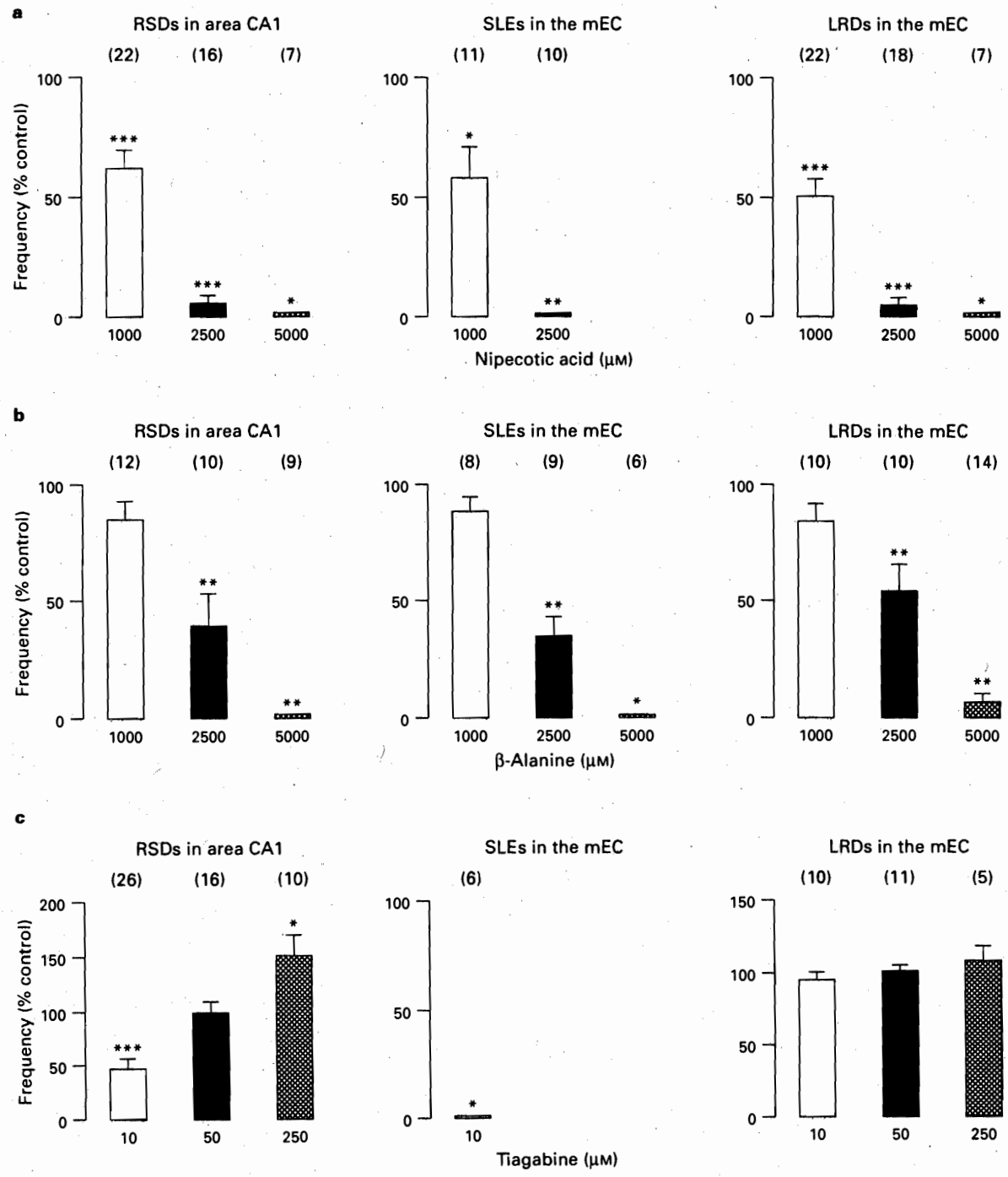

Figure 4 Summary of the effects of GABA uptake inhibitors on the different forms of epileptiform activity. (a) Nipecotic acid suppressed all forms of epileptiform activity concentration-dependently. (b) β-Alanine was slightly less potent than nipecotic acid. (c) Tiagabine (10 μM) reduced the frequency of the recurrent short discharges (RSDs). At 50 μM, no effect was seen and at 250 μM the frequency of the epileptiform discharges was significantly increased. Tiagabine blocked the seizure-like events (SLEs) at 10 μM in all experiments. At none of the concentrations tested did tiagabine suppress the late recurrent discharges (LRDs). Asterisks indicate the significance level of the discharge frequencies under GABA uptake blockers in comparison with control; $*P<0.05$; $**P<0.01$.

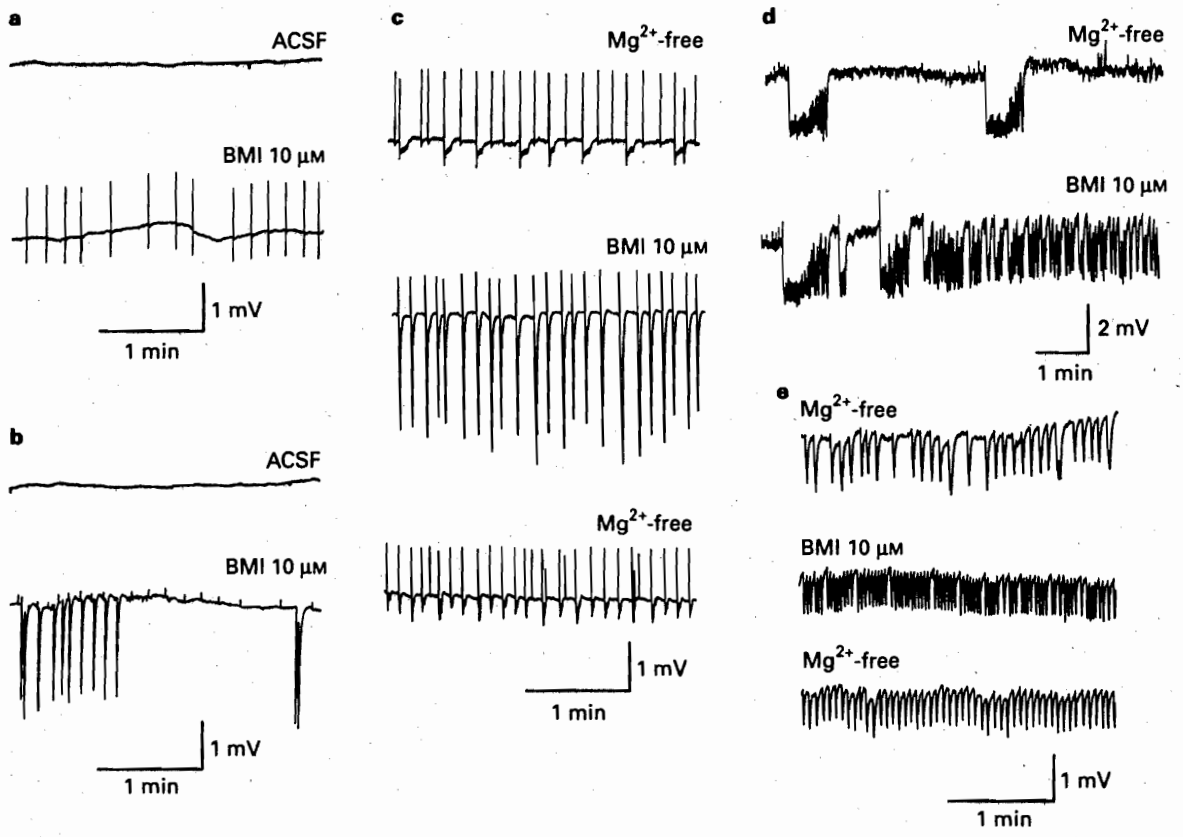

Figure 5 The $GABA_A$ receptor antagonist bicuculline methiodide (BMI) aggravated all forms of epileptiform activity. In normal ACSF, bicuculline (10 μM) induced interictal-like activity in the hippocampal area CA1 (a) and in the medial entorhinal cortex (b). (c) Bicuculline (10 μM for 9 min) reversibly aggravated low-Mg^{2+}-induced recurrent discharges (RSDs) in CA1. (d) In the medial entorhinal cortex, application of 10 μM BMI (4 min) resulted in a fast transition of seizure-like events (SLEs) to late recurrent discharges (LRDs). (e) At 10 μM bicuculline (16 min) the frequency of the LRDs was increased. This effect was reversible upon washout of the substance.

Baclofen was clearly more potent against RSDs in area CA1 than against the seizure-like events in the mEC. Recent evidence suggests that $GABA_B$ receptors mediate a strong heterosynaptic inhibition of excitatory synaptic transmission in CA1 (Isaacson *et al.*, 1993). This mechanism might explain the highly potent action of baclofen on RSDs. All uptake inhibitors tested effectively inhibited SLEs and RSDs. Most likely, this is caused by the prolongation of inhibitory postsynaptic potentials (Dingledine & Korn, 1985; Rekling *et al.*, 1990; Thompson & Gähwiler, 1992; Oh & Dichter, 1994; Roepstorff & Lambert, 1994; Draguhn & Heinemann, unpublished). The effect of tiagabine on the pharmacosensitive patterns of activity resembles the antiepileptic potency of this substance in animal models (Walton *et al.*, 1994; Smith *et al.*, 1995; Suzdak & Jansen, 1995) and man (Gram, 1994; Richens *et al.*, 1995). However, higher doses of tiagabine (50 and 250 μM) were ineffective against RSDs in CA1 and even enhanced the frequency of the events. The underlying mechanism for this inverted concentration-response relationship remains to be elucidated. We cannot exclude the possibility that exposure to high doses of tiagabine finally results in a metabolic depletion of GABA, as suggested for the late entorhinal activity.

Pharmacological relevance

In summary, our experiments show that GABA receptor agonists and GABA uptake blockers potently suppress epileptiform activity in the hippocampus and entorhinal cortex. Upon prolonged synchronized hyperactivity, the selective neuronal GABA uptake blocker tiagabine loses its efficacy. This finding might point towards a loss of synaptically available GABA in axonal endings of interneurones. In this case, a selective block of the glial GABA uptake and thereby a redirection of GABA to the neuronal pool would be a more adequate strategy for antiepileptic drugs.

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