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Kindling enhances kainate receptor-mediated depression of GABAergic inhibition in rat granule cells

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Abstract

Several lines of evidence indicate a substantial contribution of kainate receptors to temporal lobe seizures. The activation of kainate receptors located on hippocampal inhibitory interneurons was shown to reduce GABA release. A reduced GABA release secondary to kainate receptor activation could contribute to an enhanced seizure susceptibility. As the dentate gyrus serves a pivotal gating function in the spread of limbic seizures, we tested the role of kainate receptors in the regulation of GABA release in the dentate gyrus of control and kindled animals. Application of glutamate (100 μM) in the presence of the NMDA receptor antagonist D-APV and the AMPA receptor antagonist, SYM 2206 caused a slight depression of evoked monosynaptic inhibitory postsynaptic currents (IPSCs) in control, but a substantial decrease in kindled dentate granule cells. The observation that kainate receptor activation altered paired-pulse depression and reduced the frequency of TTX-insensitive miniature IPSCs without affecting their amplitude is consistent with a presynaptic action on the inhibitory terminal to reduce GABA release. In kindled preparations, neither glutamate (100 μM) nor kainate (10 μM) applied in a concentration known to depolarize hippocampal interneurons led to an increase of the TTX-sensitive spontaneous IPSC frequency nor to changes of the postsynaptic membrane properties. Consistently, the inhibitory effect on evoked IPSCs was not affected by the presence of the $GABA_B$ receptor antagonist, CGP55845A, thus excluding a depression by an enhanced release of GABA acting on presynaptic $GABA_B$ receptors. The enhanced inhibition of GABA release following presynaptic kainate receptor activation favours a use-dependent hyperexcitability in the epileptic dentate gyrus.

Introduction

As seizures from the entorhinal cortex propagate to the hippocampus, the dentate gyrus plays a crucial role in epileptogenesis. It has been suggested that the dentate gyrus functions as a filter that retards the spread of seizure activity to the hippocampus (Alger & Teyler, 1976; McNaughton *et al*., 1981; Heinemann *et al*., 1992; Lothman *et al*., 1992). This gating mechanism breaks down after chronic epilepsy is induced by kindling that facilitates the propagation of epileptiform activity (Behr *et al*., 1998). Both, single cell and network alterations may be responsible for the loss of filter function (Ribak *et al*., 1992; Schwartzkroin, 1993). Thus, previous studies described changes in the glutamatergic system at the cellular level that led to an increase in excitability that facilitated synaptic transfer from the entorhinal cortex to the hippocampus (Mody & Heinemann, 1987; Köhr *et al*., 1993; McNamara, 1994; McNamara, 1995; Mody & Lieberman, 1998). In addition, as NMDA and non-NMDA glutamate receptor antagonists both delay the induction of kindling (Bowyer, 1982; Peterson *et al*., 1983; Cain *et al*., 1988; Croucher *et al*., 1988; Sato *et al*., 1988; Dennison & Cain, 1989; McNamara, 1989; Holmes *et al*., 1990) glutamatergic neurotransmission seems to be involved critically in the generation of kindling epilepsy. The functional involvement of kainate (KA) receptors in epileptogenesis is becoming more and more elucidated (Mulle *et al*., 1998; Bernard *et al*., 1999; Vissel *et al*., 2001). Kainate receptor-mediated slow excitatory synaptic responses are present in hippocampal CA3 neurons (Castillo *et al*., 1997; Vignes & Collingridge, 1997), but in the dentate gyrus, a region that also expresses KA1, KA2, GluR-6 and GluR-7 mRNA (Wisden & Seeburg, 1993; Kamphuis *et al*., 1995), no KA receptor-mediated responses were detected in granule cells of control (Lerma *et al*., 1997) or kindled rats (Behr *et al*., 2001). Hence, the role of KA receptors in the dentate gyrus remains unclear. The activation of KA receptors located on CA1 inhibitory interneurons was shown to reduce GABA release (Frerking & Nicoll, 2000; Kullmann, 2001; Lerma *et al*., 2001) but the underlying mechanism is still a matter of dispute. Reduced GABA release secondary to KA receptor activation could effectively contribute to epileptogenesis. Therefore, we tested the role of KA receptors in the regulation of GABA release in the dentate gyrus of control and kindled animals. Our results provide insights into the contribution of KA receptor activation to the chronic hyperexcitability in temporal lobe epilepsy.

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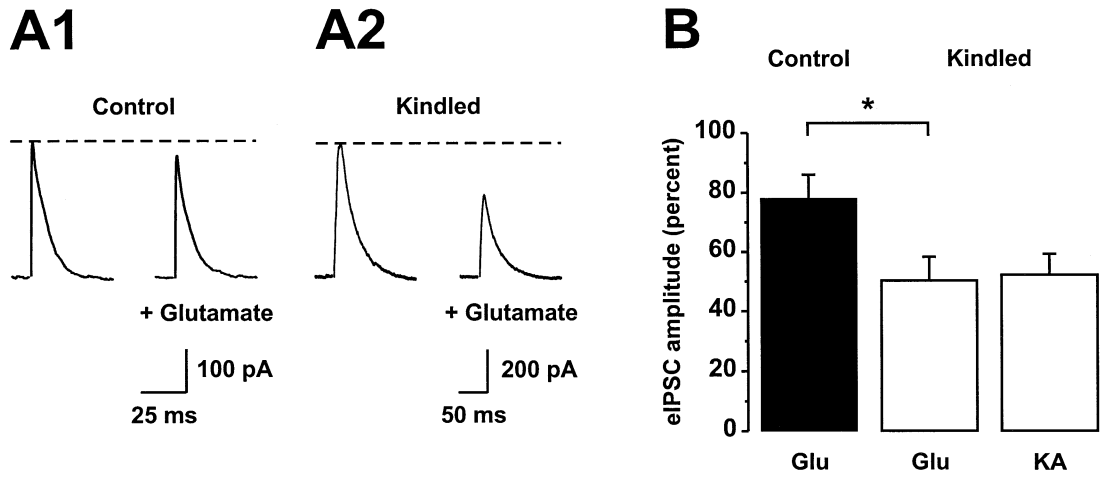

FIG. 1. Kindling enhances KA receptor-mediated depression of eIPSCs. (A1) In control rats, bath application of glutamate (100 μM) causes a slight but significant depression of $GABA_A$ receptor-mediated eIPSC amplitude in dentate granule cells (78.1 ± 7.1% of control value; $n = 7$). (A2) In kindled preparations, glutamate depresses eIPSCs significantly stronger (50.4 ± 7.9%; n = 8; $P < 0.05$). (B) A summary of experiments on KA receptor-mediated depression of eIPSCs. In kindled dentate granule cells, application of KA (10 μM) similarly causes a considerable decrease in the size of the eIPSC (52.3 ± 7.0%; $n = 4$) not significantly different from Glutamate (Glu) application (*$P < 0.05$).

Materials and methods

Kindling

Experiments were performed in 21 control hippocampal horizontal slices, obtained from four age-matched unimplanted controls and four sham-implanted controls, and 43 kindled hippocampal slices taken from 14 fully kindled 450–600 g adult Wistar rats. Animals were stimulated until ⩾15 consecutive stage 5 seizures were obtained. Bipolar stainless steel electrodes were implanted under Na-pentobarbital anaesthesia (75 mg/kg i.p) into the left amygdala (relative to bregma in mm: AP –2.5 posterior; 5 lateral; 8.5 below cortex) (Paxinos & Watson, 1986). After a postsurgical recovery period of 7–8 days, animals were stimulated daily through the implanted electrode with a train of biphasic, 150 μA pulses at 60 Hz for 1 s. Behavioural changes during kindling were scored according to the scale of Racine (Racine, 1972). All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (89/609/EEC) and approved by the regional Berlin animal ethics committee.

Slice preparation and solution

Forty-eight hours after the last seizure, the rats were decapitated under deep ether anaesthesia, the brains were removed quickly, and 400-μM-thick slices were prepared with a Campden vibroslicer (Campden, Loughborough, UK). The slices were transferred to an interface recording chamber that was perfused continuously with aerated (95% O2–5% CO_2), prewarmed (34 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 124; NaH_2PO_4, 1.25; $NaHCO_3$, 26; KCl, 3; $CaCl_2$, 1.6; $MgSO_4$, 1.8 and glucose, 10, pH 7.4.

Recording and data acquisition

Three types of inhibitory synaptic currents were studied: monosynaptic evoked inhibitory postsynaptic currents (IPSCs – eIPSCs), TTX-sensitive spontaneous IPSCs (sIPSCs) and TTX-insensitive miniature IPSCs (mIPSCs). To investigate the effect of glutamate and KA on KA receptors located on inhibitory interneurons all currents were recorded in the presence of the NMDA receptor antagonist D-APV (60 μM) and the AMPA receptor antagonist, SYM 2206 (100 μM). Activation of postsynaptic KA receptors on dentate granule cells can be neglected (Lerma *et al*., 1997; Behr *et al*., 2001). Postsynaptic $GABA_B$ receptor-mediated currents were eliminated by a Cs-based, QX-314 containing intracellular solution (Lambert & Wilson, 1993). All recordings of granule cells were done at 0 mV in whole cell voltage-clamp mode using a patch clamp amplifier (EPC-7, HEKA, Lambrecht, Germany). Electrodes were pulled from borosilicate glass (o.d., 2.0 mm, i.d., 1.0 mm) and had resistances of 6–8 MΩ when filled with intracellular solution containing (in mM): Cs-gluconate, 135; $CsCl_2$, 6; $MgCl_2$, 2; HEPES, 10; QX-314, 1; CsOH was used to adjust pH to 7.2. Whole-cell recordings were obtained using the 'blind' patch-clamp technique by lowering patch electrodes into the granule cell layer of the dentate gyrus while monitoring current responses to 10 mV voltage pulses and applying suction to form >GΩ seals. Access resistance was monitored throughout each experiment, and only recordings with access resistance of < 15 MΩ were considered acceptable for analysis. Access resistance was checked repeatedly during the experiments, and recordings showing an increase of more than 20% were rejected. Evoked IPSCs in the absence of excitatory transmission were evoked using 0.05 ms pulses every 10 s (intensity, 1–3 V) delivered through glass insulated bipolar platinum wire electrodes (tip diameter 50 μm, tip separation 100–200 μm) as described previously (Davies *et al*., 1990). Miniature IPSCs were recorded in the presence of 1 μM TTX.

Signals were filtered at 3 kHz, sampled at 9–12 kHz by an ITC–16 interface (Instrutech. Corp., Great Neck, NY, USA), and subsequently stored on an IBM-compatible computer. Peak amplitudes of eIPSCs were measured from an average of 8–10 sweeps and analysed off-line using TIDA software (HEKA, Lambrecht/Pfalz, Germany). Paired pulse depression (PPD) was expressed as the ratio of the peak amplitude of the second eIPSC to the peak amplitude of the first eIPSC. Detection of individual mIPSCs and sIPSC was performed off-line using a previously described software trigger (Otis & Mody, 1992; Soltesz *et al*., 1995) or ISO2 (MFK, Niedernhausen, Germany). Statistical evaluation was performed by applying Student's *t*-test (Origin 4.1, Microcal); data are expressed as means ± SEM. Significance level was set to $P < 0.05$.

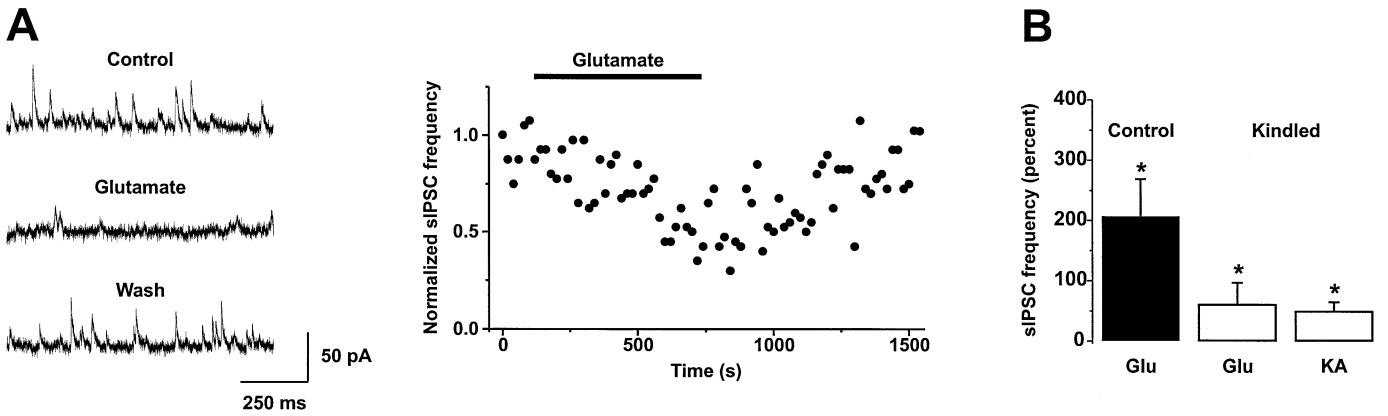

FIG. 2. In kindled rats, KA receptor activation depresses frequency of TTX-sensitive sIPSCs. (A) Recordings of sIPSCs before and after bath-application of glutamate (100 μm). While in control animals, we observed an average increase in the frequency of the sIPSC to 207 ± 61% ($n = 6$; $P < 0.05$; data not shown), in kindled animals glutamate causes a profound decrease of the discharge rate to 59 ± 34% ($n = 4$; $P < 0.05$) of control values. (B) A summary of experiments on KA receptor-mediated depression of sIPSCs. In the presence of KA (10 μm), the frequency of sIPSCs declined to 49 ± 13% ($n = 4$; $P < 0.05$), not significantly different from Glutamate (Glu) application (*$P < 0.05$).

Drugs

The following drugs were bath-applied: 60 μM 2-amino-5-phosphonovaleric acid (D-APV) (Research Biochemicals, Natick, MA, USA), 100 μM SYM 2206, 10 μM Kainic acid (KA) (both from Tocris, Bristol, UK), 2 μM 3-*N*-[1-(s)-(3,4-dichlorophenyl)ethyl]amino-2-(s)-hydroxypropyl-P-benzyl-phosphinic acid (CGP55845A) (a gift from CIBA-GEIGY, Basel, Switzerland), 100 μM L-Glutamic acid, 1 μM Tetrodotoxin (TTX) (both from Sigma-Aldrich, Deisenhofen, Germany).

Results

Voltage-clamp recordings were used to investigate the effect of the endogenous KA receptor agonist, glutamate (100 μM), on stimulus-evoked monosynaptic $GABA_A$ receptor-mediated eIPSCs in dentate granule cells. Postsynaptic $GABA_B$ receptor-mediated IPSCs were eliminated by the use of a Cs and QX-314 containing intracellular solution. NMDA receptor-mediated EPSCs were blocked by application of the selective antagonist D-APV (60 μM) and the AMPA receptor-mediated responses were eliminated by the addition of SYM 2206 (100 μM) (Pelletier *et al*., 1996; Li *et al*., 1999; Rodriguez-Moreno *et al*., 2000) (Fig. 1A and B). In control rats, bath application of glutamate caused a slight but significant depression of $GABA_A$ receptor-mediated eIPSCs amplitude in dentate granule cells [78.1 ± 7.1% of control value ($n = 7$)]. Surprisingly, in kindled preparations, the depression of eIPSCs by glutamate was significantly greater (50.4 ± 7.9%; $n = 8$; $P < 0.05$). Application of the receptor agonist, KA (10 μM), similarly caused a considerable decrease in the size of the eIPSC recorded in kindled dentate granule cells (52.3 ± 7.0% of control, $n = 4$).

As reported previously (Cossart *et al*., 1998; Frerking *et al*., 1998), activation of KA receptors induces a large increase in the frequency of TTX-sensitive sIPSCs in CA1 pyramidal cells caused by an increase in interneuronal spiking. The KA-induced increase in spontaneous GABA release may lead to an increase in the activation of presynaptic $GABA_B$ receptors and a concomitant depression of stimulus-evoked GABA release through a metabotropic-induced reduction in release probability (Davies *et al*., 1990). To test whether such a mechanism accounts for the observed eIPSC depression in dentate granule cells of kindled slices, we conducted recordings of sIPSCs before and during bath-application of glutamate (100 μM) in the presence of D-APV and SYM 2206. While in control animals we observed decreases to 61.5 ± 18.5% ($n = 2$) but also increases to 280.3 ± 63.5% ($n = 4$) of the sIPSC discharge rate with an average increase of the mean frequency from 9.1 ± 4.0 Hz to 11.5 ± 2.7 Hz ($n = 6$), in kindled rats, glutamate always caused a profound decrease of the discharge rate to 59 ± 34% of control values (the mean frequency decreased from 9.6 ± 2.3 Hz to 7.0 ± 4.3 Hz; $n = 4$; $P < 0.05$; Fig. 2A). In the presence of KA (10 μM), the frequency of sIPSCs declined to 49 ± 13% (from 13.1 ± 1.7 Hz to 6.0 ± 1.6 Hz; $n = 4$; $P < 0.05$). These results suggest that, at least in kindled animals, the depression of eIPSCs could not be explained by a substantially increased release of GABA acting on presynaptic inhibitory $GABA_B$ receptors. In agreement with this result, the depression of eIPSCs by KA (10 μM) was not affected by the presence of the $GABA_B$ receptor antagonist, CGP55845A (Fig. 3A, 55.7 ± 14.9%; $n = 6$; $P < 0.05$). In addition, an increased GABA release would cause tonic activation of postsynaptic $GABA_A$ receptors leading to changes in passive membrane properties including a decreased input resistance, a change in holding current and an increase in shunting with altered kinetics. However, as shown in a representative cell in Fig. 3B, the KA-induced depression of the eIPSC was neither paralleled by an altered kinetic of the response nor by a substantial change in holding current.

The results described thus far suggest that the increased depression of eIPSCs by KA receptor activation in kindled preparations seems not to be mediated by an increased discharge rate of inhibitory interneurons nor by a $GABA_B$ receptor-mediated metabotropic reduction in release probability. Albeit somewhat controversial, in CA1 pyramidal cells, the KA-induced depression of eIPSCs has been proposed to be mediated by presynaptic KA receptors located on terminals of inhibitory interneurons (Lerma *et al*., 2001). To determine the locus of action we first examined KA-induced changes in short-term plasticity. The KA-induced depression of eIPSCs was accompanied by an increase of the PPD (Fig. 4; $n = 6$; $P < 0.05$). Recordings of TTX-insensitive mIPSCs of control and kindled dentate granule cells also suggest a presynaptic mechanism. Miniature IPSCs are believed to result from the random release of single neurotransmitter packets, which does not require presynaptic calcium influx but seem to be regulated by Ca^{2+} release from

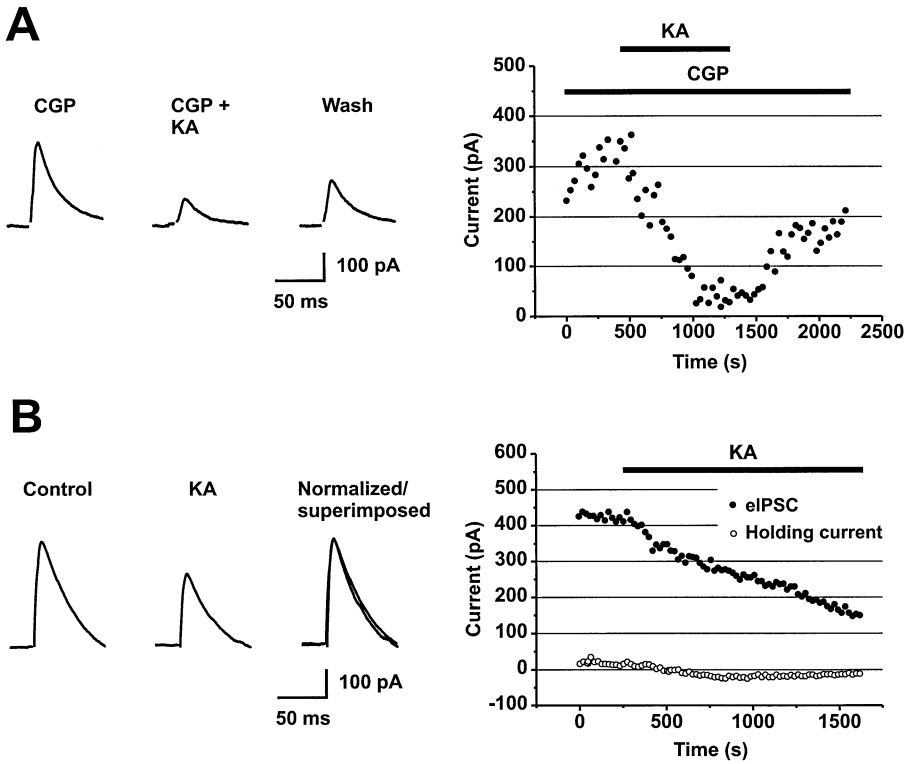

FIG. 3. Kindling induced enhancement of KA receptor-mediated eIPSC depression is independent of $GABA_B$ receptor activation and postsynaptic shunting. (A) In kindled preparations, the depression of eIPSCs by KA (10 μM) is not significantly affected by the presence of the $GABA_B$ receptor antagonist, CGP55845A (55.7 ± 14.9%; n = 6; $P < 0.05$). (B) The KA-induced depression of eIPSCs is not accompanied by changes in passive membrane properties of the postsynaptic granule cell. The inhibition of eIPSC is neither paralleled by an altered kinetic of the eIPSC nor by a change in holding current of the postsynaptic cell.

ryanodine-sensitive presynaptic Ca^{2+} stores (Llano *et al.*, 2000). Analysis of the mIPSC frequency before and during application of glutamate or KA provides information about possible changes in the presynaptic release process, while changes in the amplitudes of the miniature currents reflect postsynaptic alterations in receptor properties including their number at the synapse (Faber & Korn, 1991; Otis *et al.*, 1994; Buhl *et al.*, 1996; Nusser *et al.*, 1998). In control rats, KA (10 μM) application increased the mean inter-event interval between successive mIPSCs from 0.16 ± 0.05 s to 0.48 ± 0.31 s ($n = 8$; $P < 0.05$). Meanwhile, the amplitude distribution of mIPSCs was not affected ($P > 0.05$). In kindled rats, the mean inter-event interval similarly increased from 0.19 ± 0.03 s to 0.40 ± 0.12 s ($n = 11$; $P < 0.05$) without any significant effect on the amplitude distribution ($P > 0.05$, data not shown). Experiments in kindled preparations with glutamate (100 μM) confirmed these findings showing a prolongation of the mean inter-event interval from 0.09 ± 0.01 s to 0.13 ± 0.01 s (Fig. 5; $n = 6$; $P < 0.05$). Much like in the presence of KA, the amplitude distribution of mIPSCs remained unaltered ($P > 0.05$). These results suggest that activation of KA receptors either directly through receptors located on terminals of interneurons, or indirectly, through the release of other neuromodulators affect the GABA release machinery or the presynaptic Ca^{2+} current in both animal groups.

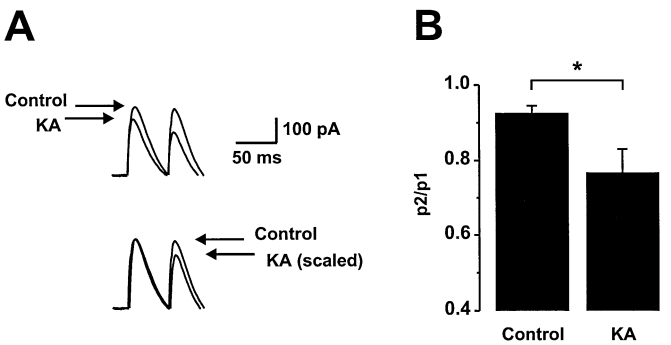

FIG. 4. KA receptor activation reduces the probability of release. (A) The KA-induced depression of eIPSCs is accompanied by an increase of the PPD ($n = 6$; $^*P < 0.05$). KA has a larger effect on the second of two eIPSCs. Bottom traces show the paired-pulse responses after scaling the first eIPSC before and during KA application. (B) A summary of experiments on PPD.

Discussion

We found that application of glutamate depresses monosynaptic eIPSCs in dentate granule cells of control and kindled rats. Interestingly, this effect was much stronger in kindled, chronic epileptic animals and could be mimicked by KA application. The profound depressant effect of KA receptor activation on inhibitory transmitter release in kindled preparations suggests the existence of KA receptors on inhibitory interneurons of the dentate gyrus. The observation that KA receptor activation altered PPD and reduced the frequency of TTX-insensitive mIPSCs in both animal groups, without affecting their amplitude, is consistent with a presynaptic action on the inhibitory terminal to reduce GABA release.

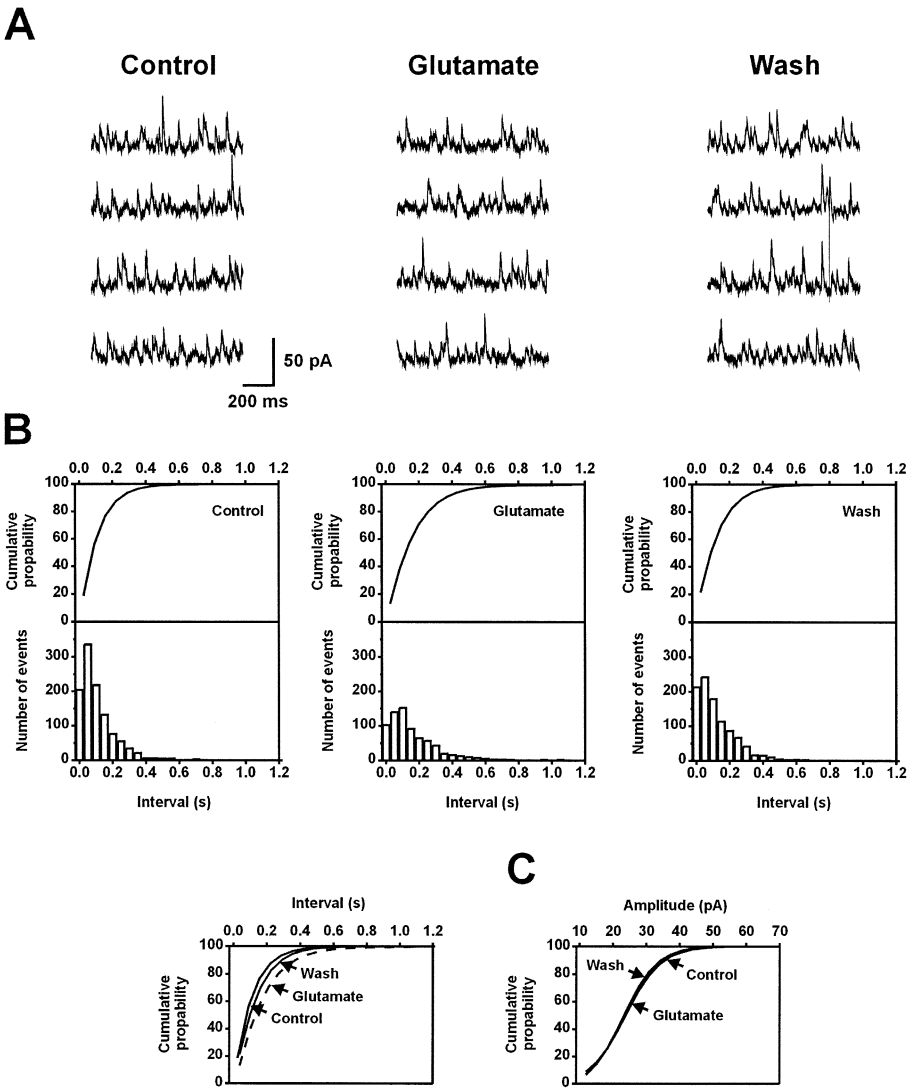

FIG. 5. In kindled rats, KA receptor activation depresses frequency of TTX-insensitive mIPSCs. (A) Consecutive recordings of mIPSCs before, during and after bath application of glutamate (100 μM). (B) Respective inter-event interval histograms. The mean mIPSC inter-event interval during the control period is 0.09 ± 0.01 s. Application of glutamate results in an increase of the inter-event interval to 0.13 ± 0.01 s ($n = 6$; $P < 0.05$) indicating a presynaptic action on KA receptors. (C) Respective cumulative probability plots of the mIPSC amplitude were not significantly altered by glutamate ($P > 0.05$).

Previous observations, that the frequency of sIPSCs in hippocampal pyramidal cells is strongly increased by the presence of KA, has prompted an alternative explanation for the KA receptor-mediated depression of eIPSCs: the increased spontaneous GABA release following dendritic or somatic KA receptor activation (Cossart *et al*., 1998; Frerking *et al*., 1998) or by triggering ectopic action potentials in axons (Semyanov & Kullmann, 2001) was shown to depress eIPSC in CA1 pyramidal cells by activation of presynaptic metabotropic $GABA_B$ receptors (Frerking *et al*., 1999). In addition, the enhanced tonic inhibition activates postsynaptic $GABA_A$ receptors causing an increased shunting with a subsequent decrease of the eIPSC (Frerking *et al*., 1999). Notably, previous studies showed no (Frerking *et al*., 1998) or just moderate (Cossart *et al*., 1998; Bureau *et al*., 1999) decreases of TTX-insensitive mIPSCs in the presence of KA. To bring together these contradictory data on the mechanisms underlying the KA receptor-mediated depression, Rodriguez-Moreno *et al*. (2000) proposed a concentration-dependent action of glutamate on different populations of KA receptors with different agonist sensitivities on stratum oriens interneurons in hippocampal area CA1. They provided evidence that glutamate, applied at low concentrations, depresses eIPSCs without a coincident increase in the interneuronal firing rate. In granule cells of control rats, we observed inconsistent effects of glutamate on spontaneous GABA release showing both, an enhancement and a depression of the sIPSCs frequency. In kindled preparations, however, neither glutamate nor KA applied in concentrations known to depolarize hippocampal interneurons led to an increase of the sIPSCs frequency or to changes of the postsynaptic membrane properties. The absence of any increase in the sIPSC frequency might be attributed to the fact that in kindled preparations, most of the sIPSCs result from a release of GABA that is independent of action potentials (Buhl *et al*., 1996). Furthermore, the inhibitory effect on eIPSCs was not affected by the presence of the $GABA_B$ receptor antagonist, CGP55845A, excluding a depression of eIPSCs by an enhanced release of GABA acting on presynaptic $GABA_B$ receptors. In addition, the depressant action of both, glutamate and KA on TTX-insensitive mIPSCs in control and

kindled preparations favours a mechanism mediated by KA receptors located at the synapse of inhibitory interneurons. Surprisingly, the change in mIPSC frequency induced by KA in control and kindled animals was about the same, whilst the depression of eIPSCs was larger in kindled rats. It is possible that the terminals responsible for generating mIPSCs are not necessarily the same as those that are stimulated by the stimulating electrode to obtain eIPSCs or not even the same as those responsible for sIPSCs. At least in dentate gyrus granule cells, mIPSCs originate mainly from synapses around the somata and proximal dendrites (Soltesz *et al*., 1995). This may be consistent with an hypothesis, that will be explored in future experiments, that the synapses formed by different interneuronal populations are affected differentially by KA. While PPD usually decreases under conditions where transmitter release is decreased, in our study, cells showed an increased PPD in the presence of KA. Presently, we do not have a conclusive explanation for the discrepancy. However, it is feasible that KA does not alter the release machinery by reducing Ca^{2+} influx alone.

What is the mechanism mediating the enhanced depressant action on eIPSCs in kindled animals? The effect was explained previously by a decreased probability of GABA release secondary to activation of presynaptic KA receptors (Rodríguez-Moreno & Lerma, 1998; Rodriguez-Moreno *et al*., 2000). This mechanism is consistent with the observation that KA receptor activation also depresses glutamate release (Chittajallu *et al*., 1996; Vignes *et al*., 1998; Frerking *et al*., 2001) presumably by a decreased presynaptic calcium influx into glutamatergic terminals (Kamiya & Ozawa, 1998; Alici *et al*., 1997). In addition, perfusion of KA onto hippocampal cultures and slices was found repeatedly to produce a sizeable reduction in the frequency of calcium entry-independent mIPSCs, a finding which, like in our study, can be regarded as an interference with presynaptic mechanisms responsible for GABA release (Rodríguez-Moreno & Lerma, 1998; Rodriguez-Moreno *et al*., 2000). The activation of second messenger systems by KA has been interpreted as evidence for the involvement of yet unidentified metabotropic KA receptors (Rodríguez-Moreno & Lerma, 1998; Rodriguez-Moreno *et al*., 2000). However, presynaptic KA receptor activation has been shown to modulate transmitter release via ionotropic mechanisms (Kamiya & Ozawa, 2000; Schmitz *et al*., 2000). A similar link between an ionotropic receptor and the transmitter release machinery has been reported for the AMPA receptor (Wang *et al*., 1997). This finding is in line with binding studies showing a functional coupling between the G_i/G_o subtype of G-protein and KA receptors (Pelletier *et al*., 1996). Extrapolating previous studies that propose a metabotropic mechanism for the depression of eIPSCs, it is feasible that kindling enhances the inhibitory action by alteration of the release machinery downstream of presynaptic depolarization via ionotropic KA receptors. Alternatively, kindling may change the expression of KA receptors and subsequently the KA receptor-mediated depolarization of the synapse because previous studies proposed a relationship between a decreased GluR Q/R site editing and an increased seizure susceptibility (Grigorenko *et al*., 1998; Bernard *et al*., 1999; Vissel *et al*., 2001).

In conclusion, we provide evidence that kindling enhances the KA receptor-mediated depression of GABAergic inhibition by activation of presynaptic KA receptors located on terminals of inhibitory interneurons. For the dentate gyrus, at present, we do not have any clues for an indirect mechanism as discussed for area CA1 (Frerking *et al*., 1999). The mechanism whereby these KA receptors inhibit GABAergic release in the kindled preparation remains unclear. As the exact process by which diverse presynaptic receptors down-regulate GABA release is not known (Thompson *et al*., 1993), the challenge ahead is to resolve fundamental mechanisms regulating the calcium entry-independent transmitter release in the CNS. Regardless of the underlying mechanism, our findings reveal an intriguing capacity of the excitatory neurotransmitter glutamate to facilitate its action by reducing GABAergic inhibition. At adjacent excitatory and inhibitory synapses, spillover of glutamate upon sustained firing of granule cells during seizures could reduce dramatically the efficacy of GABAergic interneurons in kindled animals. Hence, in kindled animals, the activation of presynaptic KA receptors on GABA cells may effectively short circuit the effects of an enhanced number of synaptic GABA receptors on granule cells (Nusser *et al*., 1998). The kindling-induced sensitivity of GABA release to KA receptor activation may produce a use-dependent hyperexcitability in the epileptic dentate gyrus facilitating the spread of limbic seizures through the entorhinal–hippocampal complex in temporal lobe epilepsy.

Acknowledgements

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Abbreviations

AMPA, RS-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionicacid; APV, 2-amino-5-phosphonovalericacid; IPSC, inhibitory post synaptic current; GABA, γ-amino-butyric acid; KA, kainate.

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