Kindling induces transient fast inhibition in the dentate gyrus–CA3 projection

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Abstract

The granule cells of the dentate gyrus (DG) send a strong glutamatergic projection, the mossy fibre tract, toward the hippocampal CA3 field, where it excites pyramidal cells and neighbouring inhibitory interneurons. Despite their excitatory nature, granule cells contain small amounts of GAD (glutamate decarboxylase), the main synthetic enzyme for the inhibitory transmitter GABA. Chronic temporal lobe epilepsy results in transient upregulation of GAD and GABA in granule cells, giving rise to the speculation that following overexcitation, mossy fibres exert an inhibitory effect by release of GABA. We therefore stimulated the DG and recorded synaptic potentials from CA3 pyramidal cells in brain slices from kindled and control rats. In both preparations, DG stimulation caused excitatory postsynaptic potential (EPSP)/inhibitory postsynaptic potential (IPSP) sequences. These potentials could be completely blocked by glutamate receptor antagonists in control rats, while in the kindled rats, a bicuculline-sensitive fast IPSP remained, with an onset latency similar to that of the control EPSP. Interestingly, this IPSP disappeared 1 month after the last seizure. When synaptic responses were evoked by high-frequency stimulation, EPSPs in normal rats readily summate to evoke action potentials. In slices from kindled rats, a summation of IPSPs overrides that of the EPSPs and reduces the probability of evoking action potentials. Our data show for the first time that kindling induces functionally relevant activity-dependent expression of fast inhibition onto pyramidal cells, coming from the DG, that can limit CA3 excitation in a frequency-dependent manner.

Introduction

In several models of experimental epilepsy, including amygdala kindling (Goddard et al., 1969), permanent and transient hippocampal changes have been described at the molecular (Dragunow & Robertson, 1987; Buzsáki et al., 1992; Mody, 1993), functional (Mody & Heinemann, 1987) and anatomical (Cavazos & Sutula, 1990; Babb et al., 1991; Isokawa et al., 1993; Qiao & Noebels, 1993; Repesa et al., 1993) level. Excitatory neurotransmission has been shown to be exacerbated (Mody & Heinemann, 1987; Martin et al., 1992; Kraus et al., 1994) while at the same time, both enhanced (Babb et al., 1989; Sloviter, 1987; Empson & Jefferys, 1993) and diminished (Obenaus et al., 1993; Najlerahim et al., 1992) functions of the GABAergic system have been reported. Some of the adaptive changes may serve to limit the risk of seizure generation while others might facilitate this process.

The granule cells of the DG are the main target of cortical input to the hippocampus and project to CA3 with their mossy fibres (MF), which form glutamatergic excitatory synapses (Crawford & Connor, 1973) on CA3 pyramidal cells and local inhibitory interneurons, which in turn inhibit pyramidal cells (Dichter & Spencer, 1969; Yamamoto, 1972; Buzsáki, 1984). Thus, the blockade of glutamatergic excitatory transmission with AMPA/kainate and NMDA receptors antagonists abolishes mono-synaptic excitatory postsynaptic potentials (EPSPs) and disynaptic inhibitory postsynaptic potentials (IPSPs) in CA3, as well as EPSPs induced by stimulation of associational × commissural inputs (Weisskopf & Nicoll, 1995).

After a series of kainic acid-induced limbic seizures, granule cells transiently express mRNA for GAD (glutamate decarboxylase, the synthesizing enzyme for GABA) and are also immunopositive for GABA (Schwarzer & Sperk, 1995; Lehman et al., 1996). There is also some evidence for the presence of both GAD and GABA in granule cells of healthy animals in which the concentration of GAD can be enhanced after electrical stimulation (Sloviter et al., 1996; Sandler & Smith, 1991). These data suggested that MF may be capable of utilizing GABA for fast neurotransmission (Sandler & Smith, 1991; Lehmann et al., 1996; Sloviter et al., 1996). Indeed it has been shown that transfection of a GAD67 promoter into fibroblasts (Ruppert et al., 1993) and astrocytes (Sacchettoni et al., 1998) induces the production of the enzyme and the formation of GABA from glutamate and its release.

Here we directly searched for functional alterations in the projection from the DG to CA3 pyramidal cells, in animals in which chronic epilepsy was induced by repetitive electrical stimulation of the amygdala (kindling). Synaptic potentials were recorded from CA3 pyramidal cells after stimulation in the DG, in slices from normal and amygdala kindled rats 24 h and 4 weeks after the last seizure. Consistent with our hypothesis, we found that temporal lobe seizures induce transient fast inhibitory transmission from the DG to CA3.
were decapitated under deep ether anaesthesia, brains were dissected and combined entorhinal cortex±hippocampus slices (400 µm) were evoked (first seizure 11.3 ± 3.7 kindling trials; n = 30). Experiments were carried out in accordance with the Guidelines of the Committee for Use of Laboratory Animals (CINVESTAV and German law for animal protection).

Twenty-four hours or 4 weeks after the last kindled seizure, the rats were anaesthetized with ketamine (60 mg/kg i.p.) and implanted with bipolar stainless steel electrodes (80 kΩ) into the left basolateral amygdala (AP 2.5; L 5; H 8.5; Paxinos & Watson, 1986). Starting 7–10 days after the operation, the animals were daily stimulated with a 2-s train of square pulses (0.1 ms pulse duration; 60 Hz; ~500 µA) until 7 ± 3 generalized convulsive seizures (stage V; Racine, 1972) were evoked (first seizure 11.3 ± 3.7 kindling trials; n = 30). Experiments were carried out in accordance with the Guidelines of the Committee for Use of Laboratory Animals (CINVESTAV and German law for animal protection).

Materials and methods

Kindling procedure

Wistar rats (230–250 g) were anaesthetized with ketamine (60 mg/kg i.p.) and implanted with bipolar stainless steel electrodes (80 kΩ) into the left basolateral amygdala (AP 2.5; L 5; H 8.5; Paxinos & Watson, 1986). Starting 7–10 days after the operation, the animals were daily stimulated with a 2-s train of square pulses (0.1 ms pulse duration; 60 Hz; ~500 µA) until 7 ± 3 generalized convulsive seizures (stage V; Racine, 1972) were evoked (first seizure 11.3 ± 3.7 kindling trials; n = 30). Experiments were carried out in accordance with the Guidelines of the Committee for Use of Laboratory Animals (CINVESTAV and German law for animal protection).

In vitro intracellular recordings

Twenty-four hours or 4 weeks after the last kindled seizure, the rats were anaesthetized with deep ether anaesthesia, brains were dissected and combined entorhinal cortex–hippocampus slices (400 µm) were cut with a vibratome (Campden Instruments, UK) submerged in oxygenated artificial cerebrospinal fluid (ACSF) at 4 °C. Slices were then transferred to an interface recording chamber and were constantly perfused with oxygenated ACSF at 35 °C containing (in mM): NaCl, 124; KCl, 3; NaH2PO4, 1.25; MgSO4, 2; CaCl2, 2; NaHCO3, 26; glucose 10; pH 7.35. The drugs used were diluted at the desired concentration in the ACSF, namely: the broad-spectrum glutamate receptor antagonist (GluRA) kynurenic acid (2 mM; Sigma, St Louis, MO, USA); the NMDA-type GluRA (DL)-2-amino-5-phosphonovaleric acid (APV; 30 µm; Research Biochemicals, Natick, MA, USA), and the non-NMDA type GluRA 6-nitro-7-sulphamoyl-benzo(f)quinoxaline-2,3-dione (NBQX; 10 µm; Novo Nordisk, Denmark); the GABAa receptor antagonist bicuculline methiodide (20 µm; Sigma); the cholinergic antagonists atropine (10 µm), and atropine (5 µm; Sigma); the metabotropic GluRA (+)-α-methyl-4-carboxyphenyl-glycine (+)-MCPG (500 µm, Tocris Neuroamin, Bristol, UK).

An hour after incubation, intracellular recordings were carried out with conventional electrophysiological techniques, using borosilicate micropipettes pulled with a Brown-Flaming horizontal puller with a resistance of 70–90 MΩ and filled with 2 M potassium acetate. The signals were sent to a computer for data acquisition and later analyzed off-line with the program pClamp6 (Axon Instruments, Foster City, CA, USA).

For electrical stimulation, a bipolar, glass-insulated platinum wire (50 µm) electrode was placed over the MF or the granular cell layer of the DG (Fig. 1A). In some experiments, the outer molecular layer of the DG was also stimulated. This was done to reduce activation of interneurons, whose fibres could perhaps project to area CA3. The results for each stimulation site are reported separately (see Results). In the CA3 region, a glass pipette (1–2 MΩ) filled with ACSF was positioned 200–300 µm apart from the recorded cell to stimulate local interneurons. Pulses had a duration of 0.1 ms and intensity was fixed to a value that evoked 70% of the EPSP amplitude needed to reach threshold for evoking action potentials. In no cases were action potentials evoked when stimulating at 3 Hz with this intensity. Repetitive stimulation was given through a programmable stimulator and consisted of trains of 2 s duration at 3 and 10 Hz, and 1 min when stimulating at 50 Hz. Each cell was tested three times with each frequency at an intertrain interval of 30 s when stimulating at 3 and 10 Hz, and 1 min when stimulating at 50 Hz.

Intracellular recordings were obtained from: 34 pyramidal cells from 17 rats 1 day after the last seizure, 19 pyramidal cells from four rats 4 weeks after the last seizure, and 40 pyramidal cells from 13 control rats within the pyramidal cell layer of area CA3. Because recordings lasted for several hours, we usually did several measurements in the same cell. Results were pooled from both hippocampi, as similar responses were seen on both sides, irrespective of the side of...
the kindling stimulation. The off-line analysis of the synaptic responses in all the cells was carried out blindly, i.e. without knowing the experimental group to which the animal belonged. Results are presented as means ± SEM.

Results

The resting membrane potentials from control and kindled pyramidal cells were: controls, −60.5 ± 0.8 mV; and kindled: −64 ± 0.8 mV (statistically significant $P < 0.006$; Student’s t-test); and the input resistances were: controls, 34 ± 1.9 MΩ; and kindled, 35.6 ± 1.8 MΩ ($n = 26$).

**Synaptic responses to single-pulse stimulation**

Single-pulse electrical stimulation of the MF or the DG evoked a fast EPSP, followed by a fast IPSP and a slow IPSP in all preparations (Fig. 1). The onset latencies (measured from the beginning of the stimulus artefact to the beginning of the synaptic potential) of the DG- and MF-evoked EPSPs are given in Table 1. The reversal potentials of the fast IPSPs elicited by DG and CA3 stimulation, respectively, are given in Table 1. The reversal potential of the EPSP could not be determined as the recordings were performed in current clamp configuration.

In the presence of antagonists to NMDA and AMPA receptors, the evoked IPSP was often followed by a slow depolarizing potential and, with increasing stimulus intensity, a slow IPSP could follow the slow EPSP. Such a slow EPSP, if present in interneurons, could account for the persistence of IPSPs in kindled animals. We therefore studied the pharmacological properties of this slow component. It was insensitive to the metabotropic glutamate receptor antagonist MCPG (500 μM). That it was not a depolarizing IPSP was confirmed by perfusion of bicuculline, which accentuated the slow depolarizing component and, at the same time blocked the fast IPSP (Fig. 3A). Also, kynurenic acid (2 mM) did not have any effect on the slow EPSP (not shown). This component was completely and reversibly blocked by simultaneous perfusion of pirenzepine or atropine ($n = 5$; Fig. 3C), indicating that it was mediated by mGlu5/6 acetylcholine receptors. The fast IPSP could still be evoked from the DG in the presence of cholinergic antagonists ($n = 5$; not shown), demonstrating that it was not dependent on cholinergic excitation of inhibitory interneurons.

To test if the presence of an IPSP in the kindled slices perfused with GluRAs was due to direct stimulation of interneurons within CA3, we looked for differences of IPSPs evoked by stimulation with a locally positioned patch-pipette (100–300 μm apart from the recording electrode). Local stimulation within area CA3 evoked, both in control and kindled animals, monosynaptic IPSPs that could be blocked by bicuculline. In contrast to the MF- or DG-induced IPSPs observed in the kindled animals, CA3-induced IPSPs were not followed by any further depolarizing component (Fig. 3B), and no synaptic responses were observed after blockade with bicuculline (not shown). Also, to avoid the possible stimulation of projections coming from inhibitory interneurons in area CA1 (Sik et al., 1994), we tested for inhibitory responses after isolating the DG and area CA3 from CA1 by cutting the hippocampus along the hippocampal fissure in three slices. Two to three hours after the cut was made, stimulation of the DG ($n = 3$) or

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>(IPSP/EPSP)</th>
<th>Onset latency (ms)</th>
<th>n</th>
<th>Latency to peak (ms)</th>
<th>n</th>
<th>Reversal potential (mV)</th>
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<tr>
<td>During normal ACSF perfusion</td>
<td></td>
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<tr>
<td>Control CA3 pyramidal cells</td>
<td>DG</td>
<td>(EPSP)</td>
<td>4.7 ± 0.4*</td>
<td>7</td>
<td>8.4 ± 0.7</td>
<td>15</td>
<td>ND</td>
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<td></td>
<td>MF</td>
<td>(EPSP)</td>
<td>3.8 ± 0.2</td>
<td>29</td>
<td>ND</td>
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<td></td>
<td>DG</td>
<td>(IPSP)</td>
<td>2.3 ± 0.4</td>
<td>17</td>
<td>13.8 ± 1.0</td>
<td>8</td>
<td>−66.3 ± 0.7</td>
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<tr>
<td>Kindled CA3 pyramidal cells</td>
<td>DG</td>
<td>(EPSP)</td>
<td>4.7 ± 0.3*</td>
<td>15</td>
<td>6.6 ± 0.6*</td>
<td>17</td>
<td>ND</td>
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<tr>
<td></td>
<td>MF</td>
<td>(EPSP)</td>
<td>3.2 ± 0.2</td>
<td>31</td>
<td>ND</td>
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<td></td>
<td>DG</td>
<td>(IPSP)</td>
<td>18.9 ± 1.5</td>
<td>17</td>
<td>−65.5 ± 0.6</td>
<td>13</td>
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<td></td>
<td>CA3</td>
<td>(IPSP)</td>
<td>2.8 ± 0.4</td>
<td>13</td>
<td>9.8 ± 0.7*</td>
<td>9</td>
<td>−68.5 ± 0.7</td>
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<td>During GluRAs perfusion</td>
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<tr>
<td>Control CA3 pyramidal cells</td>
<td>CA3</td>
<td>(IPSP)</td>
<td>1.9 ± 0.7</td>
<td>6</td>
<td>11.5 ± 0.6</td>
<td>4</td>
<td>−69 ± 1</td>
</tr>
<tr>
<td>Kindled CA3 pyramidal cells</td>
<td>DG</td>
<td>(IPSP)</td>
<td>5.3 ± 0.4*</td>
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<td>10.3 ± 0.8</td>
<td>14</td>
<td>−67.5 ± 0.5</td>
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<tr>
<td></td>
<td>MF</td>
<td>(IPSP)</td>
<td>3.6 ± 0.5*</td>
<td>10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CA3</td>
<td>(IPSP)</td>
<td>2.4 ± 0.2</td>
<td>13</td>
<td>9.3 ± 0.7*</td>
<td>8</td>
<td>−72 ± 0.8</td>
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ND, not determined. $*P < 0.05$, DG stimulus vs MF stimulus. $\ddagger P < 0.05$, control vs kindled.
MF ($n = 3$) still induced IPSPs in pyramidal cells of area CA3 during NBQX and APV perfusion, that could be blocked by bicuculline.

To test for the permanence of the expression of the DG-evoked IPSPs, we carried out MF or DG stimulation 4 weeks after the last kindled seizure. The simultaneous perfusion of NBQX and APV blocked both the EPSPs and IPSPs upon stimulation in 15 out of 19 cells ($n = 19$). In the four cells that presented IPSPs upon DG or MF stimulation, bicuculline blocked this response and, in contrast to the freshly kindled cells, no further depolarizing component was found (Fig. 3A).

The latency to the peak of the disynaptic IPSP was shortened in the kindled animals in comparison with the controls (two-way ANOVA; $F_{1,77} = 14.17; P = 0.0005$). The latencies to the peak of the synaptic responses of the control and kindled cells are grouped in Table 1.

**Synaptic responses to repetitive stimulation**

To study the behaviour of pyramidal cells in response to high-frequency stimulation, the DG was stimulated at 3, 10 and 50 Hz. The responses of control pyramidal cells to high-frequency stimulation are depicted in Fig. 4A. The intensity of the stimulation was set as described in Materials and methods and care was taken that the cells did not fire during a train of six pulses at 3 Hz. Under this condition, 3-Hz stimulation evoked EPSP/IPSP sequences that did not summate. This was true for both control and kindled preparations.

Synaptic stimulation at 10 Hz during 2 s evoked EPSP/IPSP sequences that eventually elicited action potentials. The mean number of action potentials evoked with this 2 s train in control cells was seven ($n = 12$ cells; three trials each). In the kindled cells, however, a mean of three action potentials was found ($n = 6$ cells; three trials each). The cumulative distribution of the action potentials throughout the train is plotted in Fig. 4B. Also, at this frequency, less stimuli were needed to evoke the first action potential in the control than in the kindled cells.

Trains of 1 s at 50 Hz evoked repetitive spiking due to strong summation of EPSPs, and possibly a depolarizing shift of the reversal potential for slow IPSPs due to extracellular potassium accumulation (Rausche et al., 1989). The mean number of action potentials evoked during the train of 50 stimuli in control preparations was 18 action potentials after the 50 stimuli ($n = 13$ cells; see Fig. 4B). In kindled animals, the number of action potentials evoked in CA3 pyramidal cells within the first 20 stimuli was lower than that of the control animals, similar to our observations at 10-Hz stimulation. After 25 stimuli, the number of evoked action potentials increased progressively during the second half of the train in the kindled preparations in comparison with the controls, until a mean of 25 action potentials was observed ($n = 15$). Thus, the kindled preparations presented an initial period of enhanced inhibition in response to high-frequency excitation whereas in control preparations, summation of depolarizing responses readily overrides the polysynaptic inhibitory actions. An after-hyperpolarization, due to repetitive spiking, was evoked after the 50 Hz train in both groups.

**High-frequency stimulation during application of glutamate receptor antagonists**

The initial high-frequency stimulation experiments showed that CA3 pyramidal cells in kindled preparations are under a stronger inhibitory control than control cells. To explore the behaviour of the putative, monosynaptically driven IPSPs at high-frequency stimulation, the same experiments were performed during application of GluRAs.
The DG- or MF-evoked IPSPs followed high-frequency stimulation. Figure 5A shows the effect of 10-Hz stimulation on control and kindled preparations perfused with GluRAs. In contrast to the control preparations, in which EPSPs and IPSPs were blocked, IPSPs could still be evoked in the kindled preparations (Fig. 5A). Likewise, stimulation at 50 Hz during perfusion with GluRAs did not reveal any synaptic response in the control preparations while cells from kindled preparations could follow this stimulation frequency and displayed summation leading to a net hyperpolarization (Fig. 5B).

The same kind of analysis was made on cells of animals that did not receive any kindling stimulus during a month after the last kindled seizure. As previously shown, almost none of these cells presented

the fast IPSPs upon perfusion with GluRAs. Accordingly, none of them presented synaptic responses that could be uncovered when stimulating with trains of 10 or 50 Hz (Fig. 6). In three cells with a monosynaptic IPSP in the presence of GluRAs, high-frequency stimulation produced a hyperpolarization by summation of IPSPs (Fig. 6). When blocking the IPSPs with bicuculline, however, these cells did not have the slow EPSPs revealed in the freshly kindled animals.

Discussion
Our data reflect a transient activation of a new, putatively direct inhibitory input from DG to CA3 in rats with kindling-induced temporal lobe epilepsy. The onset latency of the DG- or MF-evoked IPSPs is similar to that of the EPSPs, and is shifted by the same
amount when comparing MF vs. DG stimulation. Furthermore, the transient presence of the DG-induced IPSPs follows the time-course of the expression of GAD in granule cells of epileptic animals (Schwarzer & Sperk, 1995). The presence of a DG-evoked fast monosynaptic IPSP in pyramidal cells, that in normal conditions can only be of disynaptic nature, is in agreement with the notion that MF might not only be capable of synthesizing and storing GABA (Sandler & Smith, 1991; Schwarzer & Sperk, 1995; Sloviter et al., 1996) as it has been shown for other cell types (Cao et al., 1996), but that they might also be capable of utilizing it for fast inhibitory synaptic transmission. Apparently, a detectable inhibitory transmission from granule cells to CA3 pyramidal cells is restricted to situations of previous hyperexcitation, although GABA and GAD might normally be present in MF (Sloviter et al., 1996). The fact that 4 weeks after the last kindled seizure — at a time when GAD expression was low — the inhibitory responses are rarely seen, suggests that they are a consequence of seizures and/or of repetitive stimulation and that they are not relevant to the persistent kindled state. The increase in the transmitter pool after kindling, as well as the increase in release, is then a prerequisite for effective inhibitory transmission (for review see Waagepetersen et al., 1999).

Alternatively, one might consider the possible activation of silent inhibitory synapses (Poisbeau et al., 1997) by the seizures, but there are no hints toward a direct GABAergic projection from the DG to CA3, which might become activated by epilepsy. Several groups have described axonal sprouting in models of epilepsy. While sprouting of the MF is thought to underlie enhanced excitatory neurotransmission, our data could, in principle, be explained by sprouting of interneuronal axons (Davenport et al., 1990). This assumption is unlikely, however, as 1 month after the last kindling seizure we were almost unable to detect the aberrant IPSPs, while structural alterations remain for several months (Davenport et al., 1990; Sloviter, 1991). Moreover, rekindling these animals or inducing an acute seizure in healthy animals, which is unlikely to induce sprouting, also induces the appearance of this aberrant IPSP (Gutiérrez, 2000). That the IPSP is driven from CA1 interneurons (Sik et al., 1994) is unlikely, as we recorded the same type of inhibitory responses after isolating the DG and area CA3 by cutting the hippocampus along the hippocampal fissure, and we did not elicit similar responses in control slices.

The persistence of the DG- or MF-induced IPSPs after complete blockade of both ionotropic and metabotropic glutamate receptors in pyramidal cells from kindled animals makes it unlikely that

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**Fig. 4.** (A) Synaptic responses of CA3 pyramidal cells to dentate gyrus stimulation at 3, 10 and 50 Hz in a cell from a control rat (resting membrane potential, −63 mV) and in a cell from a kindled rat (resting membrane potential, −66 mV). The arrow shows the point above which the responses were action potentials. The dots indicate stimulus artefacts. All traces are averages of three successive trials. (B) Plots of cumulative numbers of action potentials for the 20- and 50-stimuli trains at 10 and 50 Hz, respectively, in control and kindled cells, respectively. Note that neurons in the control preparations were more likely to generate action potentials during the first 20 stimuli, both at 10 and 50 Hz, than neurons from kindled preparations. Note also that after 25 stimuli during a 50-Hz stimulus train, generation of action potentials was facilitated in kindled cells. Abscissae: stimulus number; ordinate, cumulative number of action potentials.
excitatory synapses could be capable of driving interneurons, which in turn would inhibit pyramidal cells. In no case was this noted in the control animals. The latency of the pharmacologically isolated DG-or MF-evoked IPSP is rather similar to that of the EPSP evoked by the same stimulation. Taking into account these latency values, we can rule out the possibility of activating interneurons through electrical synapses, as those existing between hilar inhibitory interneurons (Michelson & Wong, 1994). Recent data show that agents that disrupt electrical coupling do not affect the IPSPs in interneurons (Michelson & Wong, 1994). Moreover, changes in the expression of connexins following kindling- or kainate-induced epilepsy in rats were not detected (Sohl et al., 2000).

Further evidence for kindling-induced changes in transmitter and receptor distribution in the MF projection comes from the slow EPSP that follows the DG-evoked IPSP, and again it was not elicited by direct interneuron stimulation. This depolarizing potential is not sensitive to bicuculline and therefore differs from the biphasic GABA responses for which HCO₃⁻ and Cl⁻ seem to be responsible (Staley et al., 1995). Instead, cholinergic antagonists are able to block it after its isolation with the glutamatergic ionotropic and metabotropic receptor antagonists NBQX, APV, MCPG and the GABAA antagonist, bicuculline.

GABAergic neurotransmission has been shown to be diminished in the hippocampus in experimental epilepsy (Sloviter, 1987; Empson & Jefferys, 1993; Obenaus et al., 1993). A loss of GABAergic interneurons in the hilar region and DG seems to account for these observations (Sloviter, 1987; Obenaus et al., 1993). In contrast, here we demonstrate that among the synaptic plastic changes induced by kindling, fast GABAergic transmission from the DG to CA3 is induced. The new inhibitory pathway is well suited to dampen CA3 input to proximal dendrites of CA3 pyramidal cells (Gulyás et al., 1993) can only take place if the inhibitory inputs are also located close to the soma (Soltesz et al., 1995; Miles et al., 1996; Urban &
Barrionuevo, 1998). Our experiments suggest that this is the case, as we could easily determine the reversal potential of the pharmacologically isolated DG-induced IPSP. Moreover, the similar onset latencies of the EPSPs and isolated IPSPs fit with the assumption of a single pathway coming from the DG that excites and inhibits CA3 pyramidal cells at the same time. This does not necessarily imply excitatory and inhibitory signals coming from the same terminal, which has still to be proven directly. The observed suppression of generation of action potentials during repetitive stimulation in pyramidal cells from kindled animals also points to the somatic or proximal dendritic location of the inhibitory input (Miles et al., 1996). During glutamatergic blockade, we found little evidence for activation of GABA<sub>B</sub> receptors, which indeed are thought to be predominantly located at distal dendritic synapses (Miles et al., 1996) formed by lacunosum molecular interneurons (Gulyás et al., 1993).

**Functional implications**

It has been proposed that the DG serves as a filter (Heinemann et al., 1992) limiting the transfer of information from the entorhinal cortex to the pyramidal layer of the hippocampus. However, in kindled animals, this filtering mechanism seems to be altered (Behr et al., 1996, 1998). Our results indicate a compensatory change in kindled rats by the expression and release of GABA from a DG output. Moreover, high-frequency input to the pyramidal cells yields a net inhibitory effect in these cells, at least during short stimulus trains.

It has been shown, however, that activation of GABA<sub>A</sub> receptors are likely to underlie ictal activity. This effect is due to GABA-mediated depolarizations and a transient elevation of K<sup>+</sup>o, both in hippocampus and entorhinal cortex. Moreover, its involvement in the generation of ectopic action potentials has been shown (Avoli, 1996; Avoli et al., 1996a, b; Lopantsev & Avoli, 1998; Avoli et al., 1998).

The GABA-dependent depolarizations may be involved in neuronal synchronization and oscillatory activity (Köhling et al., 2000). 4-AP-induced seizures in entorhinal cortex seem to develop into pharmacoresistant epileptiform discharge patterns when bicuculline is applied together with 4-AP (Brückner et al., 1999). Whether in kindled animals, application of 4-AP can induce ictal activity — which is blocked by bicuculline or GABA<sub>B</sub> antagonists — in the absence of glutamatergic transmission has to be determined.

Finally, a summation of the slow pirenzepine-sensitive depolarization can account for the late depolarization seen at 50 Hz. Activation of muscarinic receptors is known to induce depolarizations in CA3 (Müller & Misgeld, 1986) giving rise to rhythmical bursts (Bianchi & Wong, 1994). Whether these newly described synaptic responses of CA3 to DG stimulation induced after kindling (i.e. the fast GABAergic transmission and the cholinergic excitation) act together with the recently described modulation of GABA and glutamate in this synapse (Vogt & Nicoll, 1999), can be involved in the anterograde amnesia that appears for some time after seizures, particularly after status epilepticus, so enhanced GABA transmission from DG can hamper storage of information in the hippocampal network. Although our data strongly support the possibility that this synapse might be able to corelease GABA and glutamate, further experiments are needed to prove this exciting possibility.

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**Abbreviations**

ACSF, artificial cerebrospinal fluid; APV, (D,L)-2-amino-5-phosphonovaleric acid; DG, dentate gyrus; EPSP, excitatory postsynaptic potential; GAD, glutamate decarboxylase; GluRas, glutamate receptor antagonists; IPSP, inhibitory postsynaptic potential; (+)-MCPG, (+)-methyl-4-carboxyphenylglycine; MF, mossy fibres; NBQX, 6-nitro-7-sulphamoylbenzof[2]quinoloxine-2,3-dione.

**References**


