Participation of GABA_A-Mediated Inhibition in Ictalike Discharges in the Rat Entorhinal Cortex

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Lopantsev, Valer and Massimo Avoli. Participation of GABA_A-mediated inhibition in ictalike discharges in the rat entorhinal cortex. J. Neurophysiol. 79: 352–360, 1998. The spontaneous, synchronous activity induced by 4-aminopyridine (4AP, 50 μM) in the adult rat entorhinal cortex was analyzed with simultaneous field potential and intracellular recordings in an in vitro slice preparation. Four-AP induced isolated negative-going field potentials (interval of occurrence = 27.6 ± 9.9 (SD) s; n = 27 slices) that corresponded to intracellular long-lasting depolarizations (LLDs), and ictalike epileptiform discharges (interval of occurrence = 10.4 ± 5.7 min; n = 27 slices) that were initiated by the negative field potentials. LLDs recorded with K-acetate–filled microelectrodes triggered few action potentials of variable amplitude and had a duration of 1.7 ± 0.8 s (n = 26 neurons), a peak amplitude of 11.8 ± 5.0 mV (n = 26 neurons) and a reversal potential of −66.2 ± 3.9 mV (n = 17 neurons). The ictal discharges studied with K-acetate microelectrodes consisted of prolonged depolarizations (duration = 72.9 ± 44.3 s; peak amplitude = 29.2 ± 11.4 mV; n = 25 neurons) with action-potential firing during both the tonic and the clonic phase. These depolarizations had a reversal potential of −45.3 ± 3.8 mV (n = 4 neurons). Intracellular Cl⁻ diffusion from KCl-filled microelectrodes made both LLDs and ictal depolarizations increase in amplitude (30.5 ± 8.2 mV, n = 8 and 41.8 ± 9.8 mV, n = 6 neurons, respectively). LLDs recorded with KCl and 2-[(trimethyl-amino)-N-(2,6-dimethylphenyl)-acetamide (QX-314) microelectrodes reached an amplitude of 36.3 ± 5.2 mV, lasted 12.5 ± 6.5 s, and had a reversal potential of −31.3 ± 2.5 mV (n = 4 neurons); under these recording procedures the ictal discharge amplitude was 41.5 ± 5.0 mV and the reversal potential −24.0 ± 7.0 mV (n = 4 neurons). The N-methyl-D-aspartate (NMDA) receptor antagonist 3,3′-(2-carboxy-piperazine-4-yl)-pro-yl-1-phosphonate (10 μM, n = 5 neurons) alone or concomitant with the nonNMDA receptor antagonist 6-cyano-7-nitro-quinolinic-acetic (10 μM, n = 4 neurons) abolished ictal discharges, without influencing LLDs. LLDs were blocked by the γ-aminobutyric acid-A (GABA_A) receptor antagonist bicuculline methiodide (BMI, 10 μM, n = 6 neurons) or the μ-opioid receptor agonist (D-Ala2-N-Me-Phe, Glyol) enkephalin (DAGO, 10 μM, n = 2 neurons). Application of BMI (n = 4 neurons) or DAGO (n = 2 neurons) to control the medium abolished LLDs and ictal discharges but disclosed a novel type of epileptiform depolarization that lasted 3.5 ± 1.2 s and occurred every 5.2 ± 2.6 s (n = 6 neurons). Our data indicate that 4AP induces in the rat entorhinal cortex a synchronous, GABA-mediated potential that is instrumental in initiating NMDA-dependent, ictal discharges. Moreover we present evidence for an active role played by GABA_A-mediated potentials in the maintenance and termination of these prolonged epileptiform events.

INTRODUCTION

Inhibitory potentials mediated through the postsynaptic activation of γ-aminobutyric acid (GABA) receptor subtypes control neuronal excitability in the neocortex, hippocampus, and other forebrain structures (see for review Kaila 1994; Krnjevic 1991). In several models of epilepsy the efficacy of GABA-mediated inhibition (in particular that caused by activation of the type A receptor) decreases shortly before the onset of, as well as during seizure activity, even when experimental procedures that do not antagonize the GABA_A receptor are used to induce epileptiform discharges (Avoli et al. 1995; Ben-Ari et al. 1979; Korn et al. 1987; McCarren and Alger 1985; Mody et al. 1987; Whittington et al. 1995b). However GABA-mediated inhibition is present and even potentiated in other models (McLean et al. 1996; Traub et al. 1994, 1996), including the epileptiform discharges induced in the hippocampus by low doses of the K⁺-channel blocker 4-aminopyridine (4AP) (Perreault and Avoli 1991, 1992; Rutecki et al. 1987).

Recent evidence obtained in the adult rat entorhinal cortex indicates that ictalike (thereafter referred to as ictal) discharges induced by 4AP are preceded, and thus may be initiated, by synchronous, GABA-mediated potentials that are associated with transient increases in [K⁺], (Avoli et al. 1996a). The entorhinal cortex provides the main inputs to the hippocampus proper (Bartesaghi 1994; Chrapak et al. 1995; Empson and Heinemann 1995; Lopes da Silva et al. 1990; Witter 1993) and it may play a key role in the development of limbic seizures (Avoli et al. 1996a; Heinemann et al. 1993; Nagao et al. 1996; Paré et al. 1992). A dysfunction of the entorhinal cortex was also documented in patients with temporal lobe epilepsy (Rutecki et al. 1989; Spencer and Spencer 1994), where the surgical removal of this structure may be essential for achieving seizure control (Goldring et al. 1992).

Knowledge of the mechanisms responsible for the occurrence of epileptiform activity in the entorhinal cortex is therefore important for understanding the pathogenesis of temporal lobe seizures. In this study we used conventional field potential and intracellular recordings to analyze the synchronous activity induced by 4AP in the adult rat entorhinal cortex. The epileptogenic action of 4AP results primarily from an increase in transmitter release at both excitatory and inhibitory synapses (Perreault and Avoli 1991; Rutecki et al. 1987); hence, epileptiform activity in this in vitro model is the result of the potentiation of both types of synaptic transmission, rather than to the blockade of inhibition or the enhancement of a specific excitatory mechanism, as in other models of epileptiform discharge. Our data demonstrate the occurrence of synchronous, GABA_A-mediated depolarizations that have properties similar to those seen in the hippo-
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Preparation and maintenance of the slices

Adult, male Sprague-Dawley rats (200–300 g) were used. The procedures for preparing and maintaining slices of combined hippocampus-entorhinal cortex were previously described (Avoli et al. 1996a; Nagao et al. 1996). In brief horizontal slices (450–500 μm thick) were cut with a vibratome and were transferred to a tissue chamber where they were kept at 33.5 ± 0.5°C (SD) in an interface between humidified gas (95% O₂, 5% CO₂) and oxygenated artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 124 NaCl, 2 KCl, 2 MgSO₄, 2 CaCl₂, 1.25 KH₂PO₄, 26 NaHCO₃, and 10 glucose (pH = 7.4). 4AP (50 μM, Sigma), 3.3-(2-carboxy-pi-pe-razine-4-yl)-propyl-1-phosphonate (CPP, 10 μM, Tocris Cookson), 6-cyano-7-nitro-quinoxina-line-2,3-dione (CNQX, 10 μM, Tocris Cookson), (D-Ala²-N-Me-Phe, Gly-ol) enkephalin (DAGO, 10 μM, Sigma), and bicuculline methiodide (BMI, 10 μM, Sigma) were bath applied. The rate of perfusion (0.3–1 ml/min) was kept constant in each experiment.

Recording procedures

Field potential recordings were made with glass pipettes filled with ACSF (resistance = 1–5 MΩ). Microelectrodes for intracellular recordings were filled with one of the following solutions: 1) 3 M K-acetate (resistance = 60–100 MΩ); 2) 3 M K-acetate and 50 mM 2-(trimethyl-amino)-N-(2,6-dimethylphenyl)-acetamide (QX-314, Astra; resistance = 60–100 MΩ); 3) 3 M KCl (resistance = 40–70 MΩ); or 4) 3 M KCl and 50 mM QX-314 (resistance = 50–70 MΩ). The distance between extracellular and intracellular microelectrodes ranged from 100 to 300 μm. Signals were fed to high-impedance amplifiers with internal bridge circuit (Axoclamp 2) that allowed intracellular current injection. The bridge was monitored carefully throughout the experiment and adjusted as necessary. Signals were displayed on oscilloscope and on a Gould WindoGraf recorder; they were also recorded on video cassette recorder for later analysis.

Database and analysis

The intracellular activity of 62 neurons was analyzed in the presence of 4AP in the lateral portion of the entorhinal cortex of 50 slices. The neuron depth ranged from 220 to 1,320 μm from the pia, which corresponds to layers II–VI (Köhler 1988). Their fundamental electrophysiological characteristics when recorded with K-acetate–filled microelectrodes were the following: 1) resting membrane potential (RMP) measured after withdrawal from the cell = −75.1 ± 7.0 mV (n = 41); 2) action-potential amplitude calculated from the baseline = 96.8 ± 10.5 mV (n = 39); and 3) apparent input resistance, obtained from the maximum voltage response induced by hyperpolarizing current pulses (100–200 ms, −0.4 to −0.5 nA) = 39.9 ± 13.8 MΩ (n = 33). These cells generated regular spiking activity (27/31 cells) or bursts of action potentials (4/31 cells) during injection of depolarizing current pulses (0.1–0.7 nA; 100–200 ms). Bursting cells were located 480–730 μm from the pia, which corresponds to layers III–IV (Köhler 1988).

The amplitude of the intracellular potentials was measured from the RMP, unless otherwise specified. Quantitative results are expressed as means ± SD and n indicates the number of neurons analyzed, unless otherwise specified. Statistical analysis of the data obtained under control conditions and during any experimental manipulation was performed with paired or unpaired Student’s t-tests. Data were considered significantly different if P < 0.05.

RESULTS

Spontaneous synchronous activity induced by 4AP

The spontaneous field potential activity induced by 4AP consisted of isolated, negative potentials (interval of occurrence = 27.6 ± 9.9 s, n = 27 slices) (Fig. 1A, * ) and ictal discharges (duration = 86.5 ± 52.5 s; interval of occurrence = 10.4 ± 5.7 min, n = 27 (Fig. 1A, continuous line). Each ictal discharge was usually preceded (and thus it appeared to be initiated) by a negative potential (Fig. 1A, ◊). The different types of spontaneous synchronous activity continued to occur for several hours during continuous application of 4AP. In eight slices the spontaneous synchronous activity induced by 4AP consisted of negative potentials only.

Intracellular recordings with K-acetate microelectrodes (43 cells from 35 slices) showed that the negative field potentials corresponded to long-lasting depolarizations (LLDs) that lasted 1.7 ± 0.8 s (n = 26) and attained maximal amplitude of 11.8 ± 5.0 mV (n = 26) (Fig. 1Ba, ◊ ). Action potentials of variable amplitude (4–96 mV) occurred during the LLD, but small-amplitude, action potentials were most often seen at its onset. Steady membrane depolarization obtained by injecting positive DC current made the LLD reverse in polarity at −66.2 ± 3.9 mV (n = 17, not illustrated).

The ictal discharge recorded intracellularly with K-acetate microelectrodes consisted of a prolonged depolarization (overall duration = 72.9 ± 44.3 s, n = 25) with an initial tonic phase that reached values of 29.2 ± 11.4 mV (n = 25) and a late clonic phase (Fig. 1A ). Rhythmic depolarizations (frequency = 10–12/s, amplitude measured from the sustained ictal depolarization level = 7–40 mV, duration = 20–100 ms) cAPPED by single action potentials occurred during the tonic phase (Fig. 1Bb, †† ). The amplitude of the tonic depolarization decreased over time and clonic discharges appeared (Fig. 1Bc). Each clonic depolarization increased gradually in duration and amplitude as the membrane potential returned toward RMP; however, their amplitude never reached that of the tonic depolarizations. Both tonic and clonic discharges corresponded to negative field potential transients. The ictal depolarization had largest amplitudes in cells recorded 500–900 μm from the pia. The ictal discharge onset was associated with an LLD that developed into the sustained, tonic depolarization (Fig. 1Bb ). The peak amplitude of these LLDs (16.9 ± 8.6 mV, n = 20) was significantly larger than the value of isolated LLDs recorded in the same neurons between ictal discharges (11.6 ± 6.2 mV, n = 20; Fig. 1Bb, †† ), but was smaller than the peak amplitude of the ictal depolarization (Fig. 1Bb ).

Changing the membrane potential with steady intracellular injection of hyperpolarizing or depolarizing current modified the amplitude of both ictal depolarizations and preceding LLDs without influencing their rate of occurrence. As shown in Fig. 2A (−55 mV sample) the initial LLD became hyperpolarizing at depolarized membrane potentials and thus the
ictal depolarization originated from a hyperpolarization. Both LLDs and ictal discharges increased in amplitude during steady membrane hyperpolarization (Fig. 2B; -80 mV sample). The reversal potential of the sustained ictal depolarization was $-45.3 \pm 3.8$ mV ($n = 4$, not illustrated). The input membrane resistance tested with brief hyperpolarizing current pulses ($-0.4$ to $-0.7$ nA, $30-50$ ms) decreased by $83.5 \pm 4.4\%$ ($n = 13$) during the LLDs recorded between ictal discharges (Fig. 2Ba) and by $84.4 \pm 5.3\%$ ($n = 6$) during those preceding the ictal discharge (Fig. 2Bb). The input membrane resistance also diminished during the ictal discharge (Fig. 2Bb).

Ionic mechanisms and pharmacological properties of the long-lasting depolarizations

LLDs recorded with KCl microelectrodes increased in amplitude over time and reached a steady value of $30.5 \pm 8.2$
Table 1. Electrophysiological features of the LLDs recorded in entorhinal cortex neurons with microelectrodes containing different solutions

<table>
<thead>
<tr>
<th>Intracellular Microelectrode Solution</th>
<th>LLD Amplitude, mV</th>
<th>LLD Duration, s</th>
<th>LLD Reversal Potential, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-acetate/QX-314</td>
<td>16.2 ± 4.0 (5)</td>
<td>2.0 ± 0.9</td>
<td>-31.3 ± 1.4 (2)</td>
</tr>
<tr>
<td>KCl</td>
<td>30.5 ± 8.2 (8)</td>
<td>1.6 ± 0.5</td>
<td>-61.0 ± 1.4 (2)</td>
</tr>
<tr>
<td>K-acetate/QX-314</td>
<td>16.2 ± 4.0 (5)</td>
<td>2.0 ± 0.9</td>
<td>-61.0 ± 1.4 (2)</td>
</tr>
<tr>
<td>KCl</td>
<td>30.5 ± 8.2 (8)</td>
<td>1.6 ± 0.5</td>
<td>-61.0 ± 1.4 (2)</td>
</tr>
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</table>

Values are means ± SD with number of neurons in parentheses. LLD, long-lasting depolarization; QX-314, 2-(tri-methyl-amino)-N-(2,6-dimethylphenyl)-acetamide.
sustained depolarization that was not capped by fast action potentials (Fig. 5, −72 mV sample). Under these recording procedures, the tonic ictal depolarization was associated with rhythmic depolarizing events (frequency = 10–12/s, amplitude measured from the sustained depolarization level = 10–18 mV, duration = 70–100 ms) (Fig. 5, −72 mV, a), whereas clonic depolarizations were capped by phasic events (amplitude measured from the level of the underlying clonic depolarizations = 20–44 mV, duration = 40–65 ms) (Fig. 5, −72 mV, b). Steady depolarization of the membrane revealed a reversal potential of −41 ± 3.4 mV (n = 3) for the sustained ictal event and made both tonic and clonic potentials invert in polarity (Fig. 5, −22 mV). These inverted potentials had a complex waveform consisting of an initial negative and a subsequent positive component (Fig. 5, −22 mV, \( \triangledown \) and \( \triangledown \), respectively, in a and b); they increased in duration during transition from tonic to clonic phase, while the reversal potential of the initial component became more negative (Fig. 5, −22 mV, \( \triangledown \)). The reversal potential of tonic events was −30.4 ± 5.1 mV and that of clonic events −47.9 ± 4.4 mV (n = 5).

Ictal discharges recorded at RMP with KCl/QX-314 microelectrodes (Fig. 6, −74 mV) were associated with a 41.5 ± 5.0 mV sustained depolarization (n = 4). LLDs occurring at the ictal discharge onset had amplitudes similar to those seen during the tonic phase of the ictal events and merged with them. Moreover the amplitude of the clonic depolarizations reached the level of the tonic depolarization (Fig. 6, −74 mV, \( \triangledown \)). Rhythmic, short-lasting depolarizations (frequency = 10–12/s, amplitude = 10–20 mV, duration = 100–150 ms) occurred during the tonic phase (Fig. 6, −74 mV, a). During steady membrane depolarization, the sustained ictal depolarizations inverted in polarity at −24.0 ± 7.0 mV, while tonic and clonic potentials developed a negative polarity (Fig. 6, −16 mV). These potentials had an initial negative and subsequently a more positive component (Fig. 6, −16 mV, \( \triangledown \) and \( \triangledown \), respectively) and were characterized by similar reversal values (i.e., −15.3 ± 3.5 mV for the tonic and −16.5 ± 2.4 mV for the clonic events, n = 4). Some electrophysiological features of the ictal discharges recorded with microelectrodes containing different solutions are summarized in Table 2.

As reported previously (Avoli et al. 1996a), ictal discharges were abolished by CPP (10 µM, n = 5) and/or CNQX (10 µM, n = 4) (not shown). Application of the GABAA receptor antagonist BMI (10 µM) modified the pattern of epileptiform activity in six entorhinal neurons recorded with K-acetate microelectrodes. As illustrated in Fig. 7A, an ictal event occurred 2 min after BMI onset, but it was shorter than the discharge recorded under control conditions. Later during steady application of BMI, short duration = 3.5 ± 1.2 s, n = 6) epileptiform discharges occurred regularly at intervals of 5.2 ± 2.6 s (n = 6) (Fig. 7B). In contrast to the ictal discharges induced by 4AP only, these relatively brief epileptiform events were char-
**DISCUSSION**

**GABA-mediated long-lasting depolarizations**

Our findings indicate that low concentrations of 4AP induce in the entorhinal cortex synchronous, GABA-mediated potentials with electrophysiological and pharmacological properties that resemble those described in the hippocampus (Barbarosie et al. 1994; Perreault and Avoli 1991, 1992). These similarities include 1) an increase in Cl\(^{-}\)-conductance as the main mechanism responsible for the LLD, 2) the insensitivity to ionotropic excitatory amino acid receptor antagonists, and 3) the suppression exerted by the GABA\(_A\)-receptor antagonist BMI and the \(\mu\)-opioid receptor agonist DAGO, which prevents GABA release from inhibitory terminals (Capogna et al. 1993). These findings are also in line with those obtained in the entorhinal cortex with field potential and [K\(^+\)] recordings (Avoli et al. 1996a) and further indicate that LLDs represent GABA\(_A\)-mediated postsynaptic responses to GABA released by inhibitory interneurons. These GABA\(_A\)-mediated depolarizations may be contributed by several mechanisms such as [K\(^+\)], elevations, changes in Cl\(^{-}\) driving force and a conductance increase to HCO\(_3^-\) (Alger and Nicoll 1982; Kaila 1994; Staley et al. 1995; Wong and Watkins 1982). As reported in hippocampal neurons (Perreault and Avoli 1991, 1992), the entorhinal cortex LLDs triggered only occasional, action-potential firing in spite of their large amplitude. This phenomenon results presumably from the membrane shunt because of the large conductance increase that accompanies the LLD.

NMDA-mediated postsynaptic responses were documented in the entorhinal cortex (Jones 1987, 1994; Jones and Heinemann 1988). However LLDs are not influenced by the NMDA receptor antagonist CPP, which may be because of the masking effect exerted on NMDA potentials by GABA\(_A\) receptor activation during the LLD. LLDs also occur during blockade of nonNMDA receptors, although this pharmacological procedure could cause a nonsignificant decrease of LLD amplitude. Recently we have shown participation of nonNMDA-dependent circuits in the activation of inhibitory interneurons in the entorhinal cortex (Lopantsev and Avoli 1996). The resistance of 4AP-induced, GABA-mediated potentials to excitatory amino acid receptor antagonists was described in several cortical structures (Michelson and Wong 1991; Muller and Misgeld 1990; Perreault and Avoli 1991, 1992).

**TABLE 2.** Electrophysiological characteristics of the ictal epileptiform potentials recorded in entorhinal cortex neurons with microelectrodes containing different solutions

<table>
<thead>
<tr>
<th>Intracellular Microelectrode Solution</th>
<th>Sustained Depolarization Amplitude, mV</th>
<th>Sustained Depolarization Reversal Potential, mV</th>
<th>Tonic Discharge Reversal Potential, mV</th>
<th>Clonic Discharge Reversal Potential, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-acetate*</td>
<td>31.4 ± 4.2 (9)</td>
<td>−45.3 ± 3.8 (4)</td>
<td>−30.4 ± 5.1 (5)</td>
<td>−47.9 ± 4.4 (5)</td>
</tr>
<tr>
<td>KCl*</td>
<td>41.8 ± 9.8 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-acetate/QX-314</td>
<td>30.8 ± 8.8 (5)</td>
<td>−41.3 ± 3.4 (3)</td>
<td>−15.3 ± 3.5 (4)</td>
<td>−16.5 ± 2.4 (4)</td>
</tr>
<tr>
<td>KCl/QX-314</td>
<td>41.5 ± 5.0 (4)</td>
<td>−24.0 ± 7.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD with number of neurons in parentheses. The amplitudes of sustained ictal depolarizations recorded with K-acetate and KCl-filled microelectrodes were obtained from neurons recorded at similar depths (670–930 \(\mu\)m).
Ictal discharges

4AP–induced ictal activity in the entorhinal cortex results from the powerful and synchronous activation of excitatory synaptic mechanisms as proposed in other models of interictal and ictal discharge (Gutnick et al. 1982; Johnston and Brown 1981; Nagao et al. 1996). This conclusion is based on the effects of ionotropic excitatory amino acid receptor antagonists, which blocked the ictal activity (cf. Avoli et al. 1996a) and the changes induced by intracellular injection of steady current, a procedure that modified the ictal depolarization amplitude as expected for a potential that is caused by synaptic currents. As documented in other models of entorhinal epileptiform discharge (Heinemann et al. 1993; Jones and Heinemann 1988; Nagao et al. 1996), our findings indicate that NMDA-mediated mechanisms play a primary role in the occurrence of the ictal depolarizations induced by 4AP. Moreover these prolonged epileptiform events have maximal amplitude in entorhinal cortex neurons located 500–900 μm from the pia. This evidence, along with the results obtained by using field-potential and [K⁺]₀ recordings (Avoli et al. 1996a), implies that middle and deep layer cells play a fundamental role in the generation of these ictal discharges. Inhibition may be weaker in these layers, thus allowing a greater expression of NMDA-dependent currents (Jones 1994, 1987; Jones and Heinemann 1988). Moreover, intrinsically bursting neurons were found at the depth of maximal ictal depolarization; bursting cells contribute to the generation of epileptiform discharges (Chagnac-Amitai and Connors 1989; Connors 1984; Miles and Wong 1983). However propagation of ictal activity to the hippocampus proper must involve superficial layer neurons, as these cells give rise to the perforant path projection (Champagnat et al. 1995; Empson and Heinemann 1995; Heinemann et al. 1993; Jones 1994). Laminar differences in 4AP-induced epileptiform activity were reported in the neocortex (Barkai et al. 1995).

The reversal potential of the ictal depolarization recorded with K-acetate/QX-314 microelectrodes has values more negative than what was expected for an isolated EPSP or an epileptiform depolarization induced by GABA_A-receptor antagonists (Gutnick et al. 1982; Johnston and Brown 1981). This evidence suggests that GABA_A-mediated, Cl⁻ conductances are present during the ictal activity induced by 4AP in the entorhinal cortex. Accordingly the ictal depolarization recorded with KCl or KCl/QX314 microelectrodes increased in amplitude and its reversal potential became more positive; both recording procedures lead to intracellular Cl⁻ leakage, which makes the reversal potential of GABA_A-mediated potentials more positive. Under these recording conditions clonic depolarizations increased in amplitude up to values comparable with those associated with the tonic depolarization. Similar findings were reported to occur during intracellular Cl⁻ injection in neonatal hippocampal neurons (McLean et al. 1996).

When K-acetate/QX-314 microelectrodes were used the reversal potential of the tonic and clonic depolarizations associated with the ictal event became more negative as the discharge progressed from the tonic to the clonic phase; such a shift was not observed with KCl/QX-314-filled microelectrodes. Although these reversal potential values were eventually influenced by the [K⁺]₀ elevation associated with the ictal activity (Avoli et al. 1996a,b; McCarren and Alger 1985), these data indicate that a progressive enhancement of Cl⁻-dependent inhibition occurs during the progression of the ictal discharge and may contribute to its termination.
The GABA<sub>A</sub> receptor antagonist BMI abolished both LLDs and ictal discharges, while disclosing a novel type of epileptiform activity that resembled interictal discharges induced by BMI in neocortex (Gutnick et al. 1982) and hippocampus (Schwartzkroin and Prince 1980). DAG0, which prevents GABA release from inhibitory interneurons through the activation of presynaptic µ-opioid receptors (Ca-pogna et al. 1993) had similar effects. Hence, in the entorhinal cortex CI<sup>-</sup>-dependent GABA<sub>A</sub>-mediated inhibition participates in the maintenance of the 4AP-induced ictal activity and to contribute to its termination (in addition to playing a significant role in the initiation process, see below).

Higashima et al. (1996) have shown that activation of GABAergic mechanisms is necessary for the generation of afterdischarges recorded in hippocampal slices after electrical stimuli. Experimental and computational data obtained by Traub et al. (1996) also suggest a role played by GABA<sub>A</sub>-mediated depolarizing conductances in the epileptiform synchronization that occurs in some models of epileptiform discharge (in particular that induced by 4AP application). GABAergic inhibitory networks can also synchronize principal cells in the neocortex and hippocampus (Cobb et al. 1995; Whittington et al. 1995a).

Role of the LLDs in initiation of ictal discharge

The synchronous, GABA<sub>A</sub>-mediated potential induced by 4AP represents a mechanism capable of initiating prolonged epileptiform discharges in the entorhinal cortex (cf. Avoli et al. 1996a). This conclusion rests on the finding that the ictal events were usually preceded and thus they appeared to be initiated by LLDs, as well as by the ability of pharmacological procedures that interfere with the occurrence of LLDs (i.e., application of BMI or DAGO) to abolish the ictal discharge. Moreover the amplitude of the LLD occurring at the onset of the ictal discharge had values that were larger than those seen with isolated LLDs. In the neonatal rat hippocampus a GABA<sub>A</sub> receptor antagonist potentiates giant GABAergic potentials leading to the occurrence of ictalike epileptiform discharges (McLean et al. 1996).

By employing [K<sup>+</sup>]<sub>i</sub> recordings we have shown that the larger amplitude of the LLD recorded in the entorhinal cortex at the onset of the ictal discharge corresponds to a transient [K<sup>+</sup>]<sub>i</sub> increase that is greater than that seen in coincidence with the isolated LLDs (Avoli et al. 1996a). A similar mechanism occurs in isolated hippocampal slices obtained from young rats at the onset of ictal discharges induced by 4AP (Avoli et al. 1993, 1996b). It is well known that the appearance of epileptiform activity is facilitated by high K<sup>+</sup> (Korn et al. 1987; Traynelis and Dingledine 1988). The increases in [K<sup>+</sup>]<sub>i</sub> during the GABA-mediated potential can induce a positive shift of GABA-mediated postsynaptic inhibition (Korn et al. 1987; McCarron and Alger 1985), depolarize neurons, and thus disinhibit excitatory postsynaptic interactions. All these processes can lead to the appearance of ictal epileptiform activity induced by 4AP in the entorhinal cortex. Therefore GABA<sub>A</sub>-mediated LLDs may serve as a powerful implement for the initiation and the synchronization of neuronal activity in the entorhinal cortex. This conclusion is in line with recent findings obtained in epileptic human temporal lobes, where inhibitory neuronal interac-

tions are increased in regions of seizure initiation (Colder et al. 1996).

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