Altered Inhibition in Lateral Amygdala Networks in a Rat Model of Temporal Lobe Epilepsy

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INTRODUCTION

The amygdala complex, located in the deep anteromedial part of the temporal lobe, is composed of several nuclei that are interconnected with cortical and subcortical regions in a specific manner (Lopes da Silva et al. 1990; Pitkanen et al. 2000a, b). Under normal physiological conditions, the amygdala is involved in fear conditioning and emotional learning (LeDoux 2000; Pare et al. 2004; Scott et al. 1997; Stork and Pape 2002; Wilensky et al. 2000). The amygdala is also known to be at the origin of some of the behavioral manifestations observed during seizures in temporal lobe epilepsy (TLE) patients where it is often the primary focus of seizure activity (Gloor 1992, 1997; van Elst et al. 2000). Patients with TLE can present with unilateral or bilateral damage to this structure, and in certain instances, isolated amygdalar pathology occurs in the absence of hippocampal sclerosis (reviewed by Pitkanen et al. 1998). Histochemistry of resected human epileptic tissue has revealed that the lateral and basal nuclei are the most vulnerable to injury (Yilmazer-Hanke et al. 2000). Assessment of these nuclei has disclosed that in addition to neuronal loss and gliosis, synaptic alterations—in the form of decreased dendritic branching of surviving cells—also take place (Aliashkevich et al. 2003).

Histological examination of chronically epileptic animal tissue has confirmed an overlap with the pattern of cell loss detected in humans. Specifically, these studies have confirmed that amygdala damage is nucleus-specific and that some nuclei are more resistant to injury than others (Nissinen et al. 2000; Tuunanen et al. 1996). Furthermore, in addition to loss of principal cells, decreased density of specific interneuronal populations has been documented in chronically epileptic animals (Tuunanen 1996, 1997). The basolateral amygdalar nucleus (BLA) has been the primary focus of electrophysiological evaluation of the amygdala in chronic animal models of TLE. These studies have identified various mechanisms to account for the hyperexcitability of BLA networks observed in epileptic animals including loss of spontaneously occurring inhibitory postsynaptic potentials (IPSPs), loss of feedforward inhibition, and enhanced N-methyl-D-aspartate (NMDA)- and non-NMDA-mediated excitation (Gean et al. 1989; Mangan et al. 2000; Rainnie et al. 1992; Shoji et al. 1998; Smith and Dudek 1997).

Despite such an extensive assessment of the BLA, relatively few electrophysiological studies have carefully examined other amygdalar nuclei using chronic animal models of TLE. For instance, little is known about the functional changes that take place within the lateral nucleus of the amygdala (LA) where neuronal loss and gliosis have been identified in subjects with intractable TLE (Yilmazer-Hanke et al. 2000). A recent study reported that decreased excitatory transmission occurs in the LA of epileptic rodents, probably because of a decrease in glutamate release or neurodegeneration (Niittykoski et al. 2000). However, much information is still needed to fully understand the contribution of this structure to epileptogenesis. For example, although previous studies have shown the essential role of local GABAergic circuits in controlling LA excitability (Callahan et al. 1991; Lang and Paré 1997, 1998), a detailed electrophysiological examination of how these inhibitory networks are affected in epileptic animals is still lacking.

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Assessing the contribution of the LA to hyperexcitability of limbic neuronal networks becomes essential when one considers its dense reciprocal interconnections with the hippocampus and parahippocampal cortices, structures that are highly implicated in TLE (Du et al. 1993; Pitkäkainen and Pitkänen 2001; Pitkänen et al. 1995, 2000b). In this study, we assessed the electrophysiological changes that occur in the LA of epileptic rats by using the pilocarpine chronic animal model of TLE where damage to this amygdala nucleus has been reported (Cavalheiro et al. 1987; Fujikawa 1996). Our investigations were specifically aimed at assessing the functional characteristics of inhibition within this structure.

**Methods**

**Animal preparation**

Procedures approved by the Canadian Council of Animal Care were used to induce status epilepticus (SE) in adult male Sprague-Dawley rats weighing 150–200 g at the time of injection. All efforts were made to minimize the number of animals used and their suffering. Briefly, rats were injected with a single dose of pilocarpine hydrochloride (380–400 mg/kg, ip). To reduce the discomforts caused by peripheral activation of muscarinic receptors, methyl scopolamine (1 mg/kg, ip) was administered 30 min before the pilocarpine injection. The animals’ behavior was monitored for ~4 h after pilocarpine and scored according to Racine’s classification (Racine et al. 1972). Only rats that experienced SE (stages 3–5) for >30 min (53.1 ± 9.3 (SE) min; n = 35 rats) were included in the pilocarpine group and used for in vitro electrophysiological studies ~4 mo (18 ± 1 wk; n = 35 rats) after pilocarpine injection. Because it has been previously established that all adult rats experiencing pilocarpine-induced SE will later exhibit spontaneous recurrent seizures (Cavalheiro et al. 1991; Priel et al. 1996), only a subset of pilocarpine-treated animals were video-monitored, and the presence of spontaneous behavioral seizures was confirmed in virtually all of them (n = 14/15). In this study, rats receiving a saline injection instead of pilocarpine were used as age-matched nonepileptic controls (NECs).

**Slice preparation and maintenance**

Adult rats were decapitated under halothane anesthesia; the brain was quickly removed, and a block of brain tissue containing the retrohippocampal region was placed in cold (1–3°C), oxygenated artificial cerebrospinal fluid (ACSF). The brain dorsal side was cut along a horizontal plane that was tilted by a 10° angle along a postero-superior-anteroinferior plane passing between the lateral olfactory tract and the base of the brain stem (Benini et al. 2003). Horizontal slices (400–450 μm thick) were cut from this brain block using a vibratome, and slices were transferred into a tissue chamber where they lay at the interface between ACSF and humidified gas (95% O2-5% CO2) at a temperature of 34–35°C and a pH of 7.4. We focused in this study on the most ventral slices that were comprised between −8.6 and −7.6 mm from the bregma (Paxinos and Watson 1998). These slices contained the hippocampus proper, the parahippocampal cortices, and the lateral nucleus of the amygdala (LA; Fig. 1A). Two to three of such slices could be obtained from each hemisphere. ACSF composition was (in mM) 124 NaCl, 2 KCl, 1.25 KH2PO4, 2 MgSO4, 2 CaCl2, 25 NaHCO3, and 10 glucose. (2S)-3-[[1(S)-1-(3,4-Dichlorophenyl)ethyl amino-2-hydroxypropyl] (phenyl-methyl)phosphonic acid (CGP 55845A, 10 μM), 6-cyano-7-nitroquinolxino-2,3-dione (CNQX, 10 μM), 3,3-(2-carboxy-piperazin-4-yl)-propyl-1-phosphonate (CPP, 10 μM), and picrotoxin (PTX, 50 μM) were applied to the bath. Chemicals were acquired from Sigma (St. Louis, MO) with the exception of CGP 55845A, CNQX, and CPP, which were obtained from Tocris Cookson (Ellisville, MO).

**Electrophysiological recordings and stimulation protocols**

Field potential recordings were made from the LA and deep layers of the perirhinal cortex (PC) with ACSF-filled glass pipettes (resistance = 2–10 MΩ) that were connected to high-impedance amplifiers (Fig. 1A). Sharp-electrode intracellular recordings were performed in LA with pipettes that were filled with 3 M K-acetate or with 3 M K-acetate/75 mM lidocaine, N-ethyl bromide (QX314; tip resistance = 70–120 MΩ in both cases). Intracellular signals were fed to a high-impedance amplifier with internal bridge circuit for intracellular current injection. The resistance compensation was monitored throughout the experiment and adjusted as required. The passive membrane properties of LA cells included in this study were measured as follows: 1) resting membrane potential (RMP) after cell withdrawal; 2) apparent input resistance (Ri) from the maximum voltage change in response to a hyperpolarizing current pulse (100–200 ms, < −0.6 nA); 3) action potential amplitude (APA) from baseline; and 4) action potential duration (APD) at half-amplitude. Intrinsic firing patterns of LA cells were classified from responses to depolarizing current pulses of 1,000- to 2,500-ms duration. The adaptation ratio (AR), defined as the ratio of the last interspike interval (ISI) to the first ISI, was used to quantitatively compare the firing properties of cells from pilocarpine (n = 30) and NEC groups (n = 30) (cf. Takazawa et al. 2004). For each cell, AR was obtained from a 1,200-ms depolarizing pulse at a current intensity 0.2 nA larger than that which induced threshold action potential firing.

Synaptic responses to single shock stimulation (50–100 μs; <350 μA) of local LA networks were assessed using a bipolar, stainless steel electrode placed <500 μm from the recording electrodes. “Monosynaptic” IPSPs were evoked in the presence of glutamatergic antagonists (10 μM CPP + 10 μM CNQX). The stimulation parameters used to elicit these responses were not significantly different (P > 0.05) between pilocarpine (stimulus intensity = 203 ± 21 μA; duration = 100 μs; n = 22) and NEC (stimulus intensity = 175 ± 17 μA; duration = 100 μs; n = 16) groups. Reversal potential and peak conductance values for the early and late components of the IPSPs were obtained from a series of responses evoked at membrane potentials set to different levels by intracellular current injection. Reversal potentials were computed from regression plots of response amplitude versus membrane potential. Peak conductance values were estimated using the parallel conductance model (cf. Williams et al. 1993).

Briefly, the membrane potential versus intensity of injected current was plotted (i) before the stimulation and (2) at the peak of the IPSP response. The slopes of these two regression lines were used to yield the input resistance at rest (i.e., before the stimulation) and during the response, respectively, and to ultimately determine the change in resistance that occurred during the IPSP (ΔGIPSP). ΔGIPSP was translated to peak conductance changes (ΔGIPSP) using the following formula: ΔGIPSP = 1/ΔRIPSP.

Paired stimuli (100-μs duration) at intervals from 50 to 1,600 ms were used to assess changes in synaptic depression of “monosynaptic” GABA\(_\Lambda\) receptor-mediated IPSPs by using K-acetate/QX314-filled electrodes. For the paired pulse protocols, the stimulus current strength giving >50% maximal response was used to stimulate LA interneuronal networks. Furthermore, cells were hyperpolarized by current injection to obtain depolarizing IPSPs. The membrane potential at which the test was conducted was not significantly different (P > 0.05) between the two experimental groups (pilocarpine-treated = −102 ± 2 mV, n = 10 and NEC = −105 ± 3 mV; n = 10); in addition, the absolute amplitude of the first response (P1) evoked at this membrane potential was not different in pilocarpine-treated (9 ± 0.5 mV, n = 10) and NEC (10 ± 0.5 mV, n = 10) neurons.

Field potential and intracellular signals were fed to a computer interface (Digidata 1322A, Axon Instruments) and were acquired and stored using the pClamp 9 software (Axon Instruments). Subsequent data analysis was made with the Clampfit 9 software (Axon Instru-
ments). For time-delay measurements, the onset of the field potential/intracellular signals was determined as the time of the earliest deflection of the baseline recording (e.g., Fig. 2Ca, inset). Measurements in the text are expressed as means ± SE, and n indicates the number of slices or neurons studied under each specific protocol. Data were compared with the Student’s t-test and were considered statistically significant if P < 0.05.

Neuronal labeling

Electrodes for intracellular labeling were filled with 2% neurobiotin dissolved in 2 M K-acetate. Intracellular injection of neurobiotin was accomplished by passing pulses of depolarizing current (0.5–1 nA, 3.3 Hz, 150 ms) through the recording electrode for 2–10 min. Neurobiotin injection did not have any appreciable effect on RMP, Rg, and evoked action potential properties (cf. Xi and Xu 1996). Only one neuron was filled in each slice. After the electrophysiological characterization of these neurons, slices were removed from the recording chamber and fixed in 4% paraformaldehyde and 100 mM phosphate-buffered solution overnight at 4°C. Slices were rinsed in PBS, and the endogenous peroxidase activity extinguished by incubating them in 0.1% phenylhydrazine for 20 min. After several rinses in PBS, the slices were incubated for 2 h in 1% Triton X-100 and then in vectastain ABC reagent comprising the avidin-biotinylated horseradish peroxidase complex in PBS for ≥4 h. After a wash in PBS, the sections were reacted with 0.5% 3,3'-diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in PBS, mounted on slides, dehydrated, and covered (Kita and Armstrong 1991). The intracellularly stained neurons were photographed using the Nomarski optics or a Zeiss Axiohot microscope. Neurobiotin and vectastain ABC were obtained from Vector Laboratories.

RESULTS

Intrinsic electrophysiological properties and morphology of lateral amygdala neurons

Intracellular recordings were carried out in the LA of brain slices obtained from pilocarpine-treated rats (n = 83 cells from 66 slices) and age-matched NECs (n = 54 cells from 41 slices). Morphological identification with intracellular injection of neurobiotin was also carried out in some neurons (n = 17 and
TABLE 1. Intrinsic and regular firing properties of LA neurons in NEC and pilocarpine-treated rats

<table>
<thead>
<tr>
<th></th>
<th>NEC (46)</th>
<th>Pilocarpine (68)</th>
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<tbody>
<tr>
<td>RMP, mV</td>
<td>−70.7 ± 1.0</td>
<td>−71.9 ± 0.9</td>
</tr>
<tr>
<td>Ri, MΩ</td>
<td>46.6 ± 1.3</td>
<td>47.7 ± 1.2</td>
</tr>
<tr>
<td>APA, mV</td>
<td>98.5 ± 1.3</td>
<td>99.3 ± 0.9</td>
</tr>
<tr>
<td>APD, ms</td>
<td>1.5 ± 0.03</td>
<td>1.6 ± 0.03</td>
</tr>
<tr>
<td>AR*</td>
<td>9.1 ± 1.8</td>
<td>13.5 ± 3.0</td>
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Values are RMP, resting membrane potential; Ri, input resistance; APA, action potential amplitude; APD, action potential duration at half amplitude; AR, adaptation ratio; NEC, nonepileptic control. *AR values were calculated for n = 30 cells from each group. Values are mean ± SE. Number in parentheses represents neurons.

11 in pilocarpine and NEC slices, respectively). Based on a gross visual examination of cell body shape and dendrite distribution, three main types of principal spiny neurons could be distinguished in both epileptic and NEC tissue: 1) stellate-like elements (8/17 and 6/11 of pilocarpine and NEC neurons, respectively; Fig. 1Aa); 2) bipolar-shaped cells (2/17 and 0/11 of pilocarpine and NEC neurons, respectively; Fig. 1Ab); and 3) pyramid-like neurons (7/17 and 5/11 of pilocarpine and NEC cells, respectively; Fig. 1Ac).

Analysis of the intrinsic properties of cells recorded in the two types of tissue revealed no significant differences in RMP, Ri, APA, APD, and APA (Table 1). Moreover, as previously reported by other investigators (Faber et al. 2001; Faulkner and Brown 1999), the regular firing characteristics of LA neurons consisted of a spectrum of different spike adaptations (Fig. 1, Ca–Cc). The range of adaptation ratios (AR, see METHODS) varied between 1.1 and 69.0 in pilocarpine and between 1.3 and 43.5 in NEC neurons, with no significant difference in the AR distribution between the two groups (P = 0.2). Thus no disparity in the expression of the different modalities of repetitive firing could be identified between NEC and pilocarpine-treated neurons.

Spontaneous synaptic activity in LA of pilocarpine-treated rats is altered

Field potential recordings obtained during application of normal ACSF from the LA and the PC of NEC slices (n = 41) showed the absence of any spontaneous activity (Fig. 2Aa, bottom). Moreover, when analyzed with intracellular recordings, all neurons from this group exhibited at RMP depolarizing PSPs with amplitudes of 1.71 ± 0.03 mV (range = 0.5–8.4 mV, n = 26) and rates of occurrence of 0.72 ± 0.04 s (range = 0.01–13.5 s, n = 26; Fig. 2Aa, arrows; Table 2). In addition, spontaneous hyperpolarizing PSPs (sIPSPs; amplitude: −3.4 ± 0.2 mV; rate of occurrence: 18 ± 5 s, range = 0.2–140 s, n = 14) could be recorded in 53% of NEC LA neurons (n = 25/47; Fig. 2Aa,*; Table 2). Steady hyperpolarization and depolarization of the membrane potential altered the amplitude of these two types of spontaneous activities without influencing their rate of occurrence, thereby confirming their synaptic nature (Fig. 2Ab).

Spontaneous field activity was also absent from the majority of pilocarpine-treated slices [n = 52/66; Fig. 2B, pilocarpine-treated tissue (no field activity)]. As in NEC tissue, neurons recorded intracellularly from pilocarpine-treated slices also exhibited spontaneous depolarizing PSPs with amplitudes of 2.73 ± 0.05 mV (range = 0.7–16.6 mV, n = 33) and rates of occurrence of 1.02 ± 0.07 s (range = 0.01–21.8 s, n = 33; Fig. 2B, arrows, inset; Table 2). Distribution analysis revealed that, although there was no significant difference between the two groups in their rate of occurrence, depolarizing PSPs recorded from pilocarpine-treated slices skewed toward larger amplitudes (Fig. 3A; P < 0.001; Table 2). Furthermore, in contrast to the NEC group, an appreciably lower proportion (31%) of cells (n = 21/68) in the epileptic group exhibited sIPSPs at RMP (amplitude: −3.0 ± 0.1 mV; rate of occurrence: 22 ± 4 s, range = 1–210 s, n = 16; Fig. 3B, RMP; Table 2). This difference in the expression of sIPSPs between the two experimental groups was evident even when neurons were depolarized to approximately −60 mV to unmask any reversed inhibitory potentials (Fig. 3B, depolarized MP). Thus these results suggest that pilocarpine-treated LA cells display subtle differences in synaptic activity. These alterations include the presence of larger amplitude depolarizing PSPs (Figs. 2, A and B, and 3A) and reduced incidence of sIPSPs (Fig. 3B).

In addition to these differences, more significant changes were observed in 21% of slices from the pilocarpine-treated group [n = 14/66; Figs. 2Ca and 3Da, pilocarpine-treated tissue (with field activity)]. In these slices, spontaneous field activity (duration: 786 ± 514 ms, range: 190–2,100 ms; rate of

FIG. 2. Spontaneous synaptic activity in nonepileptic control (NEC) and pilocarpine-treated tissue. Aa: simultaneous field [LA, deep perirhinal cortex (PC)] and intracellular recording (−72 mV in NEC tissue reveals I) depolarizing postsynaptic potentials (PSPs) indicated by arrows and 2) robust spontaneous hyperpolarizing inhibitory PSPs (sIPSPs) indicated by asterisks. Ab: spontaneous synaptic activity recorded during depolarization and hyperpolarization of membrane potential by steady current injection of +0.2, +0.1, −0.1, and −0.2 nA (from top to bottom). Note the larger amplitude and biphasic nature of sIPSPs at more depolarized levels (61 to −76 mV). B: simultaneous field (LA, PC) and intracellular activity (−76 mV) recorded in majority of pilocarpine-treated tissue. Note absence of field activity (LA, PC) and presence of large depolarizing PSPs indicated by arrows in the intracellular trace. Expansion of these events is depicted in the right top inset. Ca: simultaneous field (LA, PC) and intracellular activity (−60 mV) recorded in a subset of pilocarpine-treated slices reveals robust network activity (LA, PC). Expansion of an event shows initiation in LA (arrow) and spread to PC (right top inset). Cb: steady hyperpolarization and depolarization of membrane potential with current injections of +0.2, −0.2, and −0.8 nA (from top to bottom) alters amplitude of underlying excitatory PSPs (EPSPs; arrow), thereby confirming the synaptic nature of the event.
occurrence: 13.1 ± 10.7 s, 7–41 s; Table 2) could be recorded in the LA, and at times could spread to the deep layers of the PC (n = 3; Fig. 2Ca). Intracellularly, this network activity corresponded to robust neuronal firing at RMP (n = 14 cells), and in the majority of these cells (n = 11/14), no sIPSPs could be recorded (Figs. 2Ca and 3Da, control). The increasing amplitude of the underlying excitatory postsynaptic potential with hyperpolarization of the membrane potential confirmed the synaptic nature of these events (Fig. 2Cb, arrows).

Pharmacology of spontaneous activity

The NMDA receptor antagonist CPP reduced the frequency of occurrence of spontaneous depolarizing PSPs in both NEC (n = 7) and pilocarpine-treated tissue (n = 4). Further treatment with the non-NMDA receptor antagonist CNQX abolished these PSPs (n = 10 and 11 in pilocarpine and NEC slices, respectively) without affecting the occurrence of hyperpolarizing sIPSPs that were often biphasic (Fig. 3C, +CPP+CNQX, inset) and reduced by the GABA_A receptor antagonist picrotoxin (n = 4, data not shown). CPP+CNQX application to pilocarpine-treated slices exhibiting spontaneous field events completely abolished this network activity (Fig. 3Da, +CPP+CNQX) and uncovered biphasic sIPSPs (Fig. 3, Da and Db) that were diminished with picrotoxin addition.

Evidence for alterations in inhibitory networks of LA

The incidence of sIPSPs at RMP was lower in LA neurons recorded from pilocarpine-treated slices compared with those of the NEC group (Fig. 3B, RMP). This difference was also evident even when the membrane potential was depolarized to approximately −60 mV to unmask any reversed sIPSPs (Fig. 3B, depolarized MP). This observation suggested that altered inhibition occurred in the LA of pilocarpine-treated animals.

To isolate and assess the activity of local inhibitory networks within the LA of NEC and pilocarpine-treated rats, we analyzed the intracellular responses of LA neurons to single-shock stimulation in the presence of glutamatergic antagonists (CPP+CNQX). As reported by previous studies (Heinbockel and Pape 1999), these “monosynaptic” stimulus-evoked IPSPs in the NEC group (n = 13) were biphasic in nature, with an early GABA_A receptor–mediated component (Fig. 4A, NEC, early) and a late GABA_B receptor component (Fig. 4A, NEC, late). Similar observations were made in the pilocarpine group (n = 13, pilocarpine-treated), thereby suggesting that postsynaptic GABA_A and GABA_B receptor mechanisms remained intact. However, comparison of the reversal potentials of the early IPSP component revealed a significantly (P < 0.002) more depolarized value in pilocarpine-treated neurons (−65.9 ± 1.5 mV, n = 13) than NEC cells (−74.5 ± 0.7 mV, n = 13; Fig. 4B, early phase). Peak conductance of the early IPSP component was also different (P < 0.05) between the two groups, with a lower peak conductance in the pilocarpine-treated tissue (7.3 ± 1.1 nS, n = 15) compared with NEC (12.1 ± 1.6 nS, n = 15; Fig. 4C).

Similar assessment of the late GABA_B receptor–mediated component of the IPSP revealed no difference in reversal potentials between pilocarpine-treated (−95.7 ± 1.9 mV, n = 13) and NEC cells (−93.3 ± 2.0 mV, n = 11; Fig. 4B, late phase). However, the peak conductance of this late IPSP component was slightly lower in pilocarpine (2.2 ± 0.4 nS, n = 14) versus NEC neurons (4.6 ± 0.7 nS, n = 11; Fig. 4C, late phase). Altogether, these results indicate that alterations in postsynaptic GABAergic mechanisms, specifically in GABA_A receptor–mediated inhibition, occur in the LA of epileptic rats.

Functional changes also involve presynaptic alterations

To determine whether modifications in presynaptic mechanisms occurred in the LA of pilocarpine-treated rats, we delivered paired stimuli (100-μs duration; <350-μA intensity) at intervals of 50–1,600 ms with a stimulating electrode placed within 500 μm from the recording electrode. Recordings were carried out with QX-314–filled microelectrodes in the presence of CPP+CNQX. In addition to its well-known effects on voltage-gated sodium channels (Connors and Prince 1982), QX-314 blocks GABA_B receptors (Nathan et al. 1990), thus allowing the isolation of the fast GABA_A receptor–mediated component of the IPSP. Furthermore, because of the ability of this lidocaine derivative to attenuate I_h (Perkins and Wong 1995), the corresponding IPSPs could be assessed more easily in their reversed form at hyperpolarized membrane potentials (Fig. 5, A and B).

In NEC slices, paired-pulse stimulation protocols revealed a marked depression in the amplitude of the second stimulus-induced IPSP (P2) with respect to the first (P1) at interstimulus intervals between 50 and 1,000 ms (Fig. 5, A and C; n = 10). The second IPSP amplitudes recovered to initial values at intervals of ≥1,200 ms (Fig. 5C). In contrast, paired IPSPs in LA neurons recorded from pilocarpine-treated tissue tended to exhibit a less pronounced depression at interstimulus intervals between 50 and 1,600 ms compared with NEC, thereby suggesting a failure in presynaptic GABAergic interneuron auto-receptors (Fig. 5, B and C; n = 10). The difference between NEC and pilocarpine-treated groups was statistically different (P < 0.05) at interstimulus intervals between 200 and 1,000 ms (Fig. 5C, *).

Presynaptic GABA_B Receptors have been shown to contribute to the paired-pulse depression of GABA_A receptor–mediated IPSCs induced in the LA by paired stimulation of cortical and thalamic inputs in the presence of glutamatergic transmission (Szinyei et al. 2000). To determine whether the same was true for the monosynaptic IPSPs induced in our experimental
paradigm (i.e., in the absence of glutamatergic transmission), the effect of the GABAB receptor antagonist CGP 55845A on the magnitude of the IPSP paired-pulse depression (PPD) was tested at an interstimulus interval yielding maximal depressant effects (400 ms). In NEC tissue, CGP 55845A increased the P2/P1 ratio by 24.7 ± 4.7% in 6 of 12 neurons (Fig. 5D). On the other hand, PPD in pilocarpine-treated cells tended to be less affected by GABAB receptor antagonism, which increased the P2/P1 ratio in only three of nine neurons by 11.7 ± 0.9% (Fig. 5D). The difference in the extent of PPD attenuation by CGP 55845A was marginally significant between the NEC (24.7 ± 4.7%, n = 6) and pilocarpine-treated cells (11.7 ± 0.9%, n = 3) at P = 0.05. Altogether, these results suggest that presynaptic GABAB receptors may contribute to controlling neurotransmitter release from LA interneurons and point toward the possibility of altered presynaptic GABAB receptor-mediated mechanisms in chronically epileptic animals.

**DISCUSSION**

In this study, we sought to identify the functional changes that occur within the LA using the pilocarpine rodent model of TLE. The results obtained show that alterations in LA network excitability occur in chronically epileptic rats. Specifically, LA neurons exhibit larger PSPs and a lower incidence of hyperpolarizing sIPSPs than those observed in NEC animals. Moreover, in contrast to NEC, a subset of slices from the pilocarpine group displayed intense network bursting in LA. Finally, in
addition to the lower incidence of sIPSPs observed in the epileptic group, we provide for the first time evidence for both postsynaptic and, presumably, presynaptic modifications in GABA receptor–mediated mechanisms.

Synaptic alterations in LA of epileptic rats

Because of the overwhelming body of clinical evidence implicating the amygdala in the initiation and spread of limbic seizures, various studies have sought to identify the cellular

FIG. 5. Pilocarpine-treated tissue exhibits a less pronounced paired-pulse depression of monosynaptically evoked IPSPs. A: intracellular recording from a hyperpolarized LA neuron (V_m = -105 mV, 0.4 nA injected current) in NEC tissue showing paired IPSPs (P1 and P2) at interstimulus intervals of (a) 200 and (b) 600 ms. Note marked depression of P2 relative to P1 at both intervals. B: intracellular recording from a hyperpolarized LA cell (V_m = -102 mV, -0.8 nA injected current) in pilocarpine-treated tissue showing paired IPSPs (P1 and P2) at interstimulus intervals of (a) 200 and (b) 600 ms. Note that at both intervals, there is less paired-pulse depression (PPD) of the 2nd response with respect to the 1st. C: plot of P2/P1 ratios for interstimulus intervals between 50 and 1,600 ms. Note that pilocarpine-treated tissue exhibits less PPD than NEC. *Interstimulus intervals at which the 2 groups were significantly different from each other (P < 0.05). D: effect of CGP 55845A on the normalized P2/P1 ratio in NEC (n = 6) and pilocarpine-treated tissue (n = 3). Note the less pronounced effect of GABAB receptor antagonism in the pilocarpine-treated group (P = 0.05). n represents number of neurons. Error bars: means ± SE.
and network mechanisms underlying the role of this nucleated structure in epileptogenesis. In vitro studies have shown that, in the presence of convulsive agents, synaptic recruitment of amygdalar neurons through both excitatory and inhibitory mechanisms endows it with the ability to generate epileptic discharges and participate in epileptiform synchronization of limbic networks (Benini et al. 2003; Gean 1990; Gean and Shinnick-Gallagher 1988; Klueva et al. 2003; Stoop and Pralong 2000). Furthermore, studies carried out in the BLA of kindled (Gean et al. 1989; Mangan et al. 2000; Rainnie et al. 1992; Shoji et al. 1998) and kainate-treated rodents (Smith and Dudek 1997) have shown that, in addition to cellular loss and gliosis, permanent changes in synaptic transmission render this amygdaloid region epileptic. We report here similar results in the LA of pilocarpine-treated rats where spontaneous NMDA/non-NMDA–sensitive epileptiform bursting and large amplitude depolarizing PSPs occurred.

Interestingly, a reduced incidence of sIPSPs was also evident in pilocarpine-treated tissue compared with nonepileptic controls, thereby suggesting that alterations in inhibitory mechanisms had also taken place. This finding is relevant considering the extensive immunohistochemical and electrophysiological data showing that the amygdala is rich in GABAAergic cells and that inhibitory processes play an underlying role in controlling the excitability of the LA (Pitkanen and Amaral 1994; Smith et al. 1998). For instance, stimulation of various afferents in vivo results in mainly inhibitory responses within the LA (Le Gal La Salle 1976; Lang and Pare 1997, 1998). Furthermore, as shown here and by other investigators, in vitro stimulation of LA networks yields biphasic IPSP responses that are mediated by GABA_A and GABA_B receptors (Danober and Pape 1998; Martina et al. 2001; Sugita et al. 1992). Moreover, we found that the majority of LA neurons in NEC tissue exhibit robust spontaneously occurring IPSPs that are resistant to glutamatergic antagonists but are sensitive to GABA_A receptor antagonism, thus further substantiating a significant role for inhibitory networks within this nucleus.

Histological examination of epileptic tissue has shown a reduction of GABAAergic neurons within the LA (Tuunanen 1996, 1997), possibly accounting for the reduced incidence of sIPSPs observed in our pilocarpine-treated tissue. It is noteworthy to mention that, in contrast to studies reporting a complete loss of sIPSPs in the BLA of epileptic animals (Gean et al. 1989; Rainnie et al. 1992), we have shown here that inhibitory inputs onto principal cells are not completely lost in the LA of pilocarpine-treated tissue. This is evident by the continued presence of sIPSPs in epileptic tissue, albeit in a smaller proportion of cells. Our findings are in line with previous reports that show a partial loss of interneurons within the LA of chronically epileptic rats, with some studies even reporting >50% of surviving interneurons (Tuunanen et al. 1996, 1997). Finally, decreased dendritic branching of surviving principal cells in the epileptic amygdala (Aliashkevich et al. 2003) also raises the possibility that interneurons make fewer contacts onto pyramidal cells and could perhaps contribute to the reduced incidence of sIPSPs in pilocarpine-treated slices. Hence, our findings suggest that, in epileptic rats, alterations in both excitatory and inhibitory synaptic transmission contribute to the hyperexcitability of LA neuronal networks.

**Altered postsynaptic GABA_A receptor–mediated inhibition**

To further assess the changes in inhibitory inputs onto LA principal cells, IPSPs induced in the presence of glutamatergic antagonists were studied in pilocarpine-treated and NEC tissue. Interestingly, we were able to record biphasic IPSPs in the LA of both types of tissue, thereby indicating that GABA_A and GABA_B receptor–mediated mechanisms were present in the epileptic group. However, notable differences were observed in the reversal potential of the fast component of the IPSP. Specifically, the reversal potential of this GABA_A receptor–mediated component was found to be significantly more depolarized in the pilocarpine-treated tissue compared with NECs. In fact, the reversal potential in the epileptic tissue was more positive than the mean resting membrane potential by ~6 mV. Altogether, this signifies that postsynaptic GABA_A receptor–mediated potentials have a greater chance to be depolarizing at resting levels in the pilocarpine-treated group compared with NECs where the reversal potential is more negative than RMP.

GABA generally tends to induce hyperpolarization of neurons in the adult brain. However, there are several instances such as in the developing juvenile brain (Ben Ari et al. 1989) or in the adult brain under high-frequency stimulation (Lamsa and Taira 2003; Voipio and Kaila 2000) where depolarizing effects of GABA are known to occur. Furthermore, the excitatory actions of GABA have also been documented under pathological conditions such as epilepsy (Cohen et al. 2002), pain (Coull et al. 2003), and ischemia (Schwartz-Bloom and Sah 2001). Several mechanisms have been proposed to account for this polarity switch in GABA action including modified Cl−/HCO3− gradients caused by a decreased expression of the K+/Cl− cotransporter KCC2 (Rivera et al. 1999) and deafferentiation (Vale and Sanes 2000). Thus the more depolarized reversal potential and the lower peak conductance of the GABA_A-mediated IPSP denotes an excitatory effect of GABA in the LA of pilocarpine-treated tissue. These changes may potentially reduce the hyperpolarizing effect of inhibitory inputs onto principal cells, bring LA neurons closer to firing threshold, and consequently facilitate epileptiform synchronization.

**Reduced presynaptic depression of GABA release**

Alterations in presynaptic release of neurotransmitters are known to contribute to hyperexcitability of different neuronal networks (Asprodimi et al. 1992; Behr et al. 2002; Jarvis et al. 1990; Kamphuis et al. 1990). In this study, we used paired-pulse stimulation protocols in the absence of glutamatergic transmission to indirectly assess whether there were any changes in the release of GABA from LA interneurons of epileptic rats. Interneuronal inputs onto pyramidal cells generally show a frequency-dependent depression (Gupta et al. 2000). A pronounced depression in the second IPSP compared with the first would indicate that presynaptic autoreceptors are at play in controlling the release of GABA. Alternatively, additional mechanisms could include a depletion in the presynaptic vesicle pool (von Gersdorff and Borst 2002), presynaptic metabotropic receptors (Cartmell and Schoepp 2000), or even postsynaptic effects such as desensitization of receptors (Jones and Westbrook 1996) and shifts in CI− gradients (Kaila 1994; Thompson and Gahwiler 1989). Interestingly, we found in NEC tissue a pronounced PPD of GABA_A-mediated
responses that was partially reduced by GABA_B receptor antagonism. This evidence suggests that these G protein–linked receptors might play a role in controlling neurotransmitter release from LA interneurons (Miller 1998).

In contrast, we found in pilocarpine-treated tissue a depression in the paired IPSP ratio that was less pronounced and less affected by GABA_B receptor antagonism compared with NEC. Altogether, these observations suggest that presynaptic GABA_B receptors might be less efficient in controlling the release of GABA from LA interneurons of epileptic rodents. At first glance, these results are by themselves peculiar because less PPD in an excitatory network may imply more excitation, but in an inhibitory context may mean more inhibition caused by increased GABA at the synapse, specifically at high-frequency stimulation. However, the reduced PPD combined with the data suggesting a depolarizing effect of GABA could conceivably lead to hyperexcitability of LA neuronal networks in epileptic tissue.

In conclusion, until recently, most electrophysiological assessments of the amygdala and specifically of the BLA were carried out in coronal slices in which connections with other limbic structures are not maintained (Gean et al. 1989; Mangan et al. 2000; Rainnie et al. 1992; Shoji et al. 1998; Smith and Dudek 1997). However, the advent of the combined horizontal slice preparation (Stoop and Pralong 2000; von Bohlen und Halbach and Albrecht 2002) has enabled evaluation of the amygdala’s participation in intralimbic synchronization of epileptiform activity (Benini et al. 2003; Klueva et al. 2003; Stoop and Pralong 2000). The LA is heavily interconnected with hippocampal and parahippocampal structures that are highly implicated in TLE, and it is of crucial importance to determine what significance the alterations presented in this study have on the interactions of the LA with other structures like the perirhinal and entorhinal cortices.

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