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Acute desensitization of presynaptic $GABA_B$-mediated inhibition and induction of epileptiform discharges in the neonatal rat hippocampus

P. Tosetti,[1] R. Bakels,[2] I. Colin-Le Brun,[1] N. Ferrand,[1] J. L. Gaiarsa[1] and O. Caillard[1]
[1]INMED, INSERM U29, Parc Scientifique de Luminy, BP13, 13273 Marseille, France
[2]Department of Medical Physiology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

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Abstract

The consequences of sustained activation of $GABA_B$ receptors on $GABA_B$-mediated inhibition and network activity were investigated in the neonatal rat hippocampus using whole-cell and extracellular field recordings. $GABA_B$-mediated presynaptic control of γ-aminobutyric acid (GABA) release progressively diminished with time in spite of the continued presence of the agonist (100 μM baclofen, 15 min), indicating acute desensitization of presynaptic $GABA_B$-mediated inhibition on GABAergic terminals. By contrast, neither $GABA_B$-mediated inhibition of glutamate release nor postsynaptic $GABA_B$-mediated inhibition seemed to produce this desensitization. Efficacy of presynaptic $GABA_B$ receptors was still reduced by 49% 30 min after baclofen washout, suggesting a long timeframe for recovery from desensitization. The 15-min baclofen application was followed by a dramatic modification of the spontaneous network activity, with the occurrence of epileptiform events called ictal-like discharges (ILDs). Extracellular field recordings confirmed the epileptic nature of the discharges that could be recorded up to 4 h after baclofen washout. ILDs did not occur when the $GABA_B$ receptor antagonist CGP35348 was coapplied with baclofen. This indicates that ILD induction is a consequence of the sustained activation of $GABA_B$ receptors and the correlated changes in $GABA_B$-mediated inhibition. Furthermore, ILDs were also induced when blocking with CGP35348 an amount of $GABA_B$ receptors that exactly mimicked the loss of inhibition obtained with desensitization. These results show that presynaptic $GABA_B$-mediated inhibition of GABA release acutely and specifically desensitizes following a sustained application of the $GABA_B$ receptor agonist baclofen. Conditions that induce desensitization of the $GABA_B$-mediated responses also trigger persistent epileptiform discharges in the neonatal rat hippocampus.

Introduction

In the adult central nervous system, γ-aminobutyric acid (GABA) provides a major inhibitory drive and restrains excessive neuronal activity. This inhibition is achieved both pre- and postsynaptically through the activation of two pharmacologically distinct classes of receptors, termed $GABA_A$ and $GABA_B$ (for review see Sivilotti & Nistri, 1991). Presynaptically, GABA depresses transmitter release through the activation of G-protein-coupled $GABA_B$ receptors located on both glutamatergic and GABAergic terminals (Scanziani *et al*., 1992; Isaacson & Nicoll, 1993; Thompson *et al*., 1993).

Like other G-protein-coupled receptors, $GABA_B$-mediated responses have been shown to be desensitized following prolonged receptor activation (Malcangio *et al*., 1992, 1995; Wetherington & Lambert, 2002). However, the physiological consequences of $GABA_B$ receptor desensitization have not yet been investigated.

The aim of the present study was to investigate the desensitization of pre- and postsynaptic $GABA_B$-mediated inhibition and its consequences on the spontaneous network activity of the developing rat hippocampus. During the first postnatal week, hippocampal network activity is characterized by the presence of giant depolarizing potentials (GDPs, for review see Ben-Ari *et al*., 1997) that result from the synchronous discharge of the majority of pyramidal cells and interneurons. Both GABA and glutamate, acting via $GABA_A$, AMPA/kainate and NMDA receptors play an important role in the genesis of GDPs. $GABA_B$ receptors play a key inhibitory role at this developmental stage, when $GABA_A$-mediated synaptic activity is excitatory. Indeed, $GABA_B$ receptors are activated by spontaneously released GABA and control the duration and frequency of GDPs, thus preventing neonatal hippocampal cells from excessive neuronal discharge (McLean *et al*., 1996). We therefore hypothesized that $GABA_B$ receptor desensitization, if present, could affect the pattern of activity generated by the developing hippocampal network.

To test this hypothesis, we first monitored the amplitude of postsynaptic $GABA_A$ responses during constant application of a selective $GABA_B$ agonist, to detect $GABA_B$ receptor desensitization. We then investigated network activity following agonist removal. We show that presynaptic $GABA_B$ receptors located on presynaptic GABAergic terminals do desensitize relatively rapidly (<5 min) in neonates, and that this desensitization correlates with the appearance of spontaneous epileptiform discharges. The present study demonstrates that

Correspondence: Dr O. Caillard.
E-mail: caillard.o@jean-roche.univ-mrs.fr

Present address: INSERM U464, Neurobiologie des Canaux Ioniques, faculté de Medicine Secteur Nord, Boulevard P. Dramard, 13916 Marseille Cedex 20, France

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epileptiform activity can be induced by a dysfunction of $GABA_B$ receptor-mediated inhibition.

Materials and methods

Tissue preparations

All experimental procedures were carried out according to European and National guidelines for the care and the use of laboratory animals (Council Directive 86/609/EEC and French National Council). Male Wistar rats (postnatal days 2–5, P2–P5) were decapitated under hypothermic anaesthesia, and the brain was rapidly removed and immersed in ice-cold (2–4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 126; KCl, 3.5; $CaCl_2$, 2; $MgCl_2$, 1.3; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11; pH 7.4, when equilibrated with 95% O_2 and 5% CO_2. Hippocampi were dissected and either incubated in ACSF at room temperature or cut into 600-μm slices with a McIlwain tissue chopper. Both intact hippocampi and slices were incubated at room temperature in ACSF for at least 60 min before use. Prior to electrophysiological recordings, individual slices or hippocampi were transferred to a submerged recording chamber and perfused with ACSF at 34 °C at a flow rate of 2.5–3 mL/min (slices) or at 7–8 mL/min (intact hippocampi).

Electrophysiological recordings

Experiments were performed on CA3 neurons from either hippocampal slices or intact hippocampal formations (Khalilov *et al*., 1997a). Field potentials were recorded using glass micropipettes (10–20 MΩ) filled with ACSF. Whole-cell recordings of CA3 pyramidal neurons were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA). Intracellular solution comprised (in mM): KCl, 150; $MgCl_2$, 2; $CaCl_2$, 0.1; EGTA, 1; Na_2ATP, 2.5; Na_2GTP, 0.4; MgATP, 1.5; HEPES, 10; pH 7.25; osmolarity = 275 mOsmol. In addition, 5-(and-6)-tetramethylrhodamine biocytin (rhodamine) was dissolved (0.5–1%) in the recording solution to allow *post hoc* visualization of the recorded neurons. QX314 (2 mM) was added to the intracellular solution in all experiments except for the recordings of postsynaptic $GABA_B$-mediated responses. Capacitance, input and series resistances were measured online with Acquis Software (Biologic, Orsay, France) from the passive transient currents generated by a 5-mV pulse. Series resistances ranged from 5 to 25 MΩ. Cells with unstable or higher series resistances were discarded. Electrical stimulations (30–60 μs; 10–30 V) were delivered at a frequency of 0.03 Hz with a bipolar tungsten electrode placed in the stratum radiatum.

Data acquisition and analysis

Spontaneous and evoked synaptic activities were recorded on a DAT recorder and then transferred to a personal computer for subsequent analysis or directly stored via a digidata 1200 interface (Axon Instruments). Spontaneous activity was analysed with the help of Acquis (Biologic), Minianalysis (Synaptosoft, Decatur, GA, USA) and Igor programs (Wavemetrics, Lake Oswego, OR, USA). GDP duration was measured as the duration from the beginning of the event to the decay at 20% of GDP amplitude. Statistical analysis of GDP durations and percentages were performed with a Kolmogorov–Smirnov (K-S) or analysis of variance (ANOVA) tests, respectively. Differences were considered significant at $P < 0.05$.

Morphological characterization of recorded cells

After the recording session, the slices were immersed in a fixative solution containing 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, PB containing 0.9% NaCl) overnight at 4 °C. The slices were then rinsed in PBS, mounted on gelatin-coated slides and coverslipped with an aqueous mounting medium (Gel Mount, Biomeda). The rhodamine-filled cells were analysed with an Olympus confocal microscope (Fluoview BX50WI) using a helium/neon laser as the excitation source (543 nm) and an emission filter band pass (560 nm). The pinhole size was adjusted for maximum resolution along the depth axis with the lenses and wavelengths used. Series of digitized optical sections (1024 × 1024 pixels; resolution on the *z*-axis, 0.25 μm; step size, 1.5 μm; lens, ×20) were collected and maximum intensity projections were derived using Olympus Fluo-view software.

Solutions and drug delivery

Drug and recording solutions were prepared from stock solutions immediately before the experiment, and delivered to the recording chamber via a gravity-based system. Drugs used in this study were: baclofen (Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Cookson, Bristol, UK), D(-)2-amino-5-phosphovaleric acid (D-AP5; Tocris Cookson), bicuculline (Tocris Cookson), tetrodotoxin (TTX; Sigma, St Louis, MO, USA) and P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348, gift from Novartis, Basel, Switzerland).

Results

Functional desensitization of presynaptic $GABA_B$ receptor-mediated inhibition

Our first aim was to determine whether presynaptic $GABA_B$-mediated inhibition does desensitize in the neonatal rat hippocampus. To address this point, we investigated the effects of saturating concentrations of the $GABA_B$ receptor agonist, baclofen (100 μM), on GABAergic and glutamatergic synaptic transmission in the CA3 region. Whole-cell voltage-clamp recordings of pyramidal neurons were performed using hippocampal slices from P4–P6 rats. Recorded cells were dialysed with the fluorescent dye, rhodamine, for later morphological identification. $GABA_A$ postsynaptic currents (GABA-PSCs) were pharmacologically isolated in the presence of CNQX (10 μM) and D-AP5 (50 μM) to block AMPA/kainate and NMDA receptors, respectively. Glutamatergic postsynaptic currents (GLU-PSCs) were isolated in the presence of bicuculline (10 μM) and high divalent cation concentrations (6 mM Mg^{2+} 4 mM Ca^{2+}) to block polysynaptic activity (Berry & Pentreath, 1976). As illustrated in Fig. 1, bath application of baclofen led to a rapid, significant reduction in the amplitude of both GABA-PSCs and GLU-PSCs. Surprisingly, the amplitude of GABA-PSCs rapidly recovered to approximately 50% of control values in spite of the continued agonist application (Fig. 1A and B). By contrast, the decrease in GLU-PSCs remained constant during the application of baclofen (Fig. 1C and D). Indeed, the ratio of GABA-PSC amplitudes measured at 5 min and 2.5 min from the beginning of baclofen application was 2.25 ± 0.69 ($n = 8$), whereas the ratio of GLU-PSCs was 0.83 ± 0.21 ($n = 7$), indicating a selective decrease in efficacy of presynaptic $GABA_B$ receptors on GABAergic terminals (Fig. 1G). Hereafter, we refer to the decreasing effect of receptor activation while the ligand is still bound to the receptor as desensitization. Because GLU-PSCs were isolated in a high divalent cation ACSF, one could argue that lack of desensitization depended on the high Ca^{2+} and Mg^{2+} concentrations. To rule out this possibility, GABA-PSCs were also recorded in high Ca^{2+} and Mg^{2+}: the ratio of GABA-PSC amplitudes measured at 5 min and 2.5 min from the beginning of baclofen application was 2.06 ± 0.51 ($n = 7$), a value similar to the ratio measured in control ACSF ($P = 0.78$) and significantly different from the ratio of GLU-PSC amplitudes ($P < 0.05$; Fig. 1G).

The desensitization of postsynaptic $GABA_B$-mediated responses was also investigated. At −60 mV, in the presence of TTX (1 μM),

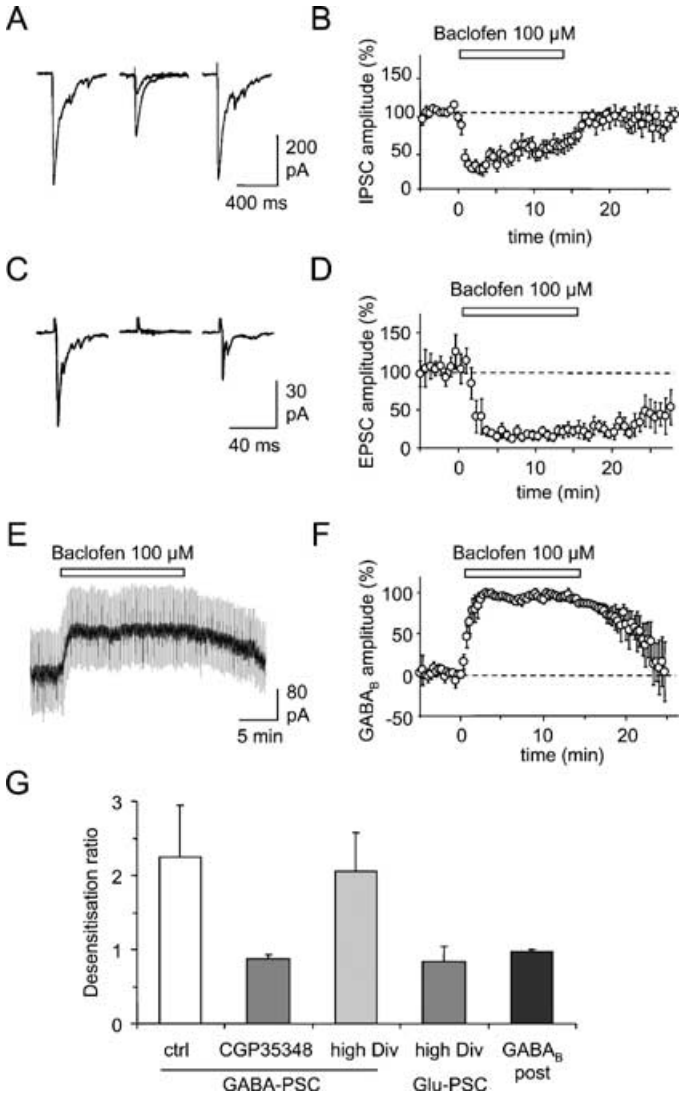

FIG. 1. Acute desensitization of presynaptic $GABA_B$-mediated control of GABA release. (A) Evoked GABAergic postsynaptic currents (GABA-PSCs) recorded in the presence of CNQX (10 µM) and D-AP5 (50 µM). From left to right, average (five trials) changes in amplitude of GABA-PSCs before, after 2.5 (grey) and 10 min application of 100 µM baclofen and 15 min following washout. (B) Average effect of 100 µM baclofen on GABA-PSC amplitude vs. time ($n = 8$). (C) Evoked glutamatergic postsynaptic currents (GLU-PSCs) recorded in the presence of bicuculline (10 µM) in a high divalent cation ACSF (6 mM Mg^{2+}; 4 mM Ca^{2+}). From left to right, average (five trials) changes in amplitude of GABA-PSC before, after 2.5 (grey) and 10 min application of 100 µM baclofen and 15 min following washout. (D) Average effect of 100 µM baclofen on GLU-PSC amplitude vs. time ($n = 7$). (E) Postsynaptic $GABA_B$-mediated response recorded in the presence of TTX at −60 mV during the application of 100 µM baclofen. Positive and negative deflections represent 5 mV depolarizing pulses used to monitor passive properties of the cell. (F) Average change of 100 µM baclofen on baseline current vs. time ($n = 7$). (G) Amplitude ratio of the current responses between 5 and 2.5 min after application of 100 µM baclofen under different conditions. From left to right, GABA-PSC recorded in control ACSF, GABA-PSC recorded in the presence of 3 mM CGP35348, GABA-PSC recorded in a high divalent cation (High Div) ACSF, GLU-PSC recorded in a High Div ACSF, and postsynaptic $GABA_B$-mediated response.

bath application of baclofen (100 µM, 15 min) induced an outward current in seven out of 17 neurons (range 50–120 pA, Fig. 1E and F). The ratio of baclofen currents measured at 5 min and 2.5 min from the beginning of baclofen application was 0.97 ± 0.02 ($n = 7$), indicating an absence of desensitization of postsynaptic $GABA_B$-mediated inhibition (Fig. 1G).

Together, these results show that presynaptic $GABA_B$-mediated inhibition of GABA release selectively undergoes acute desensitization in the neonatal rat hippocampus.

The coapplication of the selective $GABA_B$ blocker, CGP35348, antagonized the baclofen-induced desensitization of $GABA_B$-mediated control at GABAergic terminals. Indeed, in the presence of 3 mM CGP35348, baclofen inhibited GABA-PSCs to only $63 \pm 2\%$ of control values, whereas GABA-PSC amplitude was $26 \pm 6\%$ of control values in the presence of baclofen alone ($n = 5$ and 7, respectively, measured after 2.5 min baclofen application). Moreover, the ratio of GABA-PSC amplitudes at 5 min and 2.5 min from the beginning of baclofen application was 0.87 ± 0.06 in the presence of 100 µM baclofen + 3 mM CGP35348 (Fig. 1G), indicating that no change in receptor efficacy occurred during the combined drug application. Interestingly, the loss of $GABA_B$ receptor efficacy also depended on the baclofen concentration. In fact, the ratio of GABA-PSC amplitudes 15 min and 2.5 min from the beginning of baclofen application was 1.09 ± 0.17 with 10 µM baclofen ($n = 5$), 1.3 ± 0.27 with 30 µM baclofen ($n = 5$), 1.2 ± 0.06 with 50 µM baclofen ($n = 5$) and 3.07 ± 0.7 with 100 µM baclofen ($n = 7$, data not shown).

These results show that a selective decrease in efficacy of $GABA_B$-mediated inhibition at GABAergic terminals occurs within 5 min of application of 100 µM baclofen. This desensitization requires the sustained and selective activation of $GABA_B$ receptors, and its extent depends on agonist concentration.

Sustained activation of $GABA_B$ receptors leads to long-lasting modifications of spontaneous synaptic activity

Having established that presynaptic $GABA_B$-mediated inhibition does desensitize in the neonatal rat hippocampus, we next investigated the possible consequences of this desensitization on network activity.

Early in development, the spontaneous synaptic activity in the developing rat hippocampus is characterized by the presence of network-driven events termed GDPs whose duration is controlled by spontaneously released GABA via activation of $GABA_B$ receptors (McLean *et al*., 1996). We therefore hypothesized that baclofen-induced desensitization of $GABA_B$ receptors could alter the pattern of synaptic activity generated by the hippocampal network. To test this hypothesis, we investigated the consequences of a 15-min baclofen application on the spontaneous synaptic activity of neonatal hippocampal slices.

GDPs recorded in the whole-cell configuration in hippocampal slices appeared as large inward currents with a mean duration of 271 ± 8 ms ($n = 9$) and occurring at a mean frequency of $7.9 \pm 1.2\ min^{-1}$ ($n = 9$). The application of 100 µM baclofen entirely abolished GDPs. The spontaneous network activity reappeared approximately 10 min after baclofen removal, but its pattern was severely modified (Figs 2 and 3). Indeed, GDP duration was increased to $137 \pm 8\%$ of control (Fig. 3A and B), and GDP frequency reduced to $32 \pm 2\%$ of control ($n = 9$, Fig. 2C). Furthermore, after baclofen application, six of nine cells showed abnormal events not present in control conditions (Fig. 2). The events, hereafter referred to as ictal-like discharges (ILDs), were inward currents characterized by a series of slow oscillations resembling merged GDPs. They lasted approximately 50 times longer than GDPs (11.6 ± 0.9 s, range 2.9–30.4 s), and were generally followed by a period of reduced spontaneous activity (synaptic silence). Both ILDs and longer GDPs could also be induced by a shorter (5-min) baclofen application (five out of six tested cells), and could still be observed 1 h after agonist removal.

We next investigated whether the after-effects of baclofen on network activity were specifically due to the activation of $GABA_B$

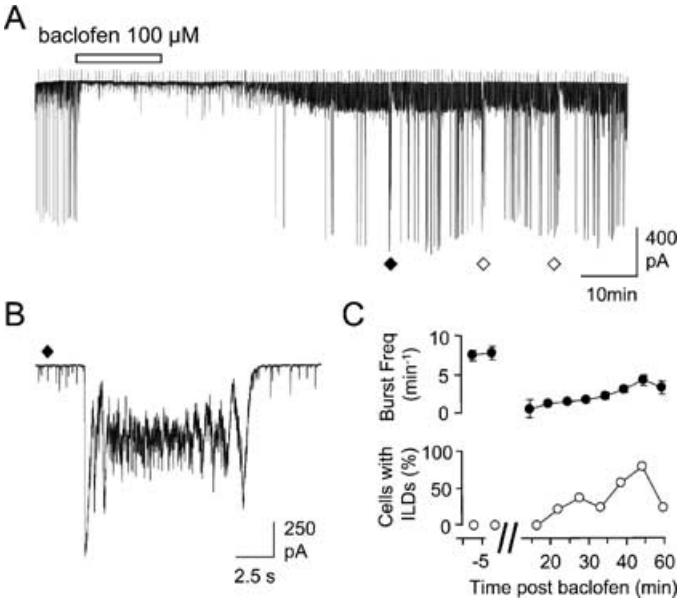

FIG. 2. Changes in the pattern of activity following sustained activation of $GABA_B$ receptors. (A) Application of baclofen (100 μM) induces a strong reduction in spontaneous activity and a complete blockade of GDPs. Upon washout of baclofen, spontaneous synaptic activity recovers but the pattern is different. Three ILDs, not present in control, can be observed (diamonds). (B) A characteristic ILD marked by the closed diamond in A and shown on an expanded time scale. (C) Time course of the frequency (up) of the total number of bursts (GDPs in control, GDPs + ILDs after baclofen) and the percentage of cells in which ILDs were recorded (down) following sustained application of baclofen (100 μM).

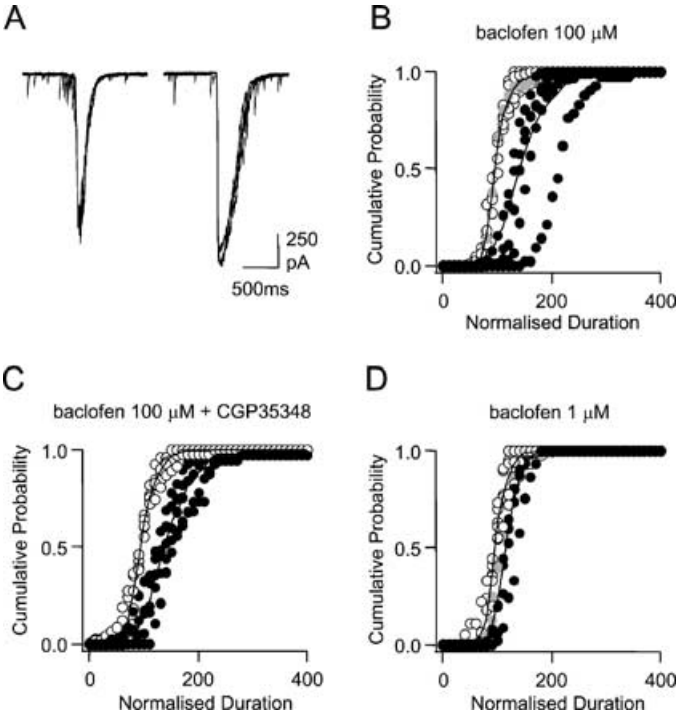

FIG. 3. Changes in the duration of GDPs following sustained activation of $GABA_B$ receptors. (A) Superimposed (five) GDPs taken from the same cell as in Fig. 3A in control conditions (left) and 30 min after a 5-min application of baclofen 100 μM (right). (B) Superimposed cumulative plots of the duration of GDPs in control and 10–60 min after application of baclofen 100 μM. Durations were normalized to an average control duration of 100 ms (white circles). Circles are black or grey when the increase in duration is significant or not (K-S test), respectively. Baclofen 100 μM induces a significant increase (K-S test) in duration in five out of six experiments. The line on the graph represents the average change in duration. (C) Same representation as in B but baclofen 100 μM was applied in the presence of CGP35348 (3 mM). An increase in duration is observed in six of six experiments. (D) Same representation as in B. Baclofen 1 μM was applied for 5 min. An increase in duration is observed in four out of six experiments.

receptors. We found that ILDs were never observed when baclofen was applied in the presence of CGP35348 (3 mM). However, GDP duration was still significantly increased ($151 \pm 8\%$, $n = 5$). This increase in GDP duration may have resulted from an incomplete blockade of $GABA_B$ receptors by the antagonist (allowing some receptor desensitization to occur). Indeed, CGP35348 did not prevent completely the reduction in GABA-PSC amplitude when 100 μM baclofen was applied. We thus simulated an incomplete receptor blockade by applying a low baclofen concentration. We observed that 1 μM baclofen indeed failed to induce ILDs but was still able to increase GDP duration significantly in four out of six cases ($120 \pm 2\%$, $n = 6$, Fig. 3D).

These data indicate that low concentrations of $GABA_B$ agonist can modify significantly the duration of network discharges. Moreover, sustained activation of $GABA_B$ receptors changes dramatically the pattern of activity received by pyramidal cells in the neonatal rat hippocampus by inducing the appearance of abnormal discharges.

Sustained activation of $GABA_B$ receptors triggers epileptiform activity

To investigate whether ILDs were network-driven events, the spontaneous synaptic activity of the intact hippocampal formation, a preparation in which the whole intrahippocampal network is preserved (Khalilov *et al*., 1997a), was recorded with field electrodes following baclofen washout. Under these conditions, GDPs appeared as a sequence of positive and negative field potentials (Leinekugel *et al*., 1998), having a mean duration of 500 ms and occurring at a frequency of $4.8 \pm 1.2\ min^{-1}$ (range 0.03–0.13 s^{-1}, Fig. 4A). Baclofen application (100 μM, 15 min) led to the complete disappearance of spontaneous GDPs. The spontaneous network activity progressively reappeared following the washout of baclofen but, as observed in slices, it was modified with respect to controls (Fig. 4B). In fact, 15–20 min after baclofen washout, the synaptic activity consisted of long discharges, occurring at a frequency of $0.6 \pm 0.9\ min^{-1}$ (range 0.05–3.48 min^{-1}), and having a mean duration of 57 ± 20 s (range 15–86 s). In most cases, these discharges started with a tonic phase characterized by rhythmic oscillations of short (50–80 ms) negative field potentials at a frequency of 10–20 Hz followed by a series of clonic discharges occurring at a frequency of 1–2 Hz (Fig. 4C).

The modification in the pattern of spontaneous synaptic activity observed following baclofen washout was present throughout the recording session (up to 4 h after agonist removal, Fig. 4D). Network discharges were totally abolished by application of CNQX (10 μM) and D-AP5 (50 μM), indicating that the epileptiform activity observed following baclofen application required glutamatergic transmission. Therefore, the ILDs observed upon washout of baclofen show a remarkable resemblance to epileptiform discharges that could be induced by convulsive agents in the intact hippocampus (Dzhala *et al*., 1999; Khalilov *et al*., 1999a, b).

The effect of baclofen on the spontaneous network activity was dose-dependent. When applied at a concentration of 100 μM, baclofen led to ictal discharges in seven out of nine cases (77%). However, induction of epileptiform activity decreased with lower baclofen concentrations; it could be observed in only 14% of cases at 10 μM

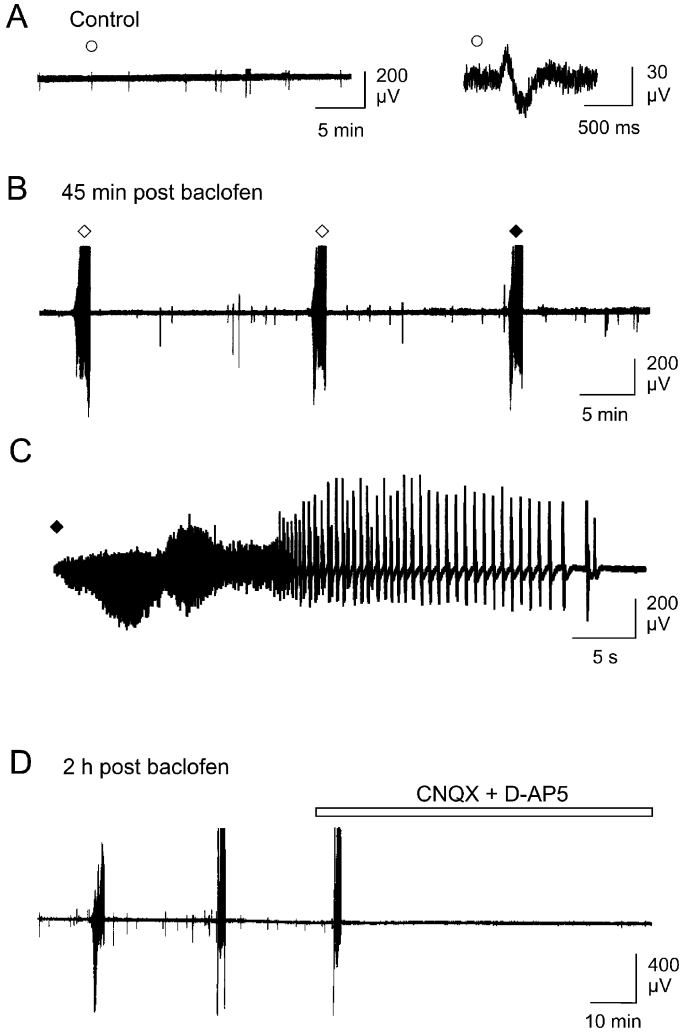

FIG. 4. Changes in the pattern of activity following desensitization in the intact hippocampal formation. (A) Spontaneous GDPs recorded with field electrodes in the area CA3 of the intact hippocampus. A GDP is depicted at an expanded time scale (right trace). (B) Spontaneous burst discharges recorded 45 min after the washout of baclofen (100 μM). Three ILDs were detected (diamonds, same hippocampus as in A). (C) The ILD marked in B with a closed diamond at an expanded time scale. (D) ILDs can still be detected 2 h after washout of baclofen (same hippocampus as in A). Application of CNQX (10 μM) and D-AP5 (50 μM) blocks these ILDs.

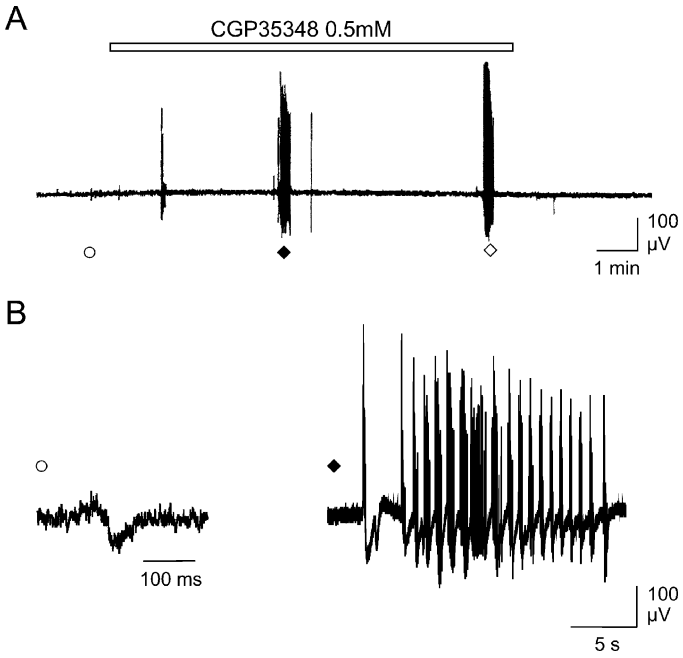

FIG. 5. Blockade of $GABA_B$ induces ILDs in the intact hippocampal formation. (A) Spontaneous burst discharges recorded with field electrodes in the area CA3 of the intact hippocampus. In the control period, biphasic events characteristic of GDPs are present (open circles). Application of an antagonist of $GABA_B$ receptors, CGP35348 (500 μM), modifies the pattern of spontaneous activity. Two ILDs (diamonds) were observed. (B) Control GDPs (left) and ILDs (right) marked in A with, respectively, an open circle and a closed diamond on an expanded time scale.

($n = 7$), 22% of cases at 30 μM ($n = 9$) and 33% of cases at 50 μM ($n = 6$).

Together, these results indicate that sustained activation of $GABA_B$ receptors leads to a profound and long-lasting modification in the synaptic activity generated by the developing hippocampal network. These changes include the appearance of epileptiform discharges following the washout of the $GABA_B$ agonist.

Persistence of $GABA_B$ receptor desensitization correlates with epileptiform activity

To strengthen further the correlation between desensitization of $GABA_B$-mediated inhibition and epileptiform discharges, we investigated the effect of the selective $GABA_B$ receptor antagonist CGP35348 on the spontaneous hippocampal activity. Indeed, if the observed epileptiform activity were due to receptor desensitization, the pharmacological blockade of $GABA_B$ receptors would mimic the loss of functional $GABA_B$ receptors and should also induce ILDs. A typical result of such an experiment, performed in an intact hippocampus, is illustrated in Fig. 5. Application of 0.5 mM CGP35348 led to the appearance of ILDs that were remarkably similar to those recorded following a 15-min baclofen application. Analogous results were obtained in six out of eight tested cells. On average, ILD duration was 41 ± 16 s ($n = 6$, range 15–60 s) and ILD frequency was $0.7 \pm 0.3\ \text{min}^{-1}$ ($n = 6$, range $0.18–1.2\ \text{min}^{-1}$). In contrast to baclofen, however, the application of CGP35348 did not have long-lasting consequences, and synaptic activity rapidly recovered to control levels upon washout of the drug.

These results therefore show that $GABA_B$-mediated inhibition plays an important role in the control of neonatal hippocampus synaptic activity, and suggest that a change in $GABA_B$ receptor efficacy, i.e. desensitization, could result in the expression of ILDs in the neonatal rat hippocampus.

For desensitization to be responsible for the expression of ILDs, it should persist after baclofen washout. We thus compared the effect of baclofen at 10 μM (a nondesensitizing concentration) on the amplitude of evoked GABA-PSCs before and 30 min after a 15-min application of baclofen 100 μM in hippocampal slices (Fig. 6A). Baclofen at 10 μM (5 min) reduced the amplitude of GABA-PSCs by $68 \pm 4\%$ in control slices, and $32 \pm 4\%$ in slices treated by 100 μM baclofen 30 min previously ($n = 5$, $P < 0.01$). Therefore, desensitization develops within 5 min from the beginning of baclofen application and lasts for at least 30 min after washout of the agonist.

In order to induce ILDs, CGP35348 was applied at saturating concentrations leading to an almost complete blockade of $GABA_B$ receptors. It is possible that desensitization, impairing only a fraction of presynaptic $GABA_B$-mediated inhibition, would not be able to induce ILDs. To exclude this possibility, we decided to reduce $GABA_B$-mediated inhibition to the level achieved following desensitization

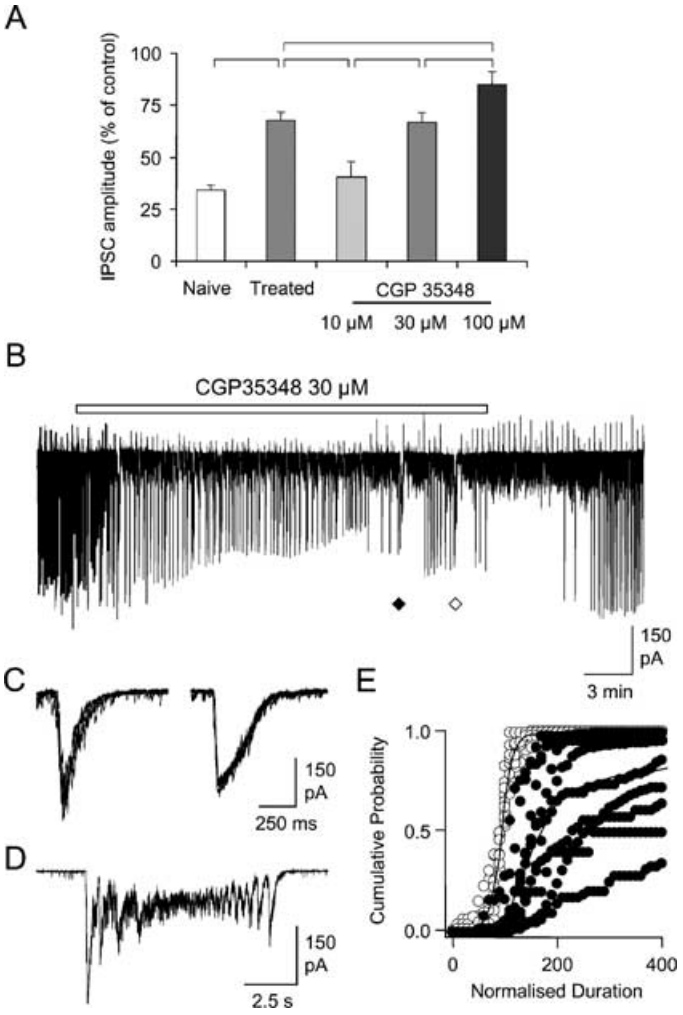

FIG. 6. Partial Blockade of $GABA_B$ receptors mimics the effects of desensitization on network activity. (A) Average reduction (in percentage of control) of GABA-PSC amplitude by baclofen 10 μM under different conditions. From left to right, naïve hippocampal slices, baclofen-treated slices (30 min after a 15-min baclofen 100 μM application), baclofen applied in the presence of 10 μM CGP35348, 30 μM CGP35348 and 100 μM CGP35348. Superimposed bars represent significant differences. (B) Application of 30 μM CGP35348 induces important changes in the pattern of synaptic activity. GDPs were increased in duration and two ILDs (diamonds) could be observed during drug application. (C) Superimposed (five) GDPs taken from the same cell as in B at control conditions (left) and during application of 30 μM CGP35348 (right). (D) One ILD, marked by a closed diamond in B, on an expanded time-scale. (E) Superimposed cumulative plots of the duration of GDPs in control (white circles) and during application of 30 μM CGP35348 (black circles). CGP35348 at 30 μM induces a significant increase (K-S-test) in duration in nine out of nine experiments. The line on the graph represents the average change in duration.

and investigate if epileptiform activity could be induced. To identify the correct CGP35348 concentration, we compared the effects of baclofen 10 μM on the amplitude of evoked GABA-PSCs in control conditions (naive slices), after 15 min of baclofen application (desensitized slices), and in the presence of various concentrations of CGP35348 (10, 30, 100 μM). The same level of presynaptic $GABA_B$-mediated inhibition observed in 'desensitized' slices could be achieved partially antagonizing $GABA_B$ receptors using 30 μM CGP35348 ($67 \pm 5\%$, $n = 8$). By contrast, the application of 10 and 100 μM CGP35348 produced a $GABA_B$-mediated inhibition that was too low ($40 \pm 7\%$, $n = 7$) or too high ($85 \pm 6\%$, $n = 8$), respectively.

We therefore investigated the effects of CGP35348 30 μM on spontaneous network activity. In the presence of 30 μM CGP35348 the network activity was severely affected. Indeed, GDP duration was significantly increased to $290 \pm 59\%$ of control ($P < 0.01$), and GDP frequency reduced to $79 \pm 10\%$ of control ($n = 10$, Fig. 6B and C). In addition, ILDs could be observed in eight out of ten cells.

These results therefore demonstrate that a partial blockade of $GABA_B$ receptors that mimics desensitization of presynaptic $GABA_B$-mediated inhibition at GABAergic synapses can induce epileptiform activity in the neonatal rat hippocampus.

Discussion

The main conclusion of the present study is that sustained activation of $GABA_B$ receptors leads to the specific and prolonged desensitization of presynaptic $GABA_B$-mediated inhibition at GABAergic terminals, and induces the appearance of ILDs in the neonatal rat hippocampus. The causal relation between the two observations is discussed below.

Sustained activation of $GABA_B$ receptors induces epileptiform activity in the neonatal rat hippocampus

We have shown that the pattern of activity generated by the neonatal rat hippocampus is profoundly altered following a prolonged activation of $GABA_B$ receptors. GDPs present in physiological conditions were replaced by aberrant epileptiform discharges following the washout of baclofen. Our data strongly support desensitization of the presynaptic $GABA_B$-mediated inhibition as the main mechanism underlying the appearance of epileptiform discharges.

The following observations are consistent with this hypothesis. (i) Desensitization of presynaptic $GABA_B$ receptor-mediated inhibition on GABAergic terminals was observed during and following baclofen application; it was blocked by CGP35348 and depended on baclofen concentration. (ii) ILDs could not be induced in conditions interfering with receptor desensitization (i.e. in the presence of low baclofen concentration or CGP35348). (iii) Mimicking the reduced receptor efficacy following desensitization by the application of a nonsaturating antagonist concentration causes the appearance of ILDs. (iv) The absence of recovery from desensitization after 30 min is consistent with the time-course of occurrence of ILDs.

CGP35348 was not able to fully prevent baclofen-mediated presynaptic inhibition of GABA release under our conditions. A similar partial effect of CGP35348 has been reported previously (Thompson & Gähwiler, 1992). The possibility of nonspecific effects of baclofen applied at high concentration can nevertheless be excluded as desensitization was not observed during baclofen and CGP35348 coapplication, nor were ILDs induced after washout of the drugs. Although $GABA_B$ receptor desensitization is the simplest and most exhaustive explanation for the appearance of ILDs following baclofen application, an alternative hypothesis can be proposed. Because $GABA_B$ receptors activate K^+ conductance (Gähwiler & Brown, 1985), and rises in $[K^+]$ are known to induce seizures (Traynelis & Dingledine, 1988), the convulsive action of baclofen may have also resulted from changes in extracellular K^+ homeostasis following sustained activation of $GABA_B$ receptors. This hypothesis is, however, unlikely because ILDs could still be recorded more than 1 h after baclofen washout, when extracellular $[K^+]$ has probably recovered to control values. The induction of epileptiform discharges could also result from a $GABA_B$ receptor-dependent, massive production of second messengers (Thompson & Gähwiler, 1992; Swartz, 1993; Tremblay *et al.*, 1995; Yoshimura *et al.*, 1995), leading to an alteration in the excitability of hippocampal neurons.

It may appear paradoxical to consider the loss of presynaptic inhibition of GABA release as a proconvulsive factor. However, it should be noted that in the neonatal rat hippocampus, GABAergic synaptic transmission exerts excitatory actions because of an inverted chloride gradient (for a review see Ben-Ari *et al*., 1997). Therefore, the loss of control of GABA release may increase neuronal excitability and lead the network to pass the threshold for epileptiform activity.

The fact that network discharges were abolished in the presence of glutamatergic antagonists indicates that epileptiform discharges induced by sustained activation of $GABA_B$ receptors share similarities with the bicuculline- or kainate-induced epileptiform activities previously described in the hippocampus (Khalilov *et al*., 1997b, 1999b). By contrast, these discharges have different electrophysiological and pharmacological properties when compared with the 4AP-induced epileptiform activities, where network-driven GABAergic discharges could be recorded in the presence of glutamatergic antagonists (Muller & Misgeld, 1990; Avoli *et al*., 1993; Michelson & Wong, 1994; McLean *et al*., 1996).

When evaluating the overall impact of a loss of $GABA_B$ receptor-mediated inhibition on tissue excitability, we must take into account $GABA_B$ receptor modulation of GABA and glutamate release as well as postsynaptic excitability (Gaïarsa *et al*., 1995; McLean *et al*., 1996; Sutor & Luhmann, 1998). Our results show functional differences between presynaptic control of GABA and glutamate release by $GABA_B$ receptors. Indeed, although we could observe acute desensitization of $GABA_B$ receptor-mediated inhibition at GABAergic terminals during a 15-min baclofen application, neither the presynaptic control of glutamate release nor the postsynaptic $GABA_B$ inhibition were acutely desensitized. Wetherington & Lambert (2002) reported similarly that presynaptic $GABA_B$ receptors located on glutamatergic terminals can be desensitized but only after more than 48 h of activation in cultured hippocampal neurons, whereas the postsynaptic $GABA_B$ receptor-mediated G-protein-activated inwardly rectifying K^+ (GIRK) currents desensitized relatively faster (2–4 h).

An interesting observation was that when $GABA_B$ receptors were blocked to a level of efficacy comparable with that observed in desensitized slices, the increase in duration of the GDPs was significantly greater ($290 \pm 59\%$ vs. 136 ± 8, $P < 0.01$). This can be explained by the fact that the application of CGP35348 partially blocked $GABA_B$ receptors on both GABAergic and glutamatergic terminals, whereas our results show that desensitization affects only $GABA_B$-mediated inhibition of GABA release. Therefore, the partial block of the presynaptic control of glutamate release could be the reason why the increase in GDP duration was potentiated in the presence of 30 μM CGP35348.

The fact that ILDs could be recorded up to 4 h following sustained activation of $GABA_B$ receptors may indicate either a long-term depression of $GABA_B$ receptor-mediated inhibition or some plastic mechanisms that take place in the hippocampal network as a consequence of the first ILDs. Alternatively, the massive release of GABA during ILDs could maintain desensitization at a level high enough to permit the generation of recurrent seizures. If the latter hypothesis is true, $GABA_B$ receptor desensitization may represent a general mechanism for the induction or maintenance of epileptiform activities in brain areas where $GABA_B$ receptors have an overall inhibitory role. Indeed, depending on the importance of these receptors in a specific brain area, and the nature of the cells expressing the receptors (glutamatergic or GABAergic cells), the consequences of desensitization could be pro- or anticonvulsive. For instance, in the thalamus, $GABA_B$ agonists have direct proconvulsive effects in the generation of absence seizure (Snead, 1992), whereas in other brain areas, such as the amygdala (Wurpel, 1994) or the hippocampus, they are anticonvulsive (Ault *et al*., 1986). The present results show that a high concentration of baclofen can be proconvulsive in the hippocampus. In the hypothesis of a causal link between desensitization and epileptiform activity, one may speculate that a decrease in $GABA_B$-mediated inhibition may indeed be critical in the neonatal rat hippocampus, because $GABA_B$ receptors are spontaneously activated by endogenous GABA and control network activity at this early stage of postnatal development (McLean *et al*., 1996; this study).

Desensitization of presynaptic $GABA_B$ receptors

The mechanisms underlying the desensitization of presynaptic $GABA_B$ receptor-mediated inhibition remain to be addressed. Several G-protein-coupled receptors (GPCRs) are phosphorylated by G-protein receptor kinases, which promote binding of β-arrestins on GPCRs and uncouple them from G-proteins. The complex GPCR–arrestin is then targeted for endocytosis (Carman & Benovic, 1998; Ferguson *et al*., 1998; Tsao & von Zastrow, 2000). The process of uncoupling of GPCRs that occurs within minutes after agonist-induced receptor activation could be the mechanism by which GABAergic synapses lose their presynaptic $GABA_B$ receptor-mediated auto-inhibition. Immunohistochemical tools were used in order to evaluate internalization as the possible cause of desensitization. Unfortunately, because of a low specificity of $GABA_B$ antibodies and/or a number of receptors that is too low to be accurately detected, such a hypothesis is at the present difficult to address (I. Colin-Le Brun, personal communication). Alternatively, desensitization could result from the uncoupling of the receptors with their targets, following activation of protein kinase C (Thompson & Gähwiler, 1992; Swartz, 1993) and protein kinase A (Yoshimura *et al*., 1995).

Conclusions

Several groups have reported that, although $GABA_B$ receptor-mediated inhibition controls seizure activity, the blockade of $GABA_B$ receptors, in the presence of intact $GABA_A$ receptor-mediated inhibition, cannot induce epileptiform activity in the hippocampus (Scanziani *et al*., 1991; Karlsson *et al*., 1992; Sutor & Luhmann, 1998). Moreover, epileptiform activity following the sole activation of $GABA_B$ receptors has, to our knowledge, never been reported *in vitro*. Here we report that, in the neonatal rat hippocampus, the continued activation of $GABA_B$ receptors can induce acute, long-lasting desensitization of the presynaptic $GABA_B$-mediated inhibition of GABA release, and results in the appearance of epileptiform activity.

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Abbreviations

GABA-PSCs, $GABA_A$ postsynaptic currents; GDPs, giant depolarizing potentials; GLU-PSCs, glutamatergic postsynaptic currents; ILDs, ictal-like discharges.

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