GABA_B Receptor Activation Promotes Seizure Activity in the Juvenile Rat Hippocampus

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GABA_B receptor activation promotes seizure activity in the juvenile rat hippocampus. J. Neurophysiol. 82: 638–647, 1999. We analyzed how the GABA_B receptor agonist baclofen (10–50 μM) influences the activity induced by 4-aminopyridine (4-AP, 50 μM) in the CA3 area of hippocampal slices obtained from 12- to 25-day-old rats. Interictal and ictal discharges along with synchronous GABA-mediated potentials occurred spontaneously in the presence of 4-AP. Baclofen abolished interictal activity (n = 29 slices) and either disclosed (n = 21/29) or prolonged ictal discharges (n = 8/29), whereas GABA-mediated potentials occurred at a decreased rate. The N-methyl-D-aspartate (NMDA) receptor antagonist 3,3-(2-carboxypiperazine-4-yl)propyl-1-phosphate (CPP, 10 μM, n = 8) did not modify the GABA-mediated potentials or the ictal events recorded in 4-AP + baclofen. In contrast ictal, activity, but not GABA-mediated potentials, was blocked by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM, n = 5). Most baclofen effects were reversed by the GABA_B receptor antagonist CGP 35348 (1 mM; n = 4). Baseline and transient increases in [K^+]_o associated with the 4-AP–induced synchronous activity were unaffected by baclofen. Baclofen hyperpolarized CA3 pyramids (n = 8) recorded with K-acetate–filled electrodes by 4.8 ± 1.3 mV and made spontaneous, asynchronous hyperpolarizing and depolarizing potentials disappear along with interictal depolarizations. GABA-mediated synchronous long-lasting depolarizations (LLDs) and asynchronous depolarizations were also studied with KCl–filled electrodes in 4-AP + baclofen. In contrast, activity, but not GABA-mediated potentials, was blocked by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM, n = 5). Most baclofen effects were reversed by the GABA_B receptor antagonist CGP 35348 (1 mM; n = 4). Baseline and transient increases in [K^+]_o associated with the 4-AP–induced synchronous activity were unaffected by baclofen. Baclofen hyperpolarized CA3 pyramids (n = 8) recorded with K-acetate–filled electrodes by 4.8 ± 1.3 mV and made spontaneous, asynchronous hyperpolarizing and depolarizing potentials disappear along with interictal depolarizations. GABA-mediated synchronous long-lasting depolarizations (LLDs) and asynchronous depolarizations were also studied with KCl–filled electrodes in 4-AP + baclofen (n = 8). Our data indicate that GABA_B receptor activation by baclofen decreases transmitter release leading to disappearance of interictal activity along with asynchronous excitatory and inhibitory potentials. By contrast, GABA-mediated LLDs and ictal events, which reflect intense action potential firing invading presynaptic inhibitory and excitatory terminals respectively, are not abolished. We propose that the proconvulsant action of baclofen results from 1) block of asynchronous GABA-mediated potentials causing disinhibition and 2) activity-dependent changes in hippocampal network excitability.

INTRODUCTION

GABA is a ubiquitous inhibitory transmitter in the CNS where it acts mainly on two receptor subtypes (termed A and B) that are located pre- and postsynaptically on both local and long-axonated cells (Bowery 1993; Kaila 1994; Macdonald and Olsen 1994; Misgeld et al. 1995). Epileptiform activity ensues when GABAergic mechanisms weaken (Krnjevic 1991), and several anticonvulsant drugs including some of the newest compounds, may enhance GABA_A-mediated inhibition (Olsen and Avoli 1997).

GABA_B receptor–mediated mechanisms are involved in the generation of focal seizures and in epileptogenesis (Haas et al. 1996; McLean et al. 1996; Scanziani et al. 1994; Velísková et al. 1996). However, the GABA_B receptor agonist baclofen may possess a surprising proconvulsant effect as documented in clinical practice (Koffler et al. 1994; Rush and Gibberd 1990) and in some models of epileptiform discharge (Lewis et al. 1989; Mott et al. 1989; Swartzwelder et al. 1987; Watts and Jefferys 1993). It has also been proposed that the proconvulsant effect of baclofen is caused by a presynaptic, GABA_B-mediated inhibition of GABA release from inhibitory interneurons leading to disinhibition (Mott et al. 1989; Watts and Jefferys 1993).

Although age-related differences in the ability of baclofen to modulate seizures have been described (Velísková et al. 1996), the evidence for a proconvulsant action of this GABA_B receptor agonist mainly derives from studies in the adult brain. Here we used extracellular and intracellular recording techniques in conjunction with [K^+]_o measurements to characterize the effects of baclofen on the activity induced by the convulsant 4-aminopyridine (4-AP) in the CA3 area of hippocampal slices obtained from young rats. At this age both interictal and ictal discharges occur in vitro along with synchronous GABA-mediated events (Avoli et al. 1993, 1996). Some of these findings have been published in abstract form (Motalli et al. 1997; Tancredi et al. 1998).

METHODS

Preparation and maintenance of the slices

Sprague-Dawley or Wistar rats (12–25 day old) were decapitated under halothane anesthesia, and the brains were quickly removed and placed in cold oxygenated artificial cerebral spinal fluid (ACSF). Isolated, 500-μm-thick hippocampal slices were cut with a vibratome and transferred to a tissue chamber where they lay in an interface between oxygenated ACSF and humidified gas (95% O_2-5% CO_2) at 32–35°C (pH 7.4). ACSF composition was (in mM) 124NaCl, 2 KCl, 1.25 KH_2PO_4, 2 MgSO_4, 2 CaCl_2, 26 NaHCO_3, and 10 glucose. 4-AP was added to the ACSF to a final concentration of 5–10 mM as necessary to produce consistent and reliable spontaneous activity. The ACSF was constantly oxygenated and maintained at 32–35°C (pH 7.4) and flowed at a rate of 2 mL/min. The slices were allowed to stabilize for 1–2 h at 32–35°C before recordings were started.
(50 μM), 4-amino-3-[4-chlorophenyl]-butanoic acid (baclofen, 10–50 μM), 3,3-(2-carboxypiperazine-4-yl)-propyl-1-phosphate (CPP, 10 μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), and CGP-35348 (1 mM) were bath applied. Chemicals were acquired from Sigma with the exception of CNQX and CPP (obtained from Tocris Cookson) and CGP 35348 (kindly donated by Novartis, Basel).

Recording procedures

Extracellular field potential recordings were made in CA3 stratum radiatum with electrodes filled with 2 M NaCl or ACSF (resistance, 2–8 MΩ). Intracellular recordings were performed with electrodes filled with 2 M K-acetate (resistance, 70–120 MΩ) or 3 M KCl (resistance, 60–90 MΩ). Signals were fed to high-impedance amplifiers with internal bridge circuit for passing intracellular current. The bridge was monitored carefully throughout the experiment and adjusted as required. Whenever necessary the resting membrane potential (RMP) during any given pharmacological test was maintained at the same value as in control by injecting intracellular current.

K⁺-selective electrodes were prepared according to the techniques described by Heinemann et al. (1977) and previously used in our laboratories (Avoli et al. 1996). For each ion-selective electrode, a double-barreled pipette was pulled to a tip diameter of 1–2 μm and silanized. One barrel was filled with NaCl and was used as a reference electrode. The other barrel was filled with a K⁺ solution, valinomycin-based K⁺ ionophore I (cocktail A), was sucked into the tip. The resin was acquired from Fluka Chemical. K⁺-selective electrodes were tested and calibrated before and after the experiment in different solutions, which were as follows (in mM): 1) 146 NaCl and 3 KCl; 2) 146 NaCl and 30 KCl; 3) 119 NaCl and 30 KCl; and 4) 59 NaCl and 90 KCl. K⁺-selective electrodes were accepted if their response to a 10-fold change in [K⁺] was ≥49 mV. Signals from the K⁺-selective electrode (that was positioned in CA3 stratum radiatum) were fed to a Meyer & Rentz amplifier (Frankfurt, Germany). In these experiments the field potential was recorded through the reference channel of the K⁺-selective electrode.

Field potential, intracellular, and [K⁺], recordings were displayed on a digital oscilloscope and on a Gould WindoGraf recorder. They
were also recorded on a videocassette recorder for later analysis. In some experiments a bipolar stainless steel electrode was used to deliver extracellular stimuli (90 μs; <1,800 μA) to the hilus of the dentate gyrus.

**Database and analysis**

Our study is based on the use of >60 slices that were analyzed with field potential, intracellular, and/or [K\(^+\)] recordings. The electrophysiological characteristics of CA3 pyramidal cells recorded with K-acetate–filled electrodes were: 1) RMP measured after electrode withdrawal was \(68.1 \pm 5.0\) mV (mean \(\pm\) SD, \(n = 11\)); 2) action potential amplitude calculated from the baseline of \(91.7 \pm 9.5\) mV (\(n = 18\)); apparent input resistance obtained from the maximum voltage response induced by small (<0.5 nA) hyperpolarizing current pulses of \(27.3 \pm 7.5\) MΩ (\(n = 13\)). Injection of depolarizing current pulses in these cells caused regular spiking activity with adaptation that was followed by a long-lasting afterhyperpolarization (160–250 ms) on pulse termination.

Throughout the paper measurements are expressed as means \(\pm\) SD, and \(n\) indicates the number of slices used for any given pharmacological protocol, unless otherwise stated. Statistical analysis of the data obtained under control conditions, and during any experimental manipulation was performed with paired or unpaired Student’s \(t\)-tests as well as with ANOVA. Data were considered significantly different if \(P < 0.01\).

**RESULTS**

**4-aminopyridine–induced synchronous activity**

Three different types of spontaneous synchronous activity were recorded in the CA3 stratum radiatum of juvenile rat hippocampal slices during 4-AP application (Avoli et al. 1993, 1996). Brief, positive interictal-like discharges (duration, 300–800 ms; rate of occurrence, 0.2–0.9 Hz; Fig. 1, A and B, arrows in Control) and negative potentials (duration, 300–900 ms; rate of occurrence, 0.007–0.23 Hz; Fig. 1, A and B, asterisks in Control) were present in all slices (\(n = 42\)). In addition, ictal-like discharges (duration, 5.4–11.0 s; rate of occurrence, 0.004–0.011 Hz) were seen in 19/42 slices (Fig. 1B, continuous line in Control). Each ictal discharge was preceded, and thus appeared to be initiated by a negative-going field potential (Fig. 1B, Control).

We have reported that both interictal-like and ictal-like (thereafter referred to as interictal and ictal) discharges induced by 4-AP are insensitive to NMDA receptor antagonists, but are abolished by CNQX (Avoli et al. 1993, 1996). We have also shown that the negative-going potentials are insensitive to excitatory amino acid receptor antagonists, but are blocked by μ-opioid receptor activation, or GABA\(_A\) receptor antagonists. Hence we shall refer to these events as synchronous, GABA-mediated potentials.

**Effects induced by baclofen on the 4-aminopyridine–induced synchronous activity**

Application of the GABA\(_B\) receptor agonist baclofen (10, 25, and 50 μM) decreased and eventually blocked the 4-AP–induced interictal activity in all experiments (\(n = 29\), Fig. 1, A and B, Baclofen). We analyzed the effects induced by increasing doses of baclofen in 12 slices. With 10 μM baclofen, a decrease in the rate of occurrence of interictal discharges occurred in 5/12 slices, whereas abolishment was seen in the remaining experiments (\(n = 17\)).

**FIG. 2.** Pharmacology of the synchronous activity recorded in the CA3 area of the juvenile rat hippocampus during application of baclofen and 4-AP. A: ictal discharge recorded in the presence of 4-AP + baclofen (50 μM) is not influenced by the N-methyl-D-aspartate (NMDA) receptor antagonist 3,3-(2-carboxypiperazine-4-yl)-propyl-1-phosphate (CPP; 10 μM), but is abolished by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM). B: ictal activity is induced by baclofen (50 μM) even when the hippocampal slice is preincubated in medium containing 4-AP and CPP (10 μM). C: application of the GABA\(_B\) receptor antagonist CGP 35348 (1 mM) to medium containing 4-AP and baclofen (50 μM) makes interictal discharges reappear and decreases the interval of occurrence of the GABA-mediated potentials; note, however, that the ictal discharges induced by baclofen continue to occur in the presence of CGP 35348.
7). When interictal activity was not fully abolished by 10 μM baclofen, increasing the concentration to 25 μM caused further reduction (n = 3) or disappearance (n = 2). Finally, no interictal discharge was observed with 50 μM baclofen (n = 2). Complete blockade of interictal discharges was also induced by application of a single concentration of baclofen (25 and 50 μM in 9 and 8 slices respectively).

Baclofen made spontaneous ictal activity appear when absent in control (n = 21/29; Fig. 1A) or increased the duration of preexisting ictal discharges. In the latter case the rate of occurrence of the ictal events decreased (n = 8/29; Fig. 1B). The dose-dependent changes induced by baclofen on the rate of occurrence and the duration of the ictal discharges are shown in Fig. 1C. Baclofen also decreased the rate of occurrence of the GABA-mediated potential/ictal discharge recorded in baclofen + 4-AP (n = 8, Fig. 2A). Moreover, baclofen disclosed ictal activity when slices were pretreated with CPP (n = 5, Fig. 2B). Ictal discharges were, however, abolished by further addition of CNQX, a procedure that did not influence the GABA-mediated potentials (n = 5; Fig. 2C). When the GABA_B receptor antagonist CGP 35348 (1 mM; n = 4) was applied to slices treated with 4-AP + baclofen, interictal activity reappeared (Fig. 2B), whereas GABA-mediated potentials occurred at a higher rate (from 0.0023 ± 0.0004 Hz during 50 μM baclofen to 0.014 ± 0.0026 Hz after adding CGP 35348, n = 4). CGP 35348 also reduced the duration of the ictal discharges that occurred more frequently than with baclofen only.

Intracellular features of the effects induced by baclofen

The intracellular counterpart of the synchronous activity induced by 4-AP in the CA3 area of juvenile hippocampal slices has been described (see Avoli et al. 1993). In agreement with these earlier experiments, whenever present, ictal discharges corresponded to prolonged depolarizations with sustained action potential firing (n = 3 cells, not shown). By

Pharmacology of the activity recorded in the presence of 4-AP and baclofen

As previously reported with 4-AP only (Avoli et al. 1993, 1996), CPP did not influence the pattern of GABA-mediated potential/ictal discharge recorded in baclofen + 4-AP (n = 8, Fig. 2A). Moreover, baclofen disclosed ictal activity when slices were pretreated with CPP (n = 5, Fig. 2B). Ictal discharges were, however, abolished by further addition of CNQX, a procedure that did not influence the GABA-mediated potentials (n = 5; Fig. 2C). When the GABA_B receptor antagonist CGP 35348 (1 mM; n = 4) was applied to slices treated with 4-AP + baclofen, interictal activity reappeared (Fig. 2B), whereas GABA-mediated potentials occurred at a higher rate (from 0.0023 ± 0.0004 Hz during 50 μM baclofen to 0.014 ± 0.0026 Hz after adding CGP 35348, n = 4). CGP 35348 also reduced the duration of the ictal discharges that occurred more frequently than with baclofen only.

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contrast, interictal events and GABA-mediated potentials (which occurred in all experiments) were mirrored by brief depolarizations with action potential burst and by long-lasting depolarizations (LLDs) with no or minimal action potential firing, respectively (n = 8 cells, Fig. 3A, Control).

Spontaneous asynchronous synaptic potentials were also recorded from CA3 pyramids between synchronous events (Avoli et al. 1993; Perreault and Avoli 1991, 1992). Recordings with K-acetate–filled electrodes revealed that these potentials consisted of depolarizing (presumptive GABA<sub>A</sub>-mediated) postsynaptic potentials (PSPs) and LLDs that lack the initial hyperpolarizing component. During baclofen the spontaneous GABA<sub>A</sub>-mediated depolarizations are virtually abolished; the LLD is only reduced in amplitude. C: plots of the peak amplitudes and of the rate of occurrence of PSPs and LLDs recorded with KCl-filled electrodes in 5 neurons under control conditions (4-AP + CNQX + CPP) and during addition of baclofen. Measurements were performed in all cases by keeping the neuron RMP at the values seen under control conditions with the injection of steady intracellular current.

Baclofen (25 μM) application to 4-AP–containing medium induced a 4.8 ± 1.3 mV, steady hyperpolarization of the RMP and disappearance of interictal activity (n = 8), whereas ictal discharges increased in duration (n = 3; not shown) or appeared whenever absent under control conditions (n = 5; Fig. 3A, Baclofen). During application of 4-AP + baclofen, ictal events were initiated by LLDs with duration that appeared to be longer than in control (not shown, but see Figs. 3A and 5A).

In all experiments baclofen reduced the occurrence of, and eventually abolished, the spontaneous asynchronous synaptic potentials; these changes paralleled the disappearance of interictal activity and either the appearance or the potentiation of ictal discharges.

To better establish the effects of baclofen on 4-AP–induced asynchronous synaptic potentials and LLDs, we used excitatory amino acid receptor antagonists while recording CA3

**Fig. 4.** A: samples of intracellular recordings performed with a K-acetate filled electrode in control (i.e., 4-AP), during addition of excitatory amino acid receptor antagonists (CNQX + CPP) and further application of baclofen (CNQX + CPP + Baclofen). Note that spontaneous excitatory postsynaptic potentials and epileptiform activity disappear during application of CNQX + CPP, while inhibitory hyperpolarizing events and LLDs continue to occur. Further addition of baclofen blocks most of these inhibitory synaptic hyperpolarizations, while the LLD can still be seen. This neuron RMP was kept throughout the experiment at the value seen under control conditions (i.e., −62 mV) by injecting steady intracellular current. B: intracellular recordings performed with a KCl-filled electrode in control (i.e., 4-AP + CNQX + CPP) and after further application of baclofen (Baclofen). Under control conditions the spontaneous activity generated by this neuron consists of depolarizing (presumptive GABA<sub>A</sub>-mediated) postsynaptic potentials (PSPs) and LLDs that lack the initial hyperpolarizing component. During baclofen the spontaneous GABA<sub>A</sub>-mediated depolarizations are virtually abolished; the LLD is only reduced in amplitude. C: plots of the peak amplitudes and of the rate of occurrence of PSPs and LLDs recorded with KCl-filled electrodes in 5 neurons under control conditions (4-AP + CNQX + CPP) and during addition of baclofen. Measurements were performed in all cases by keeping the neuron RMP at the values seen under control conditions with the injection of steady intracellular current.
pyramids with electrodes filled with K-acetate \((n = 3)\) or KCl \((n = 6)\). Both hyperpolarizing synaptic potentials and LLDs (that were initiated by a hyperpolarization) were recorded with K-acetate–filled electrodes during 4-AP and CNQX + CPP (Fig. 4A). In these experiments baclofen (25 μM) abolished the asynchronous potentials, but only caused a small reduction of the LLDs’ amplitude (Fig. 4A) while decreasing their rate of occurrence (not illustrated).

These baclofen effects were also analyzed in six cells with KCl-filled electrodes during application of 4-AP and excitatory amino acid receptor antagonists. As shown in Fig. 4B, asynchronous, presumptive GABA\(_A\)-mediated depolarizing potentials and LLDs occurred in medium containing 4-AP + CNQX + CPP. In all cases baclofen induced a steady membrane hyperpolarization \((6.0 \pm 2.1 \text{ mV}, n = 5)\) and markedly reduced the amplitude and the rate of occurrence of the asynchronous events. By contrast, it only exerted a nonsignificant change in LLD amplitude while decreasing their rate of occurrence (not illustrated).

\[ [K^+]_o \text{ and baclofen effects} \]

\([K^+]_o \text{ influences neuron excitability and modulates seizure activity (McBain et al. 1993; Traynelis and Dingledine 1988). In addition GABA}_A\text{-mediated [K}^+\text{]}_o \text{ increases contribute to ictal discharge initiation in the 4-AP model (Avoli et al. 1996). GABA}_B\text{ receptor activation induces an increase in K}^+ \text{ conductance (Dutar and Nicoll 1988; Gähwiler and Brown 1985; Newberry and Nicoll 1984). We therefore used [K}^+\text{]}_o \text{ recordings to establish whether baclofen (25 μM) effects were accompanied by changes in [K}^+\text{]}_o \text{ baseline and/or in the transient elevations associated with the GABA-mediated potential/ictal discharge (Avoli et al. 1996). [K}^+\text{]}_o \text{ baseline was unchanged with baclofen (n = 5 slices). In addition, the initial component of the transient increases in [K}^+\text{]}_o \text{ corresponding to the GABA-mediated potentials had similar peak values in control and during baclofen (Fig. 5, A and B). However, the increased duration of the ictal discharge induced by baclofen was mirrored by [K}^+\text{]}_o \text{ elevations that were more prolonged than in control (arrows in Fig. 5A).} \]

We also established whether baclofen modifies the transient \([K^+]_o \text{ increases associated with the GABA-mediated potentials recorded during application of 4-AP + CNQX + CPP (n = 5 slices). Transient [K}^+\text{]}_o \text{ elevations occurred in association with the GABA-mediated potentials recorded during blockade of ionotropic excitatory amino acid receptors (Fig. 6A) (Avoli et al. 1996). These elevations in [K}^+\text{]}_o \text{ decreased in amplitude by 15–25% during application of 25 μM baclofen, an effect that was reversed by baclofen wash out (Fig. 6A). The results obtained in the course of these experiments are summarized in Fig. 6B.} \]

\[ \text{Interictal-like stimulation during application of baclofen} \]

Ictal discharges disappear during low-frequency stimulation that elicits interictal epileptiform responses (Barbarosie and...
Avoli 1997; Bragdon et al. 1992; Swartzwelder et al. 1987). Hence we tested whether stimulation of the dentate hilus at 0.2–0.8 Hz for period >15 min could abolish the ictal activity generated by CA3 pyramidal cells during application of 4-AP baclofen (25 μM).

As illustrated in Fig. 7A, ictal events recorded during application of 4-AP + baclofen were reduced, or abolished during this type of electrical stimulation in all experiments (n = 8). This effect was also characterized by reduction (2/8) or disappearance (6/8) of the GABA-mediated synchronous potentials. Ictal discharges reappeared at intervals similar to those seen in control conditions (i.e., 4-AP + baclofen) on termination of electrical stimuli (Fig. 7B). Moreover, the inhibitory effect exerted by repetitive stimuli delivered in the dentate hilus on ictal discharge occurrence was reproduced by successive periods of stimulation in the same experiment.

**DISCUSSION**

As reported in adult brain (Lewis et al. 1989; Mott et al. 1989; Swartzwelder et al. 1987), activation of GABA<sub>B</sub> receptors by baclofen exerts a prosynaptic action in the CA3 area of juvenile rat hippocampus that is characterized by the appearance or potentiation of 4-AP–induced ictal discharges. In this study we have sought evidence for the mechanisms underlying this phenomenon and obtained data indicating that such an action 1) results from the activation of GABA<sub>B</sub> receptors leading to a reduction of spontaneous excitatory and inhibitory synaptic potentials along with the disappearance of interictal discharges; 2) is associated with the persistence of synchronous GABA-mediated potentials; 3) is characterized by excitatory amino acid receptor pharmacology for the ictal activity that is similar to what seen with 4-AP only; and 4) is not accompanied by measurable changes in baseline or transient [K<sup>+</sup>]<sub>i</sub> increases. We have also shown that the ictal activity recorded in 4-AP + baclofen is interrupted by low-frequency repetitive electrical stimuli, a procedure that induces interictal-like discharges.

**Baclofen effects on 4-AP–induced, spontaneous activity**

In our study most baclofen effects were antagonized by CGP 35348, thus indicating that they were mainly caused by the activation of GABA<sub>B</sub> receptors. In particular, we propose that baclofen abolishes 4-AP–induced asynchronous, action potential–dependent synaptic events and interictal activity by decreasing the release of transmitter from excitatory and inhibitory terminals. This may be caused by activation of presynaptic GABA<sub>B</sub> receptors inhibiting both GABA and excitatory transmitter release (Lambert and Wilkinson 1993; Lanthorn and Cotman 1981; Thompson and Gähwiler 1992), and by a postsynaptic GABA<sub>B</sub>-mediated hyperpolarization that decreases excitability of principal cells (Newberry and Nicoll 1984, 1985) and interneurons (Misselgeld et al. 1989; Williams and Lacaille 1992). Indeed, we could document this hyperpolarizing action of baclofen in all CA3 pyramidal cells that were recorded during continuous application of 4-AP.

It is unclear why the synchronous GABA-mediated potentials continued to occur (although at a reduced rate)
during application of baclofen concentrations as high as 50 μM. Baclofen may depress excitatory transmitter release to a greater extent than GABA release (Pierau and Zimmermann 1973; Potashner 1978). We are, however, inclined to exclude that GABAB receptors on inhibitory interneuron terminals may have lower affinity for GABA (and baclofen) as compared with those located on glutamatergic neurons, because both inhibitory and excitatory asynchronous potentials recorded from CA3 pyramidal cells were decreased by baclofen to a similar extent. Such a difference in sensitivity is also at odds with the proconvulsant action of baclofen because ictal discharges are excitatory amino acid–mediated synchronous events. Indeed, the resistance of 4-AP–induced GABA-mediated synchronous potentials to baclofen may reflect the intense discharge of action potential that occurs in interneurons during this type of synchronous activity (Bernardo 1997). This firing, once reaching the terminals, may overcome the inhibition of transmitter release exerted by baclofen. Moreover, this phenomenon may be facilitated by the concomitant increase in [K+]o seen during the GABA-mediated synchronous potential.

Proconvulsant action of baclofen in the juvenile rat hippocampus

A proconvulsant action of baclofen has been described in several in vitro models of epileptiform discharge in the adult hippocampus (Lewis et al. 1989; Mott et al. 1989; Swartzwelder et al. 1987; Watts and Jefferys 1993). In particular, Watts and Jefferys (1993) have reported that baclofen abolishes interictal activity and discloses ictal discharges in adult rat hippocampal slices treated with 4-AP. The similarities between our results and their findings indicate the existence of mature GABAB-mediated mechanisms of synaptic transmission in the juvenile rat hippocampus. A proconvulsant effect of baclofen has been shown in a patient treated for spasticity who did not have any previous history of seizures (Rush and Gibberd 1990) and in three patients who had previously suffered traumatic brain injury (Kofler et al. 1994).

We have also shown that the ictal activity generated by CA3 neurons in the presence of 4-AP + baclofen is insensitive to the NMDA receptor antagonist CPP, but is abolished by CNQX. These features are similar to those reported for ictal discharges in medium containing 4-AP only (Avoli et al. 1993,
1996). Hence baclofen’s proconvulsant action does not result from a novel excitatory amino acid–mediated mechanism disclosed by this GABAB-receptor agonist. In addition, the effects of baclofen did occur during continuous CPP application, which rules out the involvement of a GABAB receptor–mediated/NMDA-dependent mechanism in ictal discharge induction. Such a mechanism facilitates the occurrence of tetanus-induced long-term potentiation in the adult hippocampus (Davies et al. 1991).

GABAB receptor activation induces an increase in K\(^+\) conductance (Gähwiler and Brown 1985; Newberry and Nicoll 1984, 1985). It is also well-established that [K\(^+\)]\(_e\) modulates seizure activity (McBain et al. 1993; Traynelis and Dingledine 1988). Hence the proconvulsant action of baclofen may have resulted from changes in [K\(^+\)]\(_e\) homeostasis, and even more so because GABA-mediated [K\(^+\)]\(_e\) elevations initiate ictal discharges in the 4-AP model (Avoli et al. 1996). However, our [K\(^+\)]\(_e\) recordings indicate that the action of baclofen is not accompanied by any measurable change in either [K\(^+\)]\(_e\) baseline or [K\(^+\)]\(_e\) transient elevations. Accordingly, the increases in [K\(^+\)]\(_e\) occurring during the GABA-mediated synchronous potential recorded in the presence of baclofen were not larger than those seen in medium containing 4-AP only. Moreover, only a small, although significant decrease was appreciated when comparing the peak [K\(^+\)]\(_e\) values under control and during baclofen in slices where excitatory transmission was abolished.

It has been proposed that the proconvulsant action of baclofen is caused by disinhibition due to a decrease in GABA release (Mott et al. 1989; Watts and Jefferys 1993). In agreement with these studies, we have observed that baclofen causes a marked decrease of asynchronous GABA\(_B\)-mediated events recorded from CA3 pyramids. However, such a disinhibitory action must be accompanied by the ability of excitatory terminals to release transmitter to be expressed as a proconvulsant effect. Hence, as proposed for the synchronous GABA-mediated potentials, we are inclined to conclude that ictal activity during activation of GABA\(_B\) receptors reflects the inability of this presynaptic mechanism to inhibit transmitter release consequent to intense action potential firing, which in this case does occur at the excitatory terminals of CA3 pyramidal cells.

An important mechanism underlying the proconvulsant action of baclofen relates to activity-dependent changes in CA3 area network excitability that occur during GABA\(_B\) receptor activation. This conclusion is supported by the findings obtained with electrical stimulation of the dentate hilus, a procedure that leads to the occurrence of interictal-like responses and the concomitant suppression of the ictal activity recorded during application of 4-AP + baclofen. It is conceivable that an accumulation of glutamate-containing vesicles docking at presynaptic terminals results from the block of interictal activity and asynchronous excitatory synaptic potentials caused by baclofen. This should lead to an increased availability of excitatory transmitter. As a consequence the synchronous GABA-mediated potentials that are generated in the presence of baclofen initiate ictal discharges that are more robust than those recorded under control conditions or induce ictal events ex novo. Presynaptic factors controlling glutamate release have been proposed to regulate the probability and duration of synchronous discharges generated by the CA3 network (Staley et al. 1998).

In clinical practice, interictal events are used to localize the brain area from where ictal discharges originate. However, the temporal relation between interictal and ictal discharges remains unclear. It should be emphasized that the control exerted by interictal activity on ictal events has been shown in previous studies including those performed in combined hippocampus-entorhinal cortex slices treated with either 4-AP or low Mg\(^{2+}\) (Barbarosie and Avoli 1997; Bragdon et al. 1992; Swartzwelder et al. 1987). In these cases as well, activity-dependent changes in network excitability, leading to increased availability of excitatory transmitter, may play a role in the unexpected control exerted by interictal activity over ictal discharges.

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REFERENCES


