Ontogenesis of Presynaptic GABA\textsubscript{B} Receptor-Mediated Inhibition in the CA3 Region of the Rat Hippocampus

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Caillard, Olivier, Heather A. McLean, Yehezkel Ben-Ari, and Jean-Luc Gaiarssa. Ontogenesis of presynaptic GABA\textsubscript{B} receptor-mediated inhibition in the CA3 region of the rat hippocampus. J. Neurophysiol. 79: 1341–1348, 1998. \(\gamma\)-Aminobutyric acid-B (GABA\textsubscript{B}) receptor-dependent and -independent components of paired-pulse depression (PPD) were investigated in the rat CA3 hippocampal region. Intracellular and whole cell recordings of CA3 pyramidal neurons were performed on hippocampal slices obtained from neonatal (5–7 day old) and adult (27–34 day old) rats. Electrical stimulation in the hilus evoked monosynaptic GABA\textsubscript{B} inhibition that appears later in development stages of development. Absence of presynaptic autoinhibition of GABA release seems to be due to the small amount of transmitter that can access presynaptic regulatory sites. In adult CA3 pyramidal neurons, when a pair of identical stimuli was applied at interstimulus intervals (ISIs) ranging from 50 to 1,500 ms the amplitude of the second eIPSC was depressed when compared with the first eIPSC. This paired-pulse depression (PPD) was partially blocked by P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348, 0.5 mM), a selective GABA\textsubscript{B} receptor antagonist. In neonates, PPD was restricted to ISIs shorter than 200 ms and was not affected by CGP35348. The GABA\textsubscript{B} receptor agonist baclofen reduced the amplitude of eIPSCs in a dose-dependent manner with the same efficiency in both adults and neonates. Increasing the probability of transmitter release with high Ca\textsuperscript{2+} (4 mM)/low Mg\textsuperscript{2+} (0.3 mM) external solution revealed PPD in neonatal CA3 pyramidal neurons that was (1) partially prevented by CGP35348, (2) independent of the membrane holding potential of the recorded cell, and (3) not resulting from a change in the reversal potential of GABA\textsubscript{B} eIPSCs. In adults the GABA uptake blocker tiagabine (20 \(\mu\)M) increased the duration of eIPSCs and the magnitude of GABA\textsubscript{B} receptor-dependent PPD. In neonates, tiagabine also increased duration of eIPSCs but to a lesser extent than in adult and did not reveal a GABA\textsubscript{B} receptor-dependent PPD. These results demonstrate that although GABA\textsubscript{B} receptor-dependent and -independent mechanisms of presynaptic inhibition are present on GABAergic terminals and functional, they do not operate at the level of monosynaptic GABAergic synaptic transmission at early stages of development. Absence of presynaptic autoinhibition of GABA release seems to be due to the small amount of transmitter that can access presynaptic regulatory sites.

INTRODUCTION

In the adult vertebrate CNS, \(\gamma\)-aminobutyric acid (GABA) exerts postsynaptic inhibitory actions via at least two different classes of receptors, the GABA\textsubscript{A} receptor underlying fast postsynaptic inhibitory transmission and the GABA\textsubscript{B} receptor, which mediates slow inhibitory postsynaptic potentials (reviewed by Sivilotti and Nistri 1991). In addition to its postsynaptic inhibitory properties, GABA also reduces neurotransmitter release through the activation of presynaptic GABA\textsubscript{B} receptors (Bowery 1993; Deisz and Prince 1989; Thompson and Gähwiler 1989a). Early in development, postsynaptic GABA\textsubscript{B} receptor mediated responses can be elicited by stimulation of GABAergic afferent fibres or by exogenous application of GABA\textsubscript{A} receptor agonists (Ben-Ari et al. 1989; Lo Turco et al. 1995). Postsynaptic GABA\textsubscript{B} inhibition appears later in development and reaches adult levels by the end of the first postnatal week of life in the visual cortex (Luhmann and Prince 1991) and the hippocampus (Gaiarssa et al. 1995; Janigro and Schwartzkroin 1988). In contrast, presynaptic GABA\textsubscript{B} mediated inhibition is already functional at early stages of development. Indeed, in the cortex, both synaptically released GABA (Fukuda et al. 1993) and exogenously applied GABA\textsubscript{B} receptor agonist, baclofen (Harrison et al. 1988), depresses GABAergic transmission through a presynaptic mechanism. In the neonatal hippocampus, presynaptic GABA\textsubscript{B} receptors have a key role in the control of endogenous polysynaptic network activity (McLean et al. 1996). The importance of this presynaptic inhibition is emphasised by the fact that before postnatal day 5 postsynaptic GABA\textsubscript{A} receptor-mediated activity is excitatory (Ben-Ari et al. 1989; Khazipov et al. 1997; Leinekugel et al. 1997), while postsynaptic GABA\textsubscript{B} inhibition is not yet functional (Gaiarssa et al. 1995). Because baclofen reduces the amplitude of GABAergic inhibitory postsynaptic potentials (Gaiarssa et al. 1995), the question remains as to whether or not presynaptic GABA\textsubscript{B} receptors are operational at the level of monosynaptic GABAergic transmission. In cerebellar organotypic slice cultures for example, paired-pulse depression is not observed while application of GABA\textsubscript{B} receptor agonists reduces both spontaneous and evoked GABAergic synaptic potentials (Moguinit and Gähwiler 1996).

The aim of the present study was to investigate the presynaptic inhibition of GABAergic synaptic transmission by endogenously released transmitter in the CA3 region of the neonatal rat hippocampus by using the paired-pulse protocol paradigm (Davies et al. 1990). We report that while presynaptic GABA\textsubscript{B} receptor-dependent and -independent mechanisms are present and functional on GABAergic terminals, they do not operate in the control of monosynaptic GABAergic currents under basal conditions: the emergence of GABAergic presynaptic autoinhibition in neonates requires an increase in neurotransmitter release.
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FIG. 1. Absence of PPD in CA3 region of neonates. A: averaged (n = 5) superimposed traces representing pairs of evoked inhibitory postsynaptic potential (eIPSCs) evoked at different interstimulus intervals (ISIs) (100, 200, 300, 500, 1,000 ms) from a holding potential of −60 mV in a P27 neuron. B: grouped adult data (mean ± SE) representing % of PPD in control (n = 14) and in presence of P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348; 0.5 mM) (n = 7) at different ISIs. CGP35348 significantly reversed paired-pulse depression (PPD) at 100, 200, and 300 ms. C: averaged (n = 5) superimposed traces representing pairs of eIPSCs evoked at different ISIs (100, 200, 300, 500, 1,000 ms) from a holding potential of −60 mV in a P5 neuron. D: grouped neonatal data (mean ± SE) representing % of PPD in control (n = 23 for ISIs 50 ± 500; n = 8 for ISI 1,000 ± 1,500) and in presence of CGP35348 (0.5 mM) (n = 6 for ISIs 50–500; n = 3 for ISI 1,000–1,500) at different ISIs. CGP35348 did not significantly reverse PPD in range of ISIs studied.

METHODS

Preparation of hippocampal slices

Brains were removed from neonate (5–7 day old) and adult (27–34 day old) male Wistar rats [postnatal day (P) 5–7 or P27–34] (P0 taken as the day of birth) under ether anesthesia and submerged in cold artificial cerebrospinal fluid (ACSF) where the hippocampi were isolated. The composition of control ACSF was (in mM) 126 NaCl, 3.5 KCl, 2 CaCl2, 1.3 MgCl2, 1.2 NaH2PO4, 25 NaHCO3, and 11 glucose, pH 7.4, after equilibration with 95% O2–5% CO2. Hippocampal slices were cut at 600 μm thickness for neonates and 450 μm thickness for adults, with a McIlwain tissue chopper and incubated at room temperature in ACSF for at least 1 h before use. Individual slices were then transferred to a submerged-type recording chamber and superfused with ACSF at 2.8 ml/min at 34°C.

Electrophysiological recordings

Recordings were obtained from pyramidal neurons in the CA3b region of the hippocampus. All experiments investigating paired-pulse depression (PPD) of evoked inhibitory postsynaptic currents (IPSCs) in control conditions, in high Ca2+/low Mg2+ ACSF and in the presence of tiagabine were performed with conventional sharp microelectrodes (1.5 mm OD, Clark Electromedical) filled with 50 mM 2-(triethylamino)-N-(2,6-dimethylphenyl) acetamide (QX314) dissolved in 3 M KCl (resistance 30–55 MΩ). QX314, a derivative of lidocaine, used to block both voltage-dependent sodium channels (Flatman et al. 1982) and the GABA_A receptor activated potassium conductance (Andrade et al. 1991; Perkins and Wong 1995) was injected by positive current pulses (0.3 nA, 300 ms, 0.5 Hz). Under these conditions electrical stimulation in the hilus induced only a GABA_A-mediated potential without any slow GABA_A component (see Fig. 1). IPSCs were recorded with an Axoclamp 2A amplifier in the single electrode discontinuous voltage clamp mode with a sampling rate of 3–5 kHz, a time constant of 20 ms, and a gain of 2.5–5.0 nA/mV. To ensure correct operation of the clamp, the voltage at the head stage amplifier was monitored on a separate oscilloscope. Membrane potential was estimated from the potential observed on withdrawal of the electrode from the cell.

To test the effect of baclofen on GABA_A eIPSCs, whole cell recordings were obtained using the “blind” patch clamp technique. Recordings were performed with an Axopatch 200A (Axon Instruments) amplifier. Microelectrodes (7–10 MΩ) were filled with an internal solution of the following composition (in mM): 145 CsCl, 2 MgATP, 0.6 NaGTP, 1 ethylene glycol-bis (β-aminoethyl ether)-N,N,N’-N’-tetraacetic acid (EGTA), 10 Na-2-hydroxethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 2 QX314, pH = 7.25, osmolality = 270 mosM. During experiments, before each stimulation, series resistances were determined by an on-line fitting analysis of the transient currents during a 5 μA pulse with Acquis Software. Cells exhibiting unstable series resistances or resistances >15 MΩ were discarded.

Stimulation parameters

Electrical stimulation was performed with a bipolar electrode constructed from twisted NiCr insulated wire (50 mm diam). The stimulation electrode was positioned in the hilus. The parameters (20–40 μs, 20–60 V) were chosen to give maximal amplitude of the isolated eIPSC; paired-pulse stimulations were given at interstimulus intervals (ISI) ranging from 50 to 1,500 ms at a frequency of 0.02 Hz.

Data acquisition, storage, and analysis

Evoked synaptic potentials were stored on a personal computer and simultaneously displayed on a digital oscilloscope (Nicolet, model 3091, Madison, WI). Spontaneous and evoked potentials...
were continuously displayed on a pen recorder (Gould, model 22008, Ballainvilliers, France). Data were analyzed off-line with Acquis Software (G. Sadoc, Paris).

All data are expressed as means ± SE. Statistical analysis of percent values were performed with Wilcoxon (paired data) or analysis of variance (ANOVA) (unpaired data) tests. Numerical values were compared with the paired student t-test. Data were judged to differ when \( P < 0.05 \). In the paired-pulse protocol, PPD was defined as \( (\text{eIPSC}_2 - \text{eIPSC}_1)/\text{eIPSC}_1 \). Cells were considered to demonstrate PPD if the amplitude of eIPSC\(_2\) was reduced to at least 85% of eIPSC\(_1\) amplitude at 200 ms ISI.

Drugs

Drugs were dissolved in ACSF and bath applied to the slices through a three-way tap system. Drugs used in this study included 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris, Paris), d(-)-2-amino-5-phosphovaleric acid (t-AP5, Tocris), Baclofen (Sigma), P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348, Novartis, Basel), and Tiagabine (Novo Nordisk A/S, Bagsvarden, Denmark).

RESULTS

The present study is based on intracellular and patch-clamp recordings of neonatal (P5–P7) and adult (P27–P34) CA3 pyramidal neurons. In the intracellular recording configuration neonatal cells had higher input resistances than adult cells (105.0 ± 6.8 MΩ at P5–P7, \( n = 21 \); and 72.9 ± 10.4 MΩ, \( n = 9 \) at P27–P34) and similar resting membrane potentials (−55.2 ± 1.9 mV at P5–P7, \( n = 15 \) and −58.1 ± 3.1 mV at P25–P35, \( n = 9 \)). In the presence of CNQX (10 \( \mu \)M) and d-AP5 (50 \( \mu \)M), electrical stimulation in the hilus evoked a pure monosynaptic GABA\(_B\) receptor-mediated postsynaptic current (eIPSC) in cells recorded with QX314-filled electrodes (QX314 blocks postsynaptic GABA\(_B\) receptor-mediated potassium currents). The maximum amplitude of eIPSCs evoked from holding potentials of −65 to −75 mV was −1,021 ± 251 pA (\( n = 9 \)) and −1,150 ± 550 pA (\( n = 26 \)) for adults and neonates, respectively (\( P = 0.61 \)).

Absence of PPD in the neonatal rat hippocampus

In adults, when a pair of stimuli with an interstimulus interval (ISI) between 50 and 1,500 ms was delivered at the same intensity, the amplitude of the second eIPSC (eIPSC\(_2\)) was smaller than the first one (Fig. 1A). This paired-pulse depression (PPD) (Davies et al. 1990) was observed in 82% of the cells tested (14/17). PPD was 33.2 ± 3.2% at 200 ms ISI and 12.7 ± 4.3% at 1,500 ms ISI (Fig. 1B). The effect of the GABA\(_B\) receptor antagonist CGP35348 was tested on seven of these cells. CGP35348 (0.5 mM) had no effect on the amplitude of the first eIPSC but clearly reduced PPD at short ISIs (PPD was 18.2 ± 2.6% at 200 ms ISI, \( P = 0.028 \) compared with control, \( n = 7 \)). CGP35348 had no effect on PPD at longer ISIs (PPD was 11.0 ± 3% at 1,500 ms ISI, \( P = 0.71 \) compared with control, \( n = 7 \)) (Fig. 1B). Thus as already reported (Lambert and Wilson 1994), in the adult hippocampal CA3 region, PPD has two components: a GABA\(_B\) receptor-dependent component that occurs with ISIs <500 ms and peaks at 100–200 ms and a GABA\(_B\) receptor-independent component that occurs with longer ISIs.

The same protocol was applied to P5–P7 slices. In 23 of 30 cells, PPD was observed only at short ISIs (Fig. 1C): PPD at 50 ms was 30.2 ± 2.9%; PPD at 200 ms was 5.4 ± 1.4%. In these cells, CGP35348 had no effect on either the amplitude of the eIPSCs nor on PPD. In the presence of 0.5 mM CGP35348, PPD at 50 ms was 29 ± 3.8% (\( P = 0.893 \) compared with control, \( n = 6 \)) and PPD at 200 ms was 4.8 ± 1.8% (\( P = 0.345 \) compared with control, \( n = 6 \)). In the seven remaining cells, we observed a significant PPD at 200 ms ISI (22 ± 1.6%) that was unaffected by CGP35348 (0.5 mM, \( n = 2 \)). Taken together these results show the absence of GABA\(_A\) receptor-dependent PPD at all ISIs tested and the absence of GABA\(_B\) receptor-independent PPD at ISIs longer than 100 ms in the CA3 region of the neonatal rat hippocampus.

Baclofen reduces eIPSCs in neonates and adults with the same efficacy

Given that presynaptic GABA\(_B\) receptor complexes are present and functional at birth (Gaiarsa et al. 1995), the absence of neonatal GABA\(_B\) receptor-dependent PPD observed in this study may be explained by inefficient coupling of GABA\(_B\) receptors with their channels, the presence of a distinct type of GABA\(_A\) receptor possessing a relatively lower affinity for GABA or simply a smaller amount of GABA accessing these receptors. To determine whether absence of PPD in neonates may be due to a lower efficacy of presynaptic GABA\(_B\) receptors, a dose-response curve of the effects of baclofen on eIPSC amplitude was constructed (Fig. 2). In both adults and neonates, bath application of baclofen reversibly reduced the amplitude of eIPSCs in a dose dependent manner (Fig. 2A and B), without any effect on postsynaptic membrane resistance or holding current. EC\(_{50}\) of baclofen, determined by sigmoidal fitting, was 0.59 ± 0.81 \( \mu \)M and 0.83 ± 0.18 \( \mu \)M in adults and neonates, respectively. At concentrations that approached the EC\(_{50}\) (0.3 and 1 \( \mu \)M) baclofen reduced the amplitude of eIPSCs in a dose dependent manner (Fig. 2A and B), without any effect on postsynaptic membrane resistance or holding current. EC\(_{50}\) of baclofen was 83.0 ± 5.5% and 76.5 ± 2.3% of control in neonates and adults, respectively (\( P = 0.34, n = 3 \)). 1 \( \mu \)M baclofen reduced eIPSC amplitudes to 63.8 ± 6.0% and 65.1 ± 6.8% of control in neonates and adults, respectively (\( P = 0.55, n = 3 \)).

These results therefore show that absence of GABA\(_B\) receptor-dependent PPD in neonates cannot be attributed to a lower efficacy of presynaptic GABA\(_B\) receptors but rather is likely related to a smaller amount of GABA reaching presynaptic GABA\(_B\) receptors.

An increase in release probability reveals PPD in neonates

Activation of presynaptic GABA\(_B\) receptors requires that the initial amount of GABA released by electrical stimulation is high enough to overcome clearance mechanisms that reduce concentration of neurotransmitter in the vicinity of these receptors. Short-term changes associated with paired-pulse paradigms have been shown to be dependent on the
revealed in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF has both GABA\textsubscript{B} receptor-dependent and -independent components. The emergence of PPD in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF could be accounted for by an up-regulation of the presynaptic GABA\textsubscript{B} receptor complex (Al-Dahan and Thalmann 1989). However, in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF, baclofen (10 \mu M) reduced the amplitude of eIPSCs to 43.1 ± 5.8\% of control (\(n = 6\), data not shown). This depression was not significantly different from that observed in control ACSF (43.2 ± 3.7\%, \(P = 0.994\), \(n = 6\)).

Taken together, these results indicate that increasing transmitter release in neonates results in the emergence of both GABA\textsubscript{B} receptor-dependent and -independent PPD with no change in the efficacy of presynaptic GABA\textsubscript{B} receptors.

In neonates, PPD revealed in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF implies a presynaptic mechanism

The following set of experiments was designed to determine the mechanisms underlying the PPD observed in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF. One possible factor underlying the PPD may be desensitisation of postsynaptic GABA\textsubscript{A} receptors. Because the rate of desensitisation has been shown to depend on membrane potential (Frosch et al. 1992), paired stimuli (200 ms ISI) were given at membrane holding potentials ranging from −130 to +30 mV in high Ca\textsuperscript{2+}/low Mg\textsuperscript{2+} ACSF (Fig. 4A). As shown in Fig. 4B, although the duration of eIPSCs increased with membrane depolarization, the level of PPD remained constant suggesting that postsynaptic desensitization was not involved. Another factor underlying the PPD may be a change in the driving force for chloride ions (Thompson and Gähwiler 1989b). As such we constructed current-voltage curves of eIPSC\textsubscript{1}s and eIPSC\textsubscript{2}s. eIPSC\textsubscript{1} was evoked at membrane holding potentials ranging from −130 to +30 mV. After this, in the same cell, eIPSC\textsubscript{2} was evoked from a fixed membrane potential (−70 mV) and eIPSC\textsubscript{2} from a different membrane potential attained 50 ms before the second stimulation. The reversal potential, determined by linear fitting, was −2 ± 3.5 mV for eIPSC\textsubscript{1} and −0.6 ± 1.1 mV for eIPSC\textsubscript{2} (\(P = 0.73\), \(n = 3\)) (Fig. 4C).

These results indicate that the PPD observed under conditions favoring a high probability of neurotransmitter release was not due to postsynaptic modifications of GABA\textsubscript{A} receptor efficacy but rather involves presynaptic mechanisms.

**Blocking the GABA uptake in neonates does not reveal PPD**

The above observations confirm that presynaptic GABA\textsubscript{B} receptors are present and functional in neonates. They also suggest that a lower amount of GABA released by electrical stimulation underlies the absence of PPD in neonates, but do not rule out the involvement of a powerful GABA uptake. We therefore studied the effects of tiagabine, a specific blocker of GABA uptake (Roepstorff and Lambert 1992) on PPD and half-amplitude duration (\(t_{1/2}\)) of eIPSCs.

In adults \(t_{1/2}\) was 34.0 ± 6.0 ms for eIPSC\textsubscript{1} and 20.7 ± 2.8 ms for eIPSC\textsubscript{2}, at a holding potential of −70 mV and 200 ms ISI; PPD was 29.8 ± 11.9\% (\(n = 5\)). After a 10-min application of tiagabine (20 \mu M), \(t_{1/2}\) of eIPSC\textsubscript{1} and eIPSC\textsubscript{2} increased significantly by 269.9 ± 73.7\% and
The present study shows that although presynaptic GABAergic release.

129.2 ± 12.1%, respectively (eIPSC1: 78.4 ± 29.3 ms, P = 0.043; eIPSC2: 25.2 ± 3.2 ms, P = 0.043; n = 5) (Fig. 5A) with no change in holding current (data not shown). PPD also increased significantly to 59.2 ± 8.7% (P = 0.043 compared with control, n = 5) (Fig. 5A). Subsequent application of CGP35348 (0.5 mM) significantly increased t1/2 of eIPSC1 and eIPSC2 by 134.5 ± 22.2% and 255.6 ± 54.2%, respectively (eIPSC1: 89.5 ± 19.6 ms, P = 0.043; eIPSC2: 57.2 ± 9.8 ms, P = 0.043; n = 5) and slightly enhanced spontaneous activity (data not shown). CGP35348 also significantly decreased PPD to 19.2 ± 3.7% (P = 0.043 compared with control, n = 5) (Fig. 5, A–C). These results show that GABA uptake mechanisms shape eIPSCs and reduce access to presynaptic GABAA receptors in adult hippocampal CA3 region.

In neonates, t1/2 was 36.6 ± 3.9 ms for eIPSC1 and 31.9 ± 2.6 ms for eIPSC2 at a holding potential of −70 mV and 200 ms ISI; PPD was 9.0 ± 2.7% (n = 10). After a 10-min application of tiagabine (20 μM), t1/2 of eIPSC1 and eIPSC2 increased significantly by 135.7 ± 9.8% and 122.8 ± 5.3%, respectively (eIPSC1: 46.9 ± 4.0 ms, P = 0.005; eIPSC2: 37.6 ± 2.3 ms, P = 0.007; n = 10), with no change in holding current (data not shown). PPD increased significantly to 16.7 ± 3.6% (P = 0.005 compared with control, n = 7) (Fig. 5, B and C). Subsequent application of CGP35348 significantly increased t1/2 of eIPSC2 by 119 ± 6.5% (44.9 ± 5.3 ms, P = 0.046; n = 6) but t1/2 of eIPSC1 and PPD were not significantly affected (eIPSC1: 54.9 ± 22.8 ms, 108.6 ± 7.4%, P = 0.249; PPD: 8.9 ± 7.5%, P = 0.463; n = 6) (Fig. 5, A–C).

Thus, in the presence of the GABA uptake antagonist tiagabine, the kinetics of eIPSCs were slowed in neonates but to a lesser extent than in adults. In addition, no GABAB receptor-dependent PPD was revealed, indicating that the absence of this form of PPD in neonates was not the result of a powerful GABA uptake.

**DISCUSSION**

The present study shows that although presynaptic GABAB receptors located on GABAergic terminals impinging CA3 pyramidal neurons of neonates are present and have similar responses to exogenous applied baclofen, they cannot be activated by endogenous GABA released following monosynaptic eIPSCs in basal conditions. However, increasing probability of GABA release reveals a GABAB receptor-dependent PPD, thus indicating that, in neonates, the critical concentration of GABA required for activation of presynaptic GABAB receptors is not achieved with evoked monosynaptic GABAergic release.

PPD (Davies et al. 1990) has two components. One component is mediated by the activation of presynaptic GABAB receptors and has been termed GABAB receptor-dependent PPD (Davies et al. 1990; Lambert and Wilson 1994) and a
PPD is present in adults at ISIs ranging from 5 ms to 5 s that GABA B receptor-dependent PPD could be revealed by independent PPD (Lambert and Wilson 1994). Even though (Seress et al. 1989). In the present study, we have shown that increasing GABA release probability reveals a GABA B receptor-dependent PPD in neonates. This PPD is likely mediated through presynaptic mechanisms because the magnitude of the PPD was unaffected by membrane holding potential, thus ruling out postsynaptic voltage-dependent desensitization or a change in the chloride driving force. Together these observations demonstrate that the presynaptic mechanisms of inhibition are present and functional on GABAergic terminals in early postnatal life.

The absence of GABA B receptor-dependent PPD in CA3 region of neonates is therefore compatible with the idea that synaptically released GABA does not reach the critical concentration required for presynaptic GABA B receptor activation. Possible explanations include reduced release and/or faster clearance of GABA after electrical stimulation.

Functional synergism between synapses resulting from lateral diffusion of transmitter has been described in Mauthner cells (Faber and Korn 1988) and in the hippocampus (Isaacson and Nicoll 1993). Such spill over of GABA to neighboring presynaptic GABA B receptors may also play a crucial role in PPD (Mody et al. 1994). Indeed, GABA B receptor-dependent PPD depends on the stimulus intensity (Davies et al. 1990; Roepstorff and Lambert 1994), cannot be observed with pair-recordings of connected cells (Wilcox and Dichter 1994), and is enhanced with GABA uptake blockers (Roepstorff and Lambert 1994). Therefore, if the recruited active zones are sparse, or if clearance mechanisms are more efficient, such synergism will not occur in neonates. As a consequence, the critical concentration of GABA will not be reached in the vicinity of presynaptic GABA B receptors.

With regard to the first hypothesis, morphological studies have shown that in neonates GAD-positive terminals are less numerous, smaller, and contain less vesicles than in adults (Seress et al. 1989). In the present study, we have shown that GABA B receptor-dependent PPD could be revealed by increasing probability of GABA release. Although supporting the idea that monosynaptic GABA release is not sufficient to activate presynaptic GABA B receptors, this observation does not exclude that a faster clearance may prevent the access of released GABA to these receptors. However, although GABA uptake has been reported to peak above adult levels during the two first postnatal weeks of life (Coyle and Enna 1976; Wong and McGeer 1981), we could not reveal GABA B receptor-dependent PPD in neonates in the presence of the GABA uptake antagonist tiagabine. Thus GABA uptake does not account for the absence of PPD. In addition, as reported in the dentate gyrus (Draguhn and Heinemann 1996), GABA uptake does not play a major role in shaping eIPSC kinetics in the CA3 region of neonates. A dilution of GABA in a larger extracellular space in immature tissue could explain the absence of both tiagabine effects and PPD. Lehmenkühler et al. (1993) reported that the extracellular volume decreases as brain maturation progresses with strong reductions occurring around postnatal day 10.
In addition, in neonatal granule cells, restriction of the extracellular space prolongs eIPSCs, supporting the idea that in control conditions transmitter is rapidly eliminated from the synaptic cleft (Draguhn and Heinemann 1996). Thus the absence of PPD that we observed in neonates may be accounted for by a lower amount and/or a critical dilution of released GABA.

Given the important role of GABA<sub>B</sub> receptors in the control of network activity in immature hippocampus (McLean et al. 1996), the absence of PPD in neonates is surprising. Indeed, during the first week of postnatal life, pyramidal cells and interneurons receive endogenous polysynaptic potentials (Ben-Ari et al. 1989; Khazipov et al. 1997) resulting from the synchronous activation of GABAergic and glutamatergic afferent inputs (Khazipov et al. 1997; McLean et al. 1995). These events are regulated in duration and frequency by GABA<sub>B</sub> receptors (McLean et al. 1996). If indeed giant polysynaptic potentials in neonates fulfill satisfactory conditions for activation of these receptors, the present study shows that single electrical stimulation of the GABAergic fibers is not sufficient to activate presynaptic GABA<sub>B</sub> receptors in neonates. Emergence of a GABA<sub>B</sub> Receptor-dependent PPD can be seen only in conditions where the probability of GABA release is increased.

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