Ontogenesis of Presynaptic $GABA_B$ 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 Gaïarsa. Ontogenesis of presynaptic $GABA_B$ receptor-mediated inhibition in the CA3 region of the rat hippocampus. *J. Neurophysiol.* 79: 1341–1348, 1998. γ-Aminobutyric acid-B ($GABA_B$) 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 $GABA_A$ postsynaptic currents (eIPSCs) isolated in the presence of the ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and D(−)2-amino-5-phosphovaleric acid (D-AP5, 50 μM) with 2(triethylamino)-*N*-(2,6-dimethylphenyl) acetamine (QX314) filled electrodes. 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 $GABA_B$ receptor antagonist. In neonates, PPD was restricted to ISIs shorter than 200 ms and was not affected by CGP35348. The $GABA_B$ 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^{2+} (4 mM)/low Mg^{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 $GABA_A$ eIPSCs. In adults the GABA uptake blocker tiagabine (20 μM) increased the duration of eIPSCs and the magnitude of $GABA_B$ receptor-dependent PPD. In neonates, tiagabine also increased duration of eIPSCs but to a lesser extent than in adult and did not reveal a $GABA_B$ receptor-dependent PPD. These results demonstrate that although $GABA_B$ 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, γ-aminobutyric acid (GABA) exerts postsynaptic inhibitory actions via at least two different classes of receptors, the $GABA_A$ receptor underlying fast postsynaptic inhibitory transmission and the $GABA_B$ 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 $GABA_B$ receptors (Bowery 1993; Deisz and Prince 1989; Thompson and Gähwiler 1989a). Early in development, postsynaptic $GABA_A$ receptor mediated responses can be elicited by stimulation of GABAergic afferent fibres or by exogenous application of $GABA_A$ receptor agonists (Ben-Ari et al. 1989; Lo Turco et al. 1995). Postsynaptic $GABA_B$ 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 (Gaïarsa et al. 1995; Janigro and Schwartzkroin 1988). In contrast, presynaptic $GABA_B$ 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 $GABA_B$ receptor agonist, baclofen (Harrison et al. 1988), depresses GABAergic transmission through a presynaptic mechanism. In the neonatal hippocampus, presynaptic $GABA_B$ 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 $GABA_A$ receptor-mediated activity is excitatory (Ben-Ari et al. 1989; Khazipov et al. 1997; Leinekugel et al. 1997), while postsynaptic $GABA_B$ inhibition is not yet functional (Gaïarsa et al. 1995). Because baclofen reduces the amplitude of GABAergic inhibitory postsynaptic potentials (Gaïarsa et al. 1995), the question remains as to whether or not presynaptic $GABA_B$ 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 $GABA_B$ receptor agonists reduces both spontaneous and evoked GABAergic synaptic potentials (Mouginot 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 $GABA_B$ 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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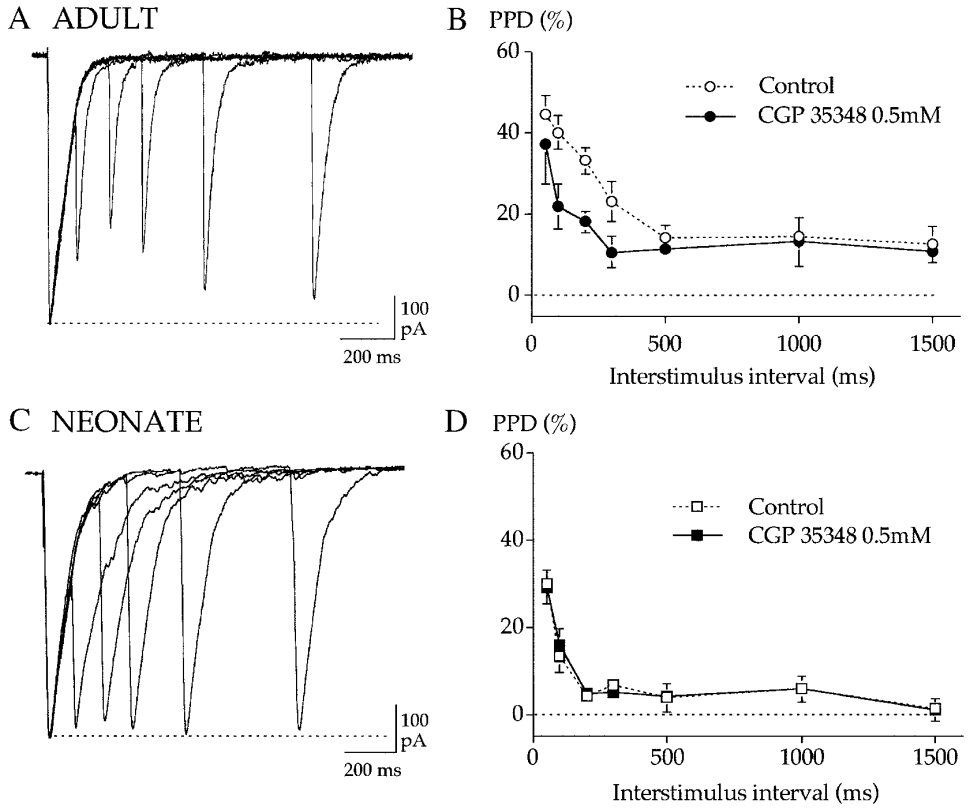

FIG. 1. Absence of PPD in CA3 region of neonates. *A*: averaged ($n = 5$) superimposed traces representing pairs of evoked inhibitory postsynaptic potential (eIPSCs) evoked at different interstimulus intervals (ISIs) (100, 200, 300, 500, 1,000 ms) from a holding potential of −60 mV in a P27 neuron. *B*: 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 neonate 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–P7 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 $CaCl_2$, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose, pH 7.4, after equilibration with 95% O_2-5% CO_2. 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 Ca^{2+}/low Mg^{2+} 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) acetamine (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 $GABA_B$ 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 $GABA_B$ 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 by 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 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 2 QX314, $pH_i = 7.25$, $osmolarity_i = 270$ mosM. During experiments, before each stimulation, series resistances were determined by an on-line fitting analysis of the transient currents during a 5 mV 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 2200S, 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 $(eIPSC_1 - eIPSC_2)/eIPSC_1$. 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 (D-AP5, Tocris), Baclofen (Sigma), P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348, Novartis, Basel), and Tiagabine (Novo Nordisk A/S, Bagsvard, 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 μM) and D-AP5 (50 μM), electrical stimulation in the hilus evoked a pure monosynaptic $GABA_A$ 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 (eIPSC2) was smaller than the first one (Fig. 1*A*). 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. 1*B*). 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. 1*B*). 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. 1*C*): 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 eIPCSs 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 $GABA_B$ 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 (Gaïarsa 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 $GABA_B$ 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. 2, *A* 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 μM and 0.83 ± 0.18 μM in adults and neonates, respectively. At concentrations that approached the EC_{50} (0.3 and 1 μM) baclofen reduced the amplitude of eIPSCs in neonates and adults to the same extent: 0.3 μM baclofen reduced eIPSC amplitudes to 83.0 ± 5.5% and 76.5 ± 2.3% of control in neonates and adults, respectively ($P = 0.34$, $n = 3$); 1 μM 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

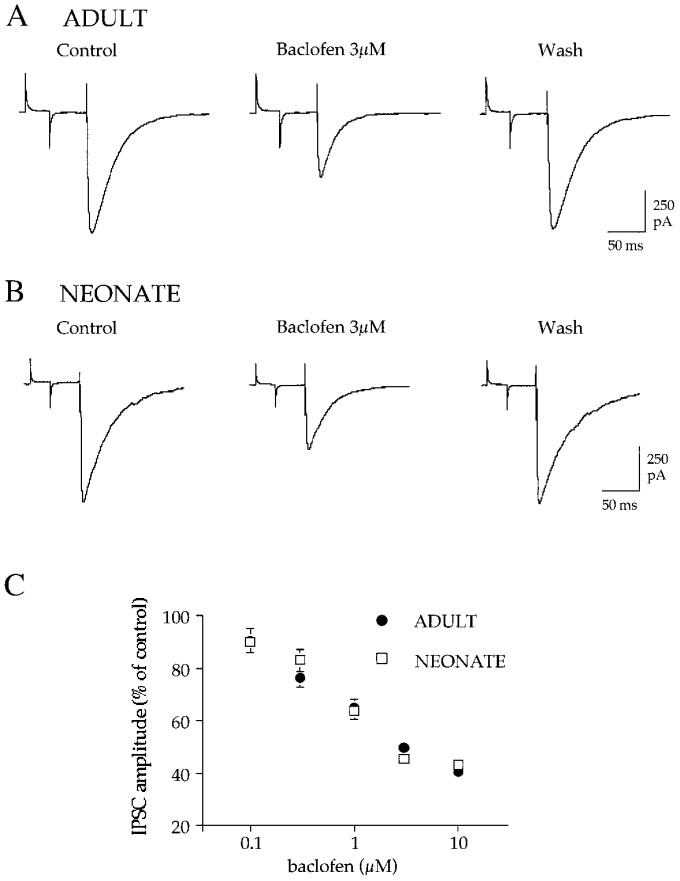

FIG. 2. Baclofen reduces amplitude of eIPSCs with same efficiency in neonates and adults. *A*: averaged ($n = 5$) traces representing eIPSCs in control conditions, 5 min after bath application of baclofen (3 μM) and 15 min after washout of baclofen in a P28 neuron held at −50 mV. *B*: averaged ($n = 5$) traces representing eIPSCs in control conditions, 5 min after bath application of baclofen (3 μM), and 15 min after washout of baclofen in a P5 neuron held at −55 mV. *C*: grouped data (mean ± SE) representing % of control eIPSC amplitude vs. concentration of baclofen (0.1–10 μM) in neonatal and adult neurons.

initial probability of release. Lowering the probability of release favors facilitation, whereas increasing the probability of release favors depression (Mallart and Martin 1968). These conditions have been reported to affect both $GABA_B$ receptor-dependent and -independent PPD in the adult hippocampus (Khazipov et al. 1995; Lambert and Wilson 1994). To test whether or not the absence of PPD was due to an insufficient amount of released GABA, we investigated the effects of an increase in the probability of transmitter release in neonates.

In neonates, in the presence of CNQX (10 μM) and D-AP5 (50 μM), raising external Ca^{2+} concentration from 2 to 4 mM and lowering external Mg^{2+} concentration from 1.3 to 0.3 mM enhanced $eIPSC_1$ amplitude to 154 ± 24% of control ($P = 0.027$, $n = 6$, Fig. 3*A*). In 63% of the cells tested (17/27), PPD was revealed. PPD was 27 ± 2.7% at 200 ms ISI and 4.0 ± 3.4% at 1,500 ms ISI ($P = 0.008$ and 0.46, respectively, when compared with control). Application of CGP35348 (0.5 mM) reduced PPD to 14.8 ± 2.8% at 200 ms ISI ($P = 0.012$, $n = 8$) but had no effect at 1,500 ms ISI (7.6 ± 4.3%, $P = 0.715$, $n = 3$) (Fig. 3). Thus PPD revealed in high Ca^{2+}/low Mg^{2+} ACSF has both $GABA_B$ receptor-dependent and -independent components.

The emergence of PPD in high Ca^{2+}/low Mg^{2+} ACSF could be accounted for by an up-regulation of the presynaptic $GABA_B$ receptor complex (Al-Dahan and Thalmann 1989). However, in high Ca^{2+}/low Mg^{2+} ACSF, baclofen (10 μM) 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 $GABA_B$ receptor-dependent and -independent PPD with no change in the efficacy of presynaptic $GABA_B$ receptors.

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

The following set of experiments was designed to determine the mechanisms underlying the PPD observed in high Ca^{2+}/low Mg^{2+} ACSF. One possible factor underlying the PPD may be desensitisation of postsynaptic $GABA_A$ 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^{2+}/low Mg^{2+} ACSF (Fig. 4*A*). As shown in Fig. 4*B*, 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 $eIPSC_1$s and $eIPSC_2$s. $eIPSC_1$ was evoked at membrane holding potentials ranging from −130 to + 30 mV. After this, in the same cell, $eIPSC_1$ was evoked from a fixed membrane potential (−70 mV) and $eIPSC_2$ 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 $eIPSC_1$ and −0.6 ± 1.1 mV for $eIPSC_2$ ($P = 0.73$, $n = 3$) (Fig. 4*C*).

These results indicate that the PPD observed under conditions favoring a high probability of neurotransmitter release was not due to postsynaptic modifications of $GABA_B$ receptor efficacy but rather involves presynaptic mechanisms.

Blocking the GABA uptake in neonates does not reveal PPD

The above observations confirm that presynaptic $GABA_B$ 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 $eIPSC_1$ and 20.7 ± 2.8 ms for $eIPSC_2$, 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 μM), $t_{1/2}$ of $eIPSC_1$ and $eIPSC_2$ increased significantly by 269.9 ± 73.7% and

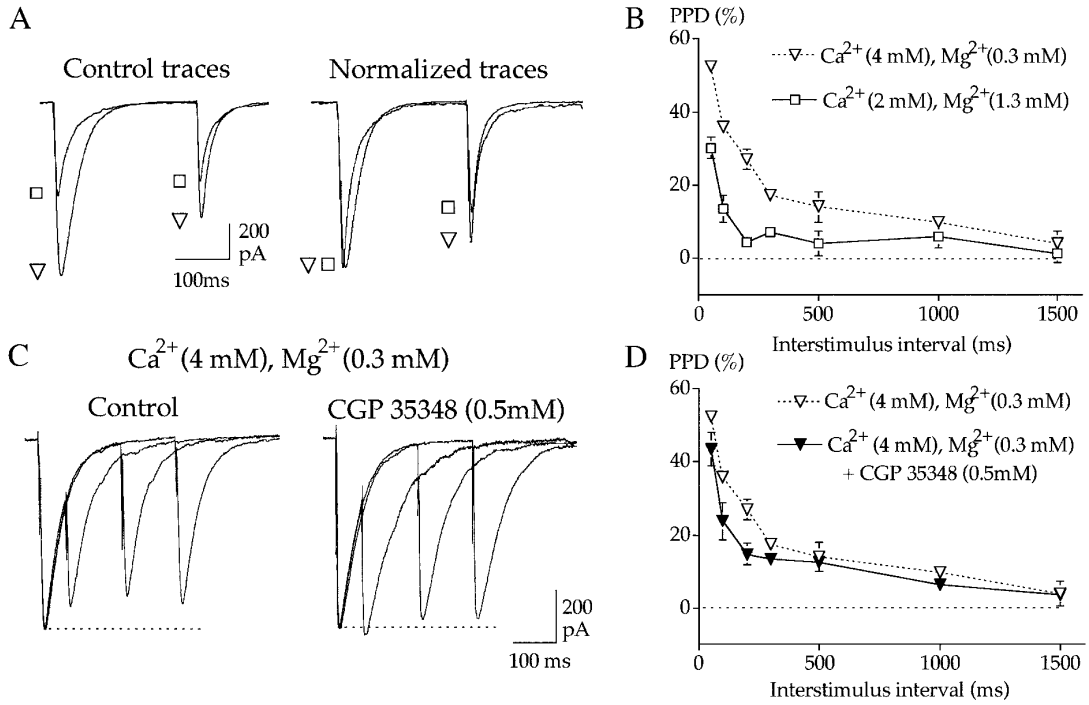

FIG. 3. Increasing probability of transmitter release reveals $GABA_B$ receptor-dependent and -independent PPD in neonates. *A, left*: averaged ($n = 5$) traces representing pairs of eIPSCs at 200 ms ISI evoked in 2 mM Ca^{2+} and 1.3 mM Mg^{2+} artificial cerebrospinal fluid (ACSF, □) and in 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF (▲) in a P5 neuron held at −60 mV. *Right*: same traces normalized to peak of 1st IPSC. *B*: grouped data (mean ± SE) representing % of PPD in 2 mM Ca^{2+} and 1.3 mM Mg^{2+} ACSF (□) and in 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF (▼) ($n = 17$) at different ISIs. In 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF, PPD is increased significantly at 50, 100, 200, and 300 ms. *C*: averaged ($n = 5$) traces representing pairs of eIPSCs at 100, 300, and 500 ms in 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF before (*left*) and after (*right*) application of CGP35348 (0.5 mM) in a P6 neuron held at −60 mV. *D*: grouped data (mean ± SE) representing % of PPD in 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF (▼) and in 4 mM Ca^{2+} and 0.3 mM Mg^{2+} ACSF containing CGP35348 (0.5 mM) ($n = 8$). CGP35348 significantly reversed PPD at 100, 200, and 300 ms ISIs.

129.2 ± 12.1%, respectively ($eIPSC_1$: 78.4 ± 29.3 ms, $P = 0.043$; $eIPSC_2$: 25.2 ± 3.2 ms, $P = 0.043$; $n = 5$) (Fig. 5*A*) 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. 5*A*). Subsequent application of CGP35348 (0.5 mM) significantly increased $t_{1/2}$ of $eIPSC_1$ and $eIPSC_2$ by 134.5 ± 2.2% and 255.6 ± 54.2%, respectively ($eIPSC_1$: 89.5 ± 19.6 ms, $P = 0.043$; $eIPSC_2$: 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 $GABA_B$ receptors in adult hippocampal CA3 region.

In neonates $t_{1/2}$ was 36.6 ± 3.9 ms for $eIPSC_1$ and 31.9 ± 2.6 ms for $eIPSC_2$ 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), $t_{1/2}$ of $eIPSC_1$ and $eIPSC_2$ increased significantly by 135.7 ± 9.8% and 122.8 ± 5.3%, respectively ($eIPSC_1$: 46.9 ± 4.0 ms, $P = 0.005$; $eIPSC_2$: 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 $t_{1/2}$ of $eIPSC_2$ by 119 ± 6.5% (44.9 ± 5.3 ms, $P = 0.046$; $n = 6$) but $t_{1/2}$ of $eIPSC_1$ and PPD were not significantly affected ($eIPSC_1$: 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 $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ receptor-dependent PPD, thus indicating that, in neonates, the critical concentration of GABA required for activation of presynaptic $GABA_B$ 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 $GABA_B$ receptors and has been termed $GABA_B$ receptor-dependent PPD (Davies et al. 1990; Lambert and Wilson 1994) and a

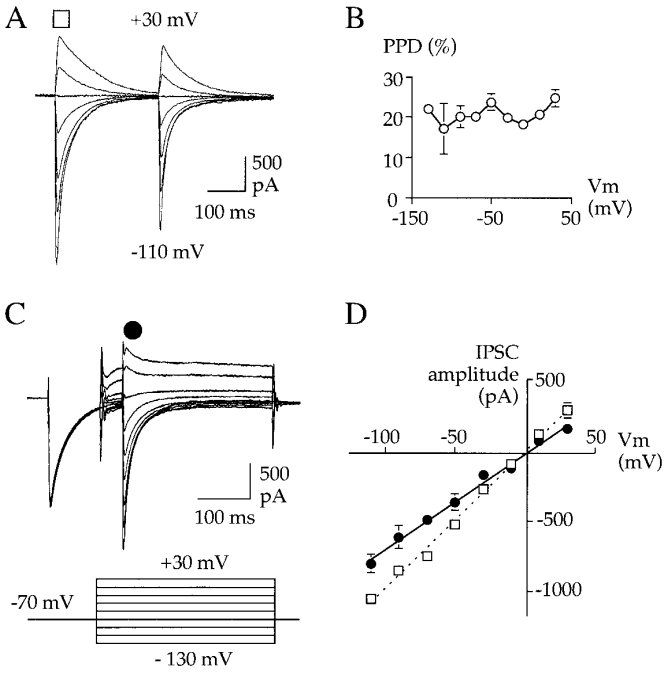

FIG. 4. PPD obtained with increased transmitter release probability is independent of membrane potential and is not the result of a change in eIPSC reversal potential. *A*: averaged ($n = 5$) traces representing pairs of eIPSCs delivered at 250 ms ISI at holding potentials of −110, −70, −50, −30, −10, 10, and 30 mV in a P7 neuron. *B*: grouped data (mean ± SE) representing % of PPD vs. membrane potential. *C*: same neuron as in *A*. Averaged ($n = 5$) traces representing pairs of eIPSC delivered at a 250 ms ISI. First eIPSC was delivered at a potential of −70 mV. Second eIPSC was delivered at a holding potential of −130, −110, −90, −70, −50, −30, −10, 10, and 30 mV attained 50 ms before stimulation. *D*: grouped data (mean ± SE) representing *I-V* curve for $eIPSC_1$ (□) normalized to 10 nS in each experiments and $eIPSC_2$ reevaluated to keep same ratio with initial IPSC conductance (●) and obtained after protocol presented in *C*. For averaging *I-V* curves, IPSC conductance of $eIPSC_1$ was determined by linear fitting for each experiment and then normalized to 10 nS. $eIPSC_2$ was then reevaluated to maintain ratio with initial IPSC conductance.

second component that does not depend on the activation of these receptors and has been termed $GABA_B$ receptor-independent PPD (Lambert and Wilson 1994). Even though PPD is present in adults at ISIs ranging from 5 ms to 5 s (Davies et al. 1990; Lambert and Wilson 1994), we show that in neonates PPD was restricted to ISIs shorter than 200 ms. This PPD was not $GABA_B$ receptor-dependent because application of CGP35348 had no effect on the amplitude of the second evoked IPSC.

There are at least two hypotheses that may account for the absence of $GABA_B$ receptor-dependent PPD in neonatal CA3 pyramidal neurons. One hypothesis is related to the presynaptic $GABA_B$ receptor complex (i.e., the absence of the receptors or the presence of a distinct subtype of receptors), whereas a second hypothesis suggests that the critical concentration of GABA required for the activation of presynaptic $GABA_B$ receptors is not achieved.

Support for the first hypothesis is lent by recent findings indicating that in the adult CA3 region presynaptic regulation of GABA release is heterogeneous; $GABA_B$-resistant GABAergic synapses are located on pyramidal cell body and $GABA_B$-sensitive GABAergic synapses are located on pyramidal dendrites (Lambert and Wilson 1993). In addition, in the hippocampus, GABAergic terminals on dendrites exhibit a higher sensitivity to the $GABA_B$ receptor agonist baclofen (Lambert and Wilson 1993; Pearce et al. 1995). However, in our conditions, baclofen depresses evoked monosynaptic GABAergic transmission with the same efficacy in neonates and adults. Finally, 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.

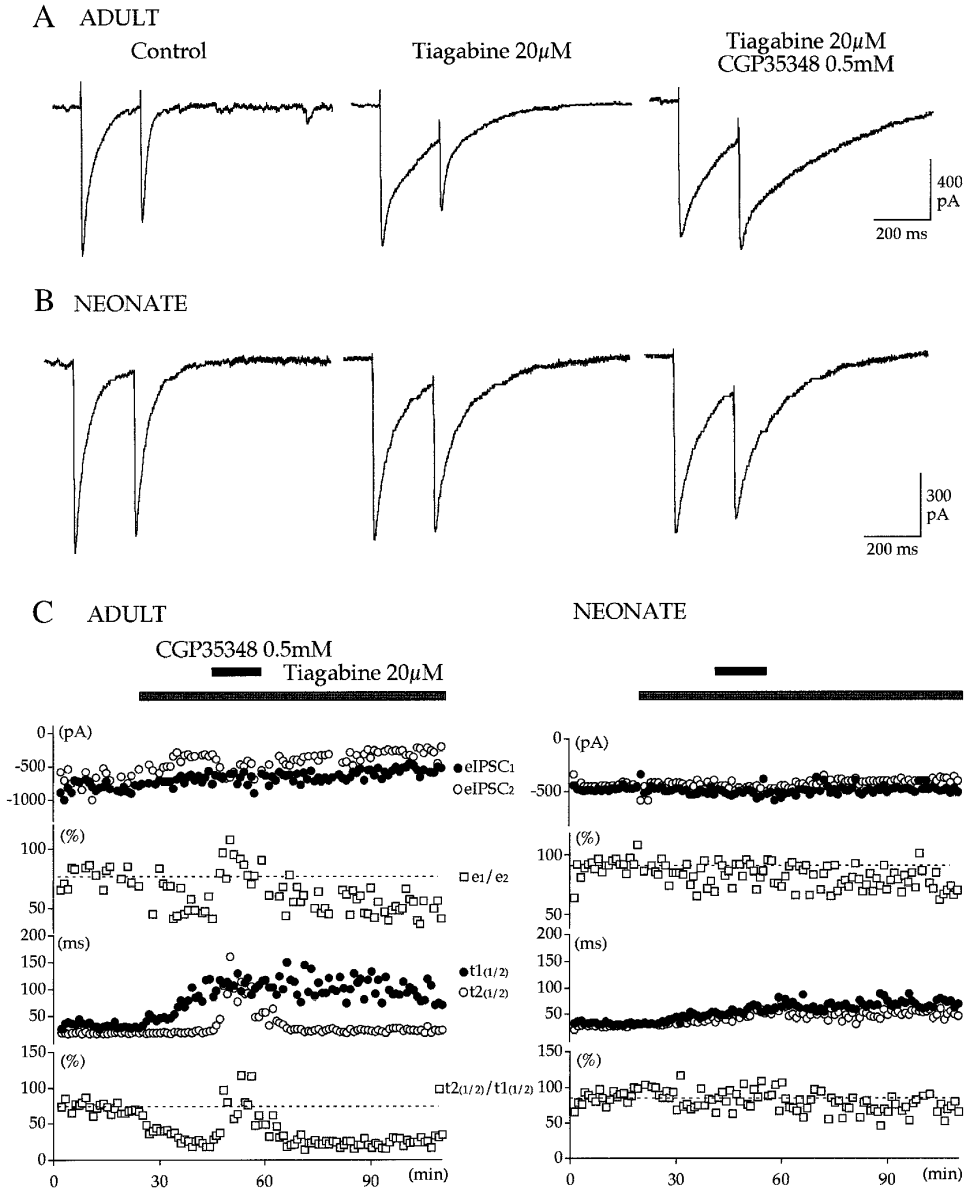

FIG. 5. Tiagabine slightly affects eIPSC kinetics but does not reveal $GABA_B$ receptor-dependent PPD in neonates. *A*: averaged ($n = 5$) traces representing pairs of eIPSC delivered at 200 ms ISI at a holding potential of −60 mV in a P28 neuron in control, after 10 min exposure of tiagabine (20 μM) and after a 3-min application of CGP35348 (0.5 mM) in presence of tiagabine (20 μM). Tiagabine prolongs kinetics of both eIPSCs and augments degree of PPD. Application of CGP35348 (0.5 mM) reverses PPD and prolongs kinetics of eIPSCs. *B*: averaged ($n = 5$) traces representing pairs of eIPSC delivered at 200 ms ISI at a holding potential of −60 mV in a P5 neuron in control, after 10 min exposure of tiagabine (20 μM) and after a 3-min application of CGP35348 (0.5 mM) in presence of tiagabine (20 μM). Tiagabine prolongs kinetics of both eIPSCs and augments degree of PPD. Application of CGP35348 (0.5 mM) does not reverse PPD but prolongs kinetics of eIPSCs. *C*: effects of application of tiagabine (20 μM; gray bar) and CGP35348 (0.5 mM; black bar) on amplitude of $eIPSC_1$ (e1), $eIPSC_2$ (e2), e2/e1 ratio, $t_{1/2}$ $eIPSC_1$ ($t1_{1/2}$), $t_{1/2}$, $eIPSC_2$ ($t2_{1/2}$) and $t2_{1/2}/t1_{1/2}$ ratio in a P28 (*left*) and in a P5 neuron (*right*). In both cases tiagabine increases $t_{1/2}$ eIPSCs but, only in P28 neuron are $eIPSC_2$ amplitude and $t_{1/2}$ increased by application of CGP35348 (0.5 mM).

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 $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ receptors in neonates. Emergence of a $GABA_B$ receptor-dependent PPD can be seen only in conditions where the probability of GABA release is increased.

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