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Dual Role of GABA in the Neonatal Rat Hippocampus

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Key Words
γ-Aminobutyric acid · Development · Hippocampus · Seizure · Bicuculline · Diazepam

Abstract
The effects of modulators of GABA-A receptors on neuronal network activity were studied in the neonatal (postnatal days 0–5) rat hippocampus in vitro. Under control conditions, the physiological pattern of activity of the neonatal hippocampal network was characterized by spontaneous network-driven giant depolarizing potentials (GDPs). The GABA-A receptor agonist isoguvacine (1–2 μ*M*) and the allosteric modulator diazepam (2 μ*M*) induced biphasic responses: initially the frequency of GDPs increased 3 to 4 fold followed by blockade of GDPs and desynchronization of the network activity. The GABA-A receptor antagonists bicuculline (10 μ*M*) and picrotoxin (100 μ*M*) blocked GDPs and induced glutamate (AMPA and NMDA)-receptor-mediated interictal- and ictal-like activities in the hippocampal slices and the intact hippocampus. These data suggest that at early postnatal ages GABA can exert a dual – both excitatory and inhibitory – action on the network activity.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult brain. Inhibitory action of GABA is achieved via GABA-A and GABA-B types of receptors. Binding of GABA to the GABA-A and GABA-B receptors opens ionic channels which are permeable to Cl^- and K^+ ions, respectively. Opening of both types of channels is associated with (i) outwardly directed currents through the postsynaptic membrane and cell hyperpolarization and (ii) an increase in the membrane conductance and shunt, resulting in reduction of the neuronal activity and inhibition of the excitatory glutamatergic inputs [Agmon and O'Dowd, 1992; Kanter et al., 1996]. GABAergic inhibition is the key element that provides the basis for the coordinated synchronized neuronal activity in neuronal networks [Freund and Buzsaki, 1996]. Removal of the GABAergic inhibition leads to paroxysmal discharges synchronized by amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and N-methyl-*D*-aspartate (NMDA) receptors in the recurrent collateral synapses between the principal neurons [Miles and Wong, 1986, 1987] and by nonsynaptic mechanisms [Jefferys, 1995].

A very different situation prevails at early stages of development of the central nervous system. Thus, in stud-

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ies in rodents, considerable evidence has been provided for the *depolarizing* and *excitatory* action of GABA at the embryonic stage and during the first week of postnatal life [for a review, see Ben-Ari et al., 1997; Cherubini et al., 1991]. Initially described in the hippocampal formation [Ben-Ari et al., 1989], excitatory effects of GABA have since been shown in developing neurons from various brain structures [Chen et al., 1996; Fiszman et al., 1990; Hales et al., 1994; LoTurco et al., 1995; Rohrbough and Spitzer, 1996; Serafini et al., 1995; Wu et al., 1992]. Activation of GABA-A receptors in immature neurons produces depolarization instead of hyperpolarization and, importantly, this depolarization is above the threshold of the sodium action potential generation. Consequently, synaptic GABA-A-receptor-mediated currents can trigger action potentials in neonatal neurons. The excitatory effect of GABA is due to elevated intracellular concentration of chloride in immature neurons and therefore depolarized reversal potential of the GABA-A-receptor-mediated responses [Hara et al., 1992; Inoue et al., 1991; Luhmann and Prince, 1991; Rohrbough and Spitzer, 1996; Serafini et al., 1995; Zhang et al., 1991]. In addition to this direct excitatory action, GABA also activates voltage-gated calcium channels [Chen et al., 1996; Connor et al., 1987; Hales et al., 1994; Leinekugel et al., 1995; LoTurco et al., 1995; Obrietan and van den Pol, 1995; Reichling et al., 1994; Yuste and Katz, 1991] and potentiates the activity of NMDA receptors via attenuation of the voltage-dependent magnesium block [Khazipov et al., 1997; Leinekugel et al., 1997].

Despite this considerable evidence for the excitatory effects of GABA in immature brain due to depolarizing action via GABA-A receptors, several reports indicated that, even while being depolarizing, GABA can also exert an *inhibitory* action via a shunting mechanism. Thus, it has been demonstrated that interaction between exogenously applied GABA and glutamate in immature neurons depends on the temporal relationship [Chen et al., 1996; Gao et al., 1998]: the glutamate-induced depolarization was shunted during the peak and potentiated during the decay phase of the GABA responses. However, the inhibitory effects of GABA with regard to the immature neuronal network activity have not been reported yet.

The pattern of activity in the neonatal rat hippocampal network is characterized by spontaneous network-driven discharges, so-called giant depolarizing potentials (GDPs) [Ben-Ari et al., 1989]. GDPs provide most of the neuronal activity in the neonatal hippocampal network, drive synchronous intracellular calcium oscillations [Leinekugel et al., 1997] and therefore have been suggested as a developmental pattern of activity implicated in the activity-dependent plasticity in the developing hippocampal network [Ben-Ari et al., 1997]. Interaction between GABA-A and glutamate receptors occurs during GDPs both in the pyramidal cells [Leinekugel et al., 1997] and interneurons [Khazipov et al., 1997], providing a basis for both synergistic excitatory action of GABA-A and glutamate receptors and inhibition of the glutamate-receptor-activated conductances by GABAergic shunt. In the present paper we have studied the effects of the GABA-A receptor modulators – the agonist isoguvacine, the allosteric modulator diazepam and the antagonist bicuculline – on the neonatal hippocampal network activity. The results of the present study are consistent with a dual – excitatory and inhibitory – action of GABA in the developing rat hippocampus that probably reflects dual depolarizing and shunting effects of GABA on immature hippocampal neurons.

Materials and Methods

The experiments were performed on the transverse hippocampal slices or intact hippocampi of neonatal (postnatal days P2–P5) Wistar rats. Slices and intact hippocampi were prepared as described previously [Ben-Ari et al., 1989; Khalilov et al., 1997]. For recordings, the hippocampi or slices were placed into a conventional fully submerged chamber superfused with artificial cerebrospinal fluid of the following composition (in m*M*): NaCl 126, KCl 3.5, $CaCl_2$ 2.0, $MgCl_2$ 2.0, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11 (pH 7.4) at 30–32 °C at a rate of 8–10 ml/min (hippocampi) and 2–3 ml/min (slices). Whole-cell and extracellular field recordings were performed in the pyramidal layer of the CA3 hippocampal area. Whole-cell recordings were made using an Axopatch 200A (Axon Instruments, USA) amplifier. Patch electrodes were made from borosilicate glass capillaries (outside diameter 1.5 mm, inside diameter 0.86 mm; type GC150F-15, Clark Electromedical Instruments) and had a resistance of 5–8 MΩ when filled with solutions containing (in m*M*): (1) 135 K-gluconate; 0.1 $CaCl_2$; 2 $MgCl_2$; 2 Na_2ATP; 1 EGTA; 10 HEPES; pH 7.25 $[Cl^-]_{in}$ = 4.2 m*M*; (2) 140 CsCl; 1 $CaCl_2$; 10 EGTA; 10 HEPES; 2 MgATP; 0.4 GTP; pH 7.25, osmolarity 280 mosm. Extracellular field potentials in the stratum pyramidale/radiatum of the CA3 area were recorded using glass microelectrodes filled with artificial cerebrospinal fluid using a DAM-80C AC differential amplifier (World Precision Instruments, USA). Tungsten bipolar electrodes disposed in the stratum radiatum of the CA3 area were used to evoke synaptic responses. The stimulation parameters were 20–60 V, 20 μs delivered at 0.033–0.05 Hz. Synaptic responses were acquired on a DAT tape recorder (Biologic, France) and into the memory of a 80486 personal computer using an analog-to-digital converter (TL1 DMA; Labmaster, USA). Axotape (Axon Instruments, USA), Acquis (Gerard Sadoc, France) and Origin 5.0 (Microcal Software, USA) programs were used for the acquisition and analysis of the synaptic activities. Group measures are expressed as means ± SEM, error bars also indicate SEM. Statistical significance of differences was assessed with Student's t test, the level of significance was set at $p < 0.05$.

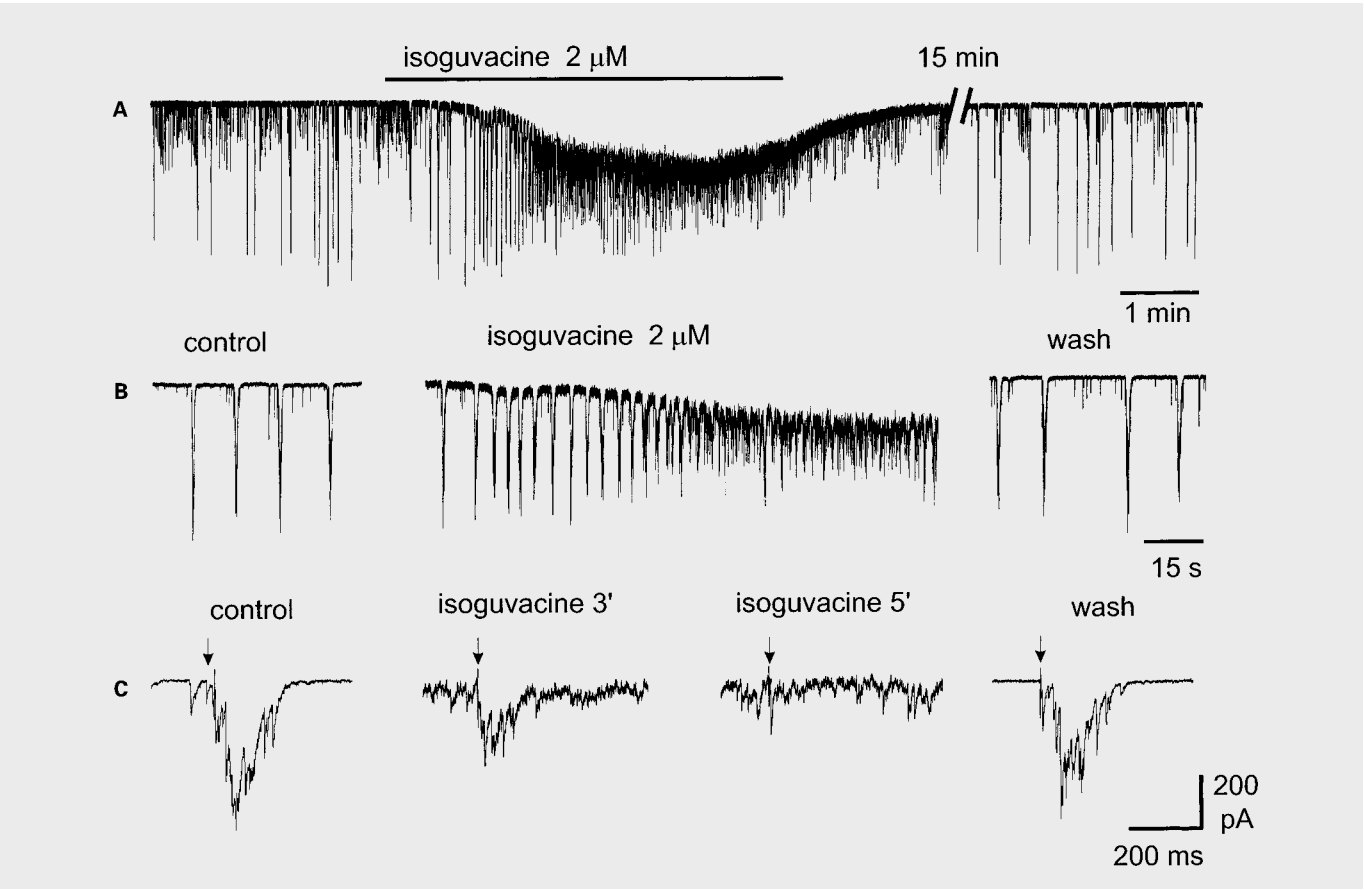

Fig. 1. Biphasic effect of the GABA-A receptor agonist isoguvacine on the network activity in the neonatal rat hippocampal slice. **A**, **B** Recording of a CA3 pyramidal cell in a 4-day-old rat hippocampal slice. In the control, the activity is characterized by spontaneous synaptic currents and network-driven events, so-called GDPs that are shown in **B** on an expanded time scale. Bath application of isoguvacine evokes an inward current in the pyramidal neuron and has a biphasic effect on the network activity – initially an increase in GDP frequency followed by suppression of GDPs and desynchronization of the synaptic activity. **C** GDPs evoked by electrical stimulation are progressively suppressed during application of isoguvacine and recover after washing out of the drug. A pyramidal cell was recorded in the whole-cell voltage-clamp mode at –50 mV with a CsCl-based pipette solution (solution 2).

Results

The present study was performed on the hippocampal slices and intact hippocampus preparation of neonatal 2- to 5-day-old (P2–5) rats using whole-cell recordings of CA3 pyramidal cells and field recordings. Under control conditions, the pattern of the physiological activity was characterized by giant depolarizing potentials (GDPs) [Ben-Ari et al., 1989; Leinekugel et al., 1998] that occurred spontaneously and could also be evoked by electrical stimulation in various regions of the hippocampal slice or intact hippocampal formation (IHF). In the present paper, we studied how the network activity will be modified by bath application of three types of modulators of GABA-A receptor activity: the agonist isoguvacine, the positive allosteric modulator diazepam and the antagonist bicuculline.

Isoguvacine

Bath application of isoguvacine (1–2 μ*M*) induced inward or outward currents in CA3 pyramidal neurons depending on the concentration of Cl^- in the recording pipette solution and an increase in the membrane conductance in CA3 pyramidal neurons. The effect of isoguvacine on the network activity was biphasic. Initially, the frequency of GDPs increased by 4-fold – from 4.2 ± 1.1 min^{-1} in controls up to 16.1 ± 2.3 min^{-1} during application of isoguvacine (n = 7; fig. 1). The increase in the

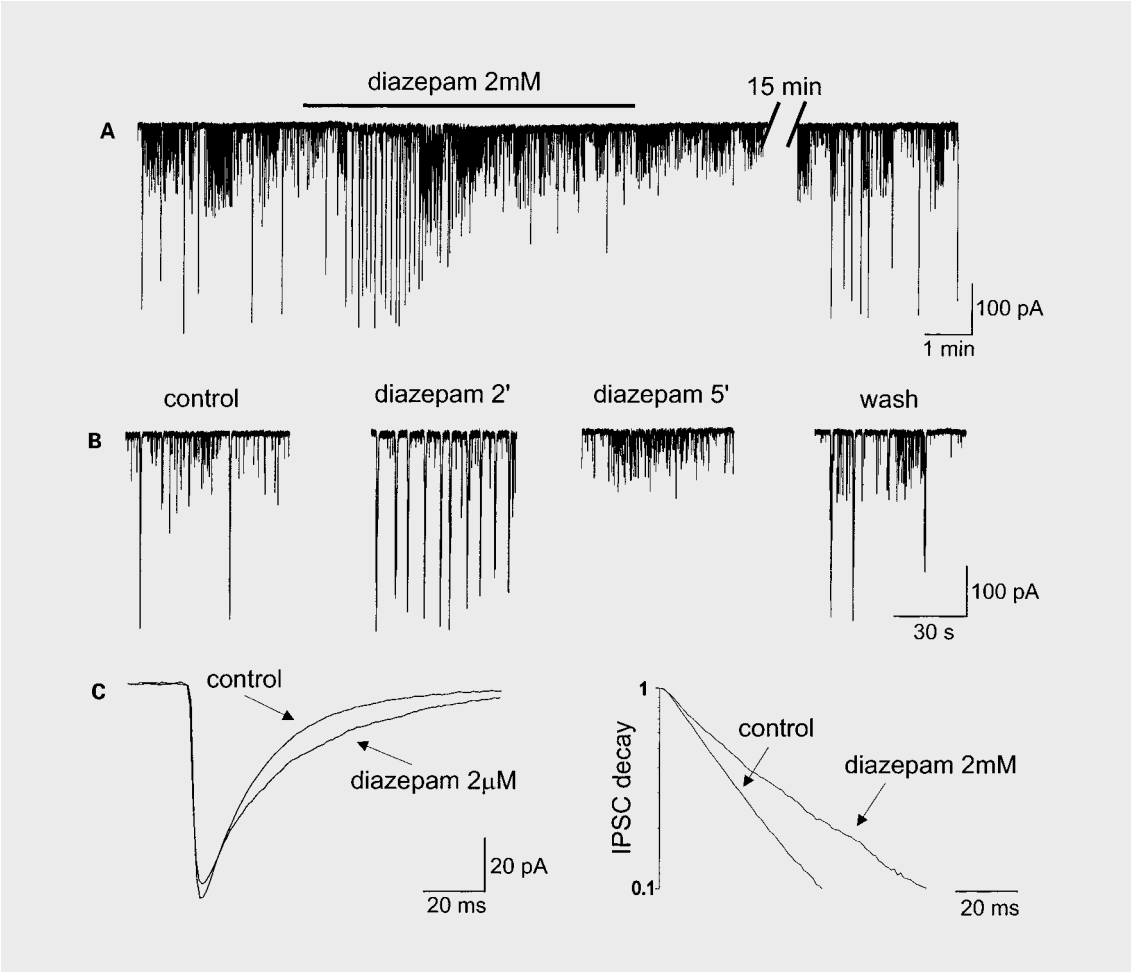

Fig. 2. Biphasic effect of the allosteric modulator of GABA-A receptor diazepam on the network activity in the neonatal rat hippocampal slice. **A**, **B** Recording of a CA3 pyramidal cell in a 3-day-old rat hippocampal slice. Note that bath application of diazepam initially induces an increase in GDP frequency followed by suppression of GDPs. **C** Effect of diazepam on the spontaneous GABA-A-receptor-mediated postsynaptic currents (PSCs). Traces represent an average of 340 and 215 GABA-A PSCs recorded under control conditions and in the presence of diazepam (2 μ*M*), respectively. On the right panel, the decay of the averaged GABA-A PSCs is plotted on the semilogarithmic scale. Note the prolongation of the decay of GABA-A PSCs in the presence of diazepam. A pyramidal cell was recorded in the whole-cell voltage-clamp mode at –50 mV with a CsCl-based pipette solution (solution 2).

GDP frequency was accompanied with a progressive decrease in GDP amplitude. This was followed by desynchronization of the network activity. During the desynchronization phase, the frequency of the synaptic noise significantly increased, but both spontaneous and evoked GDPs were suppressed (fig. 1C). After washing out of isoguvacine, the activity of the network recovered to the control level. Thus, application of GABA-A receptor agonist induces biphasic changes in the activity of the neonatal hippocampal network: GDPs are initially potentiated and then suppressed.

Diazepam

Application of diazepam (1–2 μ*M*), an allosteric modulator of GABA-A receptors known to increase the duration of opening of GABA-A receptor channels, induced also a biphasic effect on the network activity. Initially the frequency of GDPs was significantly potentiated by 3-fold (from 3.8 ± 0.6 to 12.3 ± 1.4 min^{-1}; n = 5; fig. 2). This was followed by desynchronization of the hippocampal network and suppression of GDPs (fig. 2A, B). The decay time constant of GABA-A postsynaptic currents (PSCs) was significantly prolonged in the presence of diazepam

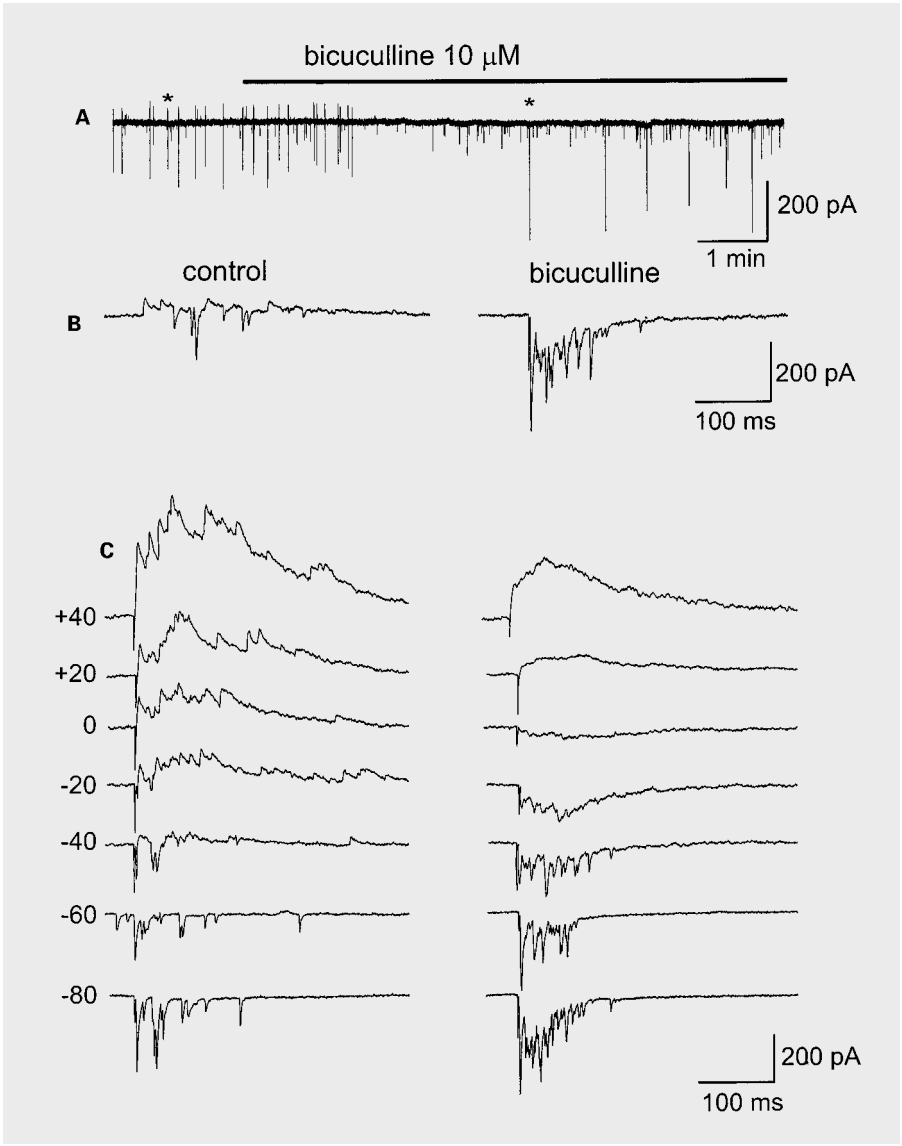

Fig. 3. The GABA-A receptor antagonist bicuculline blocks GDPs and induces glutamatergic interictal-like activity in the neonatal rat hippocampal slices. **A**, **B** Recording of a CA3 pyramidal cell in a 3-day-old rat hippocampal slice. Bath application of bicuculline blocks GDPs and induces glutamatergic interictal-like activities. **B** GDPs under control conditions and the interictal-like event are shown on an expanded time scale. **C** Current-voltage relationships of GDPs and the interictal-like events recorded in the presence of bicuculline. Note that GDPs reverse at negative potentials indicating the important contribution of GABA-A receptors and the interictal-like events reverse near 0 mV suggesting that they are mediated by glutamate receptors. Recordings were performed with a low-chloride pipette solution (solution 1) in the whole-cell voltage-clamp mode at –50 mV so that GABAergic responses were outwardly and glutamatergic responses were inwardly directed.

(2 μ*M*) from 18 ± 2 to 26 ± 3 ms (n = 4; fig. 2C). Thus, both direct activation of GABA-A receptors by isoguvacine and potentiation of the synaptically activated GABA-A receptors by diazepam had a similar biphasic effect on the activity of the neonatal hippocampal network – an initial increase in GDP frequency followed by suppression of GDPs.

Bicuculline

Bath application of the GABA-A receptor antagonist bicuculline (10 μ*M*) completely blocked GDPs and GABA-A PSCs. However, another type of network-driven activity occurred after 5–10 min of perfusion with bicuculline (n = 12). These events were less frequent than GDPs (0.2–5 min^{-1}), had a duration of 190 ± 20 ms, blocked by tetrodotoxin (n = 7) and high-divalent cation medium (n = 3). These events could be evoked by electrical stimulation in an all-or-none manner. Study of the current-voltage relationship of these events revealed reversal near 0 mV (fig. 3). Combined application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μ*M*) and 2-amino-5-phosphonovaleric acid (APV, 50 μ*M*) completely blocked both spontaneous and evoked discharges. Therefore, this activity is reminiscent of the interictal-like epileptiform activity that is observed in adult hippocampal slices after removal of GABA-A-receptor-mediated

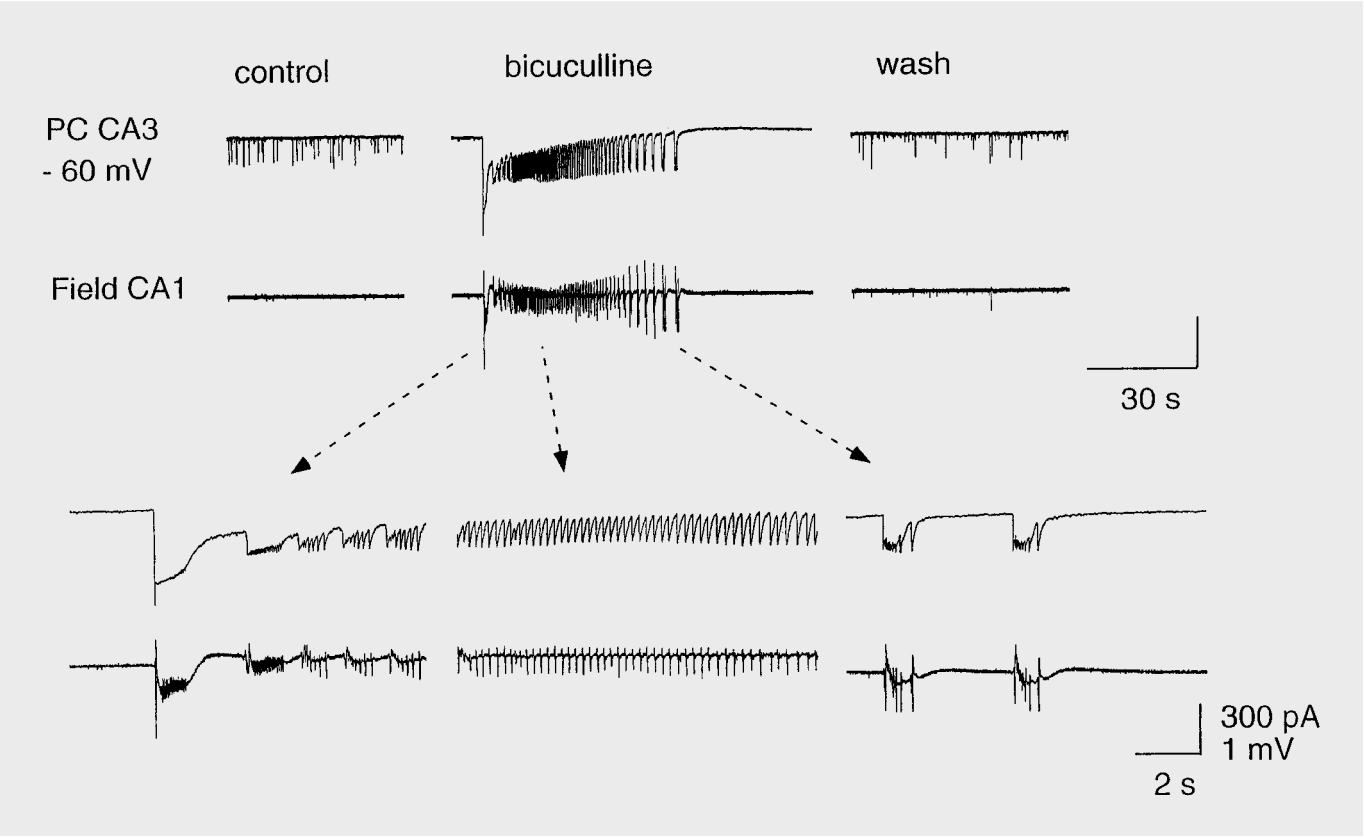

Fig. 4. Bicuculline induces ictal-like tonic-clonic epileptiform discharges in the neonatal rat intact hippocampus preparation. Simultaneous recordings of the pyramidal cell (PC; upper traces) and extracellular field (lower traces) in a CA3 subfield in a 4-day-old rat hippocampus. Bath application of bicuculline induces powerful ictal-like discharges synchronous in the extracellular and whole-cell recordings. On the traces below, phases of the ictal-like discharge are shown on an expanded time scale. The ictal-like event starts with a series of large-amplitude interictal-like discharges followed by tonic oscillations and clonic-like discharges. A pyramidal cell was recorded in the whole-cell voltage-clamp mode at –50 mV with a CsCl-based pipette solution (solution 2).

inhibition [Miles and Wong, 1987]. Another noncompetitive GABA-A receptor antagonist, picrotoxin (100 μM), exerted an action similar to that of bicuculline (n = 5).

In the next series of experiments we studied the effect of bicuculline in the intact hippocampus preparation [Khalilov et al., 1997]. Similarly to experiments in slices, bicuculline blocked GDPs and induced glutamatergic paroxysmal activity which was however much more robust in the intact hippocampus. As shown in figure 4, bicuculline-induced paroxysmal discharges developed as a sequence of interictal, tonic and clonic phases. The activity recorded in the pyramidal cells was synchronized with population activity recorded with an extracellular field electrode (fig. 4). The activity induced by bicuculline in the neonatal intact hippocampus was therefore reminiscent of the ictal-like discharges recorded in vivo [Bragin et al., 1997; Matsumoto and Akabas, 1964]. The difference in the bicuculline action in slice and intact hippocampus preparation is probably due to a better preservation of intrahippocampal connectivity [Khalilov et al., 1997] and better conditions for nonsynaptic interactions [Jefferys, 1995] in the intact hippocampus preparation. While the tonic-clonic discharges were readily induced by bicuculline in P2–5 hippocampi (n = 17), at P0–1, only interictal-like discharges have been observed (n = 6; not shown) probably reflecting yet sparse excitatory recurrent collateral connectivity between pyramidal cells at a very early postnatal age.

To study the role of the GABA-A and glutamate receptors in the excitation of the pyramidal cells, we used cell-attached recordings that do not modify the intracellular chloride concentration. At P2–5, electrical stimulation in

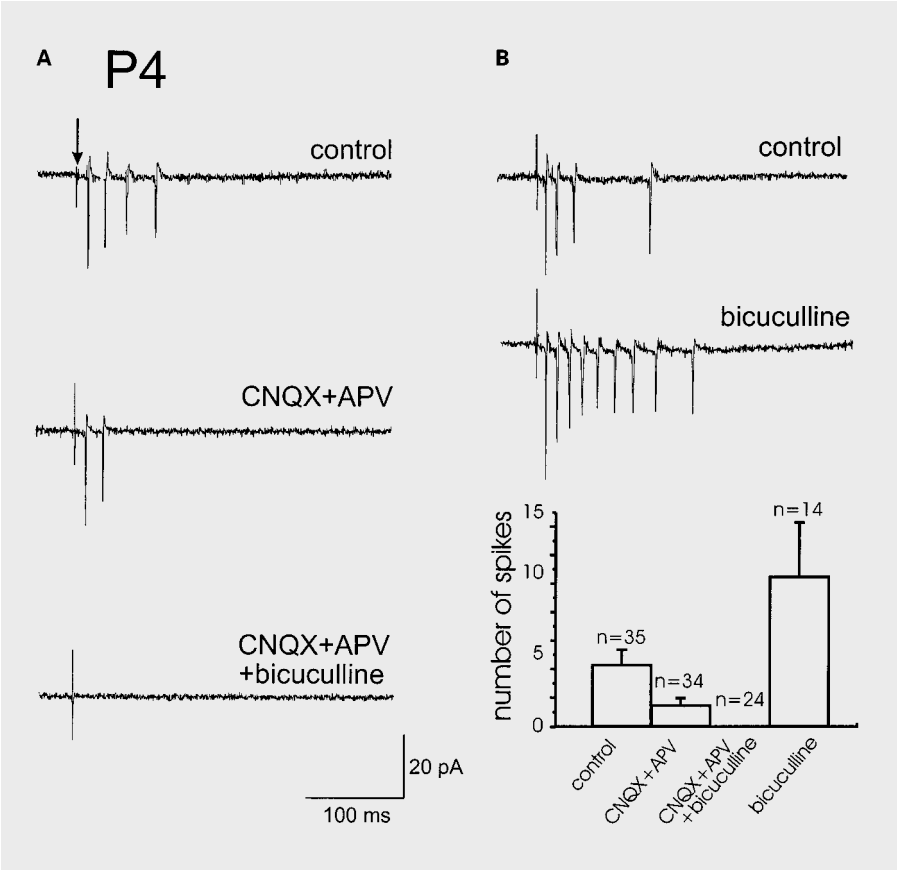

Fig. 5. Effect of bicuculline on the synaptic excitation of the pyramidal cells in the neonatal rat hippocampus. **A** Cell-attached recordings from a CA3 pyramidal neuron in a P4 hippocampal slice. Electrical stimulation in the stratum radiatum evokes a burst of action potentials in the pyramidal cell; this response is reduced in the presence of CNQX (10 μ*M*) and APV (50 μ*M*) and completely blocked by further addition of bicuculline (10 μ*M*). **B** Application of bicuculline alone significantly potentiates the response. Note that the responses under control conditions and in the presence of bicuculline correspond to evoked GDP and interictal-like discharge, respectively (see also fig. 3). **C** Statistical histogram of the number of action potentials evoked by electrical stimulation in the presence of GABA-A and glutamate receptor antagonists in P2–5 CA3 pyramidal cells.

the stratum radiatum evoked a burst of action potentials in the CA3 pyramidal cells. Combined application of the glutamate receptor antagonists CNQX (10 μ*M*) and APV (50 μ*M*) significantly reduced the number of spikes evoked by stimulation to 1–2 (fig. 5A). This response was blocked by further addition of bicuculline (10 μ*M*) suggesting that synaptically released GABA can excite CA3 pyramidal cells. However, when bicuculline was applied alone, the response was significantly potentiated, reflecting the excitation of the pyramidal cells during interictal-like discharges described above in whole-cell recordings (fig. 5B). Similar experiments performed at P10–15 revealed that the response evoked by electrical stimulation is completely blocked by CNQX (10 μ*M*) and APV (50 μ*M*) and that bicuculline significantly increases excitation of the pyramidal cells (fig. 6). These results suggest that in the neonatal rat hippocampus, both excitatory and inhibitory effects of GABA on CA3 pyramidal cells can be observed depending on the experimental conditions.

Discussion

The principal conclusion emerging from the present study is that in the neonatal rat hippocampus, GABA acting on GABA-A receptors exerts a dual – excitatory and inhibitory – action on the activity of the immature hippocampal network.

The increase in GDP frequency observed at the initial phase of isoguvacine action is likely due to GABA-A-receptor-mediated depolarization of the pyramidal cells [Leinekugel et al., 1995, 1997] and interneurons [Khazipov et al., 1997] that should increase their excitability and potentiate network-driven GDPs. A similar increase in GDP frequency has been observed after application of ionotropic [Gaïarsa et al., 1991] and metabotropic [Strata et al. 1995] glutamate receptor agonists and glycine [Gaïarsa et al., 1990b, c]. Although the mechanism of action of these drugs is evidently complex, an increase in GDP frequency can be at least partly accounted for by the depolarization and increased excitability.

The second phase of the isoguvacine-induced responses was characterized by depression of the network

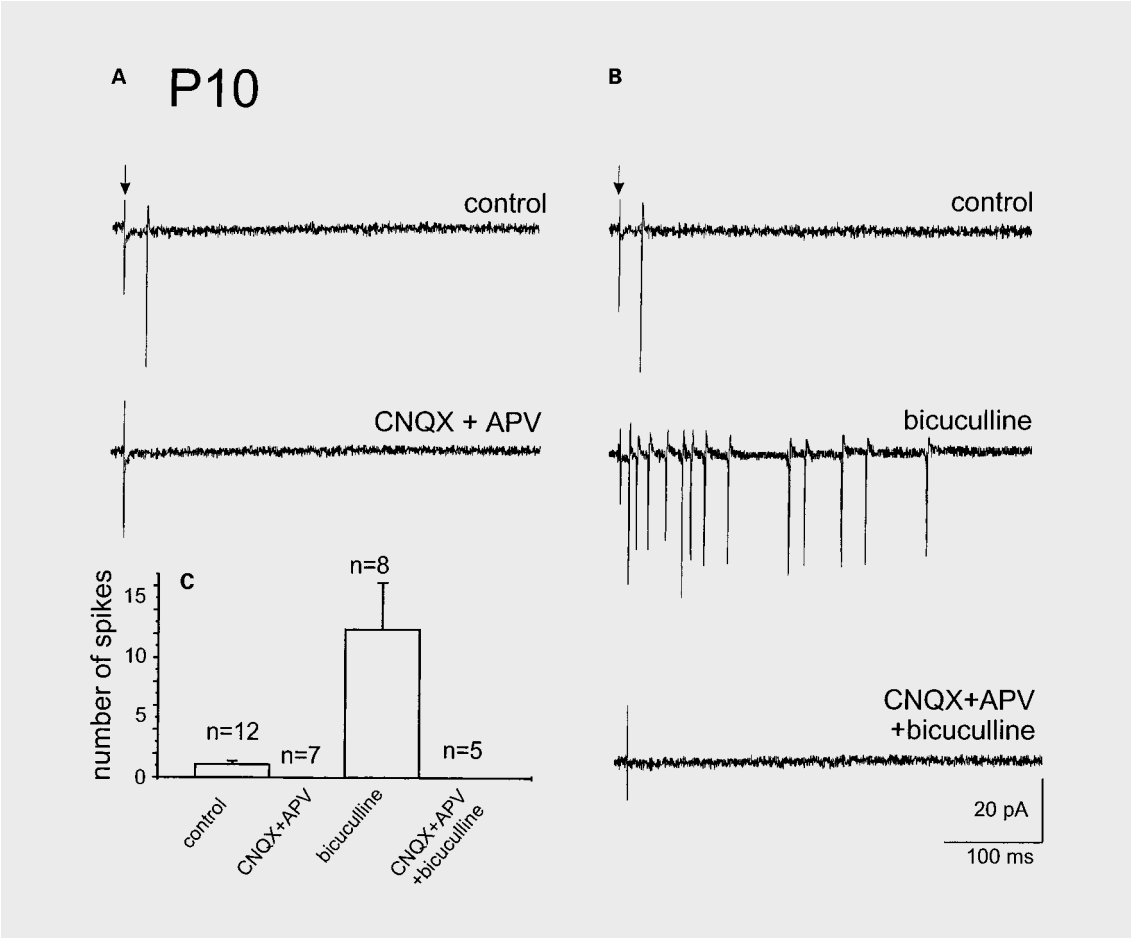

Fig. 6. Effect of bicuculline on the synaptic excitation of the pyramidal cells in an older age group (P10–15). **A** Cell-attached recordings from a CA3 pyramidal neuron in a P10 hippocampal slice. Electrical stimulation in the stratum radiatum evokes a single action potential in the pyramidal cell and the response is completely blocked by combined application of CNQX (10 μM) and APV (50 μM). **B** The response is significantly potentiated by bicuculline and is completely blocked by further addition of CNQX and APV. **C** Statistical histogram of the number of action potentials evoked by electrical stimulation in the presence of GABA-A and glutamate receptor antagonists in P2–5 CA3 pyramidal cells.

activity. Mechanisms of this depression can be complex and have not been studied so far; however, the most likely explanation is a shunt of the membrane due to the increase in GABA-A-receptor-activated Cl^- conductance which will depress the glutamate-receptor-mediated synaptic currents involved in the generation of GDPs [Ben-Ari et al., 1989; Gaïarsa et al., 1990a; Khazipov et al., 1997; Leinekugel et al., 1997] similarly to a mechanism of shunt of the responses induced by exogenous glutamate application [Gao et al., 1998] and simulated by positive current injection excitatory events [Chen et al., 1996] in developing rat hypothalamic neurons at the peak of activation of GABA-A-receptor-mediated conductance.

While the effects of isoguvacine are due to the direct activation of the GABA-A receptors, the effects of the allosteric modulator of GABA-A receptors diazepam are due to potentiation of the synaptically activated GABA-A receptors. In keeping with previous studies [Mody et al., 1994], diazepam significantly prolonged the decay phase of the GABA-A PSCs. Prolongation of GABA-A-receptor-mediated synaptic currents will potentiate the excitatory effect of GABA per se and can be responsible for the potentiation phase of the drug action. On the other hand, prolongation of the GABA-A PSCs will lead to an increase in the shunting temporal window during which other excitatory inputs will be depressed that may be responsible for the desynchronization phase of the drug action.

In keeping with previous reports, blockade of GABA-A receptors suppressed GDPs in CA3 pyramidal cells of the neonatal rat hippocampus [Ben-Ari et al., 1989; Gaïarsa et al., 1990b, c, 1991; Strata et al., 1995, 1997; Xie and Smart, 1991] and similarly to the GDP pattern of early network oscillations in CA1 pyramidal cells [Garaschuk et al., 1998]. However, in contrast to previous results, application of GABA-A receptor antagonists induced glutamatergic interictal-like activities in the slice preparation [Khazipov et al., 1997] and ictal-like tonic-clonic discharges in the preparation of the intact hippocampus in vitro [Khalilov et al., 1997], whereas the above-mentioned studies reported absence of synchronous activity in the presence of bicuculline. In one of the previous studies [Gaïarsa et al., 1991] it has been shown that although no interictal-like activity was generated in the presence of bicuculline, application of glutamate receptor agonists, high-potassium or low-magnesium solutions can induce interictal-like activities suggesting that the recurrent connections between CA3 exist but are quiescent in normal conditions. Results of the present study suggest that recurrent excitatory connections between CA3 pyramidal cells which are responsible for the generation of the synchronized epileptiform activity [Miles and Wong, 1987] exist and are functional already during the first days of life and sufficient to generate the interictal- and ictal-like paroxysmal activities after blockade of GABA-A receptors. The reasons for the discrepancy between the present and previous reports with regard to the bicuculline effect are unclear since there is no obvious difference in the experimental conditions; it may be due to the differences in the strain of animals, handling conditions, diet, etc. It will be of interest to repeat these experiments in other laboratories and elucidate the cause of this discrepancy.

Results of the pharmacological study of the responses recorded in the cell-attached configuration indicate that: (i) synaptic activation of GABA-A receptors excites immature neurons in keeping with previous studies [Ben-Ari et al., 1989; Chen et al., 1996; Fiszman et al., 1990; Hales et al., 1994; LoTurco et al., 1995; Rohrbough and Spitzer, 1996; Serafini et al., 1995; Wu et al., 1992]; (ii) blockade of GABA-A receptors potentiates the excitation of pyramidal cells mediated by glutamate (AMPA + NMDA) receptors, and (iii) GABA-A receptors potentiate the activity of the pharmacologically isolated NMDA receptors (in the presence of the AMPA receptor antagonist CNQX) that has been shown earlier under similar experimental conditions [Leinekugel et al., 1997]. The most likely explanation for this result is the following. GABA acting on GABA-A receptors depolarizes immature neurons and activates voltage-gated sodium and calcium conductances. Concerning the interaction with glutamate receptors that occurs during generation of GDPs [Khazipov et al., 1997; Leinekugel et al., 1997], depolarization produced by activation of GABA-A receptors attenuates the voltage-dependent magnesium block of NMDA channels and potentiates their activity, but the activity of AMPA receptors is inhibited probably due to a shunting effect of GABA.

Taken together, the present results suggest a dual – both excitatory and inhibitory – action of GABA in the neonatal rat hippocampus and this has a major impact on the neonatal network activity. While the excitatory action of GABA is implicated in the generation of the physiological pattern of activity (GDPs), inhibition of the AMPA receptor activity in the recurrent collateral synapses prevents paroxysmal activities similarly to the situation in adults. This dual action of GABA has to be considered in the clinical investigations of the developmental disorders including epilepsies in children.

Acknowledgements

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