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Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus

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Abstract

The immature brain is prone to seizures but the underlying mechanisms are poorly understood. We explored the hypothesis that increased seizure susceptibility during early development is due to the excitatory action of GABA. Using noninvasive extracellular field potential and cell-attached recordings in CA3 of Sprague-Dawley rat hippocampal slices, we compared the developmental alterations in three parameters: excitatory actions of GABA, presence of the immature pattern of giant depolarizing potentials (GDPs) and severity of epileptiform activity generated by high potassium. The GABA(A) receptor agonist isoguvacine increased firing of CA3 pyramidal cells in neonatal slices while inhibiting activity in adults. A switch in the GABA(A) signalling from excitation to inhibition occurred at postnatal day (P) 13.5 ± 0.4. Field GDPs were present in the form of spontaneous population bursts until P12.7 ± 0.3. High potassium (8.5 mM) induced seizure-like events (SLEs) in 35% of slices at P7–16 (peak at P11.3 ± 0.4), but only interictal activity before and after that age. The GABA(A) receptor antagonist bicuculline reduced the frequency or completely blocked SLEs and induced interictal clonic-like activity accompanied by a reduction in the frequency but an increase in the amplitude of the population spikes. In slices with interictal activity, bicuculline typically caused a large amplitude interictal clonic-like activity at all ages; in slices from P5–16 rats it was often preceded by one SLE at the beginning of bicuculline application. These results suggest that, in the immature hippocampus, GABA exerts dual (both excitatory and inhibitory) actions and that the excitatory component in the action of GABA may contribute to increased excitability during early development.

Introduction

Increased excitability constitutes an intimate feature of the immature brain. In humans, the increased excitability is manifested by a high incidence of seizures during the perinatal period (Holmes, 1994; Holmes *et al*., 2002). Critical periods of seizure susceptibility have also been documented at comparable developmental stages in lower animals. Thus, in the postnatal rat hippocampus, a bell-shaped age dependence of susceptibility to various epileptogenic agents and conditions has been documented; these include kainic acid (Albala *et al*., 1984; Tremblay *et al*., 1984), electrical stimulation (Moshe *et al*., 1981), hypoxia (Jensen *et al*., 1991), penicillin (Swann & Brady, 1984), picrotoxin (Gomez-Di Cesare *et al*., 1997), fever (Holtzman *et al*., 1981; Baram *et al*., 1997) and GABA(B) receptor antagonists (McLean *et al*., 1996).

Increased excitability is also manifested by particular physiological patterns of activity expressed in the immature brain (Ben-Ari, 2001). Thus, in the neonatal rat hippocampus *in vivo*, virtually all neuronal activity is synchronized in recurrent population bursts (Leinekugel *et al*., 2002). A similar pattern of recurrent discharges entraining the entire population of the pyramidal cells and interneurons has also been found in the neonatal rat hippocampal slices *in vitro* and described as giant depolarizing potentials (GDPs; Ben-Ari *et al*., 1989), because each population discharge was associated with, and was driven by, a remarkable depolarization and firing of pyramidal cells and interneurons. A GDP-like pattern of activity has been also observed in the intact rat hippocampus *in vitro* (Khalilov *et al*., 1997a; Leinekugel *et al*., 1998) and in hippocampal slices from neonatal rabbit (Menendez *et al*., 1998) and fetal primate (Khazipov *et al*., 2001).

Several hypothesis have been put forward in order to explain the enhanced excitability of the immature brain during certain critical periods (for reviews, see Baram & Hatalski, 1998; Holmes & Ben-Ari, 1998; Swann & Hablitz, 2000). Among various factors, developmental changes in GABAergic function have attracted particular attention. In the adult brain, GABA is the principal inhibitory neurotransmitter and plays a pivotal role in the generation of physiological patterns of activity as well as preventing the generation and propagation of paroxysmal activity (Freund & Buzsaki, 1996). Yet during early development GABA(A) receptors may exert dual (both excitatory and inhibitory) actions, depending on the context of their activation (Chen *et al*., 1996; Khalilov *et al*., 1999; Lamsa *et al*., 2000; Wells *et al*., 2000; Lu & Trussell, 2001). The excitatory effect of GABA is a probable mechanism for explaining the enhanced excitability of the immature brain. Although efforts have been made to verify this hypothesis (Michelson & Lothman, 1992), involvement of GABAergic excitation has been clearly demonstrated only in the generation of GDPs during the first postnatal week (Ben-Ari *et al*., 1989; Khazipov *et al*., 1997; Leinekugel *et al*., 1997; Garaschuk *et al*., 1998).

Using intracellular recordings it has been shown that activation of GABA(A) receptors begins to inhibit CA3 pyramidal cells by postnatal

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days (P)5–6 (Ben-Ari *et al*., 1989; Swann *et al*., 1989), yet the immature patterns of hippocampal activity disappear later in development, at the end of the second postnatal week, both *in vivo* (Leinekugel *et al*., 2002) and in hippocampal slices (Ben-Ari *et al*., 1989; Garaschuk *et al*., 1998). The peak of epileptogenesis has also been demonstrated later, around P10–12 in the cases of seizures evoked *in vivo* by hypoxia (Jensen *et al*., 1991) and fever (Baram *et al*., 1997). The apparent discrepancy in the timing of the developmental changes in GABA(A) function and excitability has substantially weakened an otherwise attractive hypothesis on the link between these two developmental phenomena. There are, however, several reasons to reconsider this hypothesis because the intracellular recordings that have been used to determine the developmental changes in the action of GABA introduce several errors. These include ionic exchange between the pipette and cytoplasm and leak conductance between the electrode and neuronal membrane that may affect neuronal excitability and internal ionic composition, particularly in small immature neurons (Barry & Lynch, 1991). Therefore, in the present study we used noninvasive extracellular recordings to determine and compare the time course of the developmental changes in the network excitability, both physiological GDPs and paroxysmal discharges, and GABA(A) signalling.

Materials and methods

Experimental system

This study followed NIH guidelines on animal care and received approval of the Animal Care and Use Committee of Harvard Medical School, Dartmouth Medical School and INSERM. Hippocampal slices were prepared from Sprague-Dawley rats of both sexes ($n = 39$). Animals were anaesthetized with chloral hydrate (350 mg/kg, intraperitoneally) or inhalation of isoflurane, and decapitated. The brain was removed and transverse hippocampal slices (500 μm) were cut using a vibroslicer Leica VT 1000S (Leica Microsystems, Nussloch GmbH, Germany). Slices were kept in oxygenated (95% O_2 : 5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl, 126; KCl, 3.5; $CaCl_2$, 2.0; $MgCl_2$, 1.3; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 11 (pH 7.4) at room temperature (20–22 °C) at least 1 h before use. For recordings, slices were placed into a conventional fully submerged thermostatic chamber (Warner Instrument Corp., Hamden, CT, USA) and superfused with ACSF (30–32 °C) at a rate of 2–3 mL/min. In the experiments with bath application of isoguvacine, a small (≈0.1 mL) volume chamber was used to provide rapid solutions exchange.

Electrophysiological recordings and data analysis

Extracellular field potential recordings were performed using metal electrodes of 50 μm diameter (California Fine Wire, Grover Beach, CA, USA). Electrodes were positioned in the pyramidal cell layer of the CA3a subfield and signals were amplified using a custom-made amplifier with enhanced electromagnetic interference noise suppression (bandpass 0.1 Hz−4 kHz; ×1000). Peak-to-peak noise was in the range of 20 μV. For single action potential detection, records were filtered with an RC (single pole) high pass filter at >200 Hz. In several experiments, extracellular action potentials were also recorded using patch electrodes filled with ACSF and patch-clamp amplifier Multiclamp 700A (Axon Instruments, Union City, CA, USA). For population spike detection, records were filtered at 1–70 Hz. Patch-clamp recordings were performed using an Axopatch 200A and Multiclamp 700A amplifiers (Axon Instruments). Patch electrodes were made from borosilicate glass capillaries (GC150F-15, Clark Electromedical Instruments). For whole-cell recordings, the patch pipette solution contained (in mM): Cs-gluconate, 135; $MgCl_2$, 2; $CaCl_2$, 0.1; EGTA, 1; and HEPES, 10; (pH 7.25); membrane potential values were corrected for a liquid junction potential of +12 mV. Whole-cell recordings were performed blindly from the CA3 pyramidal cell layer. For cell-attached recordings pipettes were filled with ACSF. Cell-attached recordings were performed from CA3 pyramidal cells under visual guidance using a differential contrast microscope Axioscope (Zeiss, Germany).

Recordings were digitized (10 kHz) online with an analogue-to-digital converter (Digidata 1322A; Axon Instruments) and analysed off-line with the Axon package MiniAnalysis program (Jaejin Software, Leonia, NJ, USA) and Origin 5.0 (Microcal Software, Northampton, MA, USA). Group measures are expressed as means ± SEM; error bars also indicate SEM. The statistical significance of differences was assessed with Students *t*-test. The level of significance was set at $P < 0.05$.

To determine the developmental changes in the GABA(A) signalling, we used isoguvacine, a specific GABA(A) receptor agonist (Krogsgaard-Larsen & Johnston, 1978). In control experiments using whole-cell recordings from CA3 pyramidal cells, we found that brief bath application of isoguvacine (10 μM for 1 min) induced a transient chloride-dependent membrane current sensitive to the GABA(A) antagonist bicuculline. In extracellular recordings, the response to isoguvacine was associated with a transient change in the frequency of multiple-unit activity (MUA), that is, action potentials recorded from tens to hundreds of neurons in the vicinity of the recording electrode (Cohen & Miles, 2000). We determined the effect of isoguvacine on MUA as a ratio of the MUA frequency at the peak of the isoguvacine response to the MUA frequency in control. For the purposes of fit we normalized the effect of isoguvacine by assigning values +1 and −1 for all values >100% (excitation) and <100% (inhibition), respectively. In cell-attached recordings from individual CA3 pyramidal cells, a picospritzer (General Valve Corporation, Fairfield, NJ, USA) was used to puff-apply isoguvacine from a glass pipette in strata pyramidale–radiatum at a distance of <100 μm from soma. The pressure was set at 2–20 kPa, and the duration of the puff varied from 20 to 200 ms.

Results

Developmental profile of the immature pattern of GDPs

We first determined the developmental profile of the immature physiological pattern of the hippocampal electrographic activity. Using a combination of whole-cell and extracellular field potential recordings, we found that hippocampal slices from neonatal Sprague-Dawley rats display spontaneous recurrent population bursts associated with sharp increases in the frequency of action potentials in the extracellular recordings from the pyramidal cell layer and large GABA(A) receptor-mediated polysynaptic events on the level of a single pyramidal cell (Fig. 1A). This pattern of activity was very similar to the pattern of GDPs described in Wistar rats (Ben-Ari *et al*., 1989), also known as giant GABAergic potentials (Strata *et al*., 1997), early network oscillations (Garaschuk *et al*., 1998) and population bursts (Palva *et al*., 2000).

Day-by-day screening of the postnatal slices revealed that field GDPs were present in all slices from P0 to P9. The period from P10 to P16 was transitory, with the proportion of slices that displayed field GDPs progressively decreasing with age. After P17 no field GDPs were observed (Fig. 1B). The occurrence of field GDPs was further approximated with the Boltzman function. The midpoint of disappearance of field GDPs, that is, the age when 50% of slices express GDPs whereas the other 50% do not, was estimated at postnatal day 12.7 ± 0.3 ($n = 71$).

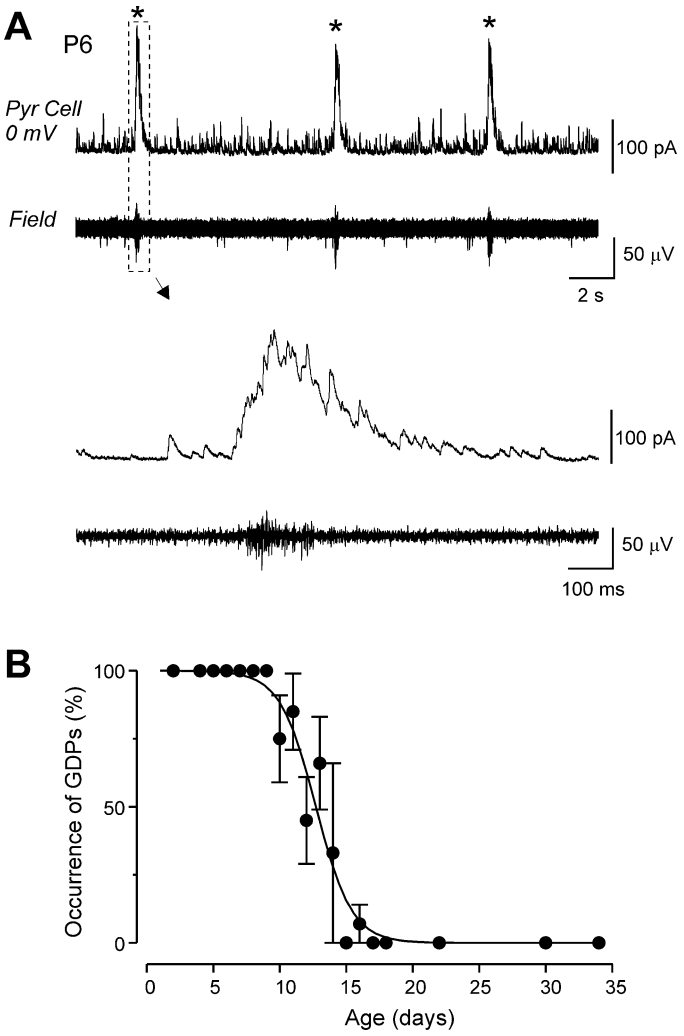


FIG. 1. Giant depolarizing potentials (GDPs) in the Sprague-Dawley rat hippocampal slices. (A) Simultaneous whole-cell recordings from a CA3 pyramidal cell and extracellular field potential recordings from the CA3 pyramidal cell layer in a P6 hippocampal slice. Spontaneous activity in the pyramidal cell is characterized by recurrent collective polysynaptic GABA(A)-mediated events (marked by asterisks), so-called GDPs (Ben-Ari *et al*., 1989), which are associated with bursts of action potentials in the extracellular field potential recordings. The GDP outlined with a dashed box is shown below on an expanded time scale. Whole-cell recordings were made with low-chloride internal solution so that at a holding potential of 0 mV the GABA(A)-mediated postsynaptic currents (PSCs) were outwardly directed. (B) Age-dependence of the occurrence of GDPs, based on recordings from 71 slices from Sprague-Dawley rats. Occurrence of GDPs is fitted by a Boltzman function. Note that GDPs are present during two postnatal weeks and the midpoint of disappearance (i.e. when only 50% of slices show GDPs) is around P12.7 ± 0.3.

Developmental profile of the high-potassium-induced epileptiform activity

In the second series of experiments, we studied the developmental profile of epileptiform activity induced in the hippocampal slices from Sprague-Dawley rats by elevation of the extracellular potassium concentration from 3.5 mM in control to 8.5 mM. We chose the high-potassium model of epileptogenesis, which is particularly attractive because elevation of $[K^+]_o$ naturally occurs during seizures and contributes to seizure generation (Zuckermann & Glaser, 1968; Rutecki *et al*., 1985; Traynelis & Dingledine, 1988; Leschinger *et al*., 1993; Jefferys, 1995; Jensen & Yaari, 1997). Figure 2 shows examples of the earliest seizure-like events (SLEs) induced by elevated potassium in P7 and P8 slices. SLEs typically started 5–15 min following exposure to high potassium and recurred with 3–4 min intervals. Each SLE started with an initial bursting discharge, followed by a tonic-like discharge phase, clonic-like afterdischarge period and postictal depression (Figs 2B and C, and 3). SLEs were observed in 35% of slices within a restricted developmental window of P7–16 ($n = 28/80$; Fig. 4). During the first postnatal week (P2–6), elevation of potassium evoked interictal-like patterns of population bursts and spikes that occurred at 0.5–1 Hz ($n = 18/18$). Interictal-like activity was also evoked in the remaining P7–16 slices that did not display SLEs ($n = 52/80$) and in all slices from the older animals (P17–37; $n = 18/18$). The relationship between age and occurrence of SLEs is shown in Fig. 4B. A period of enhanced seizure susceptibility occurred through the second and the beginning of the third postnatal week, when high potassium often induced SLEs. Before and after this period, only interictal-like activity was observed. A bell-shaped age dependence of high-potassium-induced epileptiform activity was approximated with a Gaussian function revealing a peak of paroxysmal activity attained at postnatal day 11.3 ± 0.4 and decay to baseline at the beginning of the third postnatal week ($n = 116$).

Developmental profile of the GABA(A)-mediated signalling

We next determined the time course of the developmental changes in GABA(A)-mediated signalling. To this end, using extracellular metal electrodes placed in the CA3 pyramidal cell layer, we recorded MUA (that is, action potentials from multiple neurons in the vicinity of the recording electrode). To activate GABA(A) receptors, we bath-applied the selective GABA(A) receptor agonist isoguvacine (10 μM for 1 min, bath application). The effect of isoguvacine on the MUA frequency was studied in 49 slices at different ages. In slices from neonatal rats, from P0 to P10, isoguvacine invariably induced an increase in MUA that was also associated with an increase in the frequency of field GDPs. At the end of the second week, from P10 to P14, both increases and decreases in MUA were observed in different slices. In slices obtained from rats of P15 and older, isoguvacine invariably induced a decrease in MUA. Figure 5 illustrates the typical effects of isoguvacine on MUA at P8 and P16.

Since two types of neurons, pyramidal cells and interneurons, probably contribute to the MUA recorded from the pyramidal cell layer, and the development of GABA(A)-mediated signalling may differ in these two populations (Verheugen *et al*., 1999; Hennou *et al*., 2002), we also studied the effect of isoguvacine on the activity of individual CA3 pyramidal cells using cell-attached recordings obtained under visual guidance using differential contrast microscopy (Fig. 6). In slices from younger rats, brief local pressure application of isoguvacine in the region of the soma and proximal apical dendrite caused an increase in the frequency of action potentials (11/11 cells at P4–6, 15/16 cells at P10–12). In slices from older rats, isoguvacine caused a decrease in the firing of pyramidal cells (5/5 cells at P17–19). Both excitatory and inhibitory effects of isoguvacine were reversibly suppressed by the GABA(A) receptor antagonist bicuculline (10–50 μM; $n = 7$).

Age dependence in the effect of GABA(A) receptors activation on the MUA frequency is presented in Fig. 7, in which the effect of isoguvacine is presented as a ratio between MUA frequencies at the maximum of isoguvacine-induced response and in controls prior to isoguvacine application (Fig. 7A). Age dependence of the effect of isoguvacine on MUA was approximated with a Boltzman function (Fig. 7B). A developmental switch in the action of isoguvacine from excitation to inhibition as determined by the midpoint of the Boltzman fit (i.e. the age at which in half of the slices isoguvacine induced an

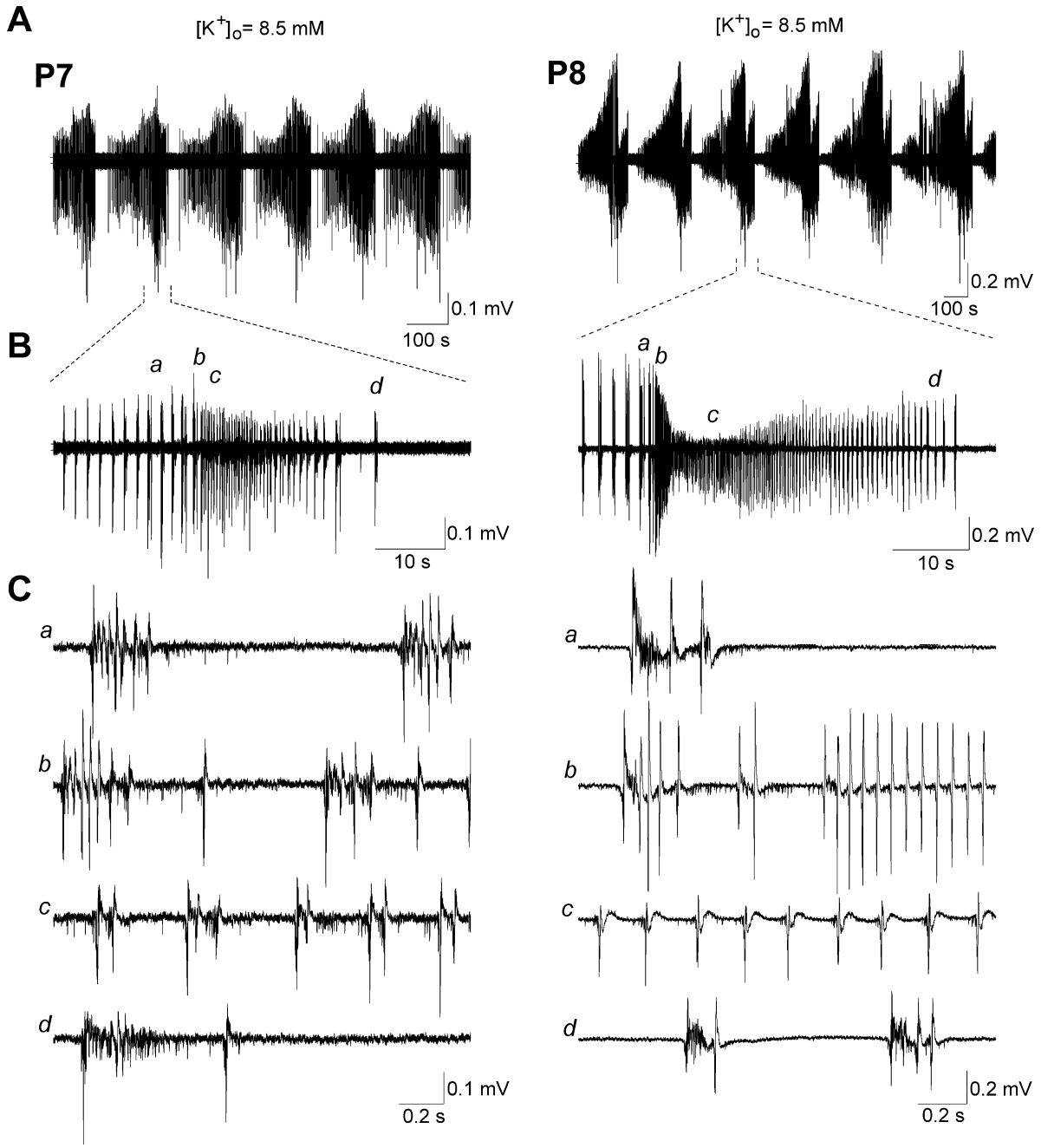


FIG. 2. Seizure-like activity induced by high potassium in the postnatal rat hippocampal slice. (A) The earliest recurrent seizure-like events (SLEs) in the presence of 8.5 mM $[K^+]_o$ were detected in (left) P7 and (right) P8 slices. (B) Examples of the earliest SLEs are shown on an expanded time scale. (C) Parts of the traces illustrating the phases of SLEs: (*a*) the initial bursting discharge; (*b*) transition to the tonic phase; (*c*) tonic-like discharge and (*d*) clonic-like afterdischarge. Note that the tonic phase (*c*) in the P7 slice is relatively short and irregular. At P8, SLEs have genuine ictal-like appearance. Extracellular field potential recordings from CA3 pyramidal cell layer.

increase in MUA and in the other half a decrease in MUA) was estimated at postnatal day 13.5 ± 0.4.

Effect of blockade of the GABA(A) receptors on the high-potassium-induced epileptiform activity

The close temporal correlation between seizure susceptibility and developmental changes in GABA(A)-mediated signalling raises the hypothesis that excitatory GABA contributes to enhanced excitability during the second postnatal week. If the assumption is correct, then blockade of GABA(A) receptors should alleviate the epileptiform activity. We therefore studied the effect of blockade of GABA(A) receptors on SLEs induced by high potassium. In this series, ictal tonic–clonic SLEs were induced by high potassium in 13 of 48 slices (average age P12.1). SLEs recurred at a frequency of $5.0 \pm 0.5 \times 10^{-3}$ Hz ($n = 13$). Addition of bicuculline (10 μM) either blocked (Fig. 8; $n = 5/13$ slices) or considerably reduced the SLEs frequency to $3.1 \pm 0.5 \times 10^{-3}$ Hz (Fig. 9; $n = 8/13$). In both cases, the incterictal activity in the presence of bicuculline was composed of regular clonic-like events.

To obtain more quantitative information on the effect of bicuculline on SLEs, we measured the frequency and amplitude of the population spikes. Blockade of GABA(A) receptors either completely (Fig. 8B) or

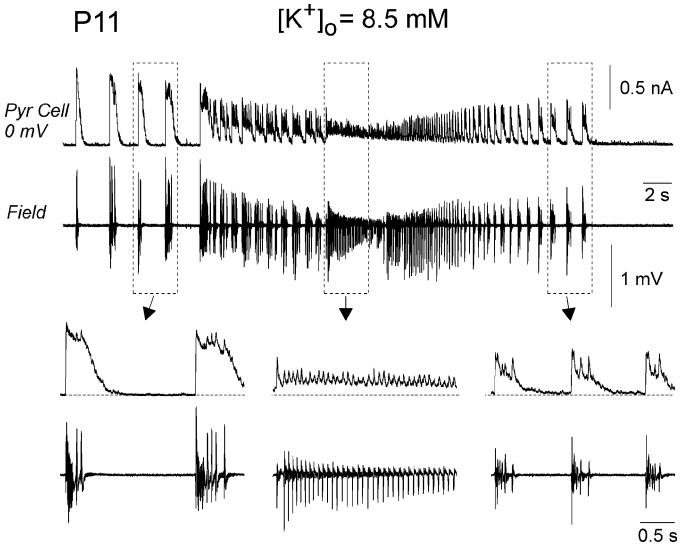


FIG. 3. GABAergic component of the seizure-like event. Simultaneous whole-cell recordings from a CA3 pyramidal cell at the glutamate reversal potential (0 mV) so that the GABA(A)-mediated postsynaptic currents are outwardly directed, and extracellular field potential recordings from CA3 pyramidal cell layer in a P11 hippocampal slice in the conditions of elevated extracellular potassium (8.5 mM). Outlined parts of seizure-like event (SLE) are shown on an expanded scale below. SLE starts with a phase of initial bursting discharge, which is followed by a tonic-like discharge phase and clonic-like afterdischarge period.

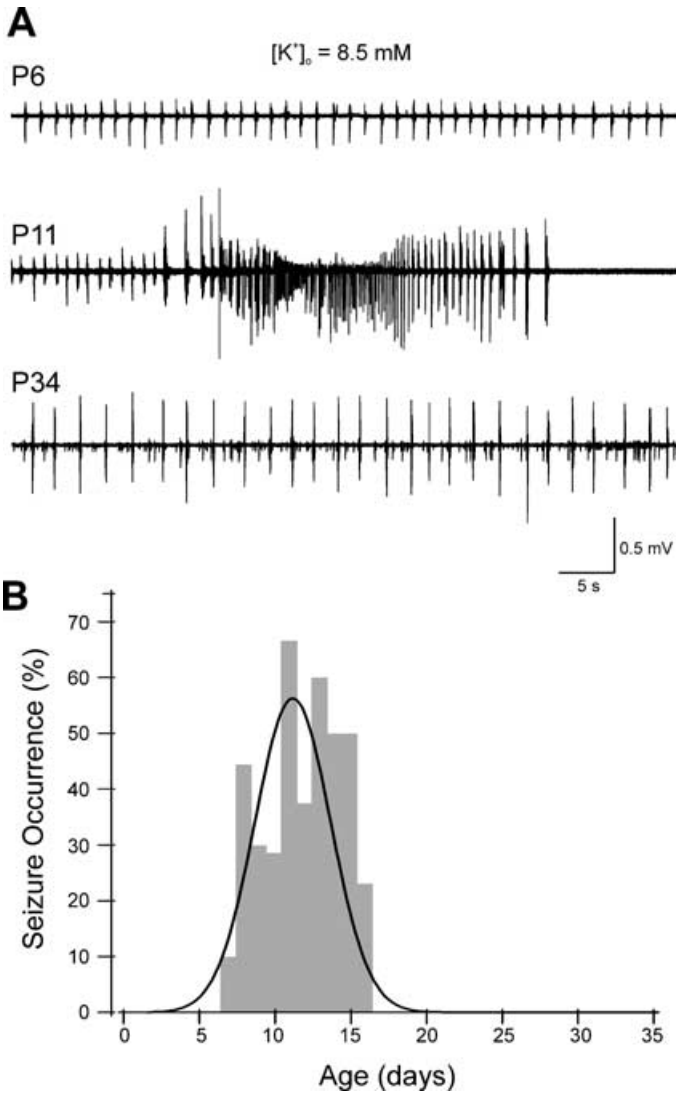


FIG. 4. Age-dependence of the occurrence of seizure-like activity evoked by high potassium. (A) Extracellular field potential recordings from the hippocampal slices in the presence of 8.5 mM $[K^+]_o$. In slices from P6 and P34 rats only interictal-like activity is observed, whereas at P11 a seizure-like event (SLE) is generated. (B) Percentage of slices generating SLEs after application of high potassium at different postnatal ages. The graph is based on extracellular recordings from CA3 pyramidal cell layer of 118 hippocampal slices of P2–37 Sprague-Dawley rats.

partly (Fig. 9B) transformed the pattern of recurrent SLEs to interictal firing. As a result, the bursts of population spikes associated with SLEs were totally suppressed or reduced in frequency. The average frequency of population spikes measured over periods of 15–20 min was also reduced after addition of bicuculline from 1.2 ± 0.3 Hz to 0.7 ± 0.1 Hz ($n = 13$; Figs 8D and 9D). Yet the amplitude of the population spikes increased after addition of bicuculline from 273 ± 45 to 414 ± 61 μV ($n = 13$; Figs 8C and E, and 9C and E). The amplitude of population spikes increased both during the SLEs and in the interictal period.

In the remaining slices from the age group P7–16, in which high potassium failed to induce SLEs and the epileptiform activity consisted of interictal population bursts or spikes, bicuculline invariably induced regular clonic-like interictal activity similar to that shown on Fig. 8 ($n = 35/48$). The interictal-like activity was often preceded by one tonic–clonic SLE at the beginning of bicuculline application (not shown).

In slices from younger (P5–6; $n = 17$) and older (P18–37; $n = 10$) rats, in which high potassium induced spontaneous population bursts or spikes but never SLEs, addition of bicuculline invariably caused regular clonic-like interictal activity (Fig. 10). In 40% of slices at P5–6 ($n = 7/17$), brief SLE occurred at the beginning of bicuculline application.

Discussion

In the present study we measured and compared the time course of the developmental changes in the excitability of the hippocampal network, both physiological and paroxysmal, and GABA(A)-mediated signalling. Figure 11 summarizes the results on age-dependence of the following parameters: (i) action of GABA(A) agonist isoguvacine on neuronal firing in the CA3 hippocampus, which characterizes the time course of the developmental switch in the GABA(A)-mediated signalling from excitation to inhibition; (ii) occurrence of GDPs, a characteristic 'immature' pattern of activity in the hippocampal slices; (iii) occurrence of the high-potassium-induced SLEs, which provides a measure of susceptibility of the hippocampus to seizures. The principal conclusion which emerges from the comparison of these three developmental curves is that the period of enhanced excitability in the rat hippocampus lies within the temporal window of the excitatory action of GABA ending with the developmental switch in the GABA(A)-mediated function from excitation to inhibition. We also showed that blockade of GABA(A) receptors reduced the frequency of or completely suppressed the high-potassium-induced SLEs, accompanied by a reduction in the frequency but an increase in the amplitude of the population spikes. Taken together with some other previous observations, these results suggest that the excitatory action of GABA contributes to enhanced excitability but that GABA also exerts inhibitory actions in the immature hippocampus.

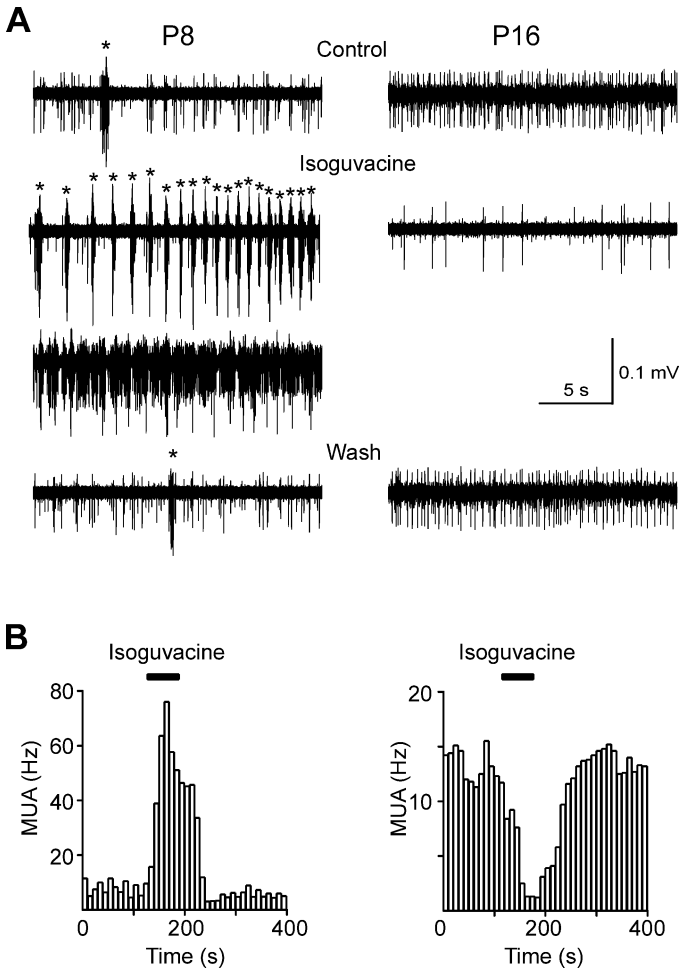


FIG. 5. Age dependence in the effect of the GABA(A) agonist isoguvacine on spontaneous neuronal firing in the rat hippocampus. (A) Bath application of the GABA(A) receptor agonist isoguvacine (10 μM for 1 min) increased the frequency of extracellularly recorded action potentials in CA3 pyramidal cell layer at P8 but decreased the frequency at P16. Field GDPs are marked with asterisks. Note that isoguvacine induced an increase in the field GDP frequency (upper trace) followed by increased asynchronous firing (lower trace). Upon wash of isoguvacine, neuronal activity returned to the control level. (B) The time course of the effect of isoguvacine on multiple unit activity (MUA) in the experiments shown above. Note that activation of GABA(A) receptors transiently increased MUA frequency (excitatory effect) at P8 and reduced MUA frequency (inhibitory effect) at P16.

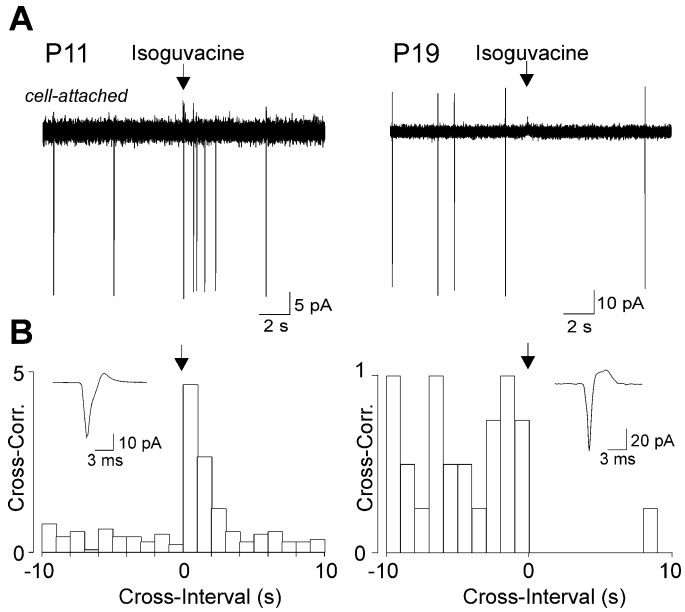


FIG. 6. Age dependence in the effect of the GABA(A) receptor agonist isoguvacine on the activity of individual CA3 pyramidal neurons. (A) Cell-attached recordings from individual CA3 pyramidal neurons from P11 (left trace) and P19 (right trace) rats. Pyramidal neurons were visualized in the hippocampal slice using differential interference contrast microscopy. Vertical deflections from the baseline are the action currents. Arrows indicate the moments of brief (<200 ms) local application of isoguvacine onto the soma and initial part of the apical dendrite of the recorded neuron. (B) Cross-correlation of the time of action currents against the time of isoguvacine applications. Averaged action currents are shown on insets. Note that isoguvacine caused an increase in firing at P11 and a decrease in firing at P19.

The developmental switch in the action of GABA via GABA(A) receptors in CA3 hippocampus from excitation to inhibition was estimated in the present study to occur at the end of the second postnatal week, at P13.5 ± 0.4. This is significantly later than the previous estimate of P5–6 which was obtained in studies using intracellular recordings (Ben-Ari *et al*., 1989). It is unlikely that this difference is due to the difference in animal species because in Wistar rats the switch also occurs later (around P10; R. Tyzio and R. Khazipov, unpublished observations). It is, however, known that intracellular recordings with sharp electrodes introduce significant error, which is due to leak conductance at the site of impalement by the electrode (Spruston & Johnston, 1992). The leak conductance artefact particularly affects neuronal excitability in small neurons (Barry & Lynch, 1991) and, in particular, causes significant neuronal depolarization in the most immature neurons (Tyzio *et al*., 2003). In addition, intracellular ionic composition is modified in intracellular recordings because of the ionic exchange between the recording electrode and cytoplasm. The noninvasive extracellular multiple unit and cell-attached recordings employed in the present study probably provide a more reliable estimate of the GABA(A)-mediated action of GABA because the neuronal membranes remain intact and neither neuronal excitability nor ionic intracellular composition are affected.

We found that the immature pattern of hippocampal electrographic activity, GDPs, is present in the hippocampal slices during the two first postnatal weeks. This observation is in agreement with the results of an *in vivo* study, in which the immature physiological pattern of recurrent spontaneous bursts was found in the neonatal rat hippocampus until the end of the second postnatal week (Leinekugel *et al*., 2002). There is considerable evidence that excitatory GABA is instrumental in the generation of GDPs (Ben-Ari *et al*., 1997; Ben-Ari, 2001, 2002). Indeed, it has been demonstrated that activation of GABA(A) receptors depolarizes and excites the hippocampal pyramidal cells and interneurons during GDPs, activates voltage-gated calcium channels and potentiates the activity of NMDA channels via removal of their voltage-dependent magnesium block (Ben-Ari *et al*., 1989; Khazipov *et al*., 1997; Leinekugel *et al*., 1997; Garaschuk *et al*., 1998; Lamsa *et al*., 2000). The present study provides further evidence that the immature hippocampal pattern is linked to the excitatory GABA by showing a remarkably tight temporal correlation between the developmental switch in the action of GABA from excitation to inhibition and disappearance of the field GDPs at the end of the second postnatal week.

Previous studies using intracellular recordings have shown that, during the second postnatal week, GDPs are transformed to GABA(A)-

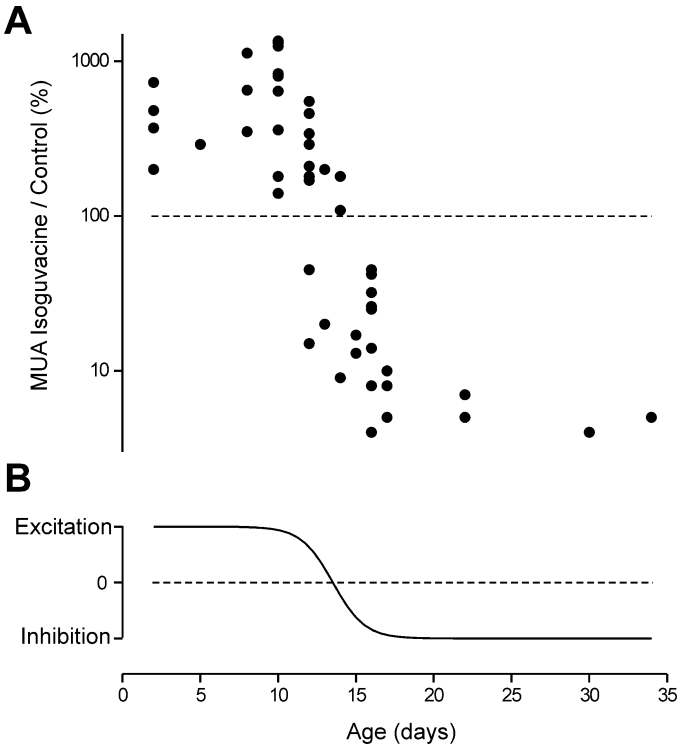


FIG. 7. Developmental switch in the GABA(A) signalling in the rat hippocampus. (A) Plot of the age dependence in the effect of isoguvacine on neuronal firing. Each point represents the ratio (as a percentage) of the MUA frequency at the peak of the isoguvacine effect to the MUA frequency in control. Pooled data from 49 slices. (B) Boltzman fit of the age dependence of the effect of isoguvacine on neuronal firing; note that the switch in GABA(A) signalling from excitation to inhibition occurs at P13.5 ± 0.4.

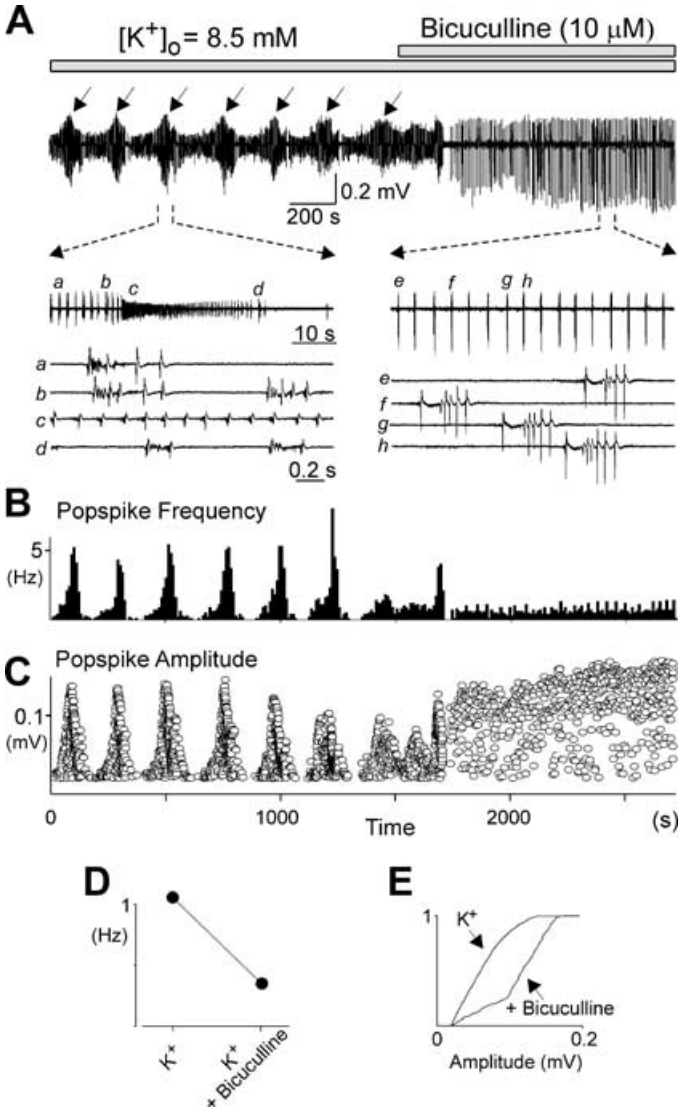


FIG. 8. Effect of the blockade of GABA(A) receptors on the high-potassium-induced epileptiform activity: suppression of SLEs. (A) Field potential recordings in P12 hippocampal slices in the presence of high potassium (8.5 mM) and following further addition of the GABA(A) receptor antagonist bicuculline (10 μM). Tonic–clonic seizure-like events (SLEs) are marked by arrows. Note that after addition of bicuculline SLEs were transformed to clonic-like interictal activity. Below, one of SLEs before the addition of bicuculline and the interictal activity after addition of bicuculline are shown on an expanded time scale. Parts of the traces illustrating the phases of (*a,b*) the initial bursting discharge, (*c*) tonic-like discharge and (*d*) clonic-like afterdischarge; (*e-h*) the stereotypic clonic-like interictal events are shown below. On the plots below are shown: (B) the corresponding frequency of the population spikes (averaged over 10 s) and (C) the corresponding amplitude of the population spikes. (D) Average frequency of the population spikes; (E) cumulative histogram of the population spike amplitude. D and E were obtained from analysis of 1000-s epochs before and after addition of bicuculline. Note that blockade of GABA(A) receptors caused a reduction in the average frequency but an increase in the amplitude of the population spikes with moderate changes in the amplitude of power of the field potential.

mediated large hyperpolarizing potentials (LHPs) that correspond in time with the switch in GABA(A) action from excitation to inhibition at P5–6 (Ben-Ari *et al*., 1989). The fact that an increase in MUA in the pyramidal cells layer is associated with the network GDPs until the end of the second week suggests that, during the second postnatal week, a significant proportion of pyramidal cells and interneurons are activated during network GDPs and contribute to their generation (GDP cells). The collective discharge of GDP cells is seen as a burst of action potentials in field potential recordings. Remaining neurons (LHP cells) are inhibited during network GDPs and therefore do not contribute to their generation. GDP cells are probably less mature neurons with excitatory GABA and LHP cells are more mature neurons with inhibitory GABA responsiveness. With age, GDP cells are transformed to LHP cells as a result of the excitatory–inhibitory switch in the action of GABA. At the end of the second week, a proportion of GDP cells with excitatory GABA responsiveness falls below the critical number required for generation of network discharge and the immature pattern of GDPs ceases. This hypothesis is in agreement with the efficiency of glutamate receptor antagonists in suppressing LHPs during the second postnatal week (Ben-Ari *et al*., 1989) and an increase in field GDP frequency in response to isoguvacine during the first and second postnatal week which has been observed in the present study. Further studies using noninvasive recordings from single neurons are required to verify this hypothesis.

Using the high-potassium model of epileptiform activity we found a bell-shaped developmental curve of the seizure susceptibility with a peak at P11.3 ± 0.4. These results obtained in the *in vitro* model correlate well with the age of hypoxia- (Jensen *et al*., 1991) and fever- (Baram *et al*., 1997) induced seizures *in vivo,* in which the peak of excitability was observed between P10 and P12. It is important to stress that both of the above-mentioned *in vivo* models and the high-potassium *in vitro* model used here are 'physiological' in the sense that none of the precipitating factors directly affects GABA(A) receptors. The developmental window of hyperexcitability, from P7 to P16, when

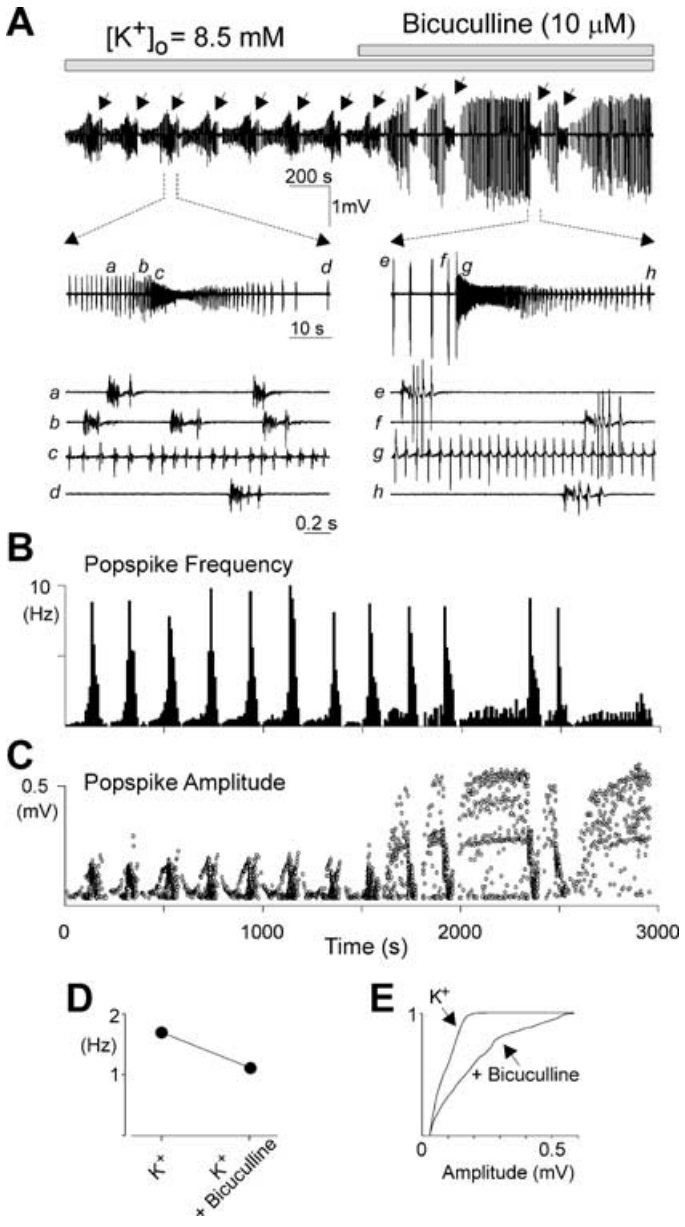


FIG. 9. Effect of the blockade of GABA(A) receptors on the high-potassium-induced epileptiform activity: persistence of SLEs. (A) Field potential recordings in P9 hippocampal slices. Note that SLEs still occurred after addition of bicuculline, yet at lower frequency. Below, two of the SLEs before and after addition of bicuculline are shown on an expanded time scale. On the plots below are shown: (B) the corresponding frequency of the population spikes (averaged over 10 s) and (C) the amplitude of the population spikes. (D) Average frequency of the population spikes; (E) cumulative histogram of the population spike amplitude. D and E were obtained from analysis of 1400-s-long epochs before and after addition of bicuculline. Note that blockade of GABA(A) receptors caused a reduction in the average frequency but an increase in the amplitude of the population spikes.

high potassium induced tonic–clonic SLEs, is close to that reported in a recent study (P4–16) using the high-potassium model in thick hippocampal slices (600–700 μm vs. 500 μm-slices used in the present study) (Dzhala & Staley, 2003). Interestingly, blockade of GABA(A) receptors in that report completely suppressed SLEs induced by high potassium. In the present study, while SLEs were completely suppressed by a GABA(A) antagonist in some slices (5/13), in many slices (8/13) SLEs persisted, although at lower frequency. We also found that after blockade of GABA(A) receptors the average frequency of the population spikes was reduced. These observations support the conclusion that excitatory GABA is causally linked to ictal activity in this developmental window. On the other hand, several lines of evidence indicate that during the early developmental stage GABA exerts inhibitory as well as excitatory actions. First, we found that the amplitude of population spikes composing the epileptiform events significantly increased after blockade of GABA(A) receptors. Second, in the proportion of slices in which high potassium induced only interictal-like population bursts or spikes but not SLEs, blockade of GABA(A) receptors caused an increase in the frequency and amplitude of the population spikes and typically caused one SLE at the beginning of bicuculline application. Third, blockade of GABA(A) receptors by itself, in the absence of any other epileptogenic agent, causes interictal-like activities from P0 and ictal-like SLEs from P2 in the intact hippocampus *in vitro* (Khalilov *et al*., 1997b; Khalilov *et al*., 1999), interictal-like activity in the hippocampal slices from P0 (Khalilov *et al*., 1999; Lamsa *et al*., 2000; Wells *et al*., 2000) and SLEs in slices at ages of P9–19 (Swann & Brady, 1984; Gomez-Di Cesare *et al*., 1997). Finally, drugs increasing GABA(A) function, such as barbiturates and benzodiazepines, suppress seizures in neonatal rats (Smythe *et al*., 1988; Kubova & Mares, 1991; Velisek *et al*., 1995; Kubova *et al*., 1999). Taken together, these observations suggest a complex contribution of GABA to the initiation and generation of paroxysmal activity in the immature hippocampus. On one hand, GABA excites the immature neurons because the GABA(A)-mediated signals depolarize the immature neurons above the action potential threshold. On the other hand, depolarizing GABA also inhibits because of a shunting mechanism due to an increase in membrane conductance as a result of opening of large number of GABA(A) channels and voltage-gated K^+ channels as well as because of Na^+ channel inactivation (Staley & Mody, 1992; Lu & Trussell, 2001). These dual effects of GABA probably explain the complexity of the observed phenomena during the developmental period of enhanced excitability.

In adult neurons, massive activation of GABA(A) receptors causes a depolarizing shift of the reversal potential of the GABA(A)-mediated responses (Barker & Ransom, 1978; Alger & Nicoll, 1979; Andersen *et al*., 1980; Alger & Nicoll, 1982), the phenomenon probably due to bicarbonate permeability of GABA(A) channels (Kaila, 1994; Staley *et al*., 1995; Perkins & Wong, 1996; Staley & Proctor, 1999) enhanced by extracellular potassium accumulation (Kaila *et al*., 1997; Smirnov *et al*., 1999). Two aspects of the present study may have relevance to this phenomenon. First, the depolarizing shift in the GABA(A)-mediated responses could have occurred in the experiments with bath application of isoguvacine. However, in the present study we did not observe an increase in multiple unit activity following isoguvacine application in adult slices. Moreover, similar age-dependence in the effect of GABA(A) receptor activation on neuronal firing was observed in the experiments with cell-attached recordings from single cells, in which local and brief applications of isoguvacine were provided from the micropipette. Therefore, the developmental curve of the changes in GABA(A) signalling is unlikely to be biased by the phenomenon of the use-dependent depolarizing shift of the reversal of GABA(A)-mediated responses. Second, there is considerable evidence that the dynamic switch in the action of GABA from inhibitory to excitatory as a result of massive release of GABA occurs during a seizure in adult animals (Avoli *et al*., 1996; Lopantsev & Avoli, 1998). Moreover, it has been demonstrated that GABA(A)-mediated excitation contributes substantially to to neuronal synchronization during SLEs in the low-magnesium model

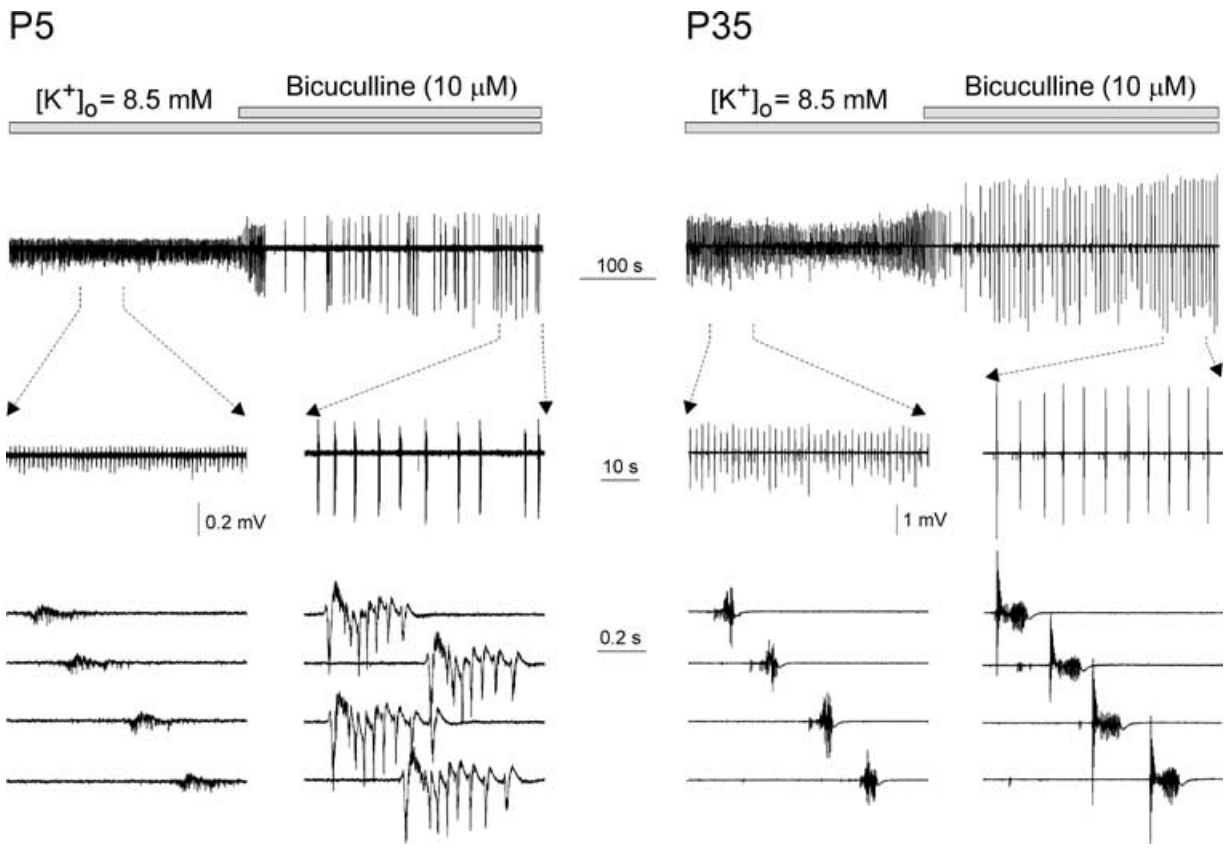
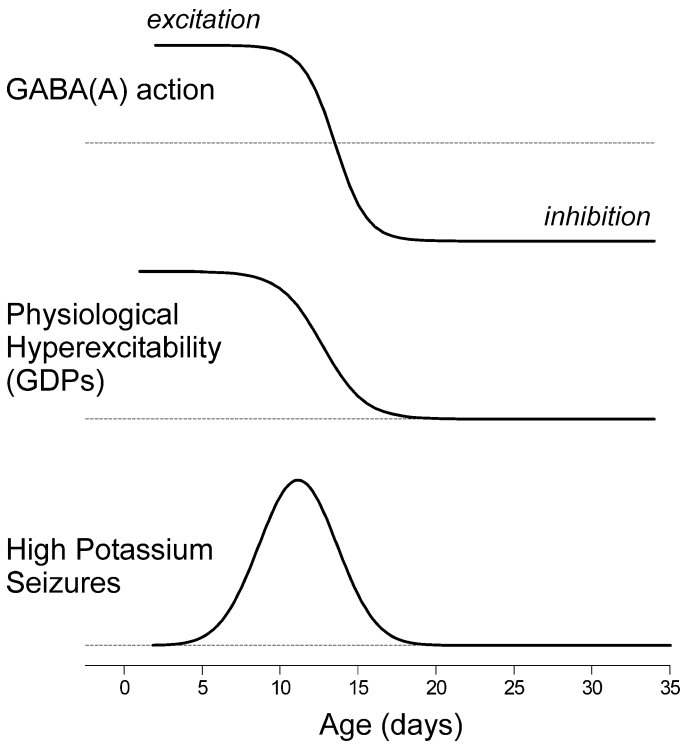


FIG. 10. Effect of the blockade of GABA(A) receptors on the high-potassium-induced interictal activity. Field potential recordings in P5 and P35 hippocampal slices in the conditions of elevated potassium and after addition of bicuculline. Middle traces show 1-min sweeps from the upper traces; the four bottom 1-s sweeps show examples of interictal events from the middle traces. Note that at P5 bicuculline transformed interictal activity from spontaneous population bursts to clonic-like events. Both at P5 and P35, after addition of bicuculline, interictal-like events increased in amplitude and duration yet their frequency decreased.



(Kohling *et al.*, 2000), during SLEs emerging following long-term exposure to the GABA(B) receptor antagonists (Uusisaari *et al.*, 2002), during the post-tetanic afterdischarges (Fujiwara-Tsukamoto *et al.*, 2003) and interictal activity in slices of subiculum from TLE patients (Cohen *et al.*, 2002). A fundamental age difference in the GABA(A)-depolarizing responses is that, in adult neurons, GABA is hyperpolarizing at the resting state in the majority of neurons (but see Staley & Mody, 1992; Wagner *et al.*, 1997; Bazhenov *et al.*, 1999; Lu & Trussell, 2001; Martina *et al.*, 2001; Gulledge & Stuart, 2003) and becomes depolarizing or excitatory only as a result of intense activation of GABA(A) receptors. In contrast, in the immature neurons, because of the delayed expression of chloride extruder KCC2 (Misgeld *et al.*, 1986; Jarolimek *et al.*, 1999; Rivera *et al.*, 1999; Payne *et al.*, 2003), GABA(A)-mediated responses are depolarizing or excitatory at rest. This is likely to be one of the major factors contributing to enhanced seizure susceptibility.

FIG. 11. Developmental profile of the GABA(A)-mediated actions and excitability in Sprague-Dawley hippocampus.The immature pattern of GDPs is present during the period of excitatory action of GABA. Age dependence of high-potassium-induced epileptiform activity is bell-shaped, with the maximum during the second postnatal week. The developmental switch in the action of GABA(A) from excitation to inhibition coincides with the end of the critical period of excitability. Curves represent the Boltzman and Gaussian fits of the data presented in Figs 1, 4 and 7.

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Abbreviations

ACSF, artificial cerebrospinal fluid; GABA, gamma-aminobutyric acid; GDP, giant depolarizing potential; LHP, large hyperpolarizing potential; MUA, multiple-unit activity; P, postnatal day; SLE, seizure-like event.

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