Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus

Olivier Caillard, Yehezkel Ben-Ari and Jean-Luc Gaiarsa

Institut National de la Santé et de la Recherche Médicale, Unité 29, Hôpital de Port-Royal, 123 Boulevard de Port-Royal, 75014 Paris cedex, France

(Received 4 January 1999; accepted after revision 15 March 1999)

1. The plasticity of GABAergic synapses was investigated in neonatal rat hippocampal slices obtained between postnatal days 3 and 6 using intracellular recording techniques. Ionotropic glutamate receptor antagonists were present throughout the experiments to isolate GABA_A receptor-mediated postsynaptic potentials (GABA_A PSPs) or currents (GABA_A PSCs).

2. Repetitive depolarizing pulses (20 pulses, 0.5 s duration, at 0.1 Hz, each pulse generating 4–6 action potentials) induced a long-term potentiation in the slope and amplitude of the evoked GABA_A PSPs and GABA_A PSCs.

3. Long-term potentiation was prevented by intracellular injection of the calcium chelator BAPTA (50 mM), or when the voltage-dependent calcium channels blockers Ni²⁺ (50 μM) and nimodipine (10 μM) were bath applied.

4. Repetitive depolarizing pulses induced a persistent (over 1 h) increase in the frequency of spontaneous GABA_A PSCs.

5. Repetitive depolarizing pulses induced a long-lasting increase in the frequency of miniature GABA_A PSCs, without altering their amplitude or decay-time constant.

6. It is concluded that the postsynaptic activation of voltage-dependent calcium channels leads to a long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. This form of plasticity is expressed as an increase in the probability of GABA release or in the number of functional synapses, rather than as an upregulation of postsynaptic GABA_A receptor numbers or conductance at functional synapses.

The activity-dependent plasticity of glutamatergic synapses has been described extensively (Nicoll & Malenka, 1995) and is believed to play a crucial role in learning and memory processes. Because activity-dependent plasticity of GABAergic synaptic transmission could also modify the input–output relationship of the neurones, study of this form of plasticity is essential.

Both long-term potentiation and long-term depression of GABAergic synaptic transmission have been reported in hippocampal (Stelzer et al. 1987; 1994; McLean et al. 1996), cortical (Komatsu, 1994) and cerebellar (Kano et al. 1992; Kano et al. 1996) neurones. While a postsynaptic rise in intracellular calcium concentration appears to be a common trigger for the induction of long-term changes in the strength of GABAergic synaptic transmission (Kano et al. 1992; Komatsu, 1996; Hashimoto et al. 1996; McLean et al. 1996; Morishita & Sastry, 1996), the mechanisms underlying the expression may differ.

Long-term changes in synaptic efficacy may be expressed as presynaptic alterations in neurotransmitter release or as postsynaptic modifications in the sensitivity to released neurotransmitter. One approach to address postsynaptic modifications is to measure the amplitude of responses induced by application of neurotransmitter agonist. This method does not, however, provide compelling evidence since exogenously applied agonist may activate extrasynaptic receptors that could be under different modulatory control than the synaptic ones (Boxall & Marty, 1997). A more direct approach is to measure the amplitude and frequency of spontaneous synaptic currents that occur independently of action potential firing. A change in the amplitude of these events, referred to as miniature postsynaptic currents, is usually considered to reflect a postsynaptic modification, while a change in their frequency is considered to reflect a presynaptic modification. This method, however, requires that a significant number of synapses impinging on the recorded neurone are affected to make postsynaptic modifications detectable.

In a previous study we reported that early in development GABAergic synaptic transmission expresses a calcium-dependent bidirectional plasticity in the neonatal rat hippocampus (McLean et al. 1996). Thus, concomitant activation of GABA_A and NMDA receptors during a high-
frequency stimulation leads to long-term depression of GABAergic synaptic transmission, while activation of only GABA_A receptors leads to a long-term potentiation of GABAergic synaptic transmission. The long-term potentiation of GABAergic synaptic transmission requires a membrane depolarization, provided by the activation of GABA_A receptors, and a rise in intracellular calcium concentration, probably resulting from an influx of calcium through voltage-dependent calcium channels. In the present study, we show that direct activation of postsynaptic voltage-dependent calcium channels in the absence of synaptic stimulation results in a long-term potentiation (LTP) of evoked and spontaneous GABA_A receptor-mediated postsynaptic potentials or currents in neonatal rat CA3 pyramidal neurones (LTP_GABA_A). The conditioning stimulus also leads to a long-lasting increase in the frequency, but not amplitude, of spontaneous and miniature GABA_A receptor-mediated postsynaptic currents. Therefore, LTP_GABA_A is expressed as an increase in the probability of GABA_A release or in the number of functional GABAergic synapses, but not as an upregulation of postsynaptic GABA_A receptors at previously functional GABAergic synapses.

METHODS

Experiments were performed on hippocampal CA3 pyramidal neurones obtained from postnatal day P3–P6 male Wistar rats. Brains were removed under ether anaesthesia and submerged in cold (0 °C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 126; KCl, 3·5; CaCl_2, 1·3; NaH_2PO_4, 1·2; NaHCO_3, 25; and glucose, 11; pH 7·4 when equilibrated with 95% O_2/5% CO_2. Hippocampal slices (600 μm thick) were cut with a McIlwain tissue chopper, incubated for at least 1 h in ACSF at room temperature (20–23 °C), and transferred individually into a submerged recording chamber superfused with ACSF (2.5–3 ml min^-1, 34 °C).

Intracellular recordings were performed with electrodes filled with 3 mM KCl (current-clamp mode, 50–60 MΩ) or 2 mM CsCl (voltage-clamp mode, 40–50 MΩ). In some experiments BAPTA (50 mM, dissolved in 3 mM KCl, 50–60 MΩ) was iontophoretically applied to the recorded cells (−0.5 nA, 500 ms, 10–30 min). Cells were considered to be BAPTA loaded when both spike-frequency stimulation leads to long-term depression of GABAergic synaptic transmission requires a membrane depolarization, provided by the activation of GABA_A receptors, and a rise in intracellular calcium concentration, probably resulting from an influx of calcium through voltage-dependent calcium channels. In the present study, we show that direct activation of postsynaptic voltage-dependent calcium channels in the absence of synaptic stimulation results in a long-term potentiation (LTP) of evoked and spontaneous GABA_A receptor-mediated postsynaptic potentials or currents in neonatal rat CA3 pyramidal neurones (LTP_GABA_A). The conditioning stimulus also leads to a long-lasting increase in the frequency, but not amplitude, of spontaneous and miniature GABA_A receptor-mediated postsynaptic currents. Therefore, LTP_GABA_A is expressed as an increase in the probability of GABA_A release or in the number of functional GABAergic synapses, but not as an upregulation of postsynaptic GABA_A receptors at previously functional GABAergic synapses.

RESULTS

Repetitive depolarizing pulses induce a long-term potentiation of GABA_A receptor-mediated synaptic potentials

To investigate the effects of depolarizing pulses on GABAergic synaptic transmission the following protocol was used. Evoked monosynaptic GABA_A receptor-mediated postsynaptic potentials or currents, hereafter referred to as GABA_A PSPs or GABA_A PSCs, were isolated in the presence of the ionotropic glutamatergic receptor antagonists CNQX (10 μM) and d-AP5 (50 μM) (Fig. 1A). After stable baseline responses were obtained for at least 10 min, 20 depolarizing pulses (from −90 to −40 ± 5 mV, 0·5 s duration), each pulse generating 3–6 action potentials, were applied to CA3 pyramidal cells at a frequency of 0·1 Hz. The depolarizing pulses were applied in the absence of synaptic stimulation (Fig. 1B). This conditioning stimulus induced a long-term increase in both the amplitude and slope of evoked GABA_A PSPs. A typical result of such an experiment is shown in Fig. 1A–D: the slope of the evoked GABA_A PSPs slowly increases to reach a maximal value of about 150% of the control value 2 min after the conditioning stimulus and then remains increased for 60 min (Fig. 1D). The effect of depolarizing pulses on evoked GABA_A PSPs was highly reproducible, occurring in 10 out of 13 cells tested. On average, the slope of the evoked GABA_A PSPs was increased by 165 ± 5% (range, 130–180%) of the control value 60 min after the depolarizing pulses (4·1 ± 0·8 mV ms⁻¹ vs. 6·9 ± 0·6 mV ms⁻¹, n = 10, P = 0·013; Fig. 1E) without any changes in membrane input resistance (97 ± 5 MΩ).
96 ± 3 MΩ, n = 10, P = 0.88; Fig. 1A), reversal potential (0.1 ± 1.3 vs. 0.8 ± 1.2 mV, n = 5, P = 0.34, not shown but see Fig. 3D and E) nor latency to peak (16.4 ± 2.1 vs. 15.7 ± 1.8 ms, n = 10, P = 0.4; Fig. 1A) of GABA<sub>A</sub> PSPs. As shown in Fig. 1A and D, the potentiated PSPs were completely blocked by bath application of bicuculline (10 μM), showing that they were entirely mediated by the activation of GABA<sub>A</sub> receptors. The long-term potentiation of GABA<sub>A</sub> PSPs induced by repetitive depolarizing pulses will be referred to hereafter as LTP<sub>gaba</sub>.

LTP<sub>gaba</sub> requires the activation of voltage-dependent calcium channels

Most forms of synaptic plasticity require a postsynaptic rise in intracellular calcium concentration ([Ca<sup>2+</sup>]) (Kano et al. 1992; Nicoll & Malenka, 1995; Komatsu, 1996; McLean et al. 1996). To test the involvement of a postsynaptic calcium rise in the induction of LTP<sub>gaba</sub>, the calcium chelator BAPTA (50 μM) was added to the recording solution. The effectiveness of the injection into the recorded neuron was monitored by observation of the spike frequency adaptation and the slow after-hyperpolarizing potential (Fig. 2A). When CA3 pyramidal neurons were loaded with BAPTA, repeated depolarizing pulses (inset in Fig. 2C) failed to induce an increase in the slope of evoked GABA<sub>A</sub> PSPs (3.6 ± 0.7 vs. 4.0 ± 1.6 mV ms<sup>−1</sup> 30 min after the conditioning stimulus, n = 5, P = 0.39) (Fig. 2B and C). Therefore a postsynaptic rise in [Ca<sup>2+</sup>] is required for the induction of LTP<sub>gaba</sub>.

Since NMDA receptors were blocked, a likely mechanism for the rise in [Ca<sup>2+</sup>] is the activation of voltage-dependent calcium channels (VDCCs) during depolarization of the neurons. To test this hypothesis, we investigated the effect of the VDCC blockers Ni<sup>2+</sup> (50 μM) and nimodipine (10 μM), on the induction of LTP<sub>gaba</sub>. In the presence of

---

Figure 1. LTP<sub>gaba</sub> is induced by repeated depolarizations

A, superimposed mean of 10 consecutive evoked GABA<sub>A</sub> PSPs recorded at the times marked by the numbers on the graph (D). In this and the following figure, CNQX (10 μM) and d-AP5 (50 μM) were present throughout the experiment. The input resistance of the cell was monitored as a negative deflection to a −0.3 nA current injection. Membrane potential, −80 mV. B, standard protocol used to elicit LTP. Twenty depolarizing pulses were applied at a frequency of 0.1 Hz. The stimulation was stopped during the conditioning stimulus. C, superimposed evoked GABA<sub>A</sub> PSPs show a clear increase in the initial slope following depolarizing pulses. D, time course of changes in the slope of evoked GABA<sub>A</sub> PSPs following depolarizing pulses and during the application of bicuculline (open bar, 10 μM Bicu) (same cell as in A and B). E, mean time course of changes in the slope of evoked GABA<sub>A</sub> PSPs following depolarizing pulses (n = 10).
the VDCC blockers, the slope of the evoked GABA_A PSPs remained unchanged 30 min after the conditioning stimulus: 99.3 ± 3% of the control value (4.1 ± 0.5 vs. 3.8 ± 0.5 mV ms⁻¹, n = 5, P = 0.3) (Fig. 2D and E). We observed, however, a short-lasting (around 5 min) transient depression of evoked GABA_A PSP amplitude (68 ± 5%, 2 min after the conditioning stimulus). These results contrast with the usual increase in the slope of evoked GABA_A PSPs observed in interleaved control slices (154 ± 6%, 30 min after depolarizing steps, n = 3).

**LTP**<sub>GABA_A</sub> is associated with a long-lasting increase in the frequency of spontaneous GABA_A PSCs

To study the locus of **LTP**<sub>GABA_A</sub> expression, we investigated the effect of depolarizing pulses on spontaneous GABA_A PSCs. We first verified that **LTP**<sub>GABA_A</sub> could be induced in the voltage-clamp mode. Neurones were held at −80 mV in the presence of CNQX (10 μM) and d-AP5 (50 μM) (Fig. 3A) except during the conditioning stimulus, which was applied in the current-clamp mode (Fig. 3B). An example of such an experiment is shown in Fig. 3A–C. Three minutes after the application of depolarizing pulses, a stable long-lasting increase in amplitude of the evoked GABA_A PSCs was observed (120 ± 7 to 160 ± 2 pA, 40 min after the depolarizing pulses; Fig. 3C). The long-lasting increase (over 1 h) in the amplitude of the evoked GABA_A PSCs was not associated with a significant change in their reversal potential as shown in Fig. 3D and E. The group data from five neurones revealed that the amplitude of evoked GABA_A PSCs increased to 139 ± 8% of control values (range, 124–166%) and that their reversal potential was −1 ± 1.3 mV before and 0.72 ± 1 mV 30 min after repeated depolarizing pulses (P = 0.2).

**Figure 2.** **LTP**<sub>GABA_A</sub> requires the activation of postsynaptic voltage-dependent calcium channels

A, soon after impalement (left panel, control) a depolarizing current pulse evoked a burst of action potentials followed by an after-hyperpolarizing potential (AHP). Fifteen minutes after 50 mM intracellular BAPTA iontophoresis (right panel, BAPTA), the same current injection induced a sustained spike discharge with an almost undetectable AHP. Membrane potential, −65 mV. B, superimposed mean of 10 consecutive evoked GABA_A PSPs before (1) and 30 min after (2) the application of depolarizing pulses in a BAPTA-loaded cell. Membrane potential, −60 mV. C, mean time course of changes in the slope of evoked GABA_A PSPs following depolarizing pulses in BAPTA-loaded cells (n = 5). The inset illustrates the response of a cell to one of the 20 depolarizing pulses applied to elicit change in the slope of GABA_A PSPs. D, superimposed mean of 10 consecutive evoked GABA_A PSPs before (1) and 30 min after (2) the application of repeated depolarizing pulses in the presence of Ni²⁺ (50 μM) and nimodipine (10 μM). Membrane potential, −75 mV. E, mean time course of changes in the slope of evoked GABA_A PSPs after depolarizing pulses in the presence of Ni²⁺ (50 μM) and nimodipine (10 μM) (n = 5). The inset illustrates the response of a cell to one of the 20 depolarizing pulses applied to elicit change in the slope of GABA_A PSPs.
Concurrent with the LTP of evoked GABA \(_A\) PSC amplitude, depolarizing pulses also resulted in a persistent increase in the frequency of spontaneous GABA \(_A\) PSCs (see Fig. 4A; \(n = 6\)). On average, the inter-events interval decreased from 0·12 ± 0·03 to 0·07 ± 0·01 s 60 min after the depolarizing pulses (\(n = 6\), \(P = 0·03\)). Due to the low incidence of single events it was possible to quantify the effect of depolarizing pulses on the amplitude of spontaneous GABA \(_A\) PSCs in only three cells. Results from one of these cells are illustrated in Fig. 4. Following depolarizing steps, the inter-events interval decreased from 0·177 ± 0·008 s (\(n = 332\)) to 0·097 ± 0·003 s (\(n = 662\)) \((P = 0·0001)\) (Fig. 4A) as illustrated by the shift to the left of the cumulative inter-events interval distribution (Fig. 4B). In the same cell, the mean amplitude of spontaneous GABA \(_A\) PSCs was not statistically increased: from 44·3 ± 1·6 pA (\(n = 332\)) to 46·8 ± 0·9 pA (\(n = 662\)) \((P = 0·53)\) (Fig. 4C). Pooled data from the three cells in which analysis of amplitude was possible gave a mean decrease in the inter-events interval of 69 ± 5% of the control value (range, 55–81%), whereas the amplitude remained constant (103 ± 4% of control value). The observation that depolarizing pulses increased the frequency, but not the amplitude, of spontaneous GABA \(_A\) PSCs suggests that a postsynaptic upregulation of GABA \(_A\) receptors is not involved in the expression of LTP\(_{\text{GABA}_A}\).

**LTP\(_{\text{GABA}_A}\) is associated with a long-lasting increase in the frequency, but not the amplitude, of miniature GABA \(_A\) PSCs**

The fact that depolarizing pulses affect spontaneous GABA \(_A\) PSCs strongly suggests that the majority, if not all of the GABAergic synapses impinging on the recorded neurones were potentiated. Therefore, going a step further in elucidating the mechanisms of LTP\(_{\text{GABA}_A}\) expression, we investigated the effect of depolarizing pulses on the amplitude and frequency of miniature GABA \(_A\) PSCs (mGABA \(_A\) PSCs). mGABA \(_A\) PSCs were isolated in the

**Figure 3. LTP\(_{\text{GABA}_A}\) is not associated with a change in the reversal potential of GABA \(_A\) PSCs**

A, low time scale recording of the GABA \(_A\) PSCs. The arrow marks the time during which the conditioning protocol was applied. B, same cell as in A. Standard protocol was used to elicit LTP. Twenty depolarizing pulses (marked by the arrow in A) were applied in current-clamp mode at a membrane potential of −90 mV and a frequency of 0·1 Hz. Stimulation was stopped during depolarization. C, same cell as in A and B showing the mean of 5 consecutive evoked GABA \(_A\) PSCs recorded at the times marked by the numbers in A. D, superimposed evoked GABA \(_A\) PSCs recorded at different holding potentials (0 mV, −20 mV, −80 mV, −100 mV and −120 mV) before (control) and 40 min after (potentiated) the application of depolarizing pulses. E, current–voltage curve of the traces depicted in D. The control and potentiated GABA \(_A\) PSCs reversed polarity at −1 and −2 mV, respectively.
presence of TTX (1 μM) and the ionotropic glutamatergic receptor antagonists CNQX (10 μM) and d-AP5 (50 μM). In order to activate VDCCs in the presence of TTX, the experiments were performed with CsCl-filled electrodes and potassium channel blockers (2 mM Cs⁺ and 10 mM TEA) were added to the ACSF. mGABA_A PSCs ranged in amplitude from 15 to 115 pA at a holding potential of −100 mV, occurred at a frequency of approximately 0.5 Hz, and were completely abolished by bicuculline (10 μM; not shown). Repeated depolarizing voltage pulses (20 pulses, 0.1 Hz, 500 ms duration) were applied from −90 to −10 mV at a holding potential of −100 mV.

**Figure 4. LTP_GABA_A is associated with an increase in spontaneous GABA_A PSC frequency**

A, spontaneous GABA_A PSCs recorded before (left, control) and 30 min after (right, potentiated) repeated depolarizations. Holding potential, −100 mV. B, cumulative probability plots of inter-events interval of control (●) and potentiated (○) spontaneous GABA_A PSCs (same cell as in A). C, mean of 30 consecutive spontaneous GABA_A PSCs before (1) and 30 min after (2) repeated depolarizations (same cell as in A).

**Figure 5. LTP_GABA_A is associated with an increase in the frequency of mGABA_A PSCs**

A, standard protocol used to investigate the effect of repeated depolarizing pulse on mGABA_A PSCs. The recording was performed at a holding potential of −100 mV in the presence of TTX (1 μM), CNQX (10 μM), d-AP5 (50 μM), Cs⁺ (2 mM) and TEA (10 mM). Twenty depolarizing voltage pulses were applied from −90 to −10 mV (500 ms duration) at a frequency of 0.1 Hz. B and C, current sweeps collected from the same cell before (B) and 30 min after (C) application of depolarizing pulses.
from −90 to 0 mV, 0.5 s duration, at 0.1 Hz) induced a long lasting (at least 30 min) increase in the frequency, but not amplitude, of mGABA_A PSCs. This effect is illustrated for a representative neurone in Fig. 5. The standard protocol used is shown in Fig. 5A, and the current sweeps in control conditions and 30 min after depolarizing pulses are shown in Fig. 5B and C, respectively. We could not detect significant changes in the amplitude of mGABA_A PSCs following depolarizing pulses, as determined by analysis of amplitude histograms (Fig. 6A) and cumulative amplitude distribution (Fig. 6C) obtained from the same cell. The mean amplitude of mGABA_A PSCs was 35.5 ± 0.9 pA (n = 167) in control and 37.0 ± 0.6 pA (n = 350) 30 min after depolarizing pulses (P = 0.47) (Fig. 6B). Neither could we detect changes in the decay time constant of mGABA_A PSCs (6.67 ± 0.3 ms in control (n = 100) vs. 6.63 ± 0.4 ms 30 min after depolarizing pulses (n = 100; P = 0.3) (Fig. 6B). The mGABA_A PSC frequency was, however, increased, as is evident from the cumulative inter-events interval distribution (Fig. 6D). The inter-events interval of mGABA_A PSCs recorded in this cell decreased from 1.56 ± 0.24 to 0.64 ± 0.05 s (P = 0.037). Similar effects were observed in four out of six neurones. On average, the inter-events interval decreased from 1.7 ± 0.18 to 0.92 ± 0.19 s (P = 0.0013) (mean of 55 ± 5 % of control value, range from 42 to 67 %), whereas the amplitude and decay time constant of mGABA_A PSCs remained unchanged (27.4 ± 4 vs. 29.5 ± 5 pA (P = 0.15) and 8.77 ± 2.35 vs. 8.66 ± 2.49 ms (P = 0.47)). In one neurone, depolarizing pulses led to a decrease in the inter-events interval from 1.9 ± 0.2 (n = 139) to 1.1 ± 0.1 (n = 229, P = 0.035), associated with a significant increase in the mean amplitude of mGABA_A PSCs from 33.5 ± 1.5 to 42.5 ± 1.8 pA (P = 0.046), but a change in their decay time constant was observed (from 6.17 ± 0.3 to 6.39 ± 0.2 ms, P = 0.31). This increase in the mean amplitude appeared to be due to an increase in the number of larger amplitude mGABA_A PSCs (data not shown). In the remaining cell neither the amplitude nor the frequency of mGABA_A PSCs was changed.

The increase in the frequency of mGABA_A PSCs was not due to the external application of potassium channel blockers. Thus in control experiments, long-term recordings (1 h, n = 3) in ACSF containing TTX, Cs⁺ (2 mM) and TEA (10 mM) did not alter the frequency of mGABA_A PSCs if the postsynaptic neurone was not repetitively stimulated with depolarizing pulses (data not shown).

An increase in the frequency of mGABA_A PSCs might have arisen from an increase in their amplitude, enabling

---

**Figure 6.** The frequency, but not the amplitude of mGABA_A PSCs is increased during LTP_GABA_A. A, superimposed control (□) and potentiated (●) amplitude histograms obtained from the cell depicted in Fig. 5. The control and potentiated histograms are obtained from 167 and 350 events recorded during a 3 min period before and 30 min after depolarizing pulses, respectively. N indicates the noise histogram. B, superimposed mean of 20 consecutive mGABA_A PSCs before (1) and 30 min after (2) application of depolarizing pulses. C and D, cumulative probability plots of amplitude (C) and inter-events interval (D) before (O) and 30 min after (●) depolarizing pulses.
previously undetectable events to cross the detection threshold. To address this point, mGABA$\text{A}$ PSCs were recorded at two different holding potentials. As illustrated in Fig. 7, changing the holding potential from $-50$ to $-100$ mV resulted in an increase in the amplitude of mGABA$\text{A}$ PSCs (Fig. 7A) from $16.7 \pm 0.57$ (n = 153) to $22.3 \pm 0.9$ pA (n = 142, P = 0.0069). The increase in the amplitude of mGABA$\text{A}$ PSCs is further illustrated by the shift to the right of the amplitude histograms (Fig. 7B) and cumulative amplitude distribution (Fig. 7D). This increase in amplitude of the mGABA$\text{A}$ PSCs was not associated with a significant change in their frequency. The mean inter-events interval of mGABA$\text{A}$ PSCs was $0.75 \pm 0.06$ s at $-50$ mV and $0.83 \pm 0.08$ s at $-100$ mV (P = 0.4). Similar experiments were repeated on five neurones. On average the amplitude of mGABA$\text{A}$ PSCs increased from $17.8 \pm 1.6$ to $27.0 \pm 2.9$ pA (P = 0.006) without any significant change in the mean inter-events interval ($0.61 \pm 0.1$ s vs. $0.65 \pm 0.1$ s, n = 5, P = 0.3). In contrast, application of hypertonic ACSF (50 mM sucrose) significantly decreased the inter-events interval of mGABA$\text{A}$ PSCs ($0.56 \pm 0.08$ s vs. $0.35 \pm 0.05$ s, n = 5, P = 0.034), without any effect on their amplitude.

Figure 7. Genuine increase in the number of mGABA$\text{A}$ PSCs during LTP. To determine whether the increase in the frequency of mGABA$\text{A}$ PSCs is due to the detection of previously undetected events, mGABA$\text{A}$ PSCs were recorded at two different holding potentials, $-50$ and $-100$ mV in the presence of TTX (1 $\mu$M), CNQX (10 $\mu$M), d-AP5 (50 $\mu$M), Cs$^+$ (2 mM) and TEA (10 mM). B, superimposed amplitude histograms of mGABA$\text{A}$ PSCs recorded at $-50$ mV and $-100$ mV. The histograms were obtained from, respectively, 153 and 142 events recorded during a 3 min period. C, superimposed mean of 20 consecutive mGABA$\text{A}$ PSCs recorded at $-50$ mV and $-100$ mV. D and E, cumulative probability plots of amplitude (D) and inter-events interval (E) of mGABA$\text{A}$ PSCs recorded at $-100$ mV (○) and $-50$ mV (●).
DISCUSSION

The principal conclusions of the present study are: (i) repeated depolarizations lead to a long-term potentiation of GABA_A receptor-mediated synaptic transmission (LTP_GABA_A) in neonatal rat hippocampus; (ii) the induction of LTP_GABA_A involves postsynaptic calcium-dependent mechanisms; and (iii) LTP_GABA_A is expressed as an increase in the probability of GABA release or as the functional expression of previously 'silent' GABAergic synapses.

Induction of LTP_GABA_A

The observation that LTP_GABA_A does not develop when buffering postsynaptic calcium with BAPTA indicates that the induction involves the activation of postsynaptic calcium-dependent mechanisms. An increase in intracellular calcium concentration ([Ca^{2+}]_i) could be produced by an influx of calcium through voltage-dependent calcium channels (VDCCs) or NMDA channels, or by a release of calcium from internal stores. In the present study, D-AP5 was present throughout the experiment, thus ruling out the involvement of NMDA channels. In fact, in a previous study we have shown that activation of NMDA receptors following high-frequency stimulation leads to a long-term depression of GABA_A PSPs in neonates (McLean et al. 1996).

In contrast, LTP_GABA_A was prevented in the presence of the VDCC blockers Ni^{2+} and nimodipine. In the presence of these blockers, we observed a short-lasting depression of evoked GABA_A PSPs following the conditioning stimulus. A likely mechanism for this transient decrease could be the phenomenon described in the cerebellum (Llano et al. 1991; Vincent & Marty, 1993) and the hippocampus (Pitler & Alger, 1994) termed depolarization-induced suppression of inhibition (DSI). In a recent study, Lenz and collaborators (1998) have shown that DSI is induced by the activation of N- and L-type channels. Thus, the transient depression observed in the presence of Ni^{2+} and nimodipine may be due to the activation of N-type calcium channels that are not blocked by these VDCC blockers. Alternatively, the transient depression may be due to a calcium-dependent downregulation of postsynaptic GABA_A receptors (Chen & Wong, 1995).

The types of VDCCs that are involved in or play the greater role in the induction of LTP_GABA_A remain to be investigated. This question may be of particular interest if a differential distribution (soma vs. dendrites) of calcium-type channels exists in neonatal pyramidal neurones, as has been described in adult neurones (Christie et al. 1995; Magee & Johnston, 1995). In the present study, the protocol applied to induce LTP_GABA_A is likely to activate both high-voltage- and low-voltage-activated calcium channels. At the concentration used, Ni^{2+} blocks T- and R-type channels, and nimodipine blocks L-type channels, while not significantly affecting other calcium channels (Mogul & Fox, 1991; Christie et al. 1995; Randall & Tsién, 1993). These calcium channels are present in the neonatal hippocampal pyramidal neurone, with kinetic and pharmacological properties comparable to those described in adult neurones (Thompson & Wong, 1991). The role of internal calcium stores has not been investigated in the present study, but their possible contribution in the induction of LTP_GABA_A, as reported in cortical (Komatsu, 1996) and cerebellar neurones (Hashimoto et al. 1996), cannot be excluded.

Even if the depolarizing pulses were applied in the absence of electrical stimulation of GABAergic fibres, it could be argued that a pairing-like protocol occurs because of the high-frequency barrage of spontaneous GABA_A PSPs present in neonatal CA3 pyramidal neurones. The potentiation of miniature GABA_A PSPs, which occurred at a 10 times lower frequency, strongly argues against such a mechanism. This finding stands in clear contrast with the data described for glutamatergic synaptic transmission. In adult CA1 pyramidal neurones and dentate gyrus granule cells, direct activation of VDCCs only leads to a short-term potentiation of evoked (Kullmann et al. 1992; Wang et al. 1997) and miniature excitatory postsynaptic currents (Wyllie et al. 1994). Thus, while the induction of LTP at hippocampal glutamatergic synapses requires both a postsynaptic influx of calcium and the activation of a presynaptic element (Isaac et al. 1995; Durand et al. 1996; Wang et al. 1997), a postsynaptic increase in [Ca^{2+}] appears to be a minimal and sufficient requirement to trigger LTP at GABAergic synapses.

Locus of LTP_GABA_A expression

Locus of LTP expression has been subject to numerous investigations for both excitatory and inhibitory synaptic transmission. LTP can be expressed presynaptically, manifested as an increased transmitter release, or postsynaptically, manifested as an increased sensitivity to released transmitter. In cerebellar Purkinje cells, activation of VDCCs leads to an increase in spontaneous IPSC amplitude (Kano et al. 1992) due to an upregulation of postsynaptic GABA_A receptors (Kano et al. 1992, 1996). Similarly, in the kindling-induced epilepsy, the long-lasting potentiation of inhibitory synapses in the dentate gyrus involves an increase in the number of postsynaptic GABA_A channels at functional synapses (Nusser et al. 1998). Conversely, a retrograde inhibitory control of GABA release following a postsynaptic rise in [Ca^{2+}] has been clearly observed in both cerebellum (Vincent & Marty, 1993) and hippocampus (Pitler & Alger, 1994).

In the present study we observed a clear increase in the spontaneous GABAergic synaptic activity. This observation

\[
(26.8 \pm 3.5 \text{ vs. } 25.8 \pm 2.4 \text{ pA, } n = 5, P = 0.3) \text{ (data not shown). Thus, under our recording conditions, changes in the amplitude of mGABA_A PSCs would not significantly influence their apparent frequency. These data therefore suggest that the depolarization-induced increase in mGABA_A PSC frequency is likely to be due to a real increase in the number of events rather than an increase in amplitude of previously undetectable events.}
\]
strongly supports the idea that a significant number of the GABAergic synapses impinging on the recorded neurone were potentiated, probably by the calcium influx induced by back-propagating action potentials (Christie et al. 1995). We therefore investigated the effect of depolarizing pulses on mGABA_A PSCs to determine the mechanism of LTP_{GABA_A} expression. We observed a nearly 2-fold increase in the frequency of mGABA_A PSCs without any significant effect on their amplitude or decay-time constant. We were concerned that our measured depolarization-induced increase in mGABA_A PSCs might be related to the detection of events that were below the detection threshold before voltage pulses. We showed, however, that our analysis routine could reliably detect changes in mGABA_A PSC amplitude, if they have to occur, without altering their apparent frequency. Thus the increase in mGABA_A PSC frequency observed after depolarizing pulses results from a real increase in the number of events, without any change in their amplitude.

An increase in the frequency of mGABA_A PSCs is consistent with a presynaptic increase in the probability of GABA release or with an increase in the number of functional GABAergic synapses. Such an increase in the number of functional GABAergic synapses could again be explained by either (i) an all-or-none upregulation of postsynaptic GABA_A receptors at previously non-functional synapses, as proposed for glutamatergic synapses on CA1 pyramidal neurones (Isaac et al. 1995; Liao et al. 1995) or (ii) alternatively, by the presynaptic switching on of previously silent synaptic connections, as proposed for glycinerergic synapses on Mauthner cells (Champier et al. 1995; Oda et al. 1995) and mossy fibre synapses on CA3 pyramidal neurones (Tong et al. 1996).

Although the mechanisms of LTP_{GABA_A} expression have not been fully elucidated, the lack of effect on the amplitude of mGABA_A PSCs suggests that it cannot be accounted for by an upregulation in the number of postsynaptic GABA_A receptors at previously functional GABAergic synapses as recently shown in the adult hippocampus (Nusser et al. 1998). In addition, in adult dentate granule cells, a transient postsynaptic rise in [Ca^{2+}]_i induced a long-lasting prolongation in the decay of miniature IPSCs (Soltész & Mody, 1995). Such an effect on the mGABA_A PSC decay was also not observed in the present study. This difference raises the interesting possibility that different mechanisms can generate persistent alterations in the efficacy of GABAergic synaptic transmission depending on the developmental stage, the neuronal type or the protocol used.

An important issue concerns the possible function that LTP_{GABA_A} might serve in the developing hippocampus. A growing body of evidence points to the link between activity-dependent plasticity and functional maturation of the neuronal network (Crair & Malenka, 1995; Kirkwood et al. 1995; Durand et al. 1996; Fitzsimonds & Poo, 1998). In neonatal rat hippocampus, activation of GABA_A receptors by endogenous GABA induces a membrane depolarization leading to the generation of action potential associated with a subsequent rise in [Ca^{2+}]_i (Ben-Ari et al. 1997). In addition, in a previous study we have reported that, early in development, high-frequency stimulation of GABAergic interneurones leads to a calcium-dependent LTP_{GABA_A} only when GABA depolarizes the pyramidal cells (McLean et al. 1996). Thus, as development progresses, spontaneously released GABA may lead to a functional maturation of GABAergic synaptic transmission in the form of long-term changes in synaptic efficacy.


Acknowledgements

We thank Drs L. Aniksztejn, C. Bernard, H. Gozlan and J. Hirsch for the helpful comments on the manuscript. This work was supported by the Institut National de la Santé et de la Recherche Médicale. O. Caillard was the recipient of a doctoral fellowship from the Ministère de l’Enseignement Supérieur et de la Recherche.

Corresponding author

J.-L. Gaiarsa: INSERM U29, Hôpital de Port-Royal, 123 Boulevard de Port-Royal, 75014 Paris cedex, France.

Email: gaiarsa@u29.sochin.inserm.fr
