Neuroscience Letters, 80 (1987) 283–288
Elsevier Scientific Publishers Ireland Ltd.

NSL 04827

Is activation of *N*-methyl-D-aspartate receptor gated channels sufficient to induce long term potentiation?

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(Received 22 January 1987; Revised version received and accepted 3 June 1987)

Key words: Hippocampal slice; CA_1; CA_3; Magnesium; *N*-Methyl-D-aspartate receptor; Schaffer collateral

Superfusion of hippocampal slices with Mg^{2+} free medium (20–30 min) produced in CA_1 interictal bursts and an enhancement of the Schaffer collateral synaptic response which persisted for over 2 h after return to control media. This long lasting effect, which was blocked by *N*-methyl-D-aspartate (NMDA) antagonists, was not associated with changes in postsynaptic cell excitability. A cut between CA_3 and CA_1, which blocked the bursts in CA_1 (but not in CA_3), also abolished the long lasting effects of Mg^{2+} free medium. It is concluded that the activation of NMDA receptors gated ionic channels is insufficient per se to induce a long term potentiation of synaptic transmission.

Several lines of evidence suggest that *N*-methyl-D-aspartate (NMDA) receptors participate in the initiation of long-term potentiation (LTP) of the Schaffer collateral synaptic response. This region is rich in NMDA receptors [9, 11] and blockade of these receptors with D-2-amino-5-phosphonovalerate (APV) appears to antagonize the induction of LTP by tetanic stimulation without reducing the amplitude of control EPSPs [3, 7, 15]. These and other observations have been offered as evidence that activation of NMDA receptor-gated ionic channels may be necessary for the induction of LTP [4, 14]. These channels show a voltage-dependent blockade by Mg^{2+} [1, 10]. It has been suggested that the intense synaptic activation, which occurs with tetanic stimulation, may overcome the Mg^{2+} blockade resulting in current flow through these channels; this in turn initiates LTP [4, 8, 14]. In the present study we have examined whether removal of the Mg^{2+} blockade, by superfusion with Mg^{2+}-free medium is sufficient to induce a long-term enhancement of synaptic transmission.

Male Wistar rats (100–150 g) were used. Transverse 450 μm hippocampal slices were cut and transferred to a completely submerged chamber as described previously [6]. Briefly, slices were maintained at a temperature of 33–34°C and superfused (2

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ml/min) with an artificial cerebrospinal fluid containing (mM): NaCl 126, KCl 3.5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $CaCl_2$ 2, glucose 11, $NaHCO_3$ 25 and gassed with 95% O_2–5% CO_2 (pH 7.3). Schaffer collaterals were stimulated with bipolar tungsten electrodes at a frequency of 0.05 Hz (pulses of 10–65 V, 15 μs duration). Extracellular and intracellular recordings were made with micropipettes filled respectively with 2 M NaCl and 4 M potassium acetate (resistance 70–150 MΩ). Bridge balance was checked repeatedly during the impalement. Potentials were displayed on a digital oscilloscope from which records were made using a computer-driven chart recorder. The effects of Mg^{2+}-free medium were tested in 22 slices from which stable intracellular recordings (for over 2 h) were made from 15 neurones. Membrane potentials were greater than -63 mV with action potentials above 75 mV.

Switching the perfusion to Mg^{2+}-free medium rapidly resulted in an increased amplitude and frequency of spontaneous EPSPs and IPSPs. The membrane potential initially hyperpolarized by 3–6 mV but gradually returned to control levels over the next 15–20 min. Occasionally a membrane depolarization (3–5 mV) was observed. In 4 cells the apparent membrane input resistance as measured and was found to increase in 3 (14–43%) and to decrease in one (30%) during perfusion with Mg^{2+}-free

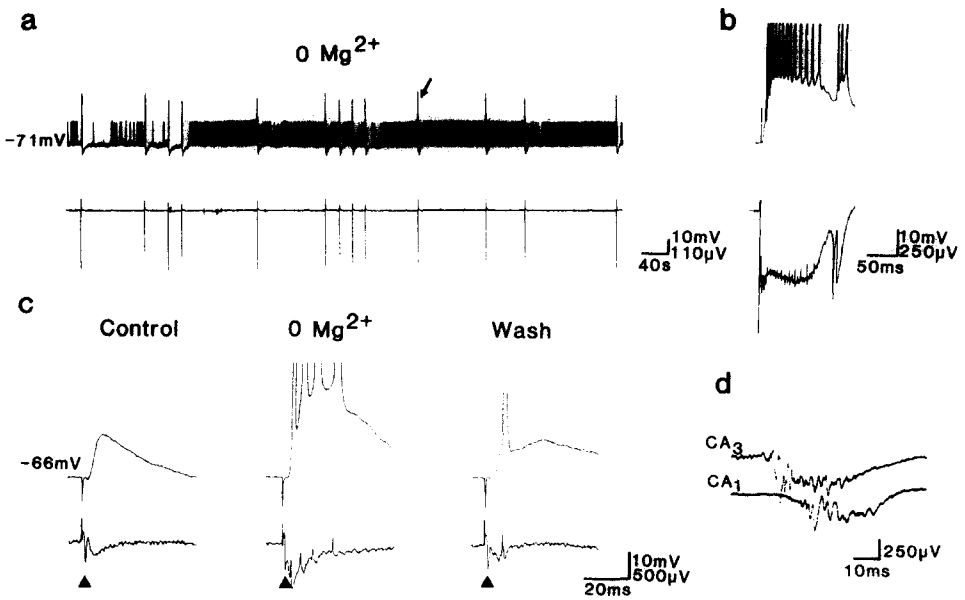

Fig. 1. Spontaneous bursts and long-lasting enhancement of the EPSP induced by Mg^{2+}-free medium. Upper traces (a–c), intracellular recordings from a CA_1 pyramidal neuron; lower traces, concomitant extracellular recordings from the stratum radiatum. a: bursts and single spikes, arising from spontaneous EPSPs, following 15 min perfusion in Mg^{2+}-free medium. The burst marked by an arrow is shown in b. c: stimulus (solid triangle) evoked only an EPSP in control medium, induces a burst of spikes in Mg^{2+}-free medium. After 1 h wash, the same stimulus evokes an increased EPSP leading to an action potential. Note that the afferent volley is unchanged. d: simultaneous field recordings from CA_3 and CA_1 reveal that spontaneous bursts in CA_3 precede those recorded in CA_1. Spikes in b and c were truncated by the recorder.

medium. Cell excitability was always increased (Fig. 2b). Within 10–15 min of superfusion, spontaneous, repetitive bursts developed (Fig. 1a, b). Concomitant extracellular recordings in the stratum radiatum showed that the intracellular burst was synchronous with negative field potentials (Fig. 1a, b). The intracellular paroxysmal events consisted of a high-frequency burst of action potentials (5–16) riding on a (15–30 mV) paroxysmal depolarizing shift followed by an afterhyperpolarization (Figs. 1a, b). Superfusion with Mg^{2+}-free medium also increased the amplitude and duration of the Schaffer collateral EPSP, which as the perfusion continued, led to evoked bursts (Fig. 1c). The duration of the evoked bursts was always shorter than that of the spontaneous bursts. These observations are in keeping with previous re-

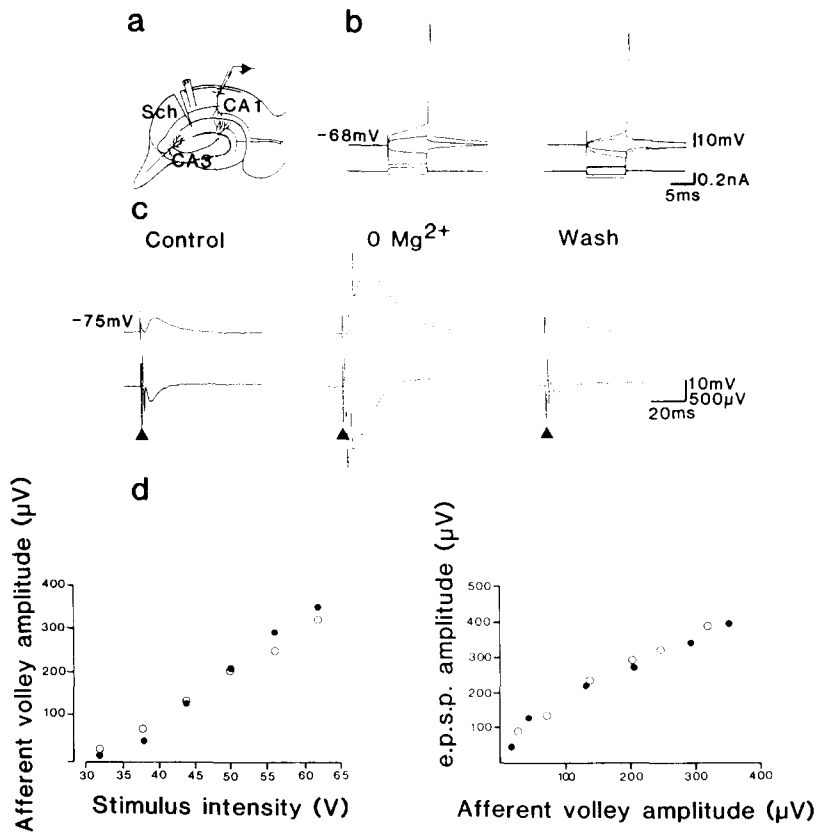

Fig. 2. Isolating CA_1 from CA_3, by a knife cut, eliminates the long-lasting enhancement of the EPSP. a: schematic diagram of the experimental procedure. b: during perfusion with Mg^{2+}-free medium, cell excitability was increased despite the reduction of input resistance (right) compared to control (left). The upper and lower traces are voltage and current recordings respectively. c: records from a cut slice illustrating that both the intracellular (upper trace) and extracellular (lower trace) EPSP are enhanced in Mg^{2+}-free medium. However, the EPSP rapidly returned to control value during the wash. d: input–output curves from a cut slice before (open symbols) and 50 min after (filled symbols) superfusion with Mg^{2+}-free medium. During superfusion with Mg^{2+}-free medium the amplitude of the EPSP was 220% of the control response (not shown).

ports [2, 13]. Both the enhancement of the EPSP and the bursts were antagonized by 20 μM DL-2-amino-7-phosphonoheptanoic acid (AP7) or 20 μM APV (not shown; see also refs. 2, 13). Upon return to control medium, the spontaneous and evoked bursts disappeared within 3–5 min. However, the EPSP remained enhanced (range 33–280%) in 4 out of 5 slices for at least 1 h. Often an action potential was triggered by the previous subthreshold stimulus (Fig. 1c). This long-term effect was associated neither with an increased amplitude of the afferent volley (Fig. 1c) nor with a change in postsynaptic cell excitability (in terms of membrane potential and threshold for intracellular spike generation; not shown). Therefore, this long-term enhancement bears several similarities with the classical LTP.

Concomitant field recordings in CA_1 and CA_3 showed that burst activity induced by Mg^{2+}-free medium appeared first in CA_3 and then propagated to CA_1 (Fig. 1d; $n=3$). The same phenomenon has been described for penicillin and bicuculline [12, 16]. As in these studies, cutting the connections between CA_1 and CA_3 eliminated the spontaneous bursts in CA_1 but not in CA_3 (not shown; see also ref. 13). This procedure also abolished the long-term enhancement of the EPSP in CA_1 (Fig. 2c, d). In cut slices, superfusion with Mg^{2+}-free medium increased the EPSP; however, this

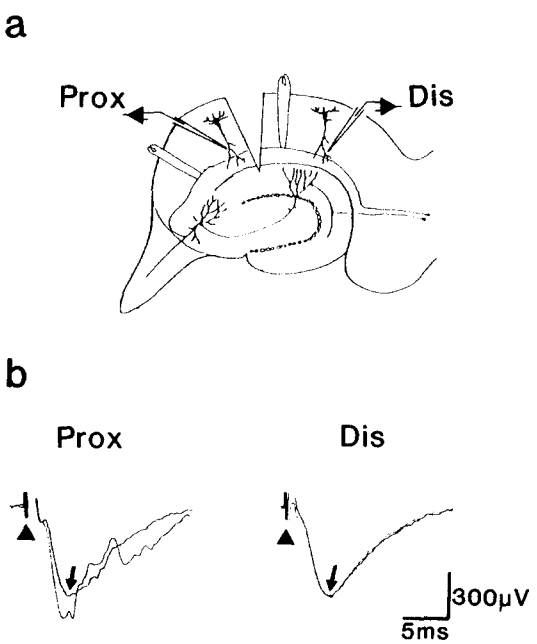

Fig. 3. Long-term enhancement of the population EPSP on the proximal (to CA_3) but not distal side of cut in CA_1. The diagram illustrates the knife cut and the stimulating and recording electrodes on both sides. The lower panel represents field potential recordings of the population EPSPs, evoked by Schaffer collateral stimulation (triangles), from proximal (Prox) and distal (Dis) sites. Two traces from each site are overlaid: (1) control (arrow), taken before superfusion with Mg^{2+}-free medium; (2) wash, taken 130 min after return to control medium. Mg^{2+}-free medium was superfused for 25 min. Note that only the EPSP from the proximal site showed a long-lasting enhancement (25% increase in amplitude and 46% increase in dV/dt of rising phase).

returned to control values after reintroducing control medium. Similar observations were made in 6 slices. In 4 additional intact slices, Mg^{2+}-free medium produced neither spontaneous bursts in CA_1 nor long-term enhancement of the EPSP further stressing the correlation between bursts and the long-term effects.

In an additional experimental series, two pairs of stimulating and recording electrodes were used to evoke and record field EPSPs from both sides of a cut bisecting CA_1 (Fig. 3). Thus, the effects of Mg^{2+}-free medium could be tested under conditions in which both intact and cut pathways were present in the same slice. Bursts and the long-term enhancement of the EPSP involved exclusively the proximal (to CA_3) but not the distal side which was unchanged (Fig. 3; $n=3$). The long-term enhancement, which ranged from 25 to 269%, lasted over 2 h after return to control medium (at this point recording was terminated).

The present data shows that during superfusion with Mg^{2+}-free medium, the amplitude of the Schaffer collateral EPSP is increased in both intact and cut slices. Although other factors may contribute to the increase in the EPSP observed during superfusion with Mg^{2+}-free medium [5], NMDA receptors are clearly involved, since the NMDA antagonists, AP7 and APV, reduced the amplitude and duration of the augmented EPSP [2, 13].

In contrast, removal of the Mg^{2+} blockade and the activation of these channels is insufficient to induce a long-term enhancement of synaptic transmission. The long-term effects observed following superfusion with Mg^{2+}-free medium are only present in slices in which the procedure also induces spontaneous bursts. In this regard, our results cannot be reconciled with the suggestion that in Mg^{2+}-free medium, low-frequency electrical stimulation is sufficient to induce long-lasting enhancement of the EPSP [2]. We suggest that the observations of Coan and Collingridge [2] resulted instead from bursts generated in CA_3. Clearly, further experiments are required to elucidate the mechanism by which bursts and trains of electrical stimulation can generate LTP.

We wish to acknowledge the gift of DL-2-amino-7-phosphonoheptanoic acid from Dr. Paul Herrling. R.N. was the recipient of a Canadian MRC traveling scientist award and an INSERM Fellowship.

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