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Non-involvement of the redox site of NMDA receptors in bidirectional synaptic plasticity in the CA1 area of the rat hippocampus in vitro

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Abstract

We have examined the effects of the redox reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) on synaptic potentials recorded extracellularly from the CA1 area in hippocampal slices following low frequency stimulation (LFS) and tetanic stimulation (TS). Application of DTNB (200 μM) neither changed synaptic responses, nor prevented the expression of TS-induced long-term potentiation of synaptic responses and their depotentiation by LFS. Conversely, in naive slices, LFS still induced long-term depression of synaptic responses following application of DTNB. This depression could be subsequently reversed with a TS. It is concluded that the redox state of *N*-methyl-D-aspartate receptors does not affect the expression of long-term potentiation and depression of synaptic responses.

Keywords: Synaptic plasticity; Long-term depression; Long-term potentiation; Redox site; Hippocampus; Extracellular recording

Long-term potentiation (LTP) and depression (LTD) of synaptic responses are commonly used as experimental models of learning and memory. In the CA1 area of the hippocampus, tetanic stimulation (TS) can lead to LTP [2] whereas low frequency stimulation (LFS) can induce LTD [4,11] of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor-mediated responses. This synaptic plasticity is dependent upon the activation of *N*-methyl-D-aspartate (NMDA) receptors [3,4,11]. NMDA receptors are sensitive to S–H and S–S reagents [1,9,12] and in the presence of the redox reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), pharmacologically isolated NMDA receptor-mediated synaptic responses are reduced by 50% due to the oxidation of S–H groups into S–S bonds [7]. Moreover, these isolated responses do not show LTP following TS whereas the expression of TS-induced LTP of AMPA receptor-mediated responses remains unaffected [7]. Since the expression of LTP or LTD appears to be controlled by the pattern of calcium influx through NMDA receptors [11] we have investigated the consequences of complete oxidation of NMDA receptors on the expression of LTD and LTP of synaptic responses using LFS and TS conditioning stimuli applied in succession. We report that bidirectional changes of synaptic responses (LTP followed by LTD or LTD followed by LTP) occur even when NMDA receptors are fully oxidized.

Slices were prepared from male Wistar albino rats (180 g, 1 month old). The animals were anaesthetized with ether before decapitation and removal of the brain. Both hippocampi were removed and sliced (400 μm). Slices were incubated in artificial cerebrospinal fluid (ACSF) for at least 1 h before recording and then placed in a submerged-type in vitro slice chamber. The ACSF was composed of (in mM) NaCl, 126; KCl, 3.5; NaH_2PO_4, 1.2; $NaHCO_3$, 25; $MgCl_2$, 1.3; $CaCl_2$, 2 and D-glucose, 11 (pH 7.4) and perfused with a mixture of 95% O_2 and 5% CO_2. The temperature in the recording chamber was maintained at 30–32°C. Extracellular population excitatory postsynaptic potentials (pEPSPs) were recorded from the stratum radiatum of CA1 in hippocampal slices, using 5–10 MΩ resistance glass microelectrodes filled with 3 M NaCl. Bipolar stimulating electrodes (twisted-wire, trimel insulated, 50 μm apart at the tip) were placed in the stratum radiatum of CA1 area proximally to stratum pyramidale and near the recording electrode. Test stimuli in the form of 50 μs pulses, 5–50 μA in magnitude were delivered at 30 s (0.33 Hz) intervals.

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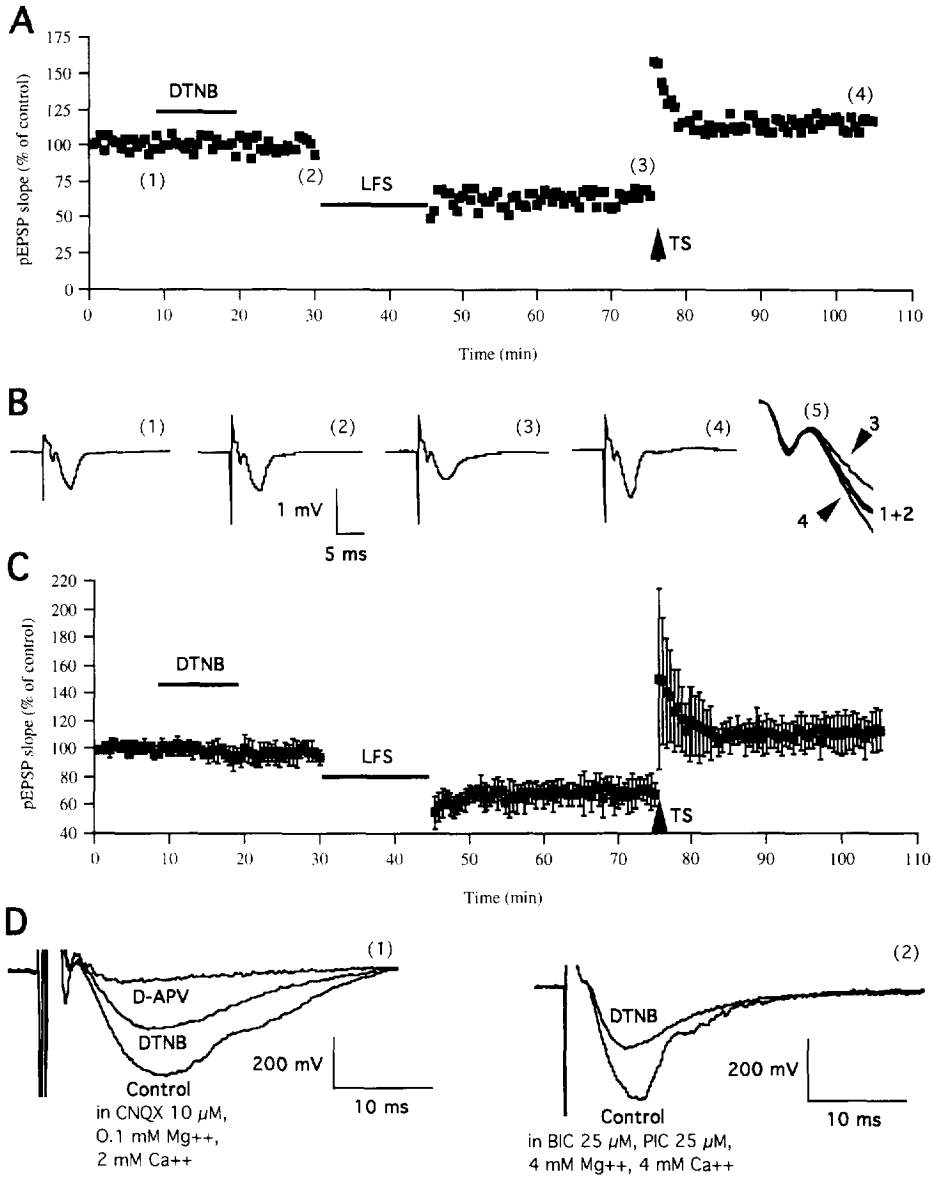

Fig. 1. DTNB does not prevent the expression of low frequency stimulation-induced long-term depression of synaptic responses. (A) Plot of population EPSP initial slopes measured every 30 s in a typical experiment. The baseline was measured during the first 10 min. DTNB (200 μM) was applied for 10 min and control ACSF was then reintroduced. The initial slope of the population EPSP was not affected either during the application of DTNB or 10 min following the reintroduction of control ACSF. 1 Hz stimulation was then applied during 15 min (LFS). Note the long-term depression of the response when compared to baseline levels. Thirty minutes after the completion of the first conditioning stimulus a tetanic conditioning stimulus (arrow) was applied which reversed the depression of the response. (B) Field potentials (averages of 5 consecutive records) taken before application of DTNB (1), 10 min after the end of DTNB application (2), (3) 30 min after LFS and (4) after TS. The times at which the illustrated sweeps were taken are indicated in (A). (5) shows the same traces superimposed with an expanded time scale. (C) Normalized means (±SD) of five experiments in which the bidirectional long-term modification of synaptic responses was monitored. DTNB (200 μM) did not significantly change the pEPSP slope (97 ± 6%). Following LFS synaptic responses were reduced to 69 ± 9% of control ($P < 0.0001$). Following TS synaptic responses were 111 ± 14% of control ($P < 0.0001$ when compared to the values prior to TS). (D) Example of the effect of DTNB (200 μM) on isolated NMDA receptor-mediated synaptic responses in the presence of 10 μM of CNQX. (1) Superimposed traces before and 15 min following bath application of DTNB (200 μM). Note the reduction in the initial slope of the pEPSP. Bath application of D-APV (50μM) abolished the remaining response. In the latter case we have used a higher stimulus intensity in order to rule out any remaining AMPA and NMDA component, hence a larger afferent volley. Both AMPA and NMDA receptor-mediated synaptic responses components can be revealed in the presence of high concentrations of divalent cations and $GABA_A$-blockers (2). DTNB (200 μM) reduced both the initial slope and the late phase of the pEPSP. Note that the time-to-peak is also decreased characterizing the reduction of the contribution of NMDA receptors to the overall response.

TS was applied through the same bipolar electrode and consisted of 4 trains of 10 pulses at 100 Hz every 1 s given at twice the amplitude used for the test responses. LFS consisted of 900 stimulations given every 1 s at test stimulus intensity. The slope of the pEPSP was measured during the first 500 μs. Changes in the slope of pEPSPs were considered as long-term if they were sustained for 30 min or longer. The redox reagent DTNB was applied via the perfusion medium. Responses were digitized, stored and analyzed on-line with locally developed software (ACQUIS1; G. Sadoc). All values are expressed as mean ± SD. Parameters were compared with a Student's *t*-test.

Bath application of DTNB (200 μM) during 10 min did not change the pEPSP slope significantly (97 ± 6%, $n = 5$). Thirty minutes after LFS, synaptic responses were reduced to 69 ± 9% of control ($n = 5$, Fig. 1). This long-term depression of synaptic responses could be reversed: following subsequent TS, synaptic responses were 111 ± 14% of control, $n = 5$. In all cases the afferent volley remained unchanged following application of DTNB, LFS or TS (see Fig. 1,B-5).

In order to show that DTNB (200 μM) reduces NMDA receptor-mediated responses, we have used a solution containing a low concentration of Mg^{2+} (0.1 mM) and 10 μM of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in order to block AMPA receptor-mediated synaptic responses. Isolated NMDA receptor-mediated synaptic responses could thus be measured (Fig. 1D). Bath application of DTNB (200 μM) reduced the NMDA receptor-mediated synaptic responses by 45 ± 6% ($n = 5$). The effect of DTNB (200 μM) was also measured when AMPA and NMDA receptors are activated. In these experiments 25 μM of bicuculline and 25 μM of picrotoxin were used in order to block $GABA_A$ receptor-mediated responses. Epileptiform activity was prevented by surgically isolating the CA3 area of the hippocampus from the CA1 area and by using high concentration of cations (4 mM of Ca^{2+} and 4 mM of Mg^{2+}) in order to reduce the activation of polysynaptic pathways. DTNB (200 μM)

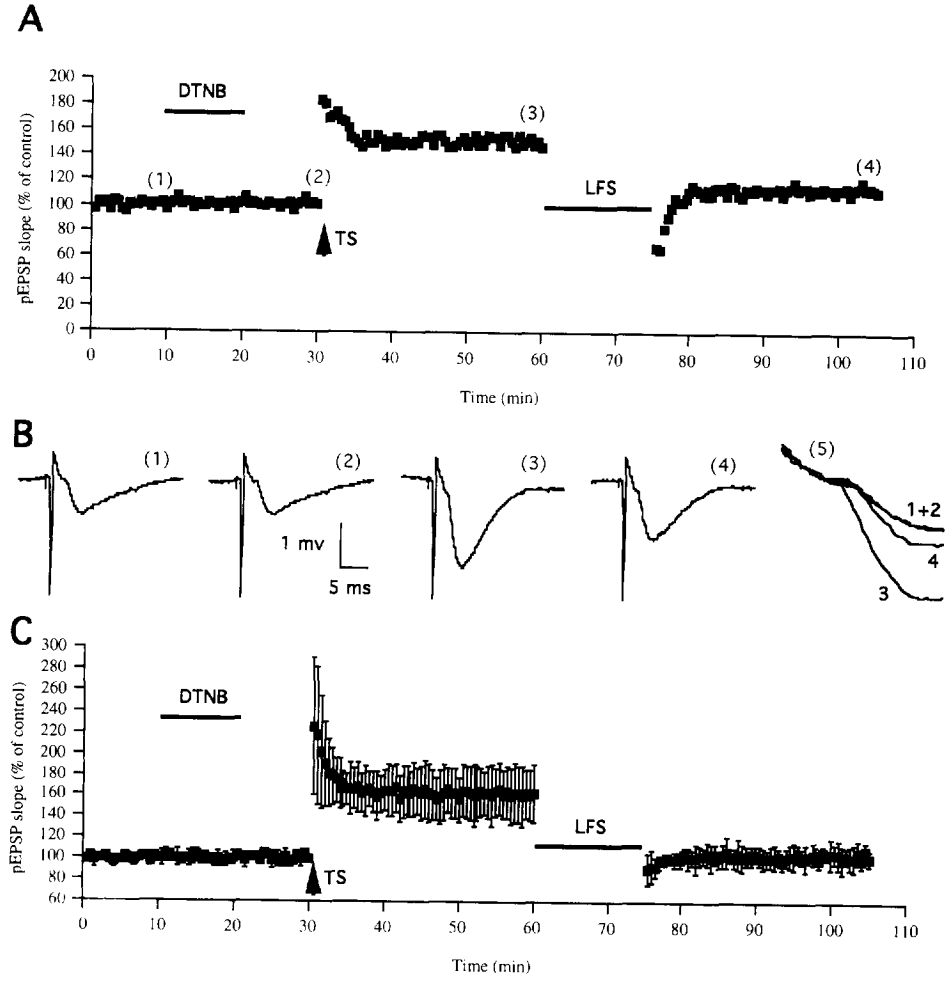

Fig. 2. DTNB does not prevent the expression of TS-induced long-term potentiation of synaptic responses and its reversal with LFS. Same as Fig. 1 except for the order of the conditioning stimuli. DTNB (200 μM) and synaptic responses were identical to control (101 ± 6% of control). We first applied a TS which resulted in a LTP of synaptic responses (161 ± 26% of control, $P < 0.0001$). This potentiation could be reversed by subsequent LFS (104 ± 10% of control, $P < 0.0001$ when compared to the values prior to LFS)

reduced the responses by 30 ± 9% ($n = 5$) showing that DTNB was also effective even when AMPA receptors were co-activated with NMDA receptors (Fig. 1D).

In the second set of experiments (Fig. 2), DTNB (200 μM) was also applied for 10 min. Synaptic responses were identical to control (101 ± 6% of control, $n = 5$). We first applied a TS which resulted in a LTP of synaptic responses (161 ± 26% of control, $n = 5$). This potentiation could be reversed by subsequent LFS (104 ± 10% of control, $n = 5$).

These results can be compared with those obtained in the absence of DTNB. In naive preparations LFS resulted in synaptic LTD (64 ± 11%, $n = 5$, data not shown). This LTD could be reversed following TS (115 ± 11%, $n = 5$). Conversely, in another set of experiments TS resulted in LTP (172 ± 30%, $n = 5$, data not shown) which was reversed following LFS (99 ± 12%, $n = 5$). All these values were not statistically different from those obtained when NMDA receptors are oxidized following application of DTNB.

Our results suggest that complete oxidation of NMDA receptors prevents neither the expression of LFS-induced synaptic LTD and its subsequent reversal nor the expression of TS-induced synaptic LTP and its subsequent depotentiation. It has been suggested that TS-induced LTP and LFS-induced LTD of AMPA receptor mediated responses share common mechanisms [10]. The key step in this mechanism would be the pattern of Ca^{2+} entry into the cell [11]. In our study, LTD of AMPA receptor-mediated responses was not prevented by a complete oxidation of NMDA receptors. This is in keeping with recent results suggesting that the redox state of NMDA receptors does not modulate the expression of TS-induced LTP [7,8]. Furthermore, the synaptic depression could be reversed in the same way as that described in the absence of drug [5]. Since DTNB reduces the isolated NMDA receptor-mediated responses by 50% in low Mg^{2+}, the Ca^{2+} entry through NMDA channels into the cells should be reduced during LFS. Nevertheless, this influx is still sufficient to induce LTD. However, it is not known whether or not the effect of DTNB in control ACSF has a similar effect to that measured in reduced Mg^{2+}. Moreover, it has been suggested that LFS can induce LTD of NMDA receptor-mediated responses [6,13]. If this is also the case in the presence of DTNB, the influx of calcium through NMDA channels will be further reduced. Nevertheless LFS-induced LTD of AMPA receptor-mediated responses could be reversed following TS. This reinforces the idea that TS-induced LTP and LFS-induced LTD share common mechanisms. This issue is still controversial since it seems that LTD, contrary to LTP, is associated with equal changes in AMPA and NMDA receptor-mediated responses [13]. Nevertheless, the consequences of LFS on NMDA responses mediated by fully oxidized receptors remain to be investigated.

These results can be compared with those obtained with a different form of LTP, i.e. anoxic LTP. Following an anoxic episode, LTP is expressed exclusively by NMDA receptors and is dependent upon the activation of NMDA receptors. In this case a full oxidation of NMDA receptors, following application of DTNB, prevents the induction and the expression of anoxic LTP of isolated NMDA receptor-mediated responses [8]. This suggests that the redox site may play a role during insults rather than in a fine tuning of LTP in control conditions.

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