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BRAIN RESEARCH

Research Report

# Emetine treatment masks initial LTP without affecting long-term stability

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ABSTRACT

Applying emetine, a protein synthesis inhibitor, at 20–40 μM for 90–120 min prior to LTP induction in hippocampal slices from young rats (2–3 weeks) and washing it out afterwards revealed a slowly developing potentiation that reached maximum after 20–30 min, distinct from the LTP observed under normal conditions. Nevertheless, the later phase of this potentiation was similar to standard LTP as judged by experiments lasting up to 8 h after induction. Emetine preapplication for 3 h without subsequent washout resulted in a substantial decay of evoked responses. By comparison between test and control pathways, LTP could still be assessed in these experiments for up to 4–6 h after induction and was found not to differ from normal, except for the slow onset. The NMDA-R blocker AP5 fully blocked LTP; however, with emetine pretreatment there was an initial depression of responses with a gradual recovery during 20–30 min. This depression involved not only the field EPSP but also the presynaptic fiber volley. However, when using the protein synthesis inhibitors cycloheximide and anisomycin there was essentially no such depression. In conclusion, the present results support the idea that preexisting proteins are sufficient for inducing stable LTP. Moreover, emetine but not anisomycin or cycloheximide impairs presynaptic action potentials, leading to an apparent slow onset of LTP. The emetine-dependent effect could be due to a characteristic blocking spectrum of the drug, preferred targeting of presynaptic compartments or effects unrelated to protein synthesis.



## 1. Introduction

NMDA receptor (NMDA-R) dependent long-term potentiation (LTP), as displayed in the hippocampus and several other brain regions, comprises two essential processes: induction and maintenance. The induction occurs by postsynaptic $Ca^{2+}$ influx through NMDA-Rs and subsequent triggering of biochemical events leading up to the maintenance process,

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*Abbreviations:* ACSF, artificial cerebrospinal fluid; AMPA-R, AMPA receptor; Ani, anisomycin; AP5, D-(–)-2-amino-5-phosphonopentanoic acid; CHX, cycloheximide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; eme, emetine; CREB, cyclic AMP-responsive element-binding protein; fEPSP, field EPSP; HFS, high-frequency stimulation; LTP, long-term potentiation; NMDA-R, NMDA receptor; PTP, post-tetanic potentiation; STD, short-term depression; STP, short-term potentiation; TCA, trichloroacetic acid

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which primarily involves an increase of AMPA receptor (AMPA-R) mediated responses (Baudry and Lynch, 2001; Nicoll, 2003). The maintenance of LTP can further be differentiated into early and late phases based on the ability of protein synthesis inhibitors to block LTP later than about 3 h while preserving early LTP (Frey et al., 1988; Krug et al., 1984; Stanton and Sarvey, 1984). The late phase, often referred to as late LTP (L-LTP), might involve other changes than those directly related to receptors, such as structural alterations in terms of synaptic growth, splitting of synapses, etc (Lüscher et al., 2000; Tominaga-Yoshino et al., 2008; Trommald et al., 1996).

Although there is substantial evidence that protein synthesis is needed for L-LTP, neither the nature of the involved proteins nor their roles in the maintenance process are well known. A further issue concerns when protein synthesis must operate in order to obtain stable LTP. It is generally conceived that the synthesis is triggered by the LTP-inducing stimulus and so takes place for a limited time after the induction event (Lanahan and Worley, 1998; Otani et al., 1989; Ouyang et al., 1999). In line with this view, several studies using protein synthesis inhibitors to characterize L-LTP have applied the inhibitor for a relatively short period around induction time, lasting an hour or even less (Sajikumar et al., 2008; Scharf et al., 2002; Stanton and Sarvey, 1984). The fact that drug application was maintained for much longer times in some other studies (e.g. Bradshaw et al., 2003; Osten et al., 1996; Serrano et al., 2005) does not violate the basic idea of a brief period of essential protein synthesis following LTP induction. It has been suggested that the newly synthesized proteins are captured by the engaged synapses through activity-dependent molecular tags (Frey and Morris, 1998; Reymann and Frey, 2007; Sajikumar et al., 2005).

However, de novo protein synthesis is not always needed to produce stable LTP. In a previous study in 2–3-week-old rats, we found that LTP lasting 4–8 h could still be obtained if a protein synthesis inhibitor, either emetine or anisomycin, was applied during −30 to +30 min with respect to LTP induction (Abbas et al., 2009). It was concluded that triggered protein synthesis is not necessary for persistent LTP but that constitutive proteins are sufficient. Whether this result was due to the use of young animals and/or other experimental features is not known. The idea that constitutive proteins can support L-LTP, at least under certain conditions, is also evidenced by the fact that concurrent blockade of protein synthesis and degradation still allowed L-LTP to be produced (Fonseca et al., 2006). In further agreement with this idea, L-LTP was easily induced in transgenic mice expressing a type of active, constitutive cyclic AMP-responsive element-binding protein CREB (Barco et al., 2002).

Considering the possible role of preexisting proteins in the context of L-LTP, we were inquisitive about the effects of blocking constitutive synthesis. The latter was achieved by applying a protein synthesis inhibitor a few hours in advance of LTP induction. We reasoned that this would lead to a decrease in the levels of necessary "plasticity-related proteins" (see Reymann and Frey, 2007; Sajikumar et al., 2005) available at induction time. To our surprise, long pretreatment with emetine did not block any late part of the recorded LTP but seemed to abolish short-term potentiation (STP), as witnessed by a slow onset of the potentiation. However, further analysis showed that this phenomenon was unrelated to STP or LTP but involved a presynaptic mechanism operating via impaired action potential generation. As a further peculiarity, the effect appeared to be specific to emetine as it was not seen with two other protein synthesis inhibitors tested, cycloheximide and anisomycin.

## 2. Results

### 2.1. *Effect of long pretreatment of emetine on LTP*

The initial goal of this investigation was to test the idea that long administration of a protein synthesis inhibitor before LTP induction might influence L-LTP. As inhibitor we chose the translational blocker emetine that is frequently used in research on LTP and protein synthesis. Fig. 1A shows data from a set of experiments where emetine at a concentration of 20–40 μM was preincubated for 90–120 min before induction of LTP. Despite the long treatment with emetine, responses of the test pathway showed a clear potentiation, peaking at a level above 150% within the first hour after induction (n=7). Comparison between test and control pathways revealed that the substantial decline of potentiated fEPSPs during the 8 h post-tetanus recording period was most likely due to a general decrease of viability. Accordingly, the drift-compensated curve in Fig. 1B, obtained as test–control ratio, showed that LTP was maintained relatively stable throughout the recording period.

Comparison with control experiments performed in the absence of emetine (n=7, see Fig. 1C) revealed two essential results. Firstly, the levels of LTP were not significantly different for experiments with and without emetine, values amounting to 145±9% vs. 150±7% at 4 h and 135±6% vs. 142±7% at 8 h after induction. As L-LTP is generally assumed to occur later than 3 h, the data show that this late component was unaffected by emetine treatment. Secondly, despite the lack of late effects, there was an intriguing early difference in that LTP, under emetine treatment, appeared to develop much slower. Whereas the "normal LTP" in Fig. 1C peaked quickly after induction, the "emetine LTP" did not peak until after nearly 30 min, suggesting that an early part, perhaps corresponding to STP, was missing. This difference between "emetine-LTP" and normal LTP seemed to fade away during the first hour after induction.

### 2.2. *LTP under extended emetine treatment*

We next considered whether the lack of effect on L-LTP by 90–120 min of emetine pretreatment (see above) might be due to insufficient time of application. Hence, we carried out additional experiments with treatment starting earlier and/or ending later than in the initial series. In the most extreme case, emetine was applied 3 h before (−3 h) the induction and kept in the bath for the rest of the experiment. Fig. 2A shows that such long application time had a serious effect on slice viability, as evidenced by complete disappearance of responses at 5–6 h after induction (corresponding to 8–9 h of emetine treatment). Nevertheless, when calculating the degree of potentiation as test/control ratio, LTP could be quantified for up to about 5 h post-tetanus (Fig. 2B) and was found to be similar to standard LTP. Thus, at 4 h after induction, there was a potentiation of 141±12% (n=10) relative to the