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Release of proteins during long-term potentiation in the hippocampus of the anaesthetized rat

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Using a push pull device, the release of endogenous proteins in the extracellular space was investigated in the CA1 region of the hippocampus of anaesthetized rats. With low-frequency stimulation of the Schaffer collaterals, there was a relatively stable release of 5 proteins (64, 54, 48, 45 and 16 kDa). A train of high-frequency stimulation produced a long-lasting enhancement of the negative field EPSP and a delayed (90–120 min) enhancement of the release of these proteins. An additional 19 kDa protein was present only 90 min after the train. These observations raise the possibility that release of proteins might be involved in the maintenance of LTP.

Long-term potentiation (LTP) is a long lasting enhancement of synaptic transmission produced by a train of high-frequency electrical stimulation [8, 28]. This phenomenon has been extensively studied in the hippocampal region and provides a useful experimental model for the study of the cellular mechanisms underlying learning and memory [20, 21]. However, the contribution of pre- and postsynaptic mechanisms to LTP have not been clarified; thus, there is a disagreement as to whether LTP is [9, 13, 15] or is not [4] associated with an increased release of the transmitter candidates glutamate or aspartate. Application of phorbol ester which produces LTP, different from that seen after a train [16], is also not associated with a release of endogenous excitatory amino acids [5].

Biochemical modifications in the hippocampus as well as protein phosphorylation have been reported after LTP [1, 2, 6, 11, 18, 23]. The appearance of newly synthesized proteins [14] in the extracellular fluid (ECF) and the prevention of LTP by inhibitors of protein synthesis [26] or monoclonal antibodies [27] suggest the involvement of macromolecules in this phenomenon. In the present study, using a push–pull cannula, we have examined the release of constituent proteins of the extracellular space

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after the induction of LTP. We report here a substantial increase in the release of proteins and a new 19 kDa secreted protein into the extracellular fluid after LTP.

Experiments were performed on 17 adult male wistar rats (200–300 g) as described previously [5]. Briefly, rats were anaesthetized with urethane (2 g/kg) and placed in a stereotaxic frame. The commissural pathway was stimulated by a bipolar electrode implanted in the hippocampal commissure (A = 6.6; L = 1; H = 7). The oxygenated artificial cerebrospinal fluid was perfused in the cannula at a flow rate of 10 μl/min and samples were collected at 15 min intervals in tubes containing a mixture of protease inhibitors (0.5 mM phenyl methyl sulfonyl fluoride (PMSF); 2 mM EDTA and 1 mM benzamidine). In a control period (1 h), the commissural pathway was stimulated at a frequency of 0.033 Hz and the slope of the negative excitatory postsynaptic field potential (EPSP) measured. Then, a train of high frequency (100 Hz for 1 s) was applied and the field EPSP further quantitated for 2 h. In additional experiments, the *N*-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphonovalerate (APV) (100 μM) [12] was added to the perfusion medium for 35 min starting 30 min before the stimulation.

Proteins were concentrated according to the method of Wessel and Flugge [29], then analyzed by electrophoresis on 12.5% polyacrylamide gels in presence of sodium dodecyl sulfate (SDS) and under reducing conditions [19]. Protein standards (phosphorylase B (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa)) were co-electrophoresed and their position and molecular weights are indicated. Proteins were detected after silver staining as described by Merril et al. [22].

In a control group ($n = 3$), the stimulation of the commissural pathway at a low frequency (0.033 Hz, 0.05 ms duration) evoked in the stratum radiatum a negative field EPSP which remained stable during the 3 h of the experiment (Fig. 1A). As shown in Fig. 1B, the pattern of the released proteins remained relatively constant during the 3 h of the experiment; it mainly consisted of 5 proteins with apparent molecular weights of 64, 54, 48, 45 and 16 kDa. A similar pattern was observed in 3 rats in which the commissural pathway was stimulated at 10 Hz during 10 s, a condition which does not induce LTP (data not shown).

In 8 rats which received a high frequency stimulation (100 Hz for 1 s) there was a long-lasting enhancement of the field EPSP. Fig. 2 illustrates the effects of a high-frequency electrical stimulation, in a typical experiment. As shown in Fig. 2A, the slope of the field EPSP showed a 64% increase in the first 15 min which followed the train, probably corresponding to the post tetanic potentiation [3]; this was followed by a smaller but significant increase of the EPSP of 22 $\pm$ 16% (mean $\pm$ S.D.) for 2 h. Fig. 2B depicts the protein pattern in perfusates obtained from the same rat. During the control period which preceded the high-frequency train (lanes 1–4), the pattern was similar to that shown in Fig. 1B; however, 90–120 min after the train there was a marked increase in the 5 proteins (Fig. 2B, lane 11). Similar results were obtained in 8 rats in spite of occasionally cyclic variations (Fig. 1B, lanes 3, 5, 7 and 10; Fig. 2B, lanes 3, 6).

An additional 19 kDa protein was conspicuous in relation to the train in 8 rats.

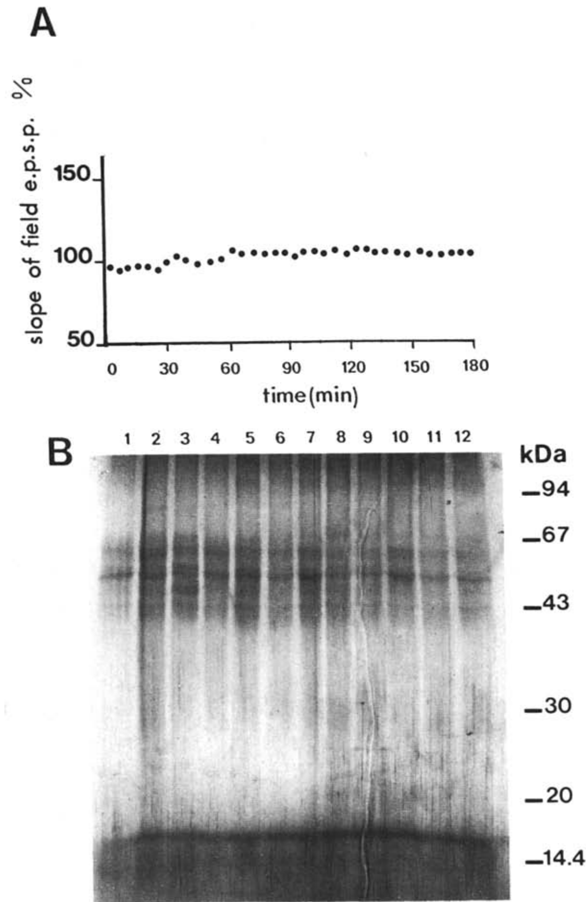

Fig. 1. Stability of the field EPSP and the pattern of released proteins in a control experiment. A: the commissural pathway was stimulated at 0.033 Hz and the slope of the negative field EPSP measured at 5 min intervals was expressed as a percentage of the mean value obtained during the control period (1 h). B: silver staining pattern of released proteins in the ECF electrophoresed in 12.5% SDS polyacrylamide gel. Each lane corresponded to proteins collected during 15 min periods. Note the release of 5 major proteins (64, 54, 48, 45 and 16 kDa) during the 3 h of the experiment.

In 6 rats this protein was not detected before the train, but was revealed 90 min after (Fig. 2B). In 2 additional rats, the 19 kDa protein was present prior to the train, but its concentration was markedly increased after it.

In 3 rats, the high frequency train failed to produce LTP; even though it was repeated twice or three times (Fig. 3A). Interestingly, there was a dramatic difference in the pattern of protein released; thus, as shown in Fig. 3B, the 28 and 19 kDa proteins which were present in high concentration before the train (lanes 1–6) were clearly reduced after it (lanes 7–12).

In 3 additional rats, we have tested the effect of the NMDA antagonist APV. APV (100 μM) prevented the LTP and did not affect the pattern of protein; there was no

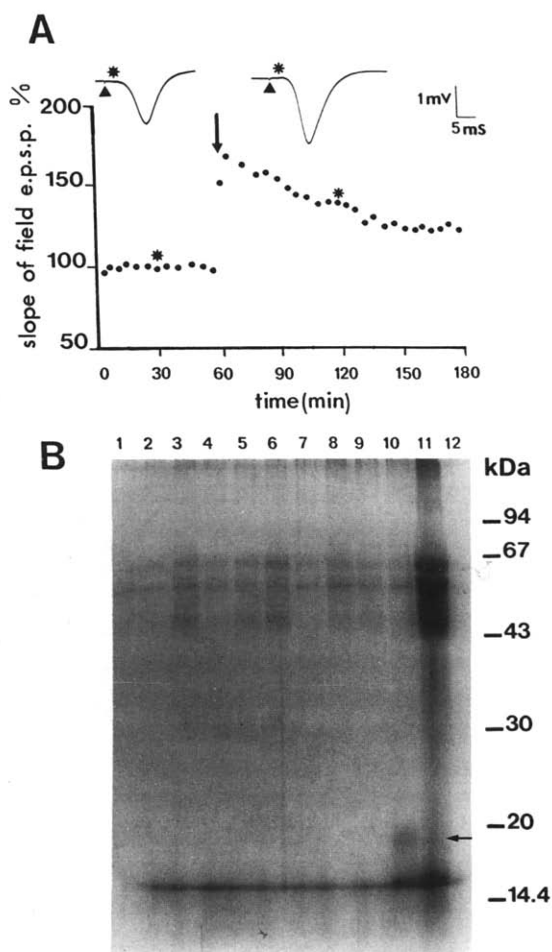

Fig. 2. Pattern of protein release in a typical experiment in which a brief high-frequency stimulation produced a LTP. Data are presented as in Fig. 1. A: the train (100 Hz for 1 s) was delivered 1 h after the start of the experiment as indicated by the arrow. It induced an enhancement of the slope of field EPSP, which persisted for 2 h. The traces represent the field EPSP before the high frequency stimulation and the potentiated EPSP 1 h after the stimulation. B: during the control period (lanes 1–4), note the typical protein pattern (64, 54, 48, 45 and 16 kDa) and note that the train induced a new 19 kDa released protein (lane 10, 90 min after the train) and an increase in the 5 proteins (lane 11).

clear change in protein concentration. It bears stressing however that the 28 kDa protein was not detected and the 19 kDa protein was only found in one case (data not shown).

The main observation of this study is that LTP induced in CA1 is associated with an increased release of proteins. Moreover, a new secreted 19 kDa protein is observed 90 min after the stimulation.

Our observations are in agreement with a recent preliminary study of Bliss et al. performed with a similar experimental design in fascia dentata [10]. Thus, in both

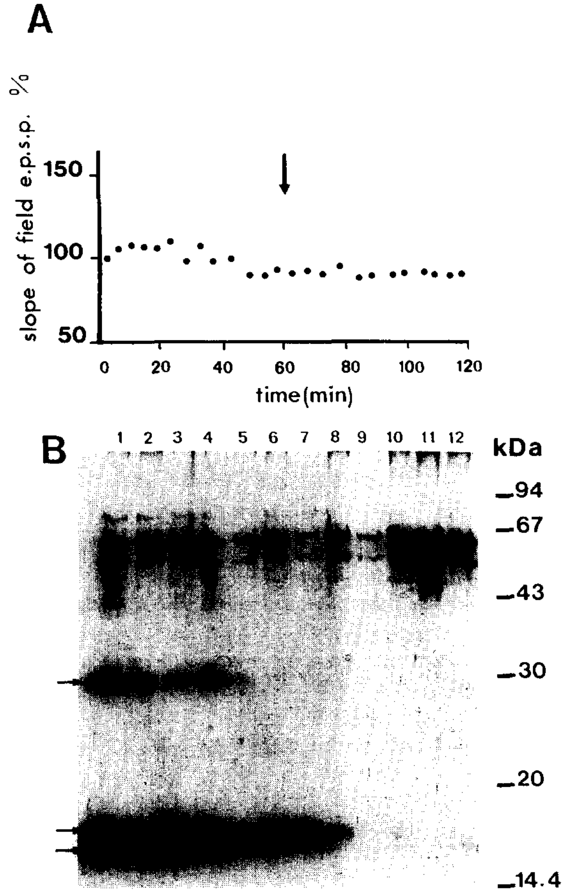

Fig. 3. Pattern of protein release in an experiment in which a brief high-frequency train did not produce a LTP. A and B, same presentation as in Fig. 1. A: the train (100 Hz for 1 s) applied 3 times 1 h after the start of the experiment (arrow) did not modify the slope of field EPSP which remained stable during 1 h. B: each lane corresponds to a 10 min sample. Note in this case, the release before the application of high-frequency train of 3 major proteins (28, 19 and 16 kDa) in addition to the 5 typical proteins (lanes 1–6). After the train, these 3 additional proteins were not secreted (lanes 7–12).

studies, there was a relatively stable release of 5 proteins (64, 54, 48, 45 and 16 kDa) during the control period which were increased 1–2 h after a brief high-frequency stimulation. This increase is only conspicuous when the train produces LTP. It is interesting to note that the molecular weights of some of these proteins (54, 48 and 45 kDa) are close to phosphorylated proteins which in rat brain synaptosomes are involved in synaptic plasticity [7] or in coated vesicles involved in endocytosis and presynaptic membrane recycling [24].

In the present study, we found in addition that two proteins (28 and 19 kDa) could also participate in LTP. The nature of these proteins and their role in the maintenance of LTP is presently elusive. However, it is intriguing that Shashoua [25] has

reported a release of a 19 kDa protein in the extracellular fluid of goldfish or Balb/c mice brains after a training paradigm. Hesse et al. [17] also described the presence of an 18 kDa protein newly synthesized in rat hippocampal slices. Further experiments are in progress to characterize the origin and nature of proteins involved in LTP.

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