Brain Research, 484 (1989) 352–356
Elsevier

BRE 23438

Influence of epidermal growth factor on long-term potentiation in the hippocampal slice

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(Accepted 20 December 1988)

Key words: Hippocampal slice; Long-term potentiation; Epidermal growth factor; Neuromodulation

Rat hippocampal slices were perfused with epidermal growth factor (EGF) at a concentration of 10^{-8} M. EGF is a well known mitogen which exhibits neurotrophic action on neurons of the CNS. During extra- and intracellular recordings in the pyramidal cell body layer of the CA1-region no influence of EGF on evoked potentials was seen if single-pulse or paired-pulse stimulation was used. Furthermore EGF has no influence on the resting membrane potential of the cells investigated. However after tetanic stimulation a significant increase in the magnitude of long-term potentiation was observed. Therefore it is concluded, that EGF might be involved in modulation of neuronal plasticity.

The epidermal growth factor (EGF) is a small peptide of 53 amino acids (MW 6045). It is well known as a mitogenic factor for several cell types. EGF immunoreactivity[10] and precursor messenger RNA[21] have been identified within the mammalian brain. Furthermore, EGF-receptor immunoreactivity was shown in rat brain[11]. The presence of EGF within the brain is, however, the subject of controversy[20]. Recently it was demonstrated that EGF exhibits neurotrophic effects on neurons of the CNS[18].

Long-term potentiation (LTP) is a long lasting increase in synaptic strength after brief repetitive activation that lasts for many hours and, in intact animals, even for weeks[4,5]. With respect to the mechanisms of LTP much is known about the initiation phase, especially concerning the involvement of *N*-methyl-D-aspartic acid (NMDA)-receptors (for review see refs. 8,19). Since activation of NMDA receptors and postsynaptic depolarisation are necessary but not sufficient for the maintenance of LTP[15], current research is focussing now on the mechanisms underlying this latter phase of LTP. Furthermore, there is evidence, that the release of glutamate is enhanced following LTP[3]. This finding suggests that the postsynaptic membrane (site of initiation) must somehow communicate in a retrograde manner with the presynaptic site (site of expression). Therefore it was proposed that soluble factor(s) might be involved in the mechanisms underlying the link between initiation phase and maintenance phase.

It was hypothesized that EGF might act as a neurotransmitter or neuromodulator or both[10]. Therefore the present study was undertaken to evaluate whether addition of EGF has an effect on neuronal excitability of the hippocampal slice especially concerning LTP.

Transverse hippocampal slices (400 μm thick) were prepared from 8–12-week-old male Wistar rats (200–300 g). After cutting, the slices were placed directly in one of two recording chambers and kept at 33 ± 0.3 °C. The slice chamber (after ref. 17, modified by H. Terlau) was constantly aerated with 5% CO_2–95% O_2 and Ringer solution (containing in mM: NaCl: 124; KCl: 5.0; KH_2PO_4: 1.25; $MgSO_4 \times 7H_2O$: 2.0; $CaCl_2 \times 2H_2O$: 3; $NaHCO_3$: 26; D-glucose: 10) was perfused continuously through both recording chambers at a rate of 0.6 ml/min.

After a preincubation period (1.5 h) one slice was

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transferred into the second recording chamber and a bipolar recording electrode (tungsten in glass) was placed under visual control in the stratum radiatum of the CA3-region. The recording electrode (glass pipettes either filled with 2M NaCl (4–16 MΩ) or filled with 4 M KAc (30–80 MΩ)) was guided into the pyramidal cell layer of the CA1-field. Stimuli consisted of a constant current pulse of 0.05 ms duration and were delivered at 0.1 Hz at all times except during high frequency stimulation (1 × 100 Hz for 2 s) to induce LTP. In part of the experiments paired pulses with an interval of 55 ms were applied.

Only slices in which a clearly visible population spike could be seen with a stimulus strength below 450 μA were used in further analysis. In one part of the experiments adjacent slices were separated into matched pairs (control slice, EGF-slice). The stimulus strength in these experiments was adjusted in such a way that before the tetanus was given the amplitude of the population spike in the control- and the EGF-treated slice was quite similar (Control: 5.10 ± 0.30 mV; EGF: 5.43 ± 0.34 mV; mean ± S.E.M., n = 21 pairs). For equivalent conditions in both slices it was tried to place the electrodes in a quite similar way, especially with respect to the distance[9]. Furthermore paired-pulse stimulation was used to get a comparable paired-pulse facilitation in the control- and the EGF-treated slice.

For intracellular recording an impaled neuron was accepted when it had no spontaneous discharges and a membrane potential below −60 mV. In these experiments the stimulus strength was adjusted so that no spike was seen.

Evoked potentials were stored with a lab computer based on a LSI11/73. During extracellular recordings 6 or 12 sweeps were averaged on line. During intracellular recordings single sweeps were stored. Off-line data analysis was done with a micro-VaxII.

Population spike amplitudes were defined as the amplitude from the negative peak to the late positive peak. Excitatory postsynaptic potential (EPSP) amplitudes were measured from baseline to peak. Lyophilized EGF from mouse submaxillary glands (Sigma) was first diluted in sterile distilled water and aliquots were stored at −20 °C. For each experiment one aliquot was taken and added to Ringer solution to final EGF-concentration of 10^{-8} M. Perfusion

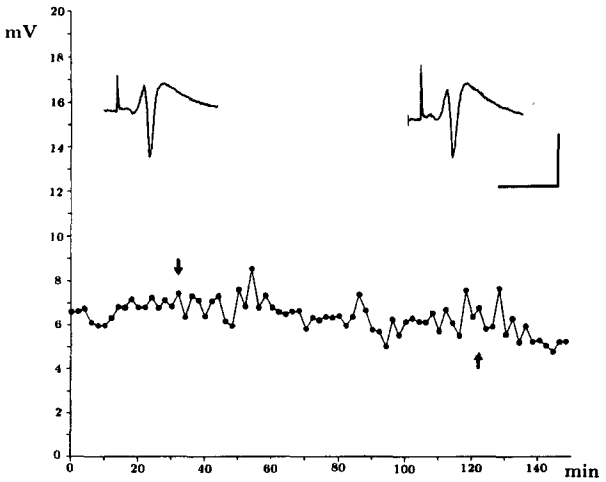

Fig. 1. Amplitude of the population spike during low-frequency stimulation and EGF perfusion. Upper panel: field potential (population spike) before addition of EGF and 90 min after addition. The calibration represents: 5 mV, 10 ms. Lower panel: changes of the population spike with the time. Each point represents an average of 12 stimuli. At ↓ perfusion with Ringer solution containing EGF was started. At ↑ the slice was perfused again with Ringer solution.

with this EGF-containing Ringer was only started after a stable potential (either field potential or EPSP) was seen. When tetanic stimulation was applied, perfusion with EGF was started 20 min before the tetanus.

Perfusion of the slices (n = 5) up to 90 min with EGF has no influence on the evoked field potential (population spike) during low-frequency stimulation (see Fig. 1). In contrast, a significant increase in the magnitude of potentiation after tetanic stimulation was observed compared to the control slices (see Fig. 2). The maximal increase was seen 1 min after tetanic stimulation. At this time point the size of the population spike in the EGF-treated slice was 323.7 ± 18.8% (mean ± S.E.M.) of the control value, while in the control slices the increase was 258.0 ± 18.9% (n = 21 pairs, $P < 0.01$). In 14 cases the magnitude of potentiation was higher in the slices treated with EGF. In 6 pairs there was only little or no difference in control and EGF-slices. In one pair of slices the control slice exhibited more potentiation than the EGF-treated slice.

The magnitude of potentiation 30 min after induction of LTP was usually reduced but still the slices, which were perfused with EGF, showed more potentiation than the control slices (Control: 248.0

± 21.4%, EGF: 307 ± 26.1%, $P < 0.02$). After this time point the observation was ended.

Intracellular recordings showed that perfusion with EGF has no influence on the resting membrane potential of the cells investigated (data not shown). The longest observation time after addition of EGF was 70 min. Furthermore there was no influence of EGF on the amplitude of the first EPSP during paired-pulse stimulation (see Fig. 3). At the beginning of the perfusion the average amplitude of the first EPSP (n = 18 cells) was: 3.55 ± 0.27 mV (mean and S.E.M.). The average of the EPSP amplitudes 20 min later (the same time point at which the tetanus was given during the experiments concerning LTP) was: 3.07 ± 0.40 mV. Both values were calculated from the average amplitude of 10 sweeps at both time points. Furthermore no changes in the average amplitude of the second EPSP during EGF perfusion were seen: 6.86 ± 0.44 mV at beginning of perfusion with EGF, 6.22 ± 0.46 mV 20 min later (n = 16, two cells started to show action potential after beginning with EGF perfusion and were discarded from analysis; see Fig. 3).

Our results demonstrate that perfusion of hippocampal slices with EGF does not lead to changes in excitability of the neurons if low frequency or paired-pulse stimulation was given as measured by population spike amplitude, resting membrane potential and EPSP amplitudes. However after tetanic stimulation a significant increase in long-term potentiation was observed. Although we have no direct evidence for a molecular mechanism of these results, there are several possibilities which might be considered: first it is well established, that EGF binding

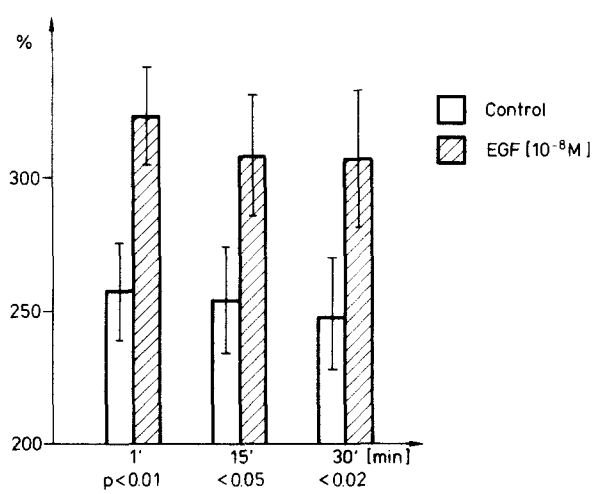

Fig. 2. Effect of EGF on long-term potentiation. Slices were stimulated and LTP was induced as described in the text. 100%: average amplitude of the population spike 10 min before tetanic stimulation. P-values: Wilcoxon signed rank test.

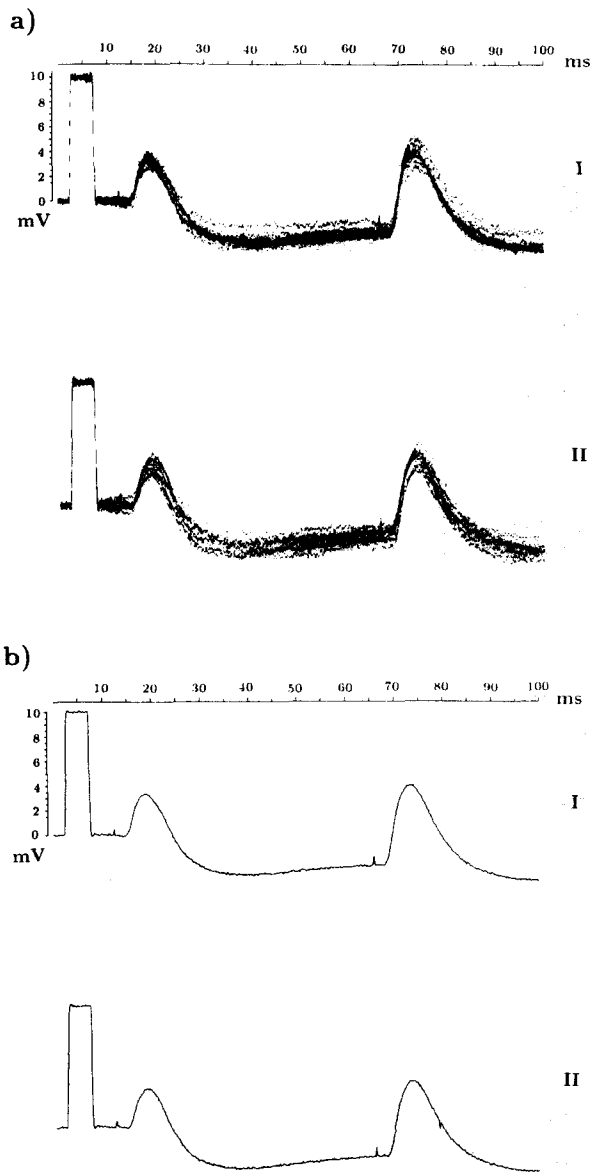

Fig. 3. EPSP's evoked by paired pulse stimulation. a), single responses at the beginning with the perfusion of the EGF-containing Ringer solution (I) and 20 min afterwards (II). b), the averages of the responses shown in a). The square wave pulse represents a calibration (5 ms, 10 mV). Since every sweep is 20 s apart, both pictures represent 200 s of recording time.

to its receptor leads to an activation of tyrosine kinase activity at the intracellular site of the receptor (for review see ref. 14). Furthermore, it has been shown that EGF addition leads to Ca^{2+}-influx[7,22,23] and to release from intracellular Ca^{2+} stores[7] resulting in an increase in cytoplasmic-free Ca^{2+}-concentration. Evidence from several laboratories points to the important role of Ca^{2+} in the mechanisms of LTP — both during the induction phase and during the maintenance phase. Furthermore it is known that Ca^{2+} activates protein kinase C (PKC) which is probably involved in the maintenance of LTP[12].

It is also known from other cell systems that addition of EGF leads to rapid induction of c-fos and c-myc proto-oncogenes[6] and resulting synthesis of c-fos protein. It has been shown, that protein synthesis is probably required for maintenance of LTP using the protein inhibitor anisomycin[16]. It might be possible that the lack of EGF is the reason that in vitro electrical or pharmacological stimulation of slices of hippocampus was without an effect on levels of c-fos within neurons[13].

The effects of EGF on Ca^{2+}-influx, Ca^{2+}-mobilisation, gene transcription and ultimate cell growth probably all depend on intact tyrosine kinase activity of the EGF-receptor[7].

Another molecular mechanism of the effect of EGF on LTP may be via the phosphoinositol pathway. It has been reported that EGF leads to an enhancement in the phospholipid turnover and production of diacylglycerol[22]. Diacylglycerol leads to an activation of PKC, which is involved in LTP too (see above). A general scheme of a possible role of growth factor(s) in signal transduction and neuromodulation related to the phospholipid turnover and resulting effects has been proposed earlier[1,2]. Interestingly, phosphorylation of EGF-receptors by PKC appears to regulate their function. Purified EGF-receptors have decreased EGF-stimulated tyrosine kinase activity if first phosphorylated by purified C-kinase. This phosphorylation may be a mechanism that can partly explain down modulation of EGF binding by EGF itself and by other extracellular factors.

Under our conditions, EGF exhibits an effect only when a high-frequency train is applied and thus the neurons are strongly depolarized. This seems feasible, since EGF leads to an increase in intracellular Ca^{2+} and therefore more Ca^{2+} may be available for both the pre- and postsynaptic site of the synapses during and after tetanic stimulation. The fact that no change in the population spike amplitudes and in the EPSP amplitudes was seen during low-frequency stimulation and paired-pulse stimulation, could be explained by the existence of some threshold for the intracellular Ca^{2+}-concentration which might be reached only under tetanic conditions. Furthermore the enhancement in potentiation by EGF is present over the whole time investigated. Since EGF addition leads to activation of several processes in parallel (as discussed above), it might be possible that the observed effect may consist of at least two or more processes.

The observation that a growth factor with neurotrophic potential like EGF modulates synaptic plasticity is highly interesting and deserves further investigation.

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