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Interleukin-6: a cytokine to forget

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SPECIFIC AIMS

Proinflammatory cytokines such as interleukin-6 (IL-6) are expressed in the CNS during disease conditions and affect brain functions such as memory and learning. We have investigated whether *1)* the cytokine interleukine-6 is produced in the "healthy" brain during changes in synaptic plasticity and *2)* IL-6 has a physiological function in synaptic plasticity and learning.

PRINCIPAL FINDINGS

1. IL-6 gene expression is increased during LTP in hippocampal slices

In a first set of experiments, extracellular recordings in the CA1 region of hippocampal slices were used to explore whether changes in IL-6 gene expression occur during LTP. Once stable baseline recordings were established for at least 30 min, robust long-term potentiation (LTP) was induced by triple high-frequency tetanic stimulation at 100 Hz for 1 s (intertrain interval: 1 min); IL-6 gene expression was quantified by RT-PCR at different times. No significant changes in the levels of IL-6 transcripts were detected 15 to 20 min after tetanic stimulation. However, a clear increase in the accumulation of IL-6 mRNA was evident by 1 h compared with control slices in which no LTP was induced. This increase in IL-6 expression persisted as long as LTP remained stable.

2. IL-6 gene expression is enhanced during LTP in freely moving rats

That IL-6 was expressed during LTP in hippocampal slices prompted us to study whether an analogous expression occurs during more physiological conditions, i.e., LTP in the hippocampus of freely moving animals that have been chronically implanted with a recording and a stimulation electrode, respectively, in the right dentate gyrus. After stable responses were obtained for 45 min, a "saturated," late-LTP was induced by strong tetanic stimulation consisting of 10 bursts of 15 pulses at 200 Hz. Eight hours after tetanic stimulation, rats were killed for examination of IL-6 mRNA expression by RT-PCR.

A clear increase in IL-6 mRNA expression was observed in the ipsilateral part of the hippocampus of animals when LTP was induced (ipsilateral hippocampus, **Fig. 1*A, B***, 4_i). In contrast, IL-6 expression in the contralateral site of the hippocampus remained at control levels (Fig. 1*A, B*, 4_c). No significant changes in IL-6 gene expression in the hippocampus were detected when induction of LTP was blocked by intracerebroventricular injection of the NMDA receptor antagonist AP-5 (Fig. 1*A, B*, 2_i) or in rats that received the same tetanic stimuli but exhibited a potentiation of only 2–3 h duration (Fig. 1*A, B*, 3_i).

3. Immunoneutralization of endogenous IL-6 in the brain reinforces LTP

The results mentioned above led to the question of whether the increase in endogenous IL-6 observed during LTP would be relevant for this process. The strategy chosen was to administer a neutralizing IL-6 antibody (IL-6Ab) to freely moving rats undergoing LTP. The antibody was administered i.c.v. 90 min after tetanic stimulation, when increased production of IL-6 is expected in the hippocampus. A weak "unsaturated" LTP was induced by applying three bursts of 15 pulses at 200 Hz, allowing detection of reinforcing and inhibitory effects on potentiation.

As shown in **Fig. 2**, LTP in rats that received the IL-6Ab clearly outlasted the potentiation observed in controls receiving the preimmune serum. Thus, rats of the IL-6Ab group displayed a significant potentiation for at least 8 h but LTP in controls decayed already after 150 min to baseline values. This effect was confined to an application time of 90 min after tetanization since no significant effects were detected when the antibody was injected either 30 min before or 5 min after tetanic stimulation.

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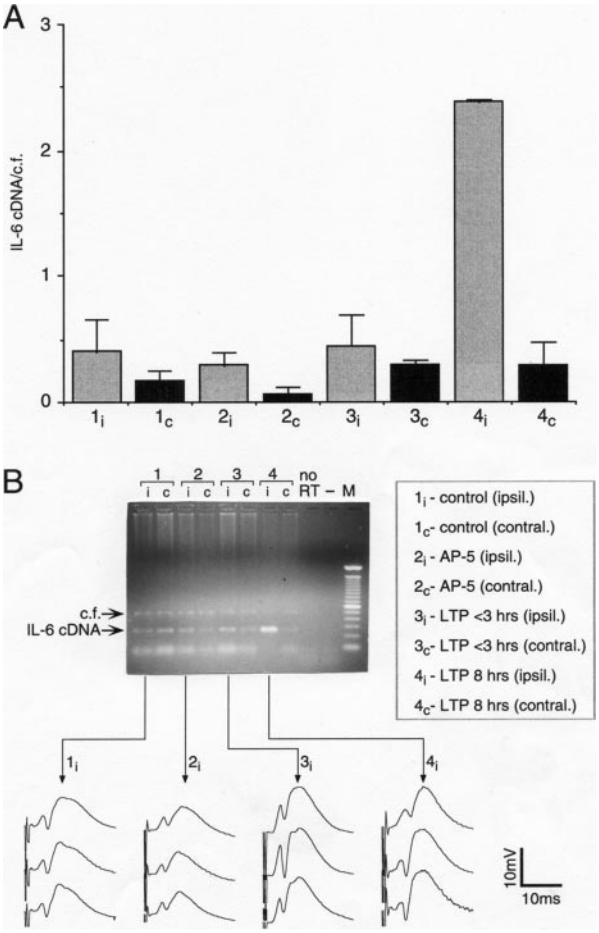

Figure 1. IL-6 gene expression during LTP in freely moving rats. *A)* IL-6 mRNA expression 8 h after tetanization. 30 min before tetanization, animals received physiological saline (groups 1, 3, and 4) or AP-5 (group 2) administered i.c.v. Gray bars indicate measurements performed in ipsilateral (i) and black bars in contralateral (c) hippocampi. Group 1: hippocampi of animals recorded under baseline conditions without tetanic stimulation. Group 2: hippocampi of animals in which LTP expression after tetanization was blocked by AP-5. Group 3: hippocampi of animals showing a potentiation that returned to baseline within 3 h. Group 4: hippocampi of animals with a robust LTP lasting for 8 h. Results of RT-PCR are expressed as mean ±SE of the ratio IL-6 cDNA and the competitive fragment (cf). $n = 4$ per group. Group 4_i differs significantly from all other groups (ANOVA followed by Fischer test for multiple comparisons). *B)* Representative ethidium bromide-stained agarose gel showing amplified transcripts of a RT-PCR obtained from ipsi- and contralateral hippocampi subjected to different experimental conditions (lane numbers correspond to groups shown in panel *A*). The line graphs display representative analog traces recorded during baseline (top), immediately after tetanization (middle), and 8 h after tetanus (bottom). M, 100 bp ladder molecular weight marker; no RT, RT-PCR without addition of reverse transcriptase; –, RT-PCR without addition of cDNA.

4. Immunoneutralization of endogenous IL-6 in the brain improves long-term memory

The potent effects of an immunoneutralization of IL-6 on hippocampal LTP in vivo prompted us to check whether the same treatment will affect hippocampus-dependent learning. We used a spatial alternation paradigm in which animals are forced by a mild foot shock to acquire an alternation between two arms of a Y-maze in complete darkness. During training on day 1, the IL-6Ab group and the control group acquired the task equally well as shown by percentage of errors, which were virtually the same. However, during the retention sessions 24 h later, IL-6Ab animals displayed significantly fewer errors compared with controls. If the data are analyzed by grouping the errors scores of the training and the retention session into blocks of 10 trials, it is obvious that the deficit of the control group results from less efficient memory storage subsequent to the acquisition of the task on day 1. Thus, during the retention session the control group started at about the same level as during acquisition whereas IL-6Ab animals displayed remembrance of the task starting with a lower percentage errors; this deficit could not be compensated for by the control group during the retention session.

CONCLUSIONS AND SIGNIFICANCE

The data presented here provide clear evidence that biologically relevant amounts of IL-6 are produced during certain types of LTP and memory formation and that these amounts have a function in the "consolidation" of these processes. It has been repeatedly shown that LTP and long-term memory require the specific

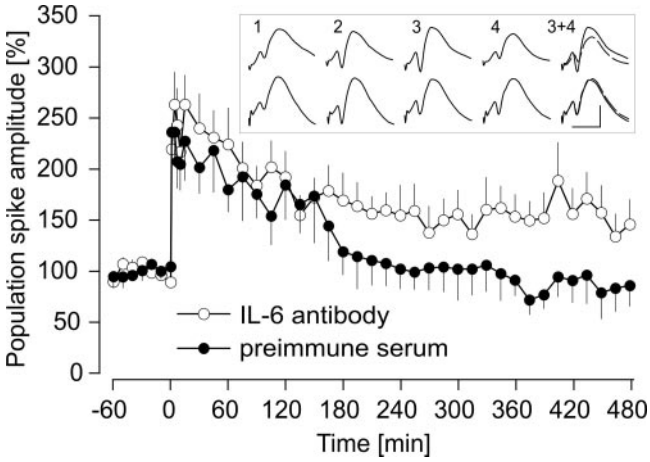

Figure 2. Immunoneutralization of IL-6 reinforces LTP in freely moving rats. 90 min after tetanic stimulation, freely moving rats were infused i.c.v. with IL-6 neutralizing antibodies (n=9) or the respective preimmune serum (control, n=8). Data are expressed as mean ±SE. LTP of rats that received the IL-6 antibody outlasted the potentiation observed in controls, where the recording returned to baseline values ~60 min after injection (P=0.034, ANOVA with repeated measures). Data are plotted as average change from baseline response (mean ±SE). Insets: representative analog traces of field recordings taken *1)* during baseline measurement, *2)* 10 min after tetanization to exclude post-tetanic potentiation, *3)* at 90 min, i.e., immediately before antibody administration, *4)* 8 h after LTP induction. Scale bars: 5 mV and 5 ms, respectively.

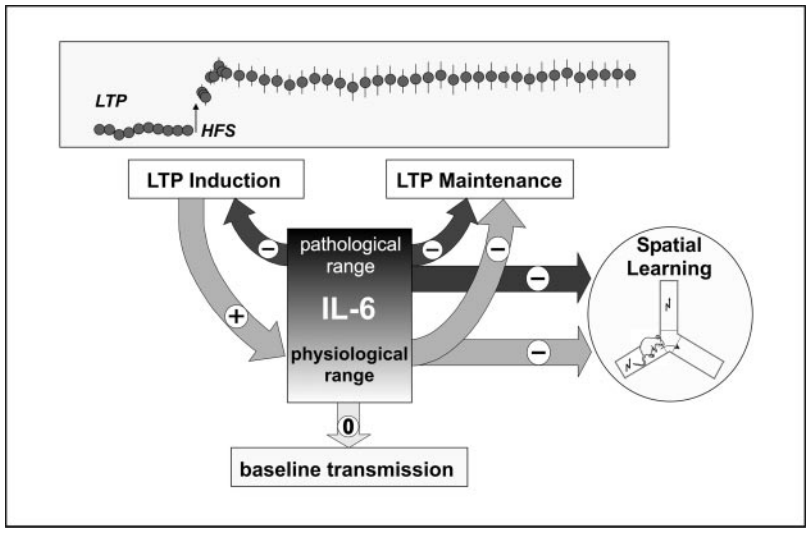

Figure 3. Scheme of the putative role of IL-6 in synaptic plasticity and learning. Although IL-6 levels in the pathological range are known to impair synaptic plasticity and learning, our study points to a physiological function of IL-6 in these processes. LTP induction by high-frequency stimulation (HFS; upper graph) increases IL-6 levels, which then exert an inhibiting effect on the maintenance of LTP. Likewise, IL-6 impairs the consolidation of spatial memory as evidenced by application of IL-6 neutralizing antibodies. In contrast, blockade of IL-6 has no overt effect on baseline transmission. Darker part of arrows indicates higher IL-6 concentration/release.

and sequential activation of distinct genes as well as the de novo synthesis of certain proteins to become consolidated. Our data indicate that activation of the IL-6 gene is a critical factor for LTP maintenance and consolidation of spatial alternation memory.

Our LTP studies provide clear evidence that the increased expression of IL-6 in the freely moving rat is triggered by mechanisms intrinsic to the potentiation, as it is NMDA receptor dependent and not caused by the tetanic stimulation per se. The increased expression of the IL-6 gene after LTP induction led us to hypothesize that translation of this gene would result in sufficient amounts of IL-6 protein to affect LTP. The results obtained support this hypothesis. When endogenous IL-6 was blocked 90 min after tetanization by i.c.v. administration of a specific IL-6 neutralizing antibody, rats showed improved maintenance of LTP that outlasted the potentiation of controls (injection of the preimmune serum). No comparable effect was obtained when the antibody was administered before or immediately after tetanic stimulation. These results indicate that basal levels of IL-6 do not affect LTP induction. Therefore, only at the time when endogenous IL-6 production is increased the cytokine participates in the regulation of LTP consolidation as a negative regulatory element. ”Strengthening” of LTP maintenance by neutralizing the IL-6 produced after tetanization indicates that this cytokine contributes to the temporal confinement of potentiation. Thus, the effect of IL-6 is opposite to the supporting role of endogenous IL-1 on LTP maintenance.

As congenial complement to the function of IL-6 in synaptic plasticity, the findings of our learning experiments indicate a similar impeding role of IL-6 in post-trial memory consolidation of spatial alternation learning, a hippocampus-dependent task. Thus, intracerebroventricular application of the same amount of neutralizing IL-6Ab used in the LTP experiments in vivo resulted in a significant improvement of retention when tested 24 h after learning.

As indicated by the missing effects of IL-6Ab infusion before or immediately after LTP induction, the susceptibility of electrophysiological and behavioral read-outs to cytokine application or any perturbation of endogenous cytokine levels depends on time. This might explain the lack of any effect when IL-6 was applied i.c.v. before water maze training and testing as reported in other studies.

Our learning data strongly suggest that physiological levels of IL-6 exert an inhibitory action on certain types of learning and memory. Similar to its role in plasticity, IL-6 might act as a filter in the fine-tuning of consolidation by limiting long-term storage of some types of information. Together, our data suggest a decisive physiological role of IL-6 in mechanisms controlling the kinetics and amount of information storage. FJ