Hippocampal Interleukin-1β Gene Expression during Long-Term Potentiation Decays with Age

DETLEF BALSCHUN,[a] ANKE RANDOLF,[b] FERNANDO PITOSSI,[c] HORST SCHNEIDER,[b] ADRIANA DEL REY,[b] AND HUGO O. BESEDOVSKY[b]

[a]*Leibniz Institute for Neurobiology, 39 118 Magdeburg, Germany*

[b]*Institute of Physiology, Division of Immunophysiology, Philipps University Marburg, 35037 Marburg, Germany*

[c]*Fundacion Instituto Leloir, University of Buenos Aires, Buenos Aires, Argentina*

KEYWORDS: cytokines; interleukin-1 (Il-1); gene expression; long-term potentiation; hippocampus

INTRODUCTION

Molecules originally described as intrinsic to the immune system are evidently also involved in physiological processes that are different from immunoregulation. A clear example is the proinflammatory cytokine interleukin-1 (IL-1). This cytokine is also synthesized in the brain by glial cells and certain neurons, and its receptors have been found in different regions of the CNS with the greatest abundance in the hippocampus.[1–4] These complementary findings indicate a physiological role of IL-1 in the brain. In further support of this, pharmacological and behavioral studies demonstrated the capacity of IL-1β to affect various neuroendocrine functions, to alter the release and turnover rate of certain neurotransmitters and modulators, and to induce changes in behavior.[5,6] (See Refs. 1, 7, and 8 for additional references.) However, in most cases the effects of IL-1 in the brain were tested either *in vitro* or after exogenous administration of pharmacological doses of the cytokine.

We previously described a function for physiological levels of IL-1 in long-term potentiation (LTP), the best-studied model for the cellular mechanisms underlying memory storage.[9,10] The expression of IL-1 in the hippocampus was significantly enhanced during LTP.[11] This increased production of IL-1 was not an epiphenomenon, because blockade of brain receptors for this cytokine resulted in significant inhibition of LTP maintenance.[11] Pathologically high levels of IL-1, however, achieved by external application of the cytokine were reported to impair LTP[12–16] and learning.[17–20] In continuation of our previous study,[11] we investigated the expression of IL-1 during LTP *in vivo* in older (12–16-month-old), freely moving

Address for correspondence: Hugo O. Besedovsky, Institute of Physiology, Division of Immunophysiology, Philipps University Marburg, Deutschhausstr. 3, 35037 Marburg, Germany.

Ann. N.Y. Acad. Sci. 992: 1–8 (2003).

rats. Here we report decay of IL-1 gene expression during LTP with age, which is not accompanied by impairment of LTP.

MATERIAL AND METHODS

Animals. Male Wistar rats were used for the experiments. Young rats were about 3 months old, and middle-aged rats were between 12 and 16 months old. Animals were fed ad libitum and housed in temperature- and light-controlled (12 h/day) rooms. Rats were treated in accordance with institutional guidelines.

Long-Term Potentiation in Vivo. Experiments were performed on freely moving rats. A monopolar recording electrode (coordinates AP −2.8, L 1.8) and a bipolar stimulation electrode (coordinates AP −6.9, L 4.1) were implanted stereotaxically under Nembutal anesthesia (40 mg/kg, ip) into the granule cell layer of the dentate gyrus and into the perforant path, respectively, of the right hemisphere. Animals were allowed to recover from surgery for at least 1 week. The stimulus intensity was adjusted to evoke 40% of the maximum population spike amplitude. Five test stimuli were applied every 10 minutes and the responses averaged for each set of the stimulus parameters. Once stable responses were obtained for 45 minutes, LTP was induced by strong tetanic bursts (10 bursts of 15 pulses 200 Hz, 0.2-ms duration of each stimulus, interburst interval 10 seconds), resulting in a "saturated," late LTP. Population spikes were recorded 1, 4, 7, 10, and 15 minutes after tetanization. Thereafter, recordings were taken in 15-minute intervals until the end of the experiment.

Interleukin-1β Gene Expression. To study IL-1β mRNA expression, rats were killed 8 hours after tetanic stimulation. After decapitation, ipsilateral and contralateral hippocampi were quickly dissected and processed for the evaluation of IL-1 mRNA. RNA was extracted from the hippocampi following standard protocols and reverse transcribed using a commercial kit (Superscript II RT kit, GIBCO/BRL). All conditions used in the semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) using a multispecific competitive fragment (pRat6) were previously described.[21] The primer sequences were: IL-1β, sense: TCCATGAGCTTTGTACAAGG and antisense: GGTGCTGATGTACCAGTTGG. Amplicons were separated by agarose gel electrophoresis in 1.5% gels containing 5 μg/ml ethidium bromide and 10% PAGE at 80 volts for 3 hours. Bands were visualized in the agarose gels by excitation at 316 nm and documented. Signals were quantified using a Phosphorimager device (Molecular Dynamics) with an Image Quant program. Background levels were subtracted from the detected signals. Each individual sample was amplified by PCR at least three times.

RESULTS AND DISCUSSION

To test whether the expression of IL-1β during LTP is subject to age-dependent changes, we evaluated IL-1β gene expression in young and middle-aged rats. As evidenced by the values of the untetanized left hippocampi (contralateral side) (FIG. 1), basal IL-1β gene expression was slightly reduced in middle-aged rats compared to young rats (0.400 ± 0.040 vs 0.224 ± 0.040; $P < 0.05$, Student's t test). Although both groups of rats displayed significant enhancement of gene expression in

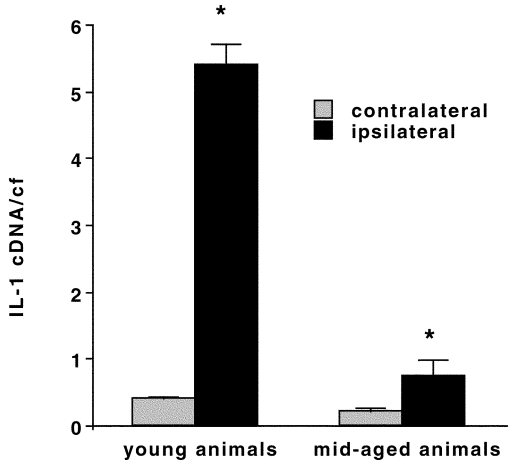

FIGURE 1. Interleukin-1β gene expression during long-term potentiation (LTP) in the dentate gyrus of freely-moving young and middle-aged rats. IL-1β gene expression during LTP i*n vivo* as assessed by RT-PCR (ratio IL-1 cDNA/competitive fragment [cf], mean ± SEM). Middle-aged rats display a lower basal level (contralateral hippocampi) and a reduced upregulation of IL-1β gene expression during LTP (ipsilateral hippocampi). Young rats: $n = 4$; middle-aged rats: $n = 5$. $^*P < 0.05$.

the right hippocampi (ipsilateral side) during LTP, this increase was more pronounced in young than in midde-aged rats (FIG. 1). Thus, young rats showed a more than 10-fold increase (ipsilateral versus contralateral hippocampus), but the rise in middle-aged animals was only threefold. Our findings apparently contradict reports of increasing hippocampal IL-1β levels during aging.[22,23] However, because these studies were conducted with older rats (22 months old), we cannot completely rule out that the decrease in IL-1β in middle-aged rats is only temporary and is followed by upregulation in older animals.

As depicted in FIGURE 2, young and middle-aged rats attained a comparable magnitude of potentiation and did not differ significantly in the maintenance of LTP. The only discernible difference was a tendency of a faster decay of potentiation in middle-aged rats. A weakened synaptic plasticity within the hippocampus had been suggested to underlie deficits in hippocampal-dependent learning and memory in aging rodents.[24–26] However, age-dependent deterioration of potentiation does not appear to manifest before the age of about 20 months in rats. Furthermore, a decline of certain forms of LTP was mostly reported for the CA1 region,[26–31] whereas the situation in the dentate gyrus is not clear. Our results confirm a continuing capacity for the induction of dentate LTP during aging in rats, as also supported by other groups.[32–34] Impairment of dentate LTP in aged animals has been described by Lynch *et al.*[23] and O'Donnell *et al.*[35] In accordance with lacking or only mild overt deficits in dentate LTP, the capacity for morphological synaptic changes during LTP was widely preserved in the dentate gyrus of aged rats.[36] Furthermore, several signaling cascades appear to operate normally in aged rats, as indicated by an

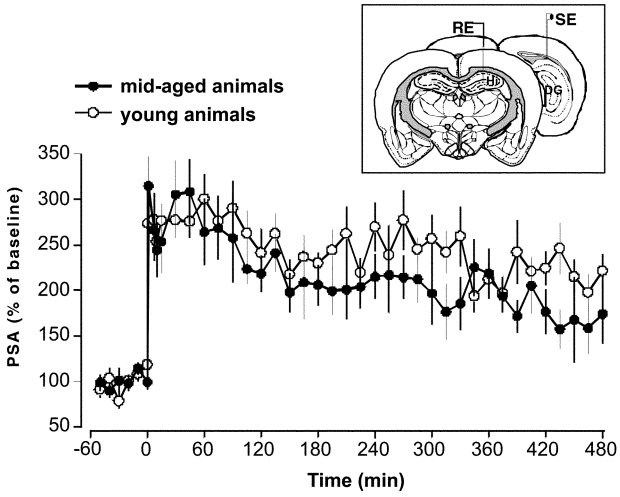

FIGURE 2. Long-term potentiation (LTP) induction in young and middle-aged rats. LTP is unchanged in middle-aged rats ($n = 5$) compared with young rats ($n = 7$). The only discernible difference was a weak tendency to faster decay of potentiation in middle-aged rats. PSA, population spike amplitude. The scheme represents placement of the stimulation electrode (SE) in the perforant path and of the recording electrode (RE) in the granule cell layer of the dentate gyrus. *DG,* dentate gyrus; *HI,* hilus of DG.

unchanged induction of an array of immediate early genes that are known to be transcriptionally activated after LTP-inducing stimuli.[37] Other pathways, however, were found to be changed in senescent animals.[25,35,37–39] Elevation of the threshold for LTP induction that has been described in aged, spatial memory-impaired rats[40] is likely to be compensated by a tetanization protocol that is sufficiently strong.

Taken together, our findings provide evidence of a reduced basal level and a decreased range of regulation of Il-1β gene expression in middle-aged animals. Since our previous investigations indicated a supportive effect of enhanced IL-1β levels on LTP maintenance, the decreased upregulation of IL-1β gene expression during LTP in middle-aged as compared to young animals would be anticipated to result in impaired maintenance of LTP in the older animals. However, this was not observed. The reasons might be the following: (1) Normal LTP only requires a "threshold" concentration of IL-1β that is also achieved in middle-aged animals. (2) The lower upregulation of IL-1β gene expression during LTP may be compensated by a greater efficacy/activity of subsequent translational cascades, resulting in about the same concentration of IL-1β protein in young and middle-aged rats. (3) The net efficacy of IL-1β in LTP does not only depend on IL-1ß concentration but is also contingent on the level of Il-1 receptors,[41] the IL-1 receptor accessory protein (IL-1RAcP),[6] and the IL-1 receptor antagonist (IL-1ra). Thus, the equilibrium of these regulatory components might be differently adjusted in older animals, while still ensuring almost normal potentiation. The preliminary findings of our laboratories support upregulation of IL-1ra during LTP. Interestingly, interleukin-6 (IL-6), which can be induced by IL-1 in the brain,[42,43] is also upregulated during hippocampal

LTP,[44,45] but it has a function opposite to that of IL-1 in LTP (Balschun *et al*., manuscript submitted). Hence, it is tempting to speculate that in the brain a cytokine network that is activated during LTP is important for the fine-tuning of mechanisms responsible for the consolidation of potentiation. Cytokines such as IL-1 and IL-6 are known to readily diffuse from their site of release in the brain.[46,47] Therefore, their increased production triggered by potentiated neurons can be expected to result in a wave of cytokines, which spreads to surrounding neurons. Since potentiation is not confined to activated synapses but propagates to synapses on neighboring neurons,[48,49] an enhanced level of diffusing IL-1 could prevent such a propagation of potentiation as suggested by inhibition of LTP by IL-1 *in vitro*[12–16] and in freely moving rats (Ref. 16 and unpublished data). By contrast, IL-6 would tend to confine an already expressed potentiation. In this way, IL-6 and IL-1 could act as diffusible messengers that augment the input-specificity of plasticity.

In conclusion, our data exclude a simple linear relation between IL-1β gene expression and the properties of synaptic plasticity in the dentate gyrus, thereby supporting the existence of fine-tuned, cytokine-mediated regulatory mechanisms. The elucidation of age-associated changes in these processes could provide a clue to the preservation of cognitive performance in old age.

SUMMARY

Interleukin-1 is a cytokine that, apart from its contribution to immunoregulation, can affect neuroendocrine mechanisms and brain functions. We have previously shown that an increase in the endogenous production of IL-1 in the dentate gyrus during LTP is relevant for the maintenance of this process. We report here that in middle-aged rats (12 to 16 months old), enhancement of IL-1 gene expression is diminished compared to that in young animals. Furthermore, basal levels of IL-1 gene expression were slightly reduced in middle-aged rats. The reduced upregulation of IL-1 gene expression during LTP was not accompanied by overt impairment of potentiation, suggesting that either the level of the cytokine is still enough to maintain synaptic plasticity in the dentate gyrus or that compensatory, probably less efficient, mechanisms develop during aging.

ACKNOWLEDGMENT

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 297) and the Nationalen Genomforschungsnetz (NGFN), NV-S14T02.

REFERENCES

1. BESEDOVSKY, H.O. & A. DEL REY. 1996. Immune-neuro-endocrine interactions: facts and hypotheses. Endocr. Rev. **17:** 64–102.
2. HAAS, H.S. & K. SCHAUENSTEIN. 1997. Neuroimmunomodulation via limbic structures: the neuroanatomy of psychoimmunology. Prog. Neurobiol. **51:** 195–222.
3. ROTHWELL, N.J. & S.J. HOPKINS. 1995. Cytokines and the nervous system. II. Actions and mechanisms of action [see comments]. Trends Neurosci. **18:** 130–136.

4. PARNET, P., K.W. KELLEY, R.M. BLUTHE & R. DANTZER. 2002. Expression and regulation of interleukin-1 receptors in the brain. Role in cytokines-induced sickness behavior. J. Neuroimmunol. **125:** 5–14.
5. MONTKOWSKI, A., R. LANDGRAF, A. YASSOURIDIS, *et al.* 1997. Central administration of IL-1 reduces anxiety and induces sickness behaviour in rats. Pharmacol. Biochem. Behav. **58:** 329–336.
6. NEVEU, P.J. & S. LIEGE. 2000. Mechanisms of behavioral and neuroendocrine effects of interleukin-1 in mice. Ann. N.Y. Acad. Sci. **917:** 175–185.
7. DUNN, A.J., J. WANG & T. ANDO. 1999. Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress. Adv. Exp. Med. Biol. **461:** 117–127.
8. ROTHWELL, N.J. & G.N. LUHESHI. 2000. Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci. **23:** 618–625.
9. BLISS, T.V. & G.L. COLLINGRIDGE. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature **361:** 31–39.
10. MALINOW, R., Z.F. MAINEN & Y. HAYASHI. 2000. LTP mechanisms: from silence to four-lane traffic. Curr. Opin. Neurobiol. **10:** 352–357.
11. SCHNEIDER, H., F. PITOSSI, D. BALSCHUN, *et al.* 1998. A neuromodulatory role of interleukin-1beta in the hippocampus. Proc. Natl. Acad. Sci. USA **95:** 7778–7783.
12. KATSUKI, H., S. NAKAI, Y. HIRAI, *et al.* 1990. Interleukin-1 beta inhibits long-term potentiation in the CA3 region of mouse hippocampal slices. Eur. J. Pharmacol. **181:** 323–326.
13. BELLINGER, F.P., S. MADAMBA & G.R. SIGGINS. 1993. Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res. **628:** 227–234.
14. CUNNINGHAM, A.J., C.A. MURRAY, L.A. O'NEILL, *et al.* 1996. Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus *in vitro*. Neurosci. Lett. **203:** 17–20.
15. COOGAN, A. & J.J. O'CONNOR. 1997. Inhibition of NMDA receptor-mediated synaptic transmission in the rat dentate gyrus *in vitro* by IL-1 beta. Neuroreport **8:** 2107–2110.
16. VEREKER, E., E. O'DONNELL & M.A. LYNCH. 2000. The inhibitory effect of interleukin-1beta on long-term potentiation is coupled with increased activity of stress-activated protein kinases. J. Neurosci. **20:** 6811–6819.
17. OITZL, M.S., H. VAN OERS, B. SCHOBITZ & E.R. DE KLOET. 1993. Interleukin-1 beta, but not interleukin-6, impairs spatial navigation learning. Brain Res. **613:** 160–163.
18. GOTZ, F., G. DORNER, U. MALZ, *et al.* 1993. Short- and long-term effects of perinatal interleukin-1 beta-application in rats. Neuroendocrinology **58:** 344–351.
19. GIBERTINI, M., C. NEWTON, H. FRIEDMAN & T.W. KLEIN. 1995. Spatial learning impairment in mice infected with *Legionella pneumophila* or administered exogenous interleukin-1-beta. Brain Behav. Immun. **9:** 113–128.
20. GIBERTINI, M. 1996. IL1 beta impairs relational but not procedural rodent learning in a water maze task. Adv. Exp. Med. Biol. **402:** 207– 217.
21. PITOSSI, F., A. DEL REY, A. KABIERSCH & H. BESEDOVSKY. 1997. Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice. J. Neurosci. Res. **48:** 287–298.
22. MURRAY, C.A. & M.A. LYNCH. 1998. Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J. Neurosci. **18:** 2974–2981.
23. LYNCH, M.A. 1998. Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? Prog. Neurobiol. **56:** 571–589.
24. DEUPREE, D.L., D.A. TURNER & C.L. WATTERS. 1991. Spatial performance correlates with in vitro potentiation in young and aged Fischer 344 rats. Brain Res. **554:** 1–9.
25. BACH, M.E., M. BARAD, H. SON, *et al.* 1999. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. Proc. Natl. Acad. Sci. USA **96:** 5280–5285.
26. TOMBAUGH, G.C., W.B. ROWE, A.R. CHOW, *et al.* 2002. Theta-frequency synaptic potentiation in CA1 *in vitro* distinguishes cognitively impaired from unimpaired aged Fischer 344 rats. J. Neurosci. **22:** 9932–9940.

27. HORI, N., I. HIROTSU, P.J. DAVIS & D.O. CARPENTER. 1992. Long-term potentiation is lost in aged rats but preserved by calorie restriction. Neuroreport **3:** 1085–1088.
28. DEUPREE, D.L., J. BRADLEY & D.A. TURNER. 1993. Age-related alterations in potentiation in the CA1 region in F344 rats Neurobiol. Aging **14:** 249–258.
29. MOORE, C.I., M.D. BROWNING & G.M. ROSE. 1993. Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. Hippocampus **3:** 57–66.
30. SHANKAR, S., T.J. TEYLER & N. ROBBINS. 1998. Aging differentially alters forms of long-term potentiation in rat hippocampal area CA1. J. Neurophysiol. **79:** 334–341.
31. WATSON, J.B., H.K HORASANI, A. PERSSON, *et al.* 2002. Age-related deficits in long-term potentiation are insensitive to hydrogen peroxide: coincidence with enhanced autophosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II. J. Neurosci. Res. **70:** 298–308.
32. CHANG, P.L., K.R. ISAACS & W.T. GREENOUGH. 1991. Synapse formation occurs in association with the induction of long-term potentiation in two-year-old rat hippocampus *in vitro*. Neurobiol. Aging **12:** 517–522.
33. DIANA, G., D.C. SCOTTI, C. FRANK, *et al.* 1994. Selective reduction of hippocampal dentate frequency potentiation in aged rats with impaired place learning. Brain Res. Bull. **35:** 107–111.
34. MAROUN, M. & G. RICHTER-LEVIN. 2002. Local circuit plasticity in the rat dentate gyrus: characterization and aging-related impairment. Neuroscience **112:** 1001–1007.
35. O'DONNELL, E., E. VEREKER & M.A. LYNCH. 2000. Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: analysis of underlying mechanisms. Eur. J. Neurosci. **12:** 345–352.
36. GEINISMAN, Y., L. TOLEDO-MORRELL, F. MORRELL, *et al.* 1992. Structural synaptic plasticity associated with the induction of long-term potentiation is preserved in the dentate gyrus of aged rats. Hippocampus **2:** 445–456.
37. LANAHAN, A., G. LYFORD, G.S. STEVENSON, *et al.* 1997. Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. J. Neurosci. **17:** 2876–2885.
38. KELLY, A., C. MAGUIRE & M.A. LYNCH. 2000. Deficits in nerve growth factor release and tyrosine receptor kinase phosphorylation are associated with age-related impairment in long-term potentiation in the dentate gyrus. Neuroscience **95:** 359–365.
39. MORI, K., H. TOGASHI, M. MATSUMOTO & M. YOSHIOKA. 2000. Deficits in nitric oxide production after tetanic stimulation are related to the reduction of long-term potentiation in Schaffer-CA1 synapses in aged Fischer 344 rats. Acta Physiol. Scand. **169:** 79–85.
40. BARNES, C.A., G. RAO & F.P. HOUSTON. 2000. LTP induction threshold change in old rats at the perforant path—granule cell synapse. Neurobiol. Aging **21:** 613–620.
41. LYNCH, A.M. & M.A. LYNCH. 2002. The age-related increase in IL-1 type I receptor in rat hippocampus is coupled with an increase in caspase-3 activation. Eur. J. Neurosci. **15:** 1779–1788.
42. REYES, T.M. & C.L. CO. 1999. The proinflammatory cytokine network: interactions in the CNS and blood of rhesus monkey. Am. J. Physiol. **274:** R139–R144.
43. ZUMWALT, J.W., B.J. THUNSTROM & B.L. SPANGELO. 1999. Interleukin-1beta and catecholamines synergistically stimulate interleukin-6 release from rat C6 glioma cells in vitro: a potential role for lysophosphatidylcholine. Endocrinology **140:** 888–896.
44. BESEDOVSKY, H.O., F. PITOSSI, H. SCHNEIDER, *et al.* 1999. Cytokines in the brain as mediators of immune-endocrine interactions. J. Immunol. 31: 2.
45. JANKOWSKY, J.L., B.E. DERRICK & P.H. PATTERSON. 2000. Cytokine responses to LTP induction in the rat hippocampus: a comparison of *in vitro* and *in vivo* techniques Learn. Mem. **7:** 400–412.
46. D'ARCANGELO, G., V. TANCREDI, F. ONOFRI, *et al.* 2000. Interleukin-6 inhibits neurotransmitter release and the spread of excitation in the rat cerebral cortex Eur. J. Neurosci. **12:** 1241–1252.
47. VITKOVIC, L., J. BOCKAERT & C. JACQUE. 2000. "Inflammatory" cytokines: neuromodulators in normal brain? J. Neurochem. **74:** 457–471.

48. SCHUMAN, E.M. & D.V. MADISON. 1994. Locally distributed synaptic potentiation in the hippocampus. Science **263:** 532–536.
49. ENGERT, F. & T. BONHOEFFER. 1997. Synapse specificity of long-term potentiation breaks down at short distances. Nature **388:** 279–284.