Investigation of Cellular Mechanisms of Hippocampal LTP and LTD

Dissertation

zur Erlangung des akademischen Grades

Doctor rerum naturalium
(Dr. rer. nat)

genehmigt durch

die Fakultät für Naturwissenschaften

der Otto-Von-Guericke-Universität Magdeburg

von Sheeja Navakkode Gangadharan, Master of Science,
Kerala, India

Eingereicht am : 26. 09. 2005

Abstract

Processes of functional plasticity, i.e. long-lasting changes of the strength of synaptic connectivity in response to relative short-lasting afferent stimulation, are the most likely mechanisms underlying memory storage in the adult brain. The best studied models of functional plasticity are long-term potentiation (LTP) and long-term depression (LTD).

It is well known that the 3'-5'-cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway is essential for the prolonged mainentance of LTP as well as LTD. Therefore, it was interesting to investigate, how substances with a direct action on cellular cAMP-regulation would affect LTP/LTD. Rolipram, a specific type IV-specific cAMP phosphodiesterase (PDE) inhibitor, was therefore used in my initial studies to investigate its effect on late plastic events during functional CA1 plasticity in rat hippocampal slices in vitro. My studies showed that, an early form of LTP which normally decays to the baseline within 2-3 h (early-LTP) can be converted to a long-lasting LTP (late-LTP) lasting up to 6 h, if rolipram was applied during a weak tetanization. This rolipram-reinforced LTP (RLTP) was NMDA-receptor- and protein synthesis-dependent. The formation of cAMP during late-LTP in region CA1 requires dopaminergic receptor activity (Frey et al., 1989;Frey et al., 1990), thus we have studied whether RLTP was influenced by inhibitors of the D1/D5-receptor. Application of the specific D1/D5 antagonist SCH23390 did not prevent RLTP, suggesting that the phosphodiesterase inhibitor acts downstream of the D1/D5-receptors. Further studies were conducted to investigate whether rolipram can interact with processes of synaptic tagging. Synaptic tagging provides a conceptual basis for characterizing the mechanisms by which newly synthesized proteins that prolong functional changes in synaptic strength may act at specific, recently activated synapses (Frey and Morris, 1997;Frey and Morris, 1998a). Inhibition of PDE and subsequent induction of RLTP in

one synaptic population S1 was able to transform early- into late-LTP in a second, independent synaptic population S2 of the same neurons. This supports our hypothesis that cAMP-dependent processes are directly involved in the synthesis of plasticity-related proteins (PRPs).

It has been reported recently that, an atypical PKC isotype PKMzeta (PKMζ) is a first LTP specific PRP which is both necessary and sufficient for long-lasting LTP maintenance, but not for LTD (Sajikumar et al., 2005b). Thus, our assumption was that rolipram may specifically activate the synthesis of PKMζ only during LTP or it is involved in a more general regulation of the synthesis of PRPs necessary for both LTP and LTD. Thus, if inhibition of PDEs can reinforce an early form of LTP, the next question was whether rolipram could reinforce an early form of LTD into a late one.

In addition to the action of rolipram on LTP, I show here, in the CA1 region of hippocampal slices from male adult rats in vitro that rolipram also converts an early form of LTD (early-LTD) that normally decays within 2-3 h, to a long-lasting LTD (late-LTD) if rolipram was applied during LTD-induction. Rolipram-reinforced LTD (RLTD) was NMDA-receptor- and protein synthesis-dependent. Furthermore, it was dependent on the synergistic co-activation of dopaminergic D1/D5- and glutamate receptors. The question arose whether synaptic tagging occurs during RLTD. I found that early-LTD in a synaptic input S1 was transformed into late-LTD, if early-LTD was induced in a second independent synaptic pathway S2 during the inhibition of PDE by rolipram, supporting the interaction of processes of synaptic tagging during RLTD.

Although the mechanism of action of different forms of LTP is well understood, signalling cascades for LTD still remain poorly understood. I therefore delineated the pathway for the possible mechanism of action of rolipram during the reinforcement of early-LTD. I could show that extracellular signal-regulated kinase (ERK1/ERK2) cascade is recruited during RLTD. Inhibition of the ERK signaling cascade with

specific inhibitors of mitogen-activated protein kinases (MAPK), U0126 or PD98059 prevented the maintenance of RLTD. I further investigated the specific pathways by which ERK1/ERK2 is activated during RLTD. Thus MAPK-activation was triggered during RLTD by the synergistic interaction of NMDA-receptor- and D1/D5-receptor-mediated Rap/B-Raf pathways but not by the Ras/Raf-1 pathway in adult hippocampal CA1 neurons, which was revealed by the use of pathway-specific inhibitors, manumycin for Ras/Raf-1 pathway and lethal toxin-82 (LT-82) for Rap/B-Raf pathway. Thus for the first time I report that PDE4B3 could represent a process-non-specific PRP which regulates the synthesis of either LTP- and/or LTD- plasticity-related proteins (PRPs).

Next, I was interested to investigate the question of what exactly is the putative nature of the synaptic tag? Are there specific ´tags´ for LTP and LTD? I studied the role of two promising candidates: Calcium/calmodulin-dependent protein kinase II (CaMKII) and mitogen- activated protein kinases (MAPK) on the setting of a synaptic tag during LTP and LTD. First I could confirm the results obtained from other laboratories that CaMKII or MAPK inhibition during the induction blocks the maintenance of LTP/LTD. However, I found that CaMKII or MAPK inhibition after the induction of LTP/LTD had no effect on the maintenance of the processes.

In a next series of experiments I have investigated whether CaMKII can mediate the setting of the synaptic tags in LTP or LTD. Induction of late-LTP in S1 followed by early-LTP in S2 and in presence of CaMKII inhibitor, KN-62 prevented processes of synaptic tagging during LTP while application of KN-62 did not affect synaptic tagging during LTD. It means setting of tags in LTP is CaMKII mediated while in LTD it is independent of CaMKII.

If CaMKII mediates the setting of synaptic tags in LTP, but not during LTD, the question was which kinase mediates the setting of the LTD-specific tags? By using

two mitogen-activated protein (MAP) kinase kinase 1 (MKK1 or MEK) inhibitors, U0126 and PD98059, I could find that setting of LTD tag is mediated by MAPK. Thus LTP tagging is specifically mediated by CaMKII and LTD tagging by MAPK.

Having determined the specifity of LTP- and LTD-specific tags I was now interested to find out the implication of tag-specificity for processes of cross-tagging. I could confirm the findings obtained in LTP/ LTD tagging, that CaMKII and MAPK mediates the setting of LTP /LTD-specific tags respectively in cross-tagging.