Neuroscience Letters 473 (2010) 82–86

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

cGMP-dependent protein kinase Type I promotes CREB/CRE-mediated gene expression in neurons of the lateral amygdala

Cindy Paul [a], Christopher Stratil [a], Franz Hofmann [a,b,c], Thomas Kleppisch [a,*]

[a] Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Straße 29, 80802 München, Germany
[b] DFG Forschergruppe 932, Germany
[c] Munich Center for Integrated Protein Science, Germany

ARTICLE INFO

Article history:
Received 22 September 2009
Received in revised form 22 January 2010
Accepted 9 February 2010

Keywords:
cGMP-dependent protein kinase
Lateral amygdala
cAMP-response element binding protein
Transcription

ABSTRACT

The process transforming newly learned information into stable long-term memory is called memory consolidation and, like the underlying long-term synaptic plasticity, critically depends on de novo RNA and protein synthesis. We have shown recently that the cGMP-dependent protein kinase Type I (cGKI) plays an important role for the consolidation of amygdala-dependent fear memory and long-term potentiation (LTP) in the lateral amygdala. Signalling downstream of cGKI at the level of transcriptional regulation remained unclear. A transcription factor of major importance for learning and memory is the cAMP-response element binding protein (CREB). The representation of fear memory in the lateral amygdala strikingly depends on the activity of CREB in individual neurons. Moreover, findings from *in vitro* experiments demonstrate CREB phosphorylation by cGK. In the hippocampus, CREB phosphorylation increases following activation of NO/cGMP signalling contributing to the late phase of LTP. To demonstrate a link from cGKI to activation of CREB and CREB-dependent transcription in neurons of the lateral amygdala as a possible mechanism for cGKI-mediated fear memory consolidation, we examined the effect of cGMP on activation of CREB/CRE using immunohistochemical staining specific for phospho-CREB and a reporter gene in control and cGKI-deficient mice, respectively. Supporting our hypothesis, marked CREB phosphorylation and CRE-mediated transcription was induced by cGMP in the lateral amygdala of control mice, but not in cGKI-deficient mice. It has been proposed that activation of cGKI is followed by its nuclear translocation that would allow direct phosphorylation of CREB. Therefore, we examined the cellular localisation of cGKI in neurons of the lateral amygdala in the presence of cGMP by double staining for cGKI and a nuclear marker in sections from areas showing prominent CREB phosphorylation, and did not observe prominent nuclear translocation of the enzyme. In summary, we provide evidence that cytosolic cGKI can support fear memory consolidation and LTP in neurons of the lateral amygdala *via* activation of CREB and CRE-dependent transcription.

© 2010 Elsevier Ireland Ltd. All rights reserved.

Auditory-cued fear memory critically depends on the lateral amygdala (LA) and is associated with long-lasting enhancement of synaptic transmission in its sensory inputs [for review see 34,36]. It is well established that memory formation is a time-dependent process by which initially labile memory is consolidated into stable long-term memory (LTM). A hall mark of memory consolidation and underlying synaptic plasticity is their dependency on de novo RNA and protein synthesis [9,11,22,33]. Nitric oxide (NO), a small gaseous molecule widely implicated in synaptic plasticity and behaviour [for review see 24], has been shown to enhance long-term potentiation (LTP) in the LA as well as the consolidation of associative fear memory *via* the cGMP signalling cascade [6,7,32]. There is evidence accumulating that the cGMP-dependent protein kinase (cGK) is an important effector of NO/cGMP signalling in the hippocampus and the amygdala [1,7,25,29,30,37]. We have shown recently that the cGKI isoform enhances LTM of auditory-cued fear and expression of LTP in the LA [30]. The downstream mechanism through which cGKI supports these transcription- and protein synthesis-dependent processes remained unclear. The role of the cAMP-response element binding protein (CREB) as a transcription factor vital for memory consolidation and synaptic plasticity is well established [2,16,19,21,23, for review see 36]. Various signalling pathways activating gene expression in the amygdala converge *via* CREB, and phosphorylation of CREB and CRE-mediated transcription is significantly increased in the LA following fear conditioning training [19,35]. Strikingly, CREB activity of neurons in the LA determines whether they are recruited into a fear memory trace [16],

* Corresponding author. Tel.: +49 89 41403288; fax: +49 89 41403261.
E-mail address: Kleppisch@ipt.med.tum.de (T. Kleppisch).
URL: http://www.ipt.med.tu-muenchen.de (T. Kleppisch).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.02.020

and targeting those neurons that exhibit significant CREB activation results in selective erasure of fear memory [17]. There is further evidence from several *in vitro* studies that the transcription factor CREB can be activated *via* the NO/cGMP/cGKI pathway [5,12,13]. For example, it has been reported that cGKI can phosphorylate CREB directly at amino acid residue Ser^{133} *in vitro* [12]. Moreover, it has been shown for hippocampal neurons that activation of the NO/cGMP signalling cascade results in augmented CREB phosphorylation important for the late phase of LTP [27]. Hence, cGKI-mediated activation of CREB/CRE-dependent transcription in neurons of the LA represents a potential mechanism for strengthening of memory consolidation by this isoenzyme observed in our recent study [30]. The present study used an *in vitro* approach to provide evidence supporting the view that CREB/CRE-dependent transcription in neurons of the LA is regulated through the cGMP/cGKI signalling cascade.

To put the *in vitro* experiments on a general basis, we initially performed a series of experiments to confirm a role of CREB/CRE signalling for fear memory formation in the LA in our experimental setting. Therefore, we tested if CREB phosphorylation occurred in the LA following auditory fear conditioning similar to the procedures used in our previous study [30]. 8-Week-old male wild type mice received three tone-foot shock pairings, were sacrificed 30 min later and the brain was removed and prepared for immunohistochemical staining. Ser^{133} of CREB has been identified as a functional phosphorylation site for PKA and cGKI [12]. To determine the phosphorylation level of CREB we used an antibody specific for this phosphorylation site of CREB (rabbit anti-$pCREB^{Ser133}$ antibody, Upstate; 1:1000) and the ABC-method for detection (Vectastain Standard ABC-kit, Vector). The density of pCREB-positive nuclei was calculated in every experiment as the number of stained nuclei normalized to the area of the LA (counted manually with ImageTool software). All data were finally expressed as the percentage of the mean value for naïve animals. Quantification of the immunohistochemical staining (Fig. 1) revealed a significant increase in the density of pCREB-positive nuclei in the LA of mice that received the foot shock (FS) in conjunction with the tone compared to naïve animals (naïve: $100 \pm 5.3\%$, $n = 4$; tone + FS: $177.2 \pm 6.9\%$, $n = 4$). In contrast, slices from animals exposed only to the conditioning context (chamber) and the tone alone did not show a significant change in pCREB density (context + tone: $95.2 \pm 6.3\%$, $n = 4$). We also observed an increase of pCREB-positive cells in the LA following application of the FS alone (FS: $124.9 \pm 5.9\%$, $n = 4$) which was significantly smaller than that after pairing the FS and the tone. These results support our hypothesis that formation of associative fear memory following the conditioning procedure used in our previous study [30] involves activation of the transcription factor CREB in neurons of the LA.

A role of NO/cGMP signalling for CREB activation has previously been implicated [4,12,13,26,27]. To address the issue whether the cGKI may contribute to CREB activation in intact tissue of the amygdala, the site crucial for consolidation of auditory-cued fear memory, we examined the effect of the cGMP analogue, 8-Br-cGMP, on the level of pCREB in sections of the LA from 4–5-week-old cGKI control (cGKI-Ctr) and cGKI-knockout (cGKI-KO) mice (Fig. 2). Protocols for mouse breeding, slice preparation and immunohistochemical staining were used as previously described and [30,38] were in agreement with the German animal protection law. Acute brain slices kept in artificial cerebrospinal fluid (ACSF) at 32 °C were treated for 30 min with 8-Br-cGMP (0.5 mM, Biolog) and fixed with 4% formaldehyde/PBS, followed by preparation for immunohistochemical staining with the pCREB antibody. All experiments mentioned below were performed 3–6 times. The treatment with cGMP elicited a marked increase of pCREB level in the LA of the control mice, while it was absent in sections from mutant mice (Fig. 2). Quantitative assessment of the staining was performed as described above, and all values were put into relation to the mean value of cGKI-Ctr specimen without cGMP treatment. This analysis revealed that cGMP increased the density of pCREB-positive nuclei significantly in slices of cGKI-Ctr mice (cGKI-Ctr unstim: $100 \pm 2.8\%$: cGKI-Ctr with 8-Br-cGMP: $148.5 \pm 4.6\%$), whereas no significant alteration was observed in cGKI-KO mice (cGKI-KO unstim: $102.3 \pm 4.3\%$; cGKI-KO with 8-Br-cGMP: $108.4 \pm 4.4\%$). In conclusion, the results support the view that in neurons of the LA, CREB phosphorylation can be induced *via* the cGMP pathway. Moreover, cGMP-induced CREB phosphorylation was mediated selectively by the cGKI in these neurons: involvement of the cGKII isoform or a cross activation of PKA can be ruled out due to the lack of a cGMP effect in cGKI-KO mice.

Although phosphorylation of the amino acid residue Ser^{133} of CREB is a prerequisite for target gene expression, as such it is not always sufficient to trigger transcription [19]. To test whether CREB phosphorylation in our experimental system indeed caused up-regulation of CRE-mediated transcription in the LA, we additionally analysed the effect of treatment with 8-Br-cGMP on the expression of the β-galactosidase reporter using a CRE-lacZ transgenic mouse line [18]. We crossed this line with cGKI-Ctr and cGKI-KO mice to obtain mice of both genotypes additionally carrying the CRE-LacZ sequence. To analyse cGMP-induced CRE-dependent gene expression, we determined expression level of the reporter

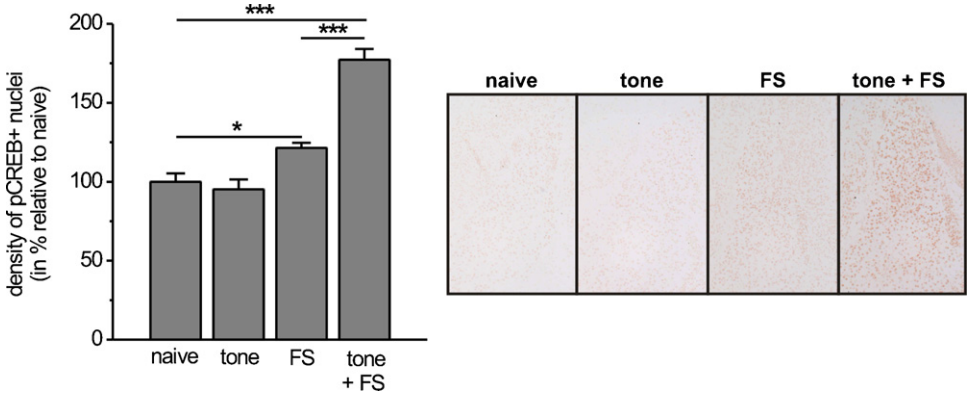

Fig. 1. Effect of fear conditioning procedure on the level of phospho-CREB in neurons of the lateral amygdala (LA). (A) An antibody specific for CREB phosphorylated at Ser^{133} and the ABC-method were used to detect pCREB-positive nuclei. Their number was counted and normalized to the area of the LA in all experiments. Slices were prepared from naïve animals, animals exposed to the context + tone (tone), to the foot shock alone (FS), and to foot shock paired with the tone (tone + FS). All data presented are expressed as the percentage of the mean value from experiments in naïve animals. Error bars display S.E.M., asterisks indicate statistical significance ($^{***}p < 0.001$; $^{*}p < 0.01$). (B) Representative images of sections with the LA stained for pCREB taken from naïve, tone alone (tone), footshock alone (FS) and conditioned (tone + FS) animals.

Download English Version:

https://daneshyari.com/en/article/4346272

Download Persian Version:

https://daneshyari.com/article/4346272

Daneshyari.com