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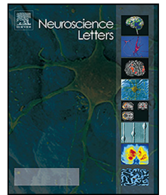

Research paper

NT-3 protein levels are enhanced in the hippocampus of PRG1-deficient mice but remain unchanged in PRG1/LPA2 double mutants

Sandra Petzold[a], Babette Sommer[a], Andrea Kröber[a], Robert Nitsch[b], Herbert Schwegler[a], Johannes Vogt[b,1], Thomas Roskoden[a,*,1]

[a] *Institute of Anatomy, Medical Faculty Otto-von-Guericke-University Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany*
[b] *Institute for Microscopic Anatomy and Neurobiology, University Medical Center, Johannes Gutenberg-University Mainz, Langenbeckstraße 1, 55131 Mainz, Germany*

HIGHLIGHTS

- In PRG1 ko mice protein level of NT-3 is increased against WT, NGF and BDNF not.
- In LPA2R knockout mice no neurotrophin (NGF, BDNF, NT-3) is changed compared to WT.
- In PRG1LPA2R double Ko mice no changes were observed in NGF, BDNF and NT-3 protein.

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ABSTRACT

The plasticity-related gene 1 (PRG1) modulates bioactive lipids at the postsynaptic density and is a novel player in neuronal plasticity and regulation of glutamatergic transmission at principal neurons. PRG1, a neuronal molecule, is highly expressed during development and regeneration processes at the post-synaptic density, modulates synaptic lysophosphatidic acid (LPA) levels and is related to epilepsy and brain injury. In the present study, we analyzed the interaction between the synaptic molecules PRG1 and LPA2R with other plasticity-related molecules the neurotrophins. The protein levels of NGF, BDNF and NT-3 were measured using ELISA in hippocampal tissue of homozygous ($PRG^{-/-}$) and heterozygous ($PRG^{+/-}$) PRG1 deficient mice and compared to their wild type ($PRG^{+/+}$/WT) littermates. In the hippocampus, protein levels of NT-3 were significantly increased in $PRG^{-/-}$ mice (compared to WT-litters) while protein levels of NGF and BDNF were not affected. Since PRG1 deficiency leads to increased neuronal excitability and higher hippocampal network activity, which may well influence neurotrophin levels, we further assessed PRG1 deficient mice on an LPA_2-receptor (LPA2R) deficient background, reported to normalize hippocampal over-excitability in $PRG1^{-/-}$ mice. However, on an LPA2R deficient background, protein levels of NT-3 in $PRG1^{-/-}$ mice ($PRG1^{-/-}$/$LPA2R^{-/-}$) were not significantly different when compared to WT animals. Since PRG1 deficient mice showed over-excitability in glutamatergic neurons. This was normalized by additional LPA2R deletion, and we conclude the increased NT3-levels were directly or indirectly attributable to increased hippocampal network activity, possibly exerting a protective effect against over-excitability.

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1. Introduction

Plasticity-related gene 1 (PRG1) was discovered and classified in 2003 as a member of the lipid phosphate phosphatase (LPP) family [5]. Detailed analysis of PRG1 revealed that PRG1 is a postsynaptic molecule, regulating synaptic bioactive lipid levels in excitatory synapses on principal neurons of the murine hippocampus [26]. PRG1 expression was also described in the cerebral cortex, olfactory bulb, cerebellar cortex, caudate-putamen and amygdala [25]. PRG1 is linked to epilepsy and was increased after brain injury [23]. Using PRG1-deficient mice Trimbuch et al. demonstrated neuronal over-excitability, though neither the expression of NMDA and AMPA

* Corresponding author.
E-mail address: thomas.roskoden@med.ovgu.de (T. Roskoden).
[1] These author contributed equally to this work.

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receptor subunits nor the ratio of these receptors differed between PRG1-deficient mice and their wild type littermates [26]. Further results provided evidence that PRG1 interferes with glutamatergic signal transduction by interacting with lysophosphatidic acid (LPA) in the synaptic cleft from the postsynaptic side thereby influencing LPA interaction with presynaptic LPA_2-receptors (LPA2R). Interestingly, when PRG1 deficient mice were assessed on an LPA2R deficient background ($LPA2R^{-/-}$), these mice did not show any sign of hippocampal excitability [26].

The hippocampus, a part of the Limbic System, is (among others) involved in spatial learning and memory abilities [1,16]. Memory abilities base on encoding, storing and receiving information via new-joining of neurons as well as neuronal plasticity including the transfer of short-term memory into long term memory [12]. This was described as synaptic plasticity and depends on different kind of mechanisms like presynaptic enhancement, post tetanic potentiation, synaptic depression and long term potentiation (LTP). The hippocampus receives afferents from different sensory systems and the entorhinal cortex, and is efferent connected with the amygdala, prefrontal cortex, hypothalamus, perirhinal cortex and entorhinal cortex. The hippocampus contains glutamatergic neurons projecting upon glutamatergic neurons thereby exhibiting LTP [17].

Hippocampal functions are modulated by neurotrophins. Neurotrophins play an important role in neuronal differentiation, survival, growth and development of the central nervous system [2]. They have been found in different subsets of neurons in the hippocampus and other brain areas [7,27,9]. Neurotrophin-3 interacts with the low affinity nerve growth receptor p75 and is capable to bind at two high affinity Trk receptors, TrkB and C. It facilitates growth and survival of existing neurons and supports development and differentiation of newly growing neurons in the central nervous system, especially of sensory neurons [24,14]. BDNF is found both in the central nervous system and in other tissues. It activates particular neurons in hippocampus, cortex, basal forebrain and cerebellum and is described as important for activity-dependent synaptic plasticity and indicated for a role of BDNF in memory acquisition and consolidation [29]. BDNF induces neuronal survival, growing and differentiation by interacting with low affinity receptor p75 like other growth factors and its high affinity receptor TrkB [18]. Several studies link occurrence or non-occurrence of BDNF with disorders like schizophrenia [28], bulimia nervosa [10], obsessive-compulsive disorder [13] and Rett-syndrome [30].

In the present study we investigated the interaction between PRG1 and altered synaptic lipid signaling, its modulation by LPA2R, and the protein level of NGF, BDNF and NT-3 in $PRG1^{-/-}$ mice. Quantitative neurotrophin levels in the hippocampus obtained from homozygous ($PRG1^{-/-}$) and heterozygous ($PRG1^{+/-}$) mice were assessed using an enzyme linked immunosorbent assay (ELISA) and compared to hippocampal protein levels of their wild type ($PRG1^{+/+}$) littermates. Moreover, we analyzed neurotrophins levels in PRG1 deficient mice on an LPA2R deficient background [PRG1//LPA2R double knockout ($PRG1^{-/-}$//$LPA2R^{-/-}$), PRG1//LPA2R Receptor knockout ($PRG1^{+/+}$//$LPA2R^{-/-}$) and their wild type littermates ($PRG1^{+/+}$//$LPA2R^{+/+}$)].

2. Materials and methods

2.1. Animals

Thirty-five female transgenic mice were delivered from the Charité (Berlin). The development of the mouse lines deficient for PRG1 or PRG1//LPA2R was described elsewhere [26]. We analyzed homozygous ($PRG^{-/-}$ $n = 6$), heterozygous ($PRG^{+/-}$ $n = 6$) PRG1 deficient mice and compared them to their wild type littermates ($PRG^{+/+}$ $n = 6$). PRG1LPA2 double knockout mice ($PRG1^{-/-}$//$LPA2R^{-/-}$ $n = 8$), PRG1LPA2 Receptor knockout mice ($PRG1^{+/+}$//$LPA2R^{-/-}$ $n = 5$) and their wild type littermates ($PRG1^{+/+}$//$LPA2R^{+/+}$ $n = 4$) were assessed in a second experimental round. Mice were singly housed in transparent cages and maintained on a 12/12 h light/dark cycle with food and water ad libitum.

2.2. Protein isolation

The left hippocampi were rapidly frozen in liquid nitrogen. Protein extraction was carried out following manufacturer's protocol using 10 mM PBS containing 0.2% Nonidet P-40 (NP40) pH 7.2 as extraction solution. Therefore, mouse brain tissue was weighed in a plastic tube and then homogenized (10.000 rpm/min) in 30-fold volume of extraction solution in an ice bath. The homogenate was centrifuged (15.000 rpm/min, 20 min) at 4 °C, and the supernant was collected and the samples were divided into test tubes in amounts of 50 μl and frozen at −80 °C until assay.

2.3. Enzyme-linked immunosorbent assay

For the ELISA of the different neurotrophins we used commercial kits (NGF: Promega Corporation, Madison, WI, USA; BDNF and NT-3: biosensis, Thebarton, SA, Australia). The particular protein was measured according the manufacturer's protocol.

NGF ELISA (Promega Corporation, Madison, WI, USA): Briefly, a 96-well plate was coated with anti-NGF polyclonal antibody (pAb; 1:1000). The plate was blocked and each sample was added in triplicate. An anti-NGF monoclonal antibody (mAb; 1:4000) and a third anti-mouse IgG horseradish peroxidase conjugate (1:100) were used. Color development was induced by TMB one solution and stopped by adding hydrochloric acid. Absorbance was measured immediately at 450 nm (SUNRISE photometer, TECAN Switzerland). A standard curve was generated according to protocol with a supplied NGF standard.

BDNF ELISA (biosensis, Thebarton, SA, Australia): Briefly, a 96-well plate coated with an anti-BDNF monoclonal capture antibody. The plate was blocked and each sample was added in triplicate. As detection antibody a biotinylated anti-BDNF and horseradish peroxidase-conjugated streptavidin were used. Color development was induced by TMB- (3,3′,5,5′-tetramethylbenzidine) one solution and stopped by adding hydrochloric acid. Absorbance was measured immediately at 450 nm (SUNRISE photometer, TECAN, Switzerland). A standard curve was generated according to protocol with a supplied BDNF standard (7.8–500 pg/ml).

NT-3 ELISA (biosensis, Thebarton, SA, Australia): Briefly, a 96-well plate coated with an anti-human NT-3 capture antibody. The plate was blocked and each sample was added in triplicate. As detection antibody a biotinylated anti-human NT-3 (1:100) and Avidin-Biotin-Peroxidase Complex (ABC) was used. Color development was induced by TMB- (3,3′,5,5′-tetramethylbenzidine) solution and stopped by adding hydrochloric acid. Absorbance was measured immediately at 450 nm (SUNRISE photometer, TECAN Switzerland). A standard curve was generated according to protocol with a supplied NT-3 standard (15.6–1000 pg/ml).

2.4. Statistical analysis

A two-way analysis of variance (ANOVA) with genotype and neurotrophins as factors were calculated for protein levels using SPSS 22 software. Separate one-way ANOVAs were calculated to distinguish NGF, BDNF and NT-3, followed by the post-hoc test (Tukey-HSD). A p value of ≤ 0.05 was taken as being statistically significant.

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