Behavioural Brain Research 296 (2016) 15–25

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

TrkB in the hippocampus and nucleus accumbens differentially modulates depression-like behavior in mice

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ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form 19 August 2015
Accepted 20 August 2015
Available online 24 August 2015

Keywords:
TrkB
Nucleus Accumbens
Hippocampus
Depression
Electroporation

ABSTRACT

Brain-derived neurotrophic factor (BDNF) exerts antidepressant-like effects in the hippocampus and pro-depressant effects in the nucleus accumbens (NAc). It is thought that downstream signaling of the BDNF receptor TrkB mediates the effects of BDNF in these brain structures. Here, we evaluate how TrkB regulates affective behavior in the hippocampus and NAc. We overexpressed TrkB by electroporating a non-viral plasmid in the NAc or hippocampus in mice. Depression- and anxiety-like behaviors were evaluated in the sucrose test (anhedonia), the forced swim test (despair) and the elevated zero maze (anxiety). Targeted brain tissue was biochemically analyzed to identify molecular mechanisms responsible for the observed behavior. Overexpressing TrkB in the NAc increased the number of young neuronal cells and decreased despair and basal corticosterone levels. TrkB overexpression in the hippocampus increased astrocyte production and activation of the transcription factor CREB, yet without altering affective behavior. Our data suggest antidepressant effects of BDNF-TrkB in the NAc, which could not be explained by activation of the transcription factors CREB or β-catenin. The effects TrkB has on depression-related behavior in different brain regions appear to critically depend on the targeted cell type.

1. Introduction

Major depression (MD) is a severe mental disorder expected to be the second leading cause of disability worldwide by 2020 [1]. According to the neurotrophin hypothesis of depression, stress-induced decreases in neurotrophic support may contribute to the pathophysiology of depression by impairing hippocampal plasticity [2,3]. Brain-derived neurotrophic factor (BDNF) is a well-studied neurotrophin directly implicated in the pathophysiology of MD and in the mechanism of action of antidepressants. Intra-hippocampal injection or viral overexpression of BDNF produce antidepressant effects in animal models for depression [4–7], while reducing BDNF by means of RNA interference in the DG or by a conditional BDNF deletion in the forebrain attenuates antidepressant efficacy [8,9]. A conditional knock-out of BDNF in the forebrain induces depression-like behavior in female mice [8], and local silencing of BDNF in the dorsal DG in male rats increases anhedonia and despair [10]. These studies provide evidence that BDNF is necessary and sufficient for anti-depressant efficacy, while BDNF reductions may be a contributing factor in MD.

Tropomyosin-related kinase B (TrkB), a member of the Trk family of receptor tyrosine kinases, is a high-affinity receptor for BDNF. Activation of TrkB is linked to multiple signaling cascades (PLCγ, Erk and Akt signaling) that increase neuronal synaptogenesis and neurogenesis, thus increasing overall plasticity in the brain [11]. These cascades are initiated by autophosphorylation of the TrkB receptor on different cytoplasmic Tyrosine residues, which recruit specific

Abbreviations: BDNF, brain-derived neurotrophic factor; DCX, doublecortin; DG, dentate gyrus; EZM, elevated zero maze; FST, forced swim test; MD, major depression; NAc, nucleus accumbens; OF, open field; SIT, sucrose intake test; TBS, tris-buffered saline; TrkB, tropomyosin-related kinase B; VTA, ventral tegmental area.

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http://dx.doi.org/10.1016/j.bbr.2015.08.027
0166-4328/

binding proteins. Phosphorylation of Tyr785 triggers activation of PLCγ, which on its turn hydrolyses phosphatidyl inositides. This causes an intracellular release of Ca^{2+} and activation of CaM and CaMKK which results in phosphorylation and activation of the transcription factor CREB. Phosphorylation of Tyr490 in the cytoplasmic domain of TrkB recruits the Shc-Grb2-SOS binding complex which on its turn activates Ras or PI-3K. Activated Ras triggers activation of the Erk/MAPK cascade which eventually results in CREB phosphorylation. Phosphorylation of PI-3K activates PI-3K/Akt signaling which phosphorylates and inhibits GSK3β. Inhibition of GSK3β leads to an accumulation of the transcription factor β-catenin and activation of Wnt-regulated gene expression. Similar outcomes to BDNF have been recorded when studying TrkB in relation to depression. Transgenic mice overexpressing full-length TrkB in the hippocampus and cortex show increased latency to immobility in the forced swim test (FST) and reduced anxiety-like behavior [12–14]. In contrast, transgenic mice overexpressing dysfunctional TrkB in these brain regions are resistant to the effects of antidepressants [15].

During chronic exposure to stress, the expression of glucocorticoid receptors is downregulated, especially at the level of the hypothalamus and the pituitary leading to disinhibition and hyperactivity of the HPA-axis. Long-lasting increased glucocorticoid levels, possibly by negatively affecting BDNF-TrkB signaling, may eventually damage the hippocampus as evidenced by decreased hippocampal volume, cell proliferation, atrophy, cell loss, and reduced neuronal turnover in the DG [16–20]. The resulting damage in the hippocampus, an important brain area involved in learning, memory and mood, may therefore cause depressive-like states. This interaction between glucocorticoids and BDNF signaling works in both directions. Acute or chronic central administration of BDNF in the lateral ventricles in rats increases hypothalamic mRNA expression of corticotrophin-releasing hormone, and activates the HPA-axis as evidenced by increased plasma corticosterone levels [21–23].

In contrast to the hippocampus, viral overexpression or infusion of BDNF into the ventral tegmental area (VTA)-nucleus accumbens (NAc) system induces depression-like phenotypes and attenuates antidepressant efficacy in rats [24,25]. Also, viral-mediated deletion of BDNF in the VTA reduces social avoidance induced by social defeat stress in mice and elicits antidepressant effects in the FST and sucrose preference test in rats [24,26]. Viral overexpression of a dominant-negative form of TrkB in the NAc produces an antidepressant phenotype in rats [25]. Additionally, rats with viral-mediated silencing of CREB in the NAc display less depression-like behavior [27]. This suggests a pro-depressant role for BDNF-TrkB-CREB signaling in the VTA-NAc, in striking contrast to the antidepressant properties in the hippocampus. Interestingly, it has not been investigated whether overexpression of functional TrkB in the NAc negatively influences affect.

1.1. Aims of the study

In order to elucidate the exact role of TrkB in the hippocampus and the NAc in relation to depression in mice, we locally transfected a non-viral plasmid encoding TrkB in the dorsal dentate gyrus (DG) of the hippocampus or NAc-shell using micro-electroporation. We hypothesized that overexpression of TrkB would have opposite effects on measures of anxiety- and depression-like behaviors, with a behavioral phenotype depending on the manipulated brain region.

2. Methods and materials

2.1. Animals

Six weeks old, male C57BL/6 mice were purchased from Charles River (L' Arbresle, France). All animals were housed under an inverted 12 h light/dark cycle (lights on at 7:00 PM) with an average temperature of 22 °C and relative humidity of 42%. All experimental procedures were approved by the local ethical committee for animal experiments, according to governmental guidelines (EAA: DGVGZ/VVP-83267).

2.2. Plasmid DNA

pEF/BOS-TrkB.TK(+) encoding rat full length TrkB with a 5′ FLAG-tag was kindly provided by Dr. Alex Krüttgen from RWTH Aachen (Aachen, Germany). pVAX-LacZ plasmid encoding β-galactosidase was from Invitrogen (San Diego, CA). Plasmid DNA was prepared using the Qiaprep Spin Maxiprep kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions. Plasmid DNA was dissolved in saline (0.9% NaCl) at 2.5 μg/μl and verified by restriction analysis and western blotting.

2.3. In vivo *micro-electroporation*

Micro-electroporation does not induce inflammation or damage and offers a safe and efficient alternative to viral overexpression [28,29]. Bilateral *in vivo* micro-electroporation was performed as described previously [28,30]. Briefly, anesthetized animals were injected with 0.5 μl plasmid DNA or saline in the NAc-shell (AP + 1.28 mm, ML ± 1.0 mm, DV −4.75 mm) or in the DG (AP −2.06 mm, ML ± 1.5 mm, DV −2.0 mm) at 0.05 μl/min. After DNA injection, electroporation was performed with a pair of needle-like electrodes (Technomed, Beek, the Netherlands). Coordinates for the electrodes in the DG were: AP −1.34 mm, ML ± 1.0 mm, DV −2.0 mm (anode); AP −3.07 mm, ML ± 2.20 mm, DV −2.0 mm (cathode). In the NAc, coordinates were: AP + 2.33 mm, ML ± 1.0 mm, DV −4.75 mm (anode); AP + 0.23 mm, ML ± 1.0 mm, DV −4.75 mm (cathode). Five unipolar square wave pulses (125 μA, 50 ms, 1 Hz) were given using a current-controlled stimulator/isolator (DS8000; WPI, Sarasota, FL). Mice were distributed over 5 experimental groups and balanced for weight: untreated ($n = 13$), HC-control ($n = 13$), HC-TrkB ($n = 13$), NAc-control ($n = 13$), NAc-TrkB ($n = 13$).

2.4. Behavior

Mice were allowed to recover for 2 weeks before behavioral testing. The sucrose intake test (SIT) and open field (OF) test were performed 2 weeks following electroporation, the elevated zero maze (EZM) test after 2.5 weeks and the FST after 3 weeks. Testing took place during the dark phase of the light/dark cycle between 7:00 AM and 7:00 PM. Mice were automatically tracked during behavioral testing in the OF, EZM and FST via a video camera connected to a video tracking system (Ethovision Pro, Noldus, the Netherlands). Outcome measures were calculated using Ethovision Pro software. In order to ensure that electroporating a vector does not induce behavioral changes itself, we electroporated the hippocampus of additional animals with a pVAX-LacZ vector expressing the reporter protein β-galactosidase. This was not the case as no differences in any of the behavioral measures were found between untreated animals, saline electroporated animals and animals electroporated with pVAX-LacZ (data not shown).

2.4.1. Open field test

As certain behavioral tasks like the FST or EZM can be affected by changes in locomotor activity, the OF was used to assess locomotor