Behavioural Brain Research 197 (2009) 371–377

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Deficits in LTP and recognition memory in the genetically hypertensive rat are associated with decreased expression of neurotrophic factors and their receptors in the dentate gyrus

Amy Hennigan[a,b], Charlotte K. Callaghan[a,b], John Kealy[b], Jennifer Rouine[a,b], Áine M. Kelly[a,b,*]

[a] Department of Physiology, School of Medicine, University of Dublin, Trinity College, Dublin 2, Ireland
[b] Trinity College Institute of Neuroscience, University of Dublin, Trinity College, Dublin 2, Ireland

ARTICLE INFO

Article history:
Received 6 August 2008
Received in revised form
24 September 2008
Accepted 26 September 2008
Available online 8 October 2008

Keywords:
LTP
Recognition memory
Dentate gyrus
NGF
BDNF
Trk

ABSTRACT

We have previously reported that a genetically hypertensive strain of Wistar rat (GH), is deficient in nerve growth factor (NGF) and Trk receptors in dentate gyrus and that these deficits are accompanied by impaired expression of long-term potentiation (LTP) in perforant path–granule cell synapses. Here we confirm this deficit in LTP and report that this strain of rat also displays impairments in long-term recognition memory when compared with normotensive controls. Further analysis of neurotrophin expression in dentate gyrus confirmed the previously-reported deficit in NGF and revealed a decrease in expression of brain-derived neurotrophic factor (BDNF), but not neurotrophin 3 (NT3) or neurotrophin 4 (NT4), in GH rats. These alterations in ligand expression were accompanied by changes in Trk receptor expression; specifically, a decrease in expression of TrkA and TrkB, but not TrkC, in the dentate gyrus of GH, compared with normotensive, rats. We conclude that the impairments in LTP and learning and memory observed in the GH strain are associated with aberrant expression of specific neurotrophic factors and their receptors in the dentate gyrus, adding weight to the evidence indicating a role for these proteins in several forms of synaptic plasticity.

1. Introduction

The roles of neurotrophins in hippocampal synaptic plasticity have been extensively investigated for over a decade. The members of this family of proteins known to be expressed in the mammalian brain include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4 (NT4). All neurotrophins bind with low affinity to the p75NTR receptor [16], and each binds preferentially and with high affinity to different isoforms of the Trk receptor family; TrkA preferentially binds NGF, TrkB preferentially binds BDNF and NT4, while NT3 is the preferred ligand of TrkC [34]. Trk receptor activation results in activation of a number of signalling cascades that have been associated with expression of synaptic plasticity, including the mitogen-activated protein kinase (MAPK) [38] and phosphatidylinositol 3'-kinase (PI3K)/Akt pathways [1]. It is thus unsurprising that neurotrophins have been suggested to play fundamental roles in such processes as long-term potentiation (LTP; e.g., [4,5,6,22]), and a variety of learning tasks (e.g., [7,32,19]).

Each of the neurotrophins and their receptors is expressed differentially in the hippocampus, with all being expressed to a greater or lesser extent in the dentate gyrus subfield [2]. Evidence exists to suggest a role for each of the neurotrophins in various forms of hippocampal plasticity. There is particularly compelling evidence for such functions for NGF and BDNF both from our laboratory [23,24,32] and others; for example, mice deficient in BDNF show impairments in object recognition and spatial learning [18] and in LTP in area CA1 of the hippocampus [27] that can be reversed by BDNF treatment [33], while NGF has been proposed to play a role in both contextual memory consolidation [42] and spatial learning [15]. There is less evidence indicating the involvement of NT3 and NT4 in hippocampal plasticity, although NT4-deficient mice have been reported to show impairments in long-term memory and in LTP in CA1 [43] and conditional NT3 knockout mice have recently been reported to display decreased neurogenesis in dentate gyrus, concomitant with impaired spatial learning and LTP [36].

While transgenic mice have proven to be a useful experimental tool, the genetically hypertensive (GH) inbred strain of rat provides another potential model with which to examine the effect of lack of neurotrophins and/or their receptors on neuronal function.

* Corresponding author at: Department of Physiology, School of Medicine, University of Dublin, Trinity College, Dublin 2, Ireland. Tel.: +353 18963794; fax: +353 16793545.
E-mail address: aikelly@tcd.ie (Á.M. Kelly).

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.09.037

The first indication of such a possibility came from a report that observed a deficit in NGF availability in the peripheral nervous system of the GH rat [28]. We subsequently extended this finding to the central nervous system, observing a decrease in expression and activity-dependent release of NGF and expression of Trk receptors in the dentate gyrus of the GH rat that was accompanied by impaired expression of LTP [21]. Since a non-specific pan-Trk antibody was used in these experiments, we did not identify whether or not expression of all Trk isoforms was altered in the GH rat.

In this study, we have extended our study of the GH strain of rat by examining the ability of the GH rat to perform a hippocampal-dependent learning task, long-term object recognition, that we have suggested may rely upon NGF-stimulated intracellular signalling [19]. We have also investigated whether the impairments in hippocampal function displayed by these rats are accompanied by alterations in expression of other neurotrophins and their receptors.

2. Materials and methods

2.1. Animals

Normotensive (N; $n = 16$) and genetically hypertensive (GH; $n = 18$) New Zealand Otago Wistar rats (300–400 g) were used. They were obtained from breeding colonies in the Bioresources unit, Trinity College Dublin and were a gift from Professor Chris Bell. The GH strain of rat was originally bred in the Department of Medicine in the University of Otago, New Zealand and was developed as a model of essential hypertension by brother sister matings of successive generations of Wistar rats that displayed high blood pressures (~140 mmHg systolic blood pressure). The N strain was bred from the ancestral stock from which the GH rats were originally bred and serve as controls for the GH rats. Rats were housed in pairs in a temperature-controlled vivarium with a 12 h light/dark cycle (lights on at 8:00 A.M.) and had access to food and water *ad libitum*. All of the experiments were performed in strict accordance with national and European Union recommendations. Blood pressure measurements of a random sample of rats from each group confirmed significantly elevated systolic blood pressure in the GH compared with the N group (data not shown).

2.2. Induction of LTP

N ($n = 4$) and GH ($n = 4$) rats were anaesthetized by intraperitoneal (i.p.) injection of urethane (1.5 g/kg); loss of consciousness was evidenced by absence of the pedal reflex. Rats were placed in a stereotaxic frame and a bipolar stimulating electrode was placed in the perforant path (4.4 mm lateral to lambda) while a recording electrode was placed in the dorsal cell body region of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to Bregma). Test shocks were delivered at 30 s intervals and recorded for 15 min in order to establish a stable baseline. This was followed by delivery of three high frequency trains of stimuli (250 Hz for 200 ms) at 30 s intervals after which recording resumed at test shock frequency for 45 min.

2.3. Object recognition task

In a separate series of experiments, N ($n = 6$) or GH ($n = 6$) rats were handled daily for 1 week and habituated to the experimental apparatus, with 20 min of exploration in the absence of objects each day for 5 days before the experiment was performed. The apparatus consisted of a black circular open field (diameter, 0.9 m; height, 0.48 m) placed in a dimly-lit room. Two objects (A and B) made from toy blocks were positioned in the arena and were firmly fixed in place to ensure they could not be moved in the course of exploration. Rats were placed into the arena at random entry points for 3×5 min trials with an inter-trial rest period of 5 min and an examiner recorded the time spent exploring each object using stopwatches. Objects were thoroughly cleaned between trials to ensure the absence of olfactory cues. 24 h later, one of the objects was exchanged for a novel object (C), placed at exactly the same coordinates, and rats were reintroduced into the open field for a single 5 min period; the time spent exploring each object was recorded as before. The time (in seconds) spent exploring each object was expressed as a percentage of the total exploration time. The criteria for exploration were strictly based on active exploration, in which rats had to be touching the object with at least their noses.

2.4. Preparation of dentate gyrus samples for analysis

In a separate series of experiments, N ($n = 6$) and GH ($n = 8$) rats were killed by cervical dislocation and decapitation. The dentate gyrus was dissected free on ice and homogenized in lysis buffer (650 μl; 50 mM NaCl, 50 mM NaF, 30 mM NaPPi, 10 mM Tris–HCl, 1 mM DTT, 500 μM benzamidine, 100 μM Na_3VO_4, 500 nM PMSF, 5 nM okadaic acid, 2.5 μg/ml aprotinin, 2.5 μg/ml pepstatin, 2.5 μg/ml leupeptin, and 1% (v/v) Triton X-100) and stored at −80 °C for later analysis of expression of NGF, BDNF, NT3, NT4, TrkA, TrkB, TrkC and actin.

2.5. Analysis of NGF, BDNF, NT3 and NT4 by ELISA

The concentrations of NGF, BDNF and NT4 in samples were quantified by ELISA (R&D Systems) according to the manufacturer's instructions. Briefly, 96-well plates (MaxiSorp; NUNC) were coated overnight at room temperature with anti-NGF, anti-BDNF or anti-NT4 antibody (100 μl; diluted in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4; pH 7.2); in the case of NGF and BDNF, 1:180; in the case of NT4, 1:360). The plates were washed three times in PBS-T (300 μl; 0.05% Tween-20 in PBS) using an automated plate washer and blocked with block buffer (300 μl; bovine serum albumin (BSA, 1% (v/v)) for 1 h at room temperature. The plates were subsequently incubated with samples and serially-diluted NGF, BDNF or NT4 standards (50 μl) for 2 h at room temperature, washed, and incubated with anti-NGF, anti-BDNF or anti-NT4 detection antibody (100 μl; diluted 1:180 in block buffer) for 2 h at room temperature. Plates were washed, incubated with streptavidin horseradish peroxidase (HRP)-conjugated antibody (100 μl) for 20 min at room temperature and washed again. TMB One solution (100 μl) was added for 20 min, the reaction was stopped with 1 N H_2SO_4 (50 μl) and the absorbance was measured at 450 nm using a 96-well plate reader (Labsystems, Multiskan, RC). Concentrations of NGF, BDNF and NT4 in samples were calculated by extrapolation from the appropriate standard curve, protein content in the samples was assessed by the method of Bradford [3] and results were expressed as ngNGF/mg protein, ngBDNF/mg protein or ngNT4/mg protein. NT3 concentration was quantified using the Emax ImmunoAssay system (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, 96-well plates (MaxiSorp; NUNC) were coated with anti-NT3 antibody (100 μl; diluted 1:500 in carbonate coating buffer (0.025 M sodium bicarbonate, 0.025 M sodium carbonate; pH 9.7)) and incubated at 4 °C overnight. Plates were washed in TBS-Tween (300 μl; 20 mM Tris–HCl, 150 mM NaCl, 0.05% (v/v) Tween-20; pH 7.6) using an automated plate washer, 1× block and sample buffer was added (200 μl; Promega) and the plates were incubated for 1 h at room temperature. Plates were washed in TBS-Tween and samples and serially-diluted standards (50 μl), were added to plates, which were incubated for 6 h at room temperature with agitation. Following washing, anti-NT3 mAb (100 μl; diluted 1:4000 in 1× block and sample buffer) was added and plates were incubated overnight at 4 °C without agitation. The plates were washed, incubated with anti-IgG HRP-conjugated antibody (100 μl; diluted 1:100 in 1× block and sample buffer) for 2.5 h at room temperature with agitation, and washed again. TMB One solution (100 μl) was added and incubated at room temperature for 10 min with agitation or until the color developed, the reaction was stopped by addition of 1N HCl (100 μl) and the absorbance was measured at 450 nm. Concentrations of NT3 in samples were calculated by extrapolation from the standard curve, protein content in the samples was assessed and results were expressed as ngNT3/mg protein.

2.6. SDS PAGE and Western immunoblotting

Samples of dentate gyrus were assayed for protein content according to the method of Bradford [3] and diluted to yield equal protein concentrations. Samples were diluted 1:2 in sample buffer (0.5 mM Tris–HCl, pH 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoethanol, and 0.05% (w/v) bromophenol blue), boiled for 5 min, and 10 μl aliquots were loaded onto 7.5% SDS gels. Proteins were separated by application of a constant voltage of 200 V for 35 min and then transferred onto nitrocellulose membranes at a constant voltage of 100 V for 1 h. After blocking in Tris-buffered saline-Tween-20 (TBS-T; 150 mM NaCl, 50 mM Tris–HCl, and 0.05% (v/v) Tween-20, pH 7.4) containing bovine serum albumin (BSA) (4%, w/v), membranes were washed and incubated in either anti-TrkA (1:800 in TBS-T containing 2% BSA; Millipore, Ireland), anti-TrkB (1:200 in TBS-T containing 2% BSA, Calbiochem, UK) or anti-TrkC (1:500 in TBS-T containing 2% BSA, Santa Cruz) overnight with agitation at 4 °C. Membranes were washed and incubated in horseradish peroxidase-conjugated secondary antibodies (in the case of TrkA and TrkC, anti-rabbit IgG diluted 1:1000 in TBS-T containing 2% BSA; Sigma, UK; in the case of TrkB, anti-mouse IgG diluted 1:500 in TBS-T containing 2% BSA; Sigma, UK) for 1 h at room temperature before being reacted with supersignal West Dura chemiluminescence reagents (Pierce, UK), exposed to photographic film and developed using a Fuji Processor. Protein bands were quantified using GeneSnap acquisition and GeneTools analysis software (GeneGenius Gel documentation and analysis system; Syngene, Cambridge, UK) to yield a figure in arbitrary units that represented the density of protein bands. Samples were assessed for actin expression to control for equal protein loading. Briefly, membranes were stripped of antibody by incubating in reblot plus solution (Chemicon, USA), washed and blocked in TBS-T containing BSA (4%, v/v). No bands were detected in stripped blots incubated in secondary antibody alone, thus verifying the efficacy of the stripping protocol. Membranes were washed in TBS-T and incubated in primary antibody (anti-β-actin diluted 1:100 in TBS-T containing 2% BSA; Millipore, Ireland) for 2 h at room temperature, after which the membranes were washed in TBS-T. Membranes were incubated with secondary antibody for 1 h (1:500 anti-rabbit IgG; Sigma, UK). The protein bands were detected using SuperSignal (Pierce Biotechnology, USA) before being exposed to photographic film and quantified using