Please cite this article in press as: Shah AP et al. Role of Trkb in the anxiolytic-like and antidepressant-like effects of vagal nerve stimulation: comparison with desipramine . neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.02.024

Neuroscience xxx (2016) xxx–xxx

ROLE OF TrkB IN THE ANXIOLYTIC-LIKE AND ANTIDEPRESSANT-LIKE EFFECTS OF VAGAL NERVE STIMULATION: COMPARISON WITH DESIPRAMINE

A. P. SHAH,[a]* F. R. CARRENO,[a] H. WU,[a] Y. A. CHUNG[a] AND A. FRAZER[a,b]

[a] *Department of Pharmacology and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, TX, USA*

[b] *South Texas Veterans Health Care System (STVHCS), Audie L. Murphy Division, San Antonio, TX, USA*

Abstract—A current hypothesis regarding the mechanism of antidepressant (AD) action suggests the involvement of brain-derived neurotrophic factor (BDNF). Consistent with this hypothesis, the receptor for BDNF (and neurotrophin 4/5 (NT-4/5)), Tropomyosin-related kinase B (TrkB), is activated in rodents by treatment with classical AD drugs. Vagal nerve stimulation (VNS), a therapy for treatment resistant depression (TRD), also activates TrkB in rodents. However, the role of this receptor in the therapeutic effects of VNS is unclear. In the current study, the involvement of TrkB in the effects of VNS was investigated in rats using its inhibitor, K252a. Anxiolytic-like and AD-like effects were analyzed using the novelty suppressed feeding test (NSFT) and forced swim test (FST), respectively. K252a blocked the anxiolytic-like effect of chronic VNS treatment and the AD-like effect of acute VNS treatment. By contrast, blocking TrkB did not prevent either the anxiolytic-like or AD-like effect of chronic treatment with desipramine (DMI), a selective noradrenergic reuptake inhibitor; it did, however, block the acute effect of DMI in the FST. To examine whether the activation of TrkB caused by either VNS or DMI is ligand-dependent, use was made of TrkB-Fc, a molecular scavenger for ligands of TrkB. Intraventricular administration of TrkB-Fc blocked the acute activation of TrkB induced by either treatment, indicating that treatment-induced activation of this receptor is ligand-dependent. The behavioral results highlight differences in the involvement of TrkB in the chronic effects of an AD drug and a stimulation therapy as well as its role in acute versus chronic effects of DMI.

Key words: antidepressants, vagal nerve stimulation, TrkB, BDNF, desipramine, depression.

INTRODUCTION

Most antidepressants (ADs) enhance transmission in serotonergic and/or noradrenergic systems (Lenox and Frazer, 2002; Morilak and Frazer, 2004) forming the basis of the monoaminergic theory of depression (Prange, 1964; Schildkraut, 1965; Gyermek, 1966). The neurogenesis theory posits that there is a stress-induced decrease in adult hippocampal neurogenesis that leads to depression and that this decrease is reversed by ADs (Jacobs et al., 2000). The neurotrophic theory is based upon studies showing opposite effects of stress and ADs on expression of certain neurotrophins in the brain (Duman et al., 1997; Duman and Monteggia, 2006). Brain-derived neurotrophic factor (BDNF) is the most widely studied neurotrophin in this regard. Along with BDNF, its receptor, Tropomyosin-related kinase B (TrkB), has also been studied. The neurotrophic and neurogenesis theories are linked; increased neurotrophin expression caused by ADs may block or reverse stress-related neuronal loss (Duman and Monteggia, 2006).

To some extent, all these theories rest upon effects produced by ADs. Although ADs are effective, about 15-30% of depressed patients do not respond to multiple treatments (Little, 2009) and are diagnosed as having treatment resistant depression (TRD); its occurrence demands better therapeutic strategies. Vagal nerve stimulation (VNS) therapy has been approved for treating TRD in several countries including the United States. Studies show promising results with VNS in TRD patients, particularly after treatment for 12 weeks, even persisting for a year or two (Schlaepfer et al., 2008; Berry et al., 2013). Previously, we reported that serotonergic and/or noradrenergic systems are involved in the behavioral effects of VNS (Furmaga et al., 2011). We also found that acute or chronic VNS treatment promotes phosphorylation of hippocampal TrkB (Furmaga et al., 2012) and that K252a, a non-specific tyrosine kinase inhibitor (Koizumi

*Corresponding author. Present address: Department of Neuroscience, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.
E-mail address: ashah69@jhmi.edu (A. P. Shah).

Abbreviations: AD, antidepressant; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; DMI, desipramine; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; FST, forced swim test; ICV, intracerebroventricular; IP, intraperitoneal; NSFT, novelty suppressed feeding test; NT-4/5, neurotrophin-4/5; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEM, standard error of the mean; TBST, Tris-buffered saline with 0.1% tween 20; TRD, treatment resistant depression; TrkB, Tropomyosin-related kinase B; VNS, vagal nerve stimulation; Y515, tyrosine residue 515; Y705, tyrosine residue 705; Y816, tyrosine residue 816.

http://dx.doi.org/10.1016/j.neuroscience.2016.02.024
0306-4522/

et al., 1988; Tapley et al., 1992), blocked TrkB phosphorylation caused by either acute VNS or desipramine (DMI) treatments (Furmaga et al., 2012). To extend these results, we first investigated the role of TrkB in the behavioral effects of VNS, using K252a, with the forced swim test (FST) providing a measure of AD-like effects (Porsolt et al., 1978) and the novelty suppressed feeding test (NSFT) a measure of anxiolytic-like effects (Bodnoff et al., 1988).

As mentioned, AD drugs activate TrkB by phosphorylation at tyrosine residue 705 (Y705), within the autophosphorylation catalytic domain of TrkB and also at tyrosine residue 816 (Y816), which leads to activation of the phospholipase C-gamma1 (PLC-γ1) pathway (Saarelainen et al., 2003; Rantamaki et al., 2007; Furmaga et al., 2012). However, only acute or chronic VNS causes phosphorylation at tyrosine residue 515 (Y515), linked to Ras/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways (Furmaga et al., 2012; Carreno and Frazer, 2014). It is currently not certain if AD-induced TrkB activation is due to BDNF release, or to a different neurotrophin or is independent of neurotrophins (Rantamaki and Castren, 2008). In conditional forebrain BDNF knockout mice, treatment with imipramine still causes phosphorylation of TrkB at Y816 (Rantamaki et al., 2011). Also, BDNF *in vitro* and *ex vivo* causes phosphorylation at all three sites (Middlemas et al., 1994; Yuen and Mobley, 1999; Huang et al., 2008; Di Lieto et al., 2012). Based on these reports, one may hypothesize that AD-induced TrkB activation is not ligand-dependent whereas that by VNS may be. Therefore, we tested whether activation of TrkB in response to DMI and VNS treatments is ligand-dependent, by using a recombinant human TrkB-Fc chimera (or TrkB-Fc) consisting of the ligand-binding domain of TrkB with the Fc region of human IgG1. TrkB-Fc acts as a false receptor and sequesters endogenous neurotrophins (both BDNF and NT-4/5) with high potency and specificity as shown *in vitro* (Shelton et al., 1995) and *in vivo* (Binder et al., 1999; Gustafsson et al., 2003).

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250–400 g, ~8 weeks old upon arrival, Harlan, Houston, TX, USA) were group-housed on a 14:10-h light/dark cycle (lights on at 0700 h) and given one week to acclimatize before any procedures. Food and water were provided *ad libitum*, unless noted otherwise. Animals were isolated after surgery. All procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the local Institutional Animal Care and Use Committee (IACUC).

AD drug administration

Acute. Desipramine hydrochloride (DMI, 15 mg/kg, SC) or vehicle (sterile physiological saline) was administered 23.5 and 1 h before testing (Fig. 1C).

Chronic. DMI (10 mg/kg/day, intraperitoneal (IP)) or vehicle (distilled water) was delivered via osmotic minipumps (2.5 μl/h, Model 2ML4, DURECT Corp., Cupertino, CA, USA) as described by Furmaga et al. (2011) for up to 24 days (Fig. 1A).

Doses of DMI are expressed as free-base.

VNS

The surgery was performed as described by us previously (Cunningham et al., 2008; Furmaga et al., 2011), using stimulators generously provided by Cyberonics, Inc. (Houston, TX, USA). Animals were allowed one-week recovery time before further procedures. Stimulation parameters used are similar to those used initially in clinical settings (Rush et al., 2005) and were as follows: current, 0.25 mA; frequency, 20 Hz; pulse width, 0.25 ms; duty cycle, 30 s ON, 300 s OFF (Furmaga et al., 2011). When given acutely, VNS was given 3 times for 2 h each time starting at −23.5, −6.5 and −2.5 h prior to testing in the FST (Cunningham et al., 2008). When given chronically, VNS was administered for periods up to 24 days using the stimulation parameters described above. Stimulators were turned off 30 min prior to the FST, switched on 5 min post-FST and turned off again before tissue collection. To avoid confounding effects from handling, the same procedure was carried out with the Sham animals except that the current setting remained at 0 mA.

Stereotaxic surgery for intracerebroventricular (ICV) administration

Rats were anesthetized with ketamine and medetomidine (75 mg/kg and 0.5 mg/kg, respectively, IM) and placed onto a stereotaxic frame. Guide cannulae (C313G, 22G, Plastics One, USA) were implanted unilaterally targeting the lateral ventricle (from skull: AP −0.8, ML −1.4, DV −3.5; Paxinos and Watson (1986)). Cannulae were cemented to the skull and capped using dummies (C313DC, Plastics One). Animals were allowed one-week recovery time before further procedures. K252a (1 μg in 1-μl dimethyl sulfoxide or DMSO, 0.25 μl/min over 4 min, Calbiochem, San Diego, CA, USA) was administered to freely moving rats via injectors (C313I, 28G, Plastics One) that fit into guide cannulae with a 1-mm projection. Injectors were left in place for 5 min post-injection to allow diffusion. The K252a administration protocol was adapted from the description by Li et al. (2011).

In the acute study with DMI, K252a was given 2 h prior to each injection of the drug (Fig. 1C). With acute VNS, it was administered at time points shown in Fig. 1D. In the chronic treatment paradigms, K252a was administered 2 h before turning on the stimulators on Day 0 to VNS-treated animals and every other day, starting on Day 1 until the end of the study to both VNS and DMI-treated animals and respective controls (Figs. 1A, B).

Behavioral tests

NSFT. The NSFT was performed on Day 10 (see Fig. 1A, B) and carried out as originally described by

Please cite this article in press as: Shah AP et al. Role of Trkb in the anxiolytic-like and antidepressant-like effects of vagal nerve stimulation: comparison with desipramine . neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.02.024