Autonomic Neuroscience: Basic and Clinical 197 (2016) 1–8

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

BDNF acting in the hypothalamus induces acute pressor responses under permissive control of angiotensin II

Chris L. Schaich, Theresa L. Wellman, Blanka Koi, Benedek Erdos *

Department of Pharmacology, University of Vermont, Burlington, VT, USA

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 25 February 2016
Accepted 28 February 2016

Keywords:
Brain-derived neurotrophic factor
Paraventricular nucleus of the hypothalamus
Angiotensin
TrkB receptors
Blood pressure
Sympathetic nervous system

ABSTRACT

Brain-derived neurotrophic factor (BDNF) expression increases in the paraventricular nucleus of the hypothalamus (PVN) during hypertensive stimuli including stress and hyperosmolarity, but its role in PVN cardiovascular regulatory mechanisms is unclear. Chronic BDNF overexpression in the PVN has been shown to elevate sympathetic tone and blood pressure in part by modulating central angiotensin (Ang) II mechanisms. However, the cardiovascular effects of short-term increases in PVN levels of BDNF and the mechanisms governing them are unknown. Therefore, we investigated whether acute BDNF microinjections into the PVN of conscious and anesthetized Sprague-Dawley rats induce blood pressure elevations and whether Ang II signaling is involved in these hypertensive responses. In conscious rats, unilateral BDNF (12.5 ng) microinjections into the PVN increased mean arterial pressure (MAP) by 27 ± 1 mm Hg ($P < 0.001$ vs vehicle), which was significantly attenuated by intracerebroventricular infusion of the Ang II-type-1 receptor (AT_1R) antagonist losartan and by ganglionic blockade with intravenous hexamethonium infusion. In anesthetized rats, unilateral PVN microinjection of BDNF increased MAP by 31 ± 4 mm Hg ($P < 0.001$ vs vehicle), which was prevented by PVN microinjection pretreatments with the high-affinity BDNF receptor TrkB antagonist ANA-12, losartan, the angiotensin converting enzyme inhibitor lisinopril, or by intravenous hexamethonium. Additional experiments in hypothalamic samples including the PVN revealed that BDNF-induced TrkB receptor phosphorylation was prevented by ANA-12 and losartan pretreatments. Collectively, these data indicate that BDNF acting within the PVN acutely raises blood pressure under permissive control of Ang II-AT_1R mechanisms and therefore may play an important role in mediating acute pressor responses to hypertensive stimuli.

1. Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, exerts diverse effects on neuronal structure and function throughout the central nervous system by regulating gene expression, neuronal structure, synaptic development and ion channel function through its high-affinity tyrosine receptor kinase B (TrkB) receptor (Andero et al., 2014). These BDNF-regulated mechanisms in neuroplasticity, memory and learning have been widely studied (Andero et al., 2014; Bekinschtein et al., 2014; Leal et al., 2015; Lu et al., 2014), but recent investigations suggest that BDNF may also be a key regulator of cardiovascular function in medullary and hypothalamic nuclei. For instance, endogenous BDNF tonically regulates blood pressure and efferent sympathetic nerve activity in the nucleus of the solitary tract (NTS) (Clark et al., 2011), and microinjections of exogenous BDNF into the NTS and rostral ventrolateral medulla (RVLM) significantly elevate blood pressure (Clark et al., 2011; Wang and Zhou, 2002).

The paraventricular nucleus of the hypothalamus (PVN) is a major neurohumoral integration site as well as a modulator of sympathetic nervous system activity via parvocellular projections to the NTS, RVLM and intermediolateral spinal column neurons (Dampney et al., 2005). Intriguingly, BDNF mRNA and protein expression in the PVN increase significantly in response to hypertensive stimuli such as stress and hyperosmolarity (Aliaga et al., 2002; Hammack et al., 2009; Smith et al., 1995a), but its role in PVN cardiovascular regulatory mechanisms is unclear. We recently demonstrated that long-term overexpression of BDNF in the PVN via adeno-associated viral vector delivery produces chronic elevations in blood pressure, heart rate and sympathetic activity (Erdos et al., 2015). However, consequences of long-term BDNF upregulation may differ considerably from the effects of short-term increases in BDNF within the PVN like those that occur during acute stress (Smith et al., 1995a).

Recent findings suggest a complex, bidirectional interaction between BDNF and angiotensin (Ang) II signaling pathways that may involve Ang II-induced upregulation of BDNF expression to mediate changes in ion channel function (Becker et al., 2015), BDNF-induced upregulation of Ang II type-1 receptor (AT_1R) expression and central AT1R-dependent elevations in sympathetic tone, blood pressure and

* Corresponding author at: Department of Pharmacology, University of Vermont, 89 Beaumont Ave, Given E315, Burlington, VT 05405, USA.
E-mail address: berdos@uvm.edu (B. Erdos).

http://dx.doi.org/10.1016/j.autneu.2016.02.011
1566-0702/

heart rate (Erdos et al., 2015). Furthermore, BDNF expression and Ang II-mediated pathways in the PVN are both stimulated by acute stress and hyperosmotic stimuli (Aliaga et al., 2002; Chen and Toney, 2001; Davern et al., 2009; Saavedra et al., 2004; Smith et al., 1995a). Based on these observations, we hypothesize that acute increases in PVN levels of BDNF elicit hypertensive and tachycardic responses that are mediated by activation of the sympathetic nervous system through an interaction with PVN Ang II signaling mechanisms.

To test this hypothesis, we performed a series of microinjection experiments in conscious and anesthetized animals. First, we investigated the effects of acute BDNF infusion into the PVN of conscious rats to evaluate cardiovascular responses free from the compounding effects of anesthesia (Brahim and Thut, 1984) with or without pretreatment by central infusion of the AT_1R inhibitor losartan or intravenous infusion of the ganglionic blocker hexamethonium. Then we examined specific Ang II and BDNF interactions within the PVN by performing microinjections in anesthetized rats using double-barreled glass capillaries to allow infusion of inhibitors and BDNF into the same region of the PVN. Finally, we studied potential involvement of AT_1Rs in BDNF-induced activation and autophosphorylation of TrkB receptors in freshly isolated hypothalamic brain punches from the area of the PVN. In this study we provide functional and biochemical evidence for a synergistic interaction between BDNF and Ang II-AT_1R signaling in the PVN that contributes to acute increases in blood pressure and establish BDNF as a novel pro-hypertensive mediator in the PVN.

2. Materials and methods

All animal housing, handling, surgical and experimental procedures were conducted within an animal care facility accredited by the Association for the Assessment and Accreditation of Laboratory Care International at the University of Vermont, in accordance with the USA Public Health Service Policy on Human Care and Use of Laboratory Animals and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Experiments were performed in male Sprague-Dawley (SD) rats obtained from Charles River (Saint-Constant, QC, Canada) at 7–9 weeks of age. Rats were housed individually with a 12-hour light/dark cycle (lights on at 6:00 am), with free access to food (standard chow) and water. Surgeries were performed under continuous isoflurane anesthesia (5% induction; 2–3% maintenance) delivered in oxygen. Depth of anesthesia was assured by close monitoring of blood pressure, heart rate, and lack of eye blink and hind paw pinch reflex responses. Survival surgeries were conducted using aseptic technique and carprofen (5 mg/kg/day s.c.) was administered for post-surgical analgesia at the beginning of surgery and for two days during post-surgical recovery. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

2.1. Experimental design and surgical procedures

2.1.1. Microinjections in conscious rats

These experiments were designed to test the effect of acute BDNF injections into the PVN on blood pressure and heart rate in conscious animals, and to investigate the involvement of central AT_1Rs and the autonomic nervous system. Under isoflurane anesthesia, intracranial guide cannulae (PlasticsOne, Roanoke, VA) targeting the right PVN (26-ga diameter; inserted 1.8 mm posterior to bregma; 1.7 mm lateral; tilted 10° laterally toward the midline and lowered to 7 mm beneath the surface of the skull) and the left lateral ventricle (22-ga diameter; inserted 0.8 mm posterior to bregma; 0.9 mm lateral and lowered to 3 mm beneath the surface of the skull) were surgically implanted and secured with stainless steel screws and dental cement. A dummy cannula was fastened into each guide. After a 5-day recovery period, rats were surgically outfitted with femoral artery (all groups) and vein (hexamethonium + BDNF group only, see below) catheters under isoflurane anesthesia 24–48 h prior to testing. Catheters were threaded out of a 0.5-cm incision in the interscapular region and sutured into place.

On the day of the experiment, the femoral artery catheter was connected to a pressure transducer (MEMSCAP, Inc., Durham, NC) using an extension catheter, while the femoral vein catheter was connected to a 1-mL syringe. The dummy cannulae were carefully removed from the PVN and intracerebroventricular (icv) guides, and injectors extending 1 mm below the guide cannulae were inserted and secured. Animals were then allowed to acclimate for a minimum of 30 min to achieve stable baseline blood pressure and heart rate levels. Rats were divided into four treatment groups: icv infusion of artificial cerebrospinal fluid (aCSF) + PVN microinjection of aCSF (n = 6); icv aCSF + PVN microinjection of BDNF (n = 7); icv losartan + PVN microinjection of BDNF (n = 7); and intravenous (iv) hexamethonium + PVN microinjection of BDNF (n = 4). Losartan (15 μg) or aCSF was infused icv in a volume of 5 μL over 20 s or hexamethonium (30 mg/kg bolus) was infused intravenously over 30 s. Fifteen minutes following icv aCSF or losartan infusion, or 5 min following intravenous hexamethonium administration, BDNF (12.5 ng) was infused into the PVN at 25 nL/s for 20 s. Blood pressure was recorded for an additional 15 min. At the end of the experiment, rats were sacrificed by decapitation under deep isoflurane anesthesia. Brains were collected, fixed in 4% paraformaldehyde, freeze-protected with 30% sucrose solution and sectioned for verification of the injection site (Fig. 1). All experiments were conducted between 1:00 pm and 5:00 pm in a quiet lit room.

2.1.2. Microinjections in anesthetized rats

To study specific intra-PVN BDNF signaling mechanisms, we performed PVN microinjection experiments in anesthetized rats. This approach allowed the use of double-barreled glass micropipettes for pretreatment injections of inhibitors followed by BDNF into the same region of the PVN. Femoral artery and vein catheters were implanted under isoflurane anesthesia. Rats were then placed in a stereotaxic apparatus and a sagittal incision was made to expose the dorsal surface of the skull. A cranial window (~3 × 4 mm) was created to expose an area of the brain surface dorsal to the PVN. As previously described (Cassaglia et al., 2014), animals were then transitioned from isoflurane to α-chloralose anesthesia (100 mg/kg/h), administered intravenously through the femoral vein catheter over a 30-min period during which isoflurane was gradually withdrawn. Blood pressure, heart rate, and toe-pinch and eye-blink reflexes were monitored closely to ensure the animal remained anesthetized. After complete withdrawal of isoflurane, anesthesia was maintained by intravenous α-chloralose infusion (25 mg/kg/h) for the remainder of testing. Oxygen flow remained constant for the duration of the experiment, and core body temperature and pO_2 were continuously monitored using a PhysioSuite system (Kent Scientific, Torrington, CT).

A minimum of 60 min passed between cessation of isoflurane and the first microinjection to achieve stable baseline measurements of blood pressure and heart rate. A double-barreled glass micropipette (World Precision Instruments; 1 mm outer diameters, ~50 μm diameter at the tip) was lowered into the left or right PVN (1.8 mm posterior to bregma; 1.7 mm lateral to the midline; 7.64 mm below the brain surface at a 10° tilt toward the midline). Each rat was injected with one of the following pretreatments: aCSF (n = 6), the TrkB-selective inhibitor ANA-12 (2 μg; n = 6), the AT_1R antagonist losartan (10 μg; n = 6), or the angiotensin converting enzyme (ACE) inhibitor lisinopril (200 ng; n = 5), followed by BDNF (12.5 ng) 15 min later. Each drug was infused at a rate of 50 nL/s over 5 s. Drug doses were based on previous studies (Busnardo et al., 2014; Cazorla et al., 2011) and our preliminary tests. An additional experimental group received intravenous hexamethonium (30 mg/kg; n = 4) through the femoral vein catheter split by a 3-way stopcock, followed by intra-PVN BDNF (12.5 ng) 5 min later. Recording continued for an additional 15 min after BDNF microinjection. At the end of experiments, rats were sacrificed by decapitation under deep isoflurane anesthesia. Brains were then collected, fixed in 4%