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Blockade of TrkB receptors in the nucleus accumbens prior to heterotypic stress alters corticotropin-releasing hormone (CRH), vesicular glutamate transporter 2 (vGluT2) and glucocorticoid receptor (GR) within the mesolimbic pathway

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ABSTRACT

Inhibition of stress-induced elevations in brain-derived neurotrophic factor (BDNF) or its primary receptor tyrosine-related kinase B (TrkB) within the reward pathway may modulate vulnerability to anxiety and mood disorders. The current study examined the role of BDNF/TrkB signaling on biochemistry and behavior under basal conditions and following exposure to a 10-day heterotypic stress paradigm in male rats. Effects of intra-accumbal administration of TrkB antagonist ANA-12 (0.25 μg/0.5 μl/min) on anxiety, and expression of Trk-B, corticotropin-releasing hormone (CRH), vesicular glutamate transporter 2 (vGluT2) and glucocorticoid receptor (GR) within the mesolimbic pathway were determined. Notably, ANA-12 attenuated anxiety-like behavior in stress rats while increasing anxiety in the non-stress group in the elevated plus maze (EPM). At the neurochemical level, ANA-12 blocked the increased vGluT2 and CRH expressions in the hypothalamic PVN and basolateral amygdala in stress rats, while it enhanced vGluT2 and CRH expressions in non-stress rats. ANA-12 also showed state-dependent effects at the NAc core, attenuating TrkB-ir in non-stress rats while reversing reduced expression in stressed rats. At the cingulate cortex, ANA-12 normalized stress-induced increase in TrkB expression. Notably, ANA-12 showed region-specific effects on GR-ir at the NAc core and shell, with increased GR-ir in non-stress rats, although the drug attenuated stress-induced GR-ir expression only in the core portion of the NAc, while having no impact at the cingulate cortex. Elevated blood CORT levels post-stress was not influenced by ANA-12 treatment. Together, these findings suggest that BDNF-mediated TrkB activation exerts differential impact in regulating emotional response under basal and stress conditions.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is widely expressed throughout the brain (Alcantara et al., 1997; Murakami et al., 2005), and is essential for proper neuronal growth, survival and differentiation during development (Leibrock et al., 1989), and for synaptic function and plasticity of CNS neurons (long-term potentiation and long-term depression) in the mature brain (Lindsay et al., 1994; Matsuda et al., 2009; McAllister et al., 1999; Thoenen, 1995). Among observed properties, BDNF is reported to protect neurons against glutamate excitotoxicity (Jiang et al., 2005), modulate *N*-methyl-D-aspartate (NMDA) receptor phosphorylation (Suen et al., 1997) and underlie learning and memory processes. In recent years, BDNF has been shown to exert a profound influence on resilience and increased susceptibility to depression (Duman and Li, 2012), with reduced BDNF levels being implicated in altered behavioral responses induced by changes in serotonin brain levels (Autry and Monteggia, 2012; Martinowich and Lu, 2008) or trophic effects on noradrenergic neurons (Fawcett et al., 1998; Saarelainen et al., 2003). Such evidence has led to the proposed neurotrophic hypothesis of depression (Duman, 2004; Duman and Aghajanian, 2012; Duman and Li, 2012; Duman and Voleti, 2012; Hosang et al., 2014), particularly suited to depressive disorders developing from stress exposure (Chao, 2003).

Notably, BDNF exerts important regulatory influences on the brain stress and reward systems (Martinowich et al., 2007) as a stress and activity-dependent neurotrophin involved in the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Jeanneteau et al., 2012). BDNF signaling is negatively regulated by glucocorticoids, which impair synaptic plasticity in the brain by inhibiting dendritic spine density, neurogenesis and long-term potentiation (Rothman and Mattson, 2013), particularly during stress. A decrease in BDNF mRNA levels

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following 6 h of daily restraint stress leads to dendritic atrophy/death of pyramidal neurons and reduction of hippocampal volume in rats (Murakami et al., 2005). Repeated stress also causes changes in the prefrontal cortex (PFC) and amygdala, which are thought to subserve cognitive and emotional impairments (de Pablos et al., 2006). In the PFC, reduced BDNF-induced glutamate release is associated with downregulated glucocorticoid receptors following stress-induced glucocorticoid release (Chiba et al., 2012). The BDNF Val66Met polymorphism is also associated with altered HPA axis reactivity (Colzato et al., 2011; Shalev et al., 2009), increasing an individual's vulnerability to alterations in mood and depression (Stein et al., 2008). Due to a broad range of physiological actions, BDNF's involvement has been suggested in local responses to various types of neuronal insults or stress (Kozisek et al., 2008; Nitta et al., 1999).

Exogenous BDNF administration within the hippocampus induces antidepressant-like behavioral effects in animal models of depression, comparable to the effects of chronic treatment with pharmacological antidepressants (Chao, 2003; Shirayama et al., 2002). BDNF-mediated TrkB receptor activation is required for the behavioral effects induced by antidepressants, and is proposed as a key mechanism of antidepressant action (Saarelainen et al., 2003). Contrasting these effects, BDNF infusion into the nucleus accumbens (NAc), a subcortical limbic region involved in mood regulation, motor function and motivational valence, triggers pro-depressant effects, whereas inhibition of its action (e.g. via adeno-associated viral infection of dominant-negative receptor TrkB-T1 (Eisch et al., 2003) or via systemic administration of selective TrkB receptor antagonist ANA-12 (Cazorla et al., 2011) promotes antidepressant effects. In this context, exposure to chronic stress enhances NAc-BDNF expression, an effect that depends on the degree of rodents' resilience (versus susceptibility) to the stress effects (e.g. anhedonia, increased anxiety and impaired coping skills) (Feder et al., 2009; Krishnan et al., 2007). Within the mesolimbic circuitry, increased spontaneous firing activity of ventral tegmental area (VTA) dopamine (DA) neurons resulting in BDNF release within the nucleus accumbens neurons has been shown to mediate susceptibility to social defeat in mice (Krishnan et al., 2007). BDNF/TrkB signaling has also been implicated in the structural and synaptic plasticity of the dopaminergic VTA-NAc reward pathway in disorders implicating stress as a predisposing factor, including depression (Christoffel et al., 2011; Eisch et al., 2003; Russo and Nestler, 2013).

Of note, the topographical organization of connections has led to proposed distinct functional attributes for the NAc core and shell portions (Deutch and Cameron, 1992; Heimer et al., 1991; Kalivas and Duffy, 1995). The NAc shell receives dense excitatory projections from the BLA (Christoffel et al., 2011; Kirouac and Ganguly, 1995) via the bed nucleus of the stria terminalis and the ventral subiculum (the major output region of the hippocampal complex) (Brog et al., 1993; Kita and Kitai, 1990; McDonald, 1991a, 1991b), together with glutamatergic projections from the PFC (Sesack et al., 1989) and dopaminergic projections from the VTA (Floresco et al., 1998). The NAc core projections from the BLA and parahippocampal regions are less dense. Within the NAc shell, anatomical coding plays an important role in the motivational valence of a stimulus (appetitive versus aversive), a phenomenon that involves dopamine/glutamate interaction in the NAc and local D1 and D2 signaling (Richard and Berridge, 2011), with selective activation of DA transmission being reported in the NAc shell, but not core portion, following 15 min of restraint stress or mild footshock (Deutch and Cameron, 1992; Kalivas and Duffy, 1995). In addition, indirect innervation of the NAc shell to the PVN is reported via efferent connections to the lateral hypothalamus (LH), which sends projections to the medial hypothalamus (Stratford, 2005).

At present, no studies have explored the anti-depressant effects of the TrkB receptor antagonist ANA-12 in an animal model of repeated heterotypic stress. Therefore, particular interest was directed toward BDNF/TrkB signaling in the brain's reward pathway and the mechanisms involved in mediating stress-induced physiological responses and neurochemical changes. The current study examined the role of BDNF/TrkB signaling in neuroendocrine responses under basal conditions and following a 10-day exposure to a heterotypic stress paradigm (alternating exposure to 30 min of restraint and 15 min of forced swim stressors), using NAc shell micro-infusions of ANA-12. In adult male Wistar rats, we determined 1) effect on endogenous glucocorticoid levels in rats, by assessing changes in blood corticosterone (CORT), 2) lasting impact on behavioral abnormalities related to anxiety, locomotor activity and anhedonia in the open field test (OFT), elevated plus maze (EPM) and forced swim test (FST) and, 3) assessed whether alterations in BDNF/TrkB signaling triggered neurochemical changes in the post-mortem brain ~9 days following cessation of the stress paradigm via immunohistochemical detection of corticotropin-releasing hormone (CRH) and vesicular glutamate transporter 2 (vGluT2) expression in the PVN and BLA, in addition to TrkB and GR expression in the PFC (anterior cingulate cortex) and NAc shell and core. This pharmacological study may enhance understanding of the modalities by which TrkB activation within the NAc shell regulates DA and glutamate release, and CRH-mediated stress response.

2. Methodology

2.1. Animals and housing

Adult male Wistar rats weighing between 250 and 275 g were obtained from Charles River Laboratories (Rochefort, Quebec, Canada), housed two to a cage and handled daily to minimize stress. They were maintained on a 12 h light/dark cycle (lights on at 7:00 am), room temperature (21–23 °C) with 60% relative humidity, and ad libitum access to water and standard Purina rat chow. Animals acclimated five days to the animal facility prior to intra-NAc guide cannula implantation surgery. All procedures were carried out in accordance with the Canadian Council of Animal care and approved by the University of Ottawa Animal Care Committee. All efforts were made to minimize animals' suffering.

2.2. Guide cannula implantation surgery

Experimentally naïve male rats were anaesthetized in a small chamber by inhalation of 2 to 3% isoflurane in oxygen and then transferred to a stereotaxic apparatus under the same anaesthesia. The head was shaved and the area disinfected prior to placement on the stereotaxic frame and skin incision. Body core temperature was kept constant at ~37 °C throughout the surgery using a feedback regulated heating blanket (Homeothermic Blanket Control Unit, Harvard Instruments, Natick, MA). A stainless steel 22-gauge guide cannula was unilaterally implanted in the NAc shell of the right hemisphere, according to the Paxinos and Watson (1998) rat brain atlas. Stereotaxic coordinates were as follows: antero-posterior, 1.2 mm from Bregma; lateral, −1.5 mm from the midline (angled at 6°); and dorsoventral, 7.0 mm below dura (Li et al., 2013). Intra-NAc internal injectors (28-gauge) extended 0.5 mm past the end of the guide cannula, for intra-NAc injections at a final depth of 7.5 mm below dura. Guide cannulas were anchored to the skull with 3 stainless steel screws (Plastics One, Roanoke, VA, USA) using dental acrylic cement and stainless steel obturators (28-gauge) secured into the guide cannulas to prevent occlusion. Post-surgery, rats received a subcutaneous injection of the non-steroidal anti-inflammatory drug Meloxicam (1.5 mg/kg, Boehringer Ingelheim Ltd.). They were then placed in their cage resting on a heating pad in an air-controlled incubator for a few hours post-op prior to returning to their vivarium room. Rats were individually housed and allowed to recover for 10 days post-surgery, with gentle handling for at least 2 min daily after the fourth recovery day (Rodd et al., 2004). A standard operating procedure chart was used to evaluate daily the animals' health and recovery. Unilateral injections were chosen as this injection method has yielded similar effects as bilateral injections, and the former minimize stress to the animal (Olson et al., 2005); also see (Bolanos et al., 2003; Carlezon et al., 1997).