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Research Paper

Activation of CRHR1 receptors regulates social and depressive-like behaviors and expression of BDNF and TrkB in mesocorticolimbic regions following global cerebral ischemia

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ABSTRACT

Increased HPA axis activation and CRH release characterize the brain's response to global cerebral ischemia. Recently, CRH via activation of CRH type 1 receptors (CRHR1) has been shown to regulate Brain Derived Neurotrophic Factor (BDNF) secretion and emotional behavior. The current study investigates the impact of CRHR1 blockade on BDNF/TrkB signaling expression in the mesolimbic circuitry, and social and depressive-like behavior following global ischemia. Adult male Wistar rats were injected with Antalarmin (2 μg/μl) or a vehicle 30 min prior to 10 min global cerebral ischemia (4VO model) or sham occlusion. The Three Chamber Social Approach Test (SIT) assessed sociability and preference for social novelty, and the novelty suppressed feeding test (NSFT), forced swim test (FST), and sucrose preference test characterized anxiety and depression. Corticosterone levels and organ (thymus, seminal and adrenal glands) weights were determined as additional physiological indices of stress. Immunohistochemistry, Western blot and Rt-PCR were used to assess BDNF and TrkB receptor levels in subregions of the medial prefrontal cortex (mPFC), nucleus accumbens (NAc) and ventral tegmental area (VTA) 30 days post-ischemia. Our findings indicate reduced BDNF and TrkB protein and mRNA expression in the mPFC post-ischemia, while heightened levels were found in the NAc. Ischemia increased immobility in the FST and reduced sucrose preference and led to reduced latency to feed in the NSFT and heightened sociability and social novelty preference in the SIT. Antalarmin treatment normalized post-ischemic biochemical/behavioral changes. Our findings support lasting effects of CRHR1 activation on brain plasticity markers, likely playing a role in emotional impairments following cardio- or cerebro-vascular accidents.

1. Introduction

Adverse social consequences post-stroke include emotional impairments and depression, manifested by a low self-esteem, an altered self-image, and a redefinition of one's social role depending on cognitive or physical changes (Barrett, 2010). Dysregulation of HPA axis activation in major depression includes impaired inhibition of cortisol release, higher baseline cortisol values, and an overactive response to psychological stressors, which have been related to abnormalities in activation of corticotropin-releasing hormone (CRH) (Sher et al., 2013), and CRHR1 blockade has beneficial effects in the treatment of anxiety and depression in humans (Holsboer and Ising, 2008). Similarly, hypersecretion of plasma CORT upon exposure to an acute restraint stress has been reported 27 days post-reperfusion in ischemic rats, which is prevented by pretreatment with Antalarmin, a specific CRH type 1 receptors (CRHR1) antagonist (de la Tremblaye et al., 2014). CRHR1-ir expression also remains elevated in the hypothalamic paraventricular nucleus (PVN) at remote intervals post-ischemia, while reduced receptor expression is observed in the hippocampal CA1 subfield, supporting its contribution to ischemia-induced HPA axis dysregulation (de la Tremblaye et al., 2014).

Recently, CRHR1 activation has been shown to modulate the expression of brain-derived neurotrophic factor (BDNF) and its high-affinity tropomyosin-related kinase B (TrkB) receptor (Bayatti et al., 2005), which are critical for neuronal survival, synaptic plasticity and memory function, the CRHR1/BDNF gene–gene interaction also being identified as an important pathophysiological factor contributing to recurrent major depressive disorder and stroke (Bennett and Lagopoulos, 2014; Platenik et al., 2013; Xiao et al., 2011). Serum BDNF levels are decreased in depressed patients (Karege et al., 2002), and reduced hippocampal BDNF expression along with depressive behaviors resulting from severe stress exposure can be restored by antidepressant treatment (Duman and Monteggia, 2006). BDNF gene methylation has been observed in

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depressive stroke survivors, and its persistence associated with worsening depressive symptoms over one year follow-up (Kim et al., 2013). Accordingly, BDNF serum levels are decreased in post-stroke depressive patients (Zhou et al., 2011) while lower TrkB mRNA and protein levels have been reported in the prefrontal cortex of suicide subjects (Dwivedi et al., 2003). In rodents, chronic stress models induce depression-like behaviors, which mimic several aspects of depressive symptoms observed in humans, including despair, anhedonia, and HPA axis hyperactivity (Ge et al., 2014). Chronic stress decreases BDNF mRNA in the hippocampus while concentrations increases in the PVN (Aliaga et al., 2002; Rage et al., 2002; Smith et al., 1995; Ueyama et al., 1997), two limbic structures regulating the HPA axis. Increased hypothalamic CRH and BDNF mRNA levels, as well as ACTH and CORT plasmatic concentrations following chronic immobilization stress are associated with increased immobility in the forced swimming test (i.e. despair) and reduced sucrose preference (i.e., anhedonia) and body weight gain (Naert et al., 2011). Of interest, single and chronic icv BDNF injections gradually increase CRH mRNA expression in the parvocellular portion of the PVN, which upregulates plasma ACTH and corticosterone concentrations (Givalois et al., 2004; Naert et al., 2006), and decreases food intake and body weight (Toriya et al., 2010). Moreover, stress-induced rapid CRH mRNA upregulation at the PVN is accompanied by increased CRH release from pre-synaptic GABAergic axon terminals within the hippocampus (Bergstrom et al., 2008; Chen et al., 2004) where it activates type 1 CRH receptors located on excitatory dendritic spines of hippocampal pyramidal neurons (Chen et al., 2012). CRHR1 deficiency prevents chronic stress-induced spatial memory impairments, reduced nectin-3 expression and dendritic atrophy in CA3 neurons (Wang et al., 2011). Notably, chronic exposure to unpredictable stress in rats reduces BDNF and TrkB protein and mRNA levels in the hippocampus and the frontal cortex while impairing sucrose consumption (Banerjee et al., 2014).

Contrary to observations at the hippocampus and PFC, stress increases BDNF expression in the nucleus accumbens (NAc) and the ventral tegmental area (VTA) (Nikulina et al., 2012), and infusion of BDNF in the VTA or the NAc have been shown to produce depressive-like effects (Berton et al., 2006). Interestingly, BDNF also co-localizes with tyrosine hydroxylase-ir neurons in the mesocorticolimbic system (Seroogy et al., 1994). Stimulation of dopamine synthesis promotes the expression of BDNF (Okazawa et al., 1992), while BDNF infusion into the VTA and NAc increases dopamine utilization (Pierce and Bari, 2001). In this context, 30-min middle cerebral artery occlusion in mice elicited depression-like symptoms associated with degeneration of dopaminergic neurons, reduced dopamine levels and dopamine transporter density along with increased BDNF protein levels in the striatum, which could be reversed by chronic antidepressant treatment initiated 7 days after stroke (Kronenberg et al., 2012). These findings support BDNF-dopamine interplay in the development of post-stroke depression.

At present, the role of CRHR1 in depressive-like behavior and expression of neuroplasticity in the mesocorticolimbic system after global cerebral ischemia has yet to be characterized. The goals of the current study therefore aim to determine the effects of pre-ischemic CRHR1 blockade on motivation, social interaction, anhedonia and depressive-like behaviors, as well as characterize changes in TrkB and BDNF protein and mRNA expression within the mPFC and NAc, using Western blot and PCR techniques. Site-specific changes in BDNF and TrkB distribution in the subregions of the mPFC: the anterior cingulate cortex (CG1), prelimbic cortex (PL) and infralimbic cortex (IL), as well as the subregions of the NAc the core (NAcC) and shell (NAcS), and in the VTA were determined by immunohistochemistry.

2. Material and methods

2.1. Animals

Male Wistar rats (N = 96, 50 underwent behavioral testing, and 46 were used for the mRNA and protein analysis) weighing between 250 and 320 g at time of surgery were obtained from Charles River Laboratories (Rochefort, Qc, Canada) and habituated to the housing facility for minimum a week before surgery. They were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 AM) with free access to water and standard rat chow. Room temperature was maintained at 21–23 °C with 60% relative humidity. The experimenter handled all rats daily for 2–3 min for four days preceding surgery, and two days before behavioral testing. All experiments and procedures were conducted in accordance with the guidelines set by the Canadian Council of Animal Care and approved by the University of Ottawa Animal Care Committee. Efforts were made to minimize the number of animals used and their suffering.

2.2. ICV cannulation and drug preparation

One week after arrival to the animal facility (see Fig. 1 for experimental timeline), rats underwent surgery for stereotaxic implantation of a guide cannula into the third ventricle, as previously described (de la Tremblaye et al., 2014). Briefly, rats were anesthetized using isoflurane mixed with oxygen (2–3%) and positioned in a stereotaxic instrument. A stainless steel 22-gauge guide cannula (Plastics One Inc.) was stereotaxically implanted using the following coordinates: 4.3 mm posterior to Bregma, 0.0 mm lateral to the midline and 4.3 mm ventral to the skull surface according to Paxinos and Watson (1997) atlas and secured to the skull using four anchor screws and dental cement. A dummy cannula was inserted into the guide cannula to prevent occlusion. The drug was administered via a 28-gauge stainless steel injector (Plastics One Inc.) projecting 0.5 mm below the tip of the guide cannula. Cannula placement into the 3rd ventricle was confirmed in thionin-stained slices.

Antalarmin hydrochloride (Sigma-Aldrich Inc.), a selective CRHR1 antagonist, was dissolved in 0.9% saline containing 10% Cremophor (Sigma-Aldrich Inc.). Ischemic and sham rats were injected with Antalarmin (ANT; 2 μg/2 μl; icv) or the vehicle (Veh) solution 30 min prior to 10-min global ischemia or sham occlusion, creating 4 experimental groups: Ischemia-ANT (IA; n = 12), Ischemia-Veh (IV; n = 12), Sham-ANT (SA; n = 10), and Sham-Veh (SV; n = 10). A home cage (HC; n = 6) group, with no surgery or drug treatment was also included.

2.3. Four-vessel occlusion surgery

The four-vessel occlusion (4VO) model was used to induce forebrain ischemia as previously described (Pulsinelli and Brierley, 1979). Briefly, rats were anesthetized using isoflurane (2–3%) mixed with oxygen. The

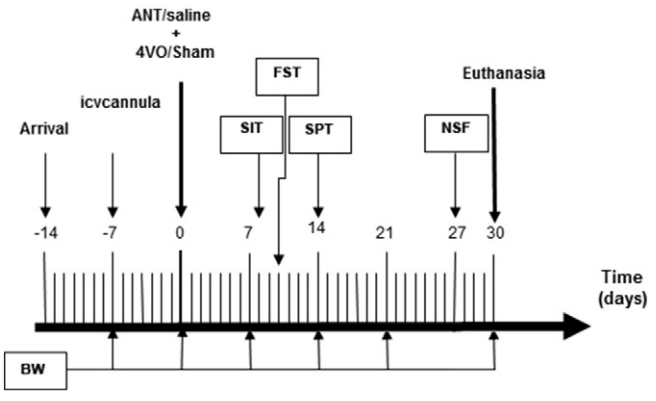

Fig. 1. Experimental timeline: Arrows indicate days at which distinct experimental procedures were conducted. Following a week acclimation to the vivarium, rats underwent surgical cannulation and 7 days later (Day 0) ischemic or sham surgeries. Antalarmin (2 μg/2 μl) or vehicle was icv administered 30 min prior to sham or carotid occlusion. Rats were tested on reperfusion day 8 in the social interaction test (SIT), in the forced swim test (FST) on day 9, in the sucrose preference test (SPT) on day 14 and in the novelty suppressed feeding test (NSFT) on day 27. Body weight (BW) was taken after each surgeries and weekly until reperfusion day 30.