Neuroscience 352 (2017) 30–38

ORAL ADMINISTRATION OF CORTICOSTERONE AT STRESS-LIKE LEVELS DRIVES MICROGLIAL BUT NOT VASCULAR DISTURBANCES POST-STROKE

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Abstract—Exposure to chronic stress following stroke has been shown, both clinically and pre-clinically, to impact negatively on the recovery process. While this phenomenon is well established, the specific mechanisms involved have remained largely unexplored. One obvious signaling pathway through which chronic stress may impact on the recovery process is via corticosterone, and its effects on microglial activity and vascular remodeling. In the current study, we were interested in examining how orally delivered corticosterone at a stress-like concentration impacted on microglial activity and vascular remodeling after stroke. We identified that corticosterone administration for two weeks following stroke significantly increased tissue loss and decreased the weight of the spleen and thymus. We also identified that corticosterone administration significantly altered the expression of the key microglial complement receptor, CD11b after stroke. Corticosterone administration did not alter the expression of the vessel basement membrane protein, Collagen IV after stroke. Together, these results suggest that corticosterone is likely to represent only one of the major stress signals responsible for driving the negative impacts of chronic stress on recovery.

Key words: corticosterone, microglia, stress, stroke, vessels.

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Abbreviations: CORT, corticosterone; HPA, hypothalamic–pituitary–adrenal.

http://dx.doi.org/10.1016/j.neuroscience.2017.03.005
0306-4522/

INTRODUCTION

Patients that have suffered from a stroke often experience severe levels of stress that can persist for many months after the initial event (Lyon, 2002; Hilari et al., 2010). While levels of distress have been investigated from a quality-of-life perspective, the impact of stress on cellular repair processes is also significant. Stress, or more accurately the stress response, refers to the coordinated physiological response triggered by unpredictable and potentially harmful events (Day and Walker, 2007). The stress response alters the activity of numerous neurotransmitter pathways within the brain, including the outflow of sympathetic and parasympathetic systems, while simultaneously modulating hormone release from the pituitary and associated peripheral glands. Collectively, these coordinated activities are considered to provide substrates, such as epinephrine and glucose that can facilitate the host's response to the stressor.

Both clinical and pre-clinical studies have identified that the prolonged stress response after stroke negatively impacts upon the recovery process (Feibel et al., 1977; Angeleri et al., 1993; Tyson, 1995; Elmstahl et al., 1996; Lyon, 2002; Kirkland et al., 2008; Jones et al., 2015; Ong et al., 2016). Kirkland et al. for instance has demonstrated that chronic restraint stress impaired motor recovery and increased infarct size after the induction of a focal lesion within the motor cortex (Kirkland et al., 2008). Similarly, studies have shown that chronic restraint stress increased infarct volume and reduced functional recovery after photothrombotic vascular occlusion in the sensorimotor cortex (Jin et al., 2010; Ong et al., 2016). Similarly, Jones et al. has demonstrated that the chronic stress following stroke exacerbated the severity of secondary neurodegenerative changes, including enhanced neuronal loss from the thalamus (Jones et al., 2015).

While the negative effects of chronic stress on post-stroke recovery are widely acknowledged, the specific mechanisms involved remain unclear. Obviously, a number of systems are influenced, however, one of the more promising candidates is the effect that stress has on vascular growth and remodeling. It is known that vascular remodeling is associated with successful brain recovery (Krupinski et al., 1993, 1994; Popa-Wagner et al., 2010). Linking stress to these findings, it has been shown that chronic stress is associated with a reduction of both VEGF and Flk-1 proteins levels in dentate gyrus

(Heine et al., 2005). Further, chronic stress disrupts the actions of microglia within the thalamus (Jones et al., 2015) and several recent studies have shown that microglial play a crucial role in modulating vascular repair (Jolivel et al., 2015; Dudvarski Stankovic et al., 2016).

Given the ability of stress to both directly and indirectly alter both microglia and vascular remodeling it would be of interest to understand exactly which of the signals generated during the stress response were most involved. Unravelling this issue is experimentally difficult due to the fact that stress alters the functioning of many different systems. Despite this, it is widely recognized that one of the key effector molecules released during the stress response in rodents is corticosterone (cortisol in humans). Corticosterone (CORT) is a steroid hormone and is released via a pathway known as the h ypothalamic–pituitary–adrenal (HPA) axis. CORT, by itself is known to be highly pleiotropic in its effect. At low concentrations CORT has been shown to be modestly neuroprotective and enhance spatial memory after stroke (Faraji et al., 2009). However, at higher levels it has been shown to inhibit angiogenesis (Shikatani et al., 2012) and endothelial cell proliferation in the hippocampus and prefrontal cortex in rats (Ekstrand et al., 2008) as well as decrease microglial activity after MPTP-induced activation (Sugama et al., 2009).

If the negative effects of chronic stress on vascular remodeling and microglia that we have already demonstrated are mediated by CORT, then future interventions could more confidently target CORT for therapeutic benefit. Given this situation, we decided to investigate whether CORT could achieve the same or similar effects to what we have previously observed with chronic stress. To achieve this we induced photothrombotic vascular occlusion which has been shown as reliable and relatively non-invasive method of stroke induction in mice (Labat-gest and Tomasi, 2013). We have chosen to induce stroke within the motor/somatosensory cortex as this area is frequently damaged by stroke (Langhorne et al., 2009). CORT was been administered 72 h after stroke, at high stress-like levels (100 μg/ml), in the animals' drinking water for 14 days. We decided to use oral delivery instead of injected, as repeated injections would result in high levels of stress in our control groups. Further, it has been shown previously that oral delivery of CORT is a very efficient route to increase circulating levels (Gourley et al., 2009; Gourley and Taylor, 2009; Olausson et al., 2013). We used the cylinder task to assess functional recovery. This task has been shown to be objective and able to detect chronic motor forelimb impairment (Schaar et al., 2010).

EXPERIMENTAL PROCEDURES

Materials

CD11b antibody was purchased from Millipore (Billerica, Massachusetts, USA) and Collagen IV antibody from Abcam (Cambridge, UK). Corticosterone hemisuccinate (4-PREGNEN-11β, 21-DIOL-3, 20-DIONE 21-HEMISUCCINATE) was purchased from Steraloids Inc. (Newport, RI, USA). Paraformaldehyde, rose bengal and other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Animals

All experiments were approved by the University of Newcastle Animal Care and Ethics Committee and conducted in accordance with the New South Wales Animals Research Act (1985) and the Australian Code of Practice for the use of animals for scientific purposes. This study complied with the ARRIVE guidelines. See Fig. 1 for experimental design.

C57BL/6 male mice (7 weeks old) were obtained from the Animal Services Unit at the University of Newcastle, each randomly allocated to one of the following groups: sham, sham + CORT, stroke and stroke + CORT (n = 5–8 per group). Mice were maintained at 21 °C ± 1 in a humidity controlled environment with water and food available *ad libitum*. Lighting was on 12:12-h reverse light–dark cycle (light on at 7 pm). All procedures were conducted in the dark phase under low-level red light. Mice were group-housed with 2–3 animals per cage and acclimatized for 7 days before commencing the procedures. Mice were weighed throughout the procedures.

Photothrombotic occlusion

Photothrombotic occlusion was performed as described (Ong et al., 2016). Briefly, on day 0 (defined as the day of photothrombotic occlusion) mice were injected intraperitoneally with 0.2 mL of 10 mg/mL of rose bengal eight minutes prior to 15 min of illumination, using a cold light source with a fiber optic end of 4.5-mm diameter placed 2.2 mm left lateral of bregma onto the exposed skull. Irradiation of the translucent skull using a cold light source following injection with rose bengal results in thrombotic microvessel occlusion. The illuminated area includes the motor (M1) and somatosensory (S1) cortices (see Fig. 3A). For the sham group, a similar procedure was applied except rose bengal was replaced with 0.2 mL of 0.9% saline.

Oral delivery of CORT

Animals were provided with CORT in their drinking water three days after the occlusion was induced. CORT was prepared to a final concentration of 100 μg/ml in drinking water as described (Gourley et al., 2006; Gourley and Taylor, 2009). Briefly, CORT was stirred in pH 11 water solution (using NaOH) until completely dissolved and then neutralized to pH 7 (using HCl). The CORT - water solution, henceforth referred to as CORT, was given to animals instead of drinking water. The solution was refreshed every 72 h over the 14 days of protocol.

Paw asymmetry assessment using the cylinder task

The cylinder test was adapted for use in mouse to assess forelimb use and rotation asymmetry (Schaar et al., 2010). The mouse was placed in a transparent cylinder 9-cm diameter and 15 cm in height and videotaped from both sides of the cylinder during the test. After the mouse