Sustained administration of corticosterone at stress-like levels after stroke suppressed glial reactivity at sites of thalamic secondary neurodegeneration

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Abstract
Secondary neurodegeneration (SND) is an insidious and progressive condition involving the death of neurons in regions of the brain that were connected to but undamaged by the initial stroke. Our group have published compelling evidence that exposure to psychological stress can significantly exacerbate the severity of SND, a finding that has considerable clinical implications given that stroke-survivors often report experiencing high and unremitting levels of psychological stress. It may be possible to use one or more targeted pharmacological approaches to limit the negative effects of stress on the recovery process but in order to move forward with this approach the most critical stress signals have to be identified.

Accordingly, in the current study we have directed our attention to examining the potential effects of corticosterone, delivered orally at stress-like levels. Our interest is to determine how similar the effects of corticosterone are to stress on repair and remodelling that is known to occur after stroke. The study involved 4 groups, sham and stroke, either administered corticosterone or normal drinking water. The functional impact was assessed using the cylinder task for paw asymmetry, grid walk for sensorimotor function, inverted grid for muscle strength and coordination and open field for anxiety-like behaviour. Biochemically and histologically, we considered disturbances in main cellular elements of the neurovascular unit, including microglia, astrocytes, neurons and blood vessels using both immunohistochemistry and western blotting. In short, we identified that corticosterone delivery after stroke results in significant suppression of key microglial and astroglial markers. No changes were observed on the vasculature and in neuronal specific markers. No changes were identified for sensorimotor function or anxiety-like behaviour. We did, however, observe a significant change in motor function as assessed using the inverted grid walk test. Collectively, these results suggest that pharmacologically targeting corticosterone levels in the future may be warranted but that such an approach is unlikely to limit all the negative effects associated with exposure to chronic stress.

1. Introduction

Stroke survivors often experience high levels of psychological stress (Hilari et al., 2010; Lyon, 2002). Although few clinical studies, have systematically dissected out the specific causes of the stress burden, even the slightest consideration of the changes in personal circumstances reveal a multitude of probable factors. Amongst the most compelling, is the fact that stroke survivors frequently report significant levels of motor and cognitive impairment, outcomes which can also directly impact on the survivor’s quality of social interactions. A further dimension to the stress experienced by stroke survivors is persistence, with several studies reporting that the level of stress for stroke survivors is unremitting and has detrimental effect on the recovery in both clinical and pre-clinical research (Angeleri et al., 1993; Elmstahl et al., 1996; Espinosa-Garcia et al., 2017; Feibel et al., 1977; Hilari et al.,...
Recently, our research team has directed its focus towards exploring the potential implications of chronic stress exposure during the recovery process on the severity of secondary neurodegeneration (SND) post-stroke. SND involves the progressive death of neurons in regions that are connected to but not originally damaged by the infarction. In our initial study into this phenomenon we identified that exposure to chronic stress was associated with a significant exacerbation of the neuronal loss within the thalamus after induction of a somatosensory cortex stroke (Jones et al., 2015; Ong et al., 2016). Interestingly, our team also observed that the increased loss of neurons in the thalamus co-occurred with a suppression of makers indicative of microglial activity (Jones et al., 2015). This observation, aligned well with a number of results that have suggested that microglia play a central role in brain repair following CNS trauma (Myers et al., 1991; Pappata et al., 2000; Sugama et al., 2009). We have also identified that chronic stress markedly alters the accumulation of neurotoxic proteins within the thalamus while concomitantly reducing synaptic density (Alfarez et al., 2009; Karten et al., 1999; Ong et al., 2016). Collectively, these findings suggest that chronic stress exerts a broadly negative influence over repair processes at sites of SND.

Limiting the negative effects of stress from a biomedical standpoint poses a number of significant challenges, foremost amongst which is the fact that the ‘stress response’ evokes changes in the activity of multiple subsystems. This creates a significant challenge for the development of targeted strategies that restrict the negative effects of stress. One potential solution to this challenge is to target which stress signals are the most biologically active and have the strongest evidence implicating their role in immunomodulation and immunosuppression. To this end, there is one compelling candidate; corticosterone. Corticosterone is one of the major immunomodulatory hormones released during the stress response. It’s release from the adrenal cortex is initiated by the coordinated actions of the hypothalamic–pituitary–adrenal axis (De Kloet, 2004).

In the current study, we aimed to investigate the effect of corticosterone administration on the severity of SND post-stroke within the thalamus. This study is a continuation of previously reported corticosterone impact in peri-infarct regions (Zalewska et al., 2017). In terms of the design of the current study we chose to use a photothrombotic (PT) model to induce a focal cortically directed stroke. This model has a number of advantages, including that it is highly steerable and repeatable, has low experiment to variance, and is widely used and extensively validated (Labat-gest and Tomasi, 2013). Importantly, it is also recognised to possess a number of limitations, including the fact that it produces only a relatively small penumbral area (at risk tissue around the primary infarct) and by its nature does not permit the study of cortical reperfusion, as the occlusion induced is permanent in nature (Labat-gest and Tomasi, 2013). We have previously used the PT model across a large number of studies and have consistently identified the ability of PT stroke directed towards the somatosensory cortex to robustly induced thalamic SND (Jones et al., 2015; Ong et al., 2016; Patience et al., 2015). To consider the influence of corticosterone, we used the same administration paradigm as previously reported by Zalewska et al., to simulate stress-like levels of corticosterone (Zalewska et al., 2017). Specifically, we delivered 100 μg/ml of corticosterone in the drinking water to mice following stroke. This concentration has been shown to increase level of corticosterone in blood to around 5 times that seen in sham animals (Zalewska et al., 2017). A similar level of increase has been shown after exposure of mice to a 30 min swim stress (Barriga et al., 2001) a 6 h session of restraint stress, or after 23 days of mild unpredictable stress (Gong et al., 2015) Corticosterone administration began 72 h after the induction of stroke. Three days of recovery reduced chances of surgery related infections and allows wound healing which could be delayed due to corticosterone administration (Padgett et al., 1998).

Corticosterone was administered via drinking water for 14 days. We assessed motor function using the cylinder task, grid walk and inverted grid test to determine functional recovery. Anxiety-like behaviour and locomotor activity was assessed by open field test. We examined changes of a variety of markers; neuronal (NeuN, PSD-95), astroglial (GFAP, S100b), microglial (CD68, CD11b) and vascular proteins (Collagen-IV, CD31) within the thalamus. Additionally, we also examined changes in microglial morphology. We hypothesised that exposure to corticosterone at stress-like levels would result in greater neuronal loss. Moreover, we expected that corticosterone would result in significant suppression of glial activation and that this will be linked to suppression of vascular growth.

### 2. Materials and methods

#### 2.1. Materials

A full list of antibodies used during this experiment is listed in Table 1. Corticosterone hemisuccinate (4-PREGNEN-11β, 21-DIOL-3, 20-DIONE 21-HEMISUCCINATE) was obtained from Steraloids.

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<th>Table 1</th>
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<tr>
<td>List of antibodies used for western blot and immunohistochemistry.</td>
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<td>Sources of antibodies</td>
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<td>Collagen IV</td>
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<td>GFAP</td>
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<td>Iba-1</td>
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<td>NeuN</td>
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