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Research report

Rats with altered behaviour following nerve injury show evidence of centrally altered thyroid regulation

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ABSTRACT

The co-morbidity of mood disturbance, in a proportion of patients, is now described across a wide range of chronic disease states. Similarly, a '*Low Thyroid Syndrome*' is also reported in a proportion of individuals with chronic diseases. Here, we report on central changes in an animal model of inflammatory stress in which altered social behaviour, representing social disability, persists in a sub-group of rats following injury. We showed in an earlier study that rats with social disability following injury have significantly decreased peripheral thyroid hormones, with no increase in Thyroid Stimulating Hormone (TSH). Only rats identified by behavioural change showed changes in hypothalamic gene expression. In whole hypothalamus extracted RNA, relative expression of mRNA for Thyrotrophin-releasing hormone (TRH) was significantly down-regulated in disabled rats ($p = 0.039$) and deiodinase 3 up-regulated ($p = 0.006$) compared to controls. Specifically in the paraventricular nucleus (PVN), numbers of immunoreactive cells for deiodinase 3-like and thyroid hormone receptor beta-like proteins were decreased in the sub-group with disability compared to the control group ($p = 0.031$ and $p = 0.011$ respectively). In rats with behavioural change post-injury, down-regulation of TRH provides an explanation for the failure of the hypothalamo-pituitary-thyroid (HPT) axis to respond to the post-injury decrease in thyroxine. Decreased local expression of deiodinase 3 protein, resulting in a local increase in T3, offers an explanation for down regulation of TRH in the hypophysiotrophic TRH neurons. It is possible that, in a sub-group of animals identified behaviourally, a mechanism resulting in hypothalamic down-regulation of the HPT axis persists following inflammatory injury.

Abbreviations: BC, Behavioural Controls; CCI, chronic constriction injury; Ct, threshold cycle; D, dominance; DAB, 3-3′-diaminobenzidine; Dio2, deiodinase 2; Dio3, deiodinase 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPT axis, hypothalamo-pituitary-thyroid axis; 1L-1β, interleukin 1β; IR, immunoreactive, immunoreactivity; LPS, lipopolysaccharide; MBH, medial basal hypothalamus; ND, No Disability; PBS, 0.9% NaCl in 0.1 M phosphate buffer; PBHS, phosphate buffered horse serum; PD, Persistent Disability; PVN, paraventricular hypothalamic nucleus; PaDP, PaMP, PaV, dorsal, medial and ventral parvocellular divisions of PVN; T3, triiodothyronine; T4, thyroxine; TD, Transient Disability; TNF, tumour necrosis factor; TRβ, thyroid hormone receptor β; TRH, thyrotropin-releasing-hormone; TSH, thyrotropin/thyroid stimulating hormone.

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1. Introduction

Behavioural changes which characterise "sickness behaviour" in acutely ill animals are seen as adaptive (Hart, 1988; Konsman et al., 2002). Behavioural and mood state changes are increasingly being reported across the chronic disease spectrum in humans (Herrman and Chopra, 2009; Qiu et al., 2010; Yohannes et al., 2010). The incidence of mood disorder in patients with chronic disease is higher than for the general population and this co-morbidity is associated with poorer 'Quality of Life' scores than for any of the chronic diseases alone or any combination of chronic diseases without depression (Moussavi et al., 2007). Many chronic pain patients experience not only sensory disturbance but also changes in complex behaviours, affect and cognition. These changes can include altered social behaviours, disturbed sleep, fatigue, altered appetite, reduced interest in the environment, and very often depression (Menefee et al., 2000; Meyer-Rosberg et al., 2001; Fredheim et al., 2008). However depressed mood is a feature of chronic conditions in only a subset of patients. A large World Health Organisation survey showed that, depending on the chronic condition, between 9 and 23% of survey participants report both depression and a chronic disease (Moussavi et al., 2007).

Mood disorder, cognitive impairment and behavioural change have long been associated with clinical hypothyroidism and have more recently been associated with milder levels of thyroid hypofunction (Whybrow and Hurwitz, 1976; Gold and Pottash, 1986; Esposito et al., 1997; Boelaert and Franklyn, 2005). Low thyroid syndrome, initially described in acute illness, is increasingly being reported in chronic diseases (Arnaout et al., 1994; Dimopoulou et al., 2001; Iervasi et al., 2003; Bunevicius et al., 2006; Adler and Wartofsky, 2007). In general, this syndrome presents as low or low normal peripheral triiodothyronine (T3) and thyroxine (T4) levels with TSH level in the normal range. There is a blunting of the TSH response to TRH and decrease or abolition of the early morning TSH surge (De Groot, 2006; Adler and Wartofsky, 2007). Controversy continues over the thyroid status of these patients: altered hypothalamic regulation is implicated with TSH levels inappropriately low for the thyroid hormone levels (De Groot, 2006).

Investigation of the low thyroid syndrome using animal models initially examined the situation in acute illness: there was peripheral and central depression of the hypothalamo-pituitary-thyroid (HPT) axis in the 24 h following onset of inflammatory illness (Kakucska et al., 1994; Boelen et al., 2004; Fekete et al., 2004). Down regulation of TRH gene expression in the paraventricular nucleus of the hypothalamus (PVN) was demonstrated, as well as an increase in deiodinase 2 (Dio2) in the medial basal hypothalamus (MBH). The mechanism proposed to explain the down regulation of TRH was a local thyrotoxicosis in the PVN (Fekete and Lechan, 2007): increased Dio2 increases the conversion of T4 to T3 which regulates TRH levels in the PVN (Nikrodhanond et al., 2006).

Other models have been used to investigate the effect of chronic illness: in rabbits following extensive burns (Mebis et al., 2009) and in mice following turpentine injection into leg muscles with abscess formation (Boelen et al., 2006). In both models it was demonstrated that peripheral thyroid hormones fell and TRH mRNA in the PVN was suppressed. In the burns model, subjects remain seriously ill 7 days after injury and are intended to model intensive care patients whereas in the abscess model, alterations to the HPT axis had normalised by 5 days following injury. Thus neither model adequately approximates to the ongoing chronic disease states reported in humans, nor addresses the behavioural changes associated with chronic diseases in a proportion of patients. However in the abscess model, specific down regulation of deiodinase 3 (Dio3) in the PVN was demonstrated at a time when TRH mRNA was suppressed (Boelen et al., 2006). Down regulation of Dio3, the enzyme responsible for metabolising T3 provides an alternate or additional mechanism, which would result in a local thyrotoxicosis in the PVN, leading to down regulation of TRH.

The rat CCI model of Bennett and Xie (1988) is one of the more commonly used pain models, largely due to the simplicity of the surgery and the consistency of the sensory disabilities produced. The constriction injury triggers an inflammatory response, which results in mechanical constriction of the nerve and sensory dysfunction characteristic of human peripheral neuropathic pain. Monassi et al. (2003) have shown that the model of Bennett and Xie has a stronger resemblance to the human neuropathic pain condition by demonstrating that a sub-population of rats shows changes in complex social behaviours and in their sleep-wake cycle following injury (Bennett and Xie, 1988; Monassi et al., 2003; Keay et al., 2004). In a subsequent study, in a different cohort of rats, we reported altered peripheral thyroid hormones in a subpopulation of rats following chronic constriction injury (CCI) of the sciatic nerve (Kilburn-Watt et al., 2010). Post-injury, the sub-population of rats with behavioural change had decreased peripheral thyroid hormones without a compensatory rise in TSH; there was a correlation between fall in peripheral T4 and changed behaviour (Kilburn-Watt et al., 2010). In contrast, rats that maintained social behaviours following injury demonstrated a surge in TSH secretion and maintenance of peripheral thyroid hormones. Preliminary assessment of gene expression in the hypothalamus of that cohort of rats indicated decreased relative expression of TRH, in keeping with that reported in other inflammatory stress models (Kilburn-Watt et al., 2010). In view of the reported co-morbidity of mood disorder and chronic disease states in a subset of patients together with reports of low thyroid syndrome in similar groups of patients, the purpose of the studies presented here was to determine in a new cohort of rats, characterised by disability following nerve injury, the differences in central expression of HPT factors in rats with disordered behaviour following injury.

2. Methods

2.1. Animals

All experimental procedures were carried out with the approval of the Animal Care and Ethics Committee of the University of Sydney, following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the NSW Animal Research Act 1985 and NSW Animal Research Act Regulation 2010.

Male Sprague–Dawley rats (220–345 g) were housed in a temperature-controlled room ($22 \pm 1\,^{\circ}C$) with food and water freely available and environmental enrichment provided. The rats were initially housed in groups of six and habituated to a 12-h reverse light cycle (lights on 18:00 h), for a minimum of 1 week prior to experimental procedures. They were then housed individually in perspex cages with visual, auditory and olfactory contact with at least five other rats. All rats were observed daily and weighed three times over the course of the experiment.

2.2. Disability profiling procedure

To identify rats with one of the three injury triggered disability profiles, i.e., Persistent, Transient and No disability, once a day, for 5 days before and for 6 days after sciatic nerve constriction injury (CCI), during the rats' active period, an 'intruder' rat was introduced into the home cage of each experimental rat (the 'resident') for a period of 6 min. The interactions between the resident and intruder rats were videotaped for later analysis. Resident rats never encountered the same intruder on more than two occasions and never on consecutive days. The interactions of the resident rat were scored from the video recording for Dominance (D), Social (S),