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Research report

Altered hypothalamic inflammatory gene expression correlates with heat stroke severity in a conscious rodent model

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ABSTRACT

It has been suggested that heat-induced hypothalamic damage mediates core temperature (T_c) disturbances during heat stroke (HS) recovery; this is significant as hypothermia and/or fever have been linked to severity and overall pathological insult. However, to date there has been a lack of histological evidence in support of these claims. We hypothesized that local hypothalamic cytokines and/or chemokines, known regulators of T_c, are mediating the elevation in T_c during HS recovery even in the absence of histological damage. In experiment 1, the hypothalamus of Fischer 344 rats was examined for 84 cytokine/chemokine genes (real-time PCR) at multiple time points ($T_{c,Max}$, 1, 3, and 10 days) during mild HS recovery. In experiment 2, the hypothalamus of three different HS severities (MILD, moderate [MOD], and severe [SEV]) in rats were examined for the same genes as experiment 1 as well as six oxidative damage markers, at a single intermediate time point (1 day). Systemic cytokines were also analyzed in experiment 2 across the three severities. There were significant alterations in 25 cytokines/chemokines expression at $T_{c,Max}$, but little or no changes in expression at longer time points in experiment 1. In experiment 2 there were significant changes in gene expression in SEV rats only, with MILD and MOD rats showing baseline expression at 1 day, despite an absence of systemic cytokine expression in any severity. There was also no change in any oxidative marker of damage at 1 day, regardless of severity. In conclusion, we show only limited changes during long term recovery from HS, but demonstrate differences in hypothalamic gene expression patterns that may be driving HS pathology and morbidity. These findings contribute to our overall understanding of HS pathology in the CNS, as well as providing avenues for future pharmacological intervention.

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1. Introduction

Heat stroke (HS) is the most devastating form of heat illness, and is characterized by systemic organ damage with potentially debilitating central nervous system (CNS) dysfunction (Leon and Bouchama, 2015). CNS dysfunction is characterized by cognitive and motor deficits during moderate HS cases while often resulting in permanent brain damage and coma in the most severe forms of HS (Ferris et al., 1938; Rav-Acha et al., 2007; Albukrek et al., 1997). It has been suggested that CNS damage to the preoptic anterior hypothalamus (POAH) causes temperature disturbances, such as fever and/or hypothermia in severe HS cases. While these post-HS temperature responses have been linked to severity and morbidity in animal models and humans there is no evidence that the POAH is damaged in even the most severe HS cases, including non-survivors (Ferris et al., 1938; Hart et al., 1982; Quinn et al., 1985; Leon et al., 2005; Malamud et al., 1946). For example, Malamud et al. (1946) performed autopsy on 125 military exertional HS cases and noted significant histological damage to several brain areas whereas the POAH was spared. Similarly, we have shown in rat and mouse conscious passive HS models that hypothermia and fever occur in the absence of histological damage to the hypothalamus or other brain regions (Quinn et al., 2014; Leon et al., 2010). In mice, hypothermia was associated with increased POAH and hippocampal gene expression of a number of cytokines (e.g., interleukin-6 [IL-6]) and chemokines (e.g., macrophage inflammatory protein [MIP]) suggesting this response is linked to core temperature (T_c) during recovery as well as mirroring what is occurring systemically (Biedenkapp and Leon, 2013). However, responses in the hypothalamus beyond 24 h of recovery remain unstudied in any model; therefore it is unknown what alterations are retained through longer-term recovery. In addition, what differences in hypothalamic responses are occurring between different HS severities, and how these might be driving severity, remains unknown.

The goal of this study was to determine if molecular alterations exist in the hypothalamus during 10 days of recovery from HS and examine hypothalamic inflammatory and oxidative gene responses and T_c profiles across 3 HS severities. We used targeted gene analysis to examine the inflammatory changes in the hypothalamus of rats at the onset of HS and at long-term recovery time points (3, 5, 10 days of recovery). We then compared inflammatory and oxidative hypothalamic gene responses amongst mild (MILD), moderate (MOD), and severe (SEV) HS rats, which previously showed a lack of histological brain damage (Quinn et al., 2014). This research effort aimed to identify neurological pathways that may play a part in determining HS severity, irrespective of histological damage. Our findings showed that the majority of alterations occurred early (<3 days), with a limited number of alterations still observed at 10 days post-HS. However, SEV HS rats showed greater gene expression changes at 1 day of recovery compared to MILD or MOD rats suggesting that hypothalamic gene expression could be influencing recovery.

2. Results

2.1. *Experiment 1*

Body weight (BW) was virtually identical between CON and MILD HS rats prior to experimentation at ~270 g (Table 1). CON rats experienced ~2% dehydration, presumably due to lack of food and water during experimentation, whereas MILD HS was associated with ~7% dehydration (Table 1; Students *t*-test, $P<0.05$) Fig. 1). MILD HS rats required ~174 min of heat exposure to reach $T_{c,Max}$ of 41.8 °C and incurred an ascending and descending thermal load of ~100 and ~19 °C min, respectively (Exact values found in Table 1). These responses are similar to previously reported values in MILD HS rats using our experimental protocol (Quinn et al., 2014). MILD HS rats displayed sustained hyperthermia (~1 °C) into 3D of recovery that is also typical of this level of HS severity in the rat (Fig. 2A; ANOVA, $P<0.05$; (Quinn et al., 2014)). For ease of presentation, T_c responses are shown through night three only with T_c virtually identical between CON and MILD HS rats beyond this time point (data not shown).

Plasma and brain samples were collected at $T_{c,Max}$ (most robust T_c response during recovery of MILD HS rats) and compared to CON at the same time point. Fig. 2B shows the pattern of gene expression changes from $T_{c,Max}$ through 10D of recovery of MILD HS rats when compared to CON values (Table 2). The majority of gene expression changes (29%) were observed at $T_{c,Max}$ with both up-regulated (33%) and down-regulated cytokine and chemokine responses (67%) observed at this time point (Fig. 2). Of those in the cytokine family, four were down-regulated (Integrin β-2 (Itgb2), Transforming growth factor β-1 (Tgfb1), Toll-like receptor-2 (Tlr2), Tumor necrosis factor (Tnf) and one was up-regulated (Interleukin 1β) at $T_{c,Max}$. Of the chemokine family, 12 were downregulated (MCP-1-related chemokine [CCL2], Eotaxin-2 [Ccl24], Mucosae-associated epithelial chemokine [Ccl28], Macrophage inflammatory protein-1 [Ccl4], Monocyte chemotactic protein-3 [Ccl7], Macrophage inflammatory protein 1α receptor [Ccr1], Macrophage inflammatory protein 1α receptor like-1 [Ccr1ll], Eosinophil eotaxin receptor [Ccr3], Macrophage inflammatory protein 3β receptor [Ccr7], Putative monocyte chemotactic protein receptor [Ccrl2], [Cmklrl], CKLF-like MARVEL transmembrane domain containing 5 [Cmtm5]), and 7 were upregulated (Chemokine binding protein 2 [Ccbp2], Monocyte chemotactic protein 1[Ccl2], Exodus-2 [Ccl21], Ccl6 [Ccl6], Chemokine receptor like-1 [Ccr8], CKLF-like MARVEL transmembrane domain containing 2A [Cmtm2a], Fractalkine

Table 1 – Characteristics of control and heat stroked rats.

	Control ($N=13$)	Heated ($N=26$)
Starting body weight (g)	268.3±3.8	270.2±2.6
Dehydration at $T_{c,Max}$ (%)	1.9±0.6	7.1±0.4
Time to $T_{c,Max}$ (min)	–	174±8
Ascending thermal area (° min)	–	101.0±8.8
Descending thermal area (° min)	–	18.6±1.7

Data are means ± SEM. $T_{c,Max}$, maximum core temperature