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Full-length Article

Histamine regulation of microglia: Gene-environment interaction in the regulation of central nervous system inflammation

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ABSTRACT

Microglia mediate neuroinflammation and regulate brain development and homeostasis. Microglial abnormalities are implicated in a range of neuropsychiatric pathology, including Tourette syndrome (TS) and autism. Histamine (HA) is both a neurotransmitter and an immune modulator. HA deficiency has been implicated as a rare cause of TS and may contribute to other neuropsychiatric conditions. *In vitro* studies suggest that HA can regulate microglia, but this has never been explored *in vivo*. We used immunohistochemistry to examine the effects of HA deficiency in *histidine decarboxylase* (*Hdc*) knockout mice and of HA receptor stimulation in wild-type animals. We find HA to regulate microglia *in vivo*, via the H4 receptor. Chronic HA deficiency in *Hdc* knockout mice reduces ramifications of microglia in the striatum and (at trend level) in the hypothalamus, but not elsewhere in the brain. Depletion of histaminergic neurons in the hypothalamus has a similar effect. Microglia expressing IGF-1 are particularly reduced, However, the microglial response to challenge with lipopolysaccharide (LPS) is potentiated in *Hdc* knockout mice. Genetic abnormalities in histaminergic signaling may produce a vulnerability to inflammatory challenge, setting the state for pathogenically dysregulated neuroimmune responses.

1. Introduction

Histamine (HA) is produced both by immune cells and by a group of neurons in the posterior tuberomamillary nucleus of the hypothalamus; in the central nervous system it functions as a neurotransmitter, with diverse functions in different circuitries (Haas et al., 2008). Histamine receptors are prominent in many brain regions, especially in the cortico-basal ganglia circuitry (Haas et al., 2008). Dysregulation of brain histamine is increasingly appreciated as a potential contributor to neuropsychiatric disease (Panula and Nuutinen, 2013). For example, disruption of histamine biosynthesis has been implicated as a rare genetic cause of Tourette syndrome (TS; Castellan Baldan et al., 2014; Ercan-Sencicek et al., 2010; Fernandez et al., 2012; Karagiannidis et al., 2013). Abnormalities in histaminergic signaling have also been hypothesized to contribute to Parkinson's disease (Shan et al., 2012), Huntington's disease (van Wamelen et al., 2011; Goodchild et al., 1999), Alzheimer disease's (Shan et al., 2012; Naddafi and Mirshafiey, 2013), narcolepsy (John et al., 2013; Valko et al., 2013), and drug addiction (Haas et al., 2008; Panula and Nuutinen, 2013).

Peripheral HA is an important modulator of immune, inflammatory, and allergic responses (e.g. Passani and Ballerini, 2012; Wernersson and Pejler, 2014). Dysregulated neuroinflammation has been proposed to contribute to TS and a variety of other neuropsychiatric conditions (Hornig and Lipkin, 2013; Mitchell and Goldstein, 2014; Frick et al., 2013), although these connections remain unproven in most cases. The possible role of HA as a regulator of neuroinflammatory processes in the brain has received scant attention.

Microglia are the primary inflammatory cells in the brain; inflammatory challenge, such as administration of lipopolysaccharide (LPS), induces them to produce inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). More recently it has been appreciated that microglia are required for normal brain development, homeostasis, synaptic function, and adult neurogenesis, even in the absence of inflammation (Ji et al., 2013; Schafer et al., 2013; Zhan et al., 2014). Consistent with

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this variety of functional roles, microglia can take on a range of phenotypes. Insulin-like growth factor 1 (IGF-1) is an important mediator of homeostatic microglial function. IGF-1 is upregulated in neuroprotective microglia induced by T_H2 cytokines such as IL-4 (Ueno et al., 2013). Support by IGF-1 expressing neurotrophic microglia is required for survival of cortical neurons during postnatal development (Ueno et al., 2013), for neurogenesis induced by environmental enrichment and exercise in the adult (Kohman et al., 2012; Ziv et al., 2006), and for neuroprotection after ischemic injury (Lalancette-Hebert et al., 2007; Kim et al., 2014). IGF-1-positive microglia also serve a neuroprotective role in animal models of neurodegenerative diseases such as amyotrophic lateral sclerosis (Chiu et al., 2008) and retinitis pigmentosum (Arroba et al., 2011).

Microglial dysregulation is seen in a variety of neuropsychiatric conditions, including TS, although whether such dysregulation is central to pathophysiology remains to be established (Frick et al., 2013; Siskova and Tremblay, 2013; Kumar et al., 2014; Lennington et al., 2014). Inflammation-related genes have been found to be upregulated in postmortem brain from individuals with TS (Lennington et al., 2014; Hong et al., 2004; Morer et al., 2010), and microglia in postmortem tissue have morphological hallmarks of activation, though they are normal in number (Lennington et al., 2014). A recent positron emission tomography study similarly suggested increased microglial activation in patients (Kumar et al., 2014). In mice, knockout of the HoxB8 gene, which is expressed in a subset of microglia, leads to microglial deficits and a marked increase in grooming behavior (Greer and Capecchi, 2002; Chen et al., 2010); similar grooming phenotypes have been described in other animal models of TS (Xu et al., 2015), obsessive compulsive disorder (OCD) Ahmari et al., 2013; Shmelkov et al., 2010; Welch et al., 2007, and autism (Zhan et al., 2014; Peca et al., 2011). Strikingly, these behavioral manifestations of the *HoxB8* knockout have been reported to be rescued by repopulation of the brain with wild-type microglia (Chen et al., 2010).

These observations led us to investigate the role of histamine in the regulation of microglia. A few *in vitro* studies have attempted to address this question, but much is unclear. In N9 cells, a microglia-like cell line, HA can induce migration, through activation of the H4 histamine receptor (but not the H1 receptor) (Ferreira et al., 2012). N9 cells, like brain microglia, produce pro-inflammatory cytokines such as IL-1β and TNF-α when stimulated with LPS. When they are stimulated by HA, however, N9 cells do not produce these pro-inflammatory cytokines – in fact, HA blocks LPS-induced N9 cell motility and IL-1β secretion. This leads to the hypothesis that HA might oppose the pro-inflammatory activation of microglia – a role that has also been suggested for other aminergic neurotransmitters (Biber et al., 2007). Similar findings have recently been reported in microglial cultures (Iida et al., 2015).

In contrast, other reports using primary microglial cultures from neonatal rat brain suggest that HA can have a pro-inflammatory effect. In one recent study, Dong et al. (2014) found that HA activated microglia *in vitro* in a dose- and time-dependent manner. Activation was mediated by both the H1 and the H4 receptors and led to the production of the inflammatory cytokines interleukin-6 (IL-6) and TNF-α. Zhu and colleagues reported similar results, also in primary microglial cultures (Zhu et al., 2014). HA has been reported to induce nitric oxide synthase (iNOS), another marker of an inflammatory phenotype, in cultured microglia (Rocha et al., 2014).

These divergent results may derive from the fact that all of these studies have been performed *in vitro*. *In vitro* studies have examined microglia isolated from neonates; even if they accurately recapitulate events *in vivo* in pups, this may not extrapolate to adult animals. Microglia in the adult have a less inflammatory expression profile than those seen in neonates or aging animals (Crain et al., 2013). In addition, such preparations eliminate the multitudinous cell-cell interactions that, it is increasingly recognized, fine-tune microglial phenotype and function *in vivo*. It remains unclear, therefore, how histamine modulates microglial activation *in vivo*, and whether this varies depending on brain region or animal state. We therefore used genetic and pharmacological tools to examine the regulation of microglia by HA in the intact brain.

2. Materials and methods

2.1. Mice

All experiments were conducted under the auspices of the Yale Institutional Animal Care and Use Committee, in accordance with NIH guidelines. Adult male mice aged 2–4 months were used for all experiments. C57Bl/6 wild-types were purchased from Jackson Laboratories (www.jax.org). *Hdc*-KO mice have been previously described (Castellan Baldan et al., 2014; Ohtsu et al., 2001); the mice used in these experiments were backcrossed onto C57Bl/6 for $\geqslant$9 generations. Knockout mice were generated by intercrossing heterozygotes in our vivarium; WT littermates were used as controls. Targeted depletion of histaminergic neurons was performed in *Hdc*-cre transgenic mice (Zecharia et al., 2012), which were obtained from Jackson Laboratories (jaxmice.jax.org/strain/021198) and backcrossed with C57Bl/6J mice in our vivarium. HDC-Cre negative littermates were used as negative controls in these experiments.

2.2. Drugs

Histamine dihydrochloride and VUF8430 dihydrobromide were purchased from Sigma Aldrich. 4-Methylhistamine dihydrochloride, 2-pyridylethylamine dihydrochloride and JNJ 10191584 maleate were purchased from Tocris Biosciences. Lipopolysaccharide from *Escherichia coli* (055:B5) was purchased from Santa Cruz Biotechnology. Except for JNJ 10191584, which was dissolved in 1% carboxymethylcellulose (Calbiochem), all the drugs were dissolved in sterile 0.9% sodium chloride.

2.3. Surgery

Intracerebroventricular (ICV) drug infusion was performed under anesthesia using standard sterile stereotaxic technique. Animals were anesthetized with ketamine:xylazine 100:10 mg/kg. An infusion syringe was inserted into the lateral ventricle at coordinates −0.7 mm anteroposterior, 2 mm dorsoventral, and 1.4 mm mediolateral (relative to bregma, using Paxinos and Franklin, 2004). Drugs were infused in a total volume of 1 μl using an UltraMicroPump III (World Precision Instruments Inc) at a rate of 100 nl/min.

For specific depletion of HDC cells in the tuberomammillary nucleus (TMN), we used an AAV5-flex-taCasp3-TEVp virus (Yang et al., 2013) (1×10^{12} viral particles, University of North Carolina Vector Core). Viral infusion injections were performed bilaterally in the TMN, at coordinates −2 mm anteroposterior, −5.2 mm dorsoventral, ±0.6 mm mediolateral. A volume of 0.5 μl was injected in each hemisphere (1 μl total) at a flow rate of 100 nl/min (Rapanelli et al., submitted for publication).

2.4. Perfusion and tissue preparation

For histology, animals were deeply anesthetized using ketamine/xylazine and transcardially perfused with 0.5–1 vol/wt PBS followed by 1–2 vol/wt of 4% paraformaldehyde (PFA) in PBS.