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Individual and interactive sex-specific effects of acute restraint and systemic IFN-γ treatment on neurochemistry

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ABSTRACT

Compelling evidence supports the involvement of the pro-inflammatory cytokines, IL-6, IFN-α and TNF-α in depression and related stress-associated pathologies. A role has also been suggested for the Th1-type cytokine, IFN-γ, with most mechanistic accounts focusing on the cytokine's capacity to induce indoleamine 2,3-dioxygenase (IDO), leading to diminished tryptophan and the generation of kynurenine metabolites. Beyond these IDO-dependent routes, there is surprisingly little evidence directly linking IFN-γ to alterations of brain regional monoamine activity and HPA axis functioning. Our specific aims in the present study were twofold: 1) assess the behavioural, plasma corticosterone and brain regional monoamine effects of acute systemic IFN-γ, with or without short duration restraint stress (15 min), and 2) determine the sex-specific nature of these effects. As predicted, IFN-γ stimulated monoaminergic activity within a number of stressor-sensitive limbic brain regions, most notably the paraventricular nucleus of the hypothalamus, central amygdala and prefrontal cortex. While several of these effects were sex-specific, there was little in the way of synergism between the cytokine and stressor treatments. Nonetheless, IFN-γ did synergistically interact with acute restraint stress to increase plasma corticosterone concentrations, and this effect was most pronounced in the male mice. These data are among the first to show that systemically administered IFN-γ can alone or in conjunction with psychologically relevant stressor, modify brain regional monoamine activity and the plasma corticosterone response.

1. Introduction

The pro-inflammatory cytokines, IL-1b, IL-6, IFN-α and TNF-α have repeatedly been linked to depression and related stress-associated psychiatric conditions (eg. Anisman et al., 2008a; Hayley et al., 2005). However, less attention has been devoted to IFN-γ, another cytokine that may be germane to this discussion (Maes et al., 2011). Indeed, elevated concentrations of IFN-γ have been detected in the blood and CSF of depressed patients (Dahl et al., 2014; Maes et al., 1994; Simon et al., 2008), and several different classes of antidepressants were shown to antagonize IFN-γ activity (Diamond et al., 2006; Kubera et al., 2000). Moreover, variation in the IFN-γ gene was reported to modify amygdala reactivity to emotional stimuli (Redlich et al., 2015), as well as depression risk in IFN-α-treated hepatitis C patients (Oxenkrug, 2011).

Consistently, acute exposure to an IFN-γ adenovector provoked long-lasting hedonic-like deficits (Kwant and Sakic, 2004) whereas genetic ablation of IFN-γ or its receptor attenuated some of the depressive-like neurochemical, immunological and behavioural effects of chronic stress (Litteljohn et al., 2010, 2014) or Mycobacterium bovis bacillus Calmette-Guérin infection (O'Connor et al., 2009). More recently, IFN-γ was implicated as an important contributor to the depressive-like behavioural effects of long-term LPS administration in rats (Fischer et al., 2015).

Recent attention has focused on IFN-γ's potent stimulatory action on the inflammatory enzyme indoleamine 2,3-dioxygenase (IDO), which shuttles the essential amino acid tryptophan down

Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; CeA, central amygdala; DA, dopamine; DOPAC, 3,4-Dihydroxyphenylacetic acid; HC, hippocampus; HPA, hypothalamus-pituitary-adrenal; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-gamma; IL, interleukin; LC, locus coeruleus; MHPG, 3-methoxy-4-hydroexyphenylglycol; NE, norepinephrine; PFC, prefrontal cortex; PVN, paraventricular nucleus of the hypothalamus.

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the kynurenine pathway and away from the serotonin (5-HT) synthesizing pathway (Miller et al., 2009). Specifically, it's been suggested that an increase in IFN-γ levels, as might occur in response to bacterial or viral infection (Schroder et al., 2004) or in the context of chronic stress or illness (Tian et al., 2014; Bandrés et al., 2000; Pollard et al., 2013), could influence affective states via an IDO-dependent reduction in 5-HT synthesis and/or the elaboration of neuroactive glutamatergic compounds, such as 3-hydroxykynurenine and quinolinic acid (Maes et al., 2011; Myint et al., 2013; Oxenkrug, 2011; Young et al., 2016).

Surprisingly few studies have sought to characterize the influence of IFN-γ on HPA axis and monoamine activity (Hamon and Blier, 2013; Horowitz and Zunszain, 2015). These few studies have generated somewhat contradictory results. For instance, while de Metz et al. (1999) showed that IFN-γ administration in humans augmented plasma concentrations of cortisol, Besedovsky et al. (1986) failed to observe analogous changes in mice. Our own work with IFN-γ knockout mice has indirectly implicated the cytokine in the modulation of central noradrenergic, dopaminergic and serotonergic systems (Litteljohn et al., 2010, 2014). But it's unclear whether exogenously administered IFN-γ can interact with psychologically relevant stressors to influence depression-related pathophysiological domains and behavioural states, as has been reported for TNF-α, IL-1β and IL-6 (Anisman et al., 2008a).

In the present investigation we assessed the individual and combined neuroendocrine and central monoamine effects of systemically administered IFN-γ and acute restraint stress among 4–5 month old male and female littermate mice. Our main hypotheses were as follows: 1) acutely administered IFN-γ will stimulate hypothalamus-pituitary-adrenal (HPA) axis and brain regional monoamine activity in a manner reminiscent of other depression-linked cytokines; 2) synergistic effects will be evident for IFN-γ and restraint stress; and 3) sexual dimorphism will be apparent for at least some of these effects.

2. Methods

2.1. Animals

Four-to-five month old male and female C57BL/6J mice were obtained from our breeding colony, which were originally purchased from the Jackson Laboratory, (Bar Harbor, ME). These animals were weaned at 21 days and maintained on a 12 h light/dark cycle (lights on at 0800 h) in standard polycarbonate enclosures (27 × 21 × 14 cm) as same-sex littermate groupings of 2–3. A diet of standard laboratory mouse chow (Harlan Laboratories, WI) and water was provided ad libitum, and room temperature maintained at ~ 21 °C. All experimental procedures were approved by the Carleton University Committee for Animal Care and complied with the Canadian Council on Animal Care's guidelines on the ethical use and care of animals in research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Procedure

Male and female mice were randomly assigned to one of 8 experimental conditions ($n = 7–9$), as provided for by our 2 (Sex: male, female) × 2 (Injection: vehicle, IFN-γ) × 2 (Stressor: control, restraint) experimental design ($N = 65$). The experimental timeline is shown in Fig. 1a. All mice were acclimated to the behavioural testing room for ~16 h, in their usual cages and with their usual cage-mates. Commencing at 0830 h on the day of experimentation and at an interval of every 10 min, mice were individually removed from the grouped enclosures and singly housed in freshly made cages. As before, food and water were provided ad libitum. Immediately upon being introduced into the new cage, animals were injected intraperitoneally with IFN-γ or vehicle (see below).

Over the ensuing 60 min, spontaneous locomotor activity was assessed using a Micromax infrared beam-break apparatus positioned exterior to the home-cage (AccuScan Instruments, Columbus, OH). A subset of the animals was then transferred to a nearby room and administered a 15 min restraint stressor. At 78 min post-injection (i.e., 3 min following termination of the stressor), mice were rated for sickness behaviours and immediately sacrificed by rapid decapitation; blood and brain tissue were collected for later corticosterone and neurochemical determinations. Sickness behaviours were rated on a 3-point scale with respect to curled body posture, ptosis, piloerection, lethargy, and overall non-responsiveness (where 0 = no symptom, 1 = one symptom present, 2 = two symptoms presents, 3 = three or more symptoms) (Anisman et al., 2008b). In order to minimize the effects of diurnal variations, experiments were carried out between the hours of 08:30 and 12:30.

2.3. Cytokine injections and acute restraint stress

Mouse recombinant IFN-γ (25000 IU, R&D Systems) was reconstituted in a phosphate-buffered saline (PBS) solution containing 0.1% bovine serum albumin (BSA, Sigma Alrdrich). The vehicle contained a matching amount of BSA. All injections were administered in a volume of 0.3 ml. The cytokine dose used in the present study falls within the range of IFN-γ doses previously reported to cause neurochemical, neuroendocrine and behavioural alterations in mice (Cano et al., 2005; Crnic and Segall, 1992; Hozumi et al., 2008; Saito et al., 1991). Although 25000 IU IFN-γ represents a physiologic dose that is expected to approximate tissue IFN concentrations during viral infection (Crnic and Segall, 1992; Heremans et al., 1980), the existing in vivo data are somewhat limited and a worthwhile next step will be to characterize the brain and behavioural effects of varying doses of IFN-γ, as well as different routes of delivery, dosing regimens and sampling time points.

The flat-bottom restraint stressor apparatus comprised a 4 × 12 cm semi-circular Plexiglas tube, with a tail restraint fashioned from the same material. Tails were also taped to prevent mice from turning. A 10–15 min application of this neurogenic stressor has been shown to reliably and sex-dependently stimulate HPA axis and brain regional monoamine activity (Anisman et al., 2001; Jacobson-Pick et al., 2013).

2.4. Brain dissection technique

Following rapid decapitation, brains were excised and sectioned into sequential coronal slices using chilled razor blades and a stainless steel microdissecting matrix with adjacent slots spaced ~0.5 mm apart. The paraventricular nucleus of the hypothalamus (PVN), locus coeruleus (LC) and central amygdala (CeA) were collected using hollow biopsy needles (the latter two bilaterally), and the medial prefrontal cortex (PFC) and dorsal hippocampus (HC) were excised using chilled razor blades. Tissue samples were taken with reference to the mouse brain atlas of Franklin and Paxinos (1997). Samples were snap frozen in a homogenizing solution containing 14.17 g monochloroacetic acid, 0.0186 g disodium ethylenediamine tetraacetate (EDTA), 5.0 ml methanol, and 500 ml H_2O; and stored at −80 °C until later neurochemical determinations.

2.5. Plasma corticosterone assay

Immediately upon sacrifice, trunk blood was collected in tubes