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Synergistic and antagonistic actions of acute or chronic social stressors and an endotoxin challenge vary over time following the challenge

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ABSTRACT

Acute stressor exposure and immunogenic challenges can synergistically increase behavioral, endocrine and neuroinflammatory responses, but much less is known about how chronic stressors influence the actions of immune challenges. In the present investigation we assessed the influence of bacterial endotoxin, lipopolysaccharide (LPS), administered on an acute chronic stressors backdrop, on sickness behavior, changes of circulating corticosterone and cytokine levels, and cytokine mRNA expression in the prefrontal cortex (PFC) and hippocampus. In this regard, it was of particular interest to determine whether the stressors would alter the temporal biological effects (onset and normalization) of LPS. There was a leftward shift in the temporal curve, in that sickness behavior, corticosterone and plasma IL-6 elevations among stressed mice appeared sooner after LPS treatment, but 3 h after treatment corticosterone and IL-6 were lower than in nonstressed mice. In contrast, the stressor, especially when applied chronically, diminished the effects of LPS on TNF-α over the course of measurement, whereas effects on IL-10 were enhanced. In contrast to these peripheral effects, central cytokine mRNA expression, especially IL-1β and TNF-α, were diminished 1.5 h following stressor and LPS administration, but were then synergistically enhanced at 3 h compared to non-stressed controls. Although acute and chronic stressors provoked similar behavioral and neuroendocrine responses when combined with LPS, the effects of chronic stressors and LPS on brain cytokines were generally diminished, particularly in the PFC. The implications of the temporal changes related to stressors and immune activation are discussed, and several possible mechanisms for these effects are suggested.

1. Introduction

Activation of the inflammatory immune system elicits several behavioral, hormonal, neurotransmitter and growth factor alterations that have been implicated in the development of psychological illnesses, such as major depressive disorder (Dantzer et al., 2008; Maes et al., 2011; Miller et al., 2009). In principle, activation of inflammatory processes might not only come about owing to the presence of bacterial or viral challenges, but also by non-sterile factors, such as stressors. To be sure, immune challenges and psychogenic and neurogenic stressors elicit several common biological changes, including elevated plasma corticosterone levels and brain monoamine activity, as well as altered cytokine expression in the blood and brain (Anisman et al., 2008). The similarity between the effects of stressors and immunogenic challenges has, in fact, contributed to the view that the effects of systemic insults, including those associated with bacterial or viral presence, might be interpreted by the brain much like other stressors (Anisman and Merali, 1999).

Beyond their independent effects, the impact of immunogenic challenges, may be markedly exaggerated when administered soon after acute stressor experiences, such as social disturbances or social defeat. In particular, animals displayed particularly marked sickness behavior, as well as particularly elevated corticosterone levels and turnover of brain monoamines in stress-related brain regions (Anisman et al., 2007; Gibb et al., 2008; Johnson et al., 2002; Quan et al., 2001). Likewise, in humans, neuronal activity within the anterior cingulate cortex and the insula was elevated among women in response to a combined immune and stressor challenge (Eisenberger et al., 2009) and depressive affect was increased under similar conditions (Brydon et al., 2009; Yeager et al., 2009).

In addition to the hormonal and neurotransmitter variations, stressors also influenced endotoxin-induced expression of pro-inflammatory cytokine mRNA and protein levels in stressor-related brain regions, although the nature and direction of the changes were not always consistent (cf. Gibb et al., 2008; Goujon et al., 1995; Johnson et al., 2002). This is not entirely unexpected as the effects of stressors on biological processes, even in the absence of an immune challenge, vary with the characteristics of the stressor to which animals are exposed. Typically, the influence of acute stressors on plasma corticosterone levels and monoamine levels

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and turnover are diminished following a chronic, predictable stressor regimen, whereas these outcomes develop less readily, if at all, in response to a chronic variable stressor regimen (Armario, 2006; Sabban and Serova, 2007; Wann et al., 2010).

Characteristics of the stressor may also affect immune functioning in that moderate acute stressors may promote immunoenhancement, whereas relatively strong or chronic stressors might lead to immunosuppression (Dhabhar, 2009). It was also reported that chronic stressor exposure potentiated central pro-inflammatory cytokine release as well as glial and neuronal cell loss in response to lipopolysaccharide (LPS) administered into the prefrontal cortex (PFC) (de Pablos et al., 2006). Similarly, chronic stressor exposure and intrahippocampal LPS administration synergistically enhanced neuroinflammation and brain derived neurotrophic factor (BDNF) mRNA expression, (Espinosa-Oliva et al., 2011). However, less information is available concerning the effects of acute versus chronic stressors on central cytokine expression.

Although examination of the effects of stressors on biological processes have most often focused on the magnitude of the changes observed, it is generally accepted that the temporal changes of biological processes may be relevant in predicting the pathological conditions that might ensue. Indeed, failure in the normalization of stressor-provoked neurochemical changes might be indicative of impaired regulation of those processes that might favor the development of pathological conditions. It is equally possible that stressors might influence the initiation of biochemical or immune-related processes ordinarily instigated by immune challenges (i.e., a leftward shift of the temporal course of biological changes that ordinarily occur), thus promoting earlier behavioral changes.

The present investigation was thus undertaken to assess the synergistic effects of stressors and systemic LPS administration on circulating cytokine levels and proinflammatory cytokine mRNA expression within the hippocampus and prefrontal cortex, both of which are sensitive to stressors, are affected by LPS, and have been implicated in depressive disorders (e.g., Gibb et al., 2011). Moreover, we assessed whether the synergistic effects of LPS and an acute stressor would be modified when LPS was administered to mice that had been exposed to a chronic, unpredictable stressor. In this regard, it was of particular interest to determine whether the temporal changes (1.5, 3 and 24 h) that occurred in response to an immunogenic challenge were altered when it was administered on a backdrop of a stressor. It was expected that following an immunogenic challenge, the magnitude and temporal changes of circulating cytokines as well as central mRNA expression of cytokines would vary relative to one another, and would change yet again when the immune challenge was superimposed on a stressor (acute or chronic) backdrop.

2. Materials and methods

2.1. Subjects

Male CD-1 mice were obtained from Charles River Canada (St. Constant, Quebec) at approximately 6–8 weeks of age. Upon arrival, mice were individually housed in standard polypropylene cages (27 × 21 × 14 cm) and permitted 2 weeks to acclimatize to the vivarium before being used as experimental subjects. Mice were maintained on a 12-h light–dark cycle (light phase: 0800 2000 h), with temperature (22 °C) and humidity (63%) kept constant, and were permitted free access to food (Ralston Purina, St. Louis, MO) and water. The studies met the guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

2.2. Procedures

2.2.1. Stressor and LPS conditions

Individually housed mice were randomly assigned to either an acute, chronic, or non-stressed condition. Thereafter, mice were treated with either LPS (10 μg in a volume of 0.3 ml; Sigma L-3755 from Escherichia coli serotype O26:B6) or saline, and then sacrificed either 1.5, 3 or 24 h later (n = 6/group). Chronically stressed mice were exposed to a series of different stressors each day over 6 weeks. During this time, stressors were applied twice daily, with the exception of stressors that were applied continuously over a 24 h period. Animals were returned to their home cages between the two stressor sessions each day. The chronic stressor regimen included the following stressors: 15 min restraint in a semicircular Plexiglas tubes (4 × 12 cm), 5 min forced swim in tepid water (19–21 °C), 15 min restraint in a tight-fitting triangular plastic bag (with a hole cut out for unrestricted breathing), 60 min wet bedding in home cage, 60 min exposure to dirty bedding taken from breeding animals, 15 min of regrouping mice separated by a partition, and 24 h of light. The sequence of stressors occurred on a variable and unpredictable schedule. The length of the chronic stressor regimen was longer than the more usual 2–3 week treatments as we previously observed that more chronic regimens were particularly likely to engender neuroendocrine and monoamine variations that might be reflective of allostatic overload (Tannenbaum et al., 2002). Unlike the chronically stressed mice, those in the acute and non-stressed conditions remained in their home cages and were not disturbed.

On the test day, chronically stressed mice were submitted to social disruption as their final stressor. This consisted of grouping 4 previously individually housed mice in a novel cage for 1 h. This stressor was previously shown to elicit potent behavioral and neurochemical alterations (Gandhi et al., 2007; Gibb et al., 2008) and like the effects of social defeat, the effects of the treatment on some biological changes (e.g., corticosterone) are longer lasting than those elicited by stressors such as restraint (Audet and Anisman, 2010; Audet et al., 2011). Mice in the acute group were likewise exposed to the social stressor, but they had not previously been exposed to any of the stressor treatments. The non-stressed controls remained undisturbed in their home cages.

2.2.2. Sickness behavior

Sickness behavior was scored within the home cage over a 1.5 h period immediately prior to sacrifice. These behaviors were assessed at 15 intervals for 10 s epochs. To this end, the presence or absence of the following symptoms was recorded: lethargy (diminished locomotion and exploratory activities; curled body posture), ptosis (drooping eyelids), and piloerection (ruffled and greasy fur, typically at the neck). Animals were rated on a 4-point scale with respect to the number of symptoms present (0 = no sickness symptoms, 1 = 1 symptom, 2 = 2 symptoms, 3 = three symptoms). This procedure provides <10% variability between raters blind to the treatment mice received and was highly correlated with other methods of scoring sickness (e.g., assessing severity of each symptom independently; Gandhi et al., 2007).

2.2.3. Tissue dissection

Immediately following stressor treatment, or an equivalent time for controls, mice were treated with either 10 μg of LPS or saline intraperitoneally (i.p.), and sickness behavior was monitored. Mice were sacrificed by rapid decapitation at 1.5, 3 or 24 h following drug treatment. Trunk blood was collected, centrifuged and the plasma stored at −80 °C for subsequent corticosterone and cytokine determinations. Brains were rapidly removed and placed on a stainless steel brain matrix (2.5 × 3.75 × 2.0 cm), which was positioned on a block of ice. The matrix comprised a series of