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Rapid Communication

RAPID COMMUNICATION

Low-Level Stress Induces Production of Neuroprotective Factors in Wild-Type but Not BDNF$^{+/-}$ Mice: Interleukin-10 and Kynurenic Acid

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) deficiency confers vulnerability to stress, but the mechanisms are unclear. BDNF$^{+/-}$ mice exhibit behavioral, physiological, and neurochemical changes following low-level stress that are hallmarks of major depression. After immune challenge, neuroinflammation-induced changes in tryptophan metabolism along the kynurenine pathway mediate depressive-like behaviors.
Methods: We hypothesized that BDNF$^{+/-}$ mice would be more susceptible to stress-induced neuroinflammation and kynurenine metabolism, so BDNF$^{+/-}$ or wild-type littermate mice were subject to repeated unpredictable mild stress. Proinflammatory cytokine expression and kynurenine metabolites were measured.
Results: Unpredictable mild stress did not induce neuroinflammation. However, only wild-type mice produced the neuroprotective factors interleukin-10 and kynurenic acid in response to repeated unpredictable mild stress. In BDNF$^{+/-}$ mice, kynurenine was metabolized preferentially to the neurotoxic intermediate 3-hydroxykynurenine following repeated unpredictable mild stress.
Conclusions: Our data suggest that BDNF may modulate kynurenine pathway metabolism during stress and provide a novel molecular mechanism of vulnerability and resilience to the development of stress-precipitated psychiatric disorders.

Keywords: Kynurenine, neuroinflammation, vulnerability

Introduction

In the adult brain, brain-derived neurotrophic factor (BDNF) is essential to the survival function of neurons and has profound effects on the plasticity and structural stability of synapses. Decreased levels of BDNF, particularly in the hippocampus and frontal cortex, occur with severe or chronic stress and are mechanistically linked with depressive phenotypes (Altar, 1999; Duman and Monteggia, 2006; Calabrese et al., 2009). While conventional BDNF$^{-/-}$ mice do not typically survive beyond postnatal day 14, heterozygous (BDNF$^{+/-}$) mice exhibit a 50% to 60% reduction in BDNF expression in both the frontal cortex and hippocampus (Chourbaji et al., 2004). We and others have shown that mice deficient in BDNF are more sensitive to stress than wild-type mice and exhibit behavioral, physiological, and neurochemical changes following low-level stress that are hallmarks

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of major depression (Duman et al., 2007; Advani et al., 2009; Burke et al., 2013). However, the mechanism mediating this vulnerability remains unclear.

A growing literature indicates that neuroinflammation contributes to the development of major depression (Raison et al., 2006; Capuron and Miller, 2011). We and others have found that neuroinflammation upregulates the kynurenine pathway of tryptophan metabolism and that inflammation-induced depressive-like behaviors in mice are dependent on this pathway (O'Connor et al., 2009; Walker et al, 2013). Microglia, innate immune cells of the brain, produce proinflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α when activated, and these cytokines stimulate the induction of the kynurenine pathway. Chronic or severe stress results in increased production of proinflammatory cytokines by activated microglia (Wohleb et al., 2011; Patki et al., 2013). Thus, central inflammation and kynurenine metabolism may be one of the factors linking stress with depression.

We have reported that BDNF$^{+/-}$ mice exhibit an exaggerated immune response and more severe depressive-like behavior following acute peripheral immune challenge compared with wild-type mice (Parrott et al., 2012). These observations led us to hypothesize that low-level stress results in an exaggerated increase in proinflammatory cytokines in the brain under conditions of BDNF deficiency. We report here no change in IL-1β, IL-6 or TNF-α mRNA or protein in the forebrain of wild-type or BDNF$^{+/-}$ mice following repeated unpredictable mild stress (UMS). We did, however, observe an increase in antiinflammatory cytokine IL-10 mRNA in wild-type mice, suggestive of a protective mechanism that is absent in BDNF$^{+/-}$ mice. Given the role of IL-10 in activating astrocytes and negatively modulating microglia, we focused our attention on distinct branches of kynurenine pathway localized in these nonneuronal cells (Guillemin et al., 2001; Schwarcz and Pellicciari, 2002). Here we report that measurement of kynurenine pathway enzymes and metabolites indicated that in wild-type mice, UMS induced changes in the kynurenine pathway that resulted in increased production of kynurenic acid, an NMDA receptor antagonist. By contrast, in stress-sensitive BDNF$^{+/-}$ mice, the production of the neurotoxic kynurenine metabolite 3-hydroxykynurenine predominated following UMS. Our data provide an innovative perspective on stress resilience and vulnerability and suggest a mechanism by which decreased levels of BDNF confer vulnerability to stress-precipitated psychiatric disorders such as major depression.

Methods

BDNF heterozygous (BDNF$^{+/-}$) and wild-type (BDNF$^{+/+}$) mice were bred at the University of Texas Health Science Center-San Antonio. Breeding pairs consisted of wild-type female (C57BL/6J) and heterozygote male (B6.129S4-Bdnf^{tm1Jae}/J) mice (The Jackson Laboratory). Mice were genotyped as recommended by The Jackson Laboratory. Mice were group-housed and maintained on a 12-hour-day/-night cycle (lights on 7:00 AM) with constant access to food and water. All procedures were approved by the University of Texas Health Science Center-San Antonio Institutional Animal Care and Use Committee and carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Male wild-type C57BL/6J and BDNF$^{+/-}$ mice, 4 to 5 months of age, were subjected to UMS, which consisted of: Day 1, cage shaking (2 hours); Day 2, switching cages with other mice in the cohort (1 hour); Day 3, 45° cage tilt (8 hours); Day 4, wet bedding (3 hours); Day 5, exposure to rat odor (3 hours); Day 6, crowding; Day 7, strobe light and loud noises in dark (1 hr); Day 8, restraint (2 hrs). Mice were sacrificed 24 hours after the last stress and forebrain collected for determination of cytokine mRNA by real time reverse transcription-polymerase chain reaction (RT-PCR) using the $2^{-\Delta\Delta Ct}$ method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control gene as we have previously described (Lawson et al., 2013) and protein analysis by Luminex-based multiplex array according to the manufacturer's instructions (ProcartaPlex Mouse Cytokine/Chemokine Panel 1, eBioscience; intra-assay CV <5%). For RT-PCR experiments, inventoried gene assays were purchased from Life Technologies (Grand Island, NY; indoleamine 2,3-dioxygenase1, Mm00492586; tryptophan 2,3-dioxygenase, mM00451266; kynurenine monooxygenase, Mm00505511; kynurenine aminotransferase 2, Mm00496169). In experiments examining the effect of UMS on the kynurenine pathway, mRNA for kynurenine pathway enzymes was determined by RT-PCR as previously described (Heisler et al., 2015), and metabolite levels were determined by liquid chromatography/mass spectrometry as described (Walker et al., 2013; Mass Spectrometry Core Facility, University of Texas Health Science Center-San Antonio).

Results

We found no change in proinflammatory cytokine IL-1β, IL-6, or TNF-α mRNA or protein in forebrain of wild-type or BDNF$^{+/-}$ mice following the UMS procedure. We did, however, observe an increase in antiinflammatory cytokine IL-10 mRNA in wild-type mice following UMS, but IL-10 mRNA expression in BDNF$^{+/-}$ mice was not different that wild-type controls following the UMS procedure (data expressed as fold change relative to the wild-type no-stress control: wild-type = 1.10 ± 0.21, BDNF$^{+/-}$ = 1.72 ± 0.58; mean ± SD, n = 6 per genotype; $P = .034$). We found no differences in baseline expression of IL-10 between wild-type and BDNF$^{+/-}$ mice in forebrain IL-10 mRNA (data not shown).

In naïve mice, we found no difference between wild-type and BDNF$^{+/-}$ mice in forebrain mRNA levels of kynurenine pathway enzymes. Eight days of UMS, however, resulted in increased expression of the 2 enzymes responsible for the metabolism of tryptophan to kynurenine, indoleamine 2,3-dioxygenase (IDO), and tryptophan 2,3-dioxygenase (TDO), in hippocampus of wild-type mice (Figure 1A-B, E). Concurrently, the expression of kynurenine monooxygenase (KMO) was downregulated (Figure 1C), which would be expected to decrease kynurenine metabolism in the neurotoxic branch of the kynurenine pathway in microglia (Figure 1E). Kynurenine aminotransferase (KAT) expression was not altered (Figure 1D). Collectively, these stress-induced changes in the expression of enzymes of the kynurenine pathway in wild-type mice favor the formation of kynurenic acid (Figure 1E).

In naïve mice, we found no difference between wild-type and BDNF$^{+/-}$ mice in forebrain levels of kynurenine pathway metabolites, suggesting that there is no difference in activation of the kynurenine pathway between wild-type and BDNF$^{+/-}$ mice at baseline. In wild-type mice, 8 days of UMS robustly increased kynurenine levels in brain, which is reflected in a significant increase in the kynurenine/tryptophan ratio (Figure 2A). The increase in the kynurenine/tryptophan ratio is indicative of increased metabolism of tryptophan to kynurenine and is consistent with the upregulation of IDO and TDO in wild-type mice following UMS (Figure 1). Kynurenine is not neurochemically active per se, but serves as a precursor for the synthesis of neurochemically active metabolites. Importantly, kynurenic acid levels were increased in wild-type mice following UMS

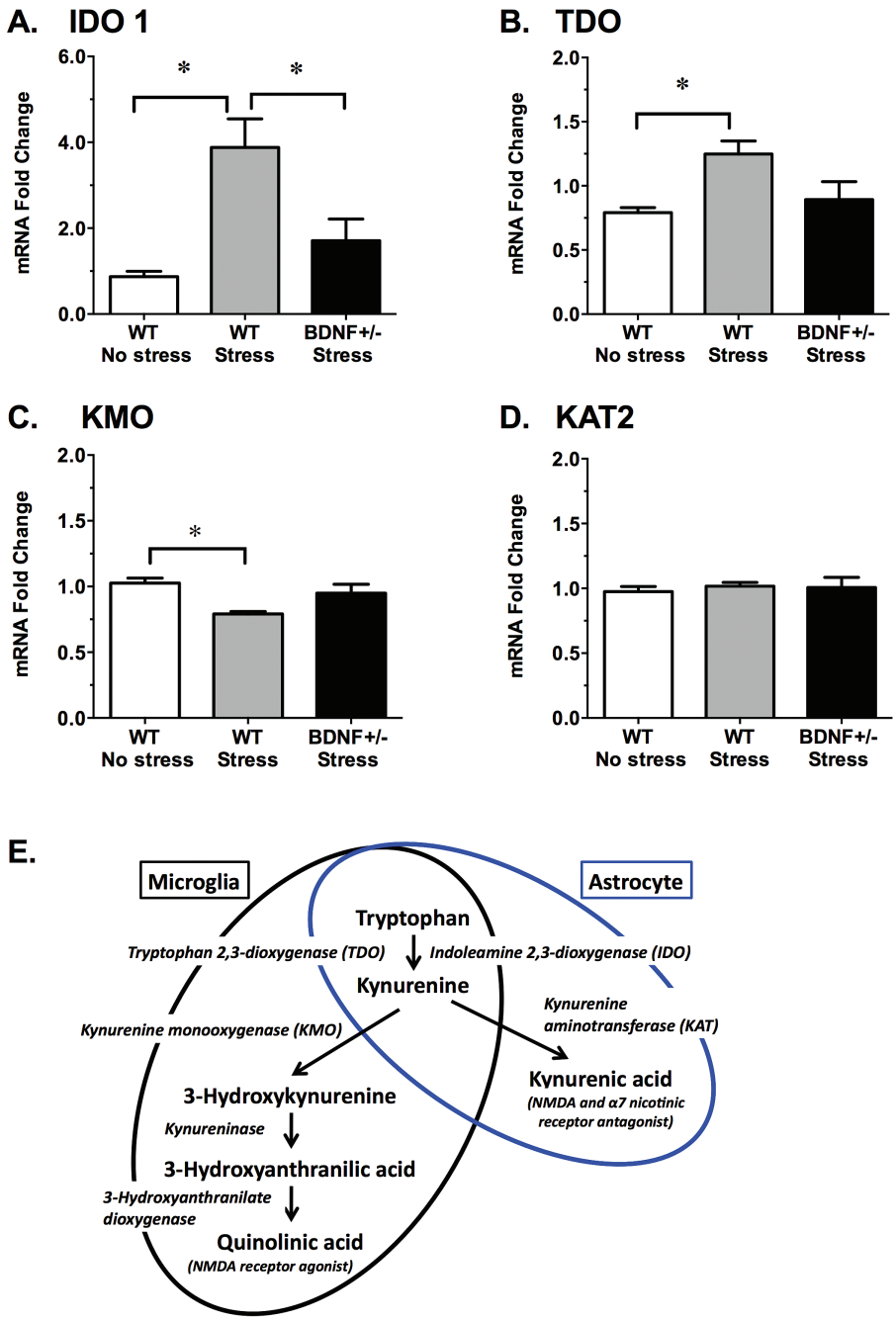

Figure 1. In wild-type (WT) mice, low-level stressor induced changes in the expression of enzymes in the kynurenine pathway that favor the formation of kynurenic acid. WT or brain-derived neurotrophic factor ($BDNF^{+/-}$) mice were subjected to 8 days of unpredictable mild stress (UMS) and sacrificed 24 hours after the last stress. RNA was isolated from forebrain. Real time reverse transcription (RT)-PCR data are expressed as relative fold change using the $2^{-\Delta\Delta Ct}$ calculation method. Mean ± SEM, n = 7/group. One-way ANOVA, *P < .05 posthoc Tukey's Multiple Comparisons Test.

(Figure 2B). This may be due in part to the downregulation of KMO in wild-type mice following UMS (Figure 1C). In striking contrast to what was observed in wild-type mice, 8 days of UMS resulted in a marked increase in 3-hydroxykynurenine in $BDNF^{+/-}$ mice (Figure 2C).

Discussion

In the present study, we found that low-level stress resulted in a marked increase in the expression of the antiinflammatory cytokine IL-10 in wild-type mice. Interestingly, this was not observed in BDNF-deficient mice following UMS. IL-10, secreted by both microglia and astrocytes (Ledeboer et al., 2002; Henry et al., 2009), negatively modulates microglia activation (Norden at al., 2014). Our data suggest that this protective mechanism present in wild-type mice is absent in $BDNF^{+/-}$ mice.

Both microglia and astrocytes express receptors for IL-10 (Ledeboer et al., 2002). While microglia activation is attenuated by IL-10, astrocytes are redirected to secrete antiinflammatory factors, which further inhibit microglia activity (Norden at al., 2014). In addition to providing negative feedback to activated microglia, astrocytes produce many regulatory factors that are

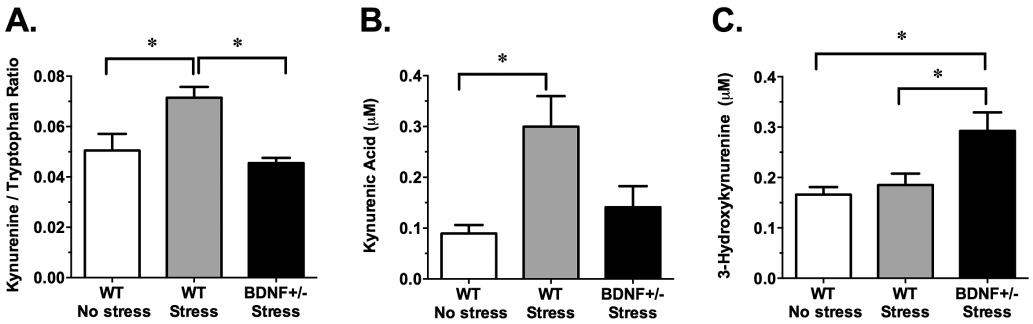

Figure 2. Low-level stressor resulted in a marked increase in (A) kynurenine and (B) kynurenic acid in wild-type (WT) mice. By contrast, low-level stressor resulted in a marked increase in (C) 3-hydroxykynurenine in brain-derived neurotrophic factor (BDNF)$^{+/-}$ mice. Mice were subjected to 8 days of unpredictable mild stress (UMS) and sacrificed 24 hours after the last stress. Forebrain samples were prepared as described (Walker et al., 2013) and kynurenine metabolites analyzed by liquid chromatography/mass spectrometry. n = 7/group. One-way ANOVA, *$P < .05$ posthoc Tukey's Multiple Comparisons Test.

neuroprotective. Astrocytes express KAT, an enzyme of the kynurenine pathway that produces kynurenic acid, an ionotropic glutamate receptor antagonist (Guillemin et al., 2001; Schwarcz and Pellicciari, 2002). By contrast, microglia do not express KAT but do express KMO and therefore preferentially produce excitotoxic metabolites 3-hydroxykynurenine and quinolinic acid when activated (Guillemin et al., 2001; Schwarcz and Pellicciari, 2002). Thus, the neuroprotective and neurotoxic branches of the kynurenine pathway are compartmentalized in astrocytes and microglia, respectively.

In wild-type mice, UMS resulted in an upregulation of IDO and TDO expression and an increase in the kynurenine/tryptophan ratio, indicating stimulation of the kynurenine pathway. UMS also resulted in the downregulation of KMO expression in wild-type mice, potentially through IL-10–mediated inhibition of microglia (Norden at al., 2014). Of note, systemic administration of kynurenine to naïve mice, without the concurrent downregulation or inhibition of KMO, produces a depressive-like behavioral phenotype (O'Connor et al., 2009; Salazar et al., 2012). Our data indicate that in wild-type mice, low-level stress results in increased kynurenine metabolism through the neuroprotective branch of the kynurenine pathway in astrocytes. Consistent with this, UMS resulted in a marked increase in the formation of kynurenic acid.

In BDNF$^{+/-}$ mice, where UMS did not upregulate IL-10 expression, we found no change in IDO or TDO expression and no increase in the kynurenine/tryptophan ratio. Following UMS, KMO was not downregulated, and we observed a marked increase in the excitotoxic metabolite 3-hydroxykynurenine. The neurotoxic metabolite 3-hydroxykynurenine contributes to oxidative stress and is a precursor of the NMDA receptor agonist quinolinic acid. Thus, in stress-sensitive BDNF$^{+/-}$ mice (Duman et al., 2007; Advani et al., 2009; Burke et al., 2013), low-level stress results in kynurenine metabolism through the neurotoxic branch of the kynurenine pathway in microglia.

A single nucleotide polymorphism in the human *bdnf* gene (rs6265; val66met) is a risk factor associated with stress-precipitated psychiatric disorders, such as major depression (Hosang et al., 2014). The met allele results in impaired cellular processing and activity-dependent secretion of BDNF (Egan et al., 2003; Chen et al., 2004). Transgenic mice expressing the met allele exhibit a depressive-like behavioral phenotype with exposure to low-level stress (Yu et al., 2012). Further mechanistic studies are necessary to firmly establish the relationship between the kynurenine pathway and stress-precipitated depressive-like phenotypes in mice as well as the role BDNF may play in the modulation of the kynurenine pathway. Such studies are necessary to increase our understanding of the cellular and molecular mechanisms by which individuals may be at risk for stress-precipitated mental illness. This work may inform clinical practice by leading to the identification of peripheral biomarkers for stress-related mental disorders and of novel therapeutic targets to restore equilibrium in the brain kynurenine pathway.

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Statement of Interest

None.

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