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Short Communication

Kynurenic acid is reduced in females and oral contraceptive users: Implications for depression

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ABSTRACT

The incidence of depression is approximately 2-fold greater in women than men but the biological mechanisms underlying this phenomenon remain unclear. One potential mechanism that has been understudied is immune function, which is modulated by sex hormones and differs considerably between males and females. The immune-regulating kynurenine pathway previously has been implicated in the pathogenesis of mood disorders. In particular, a decreased ratio of neuroprotective (kynurenic acid; KynA) to neurotoxic (3-hydroxykynurenine; 3HK and quinolinic acid; QA) kynurenine pathway metabolites has been reported in several mood disorder subtypes. Yet there is a paucity of research investigating sex differences in the kynurenine pathway in the context of depression. Similarly, oral contraceptive (OC) use has been shown to be a risk factor for depression but to our knowledge this epidemiological relationship has not been considered within the framework of immune dysfunction. Here, we compared the concentrations of c-reactive protein (CRP) and kynurenine pathway metabolites in a combined sample of subjects with major depressive disorder (MDD), bipolar disorder (BD), and healthy controls (HC) comprising 130 men and 350 women. CRP was measured in a CLIA-certified hospital laboratory. Kynurenine metabolites were quantified using high performance liquid chromatography with tandem mass spectrometry. Estradiol and progesterone were quantified with the Mesoscale Discovery (MSD) platform. After controlling for diagnosis, age, sex, BMI, analysis batch, and self-reported childhood trauma we found that women had significantly lower KynA/3HK and KynA/QA ratios than men, and that these results were driven by a decrease in KynA. There was no significant difference between males and females in the concentration of CRP. Further, women taking OC showed significantly higher levels of CRP and lower ratios of KynA/3HK and KynA/QA compared with women on no form of contraception. Moreover, among women using OC, progesterone concentrations were positively correlated with KynA, KynA/3HK, and KynA/QA. Although preliminary, our results indicate that on average, healthy women show the same pattern of kynurenine pathway metabolism as that observed in subjects with depression. This finding raises the possibility that a reduction in KynA concentrations in women may constitute a vulnerability factor that partly explains the higher incidence of depression in females. Further, the significant association between OC use and reduced KynA as well as increased CRP, could conceivably partially account for the epidemiological association between OC use and depression. Nonetheless, because of the cross-sectional nature of this study, these hypotheses need to be more rigorously tested with longitudinal designs and/or large epidemiological studies.

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1. Introduction

The lifetime rate of major depressive disorder (MDD) in women consistently has been found to be twice that of men (Kessler, 2003) but the biological mechanisms underlying this epidemiological phenomenon remain unclear. Most research has focused on hormonal differences, yet it is clear that there are significant differences in immune function between the sexes. For instance, females show greater expression of toll-like receptors (TLR) across multiple populations of immune cells, increased type-1 interferon activity of dendritic cells, increased activation and function of macrophages, neutrophils, and T-cells, as well as greater B-cell numbers and antibody production (Klein and Flanagan, 2016). Consistent with these data, females mount a stronger immune response to infection and vaccines, and are significantly more likely than males to suffer from inflammatory and autoimmune diseases (Klein and Flanagan, 2016). Given evidence that immune dysregulation plays a mechanistic role in some depressive disorders (Mechawar and Savitz, 2016; Miller and Raison, 2015), it is conceivable that sex differences in the incidence of depression may partly be related to immunological differences between males and females. Yet there is paucity of research addressing this important topic.

The mechanisms by which inflammatory mediators putatively contribute to the pathophysiology of depression remain unclear. However, activation of a key immuno-regulatory network, the kynurenine pathway, appears to be crucial. Two landmark papers showed that lipopolysaccharide does *not* cause depression-like behavior in rodents when the activation of the kynurenine pathway is genetically or pharmacologically blocked even when the levels of pro-inflammatory cytokines remain elevated (Lawson et al., 2013; O'Connor et al., 2009). The kynurenine pathway is activated by inflammatory cytokines (predominantly interferon gamma) and cortisol, increasing the production of kynurenine from tryptophan (TRP) by the enzymes *IDO* and *TDO*, respectively (Fig. S1). Kynurenine is in turn metabolized into either the putatively neuroprotective metabolite kynurenic acid (KynA), by astrocytes, or alternatively, putative neurotoxic metabolites such as 3-hydroxykynurenine (3HK) and quinolinic acid (QA) by macrophages and microglia. Under inflammatory conditions, the brain formation of QA predominates over the formation of KynA (Saito et al., 1992; Walker et al., 2013), such that the ratio of KynA/3HK and KynA/QA provides an index of the competing physiological effects of these neuroactive metabolites. We and others have previously reported reduced levels of KynA/3HK and/or KynA/QA in the serum or cerebrospinal fluid of several different mood disorder subtypes relative to controls (Bay-Richter et al., 2015; Myint et al., 2007; Poletti et al., 2016; Savitz et al., 2015a,b,c; Schwieler et al., 2016). Here, we combine these existing data with a new dataset (total n = 480) in order to perform a well-powered test of our hypothesis that, compared with males, females will have higher levels of inflammation (indexed by C-reactive protein (CRP)) and will show a greater metabolic shunt towards the neurotoxic branch of the kynurenine pathway. Given the recent report of an epidemiological relationship between depression and oral contraceptive (OC) use (Skovlund et al., 2016), we also hypothesized that serum concentrations of CRP and potentially neurotoxic metabolites would be elevated in females taking OC.

2. Methods

The current research was approved by the Western Institutional Review Board, and the study was conducted in accordance with the principles expressed in the Declaration of Helsinki. A total of 480 participants (130 men and 350 women) were included in the study (Table 1). Data from a subset of these participants (n = 345) has been previously published although the hypotheses tested herein were not also tested in these publications (Savitz et al., 2015a,b,c; Young et al., 2016). The Structured Clinical Interview for the DSM-IV-TR was used to confirm the diagnoses rendered by a clinical interview with a psychiatrist. Participants met DSM-IV-TR criteria for remitted major depressive disorder (rMDD), current major depressive disorder (dMDD), bipolar disorder (BD) or no psychiatric disorder (i.e., healthy controls (HC)) (Table 1). Exclusion criteria included serious suicidal ideation or behavior, medical conditions or concomitant medications likely to influence central nervous system or immunological function including cardiovascular, respiratory, endocrine, neurological, and known autoimmune disease, as well as a history of drug or alcohol abuse within six months or a history of drug or alcohol dependence within one year. The subjects with a mood disorder were unmedicated for at least 2 weeks (8 weeks for fluoxetine, mean time unmedicated: 3.52 years ± 3.09 years) except for a subset of the MDD and BD samples (n = 60) who were receiving treatment with psychotropic medications at the time of study (Table S1). We previously reported that unmedicated and medicated BD subjects did not differ significantly in KynA/3HK or KynA/QA (Savitz et al., 2015a). Self-reported trauma was measured with the 28-item Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). The CTQ is composed of the following Likert-type subscales: physical, sexual and emotional abuse and physical and emotional neglect. Higher scores are indicative of greater self-reported trauma. The instrument is widely used, reliable (intra-class correlation coefficients of 0.88; Cronbach's alpha, 0.79–0.94), and well validated with correlations with therapist ratings of abuse for all the CTQ subscales of between 0.36 and 0.75 (Bernstein et al., 2003).

An overnight fasting blood sample was collected via venipuncture using Becton Dickinson (BD) Vacutainer serum tubes between 8 am and 11 am, processed per standard protocol and stored at −80 °C. hs-CRP was measured in a CLIA-certified hospital lab (measurement range of 0.2–480.0 mg/L). Tryptophan (TRP) and kynurenine metabolites including kynurenic acid (KynA), 3-hydroxykynurenine (3HK), and quinolinic acid (QA) were measured blind to sex and diagnostic status by Brains Online, LLC using high performance liquid chromatography with tandem mass spectrometry. This assay had the following lower level of quantifications (LLOQ): TRP = 5 μM, Kyn = 0.50 μM, KynA = 7.5 nM, 3HK = 5 nM, and QA = 100 nM. Six participants (5 women) had KynA levels below the LLOQ and were excluded from further analysis. Estradiol (LLOQ = 0.005 ng/mL; CV = 7.01%) and Progesterone (LLOQ = 0.17 ng/mL; CV = 9.42%) were quantified blind to sex and diagnostic status using the Mesoscale Discovery MULTI-SPOT® 96 HB 4-Spot Custom Steroid Hormone Panel. Samples with estradiol (n = 9) or progesterone (n = 13) concentrations with a CV > 25% were excluded from the analyses.

Menstrual cycle was evaluated by self-report based on the first day of the last menstrual period. Naturally-cycling females were divided into two groups, follicular phase (days 0–14; low progesterone) or luteal phase (days 15–30; high estrogen/progesterone). Women on birth control or with irregular cycles (defined as shorter than 21 days or longer than 36 days, n = 36) were excluded. Phase information was only available for a subset of women (Table 1).

Statistical analyses were conducted using SPSS (Version 21). Sex differences in age, body mass index (BMI), self-reported childhood trauma, and diagnosis were assessed using independent samples t-tests and chi-squared tests. Shapiro-Wilk tests showed that kynurenine metabolites hormone levels, and CRP were non-normally distributed ($p < 0.05$), and thus these variables were natural log transformed.

The effects of sex on the *a priori* outcome variables KynA/QA, KynA/3HK, and CRP were assessed using an additive general linear

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