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Research Article

Effects of Minocycline on Changes in Brain Tryptophan Metabolism and the Behavior of Juvenile Mice Elicited By Inescapable-Predator Stress

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Abstract

Study background: Traumatic stress in early life can have long-term effects on neurobiological systems and result in more-pronounced responses to stress exposure in adulthood, which may underlie an increased risk of psychiatric disorders such as depression and anxiety disorder (including post-traumatic stress disorder). Acute stress in early life activates the brain kynurenine (KYN) pathway, the main tryptophan (TRP) metabolic pathway, which shares TRP with the serotonin (5-HT) pathway. Although the activated KYN pathway is known to play an important role in the pathophysiology of depression, it may also be related to neurobiological changes elicited by stress. In addition, early-life acute stress induces prolonged behavioral changes such as disinhibition and/or reduced anxiety. Thus, a drug that inhibits the activation of the KYN pathway elicited by early-life stress may prevent the onset of these psychiatric disorders related to traumatic events in early life. We investigated the effects of minocycline, which has powerful anti-inflammatory and neuroprotective effects, on changes in mouse behavior and brain TRP metabolism elicited by stress exposure in early life.

Methods: Four-week-old male mice were exposed to inescapable-predator stress. Minocycline (100 mg/kg) was injected intraperitoneally 1 h prior to the stress. The behavior in an elevated plus maze (EPM) and the levels of TRP, KYN, and 5-HT in the prefrontal cortex, hippocampus, amygdala, and dorsal raphe nuclei were measured 1 wk later.

Results: In the EPM, stress decreased the ratio of protected head dips (PHD) to all head dips, whereas minocycline increased the PHD. In the amygdala, stress increased the levels of KYN and 5-HT, whereas minocycline prevented the stress-induced KYN elevation and increased the level of 5-HT. Minocycline inhibited the stress-elicited increase in the KYN/TRP ratio.

Conclusion: Minocycline counteracted the stress-induced KYN pathway and behavioral changes and attenuated the influence of early-life trauma.

Keywords

Elevated plus maze; Kynurenine; Minocycline; Predator stress; Tryptophan

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Abbreviations

ANOVA: Analysis of variance; ECD: Electrochemical Detection; EPM: Elevated Plus-maze; FD: Fluorimetric Detection; HPA: Hypothalamo-Pituitary-Adrenal axis; HPLC: High-Performance Liquid Chromatography 5-HT: Serotonin; IDO: Indoleamine 2, 3-dioxygenase; IFN-γ: Interferon-gamma; IL-6: Interleukin-6; ISO: Isoproterenol; KYN: Kynurenine; LPS: Lipopolysaccharides; MANOVA: Multivariate Analysis of Variance; MDMA: 3,4-Methylenedioxymethamphetamine; NCA: Number of Closed-arm Entries; NOA: Number of Open-arm Entries; 3-NTYR, 3-Nitro-L-Tyrosine; PHD: Protected Head Dips; PND: Postnatal Days; PTEs: Potentially Traumatizing Stressful Experiences; PTSD: Post-traumatic Stress Disorder; SPF: Specific Pathogen-free; TDO: Tryptophan 2,3-dioxygenase; TNF-α: Tumor Necrosis Factor-alpha; TPH: Tryptophan Hydroxylase; TRP: Tryptophan; UHD: Unprotected Head Dips; UV: Ultraviolet

Introduction

Potentially traumatizing experiences (PTEs) in early life (e.g., maternal separation, post-weaning social isolation, post-weaning social deprivation, post-weaning social subjugation in rodents; infant abuse and neglect in non-human primates) cause long-term effects on neurobiological systems [1–4]. Animals that suffer early PTEs respond more severely to stress exposure in adulthood [5]. Evidence from clinical studies suggests that PTEs are associated with neurobiological changes in children and adults, which may underlie the increased risk of psychiatric disorders such as depression and anxiety disorder [6] including post-traumatic stress disorder (PTSD) [7]. These prolonged neurobiological changes suggest a predisposition and vulnerability to psychiatric diseases.

In respect to the underlying neurobiological changes elicited by PTEs, it is noteworthy that several studies show that activation of the kynurenine (KYN) pathway, the main tryptophan (TRP) metabolic pathway, is elicited by stress [8-11]. In our 2011 study, a PTE in juvenile mice induced prolonged behavioral changes indicating disinhibition and/or reduced anxiety, accompanied by activation of the brain's KYN pathway [11].

The activation of the KYN pathway is known to play a role in the pathophysiology of depression [10,12–17] and also that of somatization or physio-somatic symptoms [15]. The KYN pathway shares TRP with the serotonin (5-HT) pathway. Its activation deprives the 5-HT pathway of TRP. Thus, the lowered 5-HT function in depression may be derived from activation of the KYN pathway. Maes et al. were the first to show that lowered levels of plasma TRP in depression results from indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme in the KYN pathway, activation [13,14]. The following mechanisms which activate the KYN pathway are related to the pathophysiology of depression. First, the pathogenesis of depression has been extended to inflammatory responses and deficient neuroprotection [13,14,18–21]. Interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α interleukin-6 (IL-6), lipopolysaccharides (LPSs), and oxidative stress induce IDO [15,22]. Second, the KYN pathway is also activated

following the induction of tryptophan 2, 3-dioxygenase (TDO) by glucocorticoids, which are elevated in depression [15].

The lowered TRP and the increased active metabolites of the KYN pathway may attenuate the primary immune response and serve as a negative feedback loop counteracting cell meditated immune activation and inflammation in depression [15].

Although the activation of the KYN pathway has mainly been discussed with regard to the pathophysiology of depression relating to the elevated immunological activity and immunological challenges, its activation also has a close relationship with the neurological and behavioral changes elicited by stress. Because PTEs in early life induce neurobiological and behavioral changes that suggest a predisposition and vulnerability to psychiatric diseases, drugs that recover the activation of the KYN pathway and counteract the behavioral changes elicited by PTEs in early life may play an important role in protection against the onset of psychiatric diseases in adulthood.

Minocycline, an inhibitor of macrophage and microglial activities, is a potential antidepressant [23–25] with powerful anti-inflammatory [26] and neuroprotective [27] effects. Minocycline attenuated LPS-induced proinflammatory cytokines and depression-like behavior mediated by IDO activation [24,28,29]. Thus, the clinical effects of minocycline may be based on the suppression of the activated KYN pathway via indirect inhibition of IDO (targeting the production of proinflammatory cytokines) [24]. Furthermore, minocycline is an antioxidant which has direct radical scavenging property [25]. Thus, minocycline may inhibit IDO induction via attenuating oxidative stress.

Minocycline may reverse the behavioral changes via attenuation of the activated KYN pathway. The aim of the present study was to investigate minocycline's effects on changes in behavior and brain TRP metabolism elicited by early-life PTEs in our animal model.

Methods

Animals

A total of 32 male specific pathogen-free (SPF) ICR mice at 4 weeks of age were used as experimental/control animals, and 20 male Wistar rats were used as predators. At 21 postnatal days (PND), the mice were transported from the breeding company to our experimental animal center. After a 1-week habituation period, the mice were used in the experiments. The animals were divided into two stress-exposure groups: stress, exposed to a predator stress session; and nonstress, lacking such a session. The mice were also divided into two groups according to the minocycline dose: 0 mg/kg or 100 mg/kg. By combining these conditions, the mice were divided into four groups: 0 mg/kg, nonstress; 0 mg/kg, stress; 100 mg/kg, nonstress; 100 mg/kg, stress. There were eight mice per group. At 7 weeks of age, the rats were transported from the breeding company to our experimental animal center and were reared in a dedicated room. After a 1-week habituation period, the rats were used in the experiments.

The precise animal rearing conditions were as described [11]. All efforts were made to minimize both the number of animals used and the degree of their suffering. All of the experiments were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). The study was approved by the ethics committee of the Nagoya University Graduate School of Medicine on March 25, 2010.

Minocycline injection

Minocycline (Sigma, St. Louis, MO) was suspended with saline containing two drops of Tween 80 per 10 mL (100 mg/10 mL) and intraperitoneally injected into the mice (the 0 mg/kg group received the vehicle only) at 1 h prior to each predator stress session. The dose 100 mg/kg was chosen as a single dose because prior *in vivo* studies using minocycline for behavioral experiments used doses between 50 mg/kg and 80 mg/kg for repeated administrations [24,29].

Inescapable-predator stress

At 4 weeks of age, mice in the stress group were exposed to a predator stress session. The mouse was held in a 50-mL transparent plastic centrifugal tube with holes drilled in the cap and at the bottom for respiration, and the tube was fixed by adhesive tape in the center of the floor of a transparent plastic box ($28 \times 35 \times 30$ cm). A rat was placed into the box as soon as possible after the introduction of the mouse, and the rat was allowed to freely explore the box for 20 min. Thus, the mouse was held in a manner in which it could not escape from a predator rat for 20 min. Although the mouse was able to see and smell the predator, the mouse could not be directly attacked. After the stress session, the mouse was returned to its home cage. After every session, the floor of the box and the tube were wiped clean with ethanol and a wet cloth to minimize odor. For each session, one rat was selected as a predator from the group of 20 that had been reared together. The selected rat was never allocated to more than one session per day. Each rat rested at least 1 week until it was used for the next stress session. Thus, the rats used for the stress sessions ranged from 8 to 13 weeks of age. The inescapable-predator stress sessions were all performed between 13.00 and 18.00 h [11].

Elevated plus maze (EPM)

An elevated plus maze (EPM) session was performed 1 week after the mouse had been exposed to an inescapable-predator stress session, to evaluate the prolonged effects of the stress as a PTE in juvenile animals on the developing neurobiological systems. An EPM was used for the analysis of mouse behavior. The EPM ($50 \times 50 \times 8$ cm) was a black wooden apparatus shaped like a plus sign with a floor consisting of a gray resin sheet. The apparatus was raised above the ground by 0.4 m. The mouse was placed in the center of the EPM and could turn to face either an open arm or a closed arm. The mouse was allowed to freely explore the maze for 5 min. Its behavior was recorded for the 5 min session by a digital video camera (iVIS DC300, Canon, Tokyo) placed approx. 1 m above the center of the floor of the EPM. The session was carried out between 22.00 and 24.00 h in a dark room with a dim red light (7 W) placed approx. 1 m above the floor of the center of the EPM. After every session, the EPM was wiped clean with ethanol and a wet cloth to minimize the odor [11].

In addition to traditional EPM measures such as open arm time spent and/or number of open-arm entries (NOA), we measured the defensive pattern of response including risk assessment behaviors, which has been suggested to be more sensitive to anxiety [30]. Thus, we used head dips as the risk assessment behavior. The following parameters were analyzed: the number of open-arm entries, the number of closed-arm entries (NCA),the ratio of entry types (NOA/(NOA + NCA)), the number of unprotected head dips (UHDs), the number of protected head dips (PHDs), and the ratio of PHD (PHD/(UHD+PHD)). The mouse was considered to have entered an arm if it placed all four paws into the arm. Head dips were measured as

an ethological risk assessment behavior and were defined as peering over the edge of an open arm with the head, neck, and shoulders. Head dips over the sides of the maze from the center platform toward another part of the maze, or from the closed arms toward the open arms, were termed PHDs [31], whereas those performed while the four paws were placed in the open arm were termed UHDs.

Sample preparation

Four brain regions were selected [9], the first three of which possess 5-HT nerve terminals: the prefrontal cortex, which is involved in behavioral motivation; the amygdala, which is involved in emotion; and the hippocampus, which regulates the hypothalamo-pituitary-adrenal (HPA) axis, the hyperactivity of which is closely related to the etiology and pathophysiology of depression. The fourth region was the dorsal raphe nuclei. These regions were selected because they each contain the cell bodies of 5-HT neurons and are the center of brain 5-HT synthesis. The mice were sacrificed by decapitation under brief anesthesia on the day following the EPM session.

The above-mentioned brain regions were removed as quickly as possible and placed on glass plates over ice. The samples were weighed and treated with 1000 µl of an ice-cold 0.2 M trichloroacetic acid solution containing 0.2 mM sodium pyrosulfite, 0.01% EDTA-2Na, 0.5 µM isoproterenol (ISO), and 3-nitro-L-tyrosine (3-NTYR) as an internal standard per 100 mg of wet tissue [9]. The solution was sonicated and then centrifuged at 10000 g for 20 min at 4˚C. The supernatant was filtered through a Millipore HV filter (pore size, 0.45 µm) and then subjected to both high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) of 5-HT and HPLC with fluorimetric detection (FD) of TRP and with ultraviolet (UV) detection of KYN.

The standard solution was prepared using the above-mentioned ice-cold 0.2 M trichloroacetic acid solution containing 0.5 µM internal standards (ISO, 3-NTYR). The concentration was adjusted to 0.5 µM for 5-HT and KYN, and to 10 µM for TRP [9].

HPLC determination of the brain levels of 5-HT, TRP and KYN

The levels of 5-HT were measured by HPLC with electrochemical detection (ECD) [9,11,32,33]. The levels of TRP were measured by HPLC with fluorimetric (FD) detection, and those of KYN with ultraviolet (UV) detection [9,11,32,33] according to the original method [34] and an improved method [35]. The sensitivity of 5-HT measurements was 150 f mol. The sensitivity of the TRP and KYN measurements were 50 f mol and 100 f mol, respectively.

Statistical analyses

To examine the group differences in NOA and NCA in the EPM, we conducted a two-way multivariate analysis of variance (MANOVA) (Wilks's lambda) for independent measures (stress and minocycline) on the dependent measures (NOA and NCA). To examine the group differences in the entry ratio (NOA/ (NOA+NCA)), we conducted a two-way analysis of variance (ANOVA) for independent measures (stress and minocycline) on the dependent measure (entry ratio). To examine the group differences in UHD and PHD, we performed a two-way MANOVA for independent measures (stress and minocycline) on the dependent measures (UHD and PHD). To examine group differences in the ratio of PHD to all head dips (PHD/ (PHD+UHD)), a two-way ANOVA for independent measures (stress

and minocycline) was conducted on the dependent measure, the ratio (PHD/ (PHD+UHD)). To examine the group differences in the levels of TRP, 5-HT, and KYN, we conducted a two-way MANOVA for independent measures (stress and minocycline) on the dependent measures (TRP, 5-HT, and KYN). To examine the differences in the ratios of KYN/TRP, a marker for IDO/TDO activity, and KYN/5-HT, a marker for the balance between KYN and 5-HT pathways, we performed a two-way MANOVA for independent measures (stress and minocycline) on the dependent measures (KYN/TRP and KYN/5-HT).
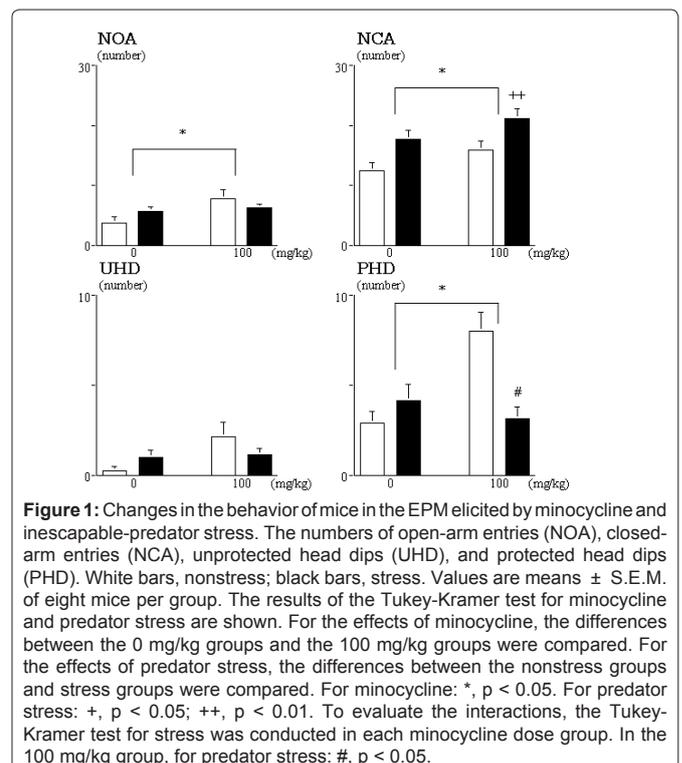
The Tukey-Kramer test was used as the post-hoc test. For the effects of stress, this test was performed to compare the differences between the two nonstress groups and the two stress groups. For the effects of minocycline, the Tukey-Kramer test was performed to compare the differences between the two 0 mg/kg groups and the two 100 mg/kg groups. To evaluate the interaction between stress and minocycline, the Tukey-Kramer test for stress was conducted in each minocycline dose group. Significance was accepted at $p < 0.05$.

Results

The main effects of MANOVA and ANOVA which showed significant differences on dependent measures are described below. The results of the Tukey-Kramer test as a post-hoc test indicating significance are described in the text and shown in the Figures and Tables using symbols indicative of significance.

Behavioral changes in the EPM

The results of the two-way MANOVA for stress and minocycline conducted on the NOA and NCA were as follows: stress (F [2, 27] = 5.752, p = 0.0083) and minocycline (F [2, 27] = 4.073, p = 0.0285) both significantly altered the dependent measures. The post-hoc

Figure 1: Changes in the behavior of mice in the EPM elicited by minocycline and inescapable-predator stress. The numbers of open-arm entries (NOA), closed-arm entries (NCA), unprotected head dips (UHD), and protected head dips (PHD). White bars, nonstress; black bars, stress. Values are means ± S.E.M. of eight mice per group. The results of the Tukey-Kramer test for minocycline and predator stress are shown. For the effects of minocycline, the differences between the 0 mg/kg groups and the 100 mg/kg groups were compared. For the effects of predator stress, the differences between the nonstress groups and stress groups were compared. For minocycline: *, p < 0.05. For predator stress: +, p < 0.05; ++, p < 0.01. To evaluate the interactions, the Tukey-Kramer test for stress was conducted in each minocycline dose group. In the 100 mg/kg group, for predator stress: #, p < 0.05.

test revealed that stress significantly increased the NCA (Figure 1), whereas minocycline significantly increased both the NCA and the NOA (Figure 1). The results of the two-way MANOVA for stress and minocycline conducted on the UHD and PHD were as follows: minocycline (F [2, 27] = 4.426, p = 0.0218) altered the dependent measures significantly.

The interaction between stress and minocycline (F [2, 27] = 7.343, p = 0.0028) was significant. The post-hoc test revealed that minocycline significantly increased the number of PHDs (Figure 1). To evaluate the interaction, we conducted the Tukey-Kramer test for stress in each minocycline dose group. In the 100 mg/kg group, stress significantly decreased the number of PHDs (Figure 1).

The results of the two-way ANOVA for stress and minocycline conducted on the ratio of PHD to all head dips were as follows: stress (F [1, 28] = 4.630, p = 0.0402) significantly altered the ratio. The post-hoc test revealed that stress significantly decreased the ratio of PHD to all head dips (Table 1).

Brain TRP metabolism

Prefrontal cortex: The results of the two-way MANOVA for stress and minocycline conducted on the levels of TRP, 5-HT, and KYN were as follows: neither stress (F [3, 26] = 1.478, p = 0.2436) nor minocycline (F [3, 26] = 2.637, p = 0.0708) altered the dependent measures. The interaction between stress and minocycline (F [3, 26] = 0.782, p = 0.5150) was not significant (Figure 2A).

The results of the two-way MANOVA for stress and minocycline conducted on the KYN/TRP and KYN/5-HT ratios were as follows: neither stress (F [2, 27] = 2.216, p = 0.1285) nor minocycline (F [2, 27] = 2.087, p = 0.1436) altered the dependent measures. The interaction

Table 1: Changes in the behavior of mice in the EPM, based on the entry ratio and the PHD ratio, elicited by minocycline and inescapable-predator stress.

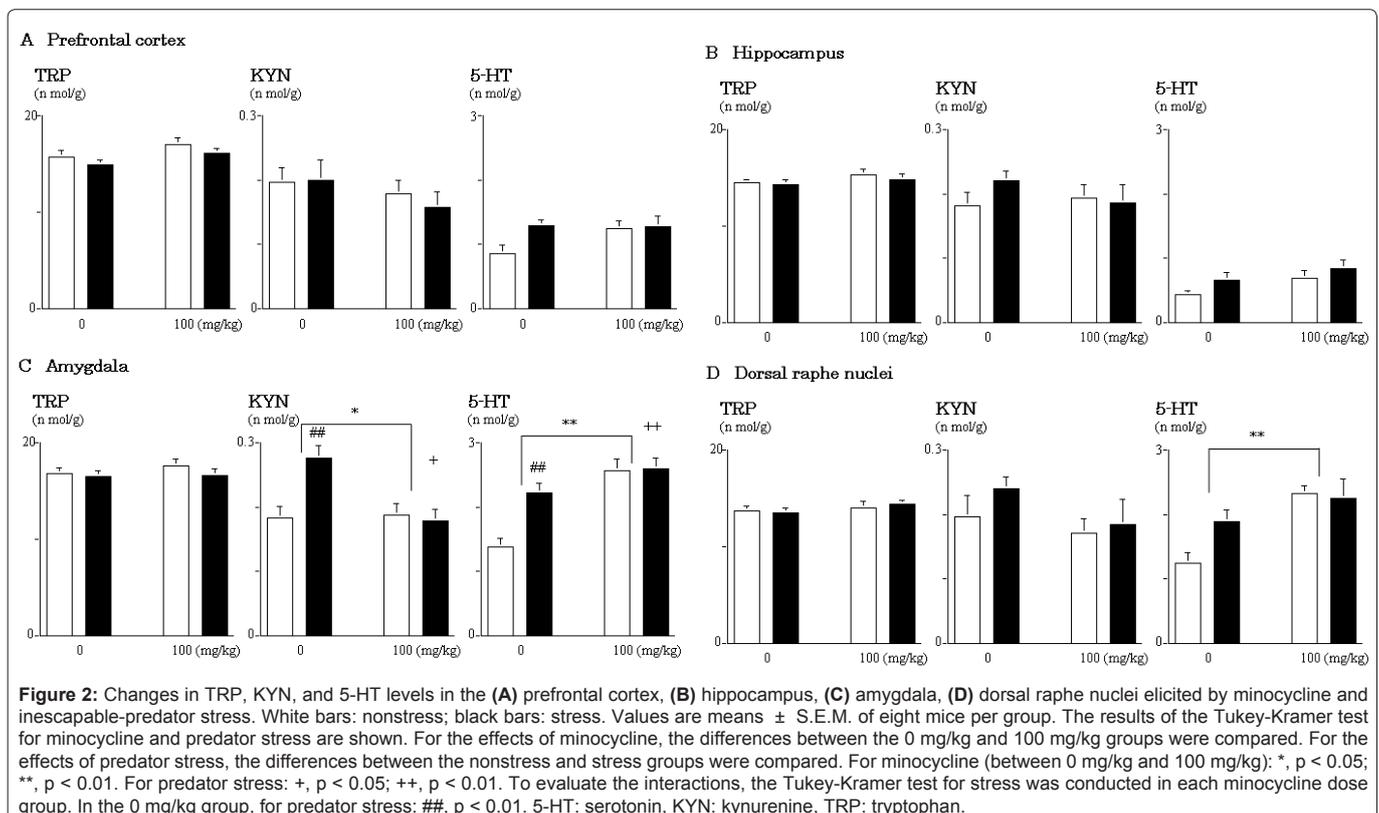| Minocycline	Predator Stress	Entry Ratio	PHD Ratio
0 mg/kg	Nonstress	0.204 ± 0.054	0.972 ± 0.028
	Stress	0.250 ± 0.034	0.746 ± 0.083
100 mg/kg	Nonstress	0.313 ± 0.038	0.830 ± 0.063
	Stress	0.231 ± 0.026	0.774 ± 0.075 +

Values are means ± S.E.M. of eight mice per group. For the effects of predator stress, the Tukey-Kramer test was performed to compare the differences between the nonstress and stress groups. +, p < 0.05. EPM, elevated plus maze; entry ratio, NOA/(NOA+NCA); NCA, number of closed-arm entries; NOA, number of open-arm entries; PHD ratio, PHD/(UHD+PHD); PHD, protected head dips; UHD, unprotected head dips.

between stress and minocycline (F [2, 27] = 2.510, p = 0.1001) was not significant.

Hippocampus: The results of the two-way MANOVA for stress and minocycline conducted on the levels of TRP, 5-HT, and KYN were as follows: neither stress (F [3, 26] = 0.861, p = 0.4739) nor minocycline (F [3, 26] = 2.518, p = 0.0801) altered the dependent measures. The interaction between stress and minocycline (F [3, 26] = 0.384, p = 0.7655) was not significant (Figure 2B).

The results of the two-way MANOVA for stress and minocycline conducted on the KYN/TRP and KYN/5-HT ratios were as follows: neither stress (F [2, 27] = 1.482, p = 0.2452) nor minocycline (F [2, 27] = 1.402, p = 0.2635) altered the ratio. The interaction between stress and minocycline (F [2, 27] = 0.635, p = 0.5376) was not significant.

Amygdala: The results of the two-way MANOVA for stress and minocycline conducted on the levels of TRP, 5-HT, and KYN in the

Figure 2: Changes in TRP, KYN, and 5-HT levels in the **(A)** prefrontal cortex, **(B)** hippocampus, **(C)** amygdala, **(D)** dorsal raphe nuclei elicited by minocycline and inescapable-predator stress. White bars: nonstress; black bars: stress. Values are means ± S.E.M. of eight mice per group. The results of the Tukey-Kramer test for minocycline and predator stress are shown. For the effects of minocycline, the differences between the 0 mg/kg and 100 mg/kg groups were compared. For the effects of predator stress, the differences between the nonstress and stress groups were compared. For minocycline (between 0 mg/kg and 100 mg/kg): *, p < 0.05; **, p < 0.01. For predator stress: +, p < 0.05; ++, p < 0.01. To evaluate the interactions, the Tukey-Kramer test for stress was conducted in each minocycline dose group. In the 0 mg/kg group, for predator stress: ##, p < 0.01. 5-HT: serotonin, KYN: kynurenine, TRP: tryptophan.

amygdala were as follows: stress (F [3, 26] = 3.040, p = 0.0468) and minocycline (F [3, 26] = 14.101, p < 0.0001) significantly altered the dependent measures. The interaction between stress and minocycline (F [3, 26] = 3.724, p = 0.0237) was significant. The post-hoc test revealed that stress significantly increased the KYN and 5-HT levels in the amygdala, whereas minocycline significantly decreased the KYN levels and increased the 5-HT levels (Figure 2C). To evaluate the interaction, we conducted the Tukey-Kramer test for stress in each minocycline dose group. In the 0 mg/kg group, stress significantly increased the KYN and 5-HT levels (Figure 2C).

The results of the two-way MANOVA for stress and minocycline conducted on the KYN/TRP and KYN/5-HT ratios were as follows: stress (F [2, 27] = 6.121, p = 0.0064) and minocycline (F [2, 27] = 16.496, p < 0.0001) significantly altered the dependent measures. The interaction between stress and minocycline (F [2, 27] = 3.647, p = 0.0396) was significant in the amygdala. The post-hoc test revealed that stress significantly increased the KYN/TRP ratio, whereas minocycline significantly decreased both ratios (Table 2). To evaluate the interaction, we conducted the Tukey-Kramer test for stress in each minocycline dose group. In the 0 mg/kg group, stress significantly increased the KYN/TRP ratio (Table 2).

Dorsal raphe nuclei: The results of the two-way MANOVA for stress and minocycline conducted on the levels of TRP, 5-HT, and KYN in the dorsal raphe nuclei were as follows: minocycline (F [3, 26] = 6.292, p = 0.0024) significantly altered the dependent measures. The post-hoc test revealed that minocycline significantly increased the 5-HT level (Figure 2D).

The results of the two-way MANOVA for stress and minocycline conducted on the KYN/TRP and KYN/5-HT ratios were as follows: minocycline (F [2, 27] = 10.416, p = 0.0004) significantly altered both ratios. The post-hoc test revealed that minocycline significantly decreased the KYN/5-HT ratio (Table 2).

Discussion

In the present study, we investigated the effects of minocycline on the prolonged changes in behavior and the brain TRP metabolism elicited by inescapable-predator stress in juvenile mice, as an early-life PTE.

Changes in behavior in the EPM

Minocycline is known to alleviate behavioral changes in animal models of depression [24,29]. In the EPM, which measures anxiety, minocycline inhibited the increase of NOA elicited by LPS [36]. It thus appears that minocycline treatment can inhibit behavioral changes such as reduced anxiety and/or disinhibition. In the present study, we found that concerning the ethological risk assessment measures, stress decreased the PHD ratio; in particular, stress decreased the number of PHDs in the 100 mg/kg minocycline group. Minocycline increased the number of PHDs. Stress reduced anxiety or elicited disinhibition, whereas minocycline increased anxiety or protected disinhibition. Thus, minocycline countered the effect of stress.

Although stress is known to induce anxiety [37], a few EPM studies showed anxiolytic behaviors elicited by foot shock, such as an increase in the percentage of open-arm time spent and the NOA [38,39]. In particular, Konno et al.'s 2007 study [38] showed that foot shock as early postnatal stress increased the percentage of time spent by rats in EPM open arms in the post-adolescence period. Further, pain elicited by a local inflammation insult [40] and or a brain LPS injection [41] in rats in early life increased the percentage of open-arm time spent and NOA. Thus, stress such as foot shock as well as local and central inflammation insult in early life substantially reduced the anxiety measured by an EPM in later life.

As an indicator of anxiety, head dipping is regarded as more sensitive than the percentage of NOA [30,31]. Although in the present

Table 2: Changes in the KYN/TRP and KYN/5-HT ratios elicited by minocycline and inescapable-predator stress.

Region	Minocycline	Predator Stress	KYN/TRP	KYN/5-HT
Prefrontal cortex	0 mg/kg	Nonstress	0.013 ± 0.002	0.263 ± 0.051
		Stress	0.013 ± 0.002	0.165 ± 0.036
	100 mg/kg	Nonstress	0.011 ± 0.001	0.149 ± 0.019
		Stress	0.010 ± 0.001	0.136 ± 0.023
Hippocampus	0 mg/kg	Nonstress	0.013 ± 0.002	0.574 ± 0.177
		Stress	0.016 ± 0.002	0.421 ± 0.075
	100 mg/kg	Nonstress	0.013 ± 0.001	0.356 ± 0.084
		Stress	0.013 ± 0.002	0.276 ± 0.062
Amygdala	0 mg/kg	Nonstress	0.011 ± 0.001	0.137 ± 0.017
		Stress	0.017 ± 0.002 ##	0.127 ± 0.011
	100 mg/kg	Nonstress	0.011 ± 0.001	0.074 ± 0.006
		Stress	0.011 ± 0.002 * +	0.068 ± 0.005 **
Dorsal raphe nuclei	0 mg/kg	Nonstress	0.015 ± 0.003	0.165 ± 0.021
		Stress	0.018 ± 0.002	0.133 ± 0.013
	100 mg/kg	Nonstress	0.012 ± 0.001	0.075 ± 0.010
		Stress	0.013 ± 0.003	0.086 ± 0.018 **

Values are means ± S.E.M. of eight mice per group. For the effects of minocycline, the differences between the 0 mg/kg and 100 mg/kg groups were compared. For the effects of predator stress, the differences between the nonstress and stress groups were compared. For minocycline (between 0 mg/kg and 100 mg/kg): *, p < 0.05; **, p < 0.01. For predator stress: +, p < 0.05. In the 0 mg/kg group, Tukey-Kramer test for predator stress: ##, p < 0.01. 5-HT, serotonin; KYN, kynurenine.

study the traditional EPM measures (i.e., NOA and NCA) may have missed changes in anxiety elicited by stress and minocycline, our risk assessment behavior of head dipping detected the changes. Thus, minocycline countered the reduced anxiety and/or the disinhibition elicited by early-life stress.

Changes in brain TRP metabolism

In the mouse amygdala examined here, the inescapable-predator stress increased the KYN and 5-HT levels and the KYN/TRP ratio, an indicator of activity in the KYN pathway. Thus, stress substantially activated the KYN pathway. In our previous study, inescapable-predator stress elicited a prolonged increase in KYN and a decrease in 5-HT [11]. Predator stress activates the brain's 5-HT system [42]. Stressors induce brain tryptophan hydroxylase (TPH) activity [43]. The reason for the differences in the direction of changes in the 5-HT level between the previous and present studies is not yet known.

Here, minocycline increased the 5-HT levels, whereas it prevented the stress-induced increases in KYN levels. Thus, minocycline decreased the KYN/TRP ratio. Minocycline also decreased the KYN/5-HT ratio. The *in vivo* systemic administration of minocycline therefore substantially suppressed the stress-induced increase in the KYN/TRP ratio and shifted the balance between the KYN and 5-HT pathways toward the 5-HT pathway.

Previous *in vivo* studies suggested that minocycline counteracts both the elevation of KYN elicited by an endotoxin (LPS) [24] and the reduction in 5-HT elicited by a neurotoxin (3, 4-methylenedioxymethamphetamine, MDMA) [44]. The effects of minocycline in the present study, i.e., the prolonged suppressions of the KYN/TRP and KYN/5-HT ratios, may be related to the results of these studies.

Because prolonged behavioral and neurochemical changes elicited by PTEs in early life [1-4] are known to become more severe in response to stress exposure in adulthood [5], these changes may suggest vulnerability and a predisposition to psychiatric disease such as depression and anxiety disorder [6] including PTSD [7]. Our present results suggest that minocycline counteracted the activation of the brain KYN pathway and the behavioral disinhibition elicited by inescapable-predator stress exposure in juvenile mice. Thus, minocycline may become a candidate drug to prevent the adult onset of psychiatric disease after exposure to PTEs such as child abuse (physical abuse, psychological abuse, and sexual abuse), neglect, infection, pain, and natural disasters.

Study limitations

The first limitation of this study is the complexity of the stress protocol. Our inescapable-predator stress included both restraint and predator stressors. Here, we chose to expose the animals to acute, life-threatening stress without inducing any immunological changes that bodily injury would elicit.

The second limitation was the measured products. We measured only TRP, KYN, and 5-HT, because the activity of the KYN pathway and the balance between the KYN and 5-HT pathways were our major concerns [9-11]. To determine the functional consequences of the alteration of KYN metabolism, it may have been useful to measure neuroprotective (such as kynurenic acid) and neurotoxic (such as quinolinic acid) products.

The last limitation is the question of which enzyme, IDO or TDO, is associated the activated KYN pathway. Because our methods were not designed to discriminate the activities of these two enzymes, the interpretation of the results may become difficult.

Conclusion

Acute inescapable-predator stress in juvenile mice decreased the ratio of PHDs to all head dips in the EPM and increased the KYN/TRP ratio in the amygdala. Thus, the stress resulted in reduced anxiety and/or disinhibition, and directly activated the KYN pathway. Minocycline counteracted these changes.

Disclosure

All authors declare that there are no conflicts of interest.

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