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Interaction of the immune-inflammatory and the kynurenine pathways in rats resistant to antidepressant treatment in model of depression

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ABSTRACT

The kynurenine pathway (KP), a major route of tryptophan catabolism, may be associated with the pathophysiology of depressive disorders. KP is responsible for *ca.* 99% of brain tryptophan metabolism *via* its degradation to kynurenine (KYN) catalyzed by indoleamine 2,3-dioxygenase (IDO). Some cytokines, such as interferon-γ (IFN-γ) and interleukin (IL)-6 are potent inducers of IDO. KYN is further converted by kynurenine aminotransferase (KAT) to the more neuroprotective kynurenic acid or by kynurenine 3-monooxygenase (KMO) to neurotoxic 3-hydroxykynurenine. The aim of the present study was to delineate whether the administration of imipramine (IMI) to rats subjected to chronic mild stress (CMS) may reverse behavioral changes induced by CMS in association with changes in immune-inflammatory markers and KP. We confirmed that the CMS procedure modeled one of the main symptoms of depression, *i.e.* anhedonia, and administration of IMI for 5 weeks resulted in a significant reduction in anhedonia in a majority of animals (CMS IMI-R animals), whereas 20% of animals did not respond to IMI treatment (CMS IMI-NR animals). We established that CMS procedure increased *IFN-γ* and *IDO* mRNA and decreased *KAT II* mRNA expression in the rat cortex. In the cortex and hippocampus, IMI treatment and non-responsiveness to IMI (in CMS IMI-NR animals) were associated with increased *IL-6* mRNA expression. In the spleen, CMS increased production of IFN-γ and IL-6 proteins, while these cytokines were decreased by IMI in CMS IMI-R animals. Chronic IMI administration to CMS rats decreased IDO and KMO mRNA and protein expression and increased KAT II/KMO mRNA and protein ratio in IMI responders (CMS IMI-R) in comparison to CMS rats. In CMS IMI-NR rats, a significant increase in IDO mRNA expression and protein level in comparison with IMI responders was observed. Our findings indicate that resistance to therapeutic action of IMI could be explained by a deficiency of the inhibitory properties of IMI on IDO, KMO and KYN synthesis in the cortex. We conclude that the antidepressant activity of IMI may, at least in part, be explained by modulatory activities on the KAT II/KMO ratio in brain areas.

1. Introduction

Depression is the leading cause of disability and the main cause of loss of productive life years worldwide according to the World Health Organization estimates of 2017. The rising prevalence and severity of depressive disorders are a derivative of increasing frequency of drug resistance and morbidity. As remission of depressive symptoms is achieved only in one-third of the major depressive disorder (MDD) patients after the first antidepressant trial, unsuccessful treatments contribute largely to the observed suffering and social costs of MDD [1]. Treatment resistant depression (TRD) is often defined as failure to achieve response or remission after at least two proven antidepressant trials with adequate dosing and duration. Given that *ca.* one-third of depressed patients suffer from TRD, this group disproportionately accounts for the largest burden of the disease, underscoring the importance of innovation and discovery in this area.

For many years the pathophysiology of depression had been restricted to a deficit of neurotransmitters. Based on the monoamine

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hypothesis, depression was associated with a decrease in 5-HT and noradrenaline synthesis and action on their receptors in certain brain regions. According to the neurotrophic hypothesis, the occurrence of depression symptoms was a consequence of decreased synthesis of neurotrophic factors (*e.g.* brain derived neurotrophic factor, BDNF) leading to disturbance of neuroplasticity and neurogenesis. Recently, many studies have suggested that depression is related to the activation of the immune system and inflammation and the first review on the inflammatory findings in depression was published by Maes in 1993 [2]. Activated immune-inflammatory pathways (IIP) may be induced by common trigger factors of depression including psychosocial stressors and medical comorbidities. High level of pro-inflammatory cytokines, such as interferon (IFN)-γ, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 have been consistently reported in plasma and brain samples of depressive patients [3]. Moreover, exogenous pro-inflammatory cytokines and bacterial endotoxins or lipopolysaccharides are able to cause depression, depression-like symptoms or depressive-like sickness behavior in humans and in animal models [4].

Dysregulation of the kynurenine pathway (KP) is also considered as a factor contributing to the pathogenesis of depression [5]. Only 1% of available tryptophan is converted to serotonin while the remaining 99% is metabolized *via* KP. The metabolic enzyme indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.52) is the initial and rate limiting extrahepatic enzyme of the kynurenine pathway. Chronic inflammation and/or stress were shown to up-regulate IDO activity and stimulate KP. IDO is known to be activated by inflammatory cytokines, *i.e.* IFN-γ, IL-6, TNF-α, IL-1β [6–9]. IDO metabolizes tryptophan to N-formylkynurenine transformed further to L-kynurenine (KYN) from which neurochemically active kynurenine metabolites are subsequently generated by kynurenine aminotransferase (KAT) or by kynurenine monooxygenase (KMO). KMO is the first enzyme of a multistep pathway that converts KYN into neurotoxic free radical generator the 3-hydroxykynurenine which is further metabolized to the excitotoxic *N*-methyl-D-aspartic acid (NMDA) receptor agonist, quinolinic acid whereas KAT yields neuroprotective kynurenic acid, which is a negative allosteric modulator of the α7 nicotinic acetylcholine receptor at endogenous concentration, and a competitive antagonist of glycine co-agonist sites of the NMDA receptor [10–12]. Whereas the synthesis of 3-hydroxykynurenine and its downstream metabolites, including quinolinic acid, takes place in microglia and other cells of monocytic origin [13], the synthesis of kynurenic acid occurs in astrocytes, neurons and oligodendrocytes [14–16]. The mammalian brain expresses four KATs. A previous study determined the relative contributions of KAT I, II, and IV to total KAT activity, and found that rat and human brains contain the highest proportion of KAT II (2-aminoadipate aminotransferase, ADA; EC 2.6.1.7) which accounts for ~60% of KAT activity with ~10% and 30% contributions of KAT I and IV, respectively, suggesting a critical role for KAT II in KYNA synthesis in the rat and human brain [17].

The fact that standard antidepressants usually require approximately one month or more to manifest their antidepressant effects, suggests that regulation of some other processes than the monoaminergic pathway, such as neuroplasticity, immune system and/or kynurenine pathway, could be involved in symptom improvement in depressed patients [18]. The effect of antidepressant drugs on the immune-inflammatory and kynurenine pathways is poorly recognized. There is also a lack of data on KP functioning in patients with treatment resistant depression.

In the present study, we used the chronic mild stress (CMS) animal model of depression in which repeated administration of the antidepressant drug, *e.g.* imipramine (IMI) antagonized anhedonia (markedly diminished interest in rewarding stimuli) in CMS IMI-reactive (CMS IMI-R) rats although some rats did not respond to the antidepressant treatment and we called this group IMI non-reactive (CMS IMI-NR) [19].

If IIP and KP disturbance plays a significant role in the formation of symptoms similar to depression in animals subjected to CMS, it is reasonable to think that chronic antidepressant treatment will reverse this disturbance in animals responding to antidepressant therapy but will be inefficient in non-responding animals.

The aim of our study was to determine whether CMS causes disturbances of IIP and KP functioning by estimating the differences in *IL-1β*, *IL-1R1*, *IL-1R2*, *IL-6*, *IL-10*, *TNF-α*, *IFN-γ*, *CD11b*, *CD40*, *GFAP*, *IDO*, *KAT II*, *KMO* mRNA expression in the cortex and/or hippocampus as well as IFN-γ, IL-6, IL-10, IDO, KAT II, KMO protein level and KYN level in the spleen or cortex between control and CMS-subjected rats. At the next step, these parameters were studied in rats responding and non-responding to antidepressant activity of IMI. We hypothesized that the lack of therapeutic effectiveness of IMI in CMS IMI-NR rats can be connected with inability of this drug to influence changes in IIP and KP caused by CMS.

2. Materials and methods

2.1. Animals

Behavioral tests were carried out on male Wistar rats (Charles River, Germany) of initial body weight 220–240 g (about 7 weeks old). Except when grouping was applied as a stress parameter, the animals were singly housed in plastic cages (40 × 25 × 15 cm) under standard laboratory conditions at room temperature of 22 °C with free access to standard laboratory food and tap water, except when food or/and water deprivation was applied as a stress parameter. The artificial day-night cycle (12/12 h, light on at 8 a.m.) was only changed in the course of the stress regime. Each experimental group consisted of 6–8 rats.

All the procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were granted an approval from the Bioethics Commission as compliant with Polish Law. All the experimental procedures were approved by the Local Bioethics Commission of the Institute of Pharmacology, Polish Academy of Sciences in Krakow, Poland.

2.2. Drug

Imipramine hydrochloride (IMI, Sigma-Aldrich, USA) was obtained commercially, dissolved in sterile 0.9% NaCl solution, and injected intraperitoneally (i.p.) at a dose of 10 mg/kg b.w. in a volume of 1 ml/kg.

2.3. Chronic Mild Stress (CMS) procedure

CMS experiments were performed according to the method described previously [19,20]. Male Wistar rats were first trained to consume a 1% sucrose solution. The training procedure lasted 6 weeks and consisted of 1-h testing sessions every week (at 10:00 a.m. on Tuesdays) in which the sucrose solution was presented to the rats in their home cages after 14 h of food and water deprivation. The sucrose intake was measured by weighing the bottles before and at the end of the test and subtraction of results. After the training period, sucrose consumption was further monitored at weekly intervals throughout the experiment. On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group of animals was subjected to the CMS procedure for a period of seven consecutive weeks. Each week the stress regime consisted of two periods of food or water deprivation, two periods of 45° cage tilt, two periods of intermittent illumination (lights on and off every 2 h), two periods of housing in a soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and three periods of no stress. All stressors lasted for 10–14 h and were applied individually and continuously. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for 14 h preceding each sucrose test, but otherwise food and water were

freely available in the home cage.

Based on their sucrose intakes following initial 2 weeks of stress, both the stressed and the control groups were each divided further into matched subgroups, and for subsequent five weeks they received once daily intraperitoneal injections of *vehicle* (sterile saline, 1 ml/kg b.w.) or IMI (10 mg/kg b.w.). IMI was administered at approx. 10.00 a.m. and the weekly sucrose tests were carried out 24 h following the last drug injection. Stress was continued throughout the entire period of treatment. After seven weeks of stress, sucrose intake was significantly lower in the stressed animals, but administration of IMI for 5 weeks to the animals subjected to CMS in most of animals resulted in a significant reduction in anhedonia (CMS IMI-R), as measured by sucrose intake, which remains in agreement with previously published data [21]. About 20% of animals did not respond to the imipramine treatment by reversal of stress-induced sucrose intake deficit and we called this group IMI non-reactive (CMS IMI-NR).

2.4. Real-time PCR

Ribonucleic acid (RNA) was extracted from tissue using the NucleoSpin®RNA II total RNA isolation kit (Macherey-Nagel, Germany). Genomic deoxyribonucleic acid (DNA) contamination was removed by the addition of DNase to the samples. RNA was reverse transcribed into cDNA using a High-Capacity cDNA Archive Kit (Applied Biosystems, Germany). Real-time PCR was performed using an ABI Prism 7300 instrument (Applied Biosystems) as previously described by [22]. Taqman Gene Expression Assays (Applied Biosystems) containing forward and reverse primers and a FAM-labelled MGB Taqman probe were used to quantify each gene of interest. Assay IDs for the genes examined were as follows: *IL-1 R1* (Rn00565482_m1), *IL-1 R2* (Rn00588589_m1), *IL-1β* (Rn00580432_m1), *IL-6* (Rn01410330_m1), *IL-10* (Rn01483988_g1), *TNF-α* (Rn93699071_m1), *IFN-γ* (Rn00594078_m1), *CD11b* (Rn00709342_m1), *CD40* (Rn01423584_g1), *GFAP* (Rn01253033_m1), *IDO* (Rn00576778_m1), *KMO* (Rn01411937_m1), *KAT II* (Rn01435645_m1),

PCR was performed using Taqman® Universal PCR Master Mix and samples were run in duplicate. The cycling conditions consisted of 90 °C for 10 min and 40 cycles at 90 °C for 15 s followed by 60 °C for 1 min. *β*-actin was used as an endogenous control to normalize gene expression data. Relative gene expression was calculated using the ΔΔCT method with Applied BioSystems RQ software (Applied BioSystems, UK).

2.5. Enzyme linked-immunosorbent assay (ELISA) (cortex)

Using ELISA Kits, quantitative determination of IDO (Cloud-Clone Corp., USA), KAT (Cusabio, USA), KMO (Cusabio, USA), KYN (BlueGene Biotech, China) in cortical supernatants was performed according to the manufacturers' instructions. The detection limits were as follows: for IDO: 0.55 ng/ml; KAT: 7.81 pg/ml; KMO: 3.9 pg/ml; and for KYN: 1.0 ng/ml. Briefly, 100 µl of standards or samples were dispensed into wells coated with rat IDO, KAT, KMO or KYN antibody, and incubated at 37 °C for 2 h. After extensive washing, 100 µl of streptavidin-HRP was added and incubated for 1 h. The chromogen in the colorimetric assay was 3,3′,5,5′-tetramethylbenzidine (TMB) (100 µl/well). The reaction was stopped after 10 min by adding 50 µl/well of stop solution. The absorbance was measured using the Infinite 200 PRO Detector system (TECAN, Switzerland) set to 450 nm. Intra-assay precision was always < 10%. Positive controls for each assay were provided by the manufacturer.

2.6. Enzyme linked-immunosorbent assay (ELISA) (splenocytes)

Rat splenocytes were tested for their ability to produce IFN-γ, IL-6 and IL-10 after mitogen stimulation. Splenocyte suspensions were seeded at a concentration of 4×10^6 cell/ml in 24-well corning tissue culture plates, and were then stimulated with a Con A solution (2.5 µg/ml). Cell-free supernatants were collected 48 h (IFN-γ, IL-6) and 72 h (IL-10) later and stored at −20 °C. ELISA was carried out as described previously [23]. IL-6 was measured using pairs of anti-cytokine monoclonal antibodies, purchased from BD Biosciences (San Jose, CA, USA), whereas IL-10 and IFN-γ were tested by DuoSet ELISA Development System (R&D Systems, Minneapolis, USA).

2.7. Proliferative response of splenocytes to mitogen stimulation in vitro

The proliferative response of spleen cells was previously described by Kubera et al. [24]. 4×10^6 splenocytes per ml were stimulated with 5 µg/ml of concanavalin A (Con A). The cells in a final volume of 0.2 ml were incubated in 96-well plates at 37 °C for 72 h. Cell proliferation was determined by adding 10 µl (0.5 µCi) of [^{3}H]thymidine per well (ICN Pharmaceuticals, USA; SpA 6.7 Ci/mmol) 16 h before the end of incubation. The cultures were harvested with an automatic cell harvester (Scatron, Norway), and [^{3}H]thymidine incorporation was assessed using a liquid scintillation counter (Beckman LS 6500).

2.8. Statistical analysis

Data from the ELISA tests are presented as pg/ml protein ± SEM, and the data from RT-PCR are presented as average fold ± SEM. The significance of the differences between the means was evaluated by one or two-way analysis of variance (ANOVA), followed by a Duncan's *post-hoc* test. A value of $p < 0.05$ was considered to be significant. The results were analyzed using the STATISTICA program.

3. Results

3.1. The effects of CMS and imipramine treatment on sucrose consumption

First, we assessed whether CMS alters animal behaviors in the sucrose consumption test. In rats subjected to CMS for 7 weeks, sucrose intake was significantly lower than in non-stressed animals, amounting to 5.2 ± 0.40 g *versus* 12.50 ± 0.7 g, respectively. In 80% of animals administration of IMI for 5 weeks to the animals continuously exposed to CMS resulted in a significant reduction in anhedonia, as measured by sucrose intake, in CMS IMI-R animals and lack of such reduction in CMS IMI-NR (Fig. 1).

The data represent the means ± SEM; $n = 6$–8 animals per group, $^{*}p < 0.05$ *versus* control; $\hat{p} < 0.05$ *versus* CMS; $^{o}p < 0.05$ *versus* CMS IMI-R.

3.2. Expression of inflammatory mediators and glial activation markers in the cortex

In rats subjected to IMI or CMS, we observed an increase *IFN-γ* mRNA expression, in comparison to *vehicle* treated control group. Also IMI was shown to decrease *IL-1β* and *CD11b* and increase *IL-6* mRNA expression in comparison with *vehicle*-treated control group. In CMS IMI-R animals we observed decrease in *TNF-α* and *CD11b* mRNA expression, as compared to CMS group. In CMS IMI-NR animals we detected diminished *CD11b* and augmented *IL-6* mRNA expression, as compared to CMS group.

In CMS IMI-NR group of animals, we observed also statistically significant decrease of *IL-1β* and *IFN-γ* and increase of *IL-6* mRNA expression, in comparison to CMS IMI-R animals (Fig. 2).

We observed no statistically significant differences in *IL-1 R1*, *IL-1 R2*, *CD40* and *GFAP* mRNA expression between groups, although the levels of *IL-10* were lower (trend $p = 0.07$) in IMI group and CMS group, as compared to control group (Table 1).

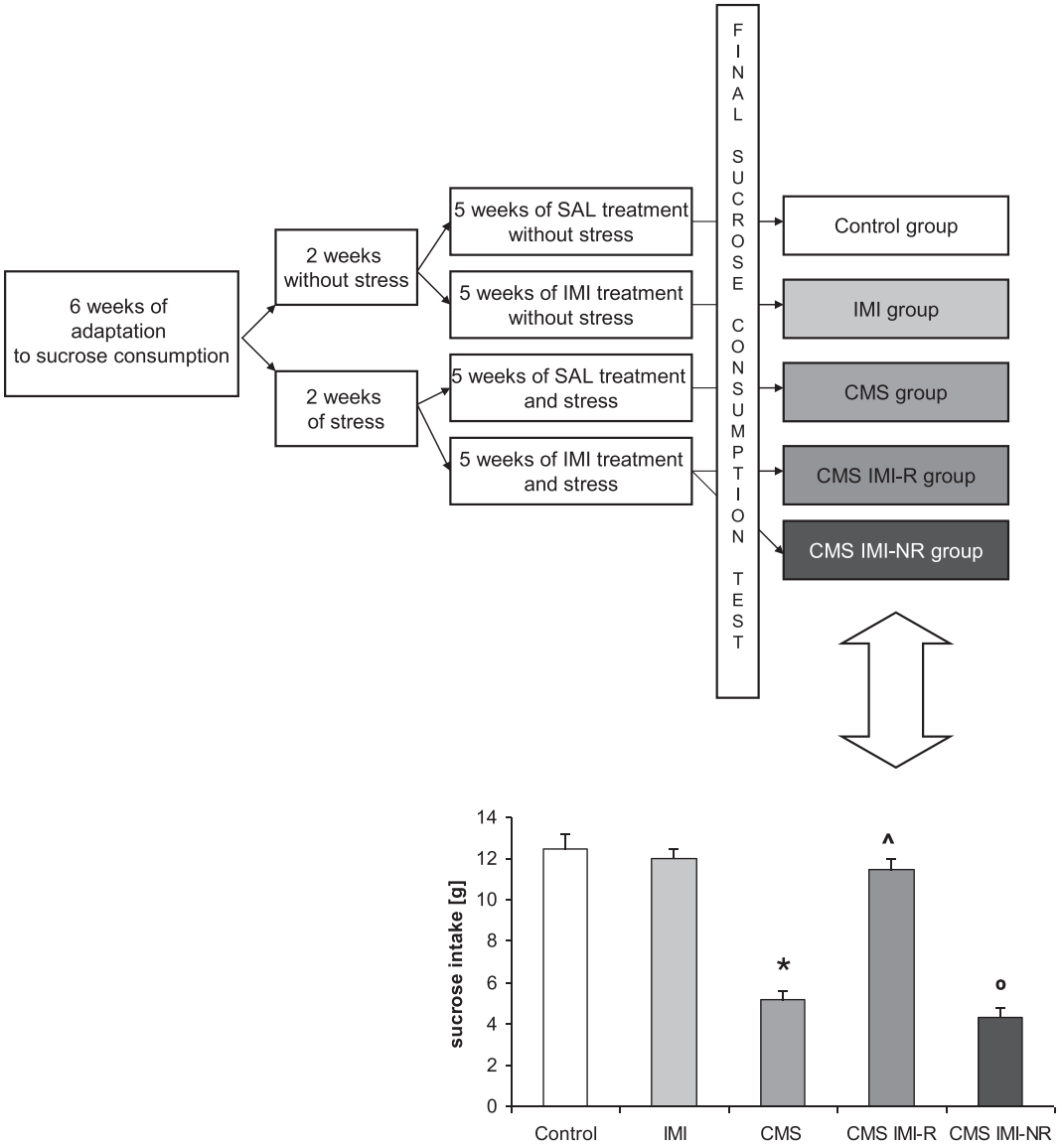

Fig. 1. The scheme of full CMS procedure. The graph shows the differences in 1% sucrose solution consumption after 7-week exposure to CMS and 5-week treatment with IMI. The results presented in Fig. 1 were obtained on 5 groups of animals: control (non-stressed animals receiving *vehicle* for 5 weeks), IMI (non-stressed animals given IMI for 5 weeks), CMS (animals stressed for 7 weeks and receiving *vehicle* for 5 weeks), CMS IMI-R (animals stressed for 7 weeks and receiving IMI for 5 weeks, which responded to antidepressant action of IMI by a significant reduction of anhedonia, as measured by sucrose intake), CMS IMI-NR (animals stressed for 7 weeks and receiving IMI for 5 weeks, which did not respond to antidepressant action of IMI). After seven weeks of stress and five weeks of IMI treatment, sucrose intake was statistically significantly different between group reactive to imipramine (CMS IMI-R) and animals resistant to the drug treatment (CMS IMI-NR).

3.3. Expression of inflammatory mediators and glial activation markers in the hippocampus

In rats subjected to IMI we observed decrease *IL-1β* and *CD11b* and increase *IL-6* mRNA expression in comparison to control group. CMS caused decrease *IL-1β* and *TNF-α* mRNA expression as compared to control group. In CMS IMI-NR stressed group we observed decrease *IL-1β versus* CMS IMI-R group and increase *IL-6* and *GFAP* mRNA expression *versus* CMS group (Fig. 2). No changes in *IFN-γ* expression in the hippocampus between the studied animal groups were observed (data not shown).

3.4. Production of cytokines by Con A stimulated splenocytes

As shown in Fig. 3A and B the production of IFN-γ and IL-6 was significantly higher in Con A stimulated splenocytes obtained from animals subjected to CMS, as compared to control group. We observed also significantly lower production of these two cytokines in CMS IMI-R group, but not in CMS IMI-NR group, in comparison to CMS group.

We detected also lower production of IL-10 (trend: $p = 0.07$) in CMS subjected rats, as compared to control group.

3.5. Proliferative activity of splenocytes unstimulated and stimulated by Con A

The proliferative activity of splenocytes unstimulated was decreased (trend: $p = 0.08$) in stressed animals responsive to imipramine in comparison to CMS-subjected rats. We observed also trend towards lower proliferative activity of splenocytes in response to Con A stimulation in CMS IMI-R group as compared to CMS group (Table 2).

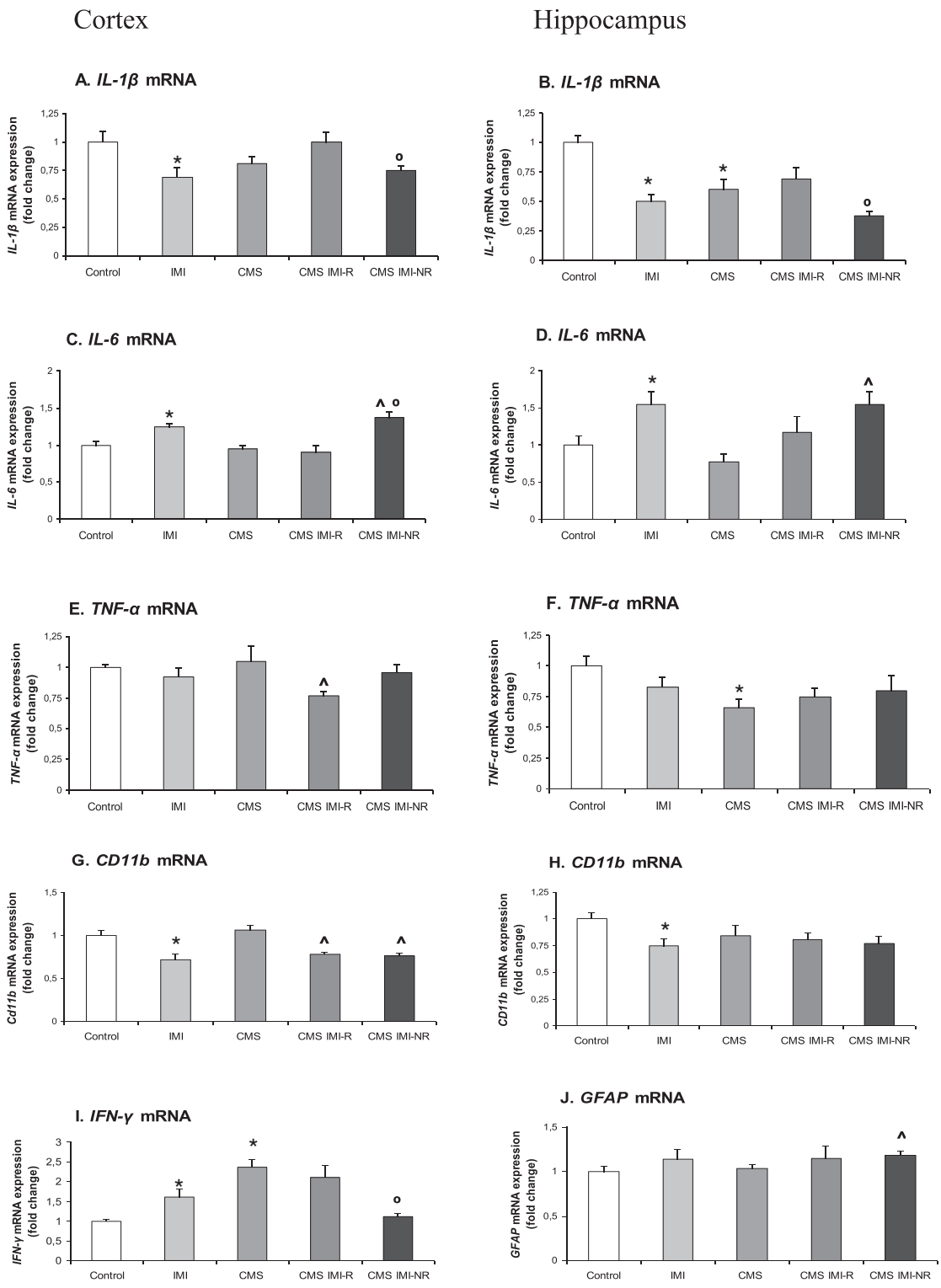

Fig. 2. The effect of 7-week exposure to CMS and 5-week IMI treatment on the *IL-1β* (A), *IL-6* (C), *TNF-α* (E), *CD11b* (G) and *IFN-γ* (I) mRNA expression in the cortex and on the *IL-1β* (B), *IL-6* (D), *TNF-α* (F), *CD11b* (H) and *GFAP* (J) mRNA expression in the hippocampus. The results are expressed as the mean fold change ± SEM; $n = 8$, $^{*}p < 0.05$ *versus* control, $^{\wedge}p < 0.05$ *versus* CMS, $^{o}p < 0.05$ *versus* CMS IMI-R.

3.6. Expression of kynurenine pathway enzymes' genes and protein levels in the cortex

3.6.1. IDO mRNA expression and protein level

In rats subjected to CMS, we observed an increased *IDO* mRNA expression (Fig. 4A), whereas in rats treated with IMI we observed decrease in IDO protein level in comparison to *vehicle* treated control group (Fig. 4B). CMS did not affect IDO protein level (Fig. 4B). Administration of IMI to stressed animals, which were responsive to this drug, caused a significant reduction in IDO mRNA expression and protein level in comparison to CMS group. In CMS IMI-NR rats, a significant increase in IDO mRNA expression and protein level in comparison with CMS IMI-R group was observed (Fig. 4A, B).

Table 1
The effect of 7-week exposure to CMS and 5-week IMI treatment on the *IL-1 R1, IL-1 R2, IL-10, CD 40* and *GFAP* mRNA expression in the cortex. The results are expressed as the mean fold change ± SEM; $n = 8$. Symbol a indicates statistical trends *versus* control.

	Control	IMI	CMS	CMS IMI-R	CMS IMI-NR
Inflammatory mediators					
IL-1 R1	1 ± 0.16	0.76 ± 0.08	0.76 ± 0.08	0.78 ± 0.12	1.1 ± 0.10
IL-1 R2	1 ± 0.25	1.125 ± 0.13	1.25 ± 0.26	0.875 ± 0.11	1.1 ± 0.125
IL-10	1 ± 0.28	0.58 ± 0.09 a	0.7 ± 0.14 a	0.56 ± 0.15	0.57 ± 0.19
Glial activation markers					
CD 40	1 ± 0.1	0.9 ± 0.1	0.95 ± 0.05	0.99 ± 0.06	1.1 ± 0.075
GFAP	1 ± 0.05	0.88 ± 0.07	0.83 ± 0.06	1.05 ± 0.07	1 ± 0.05

3.6.2. KAT II mRNA expression and protein level

In rats subjected to CMS, we observed decrease *KAT II* mRNA expression (Fig. 5A), however, we did not observe any changes in KAT II protein level in any analyzed group (Fig. 5B).

3.6.3. KMO mRNA expression and protein level

In CMS IMI-R rats, *KMO* mRNA expression was decreased in comparison to animals subjected only to CMS (Fig. 6A). We demonstrated the same effect for KMO protein level in CMS IMI-R group (Fig. 6B). Moreover, administration of IMI to non-stressed group, caused a decrease in KMO protein level in comparison to control group (Fig. 6B).

3.6.4. KAT II/KMO ratio

A decrease in the *KAT II/KMO* mRNA ratio was found after CMS exposure in comparison to control group (Fig. 7A). On the other hand, an increase of KAT II/KMO mRNA and protein ratio was observed in

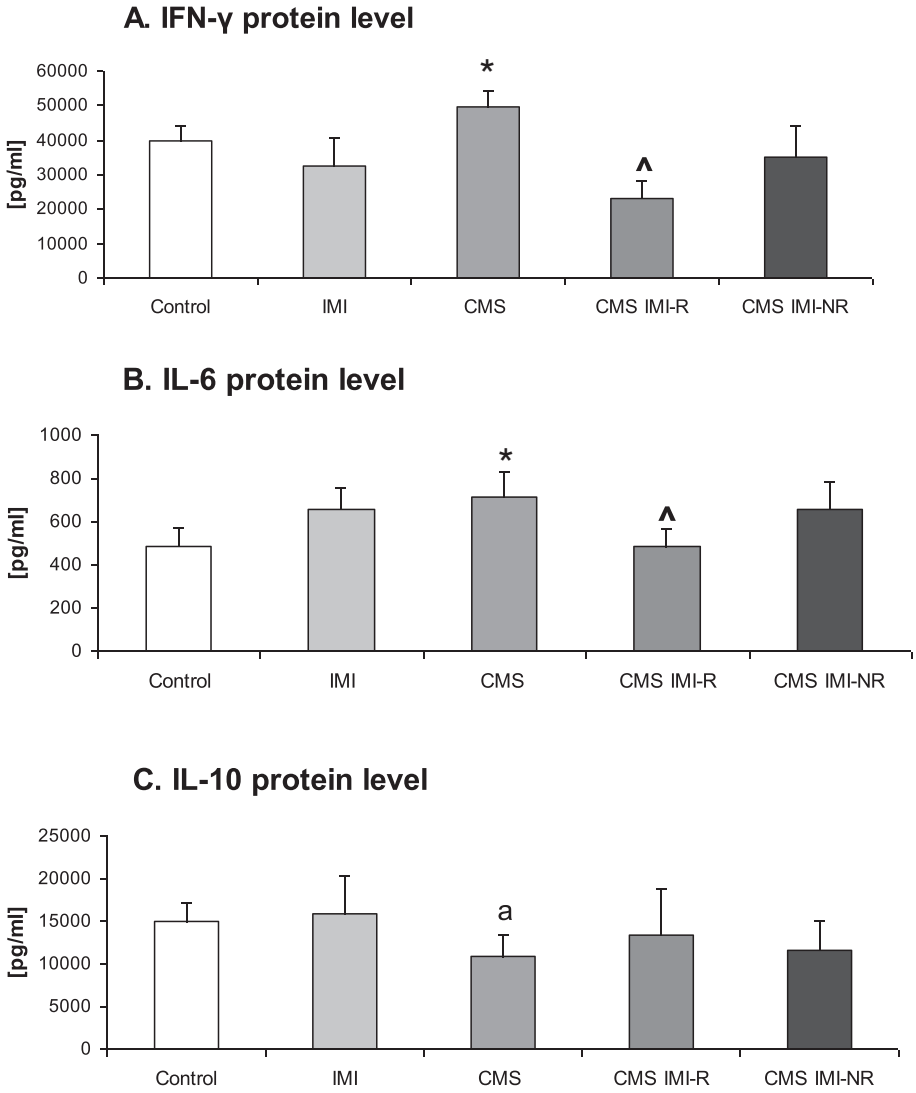

Fig. 3. The effect of 7-week exposure to CMS and 5-week IMI treatment on the IFN-γ (A), IL-6 (B) and IL-10 (C) production by stimulated splenocytes obtained from rats. The results are expressed as the mean protein levels ± SEM; $n = 8$, $^{*}p < 0.05$ *versus* control, $\hat{}p < 0.05$ *versus* CMS. Symbol a ($p = 0.07$) indicates statistical trend *versus* control. The word "trend" was used for values higher than 0.05 but lower than 0.1 to underline clear changes in cytokine levels, which, however, did not reach the level of statistical significance.

Table 2
The effect of 7-week exposure to CMS and 5-week IMI treatment on the proliferation of the unstimulated and stimulated splenocytes. The results are expressed as the mean ± SEM; $n = 8$. Symbol b ($p = 0.08$) indicates statistical trend *versus* CMS. The word "trend" was used for values higher than 0.05 but lower than 0.1 to underline clear changes in proliferative activity of splenocytes, which, however, did not reach the level of statistical significance.

	Control	IMI	CMS	CMS IMI-R	CMS IMI-NR
Unstimulated	1526 ± 508	1583 ± 510	2008 ± 500	1166 ± 160 b	1212 ± 166
Stimulated Con A 0,6	14,970 ± 875	12,516 ± 2036	12,518 ± 1507	8500 ± 920 b	13,045 ± 1250

CMS IMI-R animals, but not in CMS IMI-NR rats in comparison to CMS group (Fig. 7A, B).

3.7. Kynurenine level in the cortex

CMS induced an increase in KYN level. In CMS IMI-R rats the KYN level was significantly reduced in comparison to CMS-subjected rats. On the other hand, this effect wasn't observed in CMS IMI-NR rats (Fig. 8).

4. Discussion

The present studies confirms that the CMS procedure models one of the main symptoms of depression, namely anhedonia, and administration of IMI for 5 weeks to animals subjected to CMS results in a significant reduction in anhedonia [20,25], although around 20% of animals did not respond to IMI treatment [19,26]. In humans, the persistence of anhedonia is one of the most important symptoms of TRD, while antidepressant treatments typically result in low remission rates as low as 25–35% [1,27].

A chance to conduct studies on CMS IMI-NR animals represent a unique opportunity to better understand the mechanism of action of imipramine. The mechanisms underlying treatment resistant depression are poorly understood. TRD has been linked with disorders of drug metabolism and blood-brain barrier function, disarrayed activity of specific brain areas or neurotransmitter systems, dysregulation of hormonal and immune systems, disturbances in the related molecular targets, and also with neuroinflammation and epigenetic mechanisms.

The first main finding of our study is that CMS increased *IDO* mRNA expression, whereas chronic administration of IMI decreased IDO protein levels in cortex (*vs.* control group treated with *vehicle* and CMS subjected rats treated with *vehicle*, respectively). Since the increase in *IDO* mRNA was not accompanied by a rise in IDO protein, our results are not in agreement with previous papers showing an increase in IDO protein in stressed animals. On the other hand, our findings on reduced IDO protein levels in CMS IMI-R animals are in agreement with those of previous publications showing decreased IDO activity in association

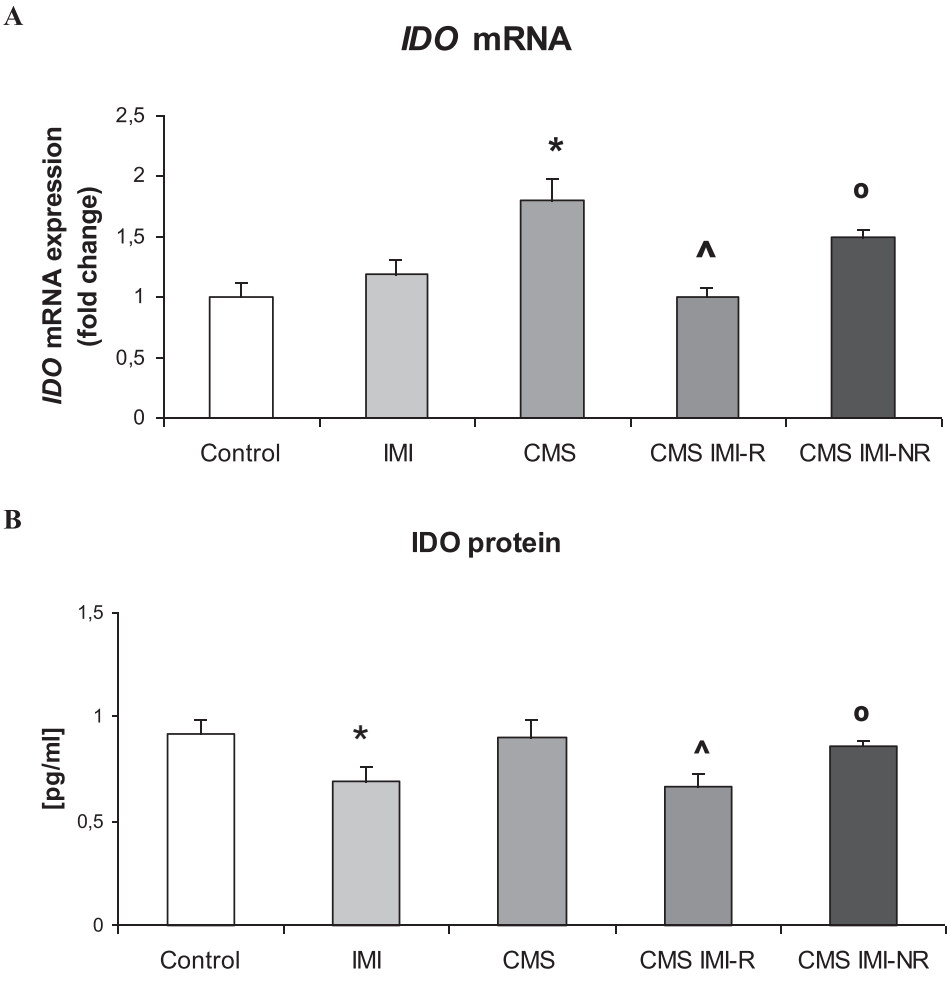

Fig. 4. The effect of 7-week exposure to CMS and 5-week IMI treatment on the IDO mRNA expression (A) and protein level (B) in the cortex. The results are expressed as the mean fold change or protein levels ± SEM; $n = 8$, $^{*}p < 0.05$ *versus* control, $\hat{p} < 0.05$ *versus* CMS, $^{o}p < 0.05$ *versus* CMS IMI-R.

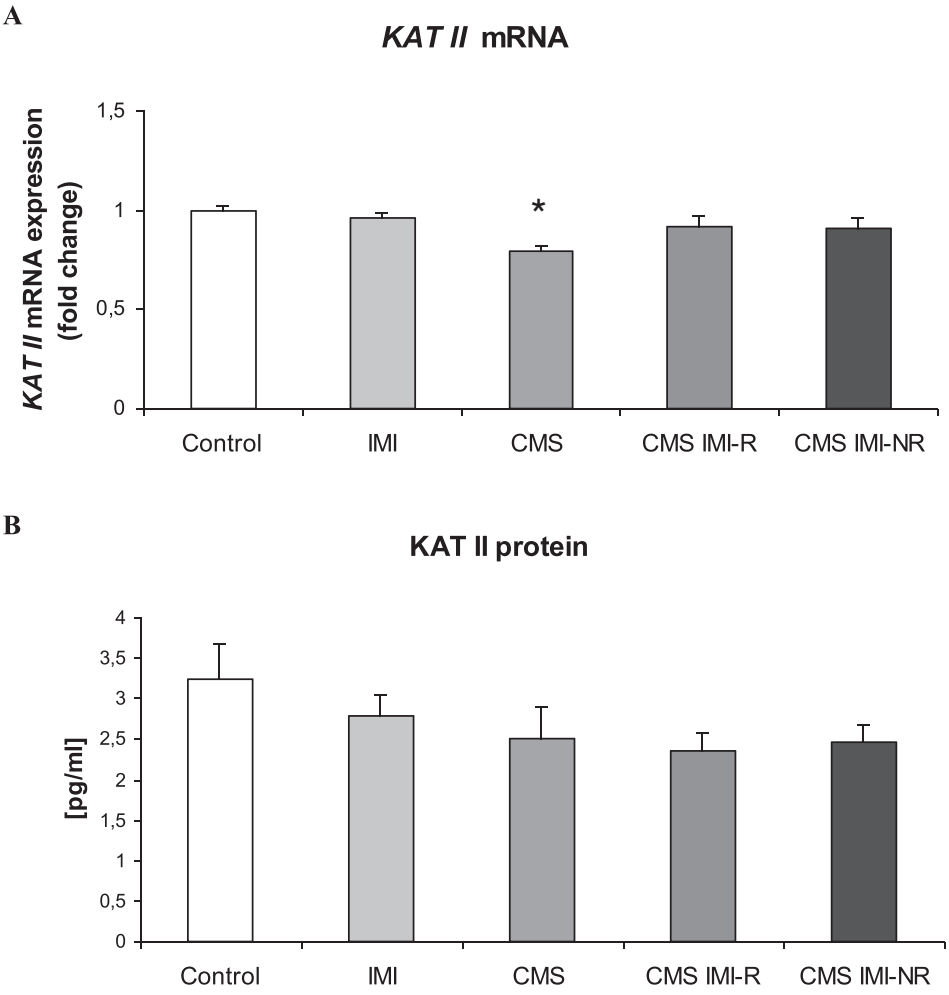

Fig. 5. The effect of 7-week exposure to CMS and 5-week IMI treatment on the KAT II mRNA expression (A) and protein level (B) in the cortex. The results are expressed as the mean fold change or protein levels ± SEM; $n = 8$, $^{*}p < 0.05$ *versus* control.

with reduced depressive-like behaviors following administration of IDO inhibitors or using IDO-knockout mice, [28–31]. Recently, IDO has been shown to participate in a number of important processes. Souza et al. [32] demonstrated for the first time that brain IDO activation may play a key role in mediating memory and emotional disturbance in the experimental model of Alzheimer's disease based on $A\beta_{1-42}$ induced neuroinflammation. IDO activation reduced tryptophan available for serotonin synthesis, which is traditionally connected with an increased risk of depression [5]. However, some pre-clinical data argued against the theory that IDO activation following immune and/or stress challenge resulted in reduced serotonin synthesis. For instance, a number of studies consistently reported increased tryptophan availability in the central nervous system following administration of bacterial lipopolysaccharide (LPS) or other inflammagens [33,34].

Also some clinical studies argue against IDO-mediated depletion of serotonin synthesis in depressed patients [35,36] and suggest that a direct action of kynurenine metabolites on the glutamatergic system may be involved in producing depressive symptoms [37].

In our opinion, the significant increase in *IDO* mRNA in CMS animals, which was not accompanied by an increased IDO protein expression, may be explained by an involvement of microRNA (miR) action. MicroRNA is a small non-coding RNA that inhibits gene expression *via* translational repression or induction of mRNA degradation. IDO was found to be a potential target protein of miR-153-3p. Recently, a direct inhibition of *IDO* mRNA translation by miR-153-3p has been reported [38] and, therefore, we may speculate that unaltered expression of IDO protein in spite of increased *IDO* mRNA expression is a result of miR-153-3p action on *IDO* mRNA in animals subjected to CMS.

The inhibiting activity of IMI on IDO mRNA and/or protein level in the control and stressed IMI-reactive groups, as observed in the current study, is in agreement with the report published by Brooks et al. [39]. They showed that desipramine (metabolite of IMI) blocked LPS-induced *IDO* expression *in vivo* in the hippocampus, astrocytes, microglia and peripheral blood mononuclear cells (PBMCs) and *ex vivo* in murine and human PBMCs. The ability to inhibit IDO activity was also described for citalopram, which exerted antidepressant-like effects and increased turnover of serotonin *via* IDO inhibition in the hippocampus, amygdala and hypothalamus of stressed rats [40]. On the other hand, Fischer et al. [41] showed that imipramine decreased the immobility time in the forced swimming test induced by IFN-α administration, but did not reverse IFN-α-induced changes in the tryptophan-kynurenine pathway (increased kynurenine/tryptophan ratio, indicative of IDO activation, and increased quinolinic acid in the hippocampus).

The second major finding of our study is the significant change in cytokines, which may influence IDO expression in the brain and in periphery. The increase in *IDO* expression in the cortex observed in the CMS group was accompanied by increased *IFN*-γ mRNA expression in the cortex and IFN-γ production by stimulated splenocytes. The lack of increase in IDO protein in the cortex, in spite of the increased *IDO* mRNA level in the CMS group, does not support the hypothesis that upregulation of IDO by IFN-γ could be a biological mediator of inflammation and/or stress–related depressive disorders [42–45].

We expected that IFN-γ expression in CMS IMI-R group would be decreased and in CMS IMI-NR increased, but this assumption was only

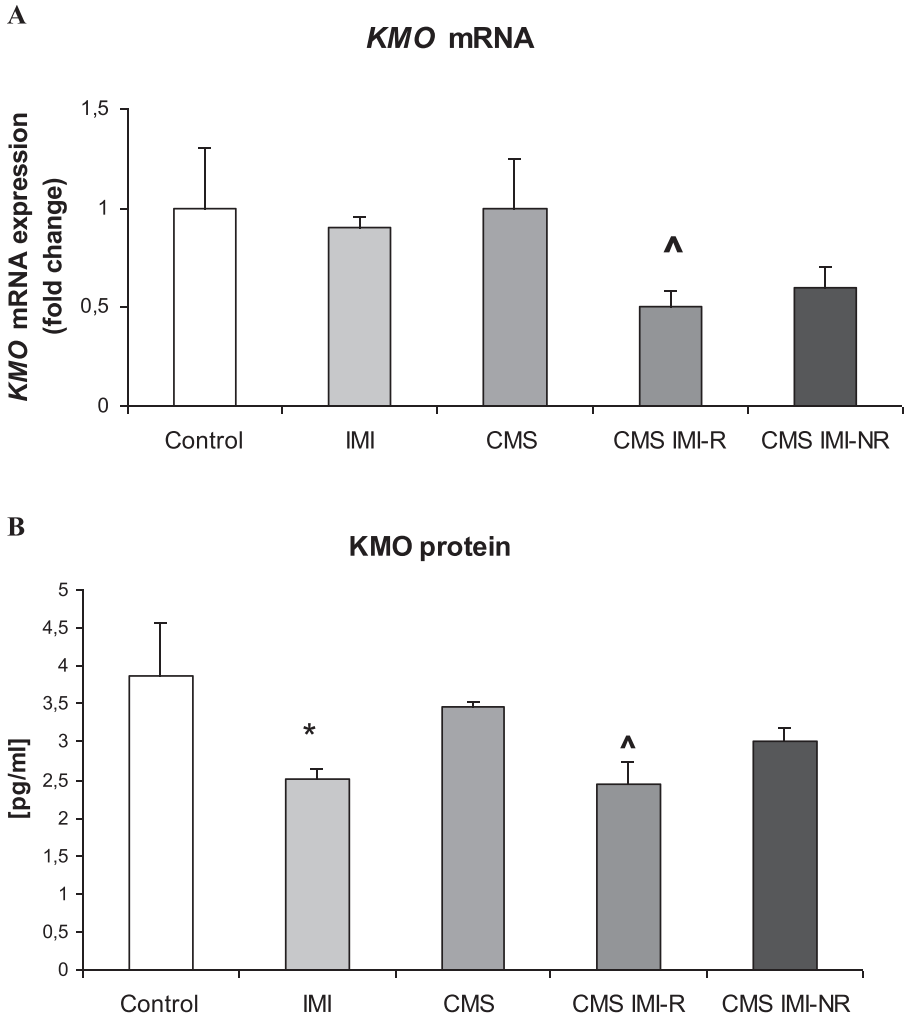

Fig. 6. The effect of 7-week exposure to CMS and 5-week IMI treatment on the KMO mRNA expression (A) and protein level (B) in the cortex. The results are expressed as the mean fold change or protein levels ± SEM; n = 8, $^{*}p < 0.05$ *versus* control, $\hat{p} < 0.05$ *versus* CMS.

partially confirmed. In the spleen, in CMS IMI-R rats we indeed observed a statistically significant inhibition of IFN-γ synthesis which dropped by > 60% *vs* CMS animals, whereas, in CMS IMI-NR animals, the reduction of IFN-γ synthesis was statistically non-significant. We were surprised to detect a very significant reduction in *IFN*-γ mRNA expression in the cortex of CMS IMI-NR rats compared with both CMS animals and CMS IMI-R group. We can assume that IMI non-responsiveness is not associated with alterations in IFN-γ levels in the cortex and that the lack of antidepressant action of IMI may be connected with some other mechanisms.

The suppression of IFN-γ and IL-6 synthesis in the spleen following IMI treatment in animals responding to antidepressant IMI action, observed in this study, indicates that a peripheral compensatory immune-regulatory reflex system (PCIRS) could participate in the treatment-induced recovery from inflammatory phases of mood disorders. Indeed, several previous studies showed that *ex vivo* antidepressants have immune-regulatory effects by attenuating the production of pro-inflammatory cytokines, such as IL-1β, TNF-α and IFN-γ [46]. These *ex vivo* findings are in agreement with the results of meta-analysis in depressed patients indicating that treatment with antidepressants attenuates IL-1β, IFN-γ and IL-6 levels and M1 polarized macrophage activity. Early studies showed that, in mood disorder patients, antidepressants and mood stabilizers reversed the acute phase response, indicating that treatment with antidepressants and mood stabilizers had immune-regulatory effects, which may partly explain their efficacy.

Another finding of the present study is that *IL-6* mRNA expression is increased in the cortex and hippocampus of individuals non-responding to antidepressant IMI action. Based on findings on an animal model of depression, sustained increases in central nervous system IL-6 are thought to play a role in the pathophysiology of TRD [47]. Moreover, it was shown that, in clinical studies, increased IL-6 levels are associated with TRD [48] and that baseline IL-6 production is significantly higher in nonresponders to antidepressants [49]. Moreover some clinical data suggest that IL-6 trans-signaling is associated with treatment resistance in depression [50].

Our findings showed lower *IL-1β* mRNA expression in the cortex and hippocampus of stressed IMI non-responding rats (CMS IMI-NR group) as compared with CMS IMI-R animals. This finding is in line with a previous study [51], which observed that IL-1β downregulation was associated with non-responder status, suggesting that pro-inflammatory response may be associated with a better prognosis. However, this conclusion should be considered with caution as mRNA levels are not necessarily correlated with protein expression. The hypothesis proposed by Belzeaux et al. [51] was confirmed by results obtained by Wamer-Schmidt et al. [52] indicating that the antidepressant effects of selective serotonin reuptake inhibitors were attenuated by anti-inflammatory drugs in mice and humans. For example, a higher percentage of patients was treatment resistant to citalopram if they had taken an NSAID than if they had not been treated with an NSAID.

The third main finding of the present study is that the level of KYN

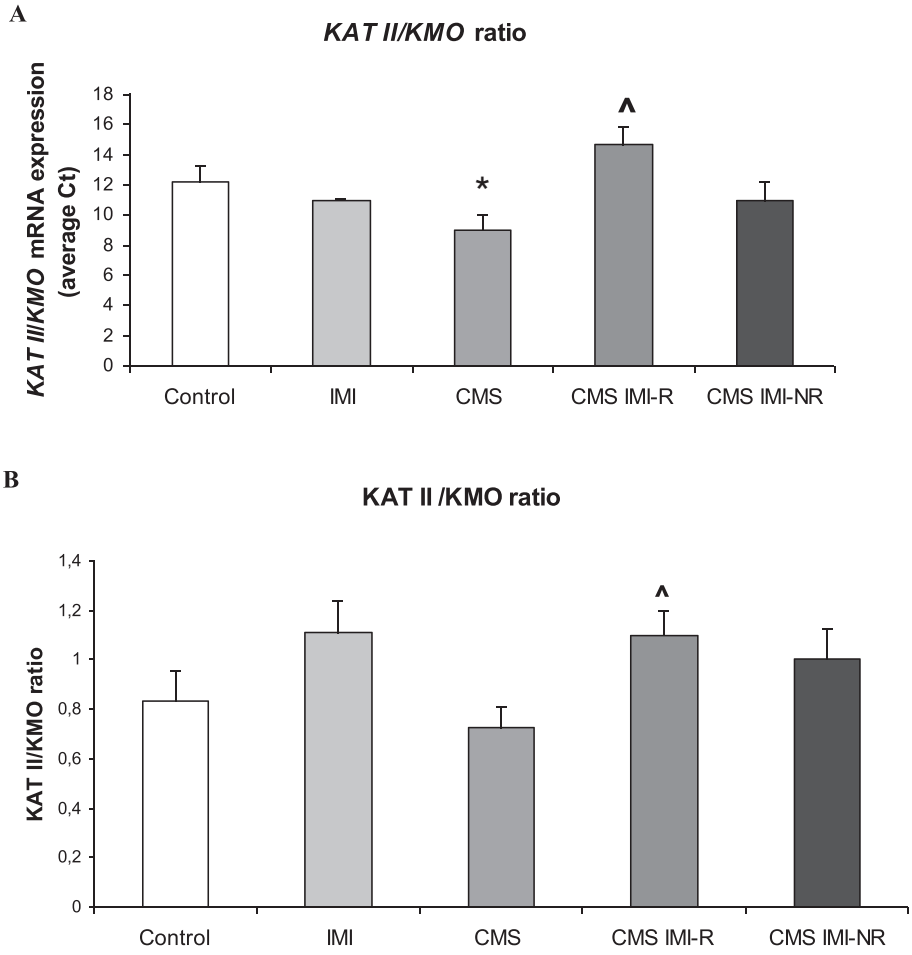

Fig. 7. The effect of 7-week exposure to CMS and 5-week IMI treatment on the KAT II/KMO mRNA (A) and protein (B) ratio in the cortex. Data are expressed as the means ± SEM; n = 8, $^{*}p < 0.05$ *versus* control, $\hat{p} < 0.05$ *versus* CMS.

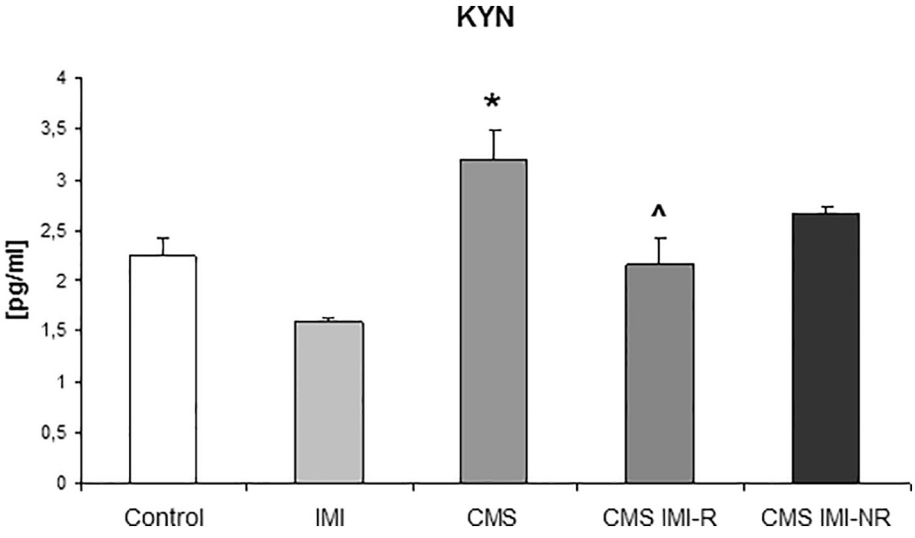

Fig. 8. The effect of 7-week exposure to CMS and 5-week IMI treatment on the KYN level in the cortex. The results are expressed as the mean protein levels ± SEM; n = 8, $^{*}p < 0.05$ *versus* control, $\hat{p} < 0.05$ *versus* CMS.

increased in rats subjected to chronic mild stress. KYN is a central KP metabolite and a branching point in the KP. Previous research has indicated that the peripheral administration of KYN triggers depressive-like behaviors in rodents in a dose-dependent manner [30]. Clinical evidence supports the hypothesis that the production of KYN may be increased in depression. Gabbay et al. [53] found higher kynurenine/tryptophan ratios in adolescent patients with depression, which reflects IDO activation. Bonaccorso et al. [54] and Wichers et al. [55] demonstrated that the onset of depressive symptoms during IFN-α-based immunotherapy was positively associated with the kynurenine/

tryptophan and kynurenine/kynurenic acid ratio. KYN has physiological roles in the CNS, including pro- and antioxidant properties. KYN also acts as an endogenous ligand of the aryl hydrocarbon receptor [56] and suppressor of antitumor immune response. KYN has been utilized as a biomarker for several neurological diseases (for details see review Fujigaki et al. [57]).

The fourth main finding of the present study is that *KAT II* mRNA expression is significantly increased in the cortex of CMS rats, while the decrease in KAT II protein level was not statistically significant. If KAT II activity was reduced in our experiment it could be expected that production of neuroprotective kynurenic acid, an endogenous NMDA receptor antagonist would be reduced and that the availability of kynurenine for production of neurotoxic 3-hydroxykynurenine by KMO would be increased. However we showed previously that expression of *KAT II* was much higher than that of *KMO* (*ca.* 8-fold higher in the cortex, and 20-fold higher in the hippocampus [22]. Since a significant reduction of kynurenic acid levels in the amygdala and striatum of mice subjected to CMS was observed by Laugeray et al. [58] we cannot exclude that CMS diverts kynurenine metabolism into neurotoxic direction. This assumption is supported by clinical study by Myint et al. [35] who found that kynurenic acid was reduced in plasma of patients with major depression. On the other hand, expression of *KAT II* and *KMO* genes as well as kynurenic acid and 3-hydroxykynurenine levels have not been studied in the central nervous system of depressed patients.

The fifth main finding of the present study is that the KMO mRNA expression and protein level decreased in stressed animals reactive to IMI, which could lead to a decrease in 3-hydroxykynurenine synthesis. 3-Hydroxykynurenine acts as a neurotoxic compound and oxidative stress generator [59] which induces apoptotic neuronal death in cortical and stratial neurons and potentiates excitotoxic neuronal loss [60,61]. Moreover, decreased *KMO* expression may deplete quinolinic acid, a potent excitotoxic compound [62]. In depression, excess production of 3-hydroxykynurenine and quinolinic acid is thought to exert toxic effects *via* numerous mechanisms including free radical formation, impairment of mitochondrial function, induction of DNA damage, potentiation of neuronal glutamate release, inhibition of the astrocytic reuptake of glutamate, disruption of the blood-brain barrier and destabilization of the cellular cytoskeleton [13,63].

We may speculate that the increased KAT II/KMO ratio (Fig. 7) in animals subjected to CMS which responded to imipramine may have a neuroprotective potential by establishing a more beneficial kynurenic acid/3-hydroxykynurenine ratio in the brain. Our findings confirm the theory proposed by Kocki et al. [64] who postulated that the action of antidepressants may involve re-establishing the above-mentioned beneficial kynurenic acid/3-hydroxykynurenine ratio in the brain. However, the investigations published by Kocki et al. [64] concerned only *in vitro* tests with primary astroglial cultures. Those authors showed that fluoxetine, citalopram, amitriptiline and imipramine increased production of kynurenic acid and diminished 3-hydroxykynurenine synthesis as well as up-regulated *KAT I* and *KAT II* and diminished *KMO* genes expression.

All in all, our findings indicate that resistance to the therapeutic action of IMI may be explained by a deficiency in the inhibiting action of IMI on IDO, KMO and KYN synthesis and *IDO* and *KMO* mRNA expression in the cortex. Non-responsiveness to imipramine can also result from elevated *IL-6* mRNA expression in the cortex and hippocampus. On the other hand, the therapeutic action of imipramine may be associated with suppressed mRNA expression and/or reduced protein level of IDO and KMO in the cortex, IFN-γ and IL-6 in the spleen, and with an increased KAT/KMO mRNA and protein ratio in the cortex. Unfortunately, there is a lack of clinical trials evaluating the expression of enzymes and KP metabolites in patients with depression. Moreover, this pathway is not explored in humans or animals resistant to the therapeutic action of antidepressants, which opens new prospects for future research.

Acknowledgments

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References

[1] R.T. de Sousa, M. V. Zanetti, A. R. Brunoni, R. Machado-Vieira, Challenging treatment- resistant major depressive disorder: a roadmap for improved therapeutics, Curr. Neuropharmacol. 13 (5) (2015) 616–635.
[2] M. Maes, A review on the acute phase response in major depression, Rev. Neurosci. 4 (4) (1993) 407–416.
[3] B. Leonard, M. Maes, Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression, Neurosci. Biobehav. Rev. 36 (2) (2012) 764–785.
[4] M. Kubera, E. Obuchowicz, L. Goehler, J. Brzeszcz, M. Maes, In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (3) (2011) 744–759.
[5] G.F. Oxenkrug, Tryptophan kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40 years later, Isr J Psychiatry Relat Sci 47 (1) (2010) 56–63.
[6] J.M. Carlin, E.C. Borden, P.M. Sondel, G.I. Byrne, Interferon-induced indoleamine 2,3-dioxygenase activity in human mononuclear phagocytes, J. Leukoc. Biol. 45 (1) (1989) 45 29–34.
[7] L.A. Pemberton, S.J. Kerr, G. Smythe, B.J. Brew, Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha, J. Interf. Cytokine Res. 17 (10) (1997) 589–595.
[8] H. Fujigaki, K. Saito, S. Fujigaki, M. Takemura, K. Sudo, H. Ishiguro, M. Seishima, The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines, J. Biochem. 139 (4) (2006) 655–662.
[9] P.A. Zunszain, C. Anacker, A. Cattaneo, S. Choudhury, K. Musaelyan, A.M. Myint, S. Thuret, J. Price, C.M. Pariante, Interleukin-1β: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis, Neuropsychopharmacology 37 (4) (2012) 939–949.
[10] M. Kessler, T. Terramani, G. Lynch, M. Baudry, A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists, J. Neurochem. 52 (1989) 1319–1328.
[11] C. Hilmas, E.F. Pereira, M. Alkondon, A. Rassoulpour, R. Schwarcz, E.X. Albuquerque, The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications, J. Neurosci. 21 (2001) 7463–7473.
[12] R. Schwarcz, R. Pellicciari, Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities, J. Pharmacol. Exp. Ther. 303 (1) (2002) 1–10.
[13] G.J. Guillemin, Quinolinic acid, the inescapable neurotoxin, FEBS J. 279 (2012) 1356–1365.
[14] F. Du, W. Schmidt, E. Okuno, R. Kido, C. Köhler, R. Schwarcz, Localization of kynurenine aminotransferase immunoreactivity in the rat hippocampus, J. Comp. Neurol. 321 (3) (1992) 477–487.
[15] G.J. Guillemin, S.J. Kerr, G.A. Smythe, D.G. Smith, V. Kapoor, P.J. Armati, J. Croitoru, B.J. Brew, Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection, J. Neurochem. 78 (4) (2001) 842–853.
[16] K. Wejksza, W. Rzeski, E. Okuno, M. Kandefer-Szerszen, J. Albrecht, W.A. Turski, Demonstration of kynurenine aminotransferases I and II and characterization of kynurenic acid synthesis in oligodendrocyte cell line (OLN-93), Neurochem. Res. 30 (8) (2005) 963–968.
[17] P. Guidetti, L. Amori, M.T. Sapko, E. Okuno, R. Schwarcz, Mitochondrial aspartate aminotransferase: a third kynurenate-producing in the mammalian brain, J. Neurochem. 102 (2007) 103–111.
[18] G.Z. Réus, K. Jansen, S. Titus, A.F. Carvalho, V. Gabbay, J. Quevedo, Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: evidences from animal and human studies, J. Psychiatr. Res. 68 (2015) 316–328.
[19] W. Duda, K. Curzytek, M. Kubera, M. Iciek, D. Kowalczyk-Pachel, A. Bilska-Wilkosz, E. Lorenc-Koci, M. Leśkiewicz, A. Basta-Kaim, B. Budziszewska, M. Regulska, J. Ślusarczyk, P. Gruca, M. Papp, M. Maes, W. Lasoń, L. Antkiewicz-Michaluk, The effect of chronic mild stress and imipramine on the markers of oxidative stress and antioxidant system in rat liver, Neurotox. Res. 30 (2) (2016) 173–184.
[20] M. Kubera, A. Symbirtsev, A. Basta-Kaim, J. Borycz, A. Roman, M. Papp, M. Claesson, Effect of chronic treatment with imipramine on interleukin 1 and interleukin 2 production by splenocytes obtained from rats subjected to a chronic mild stress model of depression, Pol. J. Pharmacol. 48 (5) (1996) 503–506.
[21] S. Monleon, P. D'Aquila, A. Parra, V.M. Simon, P.F. Brain, P. Willner, Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine, Psychopharmacology 117 (4) (1995) 453–457.
[22] T.J. Connor, N. Starr, J.B. O'Sullivan, A. Harkin, Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? Neurosci. Lett. 441 (1) (2008) 29–34.

[23] M. Kubera, A. Simbirtsev, R. Mathison, M. Maes, Effects of repeated fluoxetine and citalopram administration on cytokine release in C57BL/6 mice, Psychiatry Res. 96 (2000) 255–266.

[24] M. Kubera, A. Basta-Kaim, A. Skowron-Cendrzak, B. Mazur-Kolecka, A. Roman, J. Borycz, Effect of repeated amitriptyline administration to mice on the T lymphocyte proliferative activity and natural killer cell cytotoxicity, Pol. J. Pharmacol. 47 (1995) 321–326.

[25] M. Papp, E. Moryl, Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression, Eur. J. Pharmacol. 263 (1–2) (1994) 1–7.

[26] A. Faron-Górecka, M. Kuśmider, M. Kolasa, D. Żurawek, P. Gruca, M. Papp, K. Szafran, J. Solich, P. Pabian, I. Romańska, L. Antkiewicz-Michaluk, M. Dziedzicka-Wasylewska, Prolactin and its receptors in the chronic mild stress rat model of depression, Brain Res. 25 (1555) (2014) 48–59.

[27] A.J. Rush, M.H. Trivedi, J.W. Stewart, A.A. Nierenberg, M. Fava, B.T. Kurian, D. Warden, D.W. Morris, J.F. Luther, M.M. Husain, I.A. Cook, R.C. Shelton, I.M. Lesser, S.G. Kornstein, S.R. Wisniewski, Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study, Am. J. Psychiatry 168 (7) (2011) 689–701.

[28] M.A. Lawson, J.M. Parrott, R.H. McCusker, R. Dantzer, K.W. Kelley, J.C. O'Connor, Intracerebroventricular administration of lipopolysaccharide induces indoleamine-2,3-dioxygenase-dependent depression-like behaviors, J. Neuroinflamm. 10 (87) (2013) 1–9.

[29] Y.N. Liu, Y.L. Peng, L. Liu, T.Y. Wu, Y. Zhang, Y.J. Lian, Y.Y. Yang, K.W. Kelley, C.L. Jiang, Y.X. Wang, TNFalpha mediates stress-induced depression by upregulating indoleamine 2,3-dioxygenase in a mouse model of unpredictable chronic mild stress, Eur. Cytokine Netw.. 26 (2015) 15–25.

[30] J.C. O'Connor, M.A. Lawson, C. André, E.M. Briley, S.S. Szegedi, J. Lestage, N. Castanon, M. Herkenham, R. Dantzer, K.W. Kelley, Induction of IDO by bacille Calmette-Guerin is responsible for development of murine depressive-like behavior, J. Immunol. 182 (5) (2009) 3202–3212.

[31] B.L. Salazar, L. Gonzalez-Rivera, L. Redus, J.M. Parrott, J.C. O'Connor, Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge, Horm. Behav. 62 (2012) 202–209.

[32] L.C. Souza, C.R. Jesse, M.S. Antunes, J.R. Ruff, D. de Oliveira Espinosa, N.S. Gomes, F. Donato, R. Giacomeli, S.P. Boeira, Indoleamine-2,3-dioxygenase mediates neurobehavioral alterations induced by an intracerebroventricular injection of amyloid-β1-42 peptide in mice, Brain Behav. Immun. 56 (2016) 363–377.

[33] A.J. Dunn, M.L. Powell, C. Meitin, P.A. Small Jr., Virus infection as a stressor: influenza virus elevates plasma concentrations of corticosterone, and brain concentrations of MHPG and tryptophan, Physiol. Behav. 45 (3) (1989) 591–594.

[34] F. Moroni, P. Russi, M.A. Gallo-Mezo, G. Moneti, R. Pellicciari, Modulation of quinolinic and kynurenic acid content in the rat brain: effects of endotoxins and nicotinylalanine, J. Neurochem. 57 (5) (1991) 1630–1635.

[35] A.M. Myint, Y.K. Kim, R. Verkerk, S. Scharpé, H. Steinbusch, B. Leonard, Kynurenine pathway in major depression: evidence of impaired neuroprotection, J. Affect. Disord. 98 (1–2) (2007) 143–151.

[36] M.C. Wichers, G.H. Koek, G. Robaeys, R. Verkerk, S. Scharpé, M. Maes, IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity, Mol. Psychiatry 10 (6) (2005) 538–544.

[37] N. Müller, M.J. Schwarz, The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression, Mol. Psychiatry 12 (11) (2007) 988–1000.

[38] X.S. Zhao, Y.N. Wang, M. Lv, Y. Kong, H.Y. Luo, X.Y. Ye, Q. Wu, T.F. Zhao, H.Y. Hu, H.Y. Zhang, M.R. Huo, J. Wan, X.J. Huang, miR-153-3p, a new bio-target, is involved in the pathogenesis of acute graft-versus-host disease via inhibition of indoleamine-2,3-dioxygenase. Oncotarget. 7 (30) (2016) 48321–34.

[39] A.K. Brooks, T.M. Janda, M.A. Lawson, J.L. Rytych, R.A. Smith, C. Ocampo-Solis, R.H. McCusker, Desipramine decreases expression of human and murine indoleamine-2,3-dioxygenases, Brain Behav. Immun. 62 (2017) 219–229.

[40] I. Ara, S. Bano, Citalopram decreases tryptophan 2,3-dioxygenase activity and brain 5-HT turnover in swim stressed rats, Pharmacol. Rep. 64 (3) (2012) 558–566.

[41] C.W. Fischer, A. Eskelund, D.P. Budac, S. Tillmann, N. Liebenberg, B. Elfving, G. Wegener, Interferon-alpha treatment induces depression-like behaviour accompanied by elevated hippocampal quinolinic acid levels in rats, Behav. Brain Res. 15 (293) (2015) 166–172.

[42] J.C. O'Connor, M.A. Lawson, C. André, E.M. Briley, S.S. Szegedi, J. Lestage, N. Castanon, M. Herkenham, R. Dantzer, K.W. Kelley, Induction of IDO by bacille Calmette-Guérin isresponsible for development of murine depressive-like behavior, J. Immunol. 182 (5) (2009) 3202–3212.

[43] J.C. O'Connor, M.A. Lawson, C. André, M. Moreau, J. Lestage, N. Castanon, K.W. Kelley, R. Dantzer, Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice, Mol. Psychiatry 14 (5) (2009) 511–522.

[44] C.L. Raison, R. Dantzer, K.W. Kelley, M.A. Lawson, B.J. Woolwine, G. Vogt, J.R. Spivey, K. Saito, A.H. Miller, CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression, Mol. Psychiatry 15 (4) (2010) 393–403.

[45] R. Dantzer, J.C. O'Connor, M.A. Lawson, K.W. Kelley, Inflammation-associated depression: from serotonin to kynurenine, Psychoneuroendocrinology. 36 (3) (2011) 426–436.

[46] M. Kubera, A.H. Lin, G. Kenis, E. Bosmans, D. van Bockstaele, M. Maes, Anti-inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio, J. Clin. Psychopharmacol. 21 (2) (2001) 199–206.

[47] S.J. Sukoff Rizzo, S.J. Neal, Z.A. Hughes, M. Beyna, S. Rosenzweig-Lipson, S.J. Moss, N.J. Brandon, Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes, Transl. Psychiatry 2 (2012) e199.

[48] M. Maes, E. Bosmans, R. De Jongh, G. Kenis, E. Vandoolaeghe, H. Neels, Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression, Cytokine 9 (11) (1997) 853–858.

[49] S. Lanquillon, J.C. Krieg, U. Bening-Abu-Shach, H. Vedder, Cytokine production and treatment response in major depressive disorder, Neuropsychopharmacology 22 (4) (2000) 370–379.

[50] M. Maes, G. Anderson, M. Kubera, M. Berk, Targeting classical IL-6 signalling or IL-6 trans-signalling in depression? Expert Opin. Ther. Targets 18 (5) (2014) 495–512.

[51] R. Belzeaux, A. Bergon, V. Jeanjean, B. Loriod, C. Formisano-Tréziny, L. Verrier, A. Loundou, K. Baumstarck-Barrau, L. Boyer, V. Gall, J. Gabert, C. Nguyen, J.M. Azorin, J. Naudin, E.C. Ibrahim, Responder and nonresponder patients exhibit different peripheral transcriptional signatures during major depressive episode, Transl. Psychiatry 2 (2012) e185.

[52] J.L. Warner-Schmidt, K.E. Vanover, E.Y. Chen, J.J. Marshall, P. Greengard, Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans, Proc. Natl. Acad. Sci. U. S. A. 108 (22) (2011) 9262–9267.

[53] V. Gabbay, R.G. Klein, Y. Katz, S. Mendoza, L.E. Guttman, C.M. Alonso, J.S. Babb, G.S. Hirsch, L. Liebes, The possible role of the kynurenine pathway in adolescent depression with melancholic features, J. Child Psychol. Psychiatry 51 (8) (2010) 935–943.

[54] S. Bonaccorso, V. Marino, M. Biondi, F. Grimaldi, F. Ippoliti, M. Maes, Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus, J. Affect. Disord. 72 (3) (2002) 237–241.

[55] M.C. Wichers, G.H. Koek, G. Robaeys, A.J. Praamstra, M. Maes, IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity, Psychol. Med. 35 (3) (2005) 433–441.

[56] C.A. Opitz, U.M. Litzenburger, F. Sahm, M. Ott, I. Tritschler, S. Trump, T. Schumacher, L. Jestaedt, D. Schrenk, M. Weller, M. Jugold, G.J. Guillemin, C.L. Miller, C. Lutz, B. Radlwimmer, I. Lehmann, A. von Deimling, W. Wick, M. Platten, An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor, Nature 478 (7368) (2011) 197–203.

[57] H. Fujigaki, Y. Yamamoto, K. Saito, L-Tryptophan-kynurenine pathway enzymes are therapeutic target for neuropsychiatric diseases: focus on cell type differences, Neuropharmacology 112 (Pt B) (2017) 264–274.

[58] A. Laugeray, J.M. Launay, J. Callebert, A. Surget, C. Belzung, P.R. Barone, Peripheral and cerebral metabolic abnormalities of the tryptophan-kynurenine pathway in a murine model of major depression, Behav. Brain Res. 210 (1) (2010) 84–91.

[59] C.L. Eastman, T.R. Guilarte, The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine, Neurochem. Res. 15 (11) (1990) 1101–1107.

[60] P. Guidetti, R. Schwarcz, 3-Hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum, Eur. J. Neurosci. 11 (11) (1999) 3857–3863.

[61] S. Okuda, N. Nishiyama, H. Saito, H. Katsuki, Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine, Proc. Natl. Acad. Sci. U. S. A. 93 (22) (1996) 12553–12558.

[62] R. Schwarcz, W.O. Whetsell Jr., R.M. Mangano, Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain, Science 219 (4582) (1983) 316–318.

[63] A.L. Colin-Gonzalez, P.D. Maldonado, A. Santamaria, 3-Hydroxykynurenine: an intriguing molecule exerting dual actions in the central nervous system, Neurotoxicology 34 (2013) 189–204.

[64] T. Kocki, S. Wnuk, R. Kloc, J. Kocki, B. Owe-Larsson, E.M. Urbanska, New insight into the antidepressants action: modulation of kynurenine pathway by increasing the kynurenic acid/3-hydroxykynurenine ratio, J. Neural Transm. (Vienna) 119 (2) (2012) 235–243.