Acta Neuropsychiatrica 2017

DOI: 10.1017/neu.2016.31

ACTA NEUROPSYCHIATRICA

Decreased levels of kynurenic acid in prefrontal cortex in a genetic animal model of depression

Liu X-C, Erhardt S, Goiny M, Engberg G, Mathé AA. Decreased levels of kynurenic acid in prefrontal cortex in a genetic animal model of depression.

Objective: There is a growing interest in the role of kynurenine pathway and tryptophan metabolites in the pathophysiology of depression. In the present study, the metabolism of tryptophan along the kynurenine pathway was analysed in a rat model of depression.
Methods: Kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK) were measured by high-performance liquid chromatography (HPLC) in prefrontal cortex (PFC) and frontal cortex (FC) in a rat model of depression, the Flinders Sensitive Line (FSL) and their controls, the Flinders Resistant Line (FRL) rats. In addition, KYNA was also measured in hippocampus, striatum and cerebellum.
Results: KYNA levels were reduced in the PFC of FSL rats compared with FRL rats, but did not differ with regard to the FC, hippocampus, striatum or cerebellum. 3-HK levels in PFC and FC, representing the activity of the microglial branch of the kynurenine pathway, did not differ between the FSL and FRL strains.
Conclusion: Our results suggest an imbalanced metabolism of the kynurenine pathway in the PFC of FSL rats.

Xi-Cong Liu[1], Sophie Erhardt[1], Michel Goiny[1], Göran Engberg[1], Aleksander A. Mathé[2]

[1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; and [2]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Keywords: 3-hydroxykynurenine; depression; Flinders Sensitive Line rat; kynurenic acid

Göran Engberg, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.
Tel: +46 703526717;
Fax: +46 703526717;
E-mail: goran.engberg@ki.se

Accepted for publication May 24, 2016

First published online July 13, 2016

Significant outcomes

- A decrease in prefrontal kynurenic acid (KYNA) along with unchanged 3-hydroxykynurenine (3-HK) observed in the Flinders Sensitive Line (FSL) rats suggests that tryptophan metabolism is directed towards the microglial branch of the kynurenine pathway.
- Our results support the idea that aberrations in the kynurenine pathway participate in the pathophysiology of major depressive disorder (MDD).

Limitations

- Only one metabolite of the microglial branch of the kynurenine pathway was analysed.
- Further studies are needed to identify the mechanism(s) behind the presently shown imbalance of the kynurenine pathway.

Introduction

Depression is one of the leading causes of disability worldwide, however the underlying biochemical aberrations are essentially unknown. Although the traditional view regarding the pathophysiology of depression has focussed on perturbations in monoaminergic functions, in particular, serotonin neurotransmission, more recent findings point to an involvement also of glutamate signalling mechanisms. One of the most prominent findings in this regard come from

clinical studies where *N*-methyl-D-aspartate (NMDA) receptor antagonists, principally ketamine, generate a robust antidepressant effect already within hours after administration (1–4). Further, a large body of genetic studies and cytokine analysis of serum or cerebrospinal fluid (CSF) in humans suggests a connection between immune activation and MDD (5). In that context, the tryptophan degradation along the kynurenine pathway may emerge as a possible candidate contributing to depression symptomatology. Thus, this route of tryptophan metabolism forms at least two neuroactive metabolites; KYNA, acting as an antagonist at the glycine site of the NMDA-receptor and at the cholinergic $\alpha 7$ nicotinic receptor, and quinolinic acid (QUIN), an NMDA-receptor agonist (6). The brain kynurenine pathway, which is critically regulated by cytokines of the innate immune system, consists of two branches, KYNA is formed in astrocytes, whereas 3-HK synthesis and further downstream metabolites, such as QUIN takes place in microglia (6). In the present study, two crucial metabolites of the brain kynurenine pathway, that is KYNA and 3-HK were analysed in FSL rat, an animal model of depression.

Material and methods

Animals

Female FSL and Flinders Resistant Line (FRL) rats (age 10–12 weeks) from the breeding colonies maintained at the Karolinska Institutet were used. Animals were maintained under standard laboratory conditions with free access to chow pellet and tap water in a light-controlled room (12 h light/dark cycle, light on at 6:00 a.m.), under constant temperature (22°C) and humidity (40–55%). In total, 10 FSL rats and 10 FRL rats were used in this study. As the day of the oestrus can affect the immunological and hormonal balance in the central nerves system (CNS), measures were taken (daily vaginal smear) to control for this variable (7–8).

Brain sample preparation

Rats were guillotined, the brains taken out from the skull and immediately dissected on dry ice. A coronal slice, defined in this experiment as prefrontal cortex (PFC), was cut 2.4 mm (average wet weight 38 mg) from the tip of the frontal cortex (FC). Other brain areas were dissected according to the Glowinski and Iversen method (9) into FC, hippocampus, striatum and cerebellum. Brain samples were frozen on dry ice and stored at −80°C; 0.4 M perchloric acid was added in an amount corresponding to three times the tissue weight and homogenised (Bullet Blender® Next Advance Inc. Averill Park, NY, USA). Homogenates were centrifuged at 14 000 rpm for 5 min and supernatants were diluted ×1.1 with 70% perchloric acid and stored at −20°C for subsequent analysis of KYNA and 3-HK. Measures were taken to allow a constant low temperature (22°C) throughout the handing of all samples.

Analysis of KYNA

KYNA was analysed with an isocratic reversed-phase high-performance liquid chromatography (HPLC) system, including a dual-piston, high-pressure liquid delivery pump Shimadzu LC-10AD (Shimadzu Corporation, Kyoto, Japan), a ReproSil-Pur C18 column (4 × 100 mm; Dr. Maisch GmbH, Ammerbuch, Germany) and a fluorescence detector (Jasco Ltd, Hachioji City, Japan) with an excitation wavelength of 344 nm and an emission wavelength of 398 nm (18 nm bandwidth). A mobile phase of 50 mM sodium acetate (pH 6.2, adjusted with acetic acid) and 7.0% acetonitrile was pumped through the reversed-phase column at a flow rate of 0.5 ml/min. Samples (50 µl) were manually injected by a Rheodyne® 7725i injector (IDEX, Oak Harbor, WA, USA) into a 100 µl loop. Zinc acetate (0.5M not pH adjusted) was delivered after the column at a flow rate of 10 ml/h by a peristaltic pump (P-500; Pharmacia, Uppsala, Sweden). Signals from the fluorescence detector were transferred to a computer for analysis using Datalys Azur software (Grenoble, France). The retention time of KYNA was about 7–8 min. Inter and intra coefficients of variation were 2.3% and 2.2%, respectively, for this assay. The sensitivity of the system was verified throughout the session by analysis of KYNA standards with concentrations ranging between 0.156–10 nM, which resulted in a linear standard plot.

Analysis of 3-HK

3-HK was analysed with an isocratic reversed-phase HPLC system, coupled to an electrochemical detector (Coulochem III; ESA Inc., Chelmsford, MA, USA), similar to what is previously described (10). A mobile phase consisting of 20 mM sodium phosphate, 0.7 mM octanesulfonic acid and 10% acetonitrile (pH set to 3.2 using acetic acid) was pumped through a ReproSil-Pur C18 column (4 × 150 mm, Dr. Maisch GmbH), at a flow rate of 0.6 ml/min, delivered by a LC-20AD VP HPLC pump (Shimadzu Corporation, Kyoto, Japan). Samples of 20 µl (kept at –25°C until analysis) were manually injected through a Rheodyne® 7725i injector (IDEX) into a 100 µl loop. The retention time of 3-HK was about 8.5 min.

https://doi.org/10.1017/neu.2016.31 Published online by Cambridge University Press

Table 1. Levels of kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK) and KYNA/3-HK ratio in different brain regions of Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats

	KYNA		3-HK		KYNA/3-HK ratio	
	FSL	FRL	FSL	FRL	FSL	FRL
Frontal cortex	10.0 [4.3–15.6] (10)	8.5 [6.5–27.8] (10)	21.6 [16.3–27.8] (8)	19.0 [7.4–31.5] (10)	0.39 [0.2–0.6] (8)	0.66 [0.2–3.6] (10)
Prefrontal cortex	3.8 [3.1–5.9]* (10)	14.5 [5.1–32.1] (10)	14.2 [12.5–19.1] (10)	12.8 [9.1–17.8] (10)	0.25 [0.2–0.4]** (10)	1.0 [0.4–2.1] (10)
Striatum	3.5 [2.7–4.7] (10)	4.0 [3.1–16.6] (10)	–	–	–	–
Hippocampus	10.3 [2.5–21.1] (10)	8.5 [5.5–30.6] (10)	–	–	–	–
Cerebellum	4.9 [3.6–6.4] (10)	5.3 [3.4–7.6] (10)	–	–	–	–

Median concentrations [interquartile range] of KYNA and 3-HK are expressed in nM. Numbers of animals are given in brackets. Differences in KYNA, 3-HK levels and KYNA/3-HK ratios between FSL and FRL rats were evaluated by Mann–Whitney *U*-test followed by the Bonferroni test for multiple comparison.
*$p < 0.05$, **$p < 0.01$.

Signals from the detector were transferred to a computer for analysis with Clarity (DataApex Ltd, Prague, The Czech Republic). Inter and intra coefficients of variation were 2.7% and 2.1%, respectively. The limit of detection was at least 20 times lower than the reported values in this study. 3-HK was not detectable in two FC samples from FSL animals. Generally, values of 3-HK were similar to what is recently reported in controls rats by Schwarcz and co-workers (11–12).

Statistical analysis

The statistical software package GraphPad Prism® 6 (GraphPad Software Inc., San Diego, CA, USA) for Mac OS X was used. All data are expressed as median with interquartile range and analysed using the non-parametric Mann–Whitney *U*-test followed by Bonferroni correction for multiple comparisons. A *p*-value <0.05 was considered statistically significant throughout the study.

Results

KYNA levels in both FSL ($n = 10$) and FRL rats ($n = 10$) varied between different brain regions. In the PFC, KYNA levels were significantly lower in FSL compared with FRL animals (Table 1). However, KYNA levels did not differ between the two strains with regard to FC, striatum, hippocampus or cerebellum.

Levels of 3-HK did not differentiate between FSL and FRL rats in either PFC or FC. However, in the PFC a significant reduction in the KYNA/3-HK ratio was found in FSL compared with FRL rats. No differences were found in other brain regions (Table 1).

Discussion

FSL, a genetic rat model of depression and their controls, FRL, have been a valuable choice to explore the pathophysiology of depression, effect of gene-environment interaction and effect of antidepressant treatment (13–18). Phenotypically, the FSL rats are similar to depressed patients and exhibit dysregulation of serotonin, glutamate and neuropeptideY neurotransmission (19–20). In line with a recent study using an enzyme-based microelectrode array for glutamate detection (21), the present study suggests that FSL rats display frontal glutamatergic overactivity. Thus, a reduced concentration of the NMDA-receptor antagonist KYNA in the PFC of the FSL rat would promote endogenous activation of the NMDA-receptor. This is also in line with a recent study showing that FSL rats exhibit increased glutamatergic neurotransmission in hippocampal CA1 area concomitant with reduced expression of the glial glutamate transporter (22).

The presently shown aberration of the kynurenine metabolism, that is reduced levels of KYNA in the PFC concomitant with apparently unchanged levels of 3-HK, indicate a disproportion between the two main branches of this pathway (6). This is reflected by the decrease in KYNA/3-HK ratio in PFC suggesting that tryptophan metabolism along the kynurenine pathway is directed towards the microglial, 3-HK and QUIN containing branch. In support of an imbalanced metabolism of the kynurenine pathway in MDD, it was recently shown that antidepressant drugs like fluoxetine, citalopram, amitriptyline and imipramine increase the KYNA/3-HK ratio in primary astroglial cultures (23). Moreover, physical exercise, generally known to induce antidepressant effects in humans (24) as well as in FSL rats (25), weaken the microglial branch of the kynurenine pathway (10). Further, in a cohort of suicidal attempters low CSF KYNA was associated with severe depressive symptoms (26).

The mechanism behind the reduced PFC KYNA in FSL rats is obscure. The specific reduction in KYNA concentration in the PFC in these rats is in line with a role of this area to regulate cognitive

https://doi.org/10.1017/neu.2016.31 Published online by Cambridge University Press

functions, planning and emotional behaviour (27), in contrast to the FC, which is partly dedicated to motor functions/locomotion. Indeed, imaging studies have consistently reported neurophysiological abnormalities of the PFC in MDD patients (28–30). Clearly, the presently observed specific reduction in PFC KYNA concentration in FSL rats may affect glutamatergic and gamma-aminobutyric acid (GABA)ergic signalling in this area (31). Thus, our results are in line with previous observation pointing to a role of PFC in major depression, although the precise mechanism behind this condition is obscure. The kynurenine pathway is critically regulated by cytokines (6). Thus, several enzymes of the kynurenine pathway are known to be induced by pro-inflammatory cytokines, most importantly tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase, both being rate-limiting enzymes of the kynurenine pathway (32–33). Another enzyme of importance for the formation of KYNA is kynurenine-3-monooxygenase. An induction of this enzyme, for example, by interferon-gamma (34) would promote the downstream metabolism of kynurenine to 3-HK and QUIN, in favour of a reduced astrocytic KYNA production. Although there is mounting evidence for a relationship between depression and neuroinflammation (35), information on immune activation in FSL rats is still sparse and somewhat conflicting. Thus, FSL rats show a number of peripheral immunological aberrations compared with FRL rats (36–38). Recently though, it was shown that the expression of immune-related genes like *S100b* and complement factor *C3* is specifically down-regulated in several brain regions of FSL rats (39). Further studies on cytokine regulation of the kynurenine pathway, as well as inflammatory mechanisms to account for behavioural and biochemical aberrations in FSL rats are necessary to identify the mechanism behind the presently shown imbalance of the kynurenine pathway.

In conclusion, FSL rats show an imbalance in the two main branches of the kynurenine pathway as reflected by a reduction in KYNA concentration in the PFC compared with FRL rats. This aberration, restored by antidepressants and physical exercise, may participate in the pathophysiology of MDD.

Acknowledgements

Supported by the Swedish Medical Research Council (grants no 2838, 10414, 2009-7052 and 2013-2838), the Swedish Brain Foundation, Åhlén-stiftelsen, Petrus och Augusta Hedlunds Stiftelse and the AstraZeneca-Karolinska Institutet Joint Research Program in Translational Science. No funding sources had any role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. Authors' Contributions: A.A.M. bred the animals, harvested and dissected the brains. X-C.L. and M.G. performed the biochemical and statistical analyses, and participated in the writing of the manuscript. S.E., G.E. and A.A.M. conceived the hypothetical background and contributed to study design and writing.

Conflicts of Interest

The authors have no competing interests to declare. A.A.M. is an Associate Editor in Acta Neuropsychiatrica. However, A.A.M. did not handle the journals processing of the manuscript or was involved in any decisions related to the present work.

Ethical Standards

All experiments were approved by and performed in accordance with the guidelines from the Ethical Committee of Northern Stockholm, Sweden.

References

1. Zarate CA, Singh JB, Carlson PJ et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 2006;**63**:856–864.
2. Abdallah CG, Averill LA, Krystal JH. Ketamine as a promising prototype for a new generation of rapid-acting antidepressants. Ann N Y Acad Sci 2015;**1344**:66–77.
3. Berman RM, Cappiello A, Anand A et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 2000;**47**:351–354.
4. Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R. Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry 1996;**29**:23–26.
5. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. J Neuroinflammation 2013;**10**:43.
6. Schwarcz R, Bruno JP, Muchowski PJ, Wu H-Q. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci 2012;**13**:465–477.
7. Bogdanov VB, Bogdanova OV, Koulchitsky SV et al. Behavior in the open field predicts the number of KCl-induced cortical spreading depressions in rats. Behav Brain Res 2013;**236**:90–93.
8. Jiménez-Vasquez PA, Overstreet DH, Mathé AA. Neuropeptide Y in male and female brains of Flinders Sensitive Line, a rat model of depression. Effects of electroconvulsive stimuli. J Psychiatr Res 2000;**34**:405–412.
9. Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. J Neurochem 1966;**13**:655–669.

https://doi.org/10.1017/neu.2016.31 Published online by Cambridge University Press

10. Agudelo LZ, Femenía T, Orhan F et al. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 2014;**159**: 33–45.
11. Ceresoli-Borroni G, Guidetti P, Amori L, Pellicciari R, Schwarcz R. Perinatal kynurenine 3-hydroxylase inhibition in rodents: pathophysiological implications. J Neurosci Res 2007;**85**:845–854.
12. Notarangelo FM, Wilson EH, Horning KJ et al. Evaluation of kynurenine pathway metabolism in Toxoplasma gondii-infected mice: implications for schizophrenia. Schizophr Res 2014;**152**:261–267.
13. El Khoury A, Gruber SH, Mørk A, Mathé AA. Adult life behavioral consequences of early maternal separation are alleviated by escitalopram treatment in a rat model of depression. Prog Neuropsychopharmacol Biol Psychiatry 2006;**30**:535–540.
14. Hansson AC, Rimondini R, Heilig M, Mathé AA, Sommer WH. Dissociation of antidepressant-like activity of escitalopram and nortriptyline on behaviour and hippocampal BDNF expression in female rats. J Psychopharmacol (Oxford) 2011;**25**:1378–1387.
15. Musazzi L, Mallei A, Tardito D et al. Early-life stress and antidepressant treatment involve synaptic signaling and Erk kinases in a gene-environment model of depression. Psychiatr Res 2010;**44**:511–520.
16. Piubelli C, Vighini M, Mathé AA, Domenici E, Carboni L. Escitalopram modulates neuron-remodelling proteins in a rat gene-environment interaction model of depression as revealed by proteomics. Part I: genetic background. Int J Neuropsychopharmacol 2011;**14**:796–833.
17. Piubelli C, Vighini M, Mathé AA, Domenici E, Carboni L. Escitalopram affects cytoskeleton and synaptic plasticity pathways in a rat gene-environment interaction model of depression as revealed by proteomics. Part II: environmental challenge. Int J Neuropsychopharmacol 2011;**14**:834–855.
18. Shrestha SS, Pine DS, Luckenbaugh DA et al. Antidepressant effects on serotonin 1A/1B receptors in the rat brain using a gene x environment model. Neurosci Lett 2014;**559**:163–168.
19. Overstreet DH, Friedman E, Mathé AA, Yadid G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. Neurosci Biobehav Rev 2005;**29**:739–759.
20. Ryan B, Musazzi L, Mallei A et al. Remodelling by early-life stress of NMDA receptor-dependent synaptic plasticity in a gene-environment rat model of depression. Int J Neuropsychopharmacol 2009;**12**:553–559.
21. Hascup KN, Hascup ER, Stephens ML et al. Resting glutamate levels and rapid glutamate transients in the prefrontal cortex of the Flinders Sensitive Line rat: a genetic rodent model of depression. Neuropsychopharmacology 2011;**36**:1769–1777.
22. Gómez-Galán M, De Bundel D, Van Eeckhaut A, Smolders I, Lindskog M. Dysfunctional astrocytic regulation of glutamate transmission in a rat model of depression. Mol Psychiatry 2013;**18**:582–594.
23. Kocki T, Wnuk S, Kloc R, Kocki J, Owe-Larsson B, Urbanska EM. New insight into the antidepressants action: modulation of kynurenine pathway by increasing the kynurenic acid/3-hydroxykynurenine ratio. J Neural Transm 2012;**119**:235–243.
24. Josefsson T, Lindwall M, Archer T. Physical exercise intervention in depressive disorders: meta-analysis and systematic review. Scand J Med Sci Sports 2014;**24**: 259–272.
25. Bjørnebekk A, Mathé AA, Brené S. The antidepressant effects of running and escitalopram are associated with levels of hippocampal NPY and Y1 receptor but not cell proliferation in a rat model of depression. Hippocampus 2010;**20**:820–828.
26. Bay-Richter C, Linderholm KR, Lim CK et al. A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. Brain Behav Immun 2015;**43**:110–117.
27. Negrón-Oyarzo I, Aboitiz F, Fuentealba P. Impaired functional connectivity in the prefrontal cortex: a mechanism for chronic stress-induced neuropsychiatric disorders. Neural Plast 2016; doi: 10.1155/2016/7539065 [Epub 2016 January 19].
28. Johnstone T, van Reekum CM, Urry HL, Kalin NH, Davidson RJ. Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. J Neurosci 2007;**27**:8877–8884.
29. Liu X, Sun G, Zhang X et al. Relationship between the prefrontal function and the severity of the emotional symptoms during a verbal fluency task in patients with major depressive disorder: a multi-channel NIRS study. Prog Neuropsychopharmacol Biol Psychiatry 2014;**54**:114–121.
30. Dutta A, McKie S, Deakin JF. Resting state networks in major depressive disorder. Psychiatry Res 2014;**224**: 139–151.
31. Beggiato S, Tanganelli S, Fuxe K, Antonelli T, Schwarcz R, Ferraro L. Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. Neuropharmacology 2014;**82**:11–18.
32. Guillemin GJ, Smith DG, Smythe GA, Armati PJ, Brew BJ. Expression of the kynurenine pathway enzymes in human microglia and macrophages. Adv Exp Med Biol 2003;**527**:105–112.
33. Sellgren CM, Kegel ME, Bergen SE et al. A genome-wide association study of kynurenic acid in cerebrospinal fluid: implications for psychosis and cognitive impairment in bipolar disorder. Mol Psychiatry 2015; doi: 10.1038/mp.2015.186 [Epub ahead of print].
34. Parrott JM, O'Connor JC. Kynurenine 3-monooxygenase: an influential mediator of neuropathology. Front Psychiatry 2015;**6**:116.
35. Furtado M, Katzman MA. Examining the role of neuroinflammation in major depression. Psychiatry Res 2015;**229**:27–36.
36. Friedman EM, Irwin MR, Overstreet DH. Natural and cellular immune responses in Flinders Sensitive and Resistant Line rats. Neuropsychopharmacology 1996;**15**:314–322.
37. Caldwell CL, Irwin M, Lohr J. Reduced natural killer cell cytotoxicity in depression but not in schizophrenia. Biol Psychiatry 1991;**30**:1131–1138.
38. Carboni L, Becchi S, Piubelli C et al. Early-life stress and antidepressants modulate peripheral biomarkers in a gene-environment rat model of depression. Prog Neuropsychopharmacol Biol Psychiatry 2010;**34**: 1037–1048.
39. Strenn N, Suchankova P, Nilsson S et al. Expression of inflammatory markers in a genetic rodent model of depression. Behav Brain Res 2015;**281**:348–357.

https://doi.org/10.1017/neu.2016.31 Published online by Cambridge University Press