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Research report

Peripheral and cerebral metabolic abnormalities of the tryptophan–kynurenine pathway in a murine model of major depression

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ABSTRACT

Occurring both peripherally and centrally, the kynurenine pathway (KP) – an alternative pathway to 5-HT synthesis from tryptophan (TRP) – could be of particular value to better understand the link between peripheral changes of circulating levels of glucocorticoids (GC)/proinflammatory cytokines and altered neurotransmission observed in depressed patients. Indeed, it is activated by these mediators of stress and can produce several neuroactive compounds like quinolinic acid (QUIN) and kynurenic acid (KYNA) that can respectively increase and decrease glutamate concentration in brain. In order to characterize the role of both the peripheral and cerebral KP in the pathophysiology of depressive disorders, we used the Unpredictable Chronic Mild Stress (UCMS) to induce a depressive-like syndrome and we then measured the level of relevant TRP-KYN pathway metabolites: KYN, 3-hydroxykynurenine (3HK; precursor of QUIN) and KYNA. We also measured TRP-5HT pathway metabolites: TRP, 5-HT, 5-HIAA. We showed that UCMS increased TRP catabolism along the KP in the periphery. 5-HT and KYN were found to be strongly negatively correlated in all brain structures of control mice and of UCMS mice except in the hippocampus. More importantly we found that KYN was preferentially metabolized along the QUIN pathway at the subcortical level (amygdala/striatum) whereas, at the cortical level (cingulate cortex), the QUIN pathway was reduced. Considering the role of these metabolites on the glutamatergic neurotransmission, we propose that such KP alterations could participate to the cortical/subcortical glutamatergic alterations reported in depressed patients.

1. Introduction

The amino acid tryptophan (TRP) is metabolized mainly through two metabolic routes: serotonin (5-HT) and kynurenine (KYN) pathways. Much attention has recently been focused on the role of the kynurenine pathway (KP) in the pathophysiology of depressive disorders. Indeed, since several years, a KP up-regulation has increasingly often been reported in patients suffering from various types of depressive disorders such as major depression [39], bipolar disorders [35], major/minor depressive states related to immunotherapeutic treatments [8], pre- and post-partum depression [27,33], cardiovascular disease-related depression [46]. Such patients not only presented increased levels of KYN in plasma but also reduced levels of TRP leading to a raised KYN/TRP ratio. Interestingly, this phenomenon has been found to be positively correlated with the intensity of depressive symptoms [33]. The KP occurs both peripherally and centrally and is engaged by two enzymes: tryptophan-2,3-dioxygenase (TDO; EC 1.13.11.11) and indoleamine-2,3-dioxygenase (IDO; EC 1.13.11.52), which differ in their tissue localization and regulation [11]. TDO is predominantly expressed in the liver and constitutes the major source of KYN in non-inflammatory conditions. It is activated by glucocorticoids (GC) [10]. IDO is present both in brain (neurons, glial cells) and peripheral tissues (lung, spleen etc.) [21] and is stimulated by proinflammatory cytokines [7].

Abbreviations: UCMS, unpredictable chronic mild stress; TRP, tryptophan; KYN, kynurenine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; KP, kynurenine pathway; 3HK, 3-hydroxykynurenine; QUIN, quinolinic acid; ANA, anthranilic acid; 3-HANA, 3-hydroxyanthranilic acid; 5-HANA, 5-hydroxyanthranilic acid; PIC, picolinic acid; KYNA, kynurenic acid; IDO, indoleamine-2,3-dioxygenase; TDO, tryptophan-2,3-dioxygenase; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; NMDA, N-methyl-D-aspartate; CC, cingulate cortex; HIPPO, hippocampus; AMY, amygdala; STR, striatum; MDD, major depressive disorder; DNA, deoxyribonucleic acid; CNS, central nervous system; HPLC, high performance liquid chromatography.

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High levels of circulating GC and proinflammatory cytokines are two common features of depressive disorders [6,34], that is why it was postulated that the activation of the peripheral KP through TDO/IDO could be of importance in precipitating depressive symptoms by depleting plasma TRP and then inducing serotonin-depletion-related disorders such as depression [38,28]. But this hypothesis has never been clearly demonstrated. It has also been assumed that depressive features might likely be related to the couple of downstream neuroactive compounds further produced along the KP. Among these, the N-methyl-D-aspartate (NMDA) agonist quinolinic acid (QUIN) has been shown to enhance synaptosomal glutamate release and to inhibit the glutamate uptake into astrocytes, resulting in an increased glutamate concentration into the synaptic cleft and at the perisynaptic level [47]. On the contrary, kynurenic acid (KYNA) has been shown to significantly decrease extracellular glutamate levels in rat brain through its antagonist activity on alpha7 nicotinic acetylcholine receptors (α7AChR) [51]. The QUIN pathway can subsequently be seen as a pro-glutamatergic metabolic route whereas the KYNA pathway can exert an anti-glutamatergic effect. Given that alterations of the glutamatergic neurotransmission have been reported in the brain of depressed patients [43,22,23], it has subsequently been proposed that cerebral changes in the balance between these pathways could be implicated in such pathological processes by locally modifying neurotransmission or even by inducing neurotoxic processes [30]. In line with this are the clinical studies showing a reduced activity of the KYNA pathway in depressed patients [39,48], contributing to the recently proposed "neurodegenerative theory of depression" [30]. However, the above data were obtained from patient's blood which is probably far from what happens into the brain and, consequently does not inform about intracerebral concentrations. Given that human brain investigation is very restricted, we explored the role of the KP in corticolimbic structures involved in the regulation of emotions and mood by using an animal model of depressive syndrome: the Unpredictable Chronic Mild Stress (UCMS). UCMS is an informative model to study depression in animals [49], as it mimics the role of socio-environmental stressors in precipitating a depressive-like pathology [44]. It has been shown that the murine model satisfied several criteria for a valid model of depression [45]. In addition it has often been reported that chronic stress procedure promoted a rise in circulating GC [24] and of proinflammatory cytokines [5], two pathomechanisms of interest regarding KP activation.

In respect of the above data, the aim of this study was first to verify if UCMS promoted an accelerated catabolism of TRP along the KP in the periphery by measuring *postmortem* peripheral levels of TRP, KYN and KYN/TRP ratio. We then checked the cerebral consequences on the 5-HT metabolism by examining 5-HT and 5-HIAA contents in the following corticolimbic structures: cingulate cortex (CC), hippocampus (HIPPO), amygdala (AMY) and striatum (STR). Finally, we verified if UCMS might promote local alterations of the balance between the QUIN and KYNA pathways by measuring the cerebral level of KYNA and 3HK, the two first metabolites of each route.

2. Materials and methods

2.1. Animals

Male BALB/c mice aged of 2 months at onset of the UCMS exposure were purchased from Centre d'Elevage Janvier (Le Genest Saint Isle, France). Animals were group-housed ($n = 5$ per cage) until the beginning of the UCMS regimen and maintained under standard laboratory conditions (12/12 h light–dark cycle on at 11:00 h/off at 23:00 h, 22 ± 1 °C, food and water *ad libitum*). All animal care and treatment were in accordance with the European Community Council directive 86/609/EEC.

2.2. Unpredictable chronic mild stress

The stress regimen was previously described [45] and is a variant of the chronic mild stress procedure described by Willner in rat [50]. Mice were repeatedly subjected to various socio-environmental stressors according to a "random" schedule for a total period of 6 weeks (Fig. 1A). UCMS-exposed mice were maintained under standard laboratory conditions but were isolated in small individual cages (24 cm × 11 cm × 12 cm) while non-stressed control mice were reared (four by cage) in larger laboratory cages (42 cm × 28 cm × 18 cm). The stressors were: altered bedding (sawdust change, removal of sawdust, damp sawdust, substitution of sawdust with 21 °C water, rat feces); cage tilting (45°); cage exchange (mice were positioned in the empty cage of another male); altered length and time of light/dark cycle, forced swimming.

2.3. Coat state and body weight

Body weight and fur coat state were assessed weekly, as markers of the progression of the UCMS-evoked syndrome. The total score of the coat state resulted from the sum of scores obtained from five different body parts: head, neck, dorsal coat, ventral coat, and hindpaws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat. Thus the more elevated is the total score the nastier is the fur. Dirty state is characterized by fluffy, greasy, less dense coat or piloerection. Both of these physical parameters have been pharmacologically validated [13,17,44,45].

2.4. Tissue sampling

2.4.1. Brain tissues

Brain structures were collected 3 days after the end of the stress procedure and were microdissected by a single investigator. Brains were rapidly removed from CO_2-killed mice and placed in ice-cold slurry of 0.9% NaCl. Rostro-caudal sections (2 mm) were quickly obtained on a brain tissue blocker. Four consecutive sections from Bregma +2.4 to −3.1 were then microdissected [41]. CC was dissected from the first two sections and included prelimbic and cingulate cortices. Dorsal and ventral parts of the STR were obtained from the second slice. AMY was obtained from the third section and HIPPO from the third and the fourth sections (Fig. 1B). All samples were immediately frozen and stored at −80 °C.

2.4.2. Peripheral tissues

Lungs were removed just after the brain was extracted from the mice skull and immediately frozen and stored at −80 °C. This tissue was chosen because of its high level of IDO expression [4,37].

2.4.3. HPLC measurements of TRP metabolites

TRP, 5-HT and 5-HIAA were measured using HPLC, as described by Kema et al. [26]. Metabolites of the kynurenine pathway: KYN, KYNA and 3HK were measured using HPLC, as described by Fujigaki et al. [15]. Absolute concentrations of each metabolic intermediate were normalized to pmol/mg using wet tissue masses for each extracted sample and were then compared among UCMS and control animals. Physiologically appropriate ratios of intermediate pairs were calculated using normalized pmol/mg data. This resulted in a series of transformation ratios that can be used to estimate the activity of transformation of a precursor compound to a product [52,37]. The use of transformation ratios helps give a more extensive description of the physiological processes occurring in each metabolic pathway.

2.5. Statistics

2.5.1. Group comparisons

All data were analyzed using non-parametric procedures, especially adapted to the statistical analysis of small samples ($n < 30$). Independent two-group comparisons were analyzed with the Mann–Whitney test. As significance test only gives the probability of obtaining the observed effect and not how large the effect is, we decided to report estimates of effect sizes using a robust index: the probability of superiority (PS), also called "probabilistic index" or "measure of stochastic superiority" [1,12,19,29]. Briefly, PS is the probability that a randomly sampled score from one population is larger than a randomly sampled score from another population. It is calculated as follows: $PS = U/mn$, where U is the Mann–Whitney U statistic, m is the number of individuals in the first sample, and n is the number of individuals in the second sample. PS values, ranging from 0.5 to 1, allow putting in evidence a small effect (from 0.5 to 0.64), a medium effect (from 0.65 to 0.71) or a large effect (from 0.72 to 1) [19]. An effect was considered significant when (1) $p < 0.05$ and (2) $PS > 0.64$. Spearman's rank correlations were calculated to describe associations between metabolites of interest. All data were analyzed with Statistica 6.1 software.