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Short Communication

The impact of electroconvulsive therapy on the tryptophan–kynurenine metabolic pathway

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ABSTRACT

Background: There is still limited knowledge about the mechanism of action of electroconvulsive therapy (ECT) in the treatment of depression. Substantial evidence suggests a role for the immune-moderated tryptophan (TRP)–kynurenine (KYN) pathway in depression; i.e. a depression-associated disturbance in the balance between the TRP–KYN metabolites towards a neurotoxic process. We, therefore, aimed to investigate the impact of ECT treatment on the TRP–KYN pathway, in association with ECT-related alterations in depressive symptoms.

Method: Twenty-three patients with unipolar or bipolar depression, treated with bilateral ECT twice a week were recruited. Blood serum samples, and depression scores using the Hamilton Depression Rating Scale-17 items (HDRS) as well as the Beck Depression Inventory (BDI) were collected repeatedly during the period of ECT and until 6 weeks after the last ECT session. TRP and KYN metabolites were analyzed in serum using the High Performance Liquid Chromatography. Four patients could not complete the study; thereby yielding data of 19 patients. Analyses were performed using multilevel linear regression analysis.

Results: There was an increase in kynurenic acid (KYNA) ($B = 0.04$, $p = 0.001$), KYN/TRP ratio ($B = 0.14$, $p = 0.001$), KYNA/KYN ratio ($B = 0.07$, $p < 0.0001$), and KYNA/3-hydroxykynurenine ratio ($B = 0.01$, $p = 0.008$) over time during the study period. KYN ($B = -0.02$, $p = 0.003$) and KYN/TRP ($B = -0.19$, $p = 0.003$) were negatively associated with total HDRS over time. Baseline TRP metabolite concentrations did not predict time to ECT response.

Conclusion: Our findings show that ECT influences the TRP–KYN pathway, with a shift in TRP–KYN metabolites balance towards molecules with neuroprotective properties correlating with antidepressant effects of ECT; thereby providing a first line of evidence that the mechanism of action of ECT is (co)mediated by the TRP–KYN pathway.

1. Introduction

Evidence is scarce about the mechanism of action of ECT (Sienaert, 2014), which appears to comprise a complex network of effects on neurotransmitters (Baldinger et al., 2014), neurohormones (Haskett, 2014), and epigenetic changes (de Jong et al., 2014). Current evidence suggests differential effects on molecular mediators of the immune system by single versus repetitive sessions of ECT (Guloksuz et al., 2014); single sessions of ECT have been associated with upregulation or activation of the immune system (Fluitman et al., 2011; Lehtimaki et al., 2008), whereas repeated ECT sessions appear to lead to downregulation or suppression of the immune system (Hestad et al., 2003; Rotter et al., 2013).

The disturbed functioning of tryptophan (TRP) metabolic pathway has been proposed as one of the crucial links between the aberrant immune functioning and the neurotransmitter deregulation involved in depression (Dantzer et al., 2011; Myint and Kim, 2003; Myint et al., 2012). In physiological conditions, nearly 90% of TRP is catabolized into kynurenine (KYN) by tryptophan dioxygenase, while the activity of indoleamine 2,3-dioxygenase

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(IDO) is negligible. However, in cases of interferon (IFN)-γ and tumor necrosis factor (TNF)-α upregulation (such as is seen in inflammatory states, or with IFN-α treatment), the activity of IDO is induced, which in turn increases TRP turnover, and KYN accumulation (Myint, 2012b; Raison et al., 2010; Wichers et al., 2005). Moreover, and maybe more hazardously, it has been shown that immune activation, which induces both IDO and kynurenine-3-monooxygenase (KMO), can increase the production of several neurotoxic metabolites such as 3-hydroxykynurenine (3-HK) and quinolinic acid (QA) in microglia (see Supp Fig. S1 for a schematic illustration). 3-HK increases reactive oxygen species (ROS), lipid peroxidation; and QA is a N-methyl-D-aspartate (NMDA) receptor agonist. Another metabolite of KYN, kynurenic acid (KYNA), is produced in astrocytes and is considered to display neuroprotective properties through its antagonistic effect on NMDA receptors (Dantzer et al., 2011; Myint, 2012b). Depression has previously been related to increased TRP turnover (increased KYN/TRP) as well as an imbalance in the ratio between KYNA and KYN such that the ratio was shifted toward increased circulating neurotoxic metabolites in the KYN pathway (Myint et al., 2013, 2007). *In vitro* studies have furthermore indicated that deregulations in the KYN pathway in astroglial cultures can be partially normalized by antidepressants (Kocki et al., 2012; Myint, 2012a).

Given this suggestive evidence for a role of the TRP–KYN metabolic pathway in depression, we aimed to investigate whether ECT influences the TRP–KYN metabolic pathway in this longitudinal study, whether changes in TRP–KYN metabolites may relate to depression scores, and whether levels of TRP–KYN metabolites may predict response to ECT.

2. Methods

Twenty-three patients diagnosed with unipolar or bipolar depression according to *DSM-IV* were recruited from the Maastricht University Medical Centre. All patients were 'treatment-resistant'; in accordance with routine clinical guideline defined as the failure to produce significant clinical improvement (i.e. persistence of significant depressive symptoms) after at least two trials with antidepressants from different pharmacologic classes (adequate in dose, duration, and compliance). The study was approved by the Medical Ethics Committee, and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient prior to participation. Exclusion criteria were: age <18 and >65 years, illiteracy, major medical or psychiatric conditions that may interfere with the study procedures: cancer, cerebrovascular disorders, organic psychiatric syndromes, active drug abuse, mental retardation, dementia, neurodegenerative disorders, presence of an inflammatory condition, and regular use of immune-modulating medications (e.g. corticosteroid, non-steroid anti-inflammatory drugs). Two patients decided to leave the study on their own accord after the initial evaluation and 2 patients were excluded from the study due to (cardiovascular) side effects at first ECT session. Therefore, the final sample consisted 19 patients (12 major depression, and 7 bipolar depression). Only 3 patients had received ECT treatment previously.

Bilateral ECT with bifrontotemporal electrode placement was administered using Thymatron IV (brief pulse stimulation (0.5 ms) and fixed dose at start of 350 mC). ECT was administered twice a week, on Monday and Friday, with a mean of 6.1 sessions (range 3–11 sessions). Mean duration of EEG seizure time was 52 s (range, 10–200 s); the average charge applied was 462 mC (range, 150–900 mC). Etomidate (0.1–0.2 mg/kg) was used for anesthesia, and succinylcholine (0.5–1.0 mg/kg) for muscle relaxation. Medications used by patients, including antidepressants, were continued during ECT, with the exception of benzodiazepines (which, according to routine medical care, was stopped before the start of ECT) (Supplementary Table S1).

The Hamilton Depression Rating Scale-17 items (HDRS) and the Beck Depression Inventory (BDI) were administered before the first ECT session (baseline), and each week during the ECT treatment period. Clinical assessments were administered on days without ECT sessions to avoid possible confounding of acute and transient impact of ECT (including the effects of anesthesia) on cognition. Blood samples were collected after overnight fasting before the first ECT session (i.e. baseline) and subsequently once a week before the ECT session (i.e. every other ECT session) during the treatment period. The treating psychiatrist made the decision to stop ECT treatment when no clinical improvement was observed, or when complete remission was obtained. Thus, this resulted in a different number of treatments in each subject. After the ECT period, the clinical assessments and blood samplings were repeated every other week up to 6 weeks.

2.1. Determination of tryptophan–kynurenine metabolic pathway metabolites

Serum was separated immediately and stored at −80 °C. High performance liquid chromatography (HPLC) was used to measure serum levels of TRP, KYN, KYNA, 3-HK, 3-hydroxyanthranilic acid (3-HAA) and 5-hydroxyindoleacetic acid (5-HIAA). The measurement was performed according to the method of (Herve et al., 1996) with some modifications. The recently published method using HPLC (Oades et al., 2010a,b) was used to measure 3-HK.

KYN was detected spectrophotometrically at 365 nm. KYNA was detected fluorimetrically at an excitation wavelength of 334 nm and an emission wavelength of 388 nm. KYNA was analyzed in serum that was deproteinized using perchloric acid. 3-HK was measured at a wavelength of 365 nm by UV detection. All analyses were conducted using HPLC with a reverse phase c-18 column. The 3-HK analysis method has been validated showing an absolute recovery of 85.8%, intra-day precision of 3.9%, and inter-day precision of 7.5%; time series demonstrated perfect stability of the analyte 3-HK during our extraction and analysis steps. The intra and inter-assay coefficients of variation ranged from 5% to 7% for all of the metabolites.

The ratio between serum KYN and TRP concentrations was used to estimate TRP degradation (Myint et al., 2007). The ratio between serum KYNA and KYN concentrations was used to determine how much KYN was catabolized into KYNA (Myint et al., 2007). The ratio between serum KYNA and 3-HK concentrations was used to determine the balance between the two arms ("neuroprotective" versus "neurotoxic") of the KYN pathway (Oades et al., 2010a,b). The ratio between 5HIAA and KYN (5HIAA/KYN) was also calculated indicate the balance between TRP breakdown into KYN and synthesis of TRP into serotonin in the form of its stable metabolite, 5HIAA.

2.2. Statistical analysis

The data were analyzed using STATA version 12.0 (StataCorp, 2011). For the longitudinal analyses, multilevel linear regression analysis was applied using the XTREG command. This multilevel model takes into account that level-1 units (individual observations) are clustered into level-2 units (subjects). Effect sizes of explanatory variables were expressed as regression coefficients (*B*), which can be interpreted identically to the estimate in the unilevel linear regression analyses. To examine the effect of time in days on total HDRS, total BDI and TRP metabolite concentrations and ratios (TRP, KYN, KYNA, 3-HK, 3-HAA, 5-HIAA, KYN/TRP, KYNA/KYN, KYNA/3-HK, 5-HIAA/ KYN), cubic, quadratic and linear

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