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Research report

A ^{1}H-NMR plasma metabonomic study of acute and chronic stress models of depression in rats

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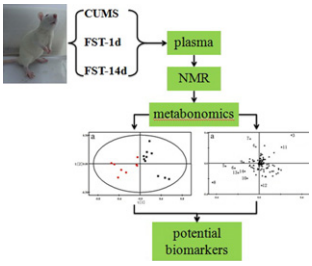
HIGHLIGHTS

- Three depression models of CUMS, FST and FST-14d were investigated.
- Behavior research and ^{1}H NMR metabonomics were used.
- CUMS model is more appropriate than FST or FST-14d for the mechanism study.
- FST-14 model can partly substitute the CUMS for the evaluation of antidepressant.

GRAPHICAL ABSTRACT



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ABSTRACT

To investigate and compare the metabonomic profiles of three stress-based models of depression, the effects of acute and chronic stress on the production of systemic endogenous metabolites were investigated. Such metabonomic analysis may provide researchers a new way of selecting appropriate animal models for the study of depression and antidepressants. Rats were subjected to one of three stress-based models: CUMS, FST-1d, or FST-14d. Endogenous metabolites excreted in plasma were analyzed using NMR in conjunction with multivariate and statistical techniques. The metabonomic study indicated that the concentration of different plasma metabolites could be used to differentiate among depression models: TMA, aspartic acid, glutamate, AcAc, NAc, alanine, lactate, Leu/Ile, lipids increased and proline, β-HB, valine decreased in the CUMS model; TMA decreased in the FST-1d model; α-glucose, β-glucose, β-HB, valine and lipids increased in the FST-14d model. The results suggested that metabonomics is a potentially appropriate method for evaluating depression models. According to the metabonomics study, CUMS model was more suitable and sensitive than the acute FST-1d model and predictable FST-14d model. The CUMS model was more appropriate for investigating both the efficacy of antidepressants and their mechanisms of action, while the FST-14d model should only be used for evaluating the efficacy of treatment.

Abbreviations: AcAc, Acetoacetic acid; BCAAs, Branch chain amino acids; β-HB, β-hydroxybutyric acid; CPMG, Carr-Purcell-Merboom-Gill; CUMS, Chronic unpredicted mild stress; D_2O, Deuterium Oxide; FST, Forced swim test; Leu/Ile, Leucine/Isoleucine; NAc, N-acetyl glycoprotein; NIH, National Institutes of Health; NMR, Nuclear magnetic resonance; PCA, Principal component analysis; PLS-DA, Partial least squares-discriminate analysis; TCA, Tricarboxylic acid cycle; TMA, Trimethylamine.

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1. Introduction

Depression is a common mental disorder characterized by pronounced and long-lasting depressed mood, as well as a variety of additional symptoms (behavioral, affective, and cognitive). Depression also carries a high risk of relapse [1,2]. The number of patients with depression has increased dramatically in recent decades. However, direct study of the neurochemical or neurobiological mechanisms of depression in the human body and the effects of antidepressants on these mechanisms remained unfeasible.

Additional studies of the development and manifestation of depressive disorders have strengthened the evidence linking depression to stress [3]. Most animal models of depression involve stress, such as the forced swim test (FST), tail suspension test (TST), learned helplessness (LH) and chronic unpredicted mild stress (CUMS) [4,5]. The CUMS model was established largely to induce depression-like behavioral changes [6], but implementing the CUMS model was both complicated and time-consuming. The FST model was established to induce despair or helpless behavior in animals by placing them within an inescapable and confined space [7]. This model was relatively easy to employ and took less time. However, it remained unknown how endogenous metabolites in plasma changed after rats were exposed to the different stressors. Whether the FST model could be substituted for the CUMS model was also an open and interesting possibility.

Metabonomics is a powerful top-down systems biological tool for studying the multivariate metabolic responses of complex organisms to stimulation or drugs [8]. The application of metabonomic techniques has been involved in the evaluation of plant species classification [9], drug discovery [10], toxicological mechanisms and disease mechanisms. Metabonomics is a perfect approach for measuring global changes in the profiles of biofluids (e.g., blood, urine and saliva) or tissues (e.g., liver and brain) in individuals or animals [11]. Metabonomic profiles can be achieved by high-yield sample analysis using technologies such as nuclear magnetic resonance (NMR) spectroscopy, gas chromatography–mass spectrometry (GC–MS) and high performance liquid chromatography–mass spectrometry (HPLC-MS), followed by pattern recognition statistics (multivariate data analysis) [12]. As a rapid, non-destructive and high-throughput method, NMR spectroscopy was used widely and frequently [13]. Multivariate statistical analysis methods including principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA) and orthogonal-projection to latent structure-discriminate analysis (O-PLS-DA) have been applied [8].

In this study, rats were used to replicate the FST and CUMS models of depression. A metabonomic study based on ^{1}H-NMR was then conducted to discriminate between the different depressive animal models. The critical metabolites that differed between models were identified and could serve as a foundation both for studying mechanisms of depression and in the selection of appropriate animal models.

2. Material and methods

2.1. Animals and housing

Male S.D. rats (weighing 160–200 g) were commercially obtained from Vital River Laboratories SCXK (Jing) 2006–0008. The animals were maintained under a standard 12 h light/dark cycle (lights on at 8:00 a.m.) at a constant temperature of 24 ± 1 °C and relative humidity of 45 ± 15%, with free access to food and distilled water except for periods of water and food deprivation. All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Maximal effort was made to minimize animal suffering and the number of animals necessary for the acquisition of reliable data.

2.2. Models and sample collection

2.2.1. Rat CUMS model

Twenty rats were randomly divided into two groups (n = 10): the control group (N1) and the CUMS model group. Rats in the CUMS model group were repeatedly exposed to a series of chronic unpredictable mild stressors that included the following [14]: 24 h food deprivation, 24 h water deprivation, exposure to an experimental room at 45 °C, swimming in cold (4 °C) water for 5 min, tail clamping for 2 min, foot shock for 2 min and exposure to ultrasonic (40 MHz) electromagnetic radiation for 3 h. One stressor was applied per day, and the entire stress procedure lasted for 4 weeks, with stressors applied in a completely random order. The rats of the control group (N1) were housed separately under the same conditions. Each week, rats were weighed, and the open-field test and sucrose preference test conducted, as previously described [15].

2.2.2. Rat FST-1d model

The FST-1d protocol was conducted as previously described [16], with slight modification. Eighteen rats were randomly divided into two groups (n = 9): the control group (N2) and the FST-1d model group. The forced swimming protocol consisted of an exposure in a cylindrical tank filled with water in which it was impossible to either touch the bottom of the tank or escape. The tank was made of transparent Plexiglas, 50 cm tall and 25 cm in diameter, and filled with water (25 ± 1 °C) to a depth of 40 cm. The water in the tank was changed after each test. Rats in the FST-1d model group were placed in the cylinder for a 5 min session, while rats in the control group (N2) were housed undisturbed under the same conditions.

2.2.3. Rat FST-14d model

The FST-14d protocol was carried out according to a previous report [17], with slight modification. Eighteen rats were randomly divided into two groups (n = 9): the control group (N3) and the FST-14d model group. The rats in the FST-14d model group were placed in the water for a 5 min session every day for 14 consecutive days, while the rats in the control group (N3) were housed undisturbed under the same conditions for 14 days.

2.2.4. Sample collection

Rats were sacrificed, and blood samples collected into centrifuge tubes 24 h after the end of the swim sessions in the FST models or 24 h after the last stressor in the CUMS model. Plasma was isolated by centrifugation for 15 min at 3000 rpm at 4 °C and stored at −80 °C for further analysis.

2.3. Plasma metabonomics

2.3.1. Sample preparation

Sample preparation was performed as previously described [18]. After thawing at 0 °C, 450 μL of plasma was mixed with 350 μL of D_2O. The mixture was then centrifuged at 13,000 rpm for 20 min at 4 °C, and a 600 μL aliquot of the supernatant was pipetted into a 5 mm NMR tube. The prepared samples were kept at 4 °C until NMR analysis.

2.3.2. ^{1}H NMR data acquisition

The ^{1}H NMR spectra of the plasma samples were recorded at 300 K on a Bruker 600-MHz AVANCE III NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at a ^{1}H NMR frequency of 600 MHz. The NMR spectrum was acquired using a Carr-Purcell-Merboom-Gill (CPMG) pulse sequence with water suppression and a total spin-spin relaxation delay (2nτ) of 320 ms to attenuate broad signals from proteins and lipoproteins due to their long transverse relation time [19].

2.3.3. Data processing

All spectra were manually phased and baseline corrected using MestReNova software (Mestrelab Research, Santiago de Compostella, Spain). Chemical shifts were referenced to creatinine at δ 3.04. The partial linear fit algorithm was applied to overcome the peak shift problem for the spectra [20]. Regions containing resonance from residual water (δ 4.7–5.0) were excluded. Each spectrum was then segmented at 0.04 ppm intervals across the chemical shift 0.50–5.50; each data point was normalized to the sum of its row and then exported as a text file for further multivariate statistical analysis.

2.3.4. Statistical analysis

The reduced and normalized NMR spectral data were imported into SIMCA-P 11.0 software. PLS-DA was performed to detect the distributions and separation of different groups, after which, scores plots and loading plots were obtained. A post hoc t-test by SPSS 17.0 was conducted on each group to determine if the average for the control group differed significantly from that of the corresponding model group using the 95% confidence interval. The result was a "t-test filtered difference spectrum" for each model group, with positive-going peaks corresponding to metabolites that increased after stress-exposure and negative-going peaks corresponding to metabolites that decreased [21].