Biochemical mechanism studies of venlafaxine by metabonomic method in rat model of depression

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Abstract. – BACKGROUND: Venlafaxine is a new antidepressant that has a chemical structure and neuropharmacological profile distinct from those of existing antidepressants. The studies about the mechanism of pharmacological action of venlafaxine mostly investigated the effects of venlafaxine on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) levels, while only few studies examined the effects on the metabolites levels and ratio of these monoamines neurotransmitters.

AIM: To study the biochemical mechanism of venlafaxine through determining the metabolism of monoamine neurotransmitters in brain tissues of rat model of depression after administration of venlafaxine using metabonomic method.

MATERIALS AND METHODS: The rat model of depression was established by using the methods of separation and chronic unpredictable stress. We have determined 5-HT, NE, DA and their metabolites, i.e., 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenylglycol (MHPG) sulfate, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat brain tissues by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) following chronic administration of different venlafaxine doses (8, 16, 32 mg·kg⁻¹) and saline solution for 14 days. Linear discriminant analysis (LDA) and principal components analysis (PCA) were used in data analysis of metabonomic.

RESULTS: Compared with saline, venlafaxine could significantly increase brain 5-HT and NE levels at middle dose (16 mg·kg⁻¹) or high dose (32 mg·kg⁻¹), especially at middle dose. These increases were greater than those seen with the comparable dose of selective serotonin reuptake inhibitor (SSRI), fluoxetine, under the same experimental conditions.

CONCLUSIONS: Venlafaxine lowers brain neurotransmitter metabolite levels by decreasing brain neurotransmitters turnover. Venlafaxine could correct the disorder of neurotransmitter’s metabolism and coordinate the balance of 5-HT, NE and DA to reach the anti-depressed function.

Key Words: Venlafaxine, Depression model, Neurotransmitter, Metabonomics

Introduction

Venlafaxine is a new antidepressant that has a chemical structure and neuropharmacological profile distinct from those of existing antidepressants, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors. The neurochemical characteristics of venlafaxine include inhibition of serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) synaptosomal reuptake and a somewhat weaker inhibition of dopamine (DA) reuptake in vitro. It is the first inhibitor that inhibits the reuptake of both 5-HT and NE (SNRIs). Because of its unique pharmacological properties, venlafaxine provides positive clinical efficacy that is comparable or superior to that of TCAs and SSRIs but has a side effect profile resembling that of SSRIs. It has become a first-line drug for the treatment of major depressive disorders and obtained more and more attention

So far, the studies about the mechanism of pharmacological action of venlafaxine mostly investigated the acute or chronic effects of venlafaxine on 5-HT, NE and DA levels in rat brain in vivo. A single administration dose of venlafaxine (1, 4 and 8 mg·kg⁻¹, i.p.) produced an increase in extracellular levels of 5-HT and NE
Another study reported that there was a significant increase in extracellular NE and 5-HT concentrations with no increase in DA extracellular concentrations in the hippocampus of rats after an acute dose of 20 mg·kg\(^{-1}\) (i.p.) venlafaxine\(^4\). Acute administration of venlafaxine (3-50 mg·kg\(^{-1}\)) produced a significant dose-dependent increase in extracellular NE in the frontal cortex following s.c. injection reaching a maximum value at 50 mg·kg\(^{-1}\). Conversely, these increases in NE were not paralleled by 5-HT, which showed no increase in extracellular concentrations at doses up to and including 50 mg·kg\(^{-1}\). While, one study\(^6\) demonstrated that the effects of acute i.p. administration of various doses (5-20 mg·kg\(^{-1}\)) of venlafaxine on extracellular 5-HT levels in frontal cortex and hippocampus were dose-dependent and chronic venlafaxine (5 mg·kg\(^{-1}\), i.p. daily for 4 weeks) did not elevate basal 5-HT levels in either cortex or hippocampus. Millan et al.\(^7\) showed that venlafaxine could elevate extracellular 5-HT and NE levels in the frontal cortex of freely moving rats at a low dose (0.63 mg·kg\(^{-1}\), s.c.), and elevate DA levels at 2.5 mg·kg\(^{-1}\). In the nucleus accumbens and striatum, venlafaxine provoked a pronounced increase in dialysis levels of 5-HT, whereas DA levels were only marginally affected at 10 mg·kg\(^{-1}\). A comparable pattern of data was obtained for venlafaxine: the influence of its acute administration upon 5-HT, NE, and DA levels in frontal cortex was not significantly modified by chronic administration of venlafaxine for 2 weeks. Another previous study\(^8\) showed that venlafaxine (10 mg·kg\(^{-1}\), i.p.) increased the efflux of 5-HT, NE and DA levels in the prefrontal cortex. The levels of 5-HT increased to 310%, an effect approximately twice the effect on NE in the hippocampus. Venlafaxine also produced a moderate increase in DA levels in the prefrontal cortex but had no effect in the hippocampus.

However, the effects of venlafaxine on the metabolites levels and ratio of these monoamines neurotransmitters have not yet been examined in the above experiments which were done in normal animals. In fact, the metabolism change can also affect the levels of monoamines neurotransmitters. 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenylglycol (MHPG), 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are the main acidic metabolites of 5-HT, NE and DA, respectively\(^9\). Only in one study\(^10\), cerebrospinal fluid (CSF) levels of 5-HIAA, 5-HT, MHPG, HVA, and DOPAC were examined in 14 never-hospitalized outpatients with unipolar depression. It found that 9 of 14 patients showed a significant decrease (42%) in their CSF 5-HIAA concentrations after at least 6 weeks treatment with venlafaxine, but no change in other CSF measures.

In the present study, we used metabonomic method to study the biochemical mechanism of venlafaxine through determining the change of the metabolism of 5-HT, NE, DA in brain tissues of rat model of depression after administration of venlafaxine, and try to find out the mechanism of pharmacological action of venlafaxine from the metabolic profile of biomarkers.

**Materials and Methods**

**Test Animals and Model Preparation**

60 male albino Sprague-Dawley rats (body weigh 170–200 g) were purchased from the Animal Department of Second Xiangya Hospital (Changsha, China). Animals were divided into normal control group (group I), model group (group II), low dose of venlafaxine group (group III), middle dose of venlafaxine group (group IV), high dose of venlafaxine group (group V) and fluoxetine group (group VI) with 10 rats in each group by randomized block design according to the results of Open-field test. All rats were housed under constant temperature environment (20–22°C) with a regular 12 h light/dark cycle and were free access to food drinking water (except accepting stress in the depression model preparation).

According to references\(^12\)–\(^13\), normal control group rats were raised in two cages (5 per cage) and not given any stress, while isolated-living condition and chronic mild unpredictable stress (CUMS) were given to the other 5 groups rats to set up depression model. 21-day stress exposure paradigm, which involved relatively harsh stressors, including immobilization (3 h), swimming in cold water (4°C, 5 min), restricted food access (24 h), restricted water access (24 h), clamp tail (1 min), alterations of the light-dark cycle (24 h), weaver (160 Hz, 5 min), hot environment (45°C, 5 min), twice feet-electric shock (36 V, 10 s per time). Following such exposure, animals exhibited a persistent reduction in responsiveness to pleasurable stimuli, measured by a decrease in their consumption of a 1 percent sucrose solution. In addition, a number of behavioral changes were seen in these animals which were assessed...
with Open-field test. There were 56 model rats used in the experiment at last because 4 rats died in the process of model preparation.

The procedures used in this study were carried out according to the Chinese Guidelines on Animals experimentation (State Science and Technology Commission, #2, November 14, 1988) and were approved by the Ethics Committee of the Second Xiangya Hospital of Central South University.

**Test Drugs and Reagents**

Venlafaxine was purchased from Chengdu Daxinan Pharmaceutical Co., Ltd. (Chengdu, Sichuan). Batch Number: 050812. Fluoxetine was purchased from Eli Lilly and Company (Madrid, Spain). Batch Number: A158883. 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (DA), 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenylglycol sulfate (MHPG sulfate), and homovanillic acid (HVA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 3, 4-dihydroxyphenylacetic acid (DOPAC) was purchased from Aldrich Chemical Co. (Steinheim, Germany).

**Measurement of Whole Brain Biogenic Amines and their Metabolites**

Quantification of levels of 5-HT, NE, DA and their metabolites 5-HIAA, MHPG sulfate, DOPAC, HVA in rat brain tissue samples was achieved by using a protocol extensively described previously. LC-MS/MS analyses were carried out on a Waters Acquity Ultra Performance LC system (Waters, Milford, MA, USA) with a Micromass Quattro Premier XE tandem quadruple mass spectrometer (Waters, Manchester, UK) equipped with electrospray ionization (ESI). The analytes were separated on a Thermo C18 column (4.6 mm × 250 mm, 5 µm, SN: 1245575T, Thermo electron corporation, USA) with a mobile phase of 0.05% formic acid/acetonitrile (92: 8 for ESI+, 82: 18 for ESI, v/v) at the flow-rate of 0.8 mL·min⁻¹. MS acquisition of 5-HT, NE and DA was performed in electrospray positive ionization multiple reaction monitoring (MRM) mode, while electrospray negative ionization MRM mode was used to monitor their metabolites. Data acquisition was carried out by MassLynx™ 4.1 software.

**Experimental Procedure**

After 21-day stress exposure, normal control group rats were continued to be raised common-ly, and rats of other groups were still in isolated-living conditions. Rats in group I and II were treated by saline and rats in group III, IV, V, VI were treated with low, middle, high dose (8, 16, 32 mg·kg⁻¹) of venlafaxine and fluoxetine (2 mg·kg⁻¹), respectively. All drugs were dissolved in saline and were intragastric administrated daily for 2 weeks. On the morning of the 15th day, all rats were decapitated simultaneously and the whole brain was separated out quickly on ice, and then thrown into liquid nitrogen immediately. All samples were frozen at -80°C until biochemical analysis.

**Statistical Analysis**

The data of all analytes are expressed as the mean ± SD (range). All data (the within group quantities of the analytes collected before administration of drug and those collected after administration) were analysed with analysis of variance (ANOVA) for completely random design followed by LSD-test. For all statistical analyses, a p value of 0.05 or less was considered statistically significant. Metabolic profiles were statistical processed by linear discriminant analysis (LDA) and principal components analysis (PCA) which use the concentration of 5-HT, NE, DA, 5-HIAA, MHPG, DOPAC and HVA as variables.

**Results**

**Comparison Between Rats in Normal Control Group and Model Group**

Compared to normal control group, just 5-HT levels in model rat brains decreased significantly (p < 0.05), while there were no significant difference of other neurotransmitters levels and the ra-
ratio of them between two groups. The two groups could not be distinguished obviously according to signal parameter. However, they could be distinguished after statistical processing by linear discriminant analysis (LDA) on metabolic profiles (Figure 1). Data of model group were scattered shown that there was individual variation in rats and depression degree caused by chronic mild unpredictable stress was different.

**Effect of Venlafaxine on 5-HT, NE, DA, 5-HIAA, MHPG, DOPAC and HVA in Rat Brain (Table I)**

After administration of drugs, compared to group II, no significant difference was found in the neurotransmitters levels in group III. In group IV, 5-HT and NE levels increased significantly ($p < 0.01$), while 5-HIAA levels decreased significantly ($p < 0.05$) and MHPG levels decreased ($p > 0.05$); DA levels increased while DOPAC and HVA levels decreased with no significant difference. In group V, 5-HT and 5-HIAA levels increased significantly ($p < 0.05$); NE levels increased while DA, MHPG, DOPAC and HVA levels decreased with no significant difference. In group VI, no significant difference was found in neurotransmitters levels. The concentrations of neurotransmitters were converted into two-dimensional data and then were analyzed by PCA (Figure 2). As is shown in the figure, the metabolic profile of model rats and rats treated with different dose of venlafaxine and fluoxetine can be clearly seen. Group III (low dose of venlafaxine), and group VI (fluoxetine) were overlapped with model group; while group IV (middle dose

**Table I. Comparison of monoamine neurotransmitters levels in rat brain tissues ($\mu$g ± SD, ng·g⁻¹).**

<table>
<thead>
<tr>
<th>Group</th>
<th>5-HT</th>
<th>NE</th>
<th>DA</th>
<th>5-HIAA</th>
<th>MHPG</th>
<th>DOPAC</th>
<th>HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>887.16 ± 362.67</td>
<td>1775.32 ± 58.39</td>
<td>680.52 ± 111.64</td>
<td>790.24 ± 131.11</td>
<td>194.68 ± 28.64</td>
<td>245.36 ± 119.35</td>
<td>133.20 ± 24.92</td>
</tr>
<tr>
<td>II</td>
<td>688.12 ± 71.51</td>
<td>1722.56 ± 225.67</td>
<td>608.12 ± 63.35</td>
<td>792.24 ± 64.10</td>
<td>195.60 ± 24.82</td>
<td>204.09 ± 39.52</td>
<td>118.68 ± 34.15</td>
</tr>
<tr>
<td>III</td>
<td>686.64 ± 79.10</td>
<td>1722.56 ± 225.67</td>
<td>608.12 ± 63.35</td>
<td>792.24 ± 64.10</td>
<td>195.60 ± 24.82</td>
<td>204.09 ± 39.52</td>
<td>118.68 ± 34.15</td>
</tr>
<tr>
<td>IV</td>
<td>939.51 ± 112.68**</td>
<td>3448.40 ± 1223.12**</td>
<td>621.30 ± 108.42</td>
<td>648.27 ± 82.95**</td>
<td>179.07 ± 28.43</td>
<td>170.09 ± 36.43</td>
<td>113.44 ± 16.23</td>
</tr>
<tr>
<td>V</td>
<td>921.80 ± 207.67*</td>
<td>2655.80 ± 411.22**</td>
<td>666.12 ± 92.22</td>
<td>933.44 ± 162.49*</td>
<td>202.00 ± 29.34</td>
<td>238.12 ± 30.61</td>
<td>113.44 ± 16.23</td>
</tr>
<tr>
<td>VI</td>
<td>843.82 ± 181.38</td>
<td>1892.93 ± 177.21</td>
<td>564.31 ± 71.36</td>
<td>705.47 ± 108.54*</td>
<td>179.07 ± 28.43</td>
<td>179.07 ± 28.43</td>
<td>113.44 ± 16.23</td>
</tr>
</tbody>
</table>

Note: In comparison with Group I, $p < 0.05$; in comparison with Group II, $p < 0.01$.
of venlafaxine) and group V (high dose of venlafaxine) could be separated from model group, especially group IV.

**Effect of Venlafaxine on the Ratio of Neurotransmitters in Rat Brain (Table II)**

After administration of drugs, compared to group II, no significant difference was found in the ratio of neurotransmitters in group III. In group IV, 5-HIAA/5-HT ratio and MHPG/NE ratio were significantly decreased ($p < 0.01$); there were no significant differences in neither (DOPAC+HVA)/DA ratio nor NE/5-HT ratio, but they both have decreasing trends. In group V, MHPG/NE ratio was significantly decreased ($p < 0.01$) and the decreasing trends of 5-HIAA/5-HT ratio and NE/5-HT ratio could be observed. In group VI, no significant difference was found in the ratio of neurotransmitters except the 5-HIAA/5-HT ratio was significantly decreased ($p < 0.01$).

### Table II. Comparison of the ratio of monoamine neurotransmitters in rat brain tissues ($\chi \pm SD$).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>5-HIAA/5-HT</th>
<th>MHPG/NE</th>
<th>(DOPAC + HVA/DA)</th>
<th>NE/5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>3.99 ± 1.47</td>
<td>0.12 ± 0.03</td>
<td>2.31 ± 0.46</td>
<td>3.40 ± 0.92</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>4.76 ± 0.50</td>
<td>0.11 ± 0.02</td>
<td>2.13 ± 0.34</td>
<td>4.10 ± 0.71</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>4.39 ± 0.65</td>
<td>0.10 ± 0.03</td>
<td>2.53 ± 0.90</td>
<td>3.68 ± 1.13</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>3.24 ± 1.20 **</td>
<td>0.06 ± 0.03 *ΔΔ</td>
<td>1.82 ± 0.62</td>
<td>3.98 ± 0.88</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>4.12 ± 0.64</td>
<td>0.08 ± 0.01 *ΔΔ</td>
<td>2.59 ± 1.42</td>
<td>3.89 ± 0.85</td>
</tr>
<tr>
<td>VI</td>
<td>9</td>
<td>3.50 ± 1.00 **</td>
<td>0.09 ± 0.02 *ΔΔ</td>
<td>2.10 ± 0.23</td>
<td>3.97 ± 0.84</td>
</tr>
</tbody>
</table>

Note: In comparison with Group I, *$p < 0.05$, **$p < 0.01$; In comparison with Group II, *$p < 0.05$, **$p < 0.01$. 

### Discussion

Metabonomics is the branch of science concerned with the quantitative understandings of the metabolite complement of integrated living systems and its dynamic responses to the changes of both endogenous factors (such as physiology and development) and exogenous factors (such as environmental factors and xenobiotics). As a holistic approach, metabonomics detects, quantifies and catalogues the time related metabolic processes of an integrated biological system, ultimately, relates such processes to the trajectories of the pathophysiological events. Now metabonomics has been established as an extremely powerful an analytical tool and hence found successful applications in many research areas including molecular pathology and physiology drug efficacy and toxicity gene modifications and functional genomics and environmental sciences\(^5\). Metabonomics can be applied at any stage of drug discovery and development processes, when used in one or more of the following setting: predictive biomarkers for drug-related effects in animal models; understanding of the biochemical mechanisms of action to target-organ or to target-organ pathologies in animal to man; developing biomarkers for toxicities in non-clinical development; and predictive biomarkers for drug-related effects in man during Phase II and Phase III clinical trials\(^6\). Metabonomics can be directly understood as the physiological and biochemical situation by its “metabolic profile” as a whole.

Depression has a lifetime prevalence of around 20%, and carries the second greatest burden of all illnesses in the industrialized countries\(^7\). The mechanism of depression is very complicated and the pathogenesis is still in the hypothesis stage. There are many theories on the aetiology of depression now, and the more received is classical
“monoamine hypothesis”

Clinical studies have not yet determined a common mechanism of action for antidepressant drugs, which have primary sites of action on a variety of different neurotransmitter systems. In our experiments, we used metabolomic method to study the metabolism changes of the neurotransmitters 5-HT, NE and DA in depression model rats after chronic administration of various doses of venlafaxine. The results showed that rats in control group and model group could be distinguished after LDA on metabolic profiles. On one hand, the quantities of neurotransmitters were insufficient, on the other hand, the metabolism of 5-HT was improved and the activity of serotonergic neurons was degraded in model rat’s brain.

In this study to examine the monoamines in rat brain after venlafaxine treatment for depression, we found that this antidepressant significantly increased brain 5-HT and NE levels at middle dose (16 mg·kg⁻¹) or high dose (32 mg·kg⁻¹), especially at middle dose. These increases were greater than those seen with the comparable dose of SSRI, fluoxetine, under the same experimental conditions. Fluoxetine just affected 5-HT system resulting in increase of 5-HT levels and decrease of 5-HIAA levels. Interestingly, venlafaxine decreased 5-HIAA levels at middle dose while increased 5-HIAA levels at high dose. In addition, there was no significant change in MHPG levels.

Our finding of decreased 5-HIAA and no change for MHPG during treatment with middle dose of venlafaxine (16 mg·kg⁻¹) stands in contrast to a large number of studies showing that regardless of whether the antidepressant inhibits reuptake of 5-HT, NE, or both, levels of the respective human CSF metabolites 5-HIAA and MHPG both decreased. But these results in consist with those of Little et al [11]. The mechanistic reason why MHPG is not decreased with venlafaxine remained to be clarified. Their explanation was that their sample size was too small to detect a difference in MHPG concentration. However, prior treatment studies with similar sample sizes showed 5-HIAA and MHPG both decreased. In fact, the MHPG levels have a certain degree decrease but not significant after chronic administration of 16 mg·kg⁻¹ venlafaxine in our experiment. It is possible that the sample size was too small as them said.

The observation of a high ratio of 5-HT/5-HIAA after venlafaxine treatment is explained by the 5-HIAA decrease and 5-HT increase with treatment. While MHPG/NE ratio is also high.
because of the NE increase and lack of change in MHPG with treatment even at high dose. It indicates that the change of NE system was much more than 5-HT system. Venlafaxine lower brain neurotransmitter metabolite levels by decreasing brain neurotransmitters turnover. Furthermore, NE/5-HT ratio decreased to approach a normal level in all treatment groups.

There was no effect on the quantity and ratio of neurotransmitters in depressed rat brain after chronic administration of low dose of venlafaxine (8 mg·kg⁻¹); the middle dose (16 mg·kg⁻¹) of venlafaxine could produce significant effect. These results could be reflected in PCA projection. It should be noted, however, that high dose of venlafaxine increased 5-HT and NE less than middle dose and even increased 5-HIAA levels significantly and increased MHPG levels more or less. Namely, the effect of venlafaxine on the quantity and ratio of neurotransmitters in depressed rat brain was not dose-dependent. It is consistent with pharmacokinetics of venlafaxine which is not dose-dependent too²⁴. One possible explanation for the observation could be that the effect of venlafaxine is saturated at high dose and the metabolism of neurotransmitters is improved due to feedback mechanisms. Another possible explanation could be that the strengthen of neurotransmitter reuptake inhibition of venlafaxine at high dose, results in large increase in extra-cellular 5-HT and NE levels, which in turn would feedback inhibit the synthetase of 5-HT (tryptophan hydroxylase, TPH) and the synthetase of NE (tyrosine hydroxylase, TOH) resulting in decrease of neurotransmitters levels.

Compared to control group, DA levels decreased despite there was no statistical significance in model group and treatment group except group of high dose of venlafaxine which suggested that DA may be associated with the onset of depression. As prior studies reported, venlafaxine is a relatively weak inhibitor of DA reuptake, so DA levels have a certain increase.

In this paper, we applied metabonomic method to study the biochemical mechanism of venlafaxine through determining the change of monoamine neurotransmitters and found that venlafaxine could adjust the relationship of monoamine neurotransmitters in many ways. It could correct the disorder of neurotransmitters’ metabolism and coordinate the balance of 5-HT, NE and DA to reach the anti-depressed function. However, the biochemical results obtained in the present study are based on the whole brain, and more researches are necessary on different part of brain, such as hypothalamus, hippocampus, striatum and cerebral cortex.

Acknowledgements

The Authors appreciate the statistical analysis assistance provided by Prof. Liang Yizeng and Doc. Chen Xian from Research center of modernization of Chinese Medicine of Central South University.

References


