Effects of Baicalin on the ATP Levels in the Prefrontal Cortex in Mice Exposed to Chronic Unpredictable Mild Stress

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Abstract

Depression is gradually becoming a primary mental disease threatening human health. It is urgent to clarify the pathogenesis of depression and find new effective natural antidepressants. This study aimed to investigate the antidepressant effects of baicalin and explore its potential mechanism in a mice model of depression induced by chronic unpredictable mild stress (CUMS). Following a 6-weeks CUMS exposure, the CUMS mice were treated with baicalin (10 mg/kg) and fluoxetine (20 mg/kg) for 4 weeks by oral gavage. The sucrose preference test (SPT) and forced swimming test (FST) were performed to evaluate the depression-like behaviors, and the levels of adenosine triphosphate (ATP) in the prefrontal cortex were detected. Moreover, the gene expression and enzyme activities related to the production of ATP and mitochondrial function were detected. The results indicated that the depression-like behaviors of mice induced by CUMS were improved by baicalin and fluoxetine. In addition, baicalin significantly increased the ATP content, the mRNA expression of hexokinase (HK), pyruvate dehydrogenase alpha (PDH α), isocitrate dehydrogenase (IDH), peroxisome proliferator-activated receptor γ coactivator-1 alpha (PGC1 α), and sirtuin 1 (SIRT1) in the prefrontal cortex. Furthermore, baicalin also increased the activity of respiratory chain complex I, V, and the level of mitochondrial membrane potential (MMP). In conclusion, the present results suggested that the antidepressant effect of baicalin may be partly mediated by accelerating the process of glycolysis and tricarboxylic acid (TCA) and improving the mitochondrial function to enhance the ATP level in the brain.

1. Introduction

Depression is mainly manifested as persistent low mood, anhedonia, energy loss, and sleep disturbance (Belmaker and Agam 2008; Willner, Scheel-Kruger, and Belzung 2013). Chronic external stresses have become the leading cause of depression in humans. The increasing number of patients with depression will inevitably become a mountain burden on the family and society (Secretariat, T 2012). Although the first-line clinical antidepressants are widely used, they have the disadvantages of slow onset, poor curative effect, low cure rate, and serious adverse reactions (Westergren, Narum, and Klemp 2019). Therefore, it is urgent to develop new effective antidepressants.

Increasing evidence showed that the pathogenesis of depression was extremely complicated (Malhi and Mann 2018). Previous studies mainly focused on the hypothesis of "monoamines deficiency", "hyperactivation of hypothalamic-pituitary-adrenal (HPA) axis", "inflammatory response", and "neurotrophic factor deficiency" (Schildkraut and Kety 1967; Barden 2004; Koo et al. 2010; Duman and Monteggia 2006). In recent years, the correlation between energy metabolism disorder and depression has been widely studied (Cao et al. 2013). In addition, increasing studies have shown that depression is closely related to mitochondrial dysfunction (Bansal and Kuhad 2016; Sharma and Akundi 2019). Recent research reported that the adenosine triphosphate (ATP) content in the brain was reduced dramatically in an animal model of depression (Detka et al. 2015; Guo et al. 2021). And the lower energy levels in the brain of depression patients and animal models could be significantly reversed by antidepressants (Glombik et al. 2020; Park et al. 2020; Weckmann et al. 2017; Lam et al. 2017). Moreover, direct intra-

cerebroventricular injection of ATP into the brain could significantly improve the depression-like behavior of chronic unpredictable mild stress (CUMS)-exposed mice (Cao et al. 2013). These findings suggested that improving the energy level in the brain may be one of the new strategies to treat depression.

Baicalin, the main active ingredient in *Scutellaria baicalensis* Georgi, possess multiple biological functions, including neuroprotective effects, anti-apoptotic and anti-inflammatory (Shen et al. 2016; Sowndhararajan et al. 2018; Li, Cheng, and Liu 2020). In addition, our previous studies have shown that baicalin can improve depression-like behaviors caused by chronic stress and repeated corticosterone injection (Li et al. 2013; Li et al. 2015). However, whether the antidepressant effects of baicalin are related to the regulation of energy metabolism in the brain is still unclear. Therefore, the present study was planned to investigate the effects of baicalin on the levels of ATP, glycolysis, tricarboxylic acid (TCA) cycle, and mitochondrial electron transport chain (ETC) in the prefrontal cortex and to explore the possible antidepressant mechanism of baicalin in CUMS-exposed mice.

2. Materials And Methods

2.1 Animals

Male C57BL/6N mice, 16–20 g, were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China). Mice were housed under a 12 h light/dark cycle with 23–25°C and 40–60 % humidity. Mice were acclimatized for one week with free access to water and food before the experiments. All animal experiments were performed following the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the Committee of Animal Care of Henan University of Chinese Medicine (DWLL16020024).

2.2 Drug administration

The mice were randomly divided into 2 groups: the control group (n = 9) and the stress group (n = 27). The stress group received CUMS for 6 weeks, and the control group was kept in a separate room without any stimulation. After week 6, the stress group was subdivided into 3 groups based on the equal sucrose preference: CUMS group, baicalin group (10 mg/kg), and fluoxetine group (10 mg/kg). The dose of baicalin was selected from our previous study, which found that 10 mg/kg of baicalin can produce an antidepressant effect in mice (Li et al. 2015). All drugs were suspended in saline and were administered by oral gavage daily in a volume of 10 mL/kg for 4 weeks. CUMS group received an equal volume of saline. The CUMS procedure still lasted during the period of drug administration.

2.3 CUMS procedure

The CUMS procedure was slightly modified from that previously described by Willner *et al.* (Willner et al. 1987). Briefly, stress group mice were individually fed in a cage and received 1–2 different stimuli every day. Stressors including day and night reversal for 24 h, food deprivation for 12 h, 45° tilting cage for 24 h, cold (4°C) or hot (45°C) water swimming for 4 min, white noise (90dB) for 12 h, soiled cage for 24 h, restraining for 3 h, flashlight (150 flashes/min) for 6 h, pair-housing for 2 h.

2.4 Sucrose preference test

The sucrose preference test (SPT) was carried out according to the protocol described in Liu *et al.* (Liu *et al.* 2018). Before the test, all mice were trained to consume two bottles of 1% sucrose solution for 24 h. Then, one bottle of sucrose solution and one bottle of freshwater were given to the mice for another 24 h. The SPT was performed after 12 h of fasting. A bottle of sucrose solution and a bottle of fresh water were weighed and given to the mice for 24 h. To avoid the position preference, two bottles were replaced every 12 h. At the end of the test, all bottles were reweighed. Sucrose preference was calculated as following: sucrose preference (%) = sucrose intake / (sucrose intake + water intake) × 100%.

2.5 Forced swimming test

The forced swimming test (FST) was carried out according to the protocol described in Porsolt *et al.* (Porsolt *et al.* 2001). The mice were placed in a 13 cm×25 cm (diameter × height) glass cylinder filled with 10 cm deep water (23–25°C). The mice were forced to swim in the water for 6 minutes, and the camera recorded the immobility time of the mice in the last 4 minutes.

2.6 Brain tissues collection

Thirty minutes after the last administration, all mice were sacrificed by decapitation. The prefrontal cortex was collected immediately and frozen by liquid nitrogen and stored at -80°C until analysis.

2.7 The extraction of the mitochondrion

The prefrontal cortex tissues were homogenized in a 10-fold volume lysis buffer (SM0020, Solarbio, Beijing, China). The homogenate was centrifuged at 1000×g for 5 min, and the supernatants were recentrifuged at 1000×g for 5 min. The supernatants were centrifuged at 12000×g for 10 min, and the supernatants were collected, which is the cytoplasmic extract. Then the precipitate was resuspended with wash buffer and centrifuged at 1000×g for 5 min. The supernatant was recentrifuged at 12000×g for 10 min, and the precipitate was resuspended with store buffer, which is the high-purity mitochondrial extract.

2.8 Biochemical analysis

The level of ATP in the prefrontal cortex was detected by ATP kit (S0027, Beyotime Biotechnology, Shanghai, China). The mitochondrial membrane potential (MMP) was detected by JC-10 kit (CA1310, Solarbio, Beijing, China), and the activity of mitochondria complex I-V were detected by kits (ab109721, ab109908, Abcam, Cambridge, UK; BC3245, BC0945, BC1445, Solarbio, Beijing, China). All procedure was carried out following the manufacturer's instructions.

2.9 Quantitative real-time polymerase chain reaction

The extraction of total RNA in the prefrontal cortex and cDNA synthesis were using commercial kits following the manufacturer's instructions (DP451, Tiangen, Beijing, China). Real-time PCR reactions were performed in the ABI-Q6 system (Applied Biosystems, USA) by SYBR Green kit (208054, Qiagen, Germany). The primers were synthesized by Invitrogen (Shanghai, China). The sequences of primers were

listed in Table 1. GAPDH was used to normalize the gene expression of SIRT1 and PGC1 α . Because GAPDH is one of the enzymes in glycolysis, β -actin were used to normalize the related gene expression involved in the glycolysis and tricarboxylic acid (TCA) cycle.

Table 1
The sequence of the primers for qRT-PCR

Gene name	Primer sequence	Accession number
HK	Forward: 5'- CTACCCGGAGTTGTTCTGCT-3' Reverse: 5'-CCCTAAGTCTCACTCCTGCC-3'	NM_013820.3
PFK	Forward: 5'- TGAGGATGGCTGGGAGAACT-3' Reverse: 5'-TGAACCACCAGATCCTTCACG-3'	NM_008826.5
PK	Forward: 5'- CTAGCGGTCCTTGGACTTCAGG-3' Reverse: 5'-ACAAATGATGCCAGTGTTGCG-3'	NM_001378869.1
PDH α	Forward: 5'- TCTGTCGGTTCCCAGTCCA-3' Reverse: 5'-CGTTTCCTTTTCACAGCACAT-3'	NM_008810.3
IDH	Forward: 5'- ATTAGACGCCAGCCAGTCG-3' Reverse: 5'-AGAAGTCGGTCCAAGAGAGC-3'	NM_001111320.1
OGDH	Forward: 5'- CCGTGCCCGCTGACATTATCT-3' Reverse: 5'-AGGCCATAGAACCCTCCTACTG-3'	NM_001252282.1
CS	Forward: 5'- TTTGTCTACCCTTCCCCTCA-3' Reverse: 5'-CAGGATGAGTTCTTGGCTCC-3'	NM_026444.4
PGC1 α	Forward: 5'- TGAAAAAGCTTGACTGGCGTC-3' Reverse: 5'-ACCAGAGCAGCACACTCTATG-3'	NM_008904.2
SIRT1	Forward: 5'- CGATGACAGAACGTCACACG-3' Reverse: 5'-ATTGTTTCGAGGATCGGTGCC-3'	NM_019812.3
GAPDH	Forward: 5'- TCTCCTGCGACTTCAACA-3' Reverse: 5'-TGTAGCCGTATTCATTGTCA-3'	NM_001289726.1
β -actin	Forward: 5'- ACTGAGCTGCGTTTTACACC-3' Reverse: 5'-GCCTTCACCGTTCCAGTTTTT-3'	NM_007393.5

2.10 Western blot

The mitochondrial and cytoplasmic proteins in the prefrontal cortex were extracted by the kit (SM0020, Solarbio, Beijing, China) and quantified by the BCA kit (CW0014S, CWBIO, Beijing, China). The protein samples were mixed with loading buffer and separated by SDS-PAGE. Then the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (IPVH00010, Millipore, Billerica, MA, USA) for blocking in 5% skim milk (BD Biosciences, Franklin Lakes, NJ, USA). The membranes were incubated with polyclonal rabbit primary antibodies in blocking solution at 4°C overnight, including anti-GAPDH (KC-5G5, Kangchen, Shanghai, China) and anti-COXIV (4850, Cell Signaling Technology, Danvers, MA, USA). The membranes were washed 3 times with PBST and incubated with goat anti-rabbit secondary antibody (KC-RB-025, Kangchen, Shanghai, China) for 30 min at room temperature. The immunoblotting bands were visualized by incubation with ECL reagent (WBLUF0100, Merck Millipore, Darmstadt, Germany) and exposed to X-ray film (6535876, Kodak, Rochester, NY, USA).

2.11 Statistical Analysis

The SPSS 24.0 software was used for statistical analysis. The *Shapiro-Wilk test* was used to analyze the normality of the data. Data were expressed as mean $\pm$ SD or median and interquartile range. After evaluating homogeneity of variance, the data were analyzed by one-way ANOVA followed by Turkey *post hoc* test. And the non-parametric data were analyzed by *Kruskal-Wallis test*. The $p < 0.05$ was considered statistically significant. The statistical analysis was performed by Ming Bai. She did not participate in the relevant experiment and was blinded to the experimental design.

3. Results

3.1 Baicalin reversed the depression-like behaviors in mice

To investigate the effects of baicalin on CUMS-induced depression-like behaviors in mice, the SPT and FST were performed. In the SPT (Fig. 1a), the sucrose preference was significantly reduced in CUMS group compared with the control group [$t(16) = 9.025, p < 0.001$], which was reversed by baicalin [$F(2, 24) = 12.818, p < 0.001$] and fluoxetine treatment [$F(2, 24) = 12.818, p = 0.023$]. As shown in Fig. 1b, the immobility time in FST was increased in CUMS group compared with the control group [$t(10.495) = -2.723, p = 0.021$]. Both baicalin and fluoxetine reduced the duration of immobility [$F(2, 24) = 13.892, p = 0.024, p < 0.001$]. These results indicated that the mice model of depression was successfully established and confirmed that baicalin can produce antidepressant effects in CUMS mice model.

3.2 Baicalin increased the ATP levels in the prefrontal cortex of mice

To investigate whether the antidepressant effects of baicalin were related to the regulation of the energy level in the brain, the ATP level in the prefrontal cortex was detected. Figure 2 showed that the level of ATP was significantly reduced in the prefrontal cortex in CUMS group [$t(8) = 4.148, p = 0.003$]. The reduced ATP level was significantly reversed by baicalin treatment [$F(2, 12) = 12.705, p = 0.007$], but not

fluoxetine [$F(2, 12) = 12.705, p = 0.604$]. These results indicated that baicalin could increase the level of ATP and then elevate the energy level in the prefrontal cortex of CUMS-induced mice.

3.3 Baicalin increased the level of glycolysis in the prefrontal cortex of mice

To investigate whether baicalin can affect the glycolysis level in the prefrontal cortex, we detected the mRNA expression of three key rate-limiting enzymes of glycolysis. As shown in Fig. 3, The mRNA expression of HK and PK were significantly down-regulated in the CUMS group [$t(8) = 7.744, p < 0.001$; $t(4.304) = 9.478, p < 0.001$] compared with the control group. And the mRNA expression of HK was significantly increased in the baicalin group and fluoxetine group [$F(2, 12) = 13.961, p = 0.048, p < 0.001$]. And the mRNA expression of PK was significantly decreased in the fluoxetine group [$F(2, 12) = 11.157, p = 0.042$]. Both baicalin and fluoxetine had no effects on the PFK mRNA levels. This result suggested that baicalin might accelerate the glycolysis process by upregulating the expression of HK to produce ATP.

3.4 Baicalin increased the TCA levels in the prefrontal cortex of mice

To investigate whether baicalin can affect the TCA level in the prefrontal cortex, the mRNA expression of four key rate-limiting enzymes in TCA was detected. The results were shown in Fig. 4. There were no significant changes between CUMS and control groups. However, the mRNA expression of PDH α and IDH were significantly up-regulated in the baicalin group compared with CUMS group {[Chi]² (2, N = 15) = 10.820, $p = 0.040$; [Chi]² (2, N = 15) = 9.420, $p = 0.033$ }. Although there was no statistical difference in the mRNA expression of CS and OGDH in the baicalin group, there was still a slight up-regulated in the mRNA expression level. The results indicated that baicalin could enhance the TCA level and thus promote the production of ATP.

3.5 Baicalin activated the electron transport chain in the prefrontal cortex of mice

To investigate whether the reduced energy levels resulted from the impairment of the mitochondrial electron transport chain, the activity of five mitochondrial respiratory chain complexes was detected. The results were shown in Fig. 5a-e. The activity of complexes II and V were significantly decreased in the CUMS group compared with the control group [$t(7.963) = 8.005, p < 0.001$; $t(6) = 15.528, p < 0.001$]. Interestingly, the activity of complex I was significantly increased by baicalin treatment [$F(2, 15) = 7.732, p = 0.013$], but the activity of complex II was further reduced in the baicalin and fluoxetine group {[Chi]² (2, N = 18) = 12.028, $p = 0.003, p = 0.032$ }. In addition, the activity of complex V was significantly increased in the baicalin and fluoxetine group {[Chi]² (2, 12) = 7.385, $p = 0.019, p = 0.019$ }. These results suggested that the effect of baicalin on complex I may be responsible for the increase of ATP level. The purity of the extracted mitochondrion was determined by western blotting (Fig. 5f, supplementary figure S2).

3.6 Baicalin increased the level of mitochondrial membrane potential in the prefrontal cortex of mice

To further investigate the effects of baicalin on mitochondrial function, the level of mitochondrial membrane potential (MMP) was detected. The result was shown in Fig. 6. The level of MMP was reduced in the CUMS-exposed mice [$t(8) = 3.409, p = 0.009$]. As expected, both baicalin and fluoxetine increased the level of MMP [$F(2, 12) = 19.807, p < 0.001, p = 0.005$]. This result indicated that baicalin could improve energy level by increasing the level of MMP in the prefrontal cortex of CUMS-induced mice.

3.7 Baicalin up-regulated the expression of mitochondrial related genes in mouse prefrontal cortex

To further clarify the energy metabolism disorder in the prefrontal cortex of depressed mice induced by CUMS at the transcription level, two key genes related to the regulation of mitochondrion were detected by qPCR. Compared with the control group, the mRNA expression of SIRT1 was significantly down-regulated in the CUMS group [$t(8) = 3.100, p = 0.015$]. Compared with the CUMS group, both PGC1 α and SIRT1 mRNA were significantly up-regulated in the baicalin group [$F(2, 12) = 7.495, p = 0.027; F(2, 12) = 9.396, p = 0.003$]. Fluoxetine had no effects on these gene expressions. The results suggested that baicalin might promote the PGC1 α and SIRT1 mRNA expression to improve the mitochondrial function in the prefrontal cortex of mice.

4. Discussion

The antidepressant effect of baicalin has been widely demonstrated in our and other previous studies and suggested that the mechanism may be related to brain-derived neurotrophic factor (BDNF), inflammation, and HPA axis (Li, Cheng, and Liu 2020; Li et al. 2013; Li et al. 2015). In the present study, the antidepressant effect of baicalin was confirmed again in CUMS-exposed mice, which was exhibited by the increased sucrose preference and the reduced immobility time in FST. Moreover, we found that baicalin significantly reversed the reduction of ATP in the prefrontal cortex induced by CUMS, which may be related to its antidepressant effect.

In recent years, the relationship between abnormal energy metabolism and depression has received increasing attention. Imaging studies have found that there were obvious disorders of energy metabolism in the brain of patients with depression (Kahl et al. 2020; Ernst et al. 2017), which were also confirmed in the brain of chronic mild stress (CMS)-exposed rats (Khan et al. 2018).

ATP is the most important energy source, and its content directly reflects the level of energy status. Previous studies have shown that the ATP content is lower in the brain of depression patients and animal models (Karabatsiakakis and Schonfeldt-Lecuona 2020; Kolar et al. 2021). And Jun *et al.* (Jun et al. 2018) reported that calcium homeostasis modulator protein 2 (Calhm2) knockout mice have a low content of ATP in the brain and exhibit depression-like behavior. Cao *et al.* (Cao et al. 2013) found that lateral

intracerebroventricular and intraperitoneal injection of ATP, or increase of endogenous ATP released by astrocytes, could significantly improve the depression-like behavior of mice induced by chronic social defeat stress (CSDS). In addition, the increasing ATP can alleviate synaptic damages, increase spine density and neural activity in the hippocampus region and improve the depression-like behavior of CUMS-exposed mice (Cao et al. 2013; Jun et al. 2018).

Together, these studies showed that increasing ATP might be a new strategy for treating depression. In the present study, we found that the ATP content in the prefrontal cortex was significantly reduced in CUMS group and was reversed by baicalin. Therefore, our results suggested that the increased ATP content by baicalin will be beneficial for maintaining the function of neurons, which may partly explain its antidepressant mechanisms.

Glycolysis is an important pathway for ATP production in organisms (Gu et al. 2021). A lower level of glycolysis was found in various neurodegenerative diseases, such as depression, Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (Xie et al. 2020; Bell et al. 2020). HK, PFK, and PK are three key rate-limiting enzymes in the glycolytic pathway (Hepkiss 2019). To observe the effect of baicalin on the glycolytic level, we detected the mRNA expression of these enzymes in the prefrontal cortex. We found that baicalin and fluoxetine significantly up-regulated the mRNA level of HK in the CUMS group, but not PK and PFK. This result indicated that the baicalin can up-regulate the expression of HK to accelerate glycolysis. Similarly, previous reports showed that the level of glycolysis was elevated by paroxetine in depressed rats (Park et al. 2020). Our results suggested that baicalin could increase the production of ATP by improving the level of glycolysis in the prefrontal cortex of mice in the CUMS group.

Under aerobic conditions, the tricarboxylic acid cycle and the coupled oxidative phosphorylation are the main ways to produce ATP in the mitochondrion. Studies have shown that mitochondrial function is closely related to depression (Streck et al. 2014). And other researchers found that chronic stress can inhibit the oxidative phosphorylation of mitochondria, destroy the mitochondrial membrane potential, and damage the mitochondrial structure of different brain regions such as the hippocampus and prefrontal cortex of mice (Gong et al. 2011; Ling-Hu et al. 2021). In addition, a study reported that disruption of the TCA cycle was shown to reduce ATP production in a rat model of depression induced by CMS (Shao et al. 2015). In this study, we found that the mRNA expression of PDH α and IDH, two key rate-limiting enzymes of the TCA cycle, were upregulated by baicalin, which suggested that the level of the TCA cycle might be increased by baicalin.

In addition, the electrons generated during the TCA cycle, which were carried by nicotinamide adenine dinucleotide (NADH) and (nicotinamide adenine dinucleotide phosphate) NADPH, were delivered to the mitochondrial membrane space by the "proton pump" in ETC, and then this proton gradient was used by ATP synthase to generate large amounts of ATP (Sousa, D'Imprima, and Vonck 2018). The dysfunction of mitochondrial ETC is closely related to depression (Rezin et al. 2009; Villa et al. 2017). In the present study, the activity of complex I and V was upregulated by baicalin, while complex II was the opposite. Mitochondrial respiratory chain complexes I and II together provide the electrons needed for the reaction

of the electron transport chain, which is the first in the energy production of mitochondria, but complex I is often the starting point of energy production (Willems et al. 2015).

Therefore, this result suggests that baicalin may increase the level of TCA and activate the activity of mitochondrial complex I, then increase the content of ATP. It is well known that the mitochondrial membrane potential (MMP) was formed by the electrons transferred in the respiratory chain. Then, the potential will be used by complex V to produce ATP. And a study reported that the MMP in the prefrontal cortex of CUMS-induced mice was significantly reduced (Gong et al. 2011). In the present study, we found that the level of MMP was increased by baicalin and fluoxetine in the CUMS group. These results suggest that the antidepressant effect of baicalin may be achieved by activating complex I and V to increase MMP and then increase ATP content.

SIRT1 and PGC1 α are two key genes closely related to mitochondrial function, biogenesis, and oxidative stress damage (Tang 2016). In previous studies, depression was associated with decreased expression of SIRT1 (Lo Iacono et al. 2015). PGC1 α is a downstream target gene of SIRT1, which is closely linked to the biogenesis of mitochondria (Li, Hou, and Hao 2017). Increasing the expression of PGC1 α can significantly improve mitochondrial dysfunction and reduce neuronal damage (Jia et al. 2020). In addition, studies have found that the activation of the SIRT1/PGC1 α pathway plays a major role in mitochondrial function and neuroprotection (Fanibunda et al. 2019). Our data showed that the baicalin could significantly increase the mRNA expression of SIRT1 and PGC1 α . This result suggested that the baicalin could activate the SIRT1/PGC pathway to increase the level of energy metabolism.

5. Conclusions

Taken together, the present study found that baicalin could improve the depression-like behavior of mice caused by CUMS and increase the ATP level in the prefrontal cortex. Moreover, baicalin could promote the glycolysis and TCA cycle by upregulating the mRNA expression of HK, PDH α , and IDH. Baicalin also upregulated the mRNA expression of PGC1 α and SIRT1, improved mitochondrial function, activated Complex I and V, and increased MMP. These findings may clarify the possible antidepressant mechanism of baicalin by increasing ATP levels through multiple pathways and provide a new strategy for the development of new antidepressant drugs.

Declarations

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Author's contribution

SFL designed and performed experiments, collected data, and wrote the manuscript. XHJ and LLZ performed experiments. JDS contributed to the data collection. MB contributed to the data analysis. YCL designed the experiments and revised the manuscript. EPX supervised and managed the experiments.

Data availability

The data are available upon request.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

All animal experiments were performed following the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the Committee of Animal Care of Henan University of Chinese Medicine (DWLL16020024).

References

- Bansal Y, and A. Kuhad (2016) Mitochondrial Dysfunction in Depression. *Curr Neuropharmacol* 14(6):610–618. <http://dx.doi.org/10.2174/1570159x14666160229114755>
- Barden N (2004) Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression. *J Psychiatry Neurosci* 29(3):185–193. <https://www.ncbi.nlm.nih.gov/pubmed/15173895>
- Bell SM, Burgess T, Lee J, Blackburn DJ, Allen SP, and H. Mortiboys., 2020. Peripheral Glycolysis in Neurodegenerative Diseases. *Int J Mol Sci* 21 (23). <http://dx.doi.org/10.3390/ijms21238924>
- Belmaker RH, and G. Agam (2008) Major depressive disorder. *N Engl J Med* 358(1):55–68. <http://dx.doi.org/10.1056/NEJMr073096>
- Cao X, Li LP, Wang Q, Wu Q, Hu HH, Zhang M, Fang YY, Zhang J, Li SJ, Xiong WC, Yan HC, Gao YB, Liu JH, Li XW, Sun LR, Zeng YN, Zhu XH, Gao. TM (2013) Astrocyte-derived ATP modulates depressive-like behaviors. *Nat Med* 19(6):773–777. <http://dx.doi.org/10.1038/nm.3162>
- Detka J, Kurek A, Kucharczyk M, Glombik K, Basta-Kaim A, Kubera M, Lason W, Budziszewska. B (2015) Brain glucose metabolism in an animal model of depression. *Neuroscience* 295:198–208. <http://dx.doi.org/10.1016/j.neuroscience.2015.03.046>
- Duman RS, Monteggia. LM (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59(12):1116–1127. <http://dx.doi.org/10.1016/j.biopsych.2006.02.013>

- Ernst J, Hock A, Henning A, Seifritz E, Boeker H, Grimm. S (2017) Increased pregenual anterior cingulate glucose and lactate concentrations in major depressive disorder. *Mol Psychiatry* 22(1):113–119. <http://dx.doi.org/10.1038/mp.2016.73>
- Fanibunda SE, Deb S, Maniyadath B, Tiwari P, Ghai U, Gupta S, Figueiredo D, Weisstaub N, Gingrich JA, Vaidya ADB, Kolthur-Seetharam U, Vaidya. VA (2019) Serotonin regulates mitochondrial biogenesis and function in rodent cortical neurons via the 5-HT_{2A} receptor and SIRT1-PGC-1 α axis. *Proc Natl Acad Sci U S A* 116(22):11028–11037. <http://dx.doi.org/10.1073/pnas.1821332116>
- Glombik K, Detka J, Kurek A, Budziszewska. B (2020) Impaired Brain Energy Metabolism: Involvement in Depression and Hypothyroidism. *Front Neurosci* 14:586939. <http://dx.doi.org/10.3389/fnins.2020.586939>
- Gong Y, Chai Y, Ding JH, Sun XL, Hu. G (2011) Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. *Neurosci Lett* 488(1):76–80. <http://dx.doi.org/10.1016/j.neulet.2010.11.006>
- Gu X, Ke S, Wang Q, Zhuang T, Xia C, Xu Y, Yang L, and M. Zhou (2021) Energy metabolism in major depressive disorder: Recent advances from omics technologies and imaging. *Biomed Pharmacother* 141:111869. <http://dx.doi.org/10.1016/j.biopha.2021.111869>
- Guo W, Tang ZY, Cai ZY, Zhao WE, Yang J, Wang XP, Ji J, Huang XX, Sun XL (2021) Iptakalim alleviates synaptic damages via targeting mitochondrial ATP-sensitive potassium channel in depression. *FASEB J* 35(5):e21581. <http://dx.doi.org/10.1096/fj.202100124RR>
- Hipkiss AR (2019) Aging, Alzheimer's Disease and Dysfunctional Glycolysis; Similar Effects of Too Much and Too Little. *Aging Dis* 10(6):1328–1331. <http://dx.doi.org/10.14336/AD.2019.0611>
- Jia L, Wang J, Cao H, Zhang X, Rong W, Xu. Z (2020) Activation of PGC-1 α and Mitochondrial Biogenesis Protects Against Prenatal Hypoxic-ischemic Brain Injury. *Neuroscience* 432:63–72. <http://dx.doi.org/10.1016/j.neuroscience.2020.02.035>
- Jun M, Xiaolong Q, Chaojuan Y, Ruiyuan P, Shukun W, Junbing W, Li H, Hong C, Jinbo C, Rong W, Yajin L, Lanqun M, Fengchao W, Zhiying W, Jianxiong A, Yun W, Xia Z, Chen Z, Zengqiang. Y (2018) Calhm2 governs astrocytic ATP releasing in the development of depression-like behaviors. *Mol Psychiatry* 23(4):1091. <http://dx.doi.org/10.1038/mp.2017.254>
- Kahl KG, Atalay S, Maudsley AA, Sheriff S, Cummings A, Frieling H, Schmitz B, Lanfermann H, Ding. XQ (2020) Altered neurometabolism in major depressive disorder: A whole brain (1)H-magnetic resonance spectroscopic imaging study at 3T. *Prog Neuropsychopharmacol Biol Psychiatry* 101:109916. <http://dx.doi.org/10.1016/j.pnpbp.2020.109916>
- Karabatsiakakis A, Schonfeldt-Lecuona C (2020) Depression, mitochondrial bioenergetics, and electroconvulsive therapy: a new approach towards personalized medicine in psychiatric treatment - a

short review and current perspective. *Transl Psychiatry* 10(1):226. <http://dx.doi.org/10.1038/s41398-020-00901-7>

Khan AR, Hansen B, Wiborg O, Kroenke CD, Jespersen. SN (2018) Diffusion MRI and MR spectroscopy reveal microstructural and metabolic brain alterations in chronic mild stress exposed rats: A CMS recovery study. *Neuroimage* 167:342–353. <http://dx.doi.org/10.1016/j.neuroimage.2017.11.053>

Kolar D, Kleteckova L, Brozka H, Vales. K (2021) Mini-review: Brain energy metabolism and its role in animal models of depression, bipolar disorder, schizophrenia and autism. *Neurosci Lett* 136003. <http://dx.doi.org/10.1016/j.neulet.2021.136003>

Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman. RS (2010) Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proc Natl Acad Sci U S A* 107(6):2669–2674. <http://dx.doi.org/10.1073/pnas.0910658107>

Lam RW, Wajsbrot DB, Meier E, Pappadopulos E, Mackell JA, Boucher. M (2017) Effect of desvenlafaxine 50 mg and 100 mg on energy and lassitude in patients with major depressive disorder: A pooled analysis. *J Psychopharmacol* 31(9):1204–1214. <http://dx.doi.org/10.1177/0269881117719261>

Li PA, Hou X, Hao. S (2017) Mitochondrial biogenesis in neurodegeneration. *J Neurosci Res* 95(10):2025–2029. <http://dx.doi.org/10.1002/jnr.24042>

Li YC, Shen JD, Li J, Wang R, Jiao S, Yi. LT (2013) Chronic treatment with baicalin prevents the chronic mild stress-induced depressive-like behavior: involving the inhibition of cyclooxygenase-2 in rat brain. *Prog Neuropsychopharmacol Biol Psychiatry* 40:138–143. <http://dx.doi.org/10.1016/j.pnpbp.2012.09.007>

Li YC, Wang LL, Pei YY, Shen JD, Li HB, Wang BY, Bai. M (2015) Baicalin decreases SGK1 expression in the hippocampus and reverses depressive-like behaviors induced by corticosterone. *Neuroscience* 311:130–137. <http://dx.doi.org/10.1016/j.neuroscience.2015.10.023>

Li Z, Cheng J, and J. Liu (2020) Baicalin Protects Human OA Chondrocytes Against IL-1 β -Induced Apoptosis and ECM Degradation by Activating Autophagy via MiR-766-3p/AIFM1 Axis. *Drug Des Devel Ther* 14:2645–2655. <http://dx.doi.org/10.2147/DDDT.S255823>

Ling-Hu T, Liu SB, Gao Y, Han YM, Tian JS, Qin. XM (2021) Stable Isotope-Resolved Metabolomics Reveals the Abnormal Brain Glucose Catabolism in Depression Based on Chronic Unpredictable Mild Stress Rats. *J Proteome Res* 20(7):3549–3558. <http://dx.doi.org/10.1021/acs.jproteome.1c00155>

Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, Chen H, Zhu DY, Zhou QG (2018) Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc* 13(7):1686–1698. <http://dx.doi.org/10.1038/s41596-018-0011-z>

- Lo Iacono L, Visco-Comandini F, Valzania A, Viscomi MT, Coviello M, Giampa A, Roscini L, Bisicchia E, Siracusano A, Troisi A, Puglisi-Allegra S, Carola. V (2015) Adversity in childhood and depression: linked through SIRT1. *Transl Psychiatry* 5:e629. <http://dx.doi.org/10.1038/tp.2015.125>
- Malhi GS, John Mann J (2018) Depression *The Lancet* 392(10161):2299–2312. [http://dx.doi.org/10.1016/s0140-6736\(18\)31948-2](http://dx.doi.org/10.1016/s0140-6736(18)31948-2)
- Park DI, Novak B, Yan Y, Kaya ME, Turck. CW (2020) Paroxetine binding and activation of phosphofructokinase implicates energy metabolism in antidepressant mode of action. *J Psychiatr Res* 129:8–14. <http://dx.doi.org/10.1016/j.jpsychires.2020.05.033>
- Porsolt RD, Brossard G, Hautbois C, Roux. S (2001) Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci* Chapter 8, Unit 8 10A. <http://dx.doi.org/10.1002/0471142301.ns0810as14>
- Rezin GT, Goncalves CL, Daufenbach JF, Fraga DB, Santos PM, Ferreira GK, Hermani FV, Comim CM, Quevedo J, Streck EL (2009) Acute administration of ketamine reverses the inhibition of mitochondrial respiratory chain induced by chronic mild stress. *Brain Res Bull* 79(6):418–421. <http://dx.doi.org/10.1016/j.brainresbull.2009.03.010>
- Schildkraut JJ, Kety. SS (1967) Biogenic amines and emotion. *Science* 156(3771):21–37. <http://dx.doi.org/10.1126/science.156.3771.21>
- Secretariat T (2012) Global burden of mental disorders and the need for a comprehensive, coordinated response from health and social sectors at the country level, EB130. World Health Organization, p 6
- Shao WH, Chen JJ, Fan SH, Lei Y, Xu HB, Zhou J, Cheng PF, Yang YT, Rao CL, Wu B, Liu HP, Xie. P (2015) Combined Metabolomics and Proteomics Analysis of Major Depression in an Animal Model: Perturbed Energy Metabolism in the Chronic Mild Stressed Rat Cerebellum. *OMICS* 19(7):383–392. <http://dx.doi.org/10.1089/omi.2014.0164>
- Sharma S, Akundi. RS (2019) Mitochondria: A Connecting Link in the Major Depressive Disorder Jigsaw. *Curr Neuropharmacol* 17(6):550–562. <http://dx.doi.org/10.2174/1570159X16666180302120322>
- Shen JD, Ma LG, Hu CY, Pei YY, Jin SL, Fang XY, Li. YC (2016) Berberine up-regulates the BDNF expression in hippocampus and attenuates corticosterone-induced depressive-like behavior in mice. *Neurosci Lett* 614:77–82. <http://dx.doi.org/10.1016/j.neulet.2016.01.002>
- Sousa JS, D'Imprima E, and J. Vonck (2018) Mitochondrial Respiratory Chain Complexes. *Subcell Biochem* 87:167–227. http://dx.doi.org/10.1007/978-981-10-7757-9_7
- Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim. S (2018) Neuroprotective and Cognitive Enhancement Potentials of Baicalin: A Review. *Brain Sci* 8. <http://dx.doi.org/10.3390/brainsci8060104>

- Streck EL, Goncalves CL, Furlanetto CB, Scaini G, Dal-Pizzol F, Quevedo. J (2014) Mitochondria and the central nervous system: searching for a pathophysiological basis of psychiatric disorders. *Braz J Psychiatry* 36(2):156–167. <http://dx.doi.org/10.1590/1516-4446-2013-1224>
- Tang BL (2016) Sirt1 and the Mitochondria. *Mol Cells* 39(2):87–95. <http://dx.doi.org/10.14348/molcells.2016.2318>
- Villa RF, Ferrari F, Bagini L, Gorini A, Brunello N, Tascedda. F (2017) Mitochondrial energy metabolism of rat hippocampus after treatment with the antidepressants desipramine and fluoxetine. *Neuropharmacology* 121:30–38. <http://dx.doi.org/10.1016/j.neuropharm.2017.04.025>
- Weckmann K, Deery MJ, Howard JA, Feret R, Asara JM, Dethloff F, Filiou MD, Iannace J, Labermaier C, Maccarrone G, Webhofer C, Teplytska L, Lilley K, Muller MB, and C. W. Turck., 2017. Ketamine's antidepressant effect is mediated by energy metabolism and antioxidant defense system. *Sci Rep.* 7 (1), 15788. <http://dx.doi.org/10.1038/s41598-017-16183-x>
- Westergren T, Narum S, Klemp. M (2019) Critical appraisal of adverse effects reporting in the 'Treatment for Adolescents With Depression Study (TADS)". *BMJ Open* 9(3):e026089. <http://dx.doi.org/10.1136/bmjopen-2018-026089>
- Willems PH, Rossignol R, Dieteren CE, Murphy MP, Koopman. WJ (2015) Redox Homeostasis and Mitochondrial Dynamics. *Cell Metab* 22(2):207–218. <http://dx.doi.org/10.1016/j.cmet.2015.06.006>
- Willner P, Scheel-Kruger J, Belzung. C (2013) The neurobiology of depression and antidepressant action. *Neurosci Biobehav Rev* 37(10):2331–2371. <http://dx.doi.org/10.1016/j.neubiorev.2012.12.007>
- Willner P, Towell A, Sampson D, Sophokleous S, Muscat. R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* 93(3):358–364. <http://dx.doi.org/10.1007/BF00187257>
- Xie X, Shen Q, Yu C, Xiao Q, Zhou J, Xiong Z, Li Z, Fu Z (2020) Depression-like behaviors are accompanied by disrupted mitochondrial energy metabolism in chronic corticosterone-induced mice. *J Steroid Biochem Mol Biol* 200:105607. <http://dx.doi.org/10.1016/j.jsbmb.2020.105607>

Figures

Figure 1

Baicalin reversed the depression-like behaviors in mice. All values are presented as means $\pm$ SEM (n = 9). (A) The sucrose preference in the SPT. (B) The immobility time in the FST. ###p < 0.001 and #p < 0.05 as compared with the control group; ***p < 0.001 and *p < 0.05 as compared with the CUMS group.

Figure 2

Baicalin increased the ATP levels in the prefrontal cortex of mice. All values are presented as means $\pm$ SEM (n = 5). ##p < 0.01 as compared with the control group; **p < 0.01 as compared with the CUMS group.

Figure 3

Baicalin increased the level of glycolysis in the prefrontal cortex of mice. The mRNA levels were normalized to β -actin, and subsequently represents as fold change relative to control group. All values are presented as means $\pm$ SEM (n = 5). ###p < 0.001 as compared with the control group; ***p < 0.001 and *p < 0.05 as compared with the CUMS group. HK: Hexokinase; PFK: Phosphofructokinase; PK: Pyruvate kinase.

Figure 4

Baicalin increased the TCA levels in the prefrontal cortex of mice. The mRNA levels were normalized to β -actin, and subsequently represents as fold change relative to control group. All values are presented as means $\pm$ SEM (n = 5). *p < 0.05 as compared with the CUMS group. PDH α : Pyruvate dehydrogenase alpha; CS: Citrate synthase; IDH: Isocitrate dehydrogenase; OGDH: Oxoglutarate dehydrogenase.

Figure 5

Baicalin activated the electron transport chain in the prefrontal cortex of mice. All values are presented as means $\pm$ SEM (n = 4-6). (A-E) Mitochondria electron transport chain complex I-V. (F) Purity of mitochondria. 1,2 Mitochondrial. 3,4 Cytoplasmic. ###p < 0.001 as compared with the control group; **p < 0.01 and *p < 0.05 as compared with the CUMS group.

Figure 6

Baicalin increased the level of mitochondrial membrane potential in the prefrontal cortex of mice. All values are presented as means $\pm$ SEM (n = 5). ##p $\leq$ 0.01 as compared with the control group; ***p $\leq$ 0.001 and **p $\leq$ 0.01 as compared with the CUMS group.

Figure 7

Baicalin up-regulated the expression of mitochondrial related genes in mouse prefrontal cortex. The mRNA levels were normalized to GAPDH, and subsequently represents as fold change relative to control group. All values are presented as means $\pm$ SEM (n = 5). #p < 0.05 as compared with the control group; **p < 0.01 and *p < 0.05 as compared with the CUMS group. PGC1 α : Peroxisome proliferator-activated receptor- γ coactivator-1 α ; SIRT1: Sirtuin-1.

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