

# Sustained AMP-activated protein kinase activation attenuates the activity of brain-derived neurotrophic factor/tyrosine kinase receptor B signaling in mice exposed to chronic stress

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*To the Editor:* Major depressive disorder is one of the most leading causes of disability worldwide. The molecular pathophysiology of depression is complicated and has already attracted intensive and considerable attention.<sup>[1]</sup> Nowadays, the neurotrophic hypothesis of depression suggests that brain-derived neurotrophic factor (BDNF)/tyrosine kinase receptor B (TrkB) receptor binding activates the downstream signaling pathways to culminate in cell survival.<sup>[2]</sup> This process is essential for neurogenesis and synaptogenesis, which are involved in the effects of antidepressants.<sup>[3]</sup>

In addition to its classical role in regulating neuronal growth and plasticity, BDNF is also suggested to play a crucial role in regulating systemic metabolism. Blockade of BDNF/TrkB signaling in the periphery caused down-regulation of several metabolic molecules, including AMP-activated protein kinase (AMPK), a protein kinase participating in cellular energy regulation homeostasis.<sup>[4]</sup> Several reports indicated that lipopolysaccharide (LPS), corticosterone, and chronic stress induced a decreased phosphorylation of AMPK in the brain. However, a controversial finding from another study showed that sustained AMPK activation participated in the process of depression induced by chronic corticosterone. The discrepancy suggests a need to further investigate the role of AMPK in the pathophysiology of depression.

Up to now, a systematic understanding of AMPK dysfunction in the neurotrophic hypothesis of depression is lacking. In particular, how AMPK mediates BDNF/TrkB signaling pathway is not well understood. In this way, the present study was aimed to demonstrate how BDNF/TrkB

signaling and AMPK interacted in depression induced by chronic stress and tried to elucidate whether BDNF/TrkB signaling exerted its function in an AMPK-dependent manner. In the present study, we used 7,8-dihydroxyflavone to activate its receptor TrkB and evaluated its effects on neurogenesis and synaptogenesis. Moreover, we applied a pharmacological intervention approach by the pretreatment of TrkB antagonist, AMPK inhibitor/activator to assess the changes in behaviors, neurogenesis, and synaptogenesis in depressive-like mice. This finding will be a crucial step toward elucidating the relationship between regulation of the BDNF/AMPK in depression.

We first evaluated the effects of 7,8-dihydroxyflavone on depressive-like behaviors. Chronic stress caused a decrease in sucrose preference, a prolongation in latency to feed, and an increase in immobility time, which can be completely reversed by 7,8-dihydroxyflavone (10 mg/kg, intraperitoneally) treatment [Figure 1A–1D]. Then we assessed BDNF and AMPK signaling activity in mice. We found that BDNF expression, TrkB phosphorylation as well as AMPK phosphorylation were significantly decreased by chronic stress, while 7,8-dihydroxyflavone restored these abnormalities [Figure 1E–1G]. We next examined whether 7,8-dihydroxyflavone altered neurogenesis and synaptogenesis in the hippocampus. The results showed that doublecortin (DCX)-positive cell and dendritic spine density were inhibited by chronic stress. On the contrary, the administration of 7,8-dihydroxyflavone reversed the reductions [Figure 1H and 1I].

Then mice were co-treated with 7,8-dihydroxyflavone and K252a, a selective antagonist of the TrkB for four weeks [Supplementary Figure 1, <http://links.lww.com/CM9/>

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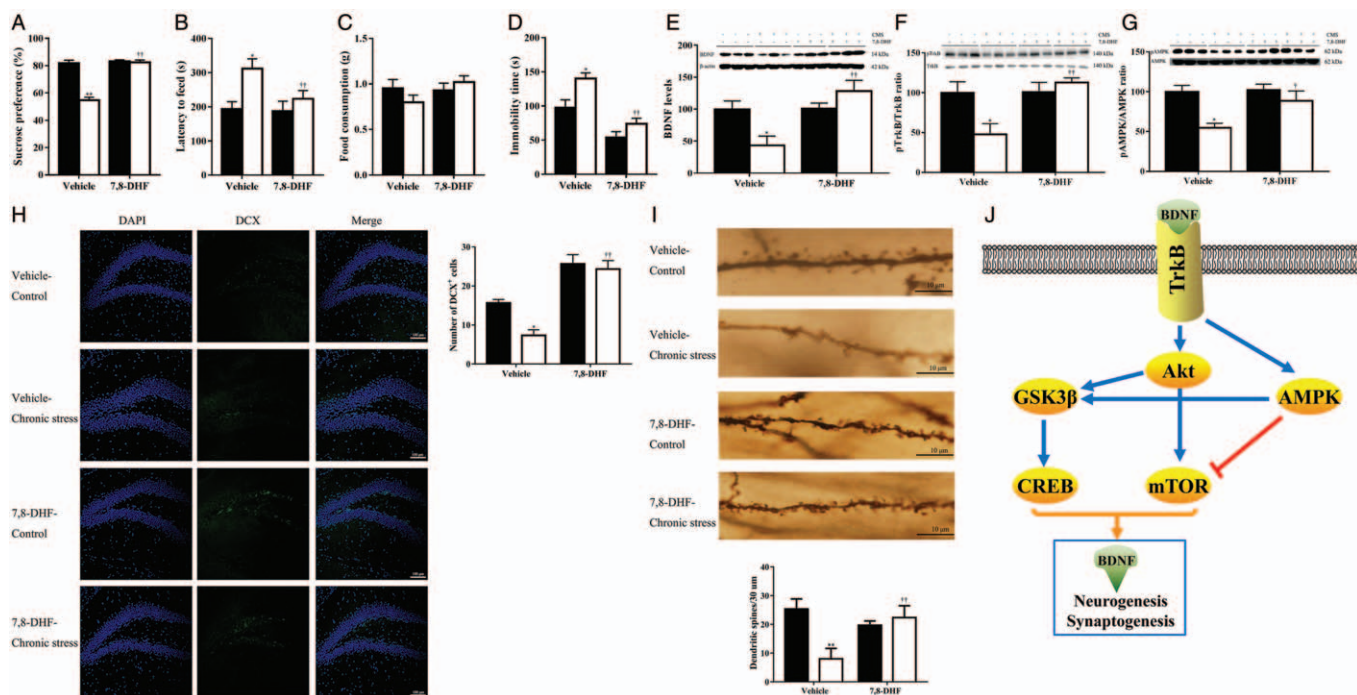
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**Figure 1:** 7,8-dihydroxyflavone reversed the depressive-like behaviors and promoted BDNF-TrkB-AMPK signaling in the hippocampus. 7,8-dihydroxyflavone increases the sucrose preference (A), decreases latency to feed (B) but does not affect home-cage feed consumption (C), and reduces the immobility time (D) in chronic stress mice. 7,8-dihydroxyflavone increases BDNF expression (E), TrkB phosphorylation (F), AMPK phosphorylation (G), DCX-positive cell (H), and density of dendritic spine (I) in the hippocampus. The results suggest a regulation of BDNF/TrkB signaling mediated neurogenesis independent with AMPK (J). The behavioral and biochemical data represented mean ± SEM from 3 to 12 mice/group, respectively. \**P* < 0.05 and \*\**P* < 0.01 vs. Control-vehicle group. †*P* < 0.05 and ††*P* < 0.01 vs. Chronic stress-vehicle group. AMPK: AMP-activated protein kinase; BDNF: Brain-derived neurotrophic factor; TrkB: Tyrosine kinase receptor B.

A432]. K252a (25 μg/kg) fully abolished the antidepressant-like effects of 7,8-dihydroxyflavone. Additionally, the effects of 7,8-dihydroxyflavone on the BDNF, TrkB, and AMPK enhancement were antagonized by the pretreatment with K252a. DCX-positive cell and dendritic spine density, which were promoted by 7,8-dihydroxyflavone, were totally blocked by K252a [Supplementary Figure 2, <http://links.lww.com/CM9/A432>]. These results were in accordance with a previous study showing that administration of 7,8-dihydroxyflavone facilitated memory performance and hippocampal functional connectivity, as well as modulated hippocampal AMPK phosphorylation in response to traumatic brain injury. Thus, AMPK activity was dependent on BDNF/TrkB activation in depressive-like animals.

To further investigate whether AMPK signaling activation is required for the antidepressant-like effects of TrkB agonist, we examined the effects of Compound C, an AMPK inhibitor, in our experiment [Supplementary Figure 3, <http://links.lww.com/CM9/A432>]. To our surprise, sucrose preference was increased and immobility time was decreased after 7,8-dihydroxyflavone treatment, no matter whether Compound C (10 mg/kg) was pre-treated or not. Subsequently, pAMPK phosphorylation was detected to be inhibited by Compound C, indicating the role of Compound C. In addition, we found that the effects of 7,8-dihydroxyflavone on BDNF and TrkB expression were not altered by Compound C. Then, the downstream signaling of AMPK was measured. Besides, AMPK, mammalian target of rapamycin (mTOR), and

glycogen synthase kinase 3 beta (GSK3β)/cAMP responsive element binding (CREB) signaling pathways are also modulated by BDNF/TrkB downstream effector Akt. The canonical BDNF/TrkB/Akt/mTOR and BDNF/TrkB/Akt/GSK3β/CREB signaling pathways are required for many antidepressants. We first found that mTOR phosphorylation was activated by either 7,8-dihydroxyflavone or Compound C. Further, co-treatment with 7,8-dihydroxyflavone and Compound C still maintained the activation of mTOR.

On the other hand, the results indicated the effects of 7,8-dihydroxyflavone on pGSK3β/GSK3β and pCREB/CREB levels were partly blocked by the pretreatment of Compound C, while pAkt/Akt levels remained activated. In parallel to the western results, immunofluorescence and Golgi staining indicated that AMPK inhibitor Compound C did not alter the effects of 7,8-dihydroxyflavone on DCX-positive cell and dendritic spine density [Supplementary Figure 4, <http://links.lww.com/CM9/A432>]. Together, these results suggested that AMPK negatively regulated mTOR signaling but positively regulated GSK3β/CREB signaling.

As AMPK inhibition did not affect the antidepressant-like effects of 7,8-dihydroxyflavone, we next used AMPK activator aminoimidazole carboxamide ribonucleotide (AICAR) to further explore the role of AMPK in our study [Supplementary Figure S5, <http://links.lww.com/CM9/A432>]. The results showed that AICAR treatment (100 mg/kg) alone did not induce antidepressant-like

effects. However, AICAR abolished the antidepressant-like effects of 7,8-dihydroxyflavone in behavioral tests. The effects of AICAR were firstly verified by the elevation of pAMPK/AMPK levels. In addition, both pGSK3 $\beta$ /GSK3 $\beta$  and pCREB/CREB levels were up-regulated after AICAR pretreatment alone. When 7,8-dihydroxyflavone was co-treated with AICAR, BDNF levels, TrkB, Akt, and mTOR phosphorylation turned to be inactivated, suggesting that sustained AMPK activation caused the inhibition of BDNF/TrkB-induced enhancement of mTOR-mediated translation. The inhibition of BDNF/TrkB signaling was confirmed by the results from immunofluorescence and Golgi staining. It can be clearly found that the effects of 7,8-dihydroxyflavone on dendritic spine density were blocked after co-treated with AICAR [Supplementary Figure 6, <http://links.lww.com/CM9/A432>], suggesting that hyperactivity of AMPK suppresses the BDNF/TrkB-dependent antidepressant-like effects.

A previous study on depressive disorders showed that the phosphorylation of AMPK was decreased in various brain regions and animals.<sup>[5]</sup> In parallel with these observations, the present study also found that chronic stress induced a decrease in AMPK phosphorylation. However, it is controversial whether activation of AMPK plays a beneficial or deleterious role in the central nervous system. On the one hand, AMPK activators such as metformin protected against depressive-like behaviors in chronic social defeat stress-induced depression in mice. AICAR produced antidepressant-like effects in olfactory bulbectomized mice.

On the other hand, phosphorylation of AMPK in the brain was increased in LPS or corticosterone-induced depression. AMPK activation decreased the expression of BDNF and AMPK inhibition increased the expression of BDNF in the hippocampus. AMPK inactivation reversed the impairments in hippocampal synaptic plasticity in mice induced by amyloid  $\beta$ . Therefore, it is rational to hypothesize that the activity of AMPK is conditional in response to stress and antidepressants. The present study showed that the activation of AMPK instead of inhibition of AMPK attenuated the effects of BDNF/TrkB signaling in chronic stress-induced mice. We found that this phenomenon was associated with the regulation of mTOR signaling. In detail, the AMPK inhibitor reversed the reduction of mTOR phosphorylation, while the AMPK activator maintained the reduction of mTOR phosphorylation in the hippocampus after chronic stress, which was consistent with the change of neurogenesis and synaptogenesis. Temporary restoration of AMPK activation is beneficial for homeostasis of energy metabolism and antidepressant-like effects. However, sustained hyperactivity of AMPK exhibits its detrimental impacts on neurons, which is due to the inhibited mTOR activity according to the results of the present study.

Overall, to our knowledge, this study, for the first time, suggests that BDNF/TrkB signaling induces antidepressant-like effects in an AMPK-independent manner in chronic stress. More importantly, the results demonstrate that sustained AMPK activation impairs BDNF/TrkB signaling activity via inhibiting mTOR, which directly inhibits protein synthesis and leads to the deficiency in neurogenesis and synaptogenesis [Figure 1J]. Our study also suggests that although AMPK is a putative target for depression therapy as its core regulatory role in energy metabolism, we should be cautious when activating AMPK in patients until we are more fully aware of the complex molecular functions involved in depression and antidepressants.

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### Conflicts of interest

None.

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