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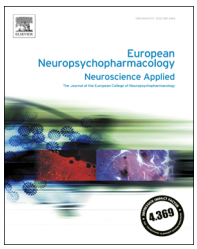

The rapid antidepressant and anxiolytic-like effects of YY-21 involve enhancement of excitatory synaptic transmission via activation of mTOR signaling in the mPFC

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Abstract
Although antidepressants have been widely prescribed to treat patients with major depressive disease (MDD), there is little disagreement over the need for improved antidepressant therapeutics as the typical treatments have a slow therapeutic onset and moderate efficacy. In the present study, we assessed a novel compound, YY-21, from timosaponin B-III derived from sarsasapogenin of *Anemarrhenae Rhizoma*. From the initial results, we found that YY-21 obviously increased presynaptic glutamate release and enhanced long-term synaptic activity within 10 min as determined by excitatory postsynaptic current (EPSC) and field excitatory postsynaptic potential (fEPSP) in medial prefrontal cortex (mPFC) slices, respectively. YY-21 demonstrated anxiolytic-like effects following acute administration in naïve animals and reversed the depressive-like and anxiety phenotypes induced by chronic unpredictable mild stress (CMS) with a relatively fast therapeutic onset. Furthermore, analysis of intracellular signaling pathways showed that YY-21 normalized the CMS-induced low protein levels of GluN2B, p-mTOR, synaptic-related proteins, such as BDNF, PSD-95 and GluA1. Pre-application of the mTOR-selective inhibitor rapamycin blocked YY-21-induced long-term synaptic enhancement. These findings suggest that the activation of BDNF-dependent mTOR signaling, which produces a rapid increase in the postsynaptic protein PSD-95 and GluA1 and further triggers the

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long-term enhancement of synaptic neurotransmission, may be the mechanism underlying the rapid antidepressant and anxiolytic effects induced by YY-21.

1. Introduction

Major depression disorder (MDD) is a mood disorder that is a leading cause of disability worldwide (Collins et al., 2011). The underlying pathophysiology of this heterogeneous disease, as well as the neuromechanisms of antidepressant therapeutics, remain poorly understood, leaving the psychiatrist with relatively few pharmacologic treatments for patients with depression. Although currently available monoaminergic antidepressants have been widely prescribed and represent moderately effective pharmacotherapy for depression, the remission rates for classic antidepressants are not optimistic. As demonstrated by the National Institute of Mental Health STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study, the average time to achieve remission for the antidepressant-responsive subgroup of patients was 7 weeks, and 40% of those required 8 or more weeks to do so (Gaynes et al., 2009). Furthermore, more than one-third of MDD patients exhibited resistance to pharmacologic treatment (Trivedi et al., 2006). These limitations highlight an urgent and clear need for more efficacious and faster-acting therapeutic agents to treat patients with MDD, especially those who are refractory to the traditional antidepressants.

The comorbidity of depression in particular with symptoms of anxiety further renders MDD challenging. Patients suffering from depression may experience anxiety and other overlapping symptoms, including fatigue, irritability, sleep disturbance, restlessness and worry (Ressler and Nemeroff, 2000). Depression had an overall rate of 57% for co-occurrence with any anxiety disorder, ranking among the top co-existing mood disorders with anxiety (Krueger and Finger, 2001; Mineka et al., 1998). The comorbidity of depression and anxiety suggests that they may share a common pathophysiology. In addition to GABA-benzodiazepines, many antidepressants, such as tricyclic antidepressants, monoamine oxidase inhibitors and SSRIs, also have anxiolytic properties (Griebel and Holmes, 2013; Nutt, 2005). It has been suggested that novel agents that have antidepressant effects may also be used as anxiolytics.

Facing the limitations of traditional antidepressants, basic research and the pharmaceutical industry are focusing on new drug targets and novel mechanisms of depression. Recently, excitatory synaptic transmission in the brain has received attention as a novel neuromechanism underlying fast-acting pharmacotherapy for MDD. One of the greatest recent advances was the elucidation of mechanisms of NMDA receptor antagonists, particularly ketamine. The mechanism of this drug's rapid action is linked to enhancement of the excitatory synaptic structure and function via the activation of mTOR signaling by increasing brain-derived neurotrophic factor (BDNF) in the prefrontal cortex (PFC) and the hippocampus (Duman and Li, 2012). Preclinical studies have also demonstrated that chronic stress exerts deleterious effects on excitatory synapses, including synaptic structures, and functions in multiple brain regions. Moreover, these dysfunctions can be reversed by the administration of certain antidepressants (Duman and Aghajanian, 2012; Pittenger and Duman, 2007). These results indicate that excitatory synaptic transmission is a distinct circuit that may play a role in mediating many symptoms of depression, which result from decreasing sensitivity to positive input with increases in negative stimuli.

Although deficits in the excitatory neurotransmission mediated by stress and genetic susceptibility are evident, currently, efficient and rapid antidepressants based on the excitatory synapses hypothesis are still rare, and the underlying mechanisms need to be further understood. In the past several years, we have focused on *Anemarrhenae Rhizoma*, which prescribed as anantipyretic, sedative, and diuretic in Chinese traditional medicine. The main active compound of *Anemarrhenae Rhizoma*, sarsasapogenin, has also been shown to have antidepressant-like effects in animals (Ren et al., 2006). We derived a series of metabolites from *Anemarrhenae Rhizoma* and artificially prepared the main metabolites, deriving new chemical structures. YY-21, a novel furostan skeleton secondary timosaponin, was obtained from timosaponin B-III via hydrolysis with hydrochloric acid. The detailed preparation methods and structure elucidation of YY-21 were described in our previous studies (Liu et al., 2013; Wu et al., 2012). Its structure is shown in Figure 1. In the initial studies, we found that YY-21 produced a strong enhancement of excitatory synaptic neurotransmission with short treatment duration (10 min) in vitro. Based on this result and the above evidence concerning rapid pharmacological mechanisms of novel antidepressants, we hypothesized that YY-21 could possibly exert a rapid antidepressant effect by modulating synaptic transmission. To address this issue, animal models were developed to assess the behavioral effects of YY-21 treatment, and the intracellular signaling pathways were further examined. The results indeed demonstrated that YY-21 had relatively fast antidepressant and anxiolytic actions. The rapid enhancement of excitatory synaptic neurotransmission may contribute to its pharmacological mechanism. Our data indicated a potentially novel antidepressant agent and confirmed that the modulation of synaptic dysfunction in depression could be a potential therapeutic target.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats, aged 12–15 days and 8 weeks, were used for electrophysiological recording and behavioral tests. Adult male C57BL/6 mice (weighing 18–20 g) were used for acute drug