The role of activation of the 5-HT1A receptor and adenylate cyclase in the antidepressant-like effect of YL-0919, a dual 5-HT1A agonist and selective serotonin reuptake inhibitor

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HIGHLIGHTS

- YL-0919 is a novel dual-targeted antidepressant.
- A 5-HT1A antagonist blocks YL-0919's antidepressant effect in animal models.
- A 5-HT1A antagonist blocks the YL-0919-raised cAMP levels in rat prefrontal cortex.
- A protein kinase A inhibitor blocks YL-0919's antidepressant effect in animal models.

ABSTRACT

This study aimed to explore the possible mechanisms underlying the antidepressant-like effect of YL-0919, a novel antidepressant candidate with dual activity as a 5-HT1A receptor agonist and a selective serotonin reuptake inhibitor. The animal models commonly used to evaluate potential antidepressants, i.e., tail suspension (TST) in mice and forced swimming test (FST) in mice were used to evaluate the antidepressant effect of YL-0919. The activity of adenylate cyclase (AC) on the synaptic membrane was determined by the homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay. The results indicated that YL-0919 (1.25–2.5 mg/kg, i.g.) significantly decreased the immobility time in both the tail suspension test and the forced swim test in a dose-dependent manner, demonstrating the antidepressant-like effect of YL-0919. Furthermore, this effect was completely antagonized by the co-administration of WAY-100635 (0.3 mg/kg, s.c.), a 5-HT1A selective antagonist. YL-0919 (10−9–10−5 mol/L) was also shown to activate AC in vitro in a dose-dependent manner in synaptic membranes extracted from the rat prefrontal cortex, and this effect (10−7–10−5 mol/L) was antagonized by WAY-100635 (10−7 mol/L). Finally, the antidepressant-like effect of YL-0919 (2.5 mg/kg, i.g.) was also blocked by the co-administration of H-89 (3 μg/site, i.c.v.), a protein kinase A (PKA) selective inhibitor. These results indicate that the activation of 5-HT1A receptors and the subsequent activation of the AC-cAMP-PKA signaling pathway in the frontal cortex play a critical role in the antidepressant-like effect of YL-0919.

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1. Introduction

Current antidepressants that are commonly prescribed in the clinic have some drawbacks, such as delayed onset of effect, evident toxicity, and a low effectiveness rate. Selective serotonin reuptake inhibitors (SSRIs), the most widely used antidepressant, can improve serotonin (5-HT) levels in the synaptic gap within a few hours, but they also activate the presynaptic 5-HT1A autoreceptor and reduce the synthesis and release of 5-HT via negative
feedback inhibition. After 2–4 weeks of drug administration, the 5-HT1A autoreceptor desensitizes, and the antidepressant-like effect thereof begins to manifest. The negative feedback inhibition induced by 5-HT1A autoreceptor activation is considered to play a key role in the delayed onset of the antidepressant [1,2]. Therefore, dual-targeted antidepressants that can modulate 5-HT1A receptor activity and inhibit 5-HT reuptake could accelerate the desensitization of 5-HT1A autoreceptor and shorten the onset time [3]. YL-0919 [1-(1-benzyl-4-hydroxypiperidin-4-ylmethyl)-2(H)-pyridinone hydrochloride (Fig. 1A)] is a structurally new compound designed and synthesized by our institute. Our previous studies indicated that YL-0919 showed high affinity and selectivity to the 5-HT1A receptor and 5-HT transporter as well as dual activities as both a 5-HT1A receptor agonist and 5-HT reuptake inhibitor [2]. Although YL-0919 exerts significant antidepressant activities [2], the role of the 5-HT1A receptor and its downstream signaling pathway remains unknown underlying the YL-0919’s behavioral effects. The cAMP signaling pathway influences neural functions and is recognized as an important mechanism mediating antidepressant-like activity [4,5]. Studies have demonstrated that activation of 5-HT1A receptors enhances the activation of AC activity [6,7]. Furthermore, many kinds of classic antidepressants activated brain CAMP-CREB signaling including hippocampus and prefrontal cortex [5,8,9]. Therefore, this study investigated the role of the 5-HT1A receptor and cAMP signaling in the antidepressant-like activity of YL-0919 by using the respective inhibitors of the 5-HT1A receptor and its downstream signaling pathway.

2. Materials and methods

2.1. Reagents

YL-0919 (white powder with purity ≥99.8%) and buspirone were synthesized by the Department of Medicinal Chemistry at our institute. WAY-100635 and H-89 were purchased from Sigma (St. Louis, MO, USA). The LANCE 384 Kit was purchased from Perkin-Elmer (Shelton, CT, USA), and the BCA Protein Assay Kit was purchased from Applygen Technologies Inc. (Beijing, China). All drugs were dissolved in indistilled water except for H-89 which was dissolved in saline.

2.2. Animals

Both male ICR mice (20 ± 2 g) and male Wistar rats (180 ± 10 g) were purchased from the Beijing SPF Laboratory Animal Technology Company (Beijing, China). The animals were maintained under the standard conditions of controlled temperature (23 ± 1°C), humidity (45%), and lighting (12 h/d). The experiments were conducted according to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996). The experimental procedures were approved by the institutional committee on animal care and use, and all efforts were made to minimize animal suffering and to reduce the number of animals used for the experiments.

2.3. Tail suspension test (TST)

The tail suspension test was performed as previously described [10] with minor modifications. Briefly, 60 min after i.g. drug administration, the mice were suspended on the top of apparatus by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility in the last 4 min of total 6 min suspension was recorded. The immobility was defined as absence of any limb or body movements, except for those caused by respiration.

2.4. Forced swim test (FST) in mice

The forced swimming test was performed following the procedures as described previously [13]. Briefly, 60 min after i.g. drug administration, the mice were individually forced to swim in an open cylindrical container (diameter 14 cm, height 20 cm, containing 12 cm of water maintained at 24 °C). The duration of immobility in the last 4 min of total 6 min test was recorded. Mice were considered immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their head above water.

To investigate the possible involvement of 5-HT1A receptors in the behavioral effects of YL-0919, mice were treated with WAY-100635 (0.1, 0.3 mg/kg, s.c.) in combination with YL-0919 (2.5 mg/kg, i.g.), and the TST or FST was carried out 60 min later. The dose of WAY-100635 used was based on our previous study.

2.5. Extraction of synaptic membrane proteins from the rat prefrontal cortex and AC activity assay

Five wistar rats were decapitated and the synaptic membrane from the prefrontal cortex was then extracted as previously described [9,14]. The concentration of obtained membrane protein
was measured by the BCA method and the proteins were maintained at −70 °C until further use.

To determine the in vitro effect of YL-0919 and other monoamine receptor inhibitors on synaptic membrane AC activity, 100 µL of reaction buffer (pH 7.5) containing 15 mM HEPES, 5 mM MgCl₂, 1 mM EGTA, 1 mM EDTA, 60 mM NaCl, 0.5 mM MgCl₂, 0.05 mM L-ATP, 0.5 g/L phosphocreatine, and 0.14 g/L creatine phosphokinase was added to the reaction tubes. IBMX was added to inhibit phosphodiesterase activity. To each tube, 5 µL of either vehicle or corresponding drugs was added. After 12 µg of synaptic membrane protein from the rat prefrontal cortex was added into each tube, the reaction tubes were immediately placed at 30 °C and incubated for 10 min, the reaction is linear within 20 min [14], after which the reaction was immediately terminated by incubating the tubes in boiling water for 3 min.

The amount of cAMP production in each reaction tube was assayed by the LANCE cAMP 384 Kit strictly following the manufacturer’s instruction. The entire assay was performed in an ice bath except for the steps requiring other temperatures as noted.

2.6. Intracerebroventricular (i.c.v.) injection of the PKA inhibitor

The PKA selective inhibitor H-89 was i.c.v. injected as previously described [15]. The mice were anaesthetized until the righting reflex was lost, after which either saline or H-89 (0.3–3 µg/site; [16]) was injected i.c.v. (5 µL/site, unilateral). After 15 min, mice were i.g. administered with either distilled water or YL-0919 (2.5 mg/kg). After an additional 45 min, the TST and FST were carried out as described above.

2.7. Statistical analysis

Data were expressed as mean ± SEM. Statistical tests used were either a one- or two-way ANOVA followed by Bonferroni’s multiple comparison test, as indicated in Section 3. For all of the tests, differences with P < 0.05 were considered to be significant.

3. Results

3.1. Effect of WAY-100635 on the antidepressant-like effects of YL-0919 in the TST and FST

YL-0919 (2.5 mg/kg, i.g.) significantly reduced the immobility time of mice in the tail suspension test or forced swim test; however, such effect was dose-dependently reversed by co-administration with WAY-100635 (0.3 mg/kg) (one-way ANOVA, compared with the YL-0919 (2.5 mg/kg)-treated group), whereas administration of WAY-100635 alone showed no effect (Student’s t-test, compared with the control) (Fig. 1B and C). These results indicated that YL-0919 exerted antidepressant-like activity, and such activity was completely blocked by co-treatment with WAY-100635.

3.2. The effect of YL-0919 on in vitro AC activity in extracted synaptic membranes

One-way ANOVA analyses revealed that, similar with the 5-HT₁A partial agonist buspirone (10⁻⁹–10⁻⁵ mol/L, Fig. 2A) and the 5-HT reuptake inhibitor FLX (10⁻⁹–10⁻⁵ mol/L, Fig. 2B), YL-0919 (10⁻⁹–10⁻⁵ mol/L) treatment also exhibited a dose-dependent stimulatory effect on the AC activity in synaptic membranes from the rat prefrontal cortex, and this effect was significant at doses of 10⁻⁵–10⁻³ mol/L (Fig. 2C).

3.3. Effect of WAY-100635 on the stimulatory effect of YL-0919 on AC activity in synaptic membranes in vitro

For further determination of 5-HT₁A receptor role in antidepressant-like effect of YL-0919, WAY-100635 was applied as a 5-HT₁A selective antagonist. As it was determined in Fig. 3, treatment with YL-0919 (10⁻⁵, 10⁻⁶ mol/L) alone significantly stimulated AC activity in synaptic membranes from the rat prefrontal cortex, as measured by increased cAMP levels. However, co-incubation with WAY-100635 (10⁻⁷ mol/L) completely abolished the stimulatory effect of YL-0919 (10⁻⁷–10⁻⁵ mol/L) on AC activity (Two-way ANOVA), which was consistent with the attenuation of the antidepressant-like effect of YL-0919 by WAY-100635 in selected behavioral tests.
3.4. Effect of H-89 on the antidepressant-like effects of YL-0919 in the TST and FST

The role of AC-cAMP-PKA signaling pathway in the antidepressant-like activity of YL-0919 was further confirmed using PKA inhibitor H-89 in the TST and FST. Compared to the control group, YL-0919 (2.5 mg/kg) caused a significant reduction in the immobility time in both behavioral tests. However, i.c.v. injection of H-89 (0.3, 1, and 3 μg/site) dose-dependently reduced the antidepressant-like effect of YL-0919 (one-way ANOVA, compared with the YL-0919 (2.5 mg/kg)-treated group) in the tail suspension (Fig. 4A) and forced swim tests (Fig. 4B), suggesting that AC-cAMP-PKA signaling pathway plays critical role in the antidepressant-like activity of YL-0919 in both behavioral tests.

4. Discussion

Our previous studies have shown that YL-0919, a structurally new compound with high affinity and selectivity for 5-HT1A receptor and serotonin transporter, exerted a significant antidepressant-like effect after acute and chronic administration in various mouse and rat models [2]. The current findings further suggested that 5-HT1A receptors and the subsequent activation of the AC-cAMP-PKA signaling pathway play a critical role in the antidepressant-like effect of YL-0919.

In present study, the tail suspension and forced swim test were used to evaluate the antidepressant-like effect of YL-0919. We observed that i.g. administration of YL-0919 exerted antidepressant-like effects which was very similar to those of the FLX (SSRI) and the 5-HT/NE dual reuptake inhibitor DLX at a low dose range in a dose dependent manner. This study used DLX instead of FLX as a positive control in the forced swim test because of the reported sensitivity of DLX in this assay [17,18,19]. Our results showed that WAY-100635 completely antagonized the antidepressant-like activity of YL-0919 in both behavioral models, suggesting that activation of the 5-HT1A receptor is critical in the antidepressant-like effect of YL-0919. The administered dose of WAY-100635 ranged from 0.1–0.3 mg/kg, which reportedly has no effect on locomotor activity and only blocks the activation of 5-HT1A receptor [11,12].

Studies have also shown that the 5-HT1A receptor plays an important role in the activities of serotonergic antidepressants [20,21]. It has been reported that FLX did not exert an antidepressant-like behavioral effect in 5-HT1A knockout mice, demonstrating the important role of 5-HT1A receptors in the antidepressant-like activity of SSRIs [22]. These results as well as data from our current study are consistent and mutually supportive.

The incidence of depression is not only limited to the changes in neurotransmitters and receptors but also involves the downstream signaling pathways. Studies have shown dysfunction of the cAMP signaling pathway in the brain of patients with depression [23]. The AC-cAMP-PKA signaling pathway initiates and involves in many intracellular biochemical mechanisms [24]. Specifically, PKA-mediated phosphorylation of the transcription factor CREB and the subsequent activation of downstream genes such as BDNF and Bcl-2 are critical in mediating the neurotrophic effect and neuroplasticity. The important role of cAMP-CREB in antidepressant treatment has been widely recognized [25,26].

Studies have demonstrated that activation of 5-HT1A receptors enhanced the subsequent activation of AC activity [6,7], and heterologous sensitization of AC has been implicated in psychiatric disorders, including depression [5,27]. To explore the mechanisms underlying the antidepressant-like effect of YL-0919, this study used the 5-HT1A receptor partial agonist buspirone or the classic antidepressant FLX as a comparison. When incubated with extracted synaptic membrane proteins, buspirone and FLX dose-dependently increased the synaptic membrane AC activity of rat cerebral cortex in vitro, which is consistent with the results of previous reports [9]. We also found that YL-0919 also directly stimulated the AC activity in synaptic membrane extracted from normal rat prefrontal cortex. The increase of AC activity
in the synaptic membranes by YL-0919 was completely blocked by WAY-100635, indicating that 5-HT1A receptor was involved in the YL-0919-mediated activation of AC in prefrontal cortex. Considering the inhibition of the behavioral effects of YL-0919 by WAY-100635, these results further suggested that activation of the 5-HT1A receptor and subsequent activation of the prefrontal cortex AC-cAMP-PKA pathway may be one of the critical mechanisms underlying the antidepressant-like effect of YL-0919. The ability of 5-HT1A receptors to induce heterologous sensitization coupled with the ability of SSRIs to enhance serotonergic neurotransmission may suggest a mechanism for enhanced CREB activity.

Injection of the selective PKA inhibitor H-89 (0.3–3 mg/kg) in combination with i.g. administration of YL-0919 significantly reduced the antidepressant-like effect of YL-0919 in both animal tests, further demonstrating that the activation of the AC-cAMP-PKA signaling pathway may be critical in the antidepressant-like effect of YL-0919. In addition, all doses of H-89 had no effect on locomotor activity when tested alone [16].

A number of studies have demonstrated that 5-HT1A receptor partial agonists (such as buspirone and tandospirone), have been used to accelerate or enhance the antidepressant effect of SSRIs [28,29,30]. Vilazodone, a drug that acts as both a 5-HT1A receptor partial agonist and 5-HT reuptake inhibitor, is the first compound that was approved on 21 January 2011 by the US Food and Drug Administration (FDA) for the treatment of Major Depressive Disorder [3]. Thus, antidepressant drugs with dual pharmacological effects as both a 5-HT reuptake inhibitor and 5-HT1A receptor agonist have become the primary focus of research and development.

5. Conclusion

In summary, this study investigated the pharmacological mechanism of the antidepressant-like activity of YL-0919 in both behavioral models and biochemical assays, and confirmed that activation of the 5-HT1A receptor and subsequent activation of the downstream AC-cAMP-PKA signaling pathway were one of the key mechanisms for the antidepressant-like effect of YL-0919. These results provide important evidence and additional clues for continued study of the precise mechanism of the antidepressant-like effect of YL-0919 as well as for the research and development of novel antidepressant.

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