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Research Paper

Augmentative effect of spinosin on pentobarbital-induced loss of righting reflex in mice associated with presynaptic 5-HT$_{1A}$ receptor

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loss of righting reflex; pentobarbital; presynaptic 5-HT$_{1A}$ receptors; spinosin

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Abstract

Objectives This study investigated whether spinosin potentiates pentobarbital-induced loss of righting reflex (LORR) in mice via 5-HT$_{1A}$ receptors.

Methods Our primary endpoint for sedation was LORR. In addition, the basal rectal temperature was measured.

Key findings The results demonstrated that the 5-HT$_{1A}$ agonist 8-OH-DPAT (s.c.) induced reductions in duration of LORR at 0.1, 0.5 and 1.0 mg/kg ($P < 0.01$), and prolongation of LORR latency at 0.5 and 1.0 mg/kg (s.c., $P < 0.01$) in pentobarbital (45 mg/kg, i.p.)-treated mice. This effect of 8-OH-DPAT was antagonized either by 5-HT$_{1A}$ antagonist *p*-MPPI (5 mg/kg, i.p.) or by spinosin (15 mg/kg, i.g.) with significance, respectively. Co-administration of spinosin and *p*-MPPI both at ineffective doses (spinosin at 5.0 mg/kg, i.g. and *p*-MPPI at 1.0 mg/kg, i.p.) showed significant augmentative effects in reducing latency to LORR, and increasing LORR duration ($P < 0.01$) in pentobarbital-treated mice. On the other hand, spinosin inhibited 8-OH-DPAT-induced hypothermia, which has been generally attributed to the activation of somatodendritic 5-HT$_{1A}$ autoreceptors in mice.

Conclusions Based on our previous results and the present data, it should be presumed that presynaptic 5-HT$_{1A}$ autoreceptor mechanisms may be involved in the inhibitory effect of spinosin on 8-OH-DPAT-induced hypothermia and also in the potentiating effect of spinosin on pentobarbital-induced LORR in mice.

Introduction

Semen *Ziziphi spinosae* is a well-known traditional tranquilizing medicine that has been extensively used in the treatment of a variety of diseases. There is abundant literature on the treatment of insomnia using Semen *Ziziphi spinosae* with good curative effect in human beings. Spinosin (2″-β-*O*-glucopyranosyl swertisin, $C_{28}H_{38}O_{15}$), one of the major constituents of semen *Zizhiphi spinozae*, is a C-glycoside flavonoid. Our previous studies showed that spinosin dose-dependently augmented pentobarbital-induced loss of righting reflex (LORR) by decreasing the latency to LORR and increasing both the rate and duration of LORR induced by sub-hypnotic dosage of pentobarbital. These effects of spinosin were promoted by 5-hydroxytryptophan (5-HTP) and inhibited by p-chlorophenylalanine (PCPA).[1] In view of these results, we supposed that the serotonerigic system may be involved in the mechanism of spinosin's potentiating effect on pentobarbital-induced LORR in mice.

Serotonin was one of the first neurotransmitters shown to be related to the physiology of the sleep/wake cycle.[2] Elucidation of the serotonergic mechanisms in sleep is complicated due to polymorphism of 5-HT receptors. In sleep research, studies have elucidated the effects of 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_7$ on sleep and wakefulness and most studies have examined the vigilance effects due to modulation of 5-HT$_1$ and 5-HT$_2$ receptors.[3] 5-HT$_{1A}$ receptors are of special interest, since there are data on their involvement in the mechanisms of sleep and hypothermia.[2,4] The results so far show that 5-HT$_{1A}$ receptors appear to play an important role in the serotonergic modulation of sleep and wakefulness. Both presynaptic somatodendritic 5-HT$_{1A}$ autoreceptors and postsynaptic 5-HT$_{1A}$ heteroreceptors may be involved.[5,6] Our recent study indicated that the effect of spinosin on rapid eye movement sleep in pentobarbital-treated rats may be related to the postsynaptic 5-HT$_{1A}$ receptors.[7]

However, we cannot exclude that presynaptic somatodendritic 5-HT$_{1A}$ autoreceptors may be involved. The goal of the present work was to study the role of 5-HT$_{1A}$ receptors in the augmentative effect of spinosin on pentobarbital-induced LORR in mice.

Materials and Methods

Drugs

Spinosin was purchased from Shanghai Tauto Biotech Co., Ltd. (purity >97% in HPLC; Shanghai, China). Other drugs, pentobarbital, 8-hydroxy-2-(di-*n*-propyl- amino) tetralin (8-OH-DPAT) and 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamido]-ethyl-piperazine (*p*-MPPI) were purchased from Sigma-Aldrich (St Louis, USA).

For oral administration, spinosin was suspended in distilled water. For intraperitoneal (i.p.) or subcutaneous (s.c.) injection, pentobarbital, 8-OH-DPAT and *p*-MPPI were dissolved in physiological saline, respectively. Spinosin was administered (i.g.) 60 min before pentobarbital administration (i.p. to the right abdomen). *p*-MPPI (i.p. to the left abdomen) and 8-OH-DPAT (s.c.) were injected 25 min before pentobarbital administration, respectively.

Animals

The animals used were ICR male mice (Grade I, purchased from Animal Center of Peking University, Beijing), 18–22 g, in groups of 12–17. Each mouse was used only for one experiment. They were housed in acrylfiber cages (440 × 270 × 178 mm, 12–17/per cage) at a controlled room (temperature 22 ± 2°C) and humidity 50 ± 10%) and were kept on a 12-h light–dark cycle. They were allowed free access to standard diet and water and acclimated for seven days before they were used. In the case of oral administration, mice were fasted for 12 h before testing. The experiments were carried out from 0800 to 1130 h in a quiet room in which the temperature was maintained at 22–24°C. All experiments were conducted in accordance with the European Community guidelines for the use of experimental animals and approved by Biomedical Ethics Committee of Peking University, Animal Welfare and Ethics Branch (Approval No. LA2010-019).

Evaluation of loss of righting reflex onset and duration time

Our primary endpoint for sedation was LORR. LORR was defined as the inability of mice to right themselves when positioned in a supine position. In the experiment measuring sleep, the righting reflex was considered restored when mice first regained an upright position, standing on their feet. Observers were blind to the drug treatment. Following the pentobarbital injection, each mouse was observed for the onset of sleep with the criterion for LORR. The LORR latency time was recorded from the injection of pentobarbital to 1 min after LORR and LORR time was recorded from 1 min after LORR to recovery.

8-OH-DPAT-induced hypothermia

After removal of mice from their home cages, basal rectal temperature was measured with a portable intelligent datalogger SN2202 (Beijing Sinan Instrument, Beijing Normal University). The probe was inserted into the rectum 1.5 cm for 40 s. Rectal temperature was determined again after the appropriate treatments with 8-OH-DPAT (0.25 mg/kg), spinosin or saline. The difference between the temperature measured before and after the administration represents an index of hypothermia. A decrease of more than 1.2°C from basal rectal temperature was considered to be a hypothermic response.

Statistical analysis

All values are expressed as mean ± SEM. For multiple comparisons, data were analysed by one-way analysis of variance followed by Student–Newman–Keuls test. $P < 0.05$ was considered statistically significant.

Results

Effect of p-MPPI and 8-OH-DPAT on pentobarbital-induced loss of righting reflex in mice

Administration of 8-OH-DPAT, a 5-HT$_{1A}$ agonist, significantly reduced LORR time at 0.1, 0.5 and 1.0 mg/kg (s.c.) ($P < 0.01$, Figure 1b) and prolonged the LORR latency with significant effect at 0.5 and 1.0 mg/kg ($P < 0.01$, Figure 1a) in pentobarbital (45 mg/kg, i.p.)-treated mice. Whereas, the selective 5-HT$_{1A}$ antagonist *p*-MPPI (2.0, 4.0 and 5.0 mg/kg, i.p.) significantly reduced LORR latency and increased LORR time in pentobarbital (45 mg/kg, i.p.)-treated mice (Figure 1c and 1d). As an antagonist, *p*-MPPI (5 mg/kg, i.p.) antagonized the effect of agonist 8-OH-DPAT (0.1 mg/kg, s.c.) on pentobarbital-induced LORR in mice ($P \leq 0.01$, Figure 2b). However, 8-OH-DPAT (0.1 mg/kg, s.c.) did not reverse the shortening effect of *p*-MPPI (5 mg/kg, i.p.) on LORR latency (Figure 2a).

Synergic effect of spinosin and p-MPPI on pentobarbital-induced loss of righting reflex in mice

To evaluate the synergistic effects of spinosin and *p*-MPPI, spinosin (5 mg/kg, i.g.) and *p*-MPPI (1 mg/kg, i.p.) were co-administered. At ineffective doses, neither spinosin (5 mg/kg, i.g., Figure 2c and 2d) nor *p*-MPPI (1 mg/kg, i.p.,

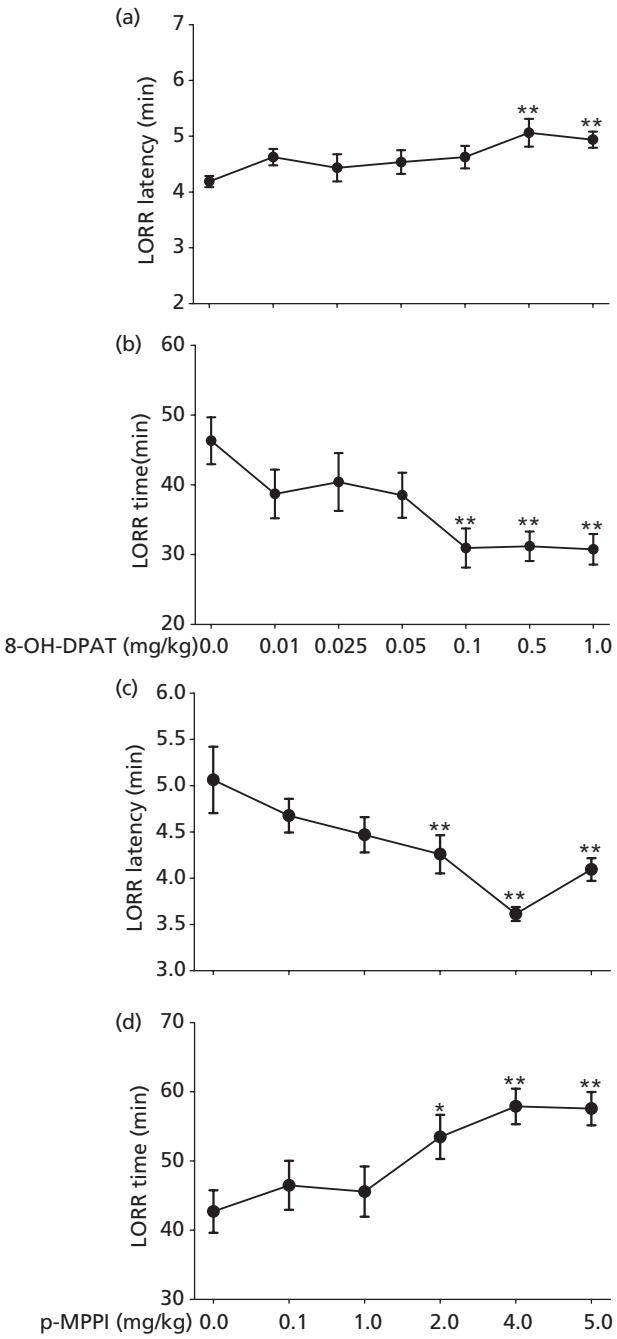

Figure 1 (a, b) Effect of 8-OH-DPAT in pentobarbital-treated mice. LORR latency (a) [$F(6,102) = 2.446$, $P < 0.03$] and LORR time (b) [$F(6,102) = 3.755$, $P < 0.002$] were assessed. All values are presented as mean ± SEM, $n = 14$–16. (c, d) Effect of p-MPPI in pentobarbital-treated mice. LORR latency (c) [$F(5,92) = 5.598$, $P < 0.001$]and LORR time (d) [$F(5,92) = 4.366$, $P < 0.001$] were assessed. All values are presented as mean ± SEM, $n = 16$–17. $^*P < 0.05$ and $^{**}P < 0.01$ vs vehicle (Student–Newman–Keuls test).

Figures 1c, 1d, 2c and 2d) exerted significant effects on pentobarbital (45 mg/kg, i.p.)-induced LORR in mice. However, co-administration of spinosin (5 mg/kg, i.g.) and *p*-MPPI (1 mg/kg, i.p.) significantly reduced LORR latency and increased LORR time ($P < 0.01$, Figure 2c and 2d) in pentobarbital (45 mg/kg, i.p.)-treated mice.

Antagonistic effect of spinosin on 8-OH-DPAT-induced reduction of loss of righting reflex in pentobarbital-treated mice

Consistent with the findings of a previous study in pentobarbital-treated mice,[1] the present result also showed that spinosin (15 mg/kg, i.g.) potentiated the anaesthetic effect of pentobarbital (45 mg/kg, i.p. Figure 2e and 2f), reflected by reduced LORR latency and increased LORR time. Meanwhile, the treatment with 8-OH-DPAT (0.1 mg/kg, s.c.) significantly antagonized the potentiating effect of spinosin (15 mg/kg, i.g.) on pentobarbital-induced LORR time ($P < 0.05$, Figure 2f). Interestingly, however, 8-OH-DPAT potentiated spinosin-induced reduction of LORR latency in pentobarbital-treated mice ($P < 0.05$, Figure 2e).

Effect of spinosin on 8-OH-DPAT-induced hypothermia in mice

Consistent with other reports, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (0.5 mg/kg, s.c.) induced a significant hypothermia in mice as observed 10 min after the injection ($P < 0.01$, Figure 3). Spinosin inhibited the hypothermia induced by 8-OH-DPAT at a dose of 15 mg/kg (i.g.).

Discussion

Serotonin has been implicated in many behavioral and physiological processes and in psychiatric disorders, such as anxiety and depression.[8] Among 5-HT receptors, special interest has been given to the 5-HT$_{1A}$ receptor subtype. Research using drugs that are selective agonists and antagonists at serotonergic receptors shows that the 5-HT$_{1A}$ receptor has an important role in modulating the serotonergic effects on sleep and waking.[9] The pharmacological stimulation of this receptor by selective 5-HT$_{1A}$ receptor agonists, such as 8-OH-DPAT, consistently inhibits the spontaneous discharge of serotonergic neurons in both anaesthetized and unanaesthetized animals.[10,11] Systemic administration of 8-OH-DPAT,[12] at doses in the range 0.1~2.0 mg/kg consistently increases waking in rats.[13–16] These effects are thought to reflect postsynaptic 5-HT$_{1A}$ receptor stimulation. *p*-MPPI, the potent and selective 5-HT$_{1A}$ antagonist both at the somatodendritic and postsynaptic 5-HT$_{1A}$ receptors,[5,6] antagonized the 8-OH-DPAT-induced increase in wakefulness in rats, indicating that *p*-MPPI acted as an effective 5-HT$_{1A}$ antagonist with respect to sleep and wakefulness.[17–20]

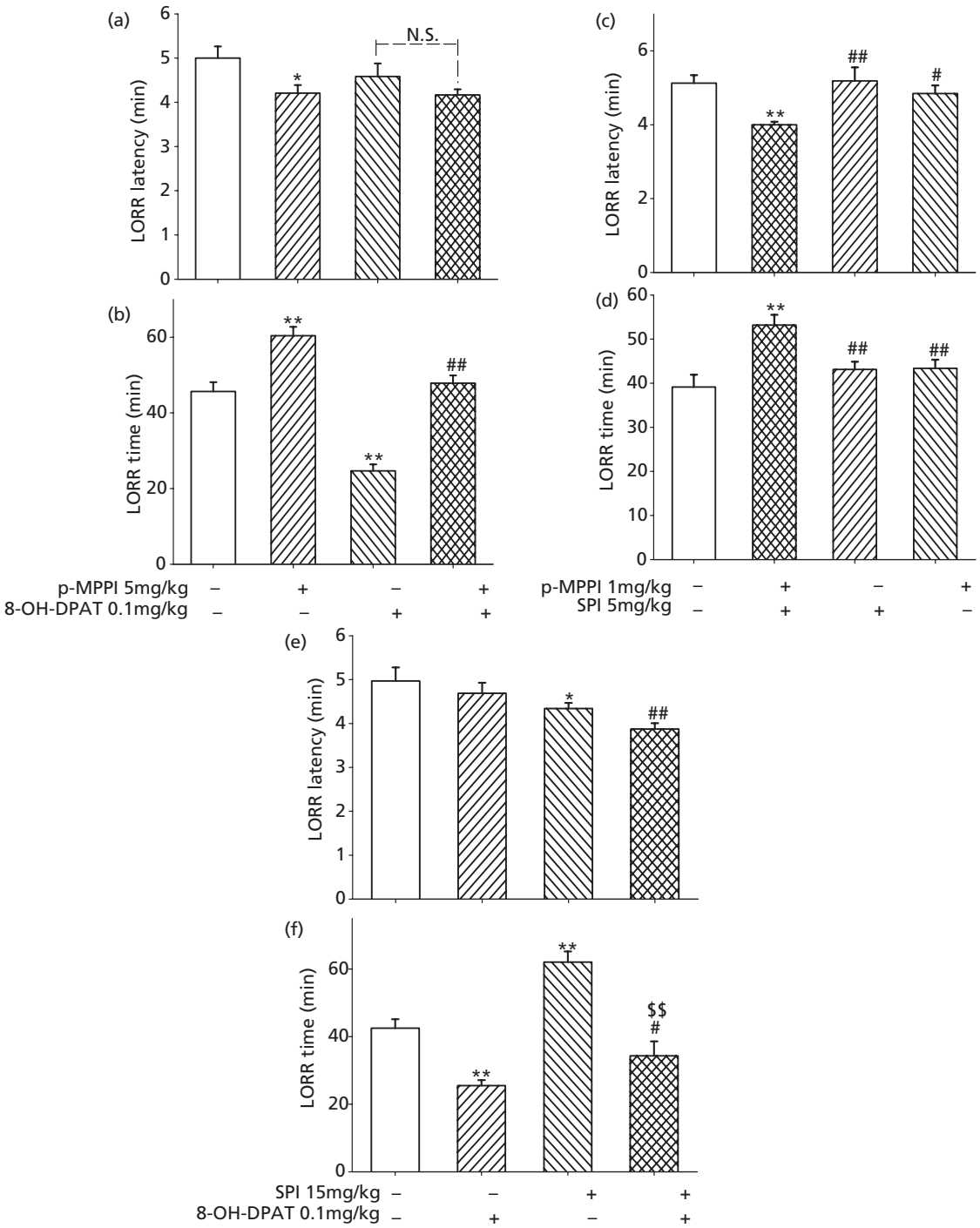

Figure 2 (a, b) Effect of p-MPPI on 8-OH-DPAT-induced insomnia in pentobarbital-treated mice. LORR latency (a) [$F(3,44) = 2.972$, $P < 0.042$] and LORR time (b) [$F(3,44) = 47.194$, $P < 0.001$] were assessed. All values are presented as mean ± SEM, $n = 12$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs vehicle and $^{\#\#}P < 0.01$ vs group treated with 8-OH-DPAT alone (Student–Newman–Keuls test). (c, d) Synergic effects of spinosin with p-MPPI on pentobarbital-induced LORR in mice. LORR latency (c) [$F(3,60) = 5.077$, $P < 0.003$] and LORR time (d) [$F(3,60) = 7.104$, $P < 0.001$] were assessed. All values are presented as mean ± SEM, $n = 16$. SPI, spinosin. $^{**}P < 0.01$ vs vehicle. $\#P < 0.05$ and $\#\#P < 0.01$ vs group treated with 1 mg/kg p-MPPI + 5 mg/kg spinosin (Student–Newman–Keuls test). (e, f) Effect of spinosin on 8-OH-DPAT-induced reduction of LORR in pentobarbital-treated mice. LORR latency (e) [$F(3,60) = 4.745$, $P < 0.005$] and LORR time (f) [$F(3,60) = 25.630$, $P < 0.001$] were assessed. All values are presented as mean ± SEM, $n = 16$. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs vehicle. $\#P < 0.05$ and $\#\#P < 0.01$ vs group treated with 0.1 mg/kg 8-OH-DPAT alone. $^{\$\$}P < 0.01$ vs group treated with 15 mg/kg spinosin alone (Student–Newman–Keuls test).

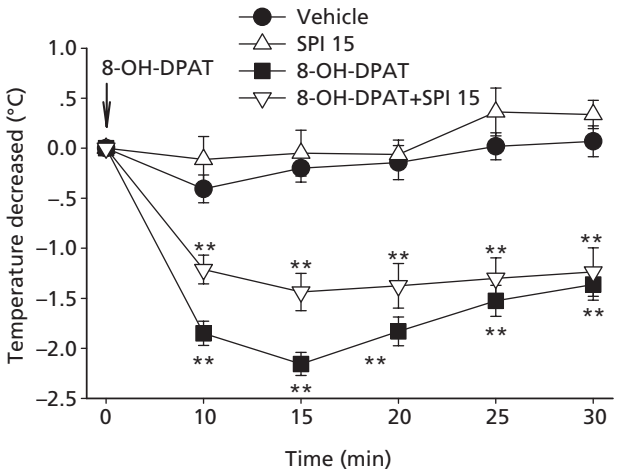

Figure 3 Effect of spinosin on 8-OH-DPAT-induced hypothermia in mice. Mice were treated orally with spinosin (15 mg/kg, i.g.) and 30 min later they were injected with 8-OH-DPAT (0.5 mg/kg, s.c.) or saline. SPI 15, spinosin 15 mg/kg. The data presented as mean ± S.E.M. of no less than eight mice. $^{**}P < 0.01$ vs initial temperature.

This study showed that 8-OH-DPAT given subcutaneously significantly increased LORR latency (Figure 1a) and decreased the LORR time (Figure 1b) in pentobarbital-treated mice. Administration of *p*-MPPI (i.p.) reduced pentobarbital-induced LORR latency (Figure 1c) and increased LORR time (Figure 1d) in mice. Nonetheless, *p*-MPPI successfully antagonized 8-OH-DPAT induced reduction of LORR time in pentobarbital-treated mice (Figure 2b). These findings are consistent with studies using systemic injection of 8-OH-DPAT in the freely moving rat,[13–16] and are also thought to reflect postsynaptic 5-HT$_{1A}$ stimulation.[20]

Consistent with our previous report,[1] this study also showed that spinosin (15 mg/kg, i.g.) significantly reduced LORR latency (Figure 2e, $P < 0.05$) and increased LORR time (Figure 2f, $P < 0.01$) in pentobarbital-treated mice. In addition, spinosin antagonized the inhibitory effect of 8-OH-DPAT on pentobarbital-induced LORR time in mice with significance (Figure 2f). Moreover, co-administration of spinosin and *p*-MPPI both at ineffective doses (spinosin at 5.0 mg/kg, i.g and *p*-MPPI at 1.0 mg/kg, i.p.) showed significant synergic effects in reducing LORR latency (Figure 2c, $P < 0.01$) and increasing LORR time (Figure 2d, $P < 0.01$) in pentobarbital (45 mg/kg, i.p.)-treated mice. 5-HT$_{1A}$ receptors are typically located on the 5-HT cell body as presynaptic autoreceptors[21] and are also found in several areas receiving serotonergic projections as postsynaptic receptors.[22,23] From the results so far, it remains unclear whether the effect of spinosin is due to presynaptic somatodendritic 5-HT$_{1A}$ receptor inhibitory activity.

The hypothermic response to 5-HT$_{1A}$ receptor stimulation has been proposed by different authors to be an index of either inhibitory somatodendritic or postsynaptic 5-HT$_{1A}$ receptor activation.[4,24,25] In rats, the hypothermic response to 8-OH-DPAT is accepted to be regulated by postsynaptic 5-HT$_{1A}$ receptor stimulation[26,27] whereas in mice the effect is thought to be presynaptically mediated[28,29] In this study, spinosin inhibited hypothermic response to 8-OH-DPAT in mice (Figure 3). This result suggested that spinosin may inhibit the 8-OH-DPAT-induced hypothermia via presynaptic 5-HT$_{1A}$ receptor pathway in mice.

PCPA is a specific, potent and irreversible inhibitor of tryptophan 5-monooxygenase, the rate-limiting enzyme of biosynthesis of serotonin. Inhibition of 5-HT synthesis with PCPA induced a severe insomnia, which could be reversed by restoring 5-HT synthesis.[30] Our previous study showed that spinosin significantly inhibited the PCPA-induced suppression of pentobarbital-induced LORR.[1] Based on these findings, it should be presumed that the presynaptic pathway of 5-HT$_{1A}$ autoreceptor, which modulates the 5-HT synthesis, release and so on, may be involved in the potentiating effect of spinosin on pentobarbital-induced LORR in mice. These findings and the data obtained in this study taken together give reasons to suppose that presynaptic 5-HT$_{1A}$ receptors may be also involved in the potentiating activity of spinosin on pentobarbital-induced LORR.

Conclusions

In summary, spinosin antagonized the effect of 8-OH-DPAT in measures of rapid eye movement sleep and non-rapid eye movement sleep in rats,[7] pentobarbital-induced LORR and hypothermia in mice associated with 5-HT$_{1A}$ activation. Thus, it should be presumed that spinosin behaved as a 5-HT$_{1A}$ receptor antagonist, acting both pre- and post-synaptically.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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