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PHARMACOLOGY

EFFECT OF RITANSERIN AND LEUPROLIDE ALONE AND COMBINED ON MARBLE-BURYING BEHAVIOR OF MICE

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Abstract: Obsessive-compulsive disorder (OCD) is characterized by absurd, recurrent thoughts (obsessions) followed by certain stereotyped actions (compulsions). OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity. The aim of present study was to evaluate the effect of ritanserin and leuprolide *per se* and in combination on marble-burying behavior of mice. The present study showed that ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*) *per se* did not show any anti-compulsive effect. Leuprolide (200 and 300 µg kg^{-1} *s.c.*) *per se* showed anti-compulsive effect, causing statistically significant inhibition of marble-burying behavior of mice. The prior treatment with ritanserin, $5HT_{2A/2C}$ antagonist (20 mg kg^{-1} *i.p.*), has effectively blocked the inhibitory influence of leuprolide (300 µg kg^{-1} *s.c.*) on marble burying behavior of mice, suggesting that leuprolide, a LHRH agonist, also requires serotonin to express its anti-compulsive effect. Further, it also suggested that the effect of leuprolide appears to be mediated through $5HT_{2A/2C}$ receptors.

Keywords: ritanserin, leuprolide, marble-burying behavior, motor activity

Obsessive-compulsive disorder (OCD) may be defined as the irruption in the mind of uncontrollable, egodystonic and recurrent thoughts, impulses or images (1, 2). In OCD, repetitive rituals serve to counteract the anxiety precipitated by obsessions (3). The thoughts and behaviors associated with OCD are viewed as senseless, and egodystonic and they stand contradictory to the individual's motives, goals, identity, and self-perception thereby creating significant subjective distress. The OCD patients realize the irrational nature of thoughts and rituals but feel helpless and hopeless about controlling them. OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. The research on OCD using animal models has exploited the expression of natural or pharmacologically induced repetitive behaviors in various species (4). The drug induced model of OCD, in which chronic treatment of rats with dopamine agonist quinpirole induces compulsive checking behavior, is partly attenuated by clomipramine (5). The observed association between OCD and a dysfunction of orbito-frontal cortex (OFC) and of the serotonergic system, namely, that OFC pathology leads to a dysregulation of serotonergic system which is manifested in compulsive behavior, was studied using a new rat model of OCD, the signal attenuation model (6). The rats and mice bury the unpleasant object able to cause aversion stimuli and fearful thoughts (7). The noxious and fearful stimuli associated with electrified prod, food of unpleasant tasting and predators such as scorpions, activates defensive behavior of animal (8–10). The marble burying test has been used as screening model for the detection of anxiolytics (11). The aim of present study was to evaluate the effect of ritanserin and leuprolide *per se* and in combination on marble-burying behavior of mice.

EXPERIMENTAL

Materials and methods

Animals

The studies were carried out in adult male albino Swiss mice (22–25 g), group housed (n = 6), under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2°C, 55 ± 2%). The animals received standard rodent

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chow (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*. Separate groups (n = 6) of mice were used for each set of experiments and each animal was used only once. Mice were acclimatized to laboratory conditions for 5 days before carrying out the experiments. The animal studies were approved by Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experiments on animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs

Ritanserin was a gift by Sun Pharma Advanced Research Centre, India. Leuprolide was purchased from Sigma-Aldrich Co., USA. Leuprolide was dissolved in 0.9% saline. Ritanserin was dissolved in a 1% concentration Tween-80.

Treatments

In the first set of experiments, ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*), leuprolide (100, 200 and 300 µg kg^{-1} *s.c.*) were administered 30 min prior to the assessment of marble-burying behavior or motor activity.

In the second set of experiments, ritanserin (20 mg kg^{-1} *i.p.*) was administered 30 min prior to leuprolide (300 µg kg^{-1} *s.c.*) and 30 min thereafter, individual mouse was tested for marble-burying behavior or motor activity. Separate groups (n = 6) of mice were used for each set of experiments.

Marble burying behavior model

In this model, mice were individually placed in separate plastic cages (21×38×14 cm) containing 5 cm thick sawdust bedding. Twenty clean glass marbles (diameter ~10 mm), were arranged on the bedding evenly spaced in four rows. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered buried if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive-compulsive behavior.

Actophotometer

Motor activity was assessed in separate group of mice using actophotometer (Techno, Lucknow), which had a circular arena of 40 cm, equipped with three infrared beams and photo-cells connected to digital counter. Motor activity was assessed in terms of total number of counts of light beams interruptions in 30 min.

Statistical analysis

The data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. The results are expressed as the mean ± SEM of six observations; $p < 0.05$ was considered to be statistically significant in all cases.

RESULTS

Effect of ritanserin on marble-burying behavior and motor activity in mice

Ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*) [F (3, 20) = 4.918, $p > 0.05$] (Fig. 1) did not reduce marble-burying behavior of mice. In another method, ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*) [F (3, 20) = 0.02655, $p > 0.05$] (Fig. 1) did not affect motor activity.

Effect of leuprolide on marble-burying behavior and motor activity in mice

Leuprolide (200 and 300 µg kg^{-1} *s.c.*) [F (3, 20) = 211.93, $p < 0.001$] (Fig. 2) significantly reduced marble-burying behavior of mice but leuprolide

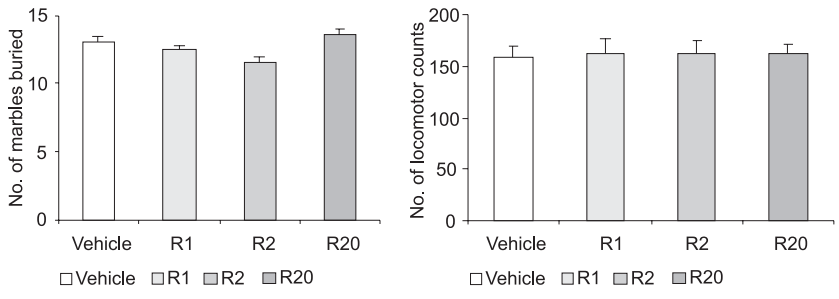

Figure 1. Effect of ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*) on marble-burying behavior and motor activity of mice tested in separate groups of mice. Each bar presents the mean ± SEM of data from 6 mice (one way ANOVA followed by Tukey test for multiple comparisons)

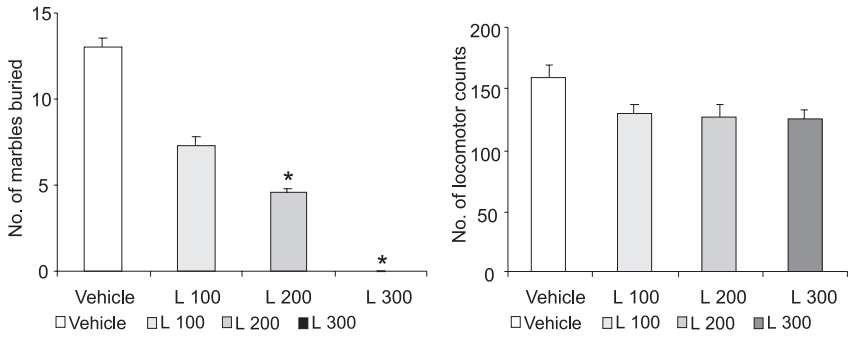

Figure 2. Effect of leuprolide (100, 200 and 300 µg kg^{-1} *s.c.*) on marble-burying behavior and motor activity of mice tested in separate groups of mice. Each bar presents the mean ± S.E.M. of data from 6 mice. *denotes $p < 0.001$ as compared to vehicle group (one way ANOVA followed by Tukey test for multiple comparisons)

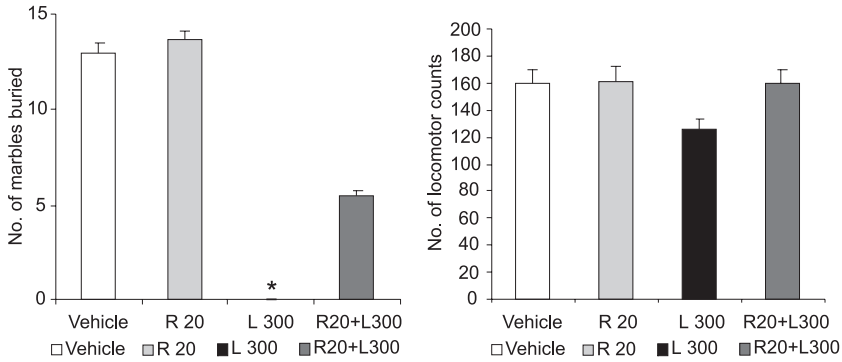

Figure 3. Effect of ritanserin (20 mg kg^{-1} *i.p.*) plus leuprolide (300 µg kg^{-1} *s.c.*) on marble burying behavior and motor activity tested in separate groups of mice. Ritanserin (20 mg kg^{-1} *i.p.*) was administered 30 min prior to leuprolide (300 µg kg^{-1} *s.c.*) and 30 min thereafter, individual mouse was tested for marble-burying behavior and motor activity. Each bar represents the mean ± SEM. of data from 6 mice; *denotes $p < 0.001$ compared to vehicle group (one way ANOVA followed by Tukey test for multiple comparisons).

(100 µg kg^{-1} *s.c.*) [F (3, 20) = 211.93, $p > 0.05$] did not reduce marble-burying behavior of mice (Fig. 2). In another method, leuprolide (100, 200 and 300 µg kg^{-1} *s.c.*) [F (3, 20) = 3.289, $p > 0.05$] (Fig. 2) did not affect motor activity.

Effect of ritanserin (20 mg kg-^{1} *i.p.*) plus leuprolide (300 µg kg-^{1} *s.c.*) on marble-burying behavior and motor activity of mice

The prior treatment with ritanserin (20 mg kg^{-1} *i.p.*), a $5HT_{2A/2C}$ antagonist, has effectively [F (3, 20) = 343.43, $p < 0.001$] (Fig. 3) blocked the inhibitory influence of leuprolide (300 µg kg^{-1} *s.c.*) on marble burying behavior of mice. It significantly increased number of marbles buried compared to leuprolide treated group. In another method, ritanserin (20 mg kg^{-1} *i.p.*) plus leuprolide (300 µg kg^{-1} *s.c.*) [F (3, 20) = 3.402, $p > 0.05$] (Fig. 3) did not affect motor activity.

DISCUSSION

OCD is a mental disorder characterized by intrusive thoughts that produce anxiety, by repetitive behaviors aimed at reducing anxiety, or by combinations of such thoughts (obsessions) and behav-

iors (compulsions) (12). OCD is classified as an anxiety disorder, and patients with OCD demonstrate a high incidence of comorbid depression (13). The brain regions impaired in OCD include basal ganglia, orbito-frontal cortex, anterior cingulate cortex, dorsolateral prefrontal cortex, amygdala, thalamus and brainstem. Entire brain functioning is disturbed in patients suffering from OCD, thereby producing devastating effects at the workplace as well as at homes of the patients (14). Burying behavior of mice consists in forward shoving the diggable material over the source of aversion using the snout and forepaws in order to avoid and protect from the localized threat (15). Marble burying behavior of mice has been used to model anxiety disorders including OCD due to the excessive nature of the behavior and due to the pharmacological effects of clinical standards (16). According to serotonin hypothesis, patients with OCD have a dysregulation in the serotonergic system, with a hypersensitivity of postsynaptic 5-HT receptors, which could account for a different mechanism of action of SSRI in OCD (17). An acute administration of certain classes of antidepressants like selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) has been shown to dose-dependently inhibit marble burying of mice (18–20) The benzodiazepine receptor agonists such as chlordiazepoxide decrease the number of marbles buried (21). The marble burying behavior is reduced by other classes of compounds such as classical antipsychotics (22). The aim of this study was to evaluate the effect of ritanserin and leuprolide alone and in combination on marble-burying behavior of mice.

In the present study, ritanserin (1, 2 and 20 mg kg^{-1} *i.p.*) alone did not significantly reduce marble-burying behavior of mice. However, ritanserin failed to produce any dose related effect on motor function. Leuprolide (200, 300 µg kg^{-1} s.c.) *per se* dose dependently attenuated marble-burying behavior of mice. Furthermore, leuprolide (100, 200 and 300 µg kg^{-1} *s.c.*) *per se* did not produce any effect on motor fuction. Incidentally, LHRH (luteinizing hormone releasing hormone) receptors have been identified in amygdala, hippocampus, anterior cingulate cortex, caudate, putamen and thalamus, that are the regions involved in obsessive-compulsive disorder (23). The prior treatment with ritanserin, $5HT_{2A/2C}$ antagonist (20 mg kg^{-1} *i.p.*), has effectively blocked the inhibitory influence of leuprolide (300 µg kg^{-1} *s.c.*) on marble burying behavior of mice. Many studies indicated that 5-HT neurons exert stimulatory or inhibitory effects on lutenizing hormone (LH) release (24). Some studies suggested that LHRH receptors are found in the brain regions, where serotonin dysfunction is believed to cause obsessive-compulsive disorder, and LHRH is reported to modulate the activity of several neurotransmitters, including serotonin (25, 26). However, the antidepressant action of LHRH and the involvement of LHRH in the action of antidepressant agents indirectly propose the modulatory role of LHRH on serotonergic or adrenergic neuronal systems (27, 28). One case study suggested that leuprolide, a LHRH agonist, has been found to benefit a patient of obsessive-compulsive disorder (29).

From the present investigations, it can be concluded that leuprolide, a LHRH agonist, exhibited significant dose dependent anti-compulsive effect, as reflected by significant inhibition of marble-burying behavior of mice. However, LHRH has been reported to exhibit anti-dopaminergic like activity (30). The prior treatment with ritanserin, $5HT_{2A/2C}$ antagonist, has effectively blocked the inhibitory influence of leuprolide on marble burying behavior of mice, suggesting that leuprolide, a LHRH agonist, also requires serotonin to express its anti-compulsive effect. Further, it also suggested that the effect of leuprolide appears to be mediated through $5HT_{2A/2C}$ receptors.

REFERENCES

1. Schwartz J.M.: J. Consciousness Stud. 6:115 (1999).
2. Rasmusen S.A., Eisen J.L.:Psychiatr. Clin, North Am. 15, 743 (1992).
3. Rasmussen S.A., Eisen J.L.: Psychiatr. Ann. 9, 67 (1989).
4. Stein D.J: Biol. Psychiatry 47, 296 (2000).
5. Joel D., Avisar A.: Behav. Brain Res. 123, 77 (2001).
6. Szechtman H, Sulis W, Eilam D.: Behav. Neurosci. 112, 1475 (1998).
7. Archer T., Fredriksson A., Lewander T., Soderberg U. S.: J. Psychol. 28, 242 (1987).
8. Treit D.: Pharmacol. Biochem. Behav. 36, 203 (1990).
9. Wilkie D.M., MacLennan A.J., Pinel J.P.: J. Exp. Anal. Behav. 31, 99 (1979).
10. Londei T., Valentini A.M., Leone V.G.: Behav. Brain Res. 94, 249 (1998).
11. Njung'e K., Handley S.L.: Biochem. Behav. 38, 63 (1991).
12. Parle M., Gaikwad U.: Int. J. Med. Sci. 2, 84 (2009).

13. Sasson Y., Zohar J., Chopra M., Lustig M., Iancu I., Hendler T.: J. Clin. Psychiat. 58, 7 (1997).
14. Parle M., Gaikwad U.: Asian J. BioSci. 4, 120 (2009).
15. Poling A., Cleary J., Monaghan M.: J. Exp. Anal. Behav. 35, 31 (1981).
16. Rapoport J.L.: J. Am. Med. Assoc. 260, 2888 (1988).
17. Billett E.A., Richter M.A., King N.: Mol. Psychiat. 2, 403 (1997).
18. Martin J.R., Bos M., Jenck F., Moreau J., Mutel V. et al.: J. Pharmacol. Exp. Ther. 286, 913 (1998).
19. Millan M.J., Dekeyne A., Papp M., La Rochelle C.D., MacSweeny C. et al.: J. Pharmacol. Exp. Ther. 298, 581 (2001).
20. Ichimaru Y., Egawa T., Sawa A.: Jpn. J. Pharmacol. 68, 65 (1995).
21. Gyertyan I.: Behav. Pharmacol. 6, 24 (1995).
22. Broekkamp C.L., Rijk H.W., Joly-Gelouin D., Lloyd K.L.: Eur. J. Pharmacol. 126, 223 (1986).
23. Rance N.E., Young III W.S., McMullen N.T.: J. Comp. Neurol. 339, 573 (1994).
24. Johnson M.D., Crowley W.R.: Endocrinology 118, 1180 (1986).
25. Aouizerate B., Guehl D., Cuny E., Rougier A., Bioulac B., Tignol J., Burbaud P.: Prog. Neurobiol. 72, 195 (2004).
26. Pan J.T., Kow L.M., Pfaff D.W.: Neuroscience 27, 623 (1988).
27. Massol J.P., Patrick M., Adesci G., Puech A.J.: Eur. J. Pharmacol. 160, 395 (1989).
28. Jain M.R, Subhedar N.K.: Brain Res. 604, 7 (1993).
29. Eriksson, T.: Am. J. Psychiat. 157,483 (2000).
30. Kadar T., Telegdy G., Schally A.V.: Physiol. Behav. 51, 601 (1992).

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