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LEUPROLIDE INHIBITS MARBLE-BURYING BEHAVIOR VIA MODULATION OF 5-HT$_{1B}$ RECEPTOR

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ABSTRACT

Obsessive compulsive disorder (OCD) is characterized by intrusive thoughts followed by repetitive behaviors. Serotonin-related genes found in OCD include those required for coding of 5-HT transporter and 5-HT receptors (5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{1B}$). Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity. The aim of this study was to evaluate the effect of leuprolide alone and it's combination with sumatriptan or ondansetron on marble-burying behavior of mice. Leuprolide (100, 200 & 300 µg kg^{-1}s.c.) dose-dependently showed anti-compulsive effect, causing statistically significant inhibition of marble-burying behavior of mice. The prior treatment with 5HT$_{1B/1D/1F}$ agonist, sumatriptan (0.1 mg kg-^{1} s.c.) potentiated the inhibitory effect of leuprolide (LHRH agonist) on marble burying behavior of mice. Furthermore, prior treatment with 5HT$_3$ antagonist, ondansetron (2 mg kg-^{1} s.c.) did not affect the inhibitory effect of leuprolide (200 µg kg^{-1}s.c.) on marble burying behavior of mice.

KEY WORDS: Leuprolide, Sumatriptan, Ondansetron, Marble-Burying Behavior

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INTRODUCTION

Obsessive–compulsive disorder (OCD) is a mental disorder characterized by intrusive thoughts (obsessions), which produce anxiety followed by repetitive behaviors (compulsions) aimed at reducing anxiety. The most common types of obsessions are related to contamination, pathological doubts, somatic dysfunctions, need for symmetry, aggression and hyper sexual drive. In OCD, senseless, repetitive rituals (such as counting, washing etc.) serve to counteract the anxiety precipitated by obsessive thoughts e.g. Symmetry and exactness pre-occupations. The thoughts and behaviors associated with OCD are viewed as senseless, and ego dystonic and they stand contradictory to the individual's motives, goals, identity, and self-respect thereby creating significant subjective distress. 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 work-places as well as at homes of the patients.[1] Abnormal 5-Hydroxytryptamine (5-HT) levels may cause OCD and changes in serotonergic transmission may have direct or indirect effects on the neuronal firing of other neuromodulators affecting thoughts, feelings and behaviors. Serotonin-related genes found in OCD include those necessary for coding of the 5-HT transporter (5-HTT), 5-HT receptors (5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{1B}$) as well the 5-HT enzyme tryptophan hydroxylase.[2] The rats and mice try to bury the unpleasant objects responsible for aversive stimuli and fearful thoughts.[3] Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity[4] The aim of this study was to evaluate the effect of leuprolide alone and the combination of leuprolide with sumatriptan or ondansetron on marble-burying behavior of mice.

MATERIALS AND METHODS

Drugs

Leuprolide was purchased from Sigma-Aldrich Ltd., USA. Sumatriptan was gifted by Natco Pharma Ltd., India, whereas, Ondansetron was gifted by Cipla Ltd., India. Leuprolide, Sumatriptan and Ondansetron were dissolved in 0.9% saline.

Animals

All the experiments were carried out in adult male Swiss mice (22–25 g), housed at an animal house under a standard alternating 12 h each light/dark cycle and controlled conditions of temperature and humidity (25± 2 °C, 55± 2%). The animals received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water ad libitum. Separate groups of mice were used for each set of experiments and each animal was used only once. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC). The care of animals was taken as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India (registration number 0436).

Experimental Design

Mice were divided in 15 groups and each group consisted of a minimum of six animals. Separate animals were used for each experiment.

Group I: It represented the control group for young mice (n=6).

Groups II, III, IV, V, VI and VII: Leuprolide (100, 200 and 300 µg kg^{-1}s.c.) was injected into young male mice. Marble-burying behavior/locomotor activity of mice were measured 30 min. after the drug administration.

Groups VIII, IX: Sumatriptan (0.1 mg kg^{-1}s.c.) was injected into young male mice. Marble-burying behavior/locomotor activity of mice were measured 30 min. after the drug administration.

Groups X, XI: Ondansetron (2 mg kg^{-1} s.c.) was injected into young male mice. Marble-burying behavior/locomotor activity of mice were measured 30 min. after the drug administration.

Groups XII, XIII : Sumatriptan (0.1 mg kg^{-1} s.c.) was given 30 min prior to the administration of leuprolide (200 µg kg^{-1}s.c.) and the effect of this combination was studied on the marble-burying behavior/locomotor activity of mice after the passage of another 30 min.

Group XIV, XV: Ondansetron (2 mg kg^{-1} s.c.) was given 30 min prior to the administration of leuprolide (200 µg kg^{-1}s.c.) and the effect of this combination was studied on the marble-burying behavior/locomotor activity of mice after the passage of another 30 min.

Marble-burying Behavioral 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 evenly on the bedding. 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

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

Statistical analysis

The data were analyzed with one-way ANOVA followed by Tukey test for multiple comparisons. The values were expressed as mean±S.E.M $p < 0.05$ was considered to be statistically significant in all the cases.

RESULTS AND DISCUSSION

Leuprolide (100, 200 & 300 µg kg^{1}s.c.) significantly reduced ($p < 0.001$) the number of marbles buried by mice (Fig. 1). Furthermore, leuprolide (100, 200 & 300 µg kg^{-1}s.c.) did not affect ($p > 0.05$) locomotor activity of mice (Fig. 2). Pre-treatment of mice with sumatriptan (0.1 mg kg^{-1}s.c.) significantly potentiated ($p < 0.001$) the inhibitory influence of leuprolide (200 µg kg^{-1} s.c.) on marble burying behavior of mice (Fig. 3). The combination of sumatriptan (0.1 mg kg^{-1}s.c.) and leuprolide (200 µg kg^{-1}s.c.) did not exhibit ($p > 0.05$) any significant effect on the locomotor function of mice (Fig. 4). Ondansetron (2 mg kg^{-1}s.c.) pre-treatment did not affect ($p > 0.05$) the inhibitory effect of leuprolide (200 µg kg^{-1}s.c) on marble burying behavior of mice (Fig. 5). The combination of ondansetron (2 mg kg^{-1}s.c.) and leuprolide (200 µg kg^{-1}s.c.) did not exhibit (Fig. 6) any significant effect on the locomotor function of mice.

Obsessive-compulsive disorder is characterized by portrayal of ambivalence, confusion of thoughts and actions that are paradoxically manifested by rigidity and abnormal behavior.[5] Obsessive-compulsive disorder is described by the presence of unconscious conflicts, which are defensive and punitive. The OCD patients realize the irrational nature of their thoughts and rituals, but feel helpless and hopeless about controlling them. Serotonin-related genes found in OCD include those required for coding of the 5-HT transporter (5-HTT) and 5-HT receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_{1B}) as well as the 5-HT enzyme tryptophan hydroxylase. Obsessive-Compulsive Disorder can impair all areas of brain functioning and produce devastating effects on patients and their families. 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.[6] Marble burying behavior of mice has been used as an

experimental model for anxiety disorders including obsessive–compulsive disorder (OCD) due to the excessive nature of the behavior and due to the pharmacological effects of clinical standards. According to serotonin hypothesis, patients with OCD have a deregulation in the serotonergic system, with hypersensitivity of postsynaptic 5-HT receptors, which could account for a different mechanism of action of SSRI in OCD.[7] An acute administration of certain classes of anti-depressants like selective serotonin re-uptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs) and tricyclic anti-depressants (TCAs) have been shown to dose-dependently inhibit marble burying of mice.[8,9,10] The marble burying behavior is also inhibited by benzodiazepine receptor agonists such as chlordiazepoxide[11] and classical antipsychotics.[12] The aim of this study was to evaluate the effect of leuprolide alone and it's combination with sumatriptan or ondansetron on marble-burying behavior of mice.

In the present study, luteinizing hormone releasing hormone (LHRH) agonist, leuprolide (100, 200, 300 µg kg^{-1}s.c.) alone dose-dependently attenuated the marble-burying behavior of mice. Furthermore, leuprolide (100, 200 & 300 µg kg^{-1}s.c.) did not produce any effect on locomotor function of mice. Incidentally, LHRH receptors have been identified in amygdala, hippocampus, anterior cingulate cortex, caudate, putamen and thalamus regions, which are affected in obsessive–compulsive disorder.[13] One case study showed that leuprolide treatment produced a remarkable benefit in a patient of obsessive–compulsive disorder.[14] Some studies suggested that the serotonergic pathways are present proximal to LHRH secreting cells of hypothalamus and that LHRH receptors are found in the brain regions, where serotonin dysfunction is believed to cause obsessive–compulsive disorder. LHRH is reported to modulate the activity of several neurotransmitters, including serotonin.[15, 16] In this study, prior treatment with $5HT_{1B/1D/1F}$ agonist, sumatriptan potentiated the inhibitory effect of LHRH agonist, leuprolide on marble burying behavior of mice. Furthermore, it has been shown that $5HT_{1B}$ receptors are present post-synaptically. Thus, it appears that the combination might have succeeded in modulating positively the postsynaptic $5HT_{1B}$ receptors present on orbitofrontal cortex and anterior cingulate gyrus. However, the anti-depressant action of LHRH and the involvement of LHRH in the action of anti-depressants indirectly propose the modulatory role for LHRH on serotonergic or adrenergic neuronal systems.[17, 18] In the present study, the combination of sumatriptan and leuprolide did not produce any significant effect on the locomotor function of mice. Furthermore, prior treatment with $5HT_3$ antagonist, ondansetron (2 mg kg^{-1}s.c.) did not affect the inhibitory effect of leuprolide on marble burying behavior of mice, suggesting that, $5HT_3$ receptors may not be playing any role in OCD.

CONCLUSION

In the present study, leuprolide (LHRH agonist) exhibited anti-OCD like effect in marble burying behavioral model of mice, probably through positive modulation of post-synaptic serotonergic receptors ($5HT_{1B}$). Such positive modulation can be either subsequent to the increased postsynaptic receptor sensitivity or increased serotonin at the synapses.

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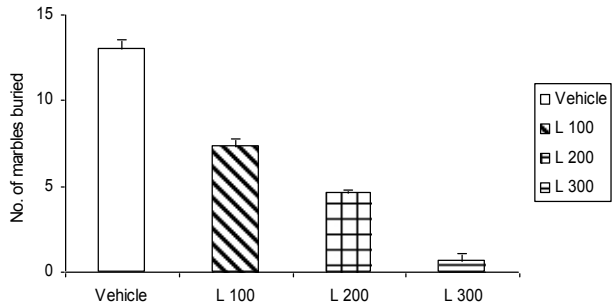

Fig. 1: Effect of L (Leuprolide 100, 200 & 300 µg kg^{-1}s.c.) on marble-burying behavior of mice

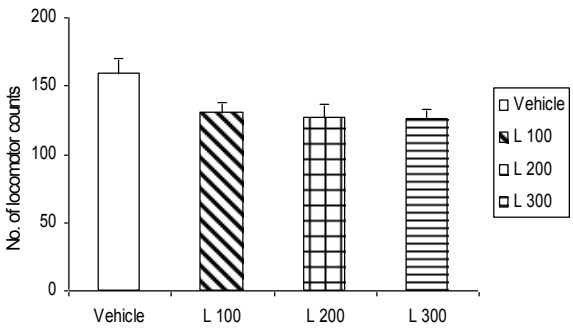

Fig. 2: Effect of Leuprolide (L) on locomotor activity of mice

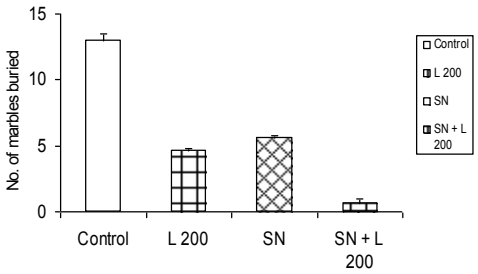

Fig. 3: Effect of SN (Sumatriptan 0.1 mg/kg) on L (Leuprolide 200 µg/kg) induced inhibition of marble-burying behavior of mice

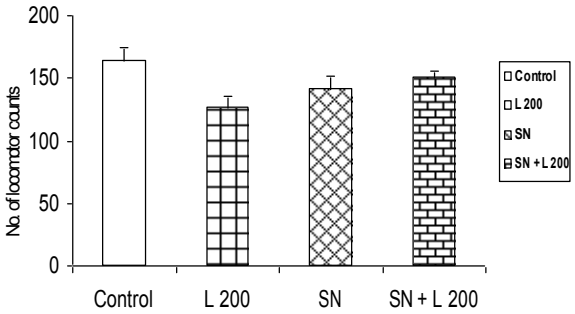

Fig. 4: Effect of SN (Sumatriptan 0.1 mg/kg) plus L (Leuprolide 200 µg/kg) on locomotor activity.

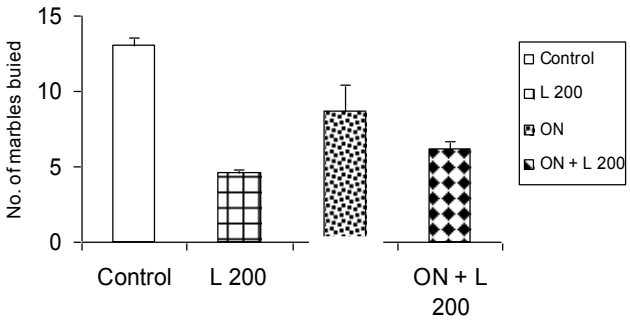

Fig. 5: Effect of ON (Ondansetron 2 mg/kg) on L (Leuprolide 200 µg/kg) induced inhibition of marble-burying behavior of mice

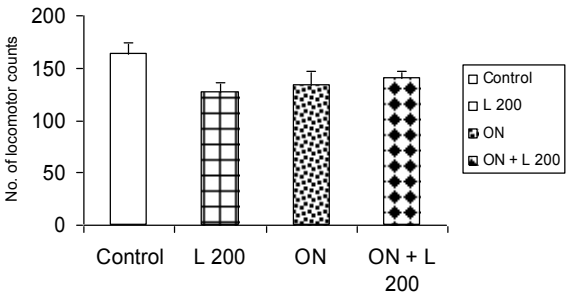

Fig. 6: Effect of ON (Ondansetron 2 mg/kg) plus L (Leuprolide 200 µg/kg) on locomotor activity of mice

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