CHAPTER 6
INVOLVEMENT OF DOPAMINE (DA) / SEROTONIN (5-HT)/SIGMA (σ) RECEPTOR MODULATION IN MEDIATING THE ANTIDEPRESSANT ACTION OF ROPINIROLE HYDROCHLORIDE, A D₂/D₃ DOPAMINE RECEPTOR AGONIST

6.1. INTRODUCTION

A deficiency of mesolimbic dopamine (DA) is a leading argument in the etiology of certain symptoms of depression. Studies have revealed that there is a decrease in dopamine and its metabolite homovanillic acid (HVA) in the cerebrospinal fluid of patients suffering from depression (Traskman et al., 1981). The drugs which increased dopamine levels in brain either by inhibiting the dopamine reuptake (e.g. bupropion or nomifensine) or dopaminergic agonistic action have been shown to be potent antidepressants (Basso et al., 2005; Lemke, 2007). Piribedil and bromocriptine, directly acting DA agonists (Dueret-Boucher et al., 1988) possessed antidepressant activity. Among all the dopamine receptors, D₂ and D₃ dopamine receptor type have been considered as particularly important in affective disorders due their localization in the limbic region of the brain along with serotoninergic transmission of the central nervous system (Basso et al., 2005; Willner et al., 2005). A recent double-blind study found that pramipexole, a very selective D₃-prefering D₂/D₃ dopamine receptor agonist to have comparable antidepressant efficacy as compared to fluoxetine, a selective serotonin reuptake inhibitor (SSRI) (Maj et al., 1997). When pramipexole was combined with fluoxetine, it enhanced its action suggesting the functional interaction between dopamine and serotonin system (Rogoz and Skuza, 2006). Standard antidepressants such as fluoxetine or imipramine are also known to modulate the dopaminergic system. In one of the studies, a single injection of 2.5 mg/kg (i.p.) of fluoxetine significantly increased the number
of spontaneously active DA neurons in rat brain suggesting the role of dopamine in mediating antidepressant action (Sekine et al., 2007).

Interestingly, a disruption of dopaminergic transmission is implicated in the depressed mood displayed both by Parkinson and by non-Parkinson patients (Lambert et al., 2000; Tremblay et al., 2002; Willner et al., 2005). Ropinirole is a nonergoline D2-D3 dopamine receptor agonist indicated in Parkinson’s disease and its use has been associated with a lower risk of dyskinesia and valvular regurgitation (Kvermo et al., 2006). Recent clinical evidences have shown that ropinirole augments the antidepressant effects of many standard drugs such as tricyclic antidepressants (TCAs) or selective serotonin reuptake inhibitors (SSRIs) (Cassano et al., 2005). Besides, ropinirole has been reported to possess anxiolytic and antidepressant profile in various animal paradigms in mice, rats and common marmoset (Rogers et al., 2000).

Various preclinical studies have indicated the role of sigma receptors in depression (Takebayashi et al., 2004; Bermack and Debonnel, 2005). Behavioral models used to test potential antidepressants have shown that ligands that bind to sigma receptors possessed “antidepressant-like” properties. Sigma ligands have potential as antidepressant medications with a fast onset of action as they produced a rapid modulation of the serotonergic system in the Dorsal Raphe Nucleus (DRN) and the glutamatergic transmission in the hippocampus (Bermack and Debonnel, 2005). One of the earlier studies from our laboratory has indicated the involvement of sigma-1 receptors in modulating the antidepressant effect of neurosteroids in forced swim test in mice and the test was carried out using NE-100, a sigma-1 receptor antagonist (Reddy et al., 1998). The earlier work reported in Chapter 3, it has been shown that sigma-1 receptors are involved in the antidepressant action of venlafaxine in mouse FST. As these effects of sigma ligands may produce antidepressant properties by completely novel mechanisms of action, they may provide an alternative to the antidepressants currently
available and may prove to be beneficial in treatment-resistant depressed patients (Bermack and Debonnel, 2005).

As sigma receptors are known to modulate the dopaminergic and serotonergic system in the brain (Kobayashi et al., 1997; Ishihara and Sasa, 2002; Bermack and Debonnel, 2005) and this interaction between dopaminergic agonists and sigma receptors may constitute a possible mechanism for their neuropharmacological effects in depression. However, there is little evidence to support this hypothesis. Therefore, it is speculated that ropinirole may be having antidepressant action possibly by acting through sigma receptors.

The antidepressant profile of ropinirole and its exact mechanism of action have not been worked out. With this background, the present study was designed to elucidate the anti-immobility profile of ropinirole in various behavioral paradigms of despair (FST and TST) and to elucidate the possible neurochemical basis of its mechanism of antidepressant activity.

6.2. MATERIALS AND METHODS

6.2.1. Animals: Refer to Chapter 1 (1.2.1.)

6.2.2. Forced Swim Test (FST): Refer to Chapter 1 (1.2.2.1.)

6.2.3. Tail Suspension Test (TST): Refer to Chapter 1 (1.2.2.2.)

6.2.4. Activity monitoring in animals: Refer to Chapter 2 (2.1.2.6.)

6.2.5. Measurement of biogenic amines: Refer to Chapter 2 (2.1.2.7.)

6.2.6. Drugs and treatment

The following drugs were used: Ropinirole hydrochloride (Panacea Biotec, Lalru, India), haloperidol (Sigma Aldrich Co., MO, USA), SCH 23390 maleate (Schering Plough Co, Bloomfield NJ, USA), (-) Sulpiride hydrochloride (Research Biochemical Inc, Natick, MA., USA), Progesterone (Sigma Aldrich Co., MO, USA), BD 1047 (Tocris Co.,
Missouri, USA), Rimcazole (Sigma Aldrich Co., MO, USA). All the drugs except progesterone were dissolved in distilled water and different doses were administered intraperitoneally in a fixed volume of 1ml per 100g of body weight. Progesterone was made in vegetable oil and administered subcutaneously. Ropinirole was administered 30 minutes before challenging the animals to behavior paradigms of despair. In case of interaction studies various antagonists were given 15 minutes before administering ropinirole. All the doses were chosen based on our previous experience and on the literature available (Chapter 2, Rogers et al., 2000; Diaz-Torga et al., 2002; Siuciak and Fujiwara, 2004; Rogoz et al., 2004; Rogoz and Skuza, 2006). The experimental protocol comprised the following groups, each consisting of minimum of six animals. Different set of animals were used for measuring the locomotor activity.

6.2.7. Statistical Analysis

Results expressed as mean (sec.) ± S.E.M and the significance of the difference in the responses of treatment groups in comparison to the control was determined by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. P<0.05 was considered statistically significant.

6.3. RESULTS

6.3.1. Effect of pretreatment of ropinirole on behavioral paradigms of despair

Ropinirole at different doses (1, 3 and 10 mg/kg., i.p.) produced S shaped curve in affecting the immobility period (in seconds) with respect to the vehicle control group in FST as well as in TST (Fig. 6.1A, 6.1B and 6.1.C). Ropinirole (3 mg/kg., i.p.) was ineffective in affecting immobility period. Ropinirole (1 and 10 mg/kg., i.p.) significantly decreased the immobility period as compared to vehicle treated group in both FST and TST (Fig. 6.1A, 6.1B and 6.1.C). Ropinirole (10 mg/kg., i.p.) was chosen for further experiment as this dose significantly decreased the immobility period in
both FST and TST without affecting the locomotor activity in mice (Table 6.1).

6.3.2. Effect of various dopaminergic modulators on the action of ropinirole (10 mg/kg., i.p.) in FST

To assess the mechanism of action, ropinirole was combined with the subeffective dose of haloperidol, SCH 23390 or sulpiride. Haloperidol (0.5 mg/kg., i.p.), SCH 23390 (0.5 mg/kg., i.p.) or sulpiride (5 mg/kg., i.p.) **per se** did not have any effect on the immobility period in FST (Fig. 6.2 and 6.3). However, when haloperidol or sulpiride (D₂ dopamine receptor antagonist) was given 15 minutes before ropinirole and subjected to FST, these dopaminergic antagonists reversed the effect of ropinirole (Fig. 6.2 and 6.3). However, SCH 23390 was not able to reverse the anti-immobility effect of ropinirole in FST (Fig 6.3). The combination treatments did not alter the locomotor activity (Table 6.1).

6.3.3. Effect of various sigma receptor modulators on the action of ropinirole (10 mg/kg., i.p.) in FST

To assess the sigma modulatory action of ropinirole, it was combined with various sigma receptor antagonists **viz.** rimcazole (non-selective sigma receptor antagonist), progesterone (non-selective sigma receptor antagonist neurosteroid), BD 1047 (selective sigma 1 receptor antagonist), respectively.

Progesterone (10 mg/kg., s.c.) (Fig.6.4), BD 1047 (1 mg/kg., i.p.) (Fig.6.5) or rimcazole (5 mg/kg., i.p.) (Fig 6.6) **per se** did not have any effect on the immobility period in FST. However, when progesterone, BD 1047 or rimcazole was given 15 minutes before ropinirole and subjected to FST, these modulators reversed the effect of ropinirole of immobility period (Fig 6.4, 6.5 and 6.6). However, progesterone was much effective in reversing the anti-immobility effect as compared to BD 1047. The combinations did not alter the locomotor activity (Table 6.1).
Fig. 6.1. Modification by ropinirole (1, 3 and 10 mg/kg, i.p) on the immobility period with respect to control during the 6 minute test in both (A) forced swim test (FST) and (B) tail-suspension test (TST). Ropinirole at different doses were administered 30 minutes before FST challenge. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. *p<0.05 as compared to vehicle treated group, *p<0.05 as compared to ropinirole (1 mg/kg, i.p.).
6.3.4. Effect of ropinirole (10 mg/kg., i.p.) on the neurotransmitter levels in the brain

When checked for the alterations in the neurotransmitter levels, ropinirole at 10 mg/kg., i.p. did not affect the dopamine levels significantly in but it markedly (160 %) increased the serotonin levels (Fig. 6.7.).

6.4. DISCUSSION

In the present experiments, ropinirole at different doses (1 and 10 mg/kg., i.p.) decreased the immobility period in both forced swim test and the suspension test in mice. The anti-immobility action of ropinirole was associated with the change in the locomotor activity. Various dopamine (particularly the D2 dopamine subtype) and sigma receptor antagonist reversed the anti-immobility action of ropinirole. Furthermore, ropinirole not affect the dopamine levels in brain but markedly increased serotonin levels showing the dopamine mediated serotonergic modulation. This is the first study which demonstrated the antidepressant profile of ropinirole (D2/D3 dopamine receptor agonist) and hypothesized to through dopaminergic, serotonergic and sigma receptor modulation.
The effects of chronically administered tricyclic antidepressants were reversed by the administration of sulpiride in the nucleus accumbens, but not in the dorsal striatum (Yamada et al., 2004). Studies on animal models suggested that many antidepressants like fluoxetine (a selective serotonin re-uptake inhibitor) or desipramine (a potent inhibitor of the noradrenaline re-uptake carrier) also increased the extracellular dopamine concentration in the prefrontal cortex by a mechanism not dependent on serotonin (Ainsworth et al., 1998). Some of the studies are consistent with the above arguments and pramipexole which is a D2/D3 dopamine receptor agonist is shown to have anti-immobility activity in various animal models of depression (Rogoz and Skuja, 2006).

**Fig. 6.2.** Effect of ropinirole (10 mg/kg., i.p.) and its modulation by haloperidol (0.5 mg/kg., i.p.) on the immobility period induced by FST. Haloperidol was administered 15 minutes before ropinirole and after 30 minutes of ropinirole injection, mice were challenged to FST. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. *p<0.05 as compared to vehicle treated group, **p<0.05 as compared to ropinirole (10 mg/kg., i.p.), V: Vehicle.
Many antidepressants are known to sensitize the mesolimbic dopaminergic pathways and "strengthen" dopaminergic signaling in the nucleus accumbens (Ainsworth et al., 1998, D'Aquila et al., 2000). In accordance with the above observations, agonists at closely related D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> dopamine receptors, could be active in experimental models of potential antidepressant activity (Willner, 1995; Lehr, 2002). Therefore, immobility in the swim test may be reversed not only by antidepressants, but also by D<sub>2</sub>/D<sub>3</sub> dopamine receptor agonists applied systemically or to the nucleus accumbens (Siuciak and Fujiwara, 2004; Basso et al., 2005).

**Fig. 6.3.** Effect of ropinirole (10 mg/kg, i.p.) and its modulation by SCH 23390 (0.5 mg/kg, i.p.) or sulpiride (5 mg/kg, i.p.) on the immobility period induced by FST. SCH 23390 or sulpiride was administered 15 minutes before ropinirole and after 30 minutes of ropinirole injection, mice were challenged to FST. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. *p<0.05 as compared to vehicle treated group, **p<0.05 as compared to ropinirole (10 mg/kg, i.p.), V: Vehicle.
Chapter 6

Conversely, a number of studies have reported that anti-immobility effects in the swim test were reversed by dopamine receptor antagonists, these include studies in which antidepressants were administered chronically (Yamada et al., 2004).

However, the studies on ropinirole hydrochloride or diethyl [2-(2-oxo-2, 3-dihydro-1H-indol-4-yl) ethyl] ammonium chloride, a non-ergoline dopamine agonist are limited and the exact mechanism regarding its antidepressant action is still debatable.

![Graph](https://via.placeholder.com/150)

**Fig. 6.4.** Effect of ropinirole (10 mg/kg i.p.) and its modulation by progesterone (10 mg/kg., s.c.) on the immobility period induced by FST. Progesterone was administered 15 minutes before ropinirole and after 30 minutes of ropinirole injection, mice were challenged to FST. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. *p<0.05 as compared to vehicle treated group, **p<0.05 as compared to ropinirole (10 mg/kg., i.p.) treated group, V: Vehicle.

Ropinirole hydrochloride has been proven to be effective in both, monotherapy and combination therapy of idiopathic Parkinson's disease (Jost and Angersbach, 2005). Ropinirole binds specifically to dopamine
D₂-like receptors with a selectivity similar to that of dopamine (D₃ > D₂ > D₄). Ropinirole has been considered as being less likely than levodopa to lead to the early development of motor fluctuations and dyskinesia in this clinical setting (Ravikumar and Sridhar, 2006).

Several studies have demonstrated that systemic administration of ropinirole produce anxiolytic-like effects in animal models (Rogers et al., 2000). This may predict an action of ropinirole in man that would provide a superior profile of action over other presently available anti-parkinsonian agents. A very recent clinical report of Cassano et al has shown that ropinirole augmented the antidepressant effect of standard drugs when used in combination (Cassano et al., 2005). In the present study, ropinirole

![Graph showing effect of ropinirole and BD1047 on immobility period](image-url)
decreased the immobility period in various behavioral paradigms of despair. Ropinirole produced S shaped curve in decreasing the immobility period in mice. In one of the earlier studies, it has been shown that ropinirole produced an inverted-U dose-response curve in the percentage period spent in the open arms (Rogers et al., 2000).

To elucidate the anti-immobility action of ropinirole, it was combined with various dopaminergic antagonists. Haloperidol, a dopamine D₂ and sigma receptor antagonist, administered prior to ropinirole reversed the anti-immobility effect of ropinirole showing dopaminergic receptor modulation. Further, when ropinirole was combined with sulpiride (a selective D₂ dopamine receptor antagonist) it exhibited similar interaction.
confirming dopaminergic interaction of the drug. However, SCH 23390, a D₁ dopamine receptor antagonist did not affect the anti-immobility effect of ropinirole. This suggests the role of D₂ dopamine receptors in mediating its anti-immobility effect in mouse FST. It is hypothesized that the postsynaptic D₂ dopamine sites in the nucleus accumbens are implicated in mediating its antidepressant action. The combination of drugs was also free of any effect on the locomotor activity. Haloperidol at 0.5 mg/kg., i.p. exhibited some depressant effect on locomotor activity but the effect was non-significant (Table 6.1.).

![Neurotransmitter Levels](image)

*Fig. 6.7. Effect of ropinirole (10 mg/kg., i.p.) on the alteration in neurotransmitter levels in the mouse whole brain. Ropinirole (10 mg/kg., i.p) was administered 30 minutes before sacrificing the animals. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. *p<0.05 as compared to vehicle treated group.*

When the brain tissue levels of dopamine and serotonin were estimated, ropinirole did not affect the dopamine levels but markedly increased the serotonin levels upto 160 % in the whole brain tissue. Dopamine and serotonin play an important role in several brain functions. Altered regulation of these two neurotransmitters in the basal ganglia is associated with various behavioral dysfunctions including motor and
obsessive-compulsive disorders (Sandyk, 1988). Moreover, numerous studies have shown neuronal connections between the dopamine and serotonin brain systems. Connections between serotonergic axons and dopaminergic cells in the substantia nigra and a nigro-raphe pathway exist, suggesting either an indirect or direct connection between the cell bodies of dopaminergic and serotonergic neurons (Lee et al., 1987; Jacobs and Azmitia, 1992).

Table 6.1. Effect of different dose of ropinirole per se and its combination with haloperidol, sulpiride, SCH 23390, rimcazole, progesterone and BD 1047 on the locomotor activity measured for total of 5 minute session in mice

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Dose (mg/kg., i.p.)</th>
<th>Mean ambulatory movement ± S.E.M.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td></td>
<td>129 ± 9.96</td>
</tr>
<tr>
<td>2</td>
<td>Ropinirole</td>
<td>1</td>
<td>117 ± 11.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>110 ± 8.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>121 ± 17.69</td>
</tr>
<tr>
<td>3</td>
<td>Haloperidol</td>
<td>0.5</td>
<td>97 ± 13.14</td>
</tr>
<tr>
<td>4</td>
<td>Haloperidol + ropinirole</td>
<td>0.5 + 10</td>
<td>107 ± 12.41</td>
</tr>
<tr>
<td>5</td>
<td>SCH 23390</td>
<td>0.5</td>
<td>122 ± 9.72</td>
</tr>
<tr>
<td>6</td>
<td>SCH 23390 + ropinirole</td>
<td>0.5 + 10</td>
<td>117 ± 17.50</td>
</tr>
<tr>
<td>7</td>
<td>Sulpiride</td>
<td>5</td>
<td>116 ± 3.55</td>
</tr>
<tr>
<td>8</td>
<td>Sulpiride + ropinirole</td>
<td>5 + 10</td>
<td>122 ± 13.79</td>
</tr>
<tr>
<td>9</td>
<td>Progesterone</td>
<td>10 (mg/kg., s.c.)</td>
<td>147 ± 9.09</td>
</tr>
<tr>
<td>10</td>
<td>Progesterone + ropinirole</td>
<td>10 (mg/kg., s.c.) + 10</td>
<td>131 ± 18.23</td>
</tr>
<tr>
<td>11</td>
<td>Rimcazole</td>
<td>5</td>
<td>128 ± 14.49</td>
</tr>
<tr>
<td>12</td>
<td>Rimcazole + ropinirole</td>
<td>5 + 10</td>
<td>115 ± 17.90</td>
</tr>
</tbody>
</table>

*n = 6–8.

Also, microdialysis studies have demonstrated interactions between the serotonergic system and dopaminergic activity. For example, serotonergic stimulation of the prefrontal cortex (Chen et al., 1992), the
striatum (West and Galloway, 1991; Parson et al., 1996), the limbic forebrain (Parson et al., 1996) or the nucleus accumbens (Parson and Justice, 1993; Boulenguez et al., 1996) potently released dopamine. Reciprocally, dopamine afferents are able to facilitate the release of serotonin in the raphe dorsalis and, at the same time, inhibit this release in the striatum (Lee and Geyer, 1984; Ferre and Artigas, 1993; Ferre et al., 1994). Previous research has demonstrated that pharmacological stimulation of postsynaptic dopamine D₂ dopamine receptors produces increases in serotonin output (Mendlin et al., 1999). Furthermore, the present findings are also in line with report of Rogoz and Sakuja in which pramipexole (D₂/D₃ dopamine receptor agonist) when combined with fluoxetine, a selective serotonin reuptake inhibitor, enhanced its action suggesting the functional interaction between dopamine and serotonin systems (Rogoz and Sakuja, 2006).

Another dopaminergic agonist, bromocriptine showed antidepressant action by modulating the serotonin and noradrenaline system secondary to the dopamine receptor stimulation (Nordin et al., 1981). We believe that this large increase in the serotonin levels (approx. 160 %) by co-administration of ropinirole suggests the strong interaction between dopaminergic and serotonergic systems in the whole brain which needs further attention. Further, it is known that blockade of 5-HT transporters may close potassium (ATP) channels through increased intracellular ATP which may in turn facilitate 5-HT release by depolarization (Mantovani et al., 2006). Therefore, it is imperative that various studies are going on to new receptor targets in the treatment of depression are to be explored to elucidate the exact mechanism of newer as well as existing antidepressant drugs.

Sigma receptors have recently been the target of drug development related to psychiatric disorders, including in schizophrenia, depression, and cognitive deficits (Ishihara and Sasa, 2002). In the present study, haloperidol, progesterone or rimcazole (all being sigma receptor antagonists) reversed the effects of ropinirole. Furthermore, BD 1047, a
newer sigma 1 receptor antagonist partially antagonized the effect of ropinirole suggesting the involvement of sigma 1 receptor activity in mediating its antidepressant action. Sigma receptors are known to modulate the serotonin system in the brain. It has been speculated that sigma receptor agonists increase the serotonin levels in the brain. In the present study, sigma antagonists reversed the effects of ropinirole. This may be due to the reason that sigma antagonists reverse the increases in serotonin levels caused by dopaminergic stimulation.

The present findings suggest that the effect of ropinirole in various behavioral paradigms of despair is via an interaction between dopamine, serotonin and sigma neurotransmission. Ropinirole could be a useful therapeutic agent in the treatment of depression associated with Parkinson's disease.