Role of adenosine in drug-induced catatonia in mice

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Parkinson’s disease is one of the most common neurodegenerative disorders affecting large majority of population who are older than age of 65. Apart from dopamine, acetylcholine and glutamate, adenosine has also been identified in the basal ganglia. Adenosine modulates the release of a variety of neurotransmitters including dopamine. In order to establish adenosine-dopamine interactions in drug-induced catatonia we studied the effect of adenosine in drug-induced catatonia in mice.

In the present study adenosine dose dependently produced catatonia when assessed on rota-rod and bar tests in mice. Adenosine also potentiated the catatonic effect of perphenazine. L-dopa plus carbidopa or OR-486 (a potent centrally acting COMT inhibitor) completely reversed adenosine-induced catatonia. Since reversal by scopolamine of adenosine-induced catatonia was not to the same extent as with l-dopa and OR-486 it appears that catecholamines particularly dopamine rather than cholinergic modulation is more important in adenosine induced catatonia. The motor dysfunction (catatonia) could be easily assessed using rota-rod test apparatus in mice.

Adenosine is known to be present in the CNS. Adenosine receptors namely A2A are present in high concentration in striatum, nucleus accumbens and olfactory tubercule (regions which are rich in dopamine)\(^1\). A2A and dopamine D2 receptors, which are co-localized in subpopulation of striatal projection neurons, the GABAergic output neurons of striatopallidal pathway have shown to be interacting antagonistically on different levels. The A2A receptor agonists are reported to reduce the affinity for D2 receptors thus modulate D2 function\(^2,3\).

Adenosine modulates the release of variety of neurotransmitters both in vivo and in vitro. Adenosine has been shown to affect the release of norepinephrine, GABA, dopamine serotonin, acetylcholine, histamine, aspartate and glutamate. Although the mechanism by which adenosine affects neurotransmission is not established it has been generally accepted that adenosine acts through presynaptic modulation of neurotransmitter release\(^4,5\).

The striatopallidal neuronal function seems to be mainly regulated not only by A2A receptors at the postsynaptic levels by direct antagonistic A2A-D2 and D2-A2A receptor-receptor interactions) but also at the presynaptic levels by an A2A receptor mediated regulation of acetylcholine release and, possibly, of GABA release. The evidence that stimulation of A2A receptors most probably localized in the striatal cholinergic interneurons has been reported to induce acetylcholine release in striatal synaptosomal preparations\(^6,7\) supports this.

With this background the present work was carried out to investigate adenosine-dopamine and adenosine-cholinergic interactions. Various models to test the motor function, including the rota-rod apparatus was used as an approach for the evaluation of catatonic moments in animals and the fall-off time in this test was compared with the other existing model of catatonia namely the bar immobility test\(^8\).

**Materials and Methods**

**Animals**—Laka mice of either sex (20-30g), bred in central animal house (CAH) of Panjab University, Chandigarh, maintained on a 12-h light and dark cycles were used in the study. Animals were housed under standard laboratory conditions, with free access to food and water. All behavioural experiments were carried out between 0900 and 1400 hrs. The experimental protocol was approved by Institutional Animals Ethics Committee.

**Assessment of catatonia**—Using the following three-test procedures assessment of catatonia was done in animals.

**Rota-rod test**—Mice were subjected to motor function evaluation by placing them individually on rota-rod\(^9\), which was adjusted to the speed of 21 r.p.m. The fall off time was recorded for each mouse and the longest time any animal was kept on the rod was 300 sec.
Bar test—In the bar test, front paw of the mice were gently placed on a horizontal metal bar with 2 mm diameter and placed 4 cm above ground level and the length of time the mouse maintained this abnormal posture with at least one paw was measured. The test was terminated when the paw of animal touched the ground or 180 sec had passed. If the animal did not hold on to the bar after three attempts, it received the score of 0 seconds.

Drug treatment—The following drugs were used in the present study. Adenosine (Loba Chemicals, Mumbai, India) was dissolved with the aid of minimum quantity of hydrochloric acid, the volume was adjusted with distilled water, and finally pH was adjusted to neutral. Perphenazine (Schering Co., Kenilworth, NJ, USA) was dissolved with the aid of minimum quantity of hydrochloric acid, the volume was adjusted with distilled water, and finally pH was adjusted to neutral. Scopolamine HBr (Merck & Co., Inc., NJ, USA) was dissolved in distilled water. L-dopa (Hi Media, Mumbai, India), carbidopa (Sun Pharmaceuticals, Mumbai, India), OR-486, (gift sample from Prof. P.T. Mannisto Finland) were suspended in 0.3% sodium CMC.

Treatment schedule—All the drugs were administered intraperitonially ip, in a constant volume of 1 ml per 100g of body weight of mice. Adenosine was administered 30 min prior to the behavioural assessment. Scopolamine, perphenazine, OR-486 was co-administered with adenosine respectively. Carbidopa was co-administered with adenosine as well as perphenazine 15 min prior to the administration of L-dopa, behavioural assessment was done 1 hr and 2 hr of L-dopa administration, respectively.

Statistical analysis—The fall-off time in rota-rod test, the time spent in bar test were expressed as mean± SE. The data was analyzed using analysis of variance (ANOVA) followed by Dunnett’s test by a statistical package STAT. In the test, the criterion for statistically significance was P<0.05.

Results

Effect of perphenazine on fall-off time from the rota-rod test and time spent on the bar in bar test—Perphenazine (1-5 mg/kg, ip) decreased the fall-off time and increased the time spent by the animal on the bar in a dose dependent manner. Significant differences were observed at all the doses compared to the non-treated control group (Figs 1 A & B).

Effect of adenosine on fall-off time and time spent on the bar in bar tests—Adenosine (10-100 mg/kg, ip) decreased the fall-off time and increased the time spent by the animal on the bar in a dose dependent manner. Significant differences were observed at dose of (50 mg/kg, ip) and (100 mg/kg, ip) as compared to the non-treated control group (Figs 2 A & B).

Adenosine (10-100 mg/kg, ip) when administered in combination with perphenazine (5 mg/kg, ip) decreased the fall-off time and increased the time spent by the animal on bar in a dose dependent manner as compared to the perphenazine (5mg/kg, ip) alone treated group. Significant differences were observed at various dose of (25, 50 and 100 mg/kg, ip) of adenosine (Figs 3 A & B).

Effect of L-dopa and carbidopa combination on adenosine induced catatonia—L-dopa (100 mg/kg, ip and 200 mg/kg, ip) and carbidopa (10 mg/kg, ip 20 mg/kg, ip) combination dose dependently increased the fall-off time when given in combination with adenosine (100 mg/kg, ip). Significant differences were found after 1 hr of L-dopa administration as compared to the control (adenosine 100 mg/kg, ip)
alone treated group. Although there was no dose dependency after 2 hr but difference was statistically significant (Fig. 4). In the bar test this combination when administered in combination with adenosine (100 mg/kg, ip) completely reversed adenosine-induced catatonia after 1 and 2 hr of l-dopa administration. The animals did not hold on to the bar in three attempts indicating a complete reversal (Table 1).

**Effect of l-dopa and carbidopa combination on adenosine plus perphenazine induced catatonia—**

L-dopa (100 mg/kg, ip and 200 mg/kg, ip) and carbidopa (10 mg/kg, ip and 20 mg/kg, ip) combination increased the fall-off time when administered along with the combination of adenosine (100 mg/kg, ip) and perphenazine (5 mg/kg, ip) treated group. Although no dose dependency was observed but the effect was statistically significant after 1 and 2 hr of l-dopa administration as compared to the control group, treated with combination of adenosine (100 mg/kg, ip) plus perphenazine (5 mg/kg, ip) (Fig. 5). In the bar test l-dopa (100 mg/kg, ip) and carbidopa (10 mg/kg, ip) combination significantly decreased the time spent by the animal on the bar when given along with the combination of adenosine (100 mg/kg, ip) plus perphenazine (5 mg/kg, ip) after 1 hr of l-dopa administration as compared with the control group treated with the combination of adenosine (100 mg/kg, ip) plus perphenazine (5 mg/kg, ip). There was a complete reversal seen after 2 hr of l-dopa administration with this dose. L-dopa (200 mg/kg, ip) and carbidopa (20 mg/kg, ip) combination when administered along with the combination of adenosine (100 mg/kg, ip) plus perphenazine (5 mg/kg, ip) completely reversed adenosine (100 mg/kg, ip) plus perphenazine (5 mg/kg, ip). Induced catatonia after 1 and 2 hr of l-dopa administration as the animal were not able to hold on to the rod in three attempts (Table 2).
Effect of OR-486 on adenosine-induced catatonia—OR-486 (30 mg/kg, ip) significantly increased the fall-off time and when it was administered in combination with adenosine (100 mg/kg, ip) as compared to adenosine (100 mg/kg, ip) alone treated group (Fig. 6). In the bar test OR-486 (30 mg/kg, ip) completely reversed adenosine (100 mg/kg, ip)-induced catatonia when it was administered in combination with adenosine (100 mg/kg, ip) as compared with adenosine (100 mg/kg, ip) treated group. The animals were not able to hold on to the rod in three attempts (Table 3).

Effect of scopolamine on adenosine-induced catatonia—Scopolamine (1 mg/kg, ip and 2 mg/kg, ip) increased the fall-off time in a dose dependent manner when administered in combination with adenosine (100 mg/kg, ip). Significant difference was observed at dose of 2 mg/kg, ip as compared to the adenosine (100 mg/kg, ip) alone treated group (Fig. 7). In the bar test scopolamine (1 mg/kg, ip) did not decrease the time spent by the animal on the bar when adminis-

Table 1—Effect of combined treatment of L-dopa and carbidopa on adenosine induced catatonia in mice as tested on bar test

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean time spent (sec ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine (100)</td>
<td>85.2 ± 11.07</td>
</tr>
<tr>
<td>Adenosine (100) after 1 hr</td>
<td>93.2 ± 13.15</td>
</tr>
<tr>
<td>Adenosine (100) + L-dopa (100) + carbidopa (10) after 1 hr</td>
<td>Reversed**</td>
</tr>
<tr>
<td>Adenosine (100) + L-dopa (200) + carbidopa (20) after 1 hr</td>
<td>Reversed**</td>
</tr>
<tr>
<td>Adenosine (100) after 2 hr</td>
<td>23.2 ± 5.2</td>
</tr>
<tr>
<td>Adenosine (100) + L-dopa (100) + carbidopa (10) after 2 hr</td>
<td>Reversed**</td>
</tr>
<tr>
<td>Adenosine (100) + L-dopa (200) + carbidopa (20) after 2 hr</td>
<td>Reversed**</td>
</tr>
</tbody>
</table>

**Animal did not hold the bar in three attempts.

Table 2—Effect of combined treatment of L-dopa and carbidopa on adenosine plus perphenazine induced catatonia in bar test

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean time spent (sec ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine (100) + PPZ (5)</td>
<td>175.8 ± 2.56</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) after 1 hr</td>
<td>68.8 ± 4.56</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) + L-dopa (100) + carbidopa (10) after 1 hr</td>
<td>Reversed **</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) + L-dopa (200) + carbidopa (20) after 1 hr</td>
<td>Reversed **</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) after 2 hr</td>
<td>154.4 ± 9.64</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) + L-dopa (100) + carbidopa (10) after 2 hr</td>
<td>Reversed **</td>
</tr>
<tr>
<td>Adenosine (100) + PPZ (5) + L-dopa (200) + carbidopa (20) after 2 hr</td>
<td>92.6 ± 2.6*</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to the control group (adenosine 100 mg/kg + perphenazine 5mg/kg) (ANOVA followed by Dunnett's test)

**Animal did not hold onto the bar in three attempts.
Fig. 5.—Effect of combination of L-dopa (100 mg/kg, 200 mg/kg) and carbidopa (10 mg/kg, 20 mg/kg) on adenosine (100 mg/kg) + perphenazine (5 mg/kg) induced catatonia in rota-rod test. *P<0.05 as compared to the control (adenosine + perphenazine) at respective time intervals (ANOVA followed by Dunnett’s test) n = 5

Fig. 6.—Effect of OR-486 on adenosine induced catatonia in rota-rod test. *P<0.05 as compared to the control (adenosine) (ANOVA followed by Dunnett’s test) n = 5

Fig. 7.—Effect of scopolamine (1-2 mg/kg) on adenosine induced catatonia in rota-rod test. *P<0.05 as compared to control (adenosine). (ANOVA followed by Dunnett’s test) n = 5

Table 3—Effect of OR-486 on adenosine induced catatonia in bar test in mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean time spent (sec ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine (100)</td>
<td>85.2 ± 11.07</td>
</tr>
<tr>
<td>Adenosine (100) + OR-486 (30)</td>
<td>88.46 ± 10.13</td>
</tr>
</tbody>
</table>

**Animal did not hold the bar in three attempts**

Response was not statistically significant as compared to their respective combinations per se (Fig 8 B).

Discussion

Since adenosine A2A and dopamine D2 receptors which are co-localized in a sub-population of striatal-projection neurons, the GABAergic output neurons of the striatopallidal pathway have been shown to interact antagonistically on different levels A2A receptor agonists are reported to reduce the affinity of dopamine agonist for D2 receptors and thus modulate D2 function.

The result of our study indicated that adenosine dose dependently (10-100 mg/kg, i.p) produced catatonia as indicated by rota-rod/ bar tests. Adenosine
also potentiated the perphenazine-induced catatonia in a dose dependent manner. In accordance with the above explanation adenosine might have acted on the adenosine A2A receptors in the striatum co-localized with D2 receptors and thus causing a decreased affinity of dopamine towards the dopamine D2 receptors leading to motor dysfunction of the dopamine D2 receptors. This would again lead to disinhibition of negative control of dopamine D2 receptors towards the indirect pathway leading to the increased activity of indirect pathway, and hence a decreased movement by indirect modulation of cortex as observed in the animals in the form of catatonia.

When adenosine is co-administered along with perphenazine, decreased function of the dopamine D2 receptors already existing due to the blockade by perphenazine, adenosine potentiated this decreased function by possibly reducing the affinity of dopamine towards the D2 receptors and causing severe catatonic effect.

The striatopallidal neuronal function seems to be mainly regulated not only by A2A receptors at the postsynaptic levels (by direct antagonistic A2A-D2 receptor-receptor interactions) but also at the pre-synaptic level by an A2A receptor mediated regulation of acetylcholine release. The evidence that stimulation of A2A receptors, most probably localized on the striatal cholinergic interneurons, has been reported to induce acetylcholine release in striatal synaptosomal preparations supports this. In accordance with the above explanation scopolamine might have acted on post-synaptic muscarinic receptors, co-localized along with dopamine D2 and adenosine A2 receptors in the striatopallidal pathway in basal ganglia. The blockade of postsynaptic muscarinic receptors would have attenuated the effects due to increased pre-synaptic acetylcholine release, resulting from the stimulation of A2 receptors by adenosine.

The mechanism by which adenosine affects neurotransmission is not fully established although it is generally accepted that adenosine acts through pre-synaptic modulation of neurotransmitter release. A1 receptors are located pre and post synaptically on cell bodies, and on axons where they mediate inhibition of the neurotransmission, by the inhibition of the neurotransmitter release, hyperpolarizing neuronal membranes, reducing excitability and firing rate and altering axonal neurotransmission. When the combination of L-dopa and a peripheral DOPA decarboxylase inhibitor, carbidopa was administered with adenosine, a complete reversal of adenosine was observed. The reversal was far better as compared to scopolamine as well as OR-486 (central COMT inhibitor), as indicated by both bar and rota-rod test. L-dopa when given in combination with peripheral DOPA decarboxylase inhibitor carbidopa gets decarboxylated into dopamine, thus increasing the levels of dopamine. These increased levels of dopamine would have restored the depleted levels of dopamine due to adenosine. OR-486, a centrally acting COMT inhibitor when administered along with the adenosine completely reversed adenosine-induced catatonia. OR-486...
might have inhibited the metabolism of catecholamines thus further increasing the levels of dopamine. When it was administered along with adenosine it completely reversed the adenosine effect. Thus this study confirms the potent adenosine-catecholamine, particularly adenosine-dopamine interactions. This study further gives clues to the use of adenosine A2A receptor antagonist as newer therapeutic agents in drug therapy of Parkinson’s disease13.

Acknowledgement
The authors appreciate Prof. P.T. Mannisto of University of Kupio, Kupio, Finland for generously providing COMT inhibitors for the study.

References