S-adenosyl-L-methionine prevents 5-HT$_{1A}$ receptors up-regulation induced by acute imipramine in the frontal cortex of the rat

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Abstract

S-adenosyl-L-methionine (SAM) has shown efficacy in speeding the onset of the antidepressant effect of imipramine in depressed patients. This effect may be related to their interactions at the serotonin$_{1A}$ (5-HT$_{1A}$) receptors. Acute imipramine up-regulated the frontal cortex 5-HT$_{1A}$ receptors ($B_{\text{max}}$, 51.5 ± 8.4 fmol/mg protein) vs. saline ($B_{\text{max}}$, 27.5 ± 5.9 fmol/mg protein), and did not show antidepressant effect. Acute SAM and imipramine + SAM did not modify frontal cortex 5-HT$_{1A}$ receptors, and showed antidepressant effects (decrease of the immobility response of 26%, $P < 0.01$; and 47%, $P < 0.001$) vs. saline. All the chronic treatments showed antidepressant effects and up-regulated the hippocampus 5-HT$_{1A}$ receptors. SAM prevents the 5-HT$_{1A}$ receptor up-regulation induced by acute imipramine in the frontal cortex. This mechanism may contribute to imipramine’s antidepressant effect. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Imipramine; S-adenosyl-L-methionine; Serotonin$_{1A}$; Frontal cortex; Hippocampus; Forced swim test

S-adenosyl-L-methionine (SAM) has shown efficacy in speeding the onset of the antidepressant effect of imipramine in depressed patients [2,5]. Both drugs increase noradrenaline and serotonin levels, but the differences between the delay in appearance of the therapeutic effect of imipramine and the rapid antidepressant effect of SAM suggest different mechanisms of action.

To find out whether SAM may speed the onset of the antidepressant effect of imipramine, the hippocampus and the frontal cortex serotonin$_{1A}$ (5-HT$_{1A}$) receptors were characterized in Wistar rats. The immobility time in the forced swimming test was used as a functional correlate. The open field test was used to distinguish the antidepressant effects from the stimulatory effects on the rat locomotor activity.

Male Wistar rats ($n = 96$, 275 ± 30 g; CRIFFA, Barcelona) were used. These were allowed to habituate to the animal facility for 10 days before the start of the experiment and were housed under standardized conditions (three per cage; constant 12 h light/dark cycle; temperature, 22 ± 2°C; water and food available ad libitum) up to the time of testing. All procedures followed the provisions and general recommendations of the European Community Council Directive (86/609/EEC) and local rules.

For acute treatments, the animals were injected intraperitoneally (i.p.) with saline (0.5 ml/kg), imipramine (10.9 mg/kg), SAM (50 mg/kg), or imipramine + SAM, 24 h, 5 h and 5 min before the start of the forced swimming test. For chronic treatments, the animals were injected i.p. with saline (0.5 ml/kg), imipramine (10.9 mg/kg), SAM (50 mg/kg), or imipramine + SAM one time/day during 20 days. The last injection was given 24 h before the start of the forced swimming test.

The forced swimming test (Porsolt method) was used to detect the antidepressant effect of the treatments. The rat was placed in a cylinder (40 cm high, 18 cm diameter) containing water to a height of 21 cm at 37°C. Rats could not support themselves by touching the bottom with their feet. Two swimming sessions were conducted. In the pre-test session, the rats were forced to swim during 15 min, then removed from the cylinder and allowed to dry for 15 min in a heated enclosure (32°C) before they were returned to their home cages. After 24 h, the animal was exposed...
again to the forced swimming test for 5 min (test). The total duration of active swim behaviour was measured during the 5 min of the test session. The depressed behaviour, according to Porsolt, is related to prolonged immobility time, and the antidepressant effect is related to increased swim time.

The rat locomotor and exploratory activity was measured by the open field test (Janssen method; arena, 85 cm diameter, 42 cm high). The only source of light in the room was a 100 W bulb attaching directly above the arena and illuminating its centre. The test was performed 15 min before the forced swimming test. The number of quadrants crossed in 10 min was counted.

After the forced swimming test, the rats were killed by decapitation. The frontal cortex and the hippocampus were dissected and homogenized in 0.32 M sucrose at 4 °C. The homogenate was centrifuged at 900 × g for 10 min. The supernatant fluid was centrifuged at 70,000 × g/15 min. The pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.5) and incubated at 37 °C for 15 min, then centrifuged again at 70,000 × g/15 min. The final pellet was resuspended in 50 mM Tris–HCl (pH 7.7), 4 mM CaCl₂, and 0.1% ascorbic acid and stored at −80 °C until use. The protein concentrations (Bradford method) were 17.58 ± 0.2 µg/100 µl in the frontal cortex and 16.69 ± 0.3 µg/100 µl in the hippocampus. Saturation experiments with 2-(N,N,Di[2,3-(3H)]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene(3H-8-OH-DPAT; 0.01–15 nM) were performed on the membranes. Specific binding of 3H-8-OH-DPAT was defined as the excess over blank values obtained in the presence of 1 µM serotonin. All binding assays were done under equilibrium conditions (30 min at 37 °C) and in triplicate. The reaction was stopped by adding 4 ml ice-cold 20 mM Tris–HCl buffer (pH 7.4), and the tube contents were immediately filtered through Whatman 24 mm GF/B filters under a vacuum (Brandel-M48 Cell Harvester filtration unit, USA). The filters were washed twice with 4 ml ice-cold Tris–HCl buffer. Liquid scintillation counting of the filters was done in 4 ml Optiphase ‘Hisafe’ 3 (LKB Wallac, UK) using an LKB beta spectrometer (counting efficiency of 70%). Saturation analysis was performed using iterative non-linear regression analysis (Radlig and Ligand software) to determine the maximal bound ($B_{max}$) and the dissociation constant ($K_d$) values.

The results are expressed as the mean and standard error of the mean (mean ± SEM). The results obtained from the behavioural test were analyzed by analysis of variance (ANOVA) test (main factors; treatment; and time of treatment). The results from the 3H-8-OH-DPAT binding to the 5-HT₁A receptors were analyzed by a three-way ANOVA test (main factors: treatment; time of treatment; and region of brain). Post-hoc testing was done with the Bonferroni method (Statistical Package for Social Sciences 9.0 software, Real State-Real Easy, 1999).

Boehringer Ingelheim, Spain provided SAM. Ciba-Geigy, Spain provided imipramine. Reagents were purchased from the Sigma Chemical Co., St Louis, MO. 3H-8-OH-DPAT (specific activity, 137 Ci/mmol) was purchased from Amersham, UK.

Table 1 shows the immobility time in the forced swimming test and the number of changes of area in the open field test. Acute SAM and acute imipramine + SAM, but not acute imipramine, significantly reduced the immobility time with respect to saline. The acute imipramine + SAM effect was 21.17% greater than that of acute SAM (P < 0.05). Chronic imipramine, SAM, and imipramine +

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Antidepressant and motor activity effects of imipramine, SAM, and imipramine + SAM vs. saline after acute and chronic i.p. injections in Wistar rat</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>Acute treatment</td>
</tr>
<tr>
<td></td>
<td>Forced swimming test Immobility time (s)</td>
</tr>
<tr>
<td></td>
<td>Open field test Changes of areas (n)</td>
</tr>
<tr>
<td>Saline</td>
<td>222 ± 10.4 55.6 ± 7.1</td>
</tr>
<tr>
<td>Imipramine</td>
<td>219 ± 8.8 (−1.35) 46.2 ± 3.7 (−16.90)</td>
</tr>
<tr>
<td>SAM</td>
<td>164 ± 10.5 (−26.12) 50.9 ± 4.2 (−8.45)</td>
</tr>
<tr>
<td>Imipramine + SAM</td>
<td>117 ± 8.1 (−47.29) 43.6 ± 2.1 (−21.58)</td>
</tr>
</tbody>
</table>

*Imipramine: 10.9 mg/kg; SAM: 50 mg/kg.  
Acute, 1 day; and chronic, 20 days. 
Data are the mean ± SEM (n = 12 animals); figures in parentheses represent the change (%) vs. saline.  
Two-way ANOVA test: P < 0.001 vs. chronic saline.  
Two-way ANOVA test: P < 0.001 vs. acute imipramine.  
Two-way ANOVA test: P < 0.001 vs. chronic SAM.  
Two-way ANOVA test: P < 0.005 vs. homologous acute treatments.  
Two-way ANOVA test: P < 0.005 vs. acute saline and acute imipramine.  
Two-way ANOVA test: P < 0.001 vs. acute saline and acute imipramine.  
Two-way ANOVA test: P < 0.005 vs. acute SAM.  
Two-way ANOVA test: P < 0.01 vs. chronic SAM.  
Two-way ANOVA test: P < 0.05 vs. acute imipramine + SAM.
SAM showed antidepressant effect in the forced swimming test. Chronic imipramine + SAM showed a greater (P < 0.05) but not synergistic effect than that of chronic imipramine (11.84%), and chronic SAM (26.31%). Imipramine shows a delay period, reducing the immobility time (antidepressant effect) only after chronic treatment. Chronic imipramine and imipramine + SAM showed a higher antidepressant effect than the homologous acute treatments (P < 0.01; Table 1).

The three acute treatments and chronic SAM did not modify the rat locomotor activity in the open field test. Chronic imipramine and imipramine + SAM diminished the locomotor activity with respect to chronic saline (P < 0.01) and homologous acute treatments (P < 0.05; Table 1).

Table 2 summarizes the 3H-8-OH-DPAT affinity (Kd) and 3H-8-OH-DPAT maximal receptor bound (Bmax) to the hippocampus and frontal cortex 5-HT1A receptors. The Hill coefficients were close to unity in all of the groups, consequently, 3H-8-OH-DPAT bound specifically and saturably to a homogeneous population of non-interacting binding sites in both tissues. The non-specific binding was 10% in the hippocampus and 15% in the frontal cortex.

Acute imipramine increased the 5-HT1A receptors’ Bmax in the frontal cortex with respect to saline (P < 0.05), but did not modify the 5-HT1A receptors’ Bmax with respect to the saline group in the hippocampus. Chronic imipramine increased 5-HT1A receptors’ Bmax in the hippocampus (P < 0.05) and diminished 5-HT1A receptors’ Bmax in the frontal cortex (−40.85%; P = 0.08). Chronic imipramine also decreased 5-HT1A receptors’ affinity in the hippocampus (Kd was 1.81-fold higher than saline; P < 0.05) and did not modify 5-HT1A receptors’ affinity in the frontal cortex. Acute SAM did not modify 5-HT1A receptor function in both tissues. Chronic SAM significantly increased the 5-HT1A receptors’ Bmax in the hippocampus (P < 0.05), but it did not affect the frontal cortex 5-HT1A receptors. In contrast to acute imipramine, acute imipramine + SAM did not modify the frontal cortex 5-HT1A receptors’ Bmax and Kd with respect to saline. Chronic imipramine + SAM significantly increased 5-HT1A receptors’ Bmax in the hippocampus (P < 0.05) and decreased the receptors’ affinity in this area (Kd was two-fold higher than the saline group; P < 0.05). In the frontal cortex, chronic imipramine + SAM significantly decreased the 5-HT1A receptors’ Bmax (P < 0.05) and did not modify the Kd with respect to saline (Table 2).

The increased frontal cortex 5-HT1A receptors’ Bmax correlated positively with increased forced swimming test immobility time (depressed behaviour) in the animals treated with imipramine (Pearson correlation coefficient, +0.719; P = 0.008), and imipramine + SAM (Pearson correlation coefficient, +0.758; P = 0.004). The increased hippocampus 5-HT1A receptors’ Bmax correlated negatively with the forced swimming test immobility time in the animals injected with imipramine (Pearson correlation coefficient, −0.735; P = 0.006), and imipramine + SAM (Pearson correlation coefficient, −0.88; P = 0.002). Consequently, imipramine and imipramine + SAM’s antidepressant effect appears to be related to a reduction of the 5-HT1A receptors’ Bmax in the frontal cortex, and an increment of the 5-HT1A receptors’ Bmax in the hippocampus.

None of the treatments increased the locomotor activity of the rats (Table 1), consequently the imipramine, SAM and imipramine + SAM antidepressant effects were not related to any stimulatory effects of the rat motor activity. On the contrary, imipramine and imipramine + SAM chronic treatments diminished the rats motor activity with respect to saline (P < 0.01) and with respect to their homologous acute treatments (P < 0.05). The reduction of the rat

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Hippocampus</th>
<th>Frontal</th>
<th>Cortex</th>
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<tbody>
<tr>
<td></td>
<td>Acute treatment</td>
<td>Chronic treatment</td>
<td>Acute treatment</td>
</tr>
<tr>
<td></td>
<td>Kd (nM)</td>
<td>Bmax (fmol/mg protein)</td>
<td>Kd (nM)</td>
</tr>
<tr>
<td>Control</td>
<td>1.785 ± 0.09</td>
<td>284 ± 19.5</td>
<td>2.473 ± 0.28</td>
</tr>
<tr>
<td>Imipramine</td>
<td>2.392 ± 0.12</td>
<td>300 ± 17.8</td>
<td>4.488 ± 0.43</td>
</tr>
<tr>
<td>SAM</td>
<td>2.107 ± 0.13</td>
<td>283 ± 25.04</td>
<td>3.723 ± 0.44</td>
</tr>
<tr>
<td>Imipramine + SAM</td>
<td>2.385 ± 0.24</td>
<td>329 ± 11.6</td>
<td>4.957 ± 0.75</td>
</tr>
</tbody>
</table>

*Acute, 1 day; and chronic, 20 days.*

*Imipramine: 10.9 mg/kg; SAM: 50 mg/kg; saline: 0.5 ml/kg.

*Data are the mean ± SEM (n = 6 animals in each group and area); figures in parentheses represent the change (%) vs. saline.

*Three-way ANOVA test: P < 0.05 vs. chronic saline and homologous acute treatments.

*Three-way ANOVA test: P < 0.05 vs. saline, SAM, and imipramine + SAM acute treatments, and homologous chronic treatments.

*Three-way ANOVA test: P < 0.05 vs. chronic saline and chronic SAM.
locomotor activity may be due to imipramine’s anti-cholinergic and anti-histaminergic-related sedative effect.

This study represents the first evidence of the relationship between imipramine’s delayed onset of clinical efficacy and the increased density of the frontal cortex 5-HT1A receptors. Stimulation of the dorsal raphe nucleus (DRN) somatodendritic 5-HT1A autoreceptors inhibits the electrical activity of the serotonergic neurons leading to a reduction of the serotonergic neurotransmission in the forebrain [17]. The activation of the post-synaptic 5-HT1A receptors in the fronto-parietal cortex would diminish the function of the DRN 5-HT1A autoreceptors by a negative feed-back mechanism leading to the inhibition of the firing activity of the DRN serotonergic neurons [10]. In relation to this effect, there are evidences indicating that these cortical 5-HT1A receptors are post-synaptic receptors [12] located on glutamatergic pyramidal output neurons which form synapses preferentially with gamma-aminobutyric acid neurons in the DRN [9,11]. As acute imipramine induces an increased 5-HT1A receptor density in the frontal cortex and increased serotonin levels in the brain, and as the antidepressant effect of chronic imipramine was correlated to a reduction of the frontal cortex 5-HT1A receptor density and to an increment of the hippocampus 5-HT1A receptor density, it may be hypothesized that acute imipramine could stimulate the frontal cortex inhibitory control of the DRN neurons leading to an inhibition of the DRN serotonergic neurons’ firing activity and diminishing the serotonergic neurotransmission in terminal areas. On the contrary, chronic imipramine diminishing the frontal cortex inhibitory control of the DRN serotonergic neurons may recover the serotonin neurons’ firing activity. This effect associated to the increment of the hippocampus 5-HT1A receptors’ Bmax induced by chronic imipramine may increase the serotonergic neurotransmission in terminal areas, and consequently induce the antidepressant effect. These data agree with those previously reported showing an increased density of the 5-HT1A receptors in the hippocampus [6], and a progressive sensitization of the post-synaptic 5-HT1A receptors in the dorsal hippocampus [4] after tricyclic chronic treatment, but disagree with another which does not show it [3].

Acute and chronic imipramine + SAM showed a higher antidepressant effect than acute and chronic SAM. The acute imipramine + SAM antidepressant effect was associated with no changes in the frontal cortex 5-HT1A receptors. Thus, SAM prevents the frontal cortex 5-HT1A receptor up-regulation induced by acute imipramine. As the antidepressant effect of acute imipramine + SAM is higher than that of acute SAM, it may be related to the absence of changes in the basal inhibitory control of the DRN by the frontal cortex. There are no direct evidences in the literature showing that increased density of the 5-HT1A receptors in the frontal cortex could prevent the antidepressant effect. However, no change [18] or increased frontal cortex 5-HT1A receptor density [1,13,19] has been reported in depressed patients. Also, chronic treatment with imipramine diminished the 5-HT1A receptor density in the rat frontal cortex [14,15], as was, in fact, observed in this study. Thus, depression appears to be related to increased density of the frontal cortex 5-HT1A receptors while the antidepressant effect appears to be related to diminished frontal cortex 5-HT1A receptor function. Chronic imipramine + SAM diminished frontal cortex 5-HT1A receptors and increased the hippocampus 5-HT1A receptors, showing similar correlations to those previously described for imipramine. These correlations are mainly related to imipramine’s effects on the 5-HT1A receptor function.

Acute SAM showed an antidepressant effect as has been reported in experimental [8] and clinical studies [2]. There were no changes in the hippocampus 5-HT1A receptor characteristics after acute SAM and imipramine + SAM treatments, indicating that the hippocampus 5-HT1A receptors are not implicated in acute SAM and imipramine + SAM antidepressant effects. The difference between acute and chronic SAM antidepressant effects was not significant. A non-correlated increased of the hippocampus 5-HT1A receptor density was linked to the SAM chronic effect, but not to the acute treatment. Thus, other mechanisms of action are implicated in SAM’s antidepressant effects. Processes, including the control of the fluidity of the membrane [7], and the regulation of the gene’s expression and transcription [20], have been related with SAM-dependent methylation reactions, and may be related to the SAM antidepressant effect. Further studies are needed to determine whether SAM prevents the frontal cortex 5-HT1A receptor up-regulation induced by acute imipramine and facilitates the frontal cortex 5-HT1A receptor down-regulation induced by chronic imipramine.

SAM prevents the up-regulation of frontal cortex 5-HT1A receptors induced by acute imipramine. This effect may contribute to imipramine’s antidepressant effect in the rat.

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