Female rats show an increased sensibility to the forced swim test depressive-like stimulus in the hippocampus and frontal cortex 5-HT$_{1A}$ receptors

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Received 21 March 2003; received in revised form 14 July 2003; accepted 16 July 2003

Abstract

Affective disorders are more common in women. The forced swim test acts like a depressive stimulus. Hippocampus and frontal cortex 5-HT$_{1A}$ receptors of female and male Wistar rats subjected to the forced swim test were compared with a sham group. The forced swim test diminishes ($P < 0.05$) the hippocampus $^3$H-8OH-DPAT bound in the female rats (184 ± 16 fmol/mg protein) with respect to the male rats (309 ± 41 fmol/mg protein) and to the female sham rats (255 ± 20 fmol/mg protein). The forced swim test increases the frontal cortex 5-HT$_{1A}$ receptors in the female rats with respect to the female sham group (40.4 ± 5 versus 24.7 ± 4 fmol/mg protein, $P < 0.05$). An increased sensibility of the 5-HT$_{1A}$ receptors to depressive-stimulus may be one mechanism underlying the higher prevalence of depression in female. © 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: Gender; 5-HT$_{1A}$; Hippocampus; Frontal cortex; Forced swim test

The life time prevalence for major depression is approximately 4% in men and 8% in women [12]. An imbalance in the dorsal raphe nucleus, hippocampus and the frontal cortex 5-HT$_{1A}$ receptors have been strongly implicated in the pathophysiology of affective disorders [6].

Our aim was to determine gender related changes of the hippocampus and fronto-parietal cortex 5-HT$_{1A}$ receptors of Wistar rats subjected to sham or to forced swim test conditions. The 5-HT$_{1A}$ receptors were characterized by $^3$H-8OH-DPAT saturation studies. The forced swim test (FST) was used as a ‘depress-like stimulus’. And the open field test was used to clearly distinguish the depressive response from possible changes in the rat locomotor and exploratory activity.

Six months-old ($n = 48$) male and female Wistar rats (CRIFFA, Barcelona, Spain) were used. In the sham groups, the rats weighed $427 \pm 10$ g in males and $358 \pm 5$ g in the females. In the groups subjected to the FST conditions the rats weighed $424 \pm 14$ g in the males, and $320 \pm 9$ g in the females. The animals were allowed to habituate to the animal facility for 5 days before the start of the experiment and were housed three per cage under standardized conditions (12 h light/dark cycle (lights on between 07:00 and 19:00 h); temperature $22 \pm 2^\circ$C; water and food available ad libitum) up to the time of testing. Thus, the estrous cycle phase related behaviour was not the main purpose of this study; vaginal cytology was not performed to avoid an additional stress to the female rats. Twenty-four rats (12 male and 12 female) were subjected to the FST conditions. The other 24 rats (12 male and 12 female) were handled but not subjected to any behavioural test. All housing and behavioural procedures followed the provisions and general recommendations of the European Communities Council Directive (86/609/EEC), and Local rules.

The forced swim test (Porsolt method) was used to induce the depressive behaviour. Each rat was placed in a vertical cylinder (40 cm height, 18 cm diameter) containing water to a height of 21 cm at 37°C. Two swimming sessions were conducted the pre-test session (15 min) and 24 h later the test session (5 min). The total duration of immobility behaviour were measured during the 5 min of the test session. The number of head waving, and the number of faecal bolus produced during the forced swim test were also counted. To avoid methodological artefacts like the existence of pheromones or other products excreted by the
rats remaining in the water after the test and weight dependent immobility response, the water was changed between testing individual animals, and a group of male rats (group male X) \((n = 6)\) with similar weight \((321 \pm 7 \, \text{g})\) to the female rats were also included in the FST studies. No weight-related differences between male X rats and female rats were observed. The group of male X rats \((4.5 \, \text{months})\) was excluded from the receptors characterization studies to avoid possible age-related influences. The open field test \((\text{Janssen method})\) was used to measure the rat locomotor and exploratory activity and was performed 15 min before the forced swim test session. The arena measured 85 cm in diameter and 42 cm in height. The only source of light in the room was a 100 W bulb hanging directly above the arenas’ center. The number of quadrants crossed, the number of stereotypes, and the number of faecal bolus produced during 10 min were counted in this test.

Afterwards the rats were killed by decapitation and their frontal cortex and hippocampus were dissected and homogenized in 0.32 M sucrose. The homogenate was centrifuged at 900 \(\times g\) for 10 min. The supernatant fluid was centrifuged at 70 \(\, 000 \times g\) for 15 min. The pellet was resuspended in 50 mM Tris–HCl buffer \((\text{pH} 7.5)\) and the suspension incubated at 37°C for 15 min then centrifuged again at 70 \(\, 000 \times g\) for 15 min. The final pellet was resuspended in buffer containing 50 mM Tris–HCl \((\text{pH} 7.7)\), 4 mM CaCl\(_2\), 0.1% ascorbic acid and stored at \(-80°C\) until use. The final protein concentrations \((\text{Bradford method})\) were 17.58 ± 0.2 \(\mu g/100 \, \mu l\) in the frontal cortex and 16.69 ± 0.3 \(\mu g/100 \, \mu l\) in the hippocampus. There were no differences between the hippocampus and frontal cortex protein concentrations of the male and female of the two groups. Saturation experiments with six concentrations of \(^3\text{H}-8\text{OH-DPAT} \, (0.01–15 \, \text{nM})\) were performed on the membranes. Specific binding of \(^3\text{H}-8\text{OH-DPAT}\) was defined as the excess over blank values obtained in the presence of 1 \(\mu M\) serotonin. All binding assays were done in triplicate in a total volume of 300 \(\mu l\) during 30 min at 37°C. The reaction was stopped by adding 4 ml ice-cold 20 mM Tris–HCl buffer, pH 7.4. The contents of the tubes were immediately filtered through Whatman 24 mm GF/B filters under a vacuum and washed twice with 4 ml ice-cold Tris–HCl buffer. Liquid scintillation counting of the filters was done in 4 ml Optiphase Hisafe 3 using an LKB beta spectrometer \((\text{counting efficiency of 70})\%\). Saturation analysis was performed using Radlig-Ligand software to determine the maximal bound \((B_{\text{max}})\) and the affinity \((K_d)\) values of the 5-HT\(_{1A}\) receptors.

The results are expressed as the mean and the standard error of the mean \((\text{mean} \pm \text{SEM})\). The results obtained from the behavioural test were analyzed by one way analysis of variance \((\text{ANOVA})\) test to determine gender related differences. The results from the \(^3\text{H}-8\text{OH-DPAT} \) bound to the 5-HT\(_{1A}\) receptors were analyzed by two-ways ANOVA test to determine swim test-sham related differences and area-related differences. Post-hoc comparisons were made by the Bonferroni methods. Pearson Correlation test was performed to show possible relationship between behaviour response and 5-HT\(_{1A}\) receptor changes. All statistics were made using the Statistical Package for Social Sciences 10.0 software. All chemicals and reagents were purchased from the Sigma Chemical Co. \(^3\text{H}-8\text{OH-DPAT} \) (specific activity 137 Ci/mmol) was purchased from Amersham.

**Fig. 1** show the result obtained in the FST and the open field test. There were no gender-related differences in the immobility response, in the number of the head waving, and in the number of the faecal bolus produced in the FST. There were also no differences between male and female rats in the number of the quadrant crossed during 10 min, in the stereotypes, and in the faecal bolus produced in the open field test.

**Table 1** summarized \(^3\text{H}-8\text{OH-DPAT} \) affinity and \(^3\text{H}-8\text{OH-DPAT} \) maximal receptor bound to the hippocampus and frontal cortex 5-HT\(_{1A}\) receptors of sham and FST groups. The \(^3\text{H}-8\text{OH-DPAT} \) specific binding showed a Hill coefficient close to unity in all the studied groups. The non-specific binding was a 10% in the hippocampus and a 15% in the frontal cortex, or less of the total binding. There were no differences between male and female hippocampus and frontal cortex 5-HT\(_{1A}\) receptors \(K_d\) and \(B_{\text{max}}\) under sham conditions.

A reduction of the \(^3\text{H}-8\text{OH-DPAT} \) \(B_{\text{max}}\) occurred in the hippocampus of the female rats with respect to the male rats.
subjected to the swim test conditions, and with respect to the female rats sham group. The female rats subjected to the swim test also showed an increment (Table 1) of the frontal cortex $^3$H-8OH-DPAT $B_{\text{max}}$ with respect to the female sham rats. No changes were detected in the $^3$H-8OH-DPAT affinity in the hippocampus and the frontal cortex 5-HT$\text{IA}_A$ receptors between male and female rats and between sham and FST conditions. The increased frontal cortex 5-HT$\text{IA}_A$ receptors' $B_{\text{max}}$ correlated negatively with the hippocampus 5-HT$\text{IA}_A$ receptors' $B_{\text{max}}$ (Pearson correlation coefficient, $-0.895; P = 0.016$).

It seems that under basal or non-depressive-like stimulus, male and female rats do not present differences in the 5-HT$\text{IA}_A$ receptors function in the hippocampus and the frontal cortex. However, female rats seem to be more sensible than the male rats to the depressive-like stimulus of the forced swim test. In this way, the female rats showed a down regulation of the hippocampus $^3$H-8OH-DPAT maximal binding leading to a reduction of the 5-HT neurotransmission in this area. On the contrary, in the frontal cortex, the female rats develop a slight up-regulation of the 5-HT$\text{IA}_A$ receptors leading to an increase of the receptors function.

The forced swim test appears to be a suitable animal model to detect the antidepressant effects because of its sensibility and specificity [11,20]. Gender related differences have been previously shown. Wistar Kyoto female rats are more active in the open field test and more immobile in the forced swim test than the male rats [16]. Even more proestrus-estrus female rats are less active in the open field test and significantly more immobile in the forced swim test than diestrus females [9,15,16]. Although this estrous cycle related differences have not been reproduced by other authors [1] it seems that gonadal hormones could affect the depress behaviour. In this sense, while progesterone has shown antidepressant effects [14], the role of estrogen on mood disorders is a subject of controversy [5]. Under the forced swim test condition the female rats develop changes of the hippocampus and frontal cortex 5-HT$\text{IA}_A$ receptors function very close to those reported for depressed patients. Actually, no changes or reduction [13,18] of the 5-HT$\text{IA}_A$ receptors in the hippocampus and an increase of the 5-HT$\text{IA}_A$ receptors in the frontal cortex [2] and in the dorsal raphe nucleus [19] of depressed patients have been shown. Long-term tricyclic antidepressants and repeated electroconvulsive shock administration induce a sensitization of the post-synaptic 5-HT$\text{IA}_A$ receptors in the dorsal hippocampus [4] while MAOIs, SSRIs, and 5-HT$\text{IA}_A$ agonists long-term treatments desensitize the somatodendritic 5-HT$\text{IA}_A$ autoreceptors of the DRN serotonergic neurons, leading to the recovery of the firing rate [3]. Consequently, it has been suggested that a diminish of the function of the 5-HT$\text{IA}_A$ receptors in the hippocampus and an increased function of the 5-HT$\text{IA}_A$ receptors in the DRN coexist with depression symptoms whereas an increase or no change of the 5-HT$\text{IA}_A$ receptors in the hippocampus and a decrease of the 5-HT$\text{IA}_A$ receptors in the DRN is related with the antidepressive effects of the different drugs.

The activation of the post-synaptic 5-HT$\text{IA}_A$ receptors in the fronto-parietal cortex may diminish the function of the DRN 5-HT$\text{IA}_A$ autoreceptors by a negative feedback mechanism [8,17] leading the inhibition of the firing activity of the DRN serotonergic neurones [7,10]. The enhanced $^3$H-8OH-DPAT maximal bound of the frontal cortex detected in the female after the forced swim test may subsequently lead to increased inhibitory effect of the fronto-parietal cortex 5-HT$\text{IA}_A$ receptors into the dorsal raphe serotonergic neurons.

It may be hypothesized that the female rats subjected to depressive-like stimulus such as the forced swim test could develop an enhanced inhibitory control of the dorsal raphe nucleus neurones by the frontal cortex leading an inhibition of dorsal raphe serotonergic neurones firing activity. Secondarily, decreased 5-HT level appears in terminal areas. Lower levels of 5-HT in the forebrain regions associated to a parallel reduction of the $^3$H-8OH-DPAT maximal bound in specific areas such as the hippocampus, which was observed in this study, may subsequently lead to a reduction of the 5-HT neurotransmission in this specific area of the forebrain.

The findings indicate that an increased sensibility of the 5-HT$\text{IA}_A$ receptors to the depressive stimulus may exist in the hippocampus and frontal cortex of female rats leading to a down-regulation of the 5-HT$\text{IA}_A$ receptors in the hippocampus, and a slight up-regulation of the 5-HT$\text{IA}_A$ receptors in the frontal cortex. An increased sensibility of the 5-HT$\text{IA}_A$

### Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Sham group</th>
<th>Forced swim test group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td>$B_{\text{max}}$ (fmol/mg prot)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>hipsocampus</td>
<td>2.46 ± 0.5</td>
<td>332 ± 30</td>
</tr>
<tr>
<td></td>
<td>2.27 ± 0.41</td>
<td>309 ± 41</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.23 ± 0.05</td>
<td>27.06 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>1.14 ± 0.07</td>
<td>40.4 ± 5.13**</td>
</tr>
</tbody>
</table>

Studies on affinity and maximal receptor density. Means ± SEM ($n = 12$ animals per group). Two-way ANOVA test followed by Bonferroni post-test: * ($P < 0.05$) with respect to male rats subjected to forced swim test, and female and male sham groups. ** ($P < 0.05$) with respect female rats sham group.
receptors to depressive-stimulus may be one mechanism underlying the higher prevalence of depression in female.

Acknowledgements

This study has been supported by the University of Malaga (Spain) I + D project 8.06/24.652. The authors thank Pauline Huang for the English corrections.

References