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ORIGINAL RESEARCH

# Trazodone effects on [$^3$H]-paroxetine and $\alpha_2$-adrenoreceptors in platelets of patients with major depression

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**Abstract:** Trazodone is an antidepressant which behaves as a selective 5-HT$_2$ antagonist and 5-HT reuptake inhibitor. The lack of information on its effects *in vivo* prompted us to evaluate $\alpha_2$-adrenoceptors by means of the specific binding of [$^3$H]-rauwolscine, and the 5-HT transporter (SERT) by means of the binding of [$^3$H]-paroxetine ([$^3$H]-Par), in platelets of depressed patients, before and after one month of treatment with trazodone (75–300 mg/day). Twenty-five outpatients of both sexes with a diagnosis of major depression, as assessed by the Structured Clinical Interview for DSM IV, were included in the study. Depressive symptoms were evaluated by means of the Hamilton Rating Scale for Depression: the total score (mean ± SD) was 20 ± 6 at baseline ($t_0$) and 7 ± 4 after one month of treatment ($t_1$). Platelet membranes, [$^3$H]-rauwolscine and [$^3$H]-Par bindings were carried out according to standardized protocols. The results showed that the $B_{max}$ values of [$^3$H]-Par were statistically lower at $t_1$ than at $t_0$ (733 ± 30 vs 1471 ± 99, $P < 0.001$), while the $K_d$ and the [$^3$H]-rauwolscine binding parameters remained unchanged. The findings of this study suggest that *in vivo* trazodone modifies the number of the SERT proteins and that, perhaps, most of its antidepressant properties are related to this activity.

**Keywords:** trazodone, depression, serotonin, platelets, $\alpha_2$-adrenoreceptors, [$^3$H]-rauwolscine, serotonin transporter, [$^3$H]-paroxetine

## Introduction

Trazodone is a compound deriving from phenylpiperazines widely-used in the United States and Europe since the beginning of the 1980s as an antidepressant characterized by lower anticholinergic properties and lower cardiac conduction effects than tricyclics.[1–4] Trazodone behaves like an antagonist of serotonin (5-HT) receptors of type 2 (5-HT$_2$) and of α-adrenoreceptors, as well as like an inhibitor of 5-HT reuptake.[5,6] Together with nefazodone, it belongs to the class of the so-called 5-HT$_2$ antagonists and 5-HT inhibitors (SARIs).

The inhibition of the reuptake increases the intrasynaptic 5-HT levels leading to indiscriminate stimulation of all 5-HT receptors. If the first activity is related to the antidepressant effect, the second is at the basis of some side-effects typically displayed by selective serotonin reuptake inhibitors (SSRIs). Indeed, raphe 5-HT$_1$ stimulation is responsible for the antidepressant efficacy, while proencefalic 5-HT$_2$ stimulation induces agitation, anxiety and sexual dysfunctions. Therefore, compounds such as trazodone that inhibit 5-HT reuptake, but antagonize only specific 5-HT receptors, seem to reduce significantly some side-effects deriving from an excessive increase of 5-HT concentrations and receptor stimulation.[7]

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The antidepressant properties of trazodone have been demonstrated in different clinical placebo-controlled studies and compared also with those of other antidepressants.[4,8,9] From such trials, it emerged that it is an effective compound with a good tolerability profile similar to that of placebo: in fact, the incidence of side-effects ranges between 15 and 30% in both trazodone and placebo users. The anticholinergic side effects, particularly dry mouth, blurred vision, constipation, and urinary retention are rare, as they are those due to α-adrenoreceptor blockade, such as hypotension and sedation. Interestingly, trazodone treatment has been seldom associated with priapism,[10,11] a characteristic that can be used to revert the sexual dysfunctions provoked by other psychotropic drugs.[12]

Although the pharmacodynamic aspects of trazodone are well known, no information is available on its effects on peripheral noradrenergic and serotonergic markers *in vivo*. For three decades, platelets have been widely used in biological psychiatry as a reliable, peripheral model of serotonergic neurons.[13] In particular, blood platelets and neurons share a similar 5-HT reuptake system, the 5-HT transporter (SERT)[14,15] and other receptors, such as $\alpha_2$-adrenoceptors. Therefore, our study aimed to evaluate $\alpha_2$-adrenoceptors, by means of the specific binding of [$^3$H]-rauwolscine, a selective antagonist of their levels, and the 5-HT transporter (SERT) by means of the binding of [$^3$H]-paroxetine ([$^3$H]-Par), in platelets of depressed patients, before and after one month's treatment with trazodone. In addition, we explored the possible correlation between kinetic parameters of [$^3$H]-rauwolscine and [$^3$H]-Par bindings and demographic or clinical characteristics of the patients.

# Materials and methods

## Subjects

Twenty-five outpatients (12 women, 13 men, between 23 and 50 years of age, mean ± SD: 32.3 ± 9.5), recruited at the Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, University of Pisa, Italy, with a diagnosis of major depression, as assessed by the Structured Clinical Interview for DSM IV,[16] were included in the study. Fifteen patients were suffering from recurrent unipolar depression and 10 from bipolar disorder (BD): in particular, three suffered from BD of type 1 and seven from BD of type 2. The [$^3$H]-Par binding assay was carried out in all patients, while the [$^3$H]-rauwolscine binding was carried out in only 21 (10 women, 11 men, age between 23 and 48 years; mean ± SD: 35.8 ± 10.5). None had taken tricyclic antidepressants in the last year, three had taken SSRIs (citalopram, sertraline, escitalopram) 30 days before the enrollment and two were currently taking mood stabilizers (valproic acid and gabapentin). Depressive symptom severity was evaluated by means of the Hamilton Rating Scale for Depression (HRSD):[17] the total score (mean ± SD) was 20 ± 6 (range: 17–32) at baseline ($t_0$) and 7 ± 4 (range: 4–13) after one month of treatment ($t_1$). The doses of trazodone ranged between 75–300 mg/day.

All patients had no concomitant medical illness, as shown by a general check-up and by blood and urine tests which were within the normal range.

The study was approved by the Ethics Committee at Pisa University and an informed written consent was completed by all patients.

## Platelet separation

Venous blood (25 mL) was collected from fasting subjects between 08.00 and 09.00 a.m, during the months of November–December and then mixed with 3 mL of anticoagulant: sodium citrate (2.2%) and citric acid (1.2%). A second sample was collected after a month of monotherapy with trazodone.

Platelets membranes were prepared according to a standardized protocol. Platelet-rich plasma (PRP) was obtained by low speed centrifugation (150 × *g* for 15 min at 20°C). Platelets were precipitated from PRP by centrifugation at 1,500 × *g* for 15 min at 20°C and stored at −80°C until binding assay which was carried out within two weeks. On the day of assay, platelets were homogenized in 10 mL buffer 50 mM Tris-HCl, 5 mM EDTA, pH 7.7, containing protease inhibitors (20 μg/mL trypsin inhibitor: 200 μg/mL bacitracine, 160 μg/mL benzamidine), with an ultrathurrax homogenizer and centrifuged at 48,000 × *g* for 15 min at 4°C. The ensuing pellet was suspended again in 10 mL buffer 50 mM Tris-HCl, 5 mM EDTA, pH 7.7 and centrifuged twice at 48,000 × *g* for 15 min at 4°C. The ensuing pellet was suspended in an assay buffer (50 mM Tris-HCl).

## [$^3$H]-Rauwolscine binding assay

The [$^3$H]-rauwolscine binding was carried out according to the methods of Corsano et al.[18] Platelet membranes (0.2–0.5 mg proteins), suspended in an assay buffer, were incubated with 0.5 nM [$^3$H]-rauwolscine (Sigma, Milan, Italy; specific activity: 71 Ci/mmol) for 60 min at 25°C in a final volume of 1 mL. The specific binding was evaluated with 10 mM cold clonidine (Sigma). To test the saturability of [$^3$H]-rauwolscine specific binding sites, the platelet membranes were incubated with eight increasing concentrations of [$^3$H]-rauwolscine, ranging between 0.1 and 5 nM. After 60 min, the incubation was halted by the addition of 5 mL of cold buffer. Samples were rapidly filtered under vacuum through glass fiber filters Whatman GF/C, washed four times with 5 mL cold buffer, and placed in vials with 4 mL of scintillation cocktail. Radioactivity was measured by means of a beta-counter (Packard 1600 TR).

## [$^3$H]-Par binding assay

The [$^3$H]-Par binding was carried out according to the method of Marazziti et al.[19] The incubation mixture consisted of 100 µL of platelet membranes (50–100 µg protein/tube), 50 µL of [$^3$H]-Par (Perkin-Elmer Life Science, Milano, Italy; specific activity: 19.1 Ci/mmol) at six concentrations ranging between 0.01 and 1 nM and 1.85 mL of assay buffer (50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, pH 7.4). Specific binding was obtained as the binding remaining in the presence of 10 µM fluoxetine (Sigma) as a displacer. All samples were assayed in duplicate and incubated at 22°C for 1 hour. The incubation was halted by adding 5 mL of cold assay buffer. The contents of the tubes were immediately filtered under vacuum through glass fibre filters GF/C and washed 3 times with 5 mL of assay buffer. Filters were then placed in vials with 4 mL of scintillation cocktail (Ready Safe scintillation cocktail; Beckman Coulter, Carlsbad, CA, USA) and radioactivity was measured by means of a beta-counter (Packard LS 1600). Proteins were measured according to the method of Peterson.[20]

## Statistical analyses

Equilibrium-saturation binding data, the maximum binding capacity ($B_{max}$, fmol/mg protein) and the dissociation constant ($K_d$, nM) were analysed by means of iterative curve-fitting computer programmes EBDA (Biosoft, Cambridge, UK).

The difference between $B_{max}$ and $K_d$, at the two assessment times was measured by means of the Student's *t*-test (two-tailed, paired). The effect of age and sex on biological parameters was evaluated by means of the covariance analysis (ANCOVA). The possible correlations between biological findings and psychopathological data were analyzed according to the Pearson's method. All analyses were carried out using SPSS; version 12.1; (SPSS Inc, Chicago, IL, USA).

# Results

No significant effects of age, sex or diagnosis on [$^3$H]-Par or [$^3$H]-rauwolscine binding were observed.

As far as the [$^3$H]-Par binding is concerned, the $B_{max}$ values (mean ± SD, fmol/mg protein) showed a statistically-significant decrease at $t_1$, as compared with $t_0$ (733 ± 30 vs 1471 ± 99, $P < 0.001$), while the $K_d$ values (mean ± SD, nM) did not change at the two assessment times (Table 1).

Similarly, no difference was detected in $B_{max}$ or $K_d$ values of [$^3$H]-rauwolscine binding of the depressed patients before and after one month of treatment with trazodone.

No correlation between biological parameters and HRSD total score or single items was observed. No difference between unipolar and bipolar patients, or drug-free or previously-treated patients was detected.

# Discussion

The present study, exploring the possible effects of one-month's treatment with trazodone on platelet SERT, as assessed by the [$^3$H]-Par binding, and $\alpha_2$-adrenoceptors, measured by means of the [$^3$H]-rauwolscine binding, of depressed patients, led to different findings. First, the binding parameters, $B_{max}$ and $K_d$, of [$^3$H]-rauwolscine, a selective $\alpha_2$-antagonist, were not modified by the treatment with trazodone. This is consistent with the pharmacological properties of trazodone: in fact, *in vitro* this compound does not provoke any effect on $\alpha_2$-adrenoceptors, while it interacts with $\alpha_1$- and 5-$HT_2$ receptors[21] and shows a certain inhibitory activity on 5-HT reuptake.[22,23] This is confirmed by our observations of a significant decrease (about 50%) of the $B_{max}$ of [$^3$H]-Par binding after one month of treatment. The change of $B_{max}$ with no modification of the $K_d$, which is the inverse of the affinity constant, suggests that trazodone provokes a decrease in the number of the SERT proteins, without affecting its affinity characteristics. Previously, it has been shown by different authors that SSRIs or tricyclics may modify [$^3$H]-Par binding parameters *in vivo*, although data are quite controversial.[24–28] In particular, the $B_{max}$ of [$^3$H]-Par binding, at $t_0$ was similar in 27 depressed patients and control subjects, albeit significantly lower in those patients at the first episode, and decreased after three months of treatment with fluoxetine or clomipramine.[27] The same result was obtained in a sample of 24 depressed children and adolescents after six weeks and also after

**Table 1** [$^3$H]-Par and [$^3$H]-Rauwolscine bindings parameters ($B_{max}$, fmol/mg protein and $K_d$, nM, mean ± SD) in platelets of depressed patients

| | $B_{max}$ (fmol/mg prot) | $K_d$ (nM) |
|---|---|---|
| [$^3$H]-Par (N = 25) | | |
| $t_0$ | 1471 ± 99 | 0.11 ± 0.4 |
| $t_1$ | 733 ± 30* | 0.1 ± 0.2 |
| [$^3$H]-Rauwolscine (N = 21) | | |
| $t_0$ | 168 ± 33 | 0.78 ± 0.2 |
| $t_1$ | 169 ± 20 | 1.02 ± 0.4 |

**Note:** *significant, $P < 0.001$.

six months of sertraline.[26] On the contrary, sertraline given for six months to a small sample (n = 10) of adult depressed patients seemed to provoke an increased $B_{max}$ of [$^3$H]-Par binding, while paroxetine led to the opposite findings in another 10 patients.[28] Although generally no change of the $K_d$ has been detected, in one study this parameter was reported to increase after six weeks of treatment with fluoxetine or lofepramine in, respectively, 22 and 18 depressed patients,[24] or to decrease in 45 depressed patients following $SSRI_S$ or tricyclics for at least one month.[29] Therefore, in spite of these controversies regarding which parameter is modified by which drug, it seems that, in any case, the interference with the platelet SERT is essential for the development of the antidepressant effect.[7] Along this line, it is interesting to highlight that all patients enrolled in our study showed a significant decrease of the $B_{max}$ of [$^3$H]-Par binding, in parallel with the improvement of depressive symptomatology, as revealed by the decrease of the HRSD total score. A significant correlation between changes in [$^3$H]-Par $B_{max}$ and change in HRSD total score after four and eight weeks of treatment with paroxetine or fluoxetine was measured in a previous study in 21 depressed patients.[25] On the contrary, we could not detect this, perhaps because of the small sample size. For the same reason, maybe, we did not find any difference between unipolar or bipolar patients, or patients who were drug-free or had been treated one month previously, or were currently taking mood stabilizers: these last drugs, however, do not seem to interfere with [$^3$H]-Par binding.[19]

In conclusion, our study shows that trazodone modifies the number of the SERT proteins in platelet membranes and suggests that these changes may underlie its short-term antidepressant properties and, perhaps, might be used as a predictor of response. Further studies should clarify whether this is the case also of long-term treatments.

## Disclosures

The authors report no conflicts of interest in this work.

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