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Chronic Effects of Triiodothyronine in Combination with Imipramine on 5-HT Transporter, 5-HT_{1A} and 5-HT_{2A} Receptors in Adult Rat Brain

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Triiodothyronine (T3) has been shown to accelerate and potentiate the clinical response to tricyclic antidepressant (TCA) treatment in depressive disorders. The neurobiological mechanisms underlying these therapeutic effects of T3 are still unknown. Since brain serotonin (5-HT) changes have been implicated in the mode of action of TCA drugs, the effects of a chronic (7 or 21 days) administration of imipramine (10 mg/kg/day) and of a low dose of T3 (4 μg/kg/day), given alone or in combination, were investigated on the density of midbrain 5-HT transporters and of hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors in adult Wistar rats. Neither single nor combined administration of imipramine and T3 for 7 days modified the density of 5-HT transporters and of 5-HT_{1A} receptors. On day 21, the combination did not change imipramine- or T3-induced decrease in 5-HT transporter density whereas it prevented imipramine-induced increase in 5-HT_{1A} receptor density. Whatever the treatment duration, imipramine-T3 combination potentiated imipramine-induced decrease in 5-HT_{2A} receptor density. On both day 7 and day 21, T3 given alone had no effects on the density of 5-HT_{1A} and 5-HT_{2A} receptors. These data indicate that T3 is able to modulate the long-term adaptive changes which occur at the postsynaptic level of 5-HT neurotransmission after antidepressant treatment.
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KEY WORDS: *5-HT transporter; 5-HT_{1A} receptor; 5-HT_{2A} receptor; Tricyclic antidepressant; Triiodothyronine; Brain*

Most of the physiological effects of thyroid hormones are mediated in periphery as well as in brain by triiodothyronine (T3), which mainly derived from the monodeiodination of thyroxine (T4), the major secretory product of the thyroid gland. Therefore studies, which have assessed the efficacy of thyroid hormones in depression, have been largely centered on T3.

More than 20 studies have examined the effects of T3-antidepressant combination in the treatment of major depressive disorders (Aronson et al. 1996; Joffe and Sokolov 1994). The doses of T3 used were usually in the physiological range and framed its daily production rate. Only six studies have examined whether the addition of T3 could accelerate the onset of action of tricyclic antidepressant (TCA) drug treatment (Coppen et al. 1972; Feighner et al. 1972; Prange et al. 1969; Steiner et al. 1978; Wheatley 1972; Wilson et al. 1970). In four of

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them, T3 has been found to shorten the latency of the therapeutic response to imipramine or amitriptyline (Coppen et al. 1972; Prange et al. 1969; Wheatley 1972; Wilson et al. 1970).

Other clinical investigations have examined whether the association of T3 to TCA could convert treatment nonresponders to responders (Aronson et al. 1996; Joffe and Sokolov 1994). A particular interest was placed on the use of T3 in euthyroid depressed patients refractory to TCA treatment with amitriptyline or imipramine, drugs which are considered to be the most effective antidepressants (Rudorfer and Potter 1989). In a recent meta-analysis including eight studies with a total of 292 euthyroid patients refractory to TCA therapy, it has been reported that approximately 50% of patients treated with T3 augmentation strategy became responsive to antidepressant treatment (Aronson et al. 1996). Interestingly, one study has compared the antidepressant permissive action of physiological doses of T3 and T4 in euthyroid depressed patients who did not respond to TCA. It has been found that the response rate to T3 was much better than that to T4 (Joffe and Singer 1990). Furthermore, preliminary evidence suggest that the addition of T3 can also enhance the response rate to other classes of antidepressant drugs such as selective serotonin (5-HT) reuptake inhibitors and monoamine oxydase inhibitors (Gupta et al. 1991; Joffe 1988, 1992).

The mechanisms that underlie the therapeutic effects of T3 in depressive disorders are still unknown. No animal studies have examined the chronic effects of physiological doses of T3 given alone or in combination with an antidepressant on the neurotransmitter systems involved in the pathophysiology and the treatment of depressive disorders. To be in the physiological range, the dose of T3 must frame its total daily production rate which is of 4 μg/kg in rats (Escobar-Morreale et al. 1996). The only previous studies that have examined the chronic effects of T3 have used it at doses which were of at least 100 μg/kg/day (Gur et al. 1999; Sandrini et al. 1996). Such doses are supraphysiologic.

The therapeutic effects of antidepressant drugs including TCA require 2 or 3 weeks to become manifest, while their pharmacological actions are prompt and occurred in less than few hours. The lag phase between the initiation of the drug treatment and the onset of clinical improvement suggests that the development of antidepressant effects requires long-term adaptive changes. Clinical investigations have provided robust evidence that the therapeutic effects of antidepressants are underlain by a sustained enhancement of the central 5-HT neurotransmission (Blier and de Montigny 1994; Heninger and Charney 1987; Maes and Meltzer 1995). Furthermore, animal studies have shown that, following their chronic administration, almost all the antidepressant drugs induce an increase in the extracellular 5-HT levels in several brain areas. However, the precise mechanism of their action on the different entities of the central 5-HT system is incompletely understood.

The 5-HT transporter, which is localized on the cell bodies of 5-HT neurons in midbrain and on terminals in projection areas, plays an important role in the regulation of central 5-HT neurotransmission. However, the importance of its involvement in the mechanism action of TCA is still a matter of debate. A number of studies using selective radioligands have examined the effects of chronic administration of TCA on the number of 5-HT transporters in projection areas of 5-HT neurons such as hippocampus, cortex and hypothalamus (Cheetham et al. 1993; Dean et al. 1997; Dewar et al. 1993; Kovachich et al. 1992; Nankai et al. 1991; Watanabe et al. 1993). However, their results are inconsistent. Following chronic administration of TCA such as imipramine or desipramine, it has been found either no change or a decrease in the number of 5-HT carriers. Otherwise, no studies using selective radioligands have examined whether a chronic treatment with TCA affects the number of midbrain 5-HT transporters. Only modification of the steady-state concentrations of the mRNA encoding for the 5-HT transporter has been studied in this brain area. Lesch et al. (1993) found a decrease in the transporter mRNA levels following long-term treatment with imipramine.

The effects of a chronic TCA administration on postsynaptic 5-HT receptors have been most studied with regard to hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors, because these receptors and their function are well characterized in these brain areas. In radioligand binding studies, prolonged administration of imipramine has always been found to increase hippocampal density of 5-HT_{1A} receptors in Wistar rats (Klimek et al. 1994; Maj et al. 1996; Papp et al. 1994). Furthermore, electrophysiological works have constantly found a sensitization of hippocampal 5-HT_{1A} receptors following chronic administration of different TCA (Chaput et al. 1991; de Montigny and Aghajanian 1978; Gallager and Bunney 1979; Gravel and de Montigny 1987). Otherwise, biochemical studies have shown that chronic administration of amitriptyline, imipramine, or desipramine leads to a decrease in the function of cortical 5-HT_{2A} receptors through a reduction in receptor density (Goodwin et al. 1984; Papp et al. 1994; Peroutka and Snyder 1980; Roth et al. 1998; Subhash and Jagadeesh 1997; Watanabe et al. 1993).

In the present article, we examined whether the addition of a low dose of T3 equivalent to its daily production rate can change the effects induced by a chronic administration of imipramine on 5-HT neurotransmission. The binding characteristics of midbrain and hypothalamic 5-HT transporters and of hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors were investigated in adult Wistar rats. Another aim of the study was to ascertain whether the hormonal association might accelerate the

onset of changes in density of 5-HT transporters and receptors induced by the TCA treatment. Therefore, we studied the influence of the different treatments after 7 or 21 days of their administration. In addition, the effects of a chronic administration of a physiological dose of T3 given alone were also compared at the same time points.

MATERIALS AND METHODS

Animals

Male Wistar rats (IFFA CREDO, L'Arbresle, France) weighing 180–200 g at the beginning of the study were used. The animals were housed 4 to 5 per cage and maintained under standard laboratory conditions (22 ± 1°C, 50–60% relative humidity, 12 hr light-dark cycle, light on at 7:00 A.M.). Rats had free access to food and drink. All the procedures were conducted in accordance with French and Economic European Community laws and policies for care and use of laboratory animals.

Treatments

Four groups of animals were treated for 7 or 21 days with imipramine (10 mg/kg/day) and T3 (4 μg/kg/day), alone or combined, or with vehicle (0.1 mg/ml bovine serum albumin), delivered in the drinking water. Fresh drinking water was provided daily around 6:00 p.m. The amount of drug delivered every day was calculated according to the mean body weight and the volume of solution drunk by each cage of treated animals. The body weight of each animal was recorded every 2 or 3 days. The volume drunk per cage was recorded every day. Animals were killed by decapitation between 10:00 A.M. and noon on day 8 or 22.

Thyroid Hormone and TCA Assays

Immediately after death, the trunk blood was collected in centrifuge tubes. After centrifugation (2200 *g*, 8 min, 4°C), serum samples were collected and stored at −70°C until assayed. Concentrations of free T3 (FT3) and T4 (FT4) were determined by radioimmunoassay and immunochemiluminiscent assay, respectively (Amerlex-MAB® FT3 and Amerlite-MAB® FT4, respectively, Johnson & Johnson Clinical Diagnostics, Les Ulis, France) and expressed in pM. Concentrations of TCA, which correspond to the concentration of imipramine plus its main metabolite desipramine, were determined by fluorescence polarization immunoassay (TDx/TDxFLx® Tricyclic Antidepressants assay, Abbott Laboratories, Chicago, USA) and expressed in ng/ml.

Preparation of Brain Homogenates

Immediately after the trunk blood collection, the brain was removed. Frontal cortex, hippocampus, hypothalamus, and midbrain were dissected out on ice. Cortex and hippocampus were homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.7, and centrifuged (20000 *g*, 10 min, 4°C). After centrifugation, the supernatant was discarded and the membrane pellet was resuspended and centrifuged as above. However, for the hippocampus homogenates, a 15 min incubation at 35°C was carried out in between the two centrifugations to remove endogenous 5-HT. Hypothalamus and midbrain homogenates were prepared as the cortical ones except that homogenization solution was buffered at pH 7.4. The final pellets were stored at −80°C until subsequent radioligand binding assays.

Radioligand Binding Assays

Radioligand binding experiments were performed as previously described (Kulikov et al. 1999).

[^{3}H]Citalopram Binding. Midbrain and hypothalamic membrane pellets were suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl and 5 mM KCl at a protein concentration of approximately 0.3 and 0.15 mg/ml, respectively. The reaction was carried out for 60 min at 25°C in the presence of 8 concentrations of [^{3}H]citalopram (0.12–16 nM).

[^{3}H]8-OH-DPAT Binding. Hippocampal membrane pellets were suspended in 50 mM Tris-HCl buffer, pH 7.7, containing 5 mM $CaCl_2$, 0.1% ascorbic acid, and 1 μM pargyline at a protein concentration of approximately 0.25 mg/ml. The reaction was carried out for 15 min at 35°C in the presence of 8 concentrations of [^{3}H]8-OH-DPAT (0.06–8 nM).

[^{3}H]ketanserin Binding. Cortical membrane pellets were suspended in 50 mM Tris-HCl buffer, pH 7.7, at a protein concentration of approximately 0.14 mg/ml. The reaction was carried out for 15 min at 35°C in the presence of 8 concentrations of [^{3}H]ketanserin hydrochloride (0.06–8 nM).

Incubations were stopped by adding 4 ml of the respective cold Tris-HCl buffer and rapid vacuum filtration through Whatman GF/B glass fiber filters presoaked in an aqueous solution of polyethylenimine (0.3 % v/v). After two additional washes with 4 ml of Tris-HCl buffer, filters were dried for 1 hr at 70°C and immersed in 5 ml of Opti-Scint "High Safe" scintillation cocktail (LKB/Pharmacia, Les Ulis, France) for radioactivity counting. All determinations were done in duplicate.

Protein concentrations were determined according

to the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

The values of the maximal specific binding (Bmax, expressed in fmol/mg protein), non-specific binding and of the dissociation constant (Kd, expressed in nM) were calculated by non linear iterative least squared regression, using the EBDA-LIGAND computerized program (Munson and Rodbard 1980). We verified that the experimental non-specific bindings defined in the presence of 1 μM paroxetine for [^{3}H]citalopram, 10 μM 5-HT for [^{3}H]8-OH-DPAT, 10 μM methysergide for [^{3}H]ketanserin, were not significantly different from those calculated by the EBDA-LIGAND program. The non-specific bindings represented about 5%, 15%, and 8% of total [^{3}H]citalopram, [^{3}H]8-OH-DPAT, and [^{3}H]ketanserin bindings, respectively.

Chemicals

[^{3}H]citalopram (85 Ci/mmol), [^{3}H]8-OH-DPAT (127 Ci/mmol), and [^{3}H]ketanserin hydrochloride (66.4 Ci/mmol) were purchased from Dupont/NEN (Les Ulis, France). 3,3prime,5-tri-iodo-L-thyronine sodium salt, 5-HT creatinine sulfate, BSA, polyethylenimine, imipramine hydrochloride, and pargyline hydrochloride were purchased from SIGMA (St. Quentin-Fallavier, France). Methysergide maleate was purchased from Research Biochem (Natick, USA). Paroxetine was a gift from SmithKline & Beecham (Harlow, UK).

Data Analysis

All data are presented as means ± SEM. For the body weight data, a repeated measures design analysis of variance was used, with different treatments as the between factor and time as the within factor. Significant treatments by time interactions were further explored using one-way analysis of variance (ANOVA) at each time point accompanied by Bonferroni-Dunn test where each treatment group was compared to the control group. TCA data in animals treated with imipramine alone or in combination with T3 were analyzed using a two-tail Student *t*-test. Hormonal and radioligand binding data were analyzed using one-way ANOVA followed by Bonferroni-Dunn test where each treatment group was compared to the control group unless mentioned.

RESULTS

Body Weight Changes

Table 1 shows the effects of a 21-day treatment with imipramine and T3, alone or in combination, on body weight gain. An overall ANOVA showed a significant treatment effect ($F_{3,41} = 2.99$, $p < .05$), a significant time effect ($F_{3,123} = 664$, $p < .0001$), and a significant interaction between time and treatment ($F_{9,123} = 5.2$, $p < .001$). Further analysis revealed that only the combination imipramine-T3 induced a significant reduction in body weight gain on day 21.

TCA Concentrations

For 7- and 21-day treatment, there were no differences in the serum concentrations of TCA between animals treated with imipramine alone and those treated with the combination imipramine-T3 ($F_{1,14} = 0.76$, NS; $F_{1,14} = 0.26$, NS, respectively) (Table 2).

Thyroid Hormone Concentrations

For 7- and 21-day treatment, one-way ANOVA gave an overall significant treatment effect on FT4 serum concentrations ($F_{3,25} = 58.5$, $p < .0001$; $F_{3,36} = 79.7$, $p = .0001$, respectively). Further analysis revealed that treatment with T3 alone and with the combination imipramine-T3 induced a significant decrease in FT4 concentrations on both days 7 and 21 (Figure 1).

For 7- and 21-day treatment, there were no differences in FT3 serum concentrations between the different groups ($F_{3,25} = 2.22$, NS; $F_{3,36} = 0.75$, NS, respectively) (Figure 1).

[^{3}H]Citalopram Binding

For 7-day treatment, there was no overall difference in Bmax of [^{3}H]citalopram binding in midbrain between the different groups ($F_{3,28} = 1.25$, NS) (Figure 2A). By contrast, one-way ANOVA on day 21 gave an overall significant effect of treatment on Bmax ($F_{3,41} = 9.83$, $p < .0001$) (Figure 2B). Further analysis revealed that imipramine and T3 treatment, alone or combined, significantly decreased Bmax. However, the decrease in Bmax induced by imipramine or T3 alone did not differ significantly from that induced by the combination of these treatments. For 7- and 21-day treatment, there were no overall differences in Kd of [^{3}H]citalopram binding in midbrain between the different groups ($F_{3,28} = 0.53$, NS; $F_{3,41} = 1.53$, NS, respectively) (Table 3).

For 7- and 21-day treatment, there were no overall differences in either Bmax ($F_{3,31} = 0.81$, NS; $F_{3,36} = 0.45$, NS) (Figure 2) or Kd ($F_{3,31} = 0.13$, NS; $F_{3,36} = 0.34$, NS) (Table 3) of [^{3}H]citalopram binding in hypothalamus between the different groups.

[^{3}H]8-OH-DPAT Binding

For 7-day treatment, one-way ANOVA did not yield to an overall significant treatment effect on Bmax of [^{3}H]8-OH-DPAT binding in hippocampus ($F_{3,33} = 0.92$, NS)

Table 1. Effects of a 21-day Treatment with Imipramine and T3, Alone or Combined, on Body Weight Gain in Adult Rats

	Body Weight (g)			
Treatment days	**Control**	**Imipramine**	**Imipramine + T3**	**T3**
0	207.7 ± 3.1	208.9 ± 2.5	208.7 ± 2.2	200.4 ± 1.8
7	254.5 ± 3.5	249.9 ± 2.2	247.9 ± 2.5	247.1 ± 2.2
14	286.5 ± 5.3	271.6 ± 6.8	269.5 ± 5.2	280.1 ± 4.0
21	324.2 ± 4.8	302.8 ± 8.9	293.8 ± 6.5*	316.6 ± 4.0

Values represent the means body weight ± SEM from 10 animals per treatment group.
*$p < .05$, significantly different from control animals, Bonferroni-Dunn test.

(Figure 3A). By contrast, one-way ANOVA on day 21 gave an overall significant treatment effect on Bmax ($F_{3,42} = 4.82$; $p < .01$) (Figure 3B). Further analysis revealed that treatment with the combination imipramine-T3 or with T3 alone had no effects on Bmax whereas treatment with imipramine alone induced a significant increase in Bmax. Furthermore, Bmax was also significantly higher in imipramine-treated animals than in those receiving the imipramine-T3-combination. For 7-day, but not 21-day treatment, one-way ANOVA showed an overall significant treatment effect on Kd of [^{3}H]8-OH-DPAT binding ($F_{3,33} = 9.53$, $p < .0001$; $F_{3,42} = 0.39$, NS, respectively) (Table 3). Further analysis revealed that on day 7, only T3 given alone produced a significant increase in Kd.

[^{3}H]ketanserin Binding

For 7- and 21-day treatment, one-way ANOVA gave an overall significant treatment on Bmax ($F_{3,33} = 12.7$, $p < 0.0001$; $F_{3,38} = 32.2$, $p < .0001$, respectively) (Figure 4) and Kd ($F_{3,33} = 6.3$, $p < .05$; $F_{3,38} = 6.90$, $p < .001$, respectively) (Table 3) of [^{3}H]ketanserin binding in frontal cortex. Further analysis revealed that Bmax of animals treated with imipramine alone or with the combination of imipramine and T3 were significantly decreased on both days 7 and 21. Treatment with T3 alone had no effect on Bmax. Comparisons of the mean Bmax values of imipramine- and imipramine-T3–treated animals yield to significant differences both on days 7 and 21. The imipramine-T3 combination induced decreases in Bmax that were of a greater extent than those produced by imipramine alone. For 7-day treatment, Kd of [^{3}H]ketanserin was decreased only in the group of animals receiving the combined treatment. However, for 21-day treatment, all the three groups of treated animals had a decrease in Kd.

Table 2. Effects of a 7- or 21-day Treatment with Imipramine Given Alone or in Combination with T3 on Serum Concentrations of TCA

	Serum concentrations of TCA (ng/ml)	
Treatment days	**Imipramine**	**Imipramine + T3**
7	68.2 ± 7.8	72.5 ± 11.3
21	88.2 ± 7.3	80.1 ± 13.9

Serum concentrations of TCA represent the sum of the concentrations of imipramine and of its metabolite, desipramine. Values represent the mean ± SEM from 8 animals per treatment group.

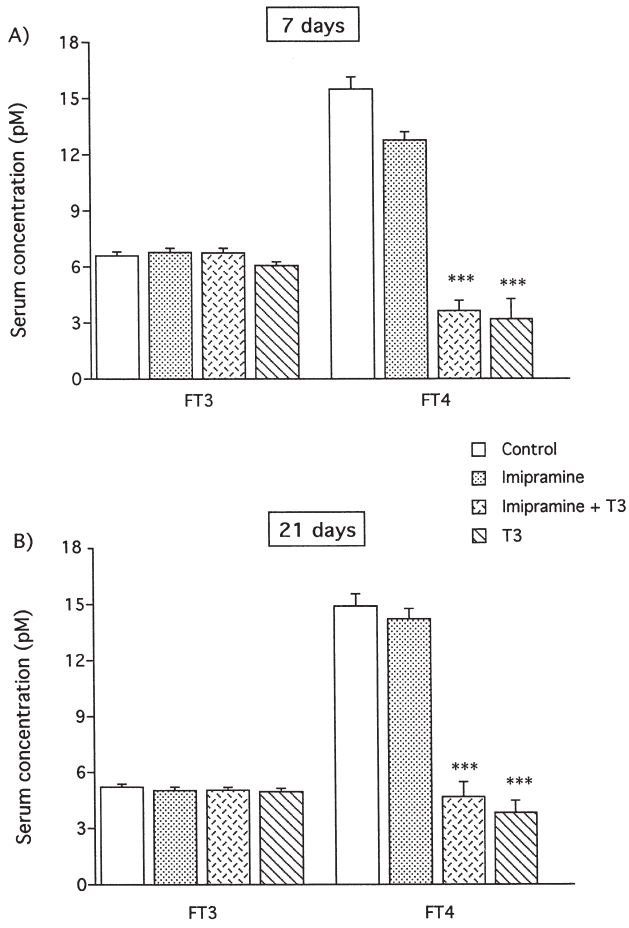

Figure 1. Effects of imipramine and T3 treatment alone or combined, for 7 **(A)** or 21 **(B)** days on serum FT4 and FT3 concentrations. Each bar represents the mean ± SEM from at least 8 independent determinations per treatment group. ***$p < .001$ when compared to control animals.

DISCUSSION

The present study was designed to evaluate whether the association of a physiological dose of T3 to imipramine treatment could potentiate and accelerate adaptive changes produced by the TCA administration on the density of midbrain and hypothalamic 5-HT transporters and of hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors in adult Wistar rats. So, the effects of imipramine and T3 given alone were compared to those induced by the imipramine-T3 combination after 1- and 3-week treatments. Three-week treatment was chosen as a conservative equivalent of the typical 3-week period required for the development of a quantitative antidepressant response in depressed patients. One-week treatment was chosen because in some clinical trials on hormonal augmentation strategy of antidepressant treatment, T3 was reported to lead to a therapeutic response as soon as the first week of initiation of TCA treatment (Joffe et al. 1995).

This is the first study to demonstrate that both chronic administrations of imipramine and T3 trigger a time-regulatory effect on the density of midbrain 5-HT transporters. Only a 3-week treatment with imipramine and T3 given alone produced a significant decrease in the binding of [^{3}H]citalopram. In addition, we found that T3 and imipramine did not have additive effects since the combination of these treatments did not enhance the downregulation of 5-HT transporters induced by T3 and imipramine given alone. These results suggest that T3 and imipramine decrease the midbrain 5-HT transporter density through different mechanisms. Further investigations are needed to identify the mechanism that underlies the effect of T3. It is possible that the decrease in the number of transporters induced by imipramine reflects a downregulation process, which is secondary to a reduction in the steady-state concentrations of their mRNA. Supporting evidence for this come from the findings of Lesch et al. (1993) who observed that 3 weeks administration of imipramine induced a large decrease in the mRNA levels of 5-HT transporters in midbrain. On the other hand, the downregulation of 5-HT transporters could have a functional impact and led to a reduction of 5-HT uptake rate. In

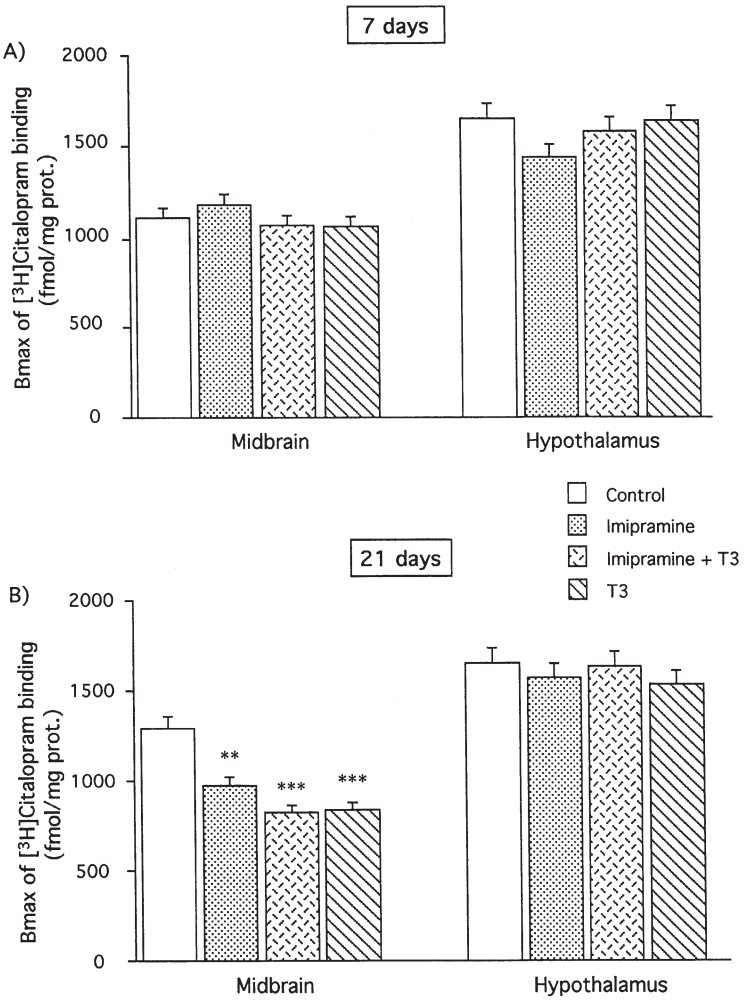

Figure 2. Effects of imipramine and T3 treatment alone or combined, for 7 **(A)** or 21 **(B)** days on Bmax of [^{3}H]-citalopram binding to 5-HT uptake sites in midbrain and hypothalamus. Each bar represents the mean ± SEM from at least 8 independent determinations per treatment group. $^{**}p < .01$, $^{***}p < .001$ when compared to control animals.

Table 3. Effects of a 7- or 21-day Treatment with Imipramine and T3, Alone or Combined, on the Kd Values (nM) for [^{3}H]citalopram Binding to 5-HT Uptake Sites, [^{3}H]8-OH-DPAT Binding to 5-HT$_{1A}$ Receptors, and [^{3}H]ketanserin Binding to 5-HT$_{2A}$ Receptors in Various Regions of Rat Brains

		Treatment			
	Days	Control	Imipramine	Imipramine + T3	T3
[^{3}H]citalopram					
Midbrain	7	1.43 ± 0.09	1.58 ± 0.20	1.48 ± 0.14	1.35 ± 0.07
	21	1.48 ± 0.08	1.42 ± 0.08	1.70 ± 0.15	1.42 ± 0.11
Hypothalamus	7	1.66 ± 0.06	1.72 ± 0.11	1.71 ± 0.10	1.74 ± 0.14
	21	1.65 ± 0.06	1.60 ± 0.07	1.67 ± 0.10	1.57 ± 0.07
[^{3}H]8-OH-DPAT					
Hippocampus	7	0.88 ± 0.05	0.87 ± 0.03	0.92 ± 0.10	1.21 ± 0.05*
	21	0.82 ± 0.08	0.88 ± 0.05	0.90 ± 0.08	0.87 ± 0.08
[^{3}H]ketanserin					
Frontal cortex	7	0.51 ± 0.06	0.43 ± 0.07	0.25 ± 0.05*	0.65 ± 0.07
	21	0.52 ± 0.05	0.33 ± 0.03**	0.28 ± 0.04**	0.30 ± 0.04**

Values represent the means ± SEM from at least 8 independent determinations.
*$p < .05$; **$p < .001$: significantly different from control animals, Bonferroni-Dunn test.

agreement with this view, Piñeyro et al. (1994) reported that chronic administration of a selective inhibitor of 5-HT uptake reduced the effectiveness of 5-HT transport by decreasing the number of transporter sites in rat hippocampus.

By contrast to their effects on the density of midbrain 5-HT transporters, neither imipramine and T3 alone nor the combination of these treatments modified the number of 5-HT transporters in hypothalamus. It is of note that no earlier works have examined the influence of T3 and of the TCA-T3 combination on the number of 5-HT transporters in this brain area. Otherwise, the present data are in line with those of Nankai et al. (1991) who observed that a 10-day treatment with desipramine (10 mg/kg/day) did not alter the number of 5-HT carriers in rat hypothalamus. A possible explanation for the lack of effect of imipramine treatment on the density of hypothalamic 5-HT transporters may be related to the fact that we used a dose of imipramine, which was not sufficient to block 5-HT transport. Supporting evidence for this come from the findings of Thomas et al. (1987) who reported that only chronic treatments with doses of imipramine greater than 20 mg/kg/day were able to inhibit 5-HT uptake rate into hypothalamic synaptosomes.

In the present study, neither 1-week administration of imipramine and T3 alone nor 1-week administration of the combination imipramine-T3 modified the binding of [^{3}H]8-OH-DPAT to hippocampal 5-HT$_{1A}$ receptors. No previous studies have examined the effect of 1-week administration of TCA given alone or in combination with a low dose of T3 on the density of hippocampal 5-HT$_{1A}$ receptors. Our results on the lack of effect of a low dose of T3 are in line with those of Sandrini et al. (1996) who reported that even a high dose of T3 administered for 1 week did not induce any change in the number of these receptors.

Our data on the effect of a 3-week administration of imipramine on the binding of [^{3}H]8-OH-DPAT to hippocampal 5-HT$_{1A}$ receptors in Wistar rats confirm the findings of previous studies. Two- to four-week treatments with different TCA have always been found to increase the density of hippocampal 5-HT$_{1A}$ receptors in Wistar rats (Klimek et al. 1994; Maj et al. 1996; Papp et al. 1994). The mechanism by which imipramine modulates the number of 5-HT$_{1A}$ receptors is poorly understood but does not seem to involve transcriptional regulation. Burnet et al. (1994) reported that a chronic treatment with imipramine increased the density of hippocampal 5-HT$_{1A}$ receptors without altering the mRNA levels of these receptors.

By contrast to the effect of 3-week administration of imipramine, 3-week administration of the combination imipramine-T3 failed to increase the density of 5-HT$_{1A}$ receptors in Wistar rats. Imipramine-T3–treated animals had a number of 5-HT$_{1A}$ receptors that was significantly less than imipramine-treated animals but that did not differ from controls. One possible explanation for the lack of effect of the imipramine-T3 combination on 5-HT$_{1A}$ receptors may be that T3 and imipramine have an opposite effect on the density of these receptors. However, this possibility seems unlikely because a 3-week administration of T3 alone did not affect the density of 5-HT$_{1A}$ receptors whereas that of imipramine increased it. Another possible explanation for the lack of effect of the imipramine-T3 combination may be that the addition of T3 alters the pharmacodynamic of imipramine. However, this possibility seems also unlikely because we found no difference in the serum concentrations of TCA between animals that received imipramine alone and those that received the combination imipramine-T3. Moreover, previous studies have shown that chronic addition of T3 to imipramine treat-

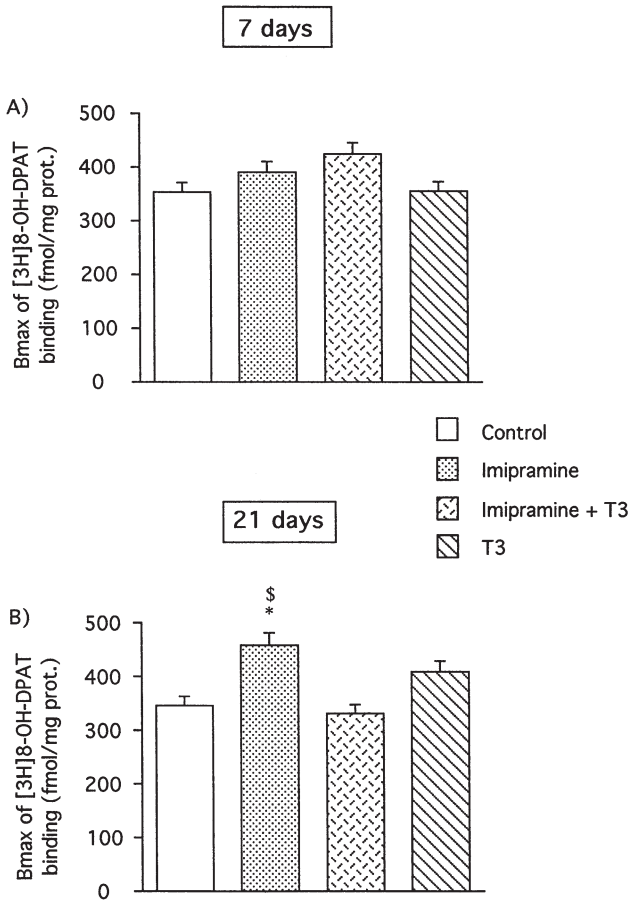

Figure 3. Effects of imipramine and T3 treatment alone or combined, for 7 **(A)** or 21 **(B)** days on Bmax of [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in hippocampus. Each bar represents the mean ± SEM from at least 8 independent determinations per treatment group. $^*p < .05$ when compared to control animals; significantly different from imipramine-T3-treated animals: $^\$p < .05$.

ment failed to alter the disposition and the metabolism of imipramine in periphery as well as in brain (Breese et al. 1972; Garbutt et al. 1979). Taken together, these data indicate that the chronic addition of a physiological dose of T3 has prevented the effect of the antidepressant on the density of 5-HT$_{1A}$ receptors. As it has been shown that an increase in the hippocampal number of these receptors could be associated with an increase in their responsiveness (Welner et al. 1989), it will be interesting to determine whether the combination imipramine-T3 has also a functional impact on 5-HT$_{1A}$ receptors by preventing the appearance of a state of supersensitivity.

In the present study, 1- and 3-week administration of imipramine reduced the binding of [^{3}H]ketanserin to cortical 5-HT$_{2A}$ receptors. These data confirm and extend the findings of previous studies. The downregulation of cortical 5-HT$_{2A}$ receptor binding after 2- to 4-week treatment with different TCA drugs is a well-established observation. However, this is the first investigation to demonstrate that 1-week treatment with imipramine is able to reduce the number of cortical 5-HT$_{2A}$ receptors. Several studies have shown that repeated administration of drugs with 5-HT$_{2A}$ antagonist properties induced a rapid downregulation of 5-HT$_{2A}$ receptors (Eison and Mullins 1996; Roth et al. 1998). Because imipramine has a relatively high affinity for 5-HT$_{2A}$ receptors and antagonist activity at these receptors, it is not surprising that its administration for 1 week decreased the number of cortical 5-HT$_{2A}$ receptors. The mechanism responsible for the paradoxical downregulation of cortical 5-HT$_{2A}$ receptors by TCA or by 5-HT$_{2A}$ antagonists has been studied incompletely (Roth et al. 1998). However, it is unlikely that transcriptional regulation of 5-HT$_{2A}$ receptors is involved. Chronic administration of imipramine and of atypical antidepressant with 5-HT$_{2A}$ receptor antagonist property such as mianserin did not alter 5-HT$_{2A}$ receptor mRNA concentrations in rat cortex (Burnet et al. 1994; Roth and Ciaranello 1991). Furthermore, it has been found that mianserin decreased the density of 5-HT$_{2A}$ receptors in stably transfected cells (Newton and Elliot 1997).

Unlike imipramine, T3 given for 1 and 3 weeks did not affect the density of cortical 5-HT$_{2A}$ receptors. Our findings are apparently discordant with those of Sandrini et al. (1996) who found a reduction in the cortical density of 5-HT$_{2A}$ receptors after 1 week of T3 administration. However, it is of note that these authors gave a supraphysiological dose of T3 whereas we used a physiological one.

The main finding of the present study is that the addition of T3 to imipramine amplified the downregulation of cortical 5-HT$_{2A}$ receptors caused by the antidepressant. Both 1- and 3-week treatment with imipramine-T3 combination enhanced the downregulation rate of 5-HT$_{2A}$ receptors (+47% and +38%, respectively). Furthermore, the extent of the downregulation induced by a 1-week administration of the combination was greater than that induced by a 3-week administration of imipramine.

All the TCA drugs downregulate 5-HT$_{2A}$ receptors with a time frame that is correlated to their clinical efficacy (Lafaille et al. 1991). In addition, it has been recently demonstrated that the sole downregulation of 5-HT$_{2A}$ receptors, when induced by an intracerebroventricular administration of a receptor-specific antisense oligonucleotide, was sufficient to achieve an antidepressant-like effect in mice (Sibille et al. 1997). These findings, together with our data, suggest that the ability of T3 to shorten the delay for the clinical efficacy of TCA drugs may lie, at least in part, in its capacity to enhance the downregulation rate of 5-HT$_{2A}$ receptors caused by the chronic administration of TCA. The potentiating effect of T3 on imipramine-induced downregulation of 5-HT$_{2A}$ receptors seems to be the conse-

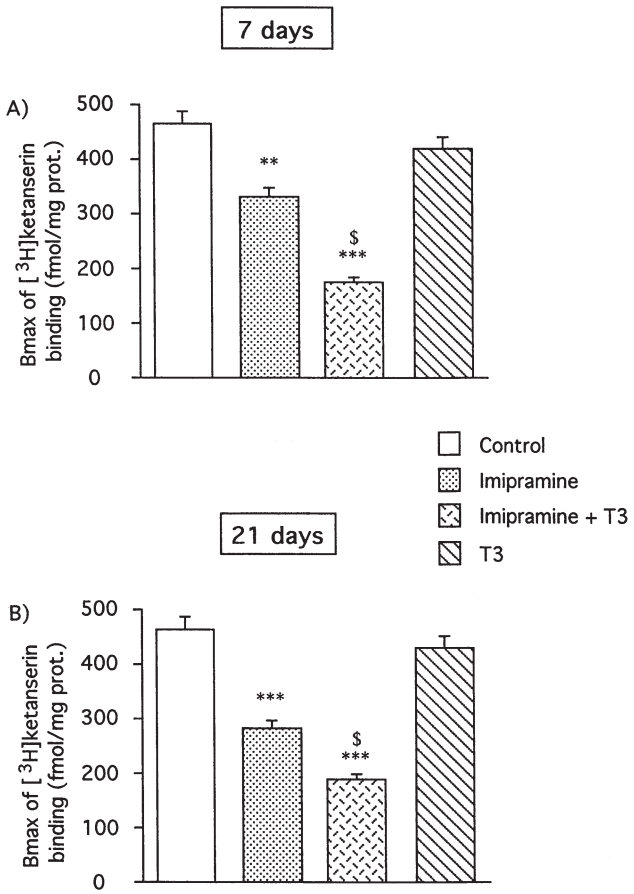

Figure 4. Effects of 7- or 21-day administration of imipramine, T3 and of their combination on Bmax of [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors in frontal cortex. Each bar represents the mean ± SEM from at least 8 independent determinations per treatment group. Significantly different from control animals: $^{**}p < .01$, $^{***}p < .001$; significantly different from imipramine-treated animals: $^{\$}p < .05$.

quence of an enhancing action of T3 on imipramine-induced increase in the extracellular 5-HT levels in cortex. Recent microdialysis studies have shown that chronic administration of imipramine and clomipramine increased the extracellular 5-HT levels in rat cortex (Bel and Artigas 1996; Gur et al. 1999). Furthermore, Gur et al. (1999) observed that in frontal cortex chronic treatment with the combination clomipramine-T3 potentiated the increase in the extracellular 5-HT levels induced by T3 or clomipramine alone.

Finally, our data on the chronic effects of single and combined imipramine and T3 administrations on the serum concentrations of thyroid hormones confirm and extend those of previous studies. As Kennedy et al. (1997), we observed that a chronic administration of imipramine did not alter the serum concentrations of FT3 and FT4. In the present study, we also reported that FT4, but not FT3, concentrations were decreased after chronic administration of the combination imipramine-T3. It is of note that no earlier animal studies have examined the effects of TCA-T3 combination of the serum availability of thyroid hormones. It is likely that the effect of the combination on these hormones results from the sole action of T3. We found that, like the administration of the imipramine-T3 combination, T3 administration decreases FT4 levels without altering those of FT3. Our data with T3 alone confirm those of Barsano et al. (1994) who observed that, in rats, the administration of T3 at a dose equivalent to its daily production rate decreased FT4 concentrations without affecting those of FT3. It is likely that the administration of a low dose of T3 has activated the mechanisms of negative feedback of the thyroid axis, which led to a decrease in the endogenous production of T4 and T3 (Barsano et al. 1994). However, no changes in the serum concentrations of T3 were observed because the reduction in the endogenous production of T3 was compensated by the administration of a low dose of T3. These findings and our data suggest that neither imipramine and T3 given alone nor the combination of these treatments have provided their effects on the central 5-HT entities by overcoming the capacity of the thyroid axis to regulate the hormone availability.

In conclusion, the present results show that T3 can modulate the effects of TCA on their serotonergic targets. Of particular interest, the action of T3 in potentiating the downregulation of cortical 5-HT$_{2A}$ receptors induced by a chronic administration of imipramine might explain, at least in part, its antidepressant properties.

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