*European Journal of Pharmacology*, 152 (1988) 347–351
Elsevier



EJP 50407

# Triiodothyroacetic acid (TRIAC) potentiation of antidepressant-induced reversal of learned helplessness in rats

Jacques Massol [1], Patrick Martin *, Philippe Soubrié and Alain J. Puech

*Département de Pharmacologie, Faculté de Médecine Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, 75634 Paris Cedex 13, and [1] Service d'Endocrinologie, CHU Minjoz, 25030 Besançon Cedex, France*

Received 22 April 1988, accepted 31 May 1988

The results of several clinical investigations have suggested that a special relationship exists between thyroid function and affective disorders and/or the therapeutic response to antidepressants. Animal studies have shown the possible β-adrenergically mediated antidepressant-like properties of triiodothyroacetic acid (TRIAC) in rodents. The present experiment showed (1) that the reversal by antidepressants (clomipramine or imipramine) of escape deficits produced by previous exposure to uncontrollable shock was significantly hastened in animals given TRIAC and (2) that L-penbutolol treatment prevented the elimination of helpless behavior induced by TRIAC, suggesting a β-adrenergic mediation of the antidepressant activity of thyroid compounds. The study confirmed that learned helplessness might be a useful model for studying in animals the neurohormonal correlates of affective disorders and the neurobiochemical basis of the enhancement of the antidepressant action produced by thyroid compounds.

Triidothyroacetic acid (TRIAC); Antidepressants; Learned helplessness; (Rat)

## 1. Introduction

Triiodothyronine (T3) has been successfully though not extensively prescribed for depressive states (see Prange, 1985 for a review). T3 may accelerate or potentiate the effects of a variety of antidepressants (Goodwin et al., 1982; Schwarcz et al., 1984). Animal studies showed the partial profile of an antidepressant for this thyroid hormone, an action presumably mediated through a β-adrenergic mechanism (Brochet et al., 1982) T3 was also shown to potentiate the behavioural response to various antidepressants. Triiodothyroacetic acid (TRIAC) is a natural metabolite of T3 that is quite devoid of the peripheral hormonal properties of the hormone. However, TRIAC was found to be as effective as T3 (Martin et al., 1985) for exerting antidepressant-like effects in rodents (Massol et al., 1987; 1988) and in particular to reverse learned helplessness in rats. Thus, it was decided to investigate whether subeffective doses of TRIAC were also able to potentiate the effects of tricyclics on learned helplessness.

## 2. Materials and methods

The experiments were carried out on male Wistar A.F. rats (Centre d'élevage R. Janvier, France) weighing 180 ± 10 g at the beginning of the experiments. The animals were housed in groups of 10/cage under standard conditions: room temperature (21 ± 1°C); light/dark cycle (12/12 h); water and food ad libitum.

* To whom all correspondence should be addressed: Département de Pharmacologie, Faculté de Médecine Pitié-Salpêtrière, 91 Bd. de l'Hôpital, 75634 Paris Cedex 13, France.

*2.1. Learned helplessness procedure*

*2.1.1. Inescapable shock preconditioning*

Electric footshocks were delivered to each rat in a plexiglass chamber (20 × 10 × 10 cm) covering a stainless -steel (1.5 cm spaced) grid. A constant-current shocker was used to deliver 60 scrambled, randomized and inescapable shocks of 15 s duration every min ± 15 s to the grid floor. Control rats were placed in identical chambers for 1 h but shock was not administered. All preconditioning trials were performed in the morning on day 1 (inescapable footshock stress is not forbidden under French law).

*2.1.2. Conditioned avoidance training*

To evaluate escape deficits, avoidance training was initiated 48 h (day 3) after inescapable shock preconditioning in automated two-way shuttle-boxes (60 × 21 × 30 cm) with a floor consisting of stainless-steel rods spaced 1.0 cm apart. Each shuttle-box was divided into two chambers of equal size by a stainless-steel partition with a gate giving access to the adjacent compartment through a 7 × 7 cm space. The animals were placed singly in the shuttle-box, allowed to habituate to the environment for 5 min (for the first session only) and were then subjected to 30 avoidance trials (intertrial intervals being 30 s). A light signal (used as a CS) was presented during the first 3 s of each trial. Crossing the gate into the other compartment of the box during this 'CS only' period (referred to as avoidance response) allowed the rats to avoid shocks. If an avoidance response did not occur within this period, a 0.8 mA shock (3 s duration) was applied via the grid floor. Crossing of the gate into the other compartment during this CS + shock period was termed an escape response. Absence of escape response during the 3 s duration CS + shock was considered to be an escape failure. Escape failure is usually defined as failure to escape within a 30-60 s period in procedures designed to assess learned helplessness. However, we took this deficit to be present in an animal did not escape within 3 s. This procedural modification was aimed at avoiding prolonged noxious stimulation and was justified on account of previous studies indicating that the very first seconds following the start of the shock seem to be critical for detecting escape deficits in animals pre-exposed to inescapable shocks (Martin et al., 1986a; Telner and Singhal, 1981). Avoidance sessions were held for 3 consecutive days (days 3, 4 and 5) in the morning, and the number of escape failures, referred to as 'no crossing' response during shock delivery, was recorded.

*2.2. Drug administration*

The rats were treated randomly according to one of the following protocols (10 rats per group): controls with no shock, given vehicle; experimental animals with inescapable shocks, injected once a day with TRIAC at the subeffective dose of 0.25 and the effective dose of 0.50 mg/kg i.p. (according to Massol et al., 1987b) for 4 consecutive days (6 h after shock pretreatment or shuttle box session). Controls with no shock, given saline; experimental animals with inescapable shocks, injected with saline, clomipramine (16 mg/kg per day) or imipramine (16 mg/kg per day) (these dose regimens being selected on the basis of their reported partial and progressive reversal effect of escape failures) or imipramine (32 mg/kg per day) (at the dose which has been shown to produce a marked significant reversal of escape failures).

The doses were selected on the basis of previous study (Martin et al., 1986a). TRIAC was given once a day either at the subeffective dose of 0.25 mg/kg per day or at the effective dose of 0.50 mg/kg per day i.p. for 4 consecutive days (6 h after shock pretreatment of shuttle-box session), either to saline-treated rats or to rats receiving antidepressants (according to Massol et al., 1987).

A $\beta$-antagonist (L-penbutolol, 0.5 mg/kg per day) was given once a day, either to the tricyclic antidepressant-treated group or to the TRIAC-treated group. The $\beta$-antagonist was administered only once, 45 min before the shuttle-box session. The dose of L-penbutolol was as described by Martin et al. (1986b).

TRIAC (Ana, France) and L-penbutolol (Hoechst) were suspended in gum acacia (5%) and imipramine or clomipramine (Ciba Geigy) as solution were injected in a volume of 0.5 ml/100 g body weight. Between group comparisons were

made with the two-way analysis of variance (group and session) and Dunnett's one-tailed t-test or Dunn's t-test.

## 3. Results

Analysis of variance revealed that daily imipramine (2 × 16 mg) or TRIAC (0.5 mg/kg per day) abolished the escape deficits, since the performance of the treated rats was not statistically different from that of unshocked controls ($t = 1.03$ for imipramine; and $t = 1.14$ for TRIAC, in the experiment with L-penbutolol). L-Penbutolol given once a day before each shuttle-box session significantly reduced ($P < 0.001$, $F(1,25) = 19.87$) the ability of daily imipramine (2 × 16 mg/kg per day) or TRIAC (0.5 mg/kg per day) to reverse the escape deficits seen during the three shuttle-box sessions with rats subjected to inescapable shock pretreatment (fig. 1).

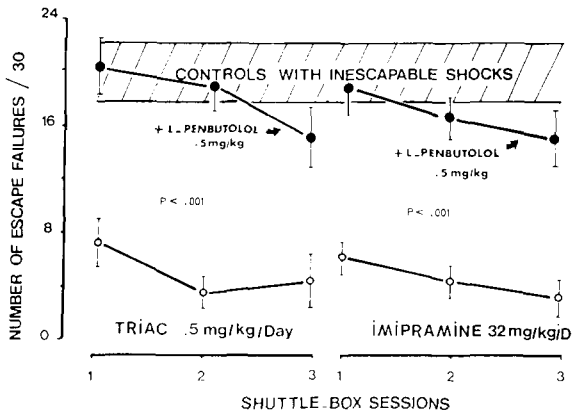


Fig. 1. Effects of daily L-penbutolol on antidepressant- and triiodothyroacetic acid (TRIAC)-induced reversal of escape deficits produced by inescapable shock pretreatment. The data shown are the mean number of escape failures (± S.E.M.) out of 30 two-way avoidance trials during the three shuttle-box sessions. Escape failure refers to failure of the rat to change compartments during the electric footshock. Circles refer to rats exposed to inescapable shock pretreatment at day 1 then treated daily with TRIAC (0.5 mg/kg per day) or imipramine alone (2×16 mg/kg) and in combination with L-penbutolol. L-Penbutolol was given once daily 45 min before testing. $P < 0.001$ indicates that rats given L-penbutolol in combination with TRIAC or imipramine differed from rats given TRIAC or antidepressants alone.

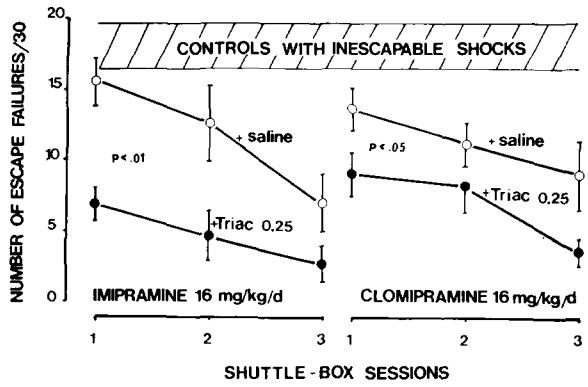


Fig. 2. Triiodothyroacetic acid (TRIAC) potentiation of imipramine- and clomipramine-induced reversal of escape failures as a function of the number of exposures to a daily shuttle-box session. The dose indicated refers to the total dose injected from shock pretreatment onwards. Rats given imipramine or clomipramine in combination with TRIAC (0.25 mg/kg per day) differed from rats given either antidepressant alone.

Analysis of variance of the data presented in fig. 2 indicated that, overall, the magnitude of the effect of each antidepressant tested (imipramine or clomipramine) was significantly enhanced in TRIAC-pretreated rats (0.25 mg/kg per day) as compared with saline-pretreated animals: clomipramine ($F(1,58) = 4.84$, $P < 0.05$) and imipramine ($F(1,58) = 8.87$, $P < 0.01$). Further analysis (Dunn's t-test) revealed that one of the most salient differences for saline vs. TRIAC-pretreated rats was that each drug produced a significant reduction ($P < 0.001$) in escape failures after only four injections in the latter group. There was no such rapid start of the effect was in rats given either antidepressant alone.

## 4. Discussion

Animal studies have demonstrated the efficacy of either T3 or TRIAC, its natural metabolite, to exert antidepressant-like properties in rodents (Brochet et al., 1982; Martin et al., 1985; Massol et al., 1987; 1988). A previous study with learned helplessness in rats showed that T3 potentiated the effects of various antidepressants by eliminating escape deficits in the shuttle-box. The present study showed that a subeffective dose of TRIAC

(0.25 mg/kg per day) was also able to enhance the same antidepressant effects under the same experimental conditions. Indeed, whereas six or eight injections of twice daily clomipramine (16 mg/kg per day) or imipramine (16 mg/kg per day) were required to produce a significant though partial reversal of escape failures, four injections of either compound, at the same doses, were sufficient to almost completely eliminate the helpless behavior in rats receiving TRIAC. These findings are in complete agreement with clinical studies indicating that thyroid hormones can speed or improve the response to tricyclic antidepressants (Goodwin et al., 1982; Prange et al., 1984).

It is unlikely that the enhancement of the effects of antidepressants by TRIAC involved the elevation of the brain levels of antidepressant drugs since rats treated with T3, which exerts a major influence on liver metabolism, showed no statistically significant differences in antidepressant brain levels compared to saline-treated rats (Martin et al., 1987). Moreover, it has been shown that, in depressive states, the ability of T3 to moderate the actions of antidepressants was not due to a change in the blood levels of tricyclic antidepressants (Garbutt et al., 1986).

It has been suggested previously that thyroid hormones may act at central synaptic sites to facilitate the action of antidepressants, perhaps by altering the density of certain brain receptors or their responsiveness (Whybrow and Prange, 1981). The possibility of crucial T3 interactions with antidepressants in the learned helplessness model is strengthened by the following facts: the expression of helpless behavior has been found to correlate with various indices suggesting a deficient noradrenergic transmission (Anisman et al., 1980; 1981; Johnson et al., 1982). Direct $\beta$-adrenoceptor stimulants such as clenbuterol or salbutamol reportedly eliminated escape deficits (Martin et al., 1986a) whereas blockade of $\alpha$- or $\beta$-receptors prevented the antidepressants from reversing escape failures (Martin et al., 1986b). Moreover, thyroid hormones have been shown to facilitate central noradrenergic transmission by inducing an increase of central $\beta$-binding sites (Fox et al., 1985). Similarly, a central $\beta$-adrenergic mechanism of action of TRIAC can be suggested by the fact that penbutolol prevented the reversal of learned helplessness at the same dose that was required to prevent the effect of imipramine. This result confirms the $\beta$-adrenergic mediation of TRIAC activity that was previously suggested for the periphery by the finding that myocardial hypertrophy induced by TRIAC could be prevented by $\beta$-blockade (Olssen et al., 1980). Whatever the mechanisms involved, our animal data suggest that noradrenergic processes could be the main substrate of the interactions of TRIAC and T3 with antidepressants that we now observed.

Although further experiments will be required to examine the possible role of serotonergic systems, the noradrenergic hypothesis is supported indirectly by the observation that depressed patients treated with L-tryptophan showed no beneficial effect of T3 (Coppen et al., 1972) and that T3 could not directly enhance serotonin-dependent behavior in experimental animals (Brochet et al., 1985).

Since both T3 (Brochet et al., 1985) and TRIAC (unpublished results) failed to potentiate the effects of antidepressants in other behavioral testing procedures such as the forced swimming test in mice, the results confirm that learned helplessness can be a useful animal model for assessing the neurohormonal correlates of affective disorders and the neurobiochemical basis of the ability of thyroid compounds to enhance the action of antidepressants.

## Acknowledgements

The authors wish to thank Dr. Richard J. Beninger for his valuable comments concerning this manuscript.

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