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Triiodothyronine Potentiation of Antidepressant-Induced Reversal of Learned Helplessness in Rats

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Abstract. Several clinical investigations have suggested that a special relationship exists between thyroid function and affective disorders and/or therapeutic response to antidepressants. The present report describes that the reversal by antidepressants (imipramine, desipramine, and nomifensine) of depressive-like behavior in rats (escape deficits produced by previous exposure to uncontrollable stress) was significantly hastened in animals given daily triiodothyronine (T_3). The learned helplessness paradigm might be a useful model for approaching in animals the neurohormonal correlates of affective disorders and the neurobiochemical bases of the reported T_3 enhancement of antidepressants.

Key Words. Triiodothyronine, antidepressants, learned helplessness, escape deficits, rats.

Affective disorders are the most commonly reported mental disturbances associated with abnormalities of thyroid function (Whybrow et al., 1969; Whybrow and Prange, 1981). L-Triiodothyronine (T_3) has been reported to enhance the efficacy of tricyclic antidepressant treatment (Prange et al., 1969; Wheatley, 1972) and to convert tricyclic nonresponders to responders (Earle, 1970; Banki, 1977; Goodwin et al., 1982). For a review of the therapeutic use of thyroid hormone in depression, see Prange et al. (1984).

These data prompted us to investigate the effect of T_3 on the activity of three antidepressants—desipramine, imipramine, and nomifensine—in an animal model—the learned helplessness paradigm. This paradigm is highly sensitive to antidepressants, and increasingly used for investigating the mechanisms of action of these agents and the neurobiology of depressive illness (Sherman and Petty, 1980, 1982; Sherman et al., 1982). Briefly, learned helplessness is a condition in which exposure to an uncontrolled aversive stimulus leads to a decreased ability to escape future aversive situations (Maier and Seligman, 1976). For instance, training rats in a grid cage with inescapable electric footshocks results in a subgroup of animals who do not learn to escape subsequent exposure to shocks, such behavioral deficit being eliminated by antidepressant drug administration.

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Methods

Male Wistar AF rats (CERJ, France), weighing 175-200 g at the beginning of the experiments, were housed in groups of 10 per cage with free access to food and water, and maintained on a 12-hour light, 12-hour dark cycle at a room temperature of 21 ± 1°C.

Inescapable Shock Pretreatment. 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, inescapable shocks (15 seconds' duration, 0.8 mA, every minute ± 15 seconds) to the grid. Control rats were placed for 1 hour in identical chambers, but no shocks were administered. Inescapable shock pretreatment was performed in the morning, on day 1.

Conditioned Avoidance Training. To evaluate escape and avoidance performances, avoidance training was initiated 48 hours after inescapable shock pretreatment 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 equal-sized chambers, a gate (7 × 7 cm) providing the access to the adjacent compartment. Animals were placed singly in the shuttle-box (allowed to habituate to the test environment for 5 minutes the first session only) and subjected to 30 avoidance trials (intertrial intervals being 30 seconds). During the first 3 seconds of each trial, a light signal (used as a conditioned stimulus) was presented, allowing the animals to avoid shock. If a response did not occur within this period, a 0.8 mA shock (3 seconds' duration) was applied via the grid floor. If no escape response occurred within the latter 3-second period, shock and light were terminated. The response (avoidance or escape) required of the rat was to cross the gate into the other compartment. Avoidance sessions were performed for 3 consecutive days in the morning, and the number of escape failures was recorded separately for each rat. No crossing response during shock delivery was referred to as escape failure.

Drug Administration. Rats were randomly treated according to one of the following protocols (16 to 20 rats per group): controls with no shock were given saline, experimental animals with inescapable shocks were injected with saline, desipramine, imipramine, or nomifensine. Imipramine is known to be rapidly converted to desipramine in rats (Dingell et al., 1964). Nevertheless, both drugs were selected in the present study since they have been used in much clinical research on T_3-antidepressant potentiation. Injections were performed on 5 consecutive days: the first administration was given 6 hours after shock pretreatment; then drugs were given twice a day; in the morning (30 minutes before shuttle-box session) and at the end of the afternoon (except on day 5). Desipramine was given at 16 mg/kg (morning: 8 mg/kg + afternoon: 8 mg/kg) and 32 mg/kg (16 + 16), imipramine at 16 mg/kg (8 + 8) and 32 mg/kg (16 + 16), and nomifensine at 0.5 mg/kg (0.25 + 0.25) and 2 mg/kg (1 + 1). These doses of drugs were chosen on the basis of previous studies (Martin et al., 1986*a*, 1986*b*) to produce either a minimal initial effect (lower doses) or a marked significant reversal of escape failures (higher doses).

T_3 was given once a day, according to Brochet et al. (1985*b*) and Martin et al. (1985), at a noneffective dose (0.03 mg/kg) on 4 consecutive days (6 hours after the shock pretreatment and each shuttle-box session) either to saline-treated rats or to rats receiving desipramine, imipramine, or nomifensine.

All drugs were injected intraperitoneally in a volume of 0.5 ml/100 g of body weight. Desipramine, imipramine (Ciba-Geigy), and nomifensine (Hoechst) were injected in bidistilled water. T_3 (Merrell Toraude) was suspended in acacia gum.

In a control experiment, brain concentrations of desipramine (daily dose 16 mg/kg) were assayed, according to Diquet et al. (1983), in six saline- and six T_3-treated rats. Rats were killed 30 min after the fourth injection of desipramine, i.e., at the time at which rats of the aforementioned experiments were subjected to the first shuttle-box session.

Between-groups comparisons were made with a two-way analysis of variance (ANOVA) followed by Dunnett's one tailed t test.

Results

Two-way ANOVA revealed that nondrugged rats preexposed to inescapable shocks ($n = 20$) exhibited significantly ($F = 10.96$, $df = 1/78$, $p < 0.01$) more escape failures than saline controls ($n = 20$) not subjected to shock pretreatment (Table 1).

Table 1. Mean (± SD) number of escape failures on 3 consecutive daily shuttle-box (SD) sessions in drug-naive rats

	Number of escape failures/30		
	SB1	SB2	SB3
Experimental rats (60 inescapable shocks)	21.2 ± 6.63	23.1 ± 7.65	24.1 ± 6.12
Controls (no shock pretreatment)	8.4 ± 6.63	8.3 ± 10.71	5.5 ± 7.65

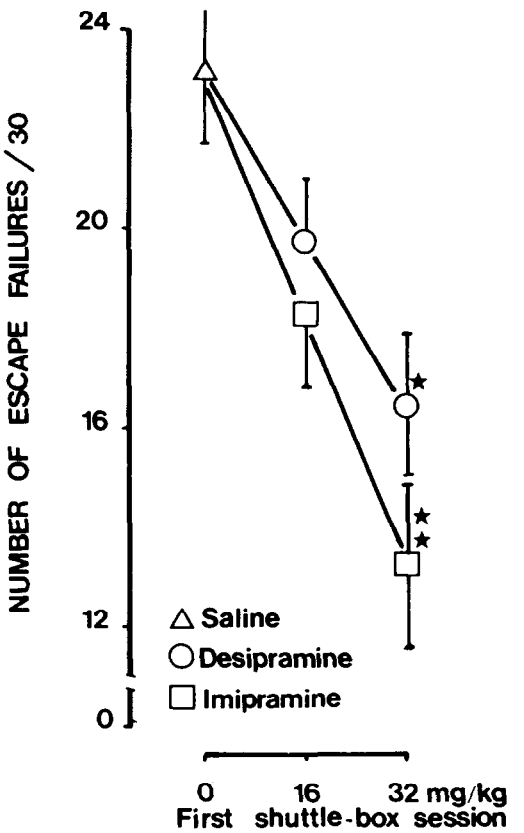
Escape failure refers to the absence of the response required of the rat to change compartments during the electric footshocks (30 trails/session).

Two-way ANOVA indicated that daily administration of desipramine, imipramine (Figs. 1 and 2) and nomifensine (Fig. 3) produced a significant ($F = 25.19$, $df = 1/246$, $p < 0.01$) treatment effect (reversal of escape deficit), the magnitude of which depended on the drug administered and the number of injections. At the highest dose studied, desipramine (total daily dose: 32 mg/kg), imipramine (32 mg/kg), and nomifensine (2 mg/kg) ($n = 18$ in each case) all markedly reduced (Dunnett's t test, $df = 7/246$, $t = 2.70$, $p < 0.05$; $t = 6.18$, $p < 0.01$; $t = 3.40$, $p < 0.01$, respectively) escape failures from the first shuttle-box session onwards (i.e., after four injections, Figs. 1 and 3). An almost complete elimination of escape failure was observed at the third shuttle-box session (i.e., after eight injections, Figs. 2 and 3). At the lowest dose studied, neither desipramine ($n = 16$), imipramine ($n = 18$), nor nomifensine ($n = 16$) significantly (Dunnett's test, $t = 1.80$, $t = 1.68$; $t = 1.57$, respectively) attenuated escape deficits on the first shuttle-box session (i.e., after four administrations). In these conditions, six and even eight injections were generally required to cause a pronounced significant (Dunnett's t test, $t = 3.89$, $p < 0.01$; $t = 4.10$, $p < 0.01$; $t = 2.48$, $p < 0.05$) reduction of escape failures that did not, however, coincide with a restoration of shuttle-box performances identical to that of nonshocked animals (Figs. 2 and 3).

As shown in Figs. 2 and 3, in rats receiving a daily injection of a noneffective dose of T_3, all antidepressants studied caused, at these latter doses, a significant (desipramine: $F = 5.15$, $df = 1/104$, $p < 0.02$; imipramine: $F = 6.13$, $df = 1/108$, $p < 0.02$; nomifensine: $F = 3.94$, $df = 1/104$, $p < 0.05$) reduction of escape failures from the first shuttle-box session onwards. Moreover, at the third shuttle-box session, a reduction of the number of escape failures to a level identical to that observed in rats given higher doses of antidepressants, or in unshocked rats, can be obtained with low doses of antidepressants in combination with T_3 (Figs. 2 and 3).

In those rats killed at the time corresponding to that of the first shuttle-box session (i.e., 30 min after the 4th injection of desipramine), brain concentrations (mean ± SD) of desipramine were not statistically different between controls ($n = 6$) (2.56 ± 0.76 μg/g fresh tissue and T_3-treated rats ($n = 6$) (2.43 ± 1.00 μg/g fresh tissue, $t = 0.25$).

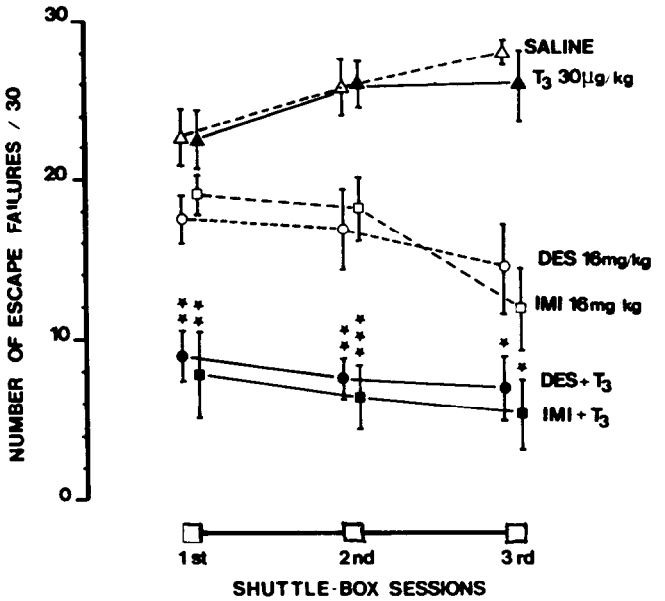
Fig. 1. Dose-related reversal of escape failures by desipramine and imipramine



Escape failure (mean ± SEM) refers to failure of rats previously exposed to inescapable shock to change compartments during electric footshock. The doses indicated refer to the total dose injected daily after the inescapable shock pretreatment.

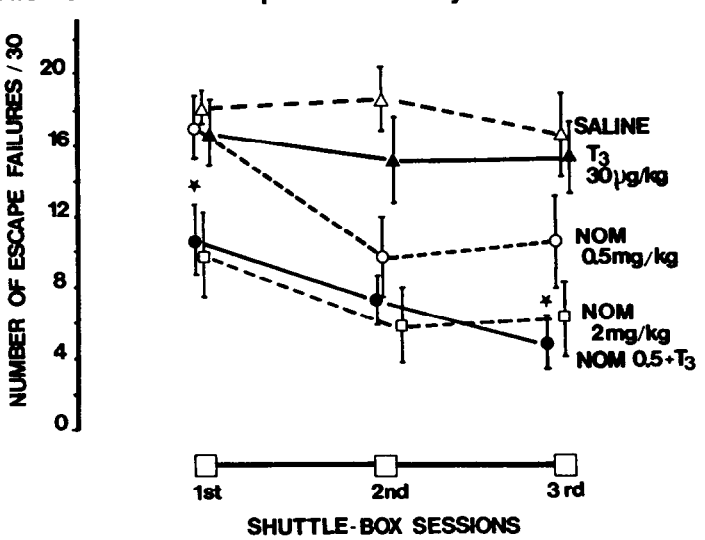
* $p < 0.05$; ** $p < 0.01$ as compared with saline-treated rats (Dunnett's t test).

Fig. 2. T_3 potentiation of IMI- and DES-induced reversal of escape failure as a function of number of exposures to daily shuttle-box session



The doses indicated (T_3 = triiodothyronine, DES = desipramine, and IMI = imipramine) refer to the total dose injected daily after the shock pretreatment. Rats given DES or IMI in combination with T_3 differ from rats given either antidepressant alone at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Dunnett's t test).

Fig. 3. T_3 potentiation of NOM-induced reversal of escape failures as a function of number of exposures to daily shuttle-box session



Rats given daily nomifensine (NOM) at 0.5 mg/kg in combination with triiodothyronine (T^4) differ from rats given NOM (0.5 mg/kg) alone at * $p < 0.05$ (Dunnett's t test).

In those rats killed at the time corresponding to that of the first shuttle-box session (i.e., 30 min after the 4th injection of desipramine), brain concentrations (mean ± SD) of desipramine were not statistically different between controls ($n = 6$) (2.56 ± 0.76 μg/g fresh tissue) and T_3-treated rats ($n = 6$) (2.43 ± 1.00 μg/g fresh tissue, $t = 0.25$).

Discussion

With the exception of one contradictory study (Feighner et al., 1972), the clinical research literature has indicated that the co-administration of T_3 to depressed patients may potentiate the action of tricyclic antidepressants, hastening the therapeutic effects of imipramine (Prange et al., 1969; Wilson et al., 1970, 1974; Coppen et al., 1972) and amitriptyline (Wheatley, 1972). Moreover, it has been shown that a good therapeutic response may be achieved in depressed patients who had formerly been refractory to tricyclic treatment and to electroconvulsive therapy. In these nonresponders, the daily addition of T_3 to imipramine (Earle, 1970; Goodwin et al., 1982), amitriptyline (Banki, 1977; Goodwin et al., 1982), desipramine (Extein, 1982; Schwarcz et al., 1984), or phenelzine (Hullett and Bidder, 1983) generally induced a rapid, and virtually complete, remission.

Using the learned helplessness paradigm in rats, we found that T_3 potentiated the effects of antidepressants in eliminating depressive-like behavior (escape deficit in the shuttle-box). Indeed, whereas six or eight injections of twice-daily desipramine (16 mg/kg), imipramine (16 mg/kg), or nomifensine (0.5 mg/kg) were required to produce a significant, though partial, reversal of escape failures, four injections of either compound, at the same doses, almost completely eliminated helpless behavior in rats receiving T_3.

It cannot be ascertained whether the few treated rats that maintained a high level of escape failures could be considered as nonresponders to antidepressants. Nevertheless, our findings as a whole are in complete agreement with the aforementioned clinical studies indicating that thyroid hormone may speed or improve the response to tricyclic antidepressants. Furthermore, the results we obtained with nomifensine, though somewhat less clear-cut than with desipramine or imipramine, suggest that the potentiating action of T_3 is not restricted to tricyclic drugs. This possibility is consonant with the reported ability of T_3 to trigger a therapeutic response in depressed patients who failed to respond to phenelzine, a monoamine oxidase inhibitor (Hullett and Bidder, 1983).

The enhancement of antidepressant action by T_3 does not appear to involve an elevation of antidepressant drug brain levels. Indeed, brain desipramine concentrations were not statistically different in saline- and T_3-treated rats, yet these rats markedly differed in their ability to escape from shocks. These data agree with previously reported clinical and experimental observations. Neither the plasma levels of imipramine nor the desipramine/imipramine ratio in imipramine-treated patients significantly differed whether patients received the antidepressant alone or in combination with T_3 (Garbutt et al., 1979; Goodwin et al., 1982). In rats, Breese et al. (1972) have shown that the amount of radioactivity found in brain after administration of ^{14}C-imipramine was not altered by a T_3 regimen (0.05 mg/kg for 5 days).

It has been proposed that T_3 may act at central synaptic sites to facilitate antidepressant actions, perhaps by altering brain receptor density or responsiveness (Whybrow and Prange, 1981). In particular, T_3 has been shown to enhance the density of central α- (Gross et al., 1981) and β-adrenoceptors (Perumal et al., 1984). The possibility of a crucial (though probably not exclusive) involvement of these receptor changes in the T_3-antidepressant interaction in the learned helplessness model is strengthened by the following facts.

Expression of helpless behavior has been found to correlate with various indexes suggesting a deficient noradrenergic transmission (Anisman et al., 1980; Johnson et al., 1982). Direct β-adrenoceptor stimulants such as clenbuterol or salbutamol reportedly eliminate escape deficits (Martin et al., 1986*a*), whereas blockade of α- or β-receptors prevents tricyclic antidepressants from reversing escape failures (Martin et al., 1986*b*). T_3-induced up-regulation of central β-adrenoceptors could not be the primary mechanism of T_3 potentiation of antidepressant action in our model. Indeed, down-regulation of these same receptors—down-regulation that can be achieved rapidly under stressful conditions—has been proposed as a critical mechanism for the action of most antidepressants (see Duncan et al., 1985). Although it is not known whether T_3 could facilitate or hasten down-regulation of β-adrenoceptors by antidepressants, the present data, in agreement with the studies listed above (Martin et al., 1986*a*, 1986*b*) suggest that indirect (via catecholamine reuptake inhibition) stimulation of adrenoceptors remains a key factor (in addition to or in spite of β-receptor down-regulation) in the attenuation of escape deficits by antidepressants. Whatever the mechanisms involved, our animal data suggest that noradrenergic processes could underlie the T_3-antidepressant interaction we observed in our model. Although further experiments are required to draw definite conclusions, and in particular with regard to the possible role of serotonergic systems, this assumption is in line with the absence of any beneficial effect of T_3 on depressed patients treated with L-tryptophan (Coppen et al., 1972) and the absence of a direct capacity of T_3 to enhance serotonin-dependent behavior in animals (Brochet et al., 1985*b*).

Since T_3 failed to potentiate the effects of antidepressants in other behavioral testing procedures such as the forced swimming test in mice (Brochet et al., 1985*a*), all these facts indicate that the learned helplessness paradigm can be a useful animal model for approaching the neurohormonal correlates of affective disorders and the neurobiochemical bases of T_3 enhancement of antidepressant action.

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