Effect of Triiodothyronine on Antidepressant Screening Tests in Mice and on Presynaptic 5-HT\textsubscript{1A} Receptors: Mediation by Thyroid Hormone \( \alpha \) Receptors

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ABSTRACT

Although triiodothyronine (T3) is widely used clinically, preclinical support for its antidepressant-like effects is limited, and the mechanisms are unknown. We evaluated 1) the antidepressant-like effects of T3 in the novelty suppressed feeding test (NSFT), tail suspension test (TST), and forced swim test (FST), 2) the role of presynaptic 5-HT\textsubscript{1A} receptors in the antidepressant-like mechanism of T3 by the hypothermic response to the 5-HT\textsubscript{1A} receptor agonist, 8-hydroxy-\( N,N \)-dipropyl-2-aminotetralin (8-OH-DPAT), 3) the thyroid hormone receptor type mediating the antidepressant-like effects by concurrent administration of the specific thyroid hormone \( \alpha \) receptor (TR\( \alpha \)) antagonist, dronedarone, and 4) the presence of these effects in both genders. Male and female BALB/c mice were administered 1) T3 (20, 50, 200, or 500 \( \mu \)g/kg per day) or vehicle or 2) T3 (50 \( \mu \)g/kg per day), dronedarone (100 \( \mu \)M/day), or the combination intraperitoneally for 21 days and then underwent a behavioral test battery. The NSFT showed a shortened latency to feed in males at the two lower T3 doses. The TST and FST showed decreased immobility in male mice at T3 doses \( >20 \) \( \mu \)g/kg per day and in females at all T3 doses. Concurrent dronedarone prevented T3 effects in males on the NSFT and in the TST and FST in both genders. Attenuation of 8-OH-DPAT-induced hypothermia was observed in males only and may be reduced by concurrent dronedarone. These findings support an antidepressant-like effect of T3. Attenuation of 8-OH-DPAT-induced hypothermia in males only suggests the need to evaluate a possible gender disparity in the role of presynaptic 5-HT\textsubscript{1A} receptors in T3 antidepressant mechanisms. Blockade by dronedarone of the antidepressant-like effects of T3 suggests that these effects are TR\( \alpha \) receptor-mediated.

Introduction

The thyroid hormone triiodothyronine (T3) is commonly used to augment antidepressant effects in patients who have not responded to treatment (Aronson et al., 1996; Nierenberg et al., 2006). T3 is also administered in conjunction with antidepressants from the initiation of treatment to accelerate the onset of therapeutic effects (Altshuler et al., 2001) or to enhance treatment outcome (Cooper-Kazaz and Lerer, 2008). Some studies have suggested that acceleration (Altshuler et al., 2001) or augmentation (Agid and Lerer, 2003) by T3 of antidepressants from the initiation of treatment to accelerate the onset of therapeutic effects (Altshuler et al., 2001) or to enhance treatment outcome (Cooper-Kazaz and Lerer, 2008).

In contrast to the extensive clinical use of T3 in major depression, little work has been done to determine whether T3 has an antidepressant-like profile in rodent models that screen for antidepressant activity. Such information would contribute to a more rational delineation of the role of T3 in the treatment of depression and to a better understanding of its antidepressant mechanism(s). Evidence from our laboratory suggests that a 10-day administration of 100 \( \mu \)g/kg per day T3 induces a (delayed) decrease in immobility on the forced swim test (FST) in female rats (Lifschytz et al., 2006) and that 50 \( \mu \)g/kg per day T3 alone or in combination with 5 \( \mu \)g/kg per day fluoxetine for 21 days, shortens latency to feed in the novelty suppressed feeding test (NSFT) in male rats (Eitan et al., 2010). Using the learned helplessness paradigm, Brochet et al. (1987) showed that the reversal by antidepressants of escape failures produced by exposure to uncontrollable stress was enhanced by T3 in male Wistar rats; conversely, reversal by tricyclic antidepressants of depres-

ABBREVIATIONS: T3, triiodothyronine; FST, forced swim test; NSFT, novelty suppressed feeding test; TR, thyroid hormone receptor; TST, tail suspension test; 8-OH-DPAT, 8-hydroxy-\( N,N \)-dipropyl-2-aminotetralin; ANOVA, analysis of variance; ANCOVA, analysis of covariance; LSD, least significant difference.
sive-like behavior in the same paradigm was attenuated in rats rendered hypothyroid by propylthiouracil in their drinking water (Martin et al., 1987). Female animals were not investigated in the above studies.

The biological actions of T3 are primarily achieved by interaction with nuclear thyroid hormone receptors (TRs) that serve as modulators of the transcription of specific target genes (Zhang and Lazar, 2000). TRα, which mediates the effects of T3 to induce noradrenergic stimulation, bone and muscle depletion, and tachycardia (Yoshihara and Scanlan, 2003; Ocasio and Scanlan, 2005), is encoded by a gene on chromosome 17, whereas the gene for TRβ is on chromosome 3. Specific modulators of TRα or TRβ have been developed. Dronedarone, a newly approved drug for suppression of cardiac arrhythmias (Patel et al., 2009), demonstrates specific TRα antagonist activity via its metabolite, debutildronedarone (Van Beeren et al., 2003). It is of considerable interest to determine which TR subtype mediates the antidepressant effects of T3. If these effects are mediated by TRβ, specific agonists of these receptors could serve as T3-like augmenting agents with advantageous side effect profiles.

After T3 becomes associated with its receptor, the hormone-receptor complex becomes attached to the promoters of target genes, modulating their transcription. In this context, we have shown (Lifschytz et al., 2010) that chronically administered T3 induces a reduction in 5-HT̂₁A (presynaptic in raphe nucleus and postsynaptic in various forebrain areas) and 5-HT̂₁B receptor expression (presynaptic in raphe and pre- and postsynaptic in forebrain areas). Effects of T3 on the serotonergic system have been studied previously in relation to the antidepressant effects of the hormone (Newman et al., 2000; Bauer et al., 2002) with evidence from functional and behavioral studies (Heal and Smith, 1988) and in vivo microdialysis experiments (Gur et al., 1999, 2002, 2004; Lifschytz et al., 2004) for a reduction in the activity of inhibitory, presynaptic 5-HT̂₁A and 5-HT̂₁B receptors.

We present for the first time a comprehensive set of experiments in which the effect of T3 administered for 21 days to mice on a battery of behavioral tests for antidepressant-like effects was evaluated. To further explore the role of desensitization of inhibitory, presynaptic serotonergic receptors in the antidepressant-like effects of T3, the functional effect of T3 treatment regimens on presynaptic 5-HT̂₁A receptor activity in the raphe nucleus was evaluated by measuring the hypothemic response to 8-OH-DPAT, a 5-HT̂₁A receptor agonist. Our third goal was to establish the TR subtype mediating the antidepressant-like effects of T3 by determining whether concurrent administration of the TRα antagonist, dronedarone, would prevent the effects of T3 on the behavioral tests and desensitization of the presynaptic 5-HT̂₁A receptors in the raphe. A further aim was to evaluate whether the effects of T3 are present in male and female animals alike.

Materials and Methods

Animals and Treatments

Male and freely cycling female BALB/c mice (3 months) were used in all experiments, which were conducted according to protocols approved by the Animal Care and Use Committee of the Hebrew University and Hadassah Medical Center. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care International accredited institute. Ten animals of each gender were allocated per cage and housed in a temperature-controlled environment (24°C) with a regular 12-h light/dark cycle. Food and water were freely available. Pharmacological treatment was administered in the context of two separate experiments. In the first T3 dose-response experiment, animals were administered intraperitoneal injections of vehicle (0.9% saline plus 0.01% NaOH) or increasing doses of T3 (3,3',5-triiodo-l-thyronine sodium salt; Sigma-Aldrich Israel, Rehovot, Israel) at 20, 50, 200, or 500 μg/kg i.p. per day, dissolved in this vehicle, for 21 days. In the second experiment (T3 plus dronedarone experiment) animals were administered the same T3 vehicle, 50 μg/kg per day T3, the specific thyroid hormone receptor α antagonist, 100 μM/day dronedarone (N-(2-buty1-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl)) dissolved in distilled water, or the combination of dronedarone and T3 in the above doses (administered immediately one after the other in randomized order), by intraperitoneal injection for 21 days. Solutions were prepared so that the injection volume was always 1 ml/100 g animal weight. Our general approach was to include 9 to 12 animals in each treatment group. There were one to two deaths of animals per group (see individual figures for numbers of animals in each treatment group).

The morning after the last injection, each animal from each gender/treatment group underwent a battery of behavioral tests followed by the 8-OH-DPAT-induced hypothermia test. The temporal sequence of the behavioral tests was adopted relative to the putative stressogenic effect of each test (from low to high: NSFT, TST, and FST) to avoid possible carryover effects (Zhang et al., 2010) and remained the same for all animals tested. Thus, the NSFT was conducted on day 22, followed by the TST and FST and by the 8-OH-DPAT-induced hypothermia test on days 23, 24, and 25, respectively. The behavioral tests were conducted at 11:00 AM, and basal body temperature was taken daily at 6:00 PM for 3 days before the 8-OH-DPAT challenge, which was conducted on day 25 at the same hour. There was an interval of 5 months between the T3 dose-response and T3 plus dronedarone experiments. Within each experiment, male and female animals were studied separately.

Behavioral Tests

Novelty Suppressed Feeding Test. After the last treatment injection, all food is removed from the cage for 24 h (water available ad libitum). At the end of this time, the animal is introduced into a 50 × 50 × 20 (height)-cm wooden arena, the floor of which is marked with equal rectangles of 10 × 10 cm. A pellet of food is placed on an elevated surface in the center of the arena. The time elapsing from the introduction of the animal into the arena until it commences eating (latency to feed) is recorded. The animal is removed from the arena immediately after it begins to eat or after not doing so for 5 min. During the test, the number of lines crossed by the animal is counted as a measure of motor activity and is calibrated for 5 min if the animal started eating before that. After the test, the animal is immediately transferred to its home cage and left to consume a previously weighed amount of food for 10 min. On completion of this period the food is weighed again to calculate the home cage food consumption.

The rating of the animals’ behavior in each of the above paradigms was conducted by two experimenters who were blind to the treatment received by each mouse. The mean of the two ratings was calculated and used for the statistical analysis.

Tail Suspension Test. Animals are suspended upside down by adhesive tape placed 1 cm from the tail tip (Cryan et al., 2005a). The elevation of the animal is 50 cm from the nearest surface. The test duration is 6 min during which the animal’s behavior is rated every 5 s as “active” or “inactive.” Statistical analysis compares the magnitude of immobility (in seconds) between different treatment groups. In this test, antidepressants characteristically cause an increase of the time fraction spent by the animal as active at the expense of the inactivity period.
Forced Swim Test. The animal is placed in a circular, transparent, Plexiglas tank measuring 21 cm in diameter and 46 cm in height containing water 15 cm high, maintained at 23–25°C (Cryan et al., 2005b). During a 6-min test period, the activity is rated every 5 s as either active (swimming) or inactive (immobile, performing only movements to keep itself from drowning). Also measured is the latency period, the time elapsing for each animal from immersion in the water tank until the first occurrence of immobility. Because climbing activity is minimal in mice, this variable was not included in the statistical analysis. Antidepressant treatment characteristically causes an increase in the time fraction spent by the animal in activity at the expense of immobility out of the total test duration. After completion of the test, the animals are towel-dried and placed for 15 min near a heating device before being returned to their home cage.

8-OH-DPAT-Induced Hypothermia

Animals have their rectal temperature taken daily at 6:00 PM for 3 days before the 8-OH-DPAT challenge. The probe is inserted to a depth of 3 cm inside the animal’s body without lubrication. The time to reach temperature equilibrium by the thermometer was approximately 5 s. The time lag between consecutive measurements for different animals was approximately 30 s. On the day of the experiment each animal has its basal body temperature measured and thereafter receives an 8-OH-DPAT injection (500 μg/kg s.c.); its temperature is measured again 30 min after challenge administration. The experiment is performed in a temperature-controlled environment with the ambient temperature held constant at 22°C.

Statistical Analysis

For the dose-response experiment, data were analyzed by one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA) followed by planned comparisons using Statistica for Windows (release 4.5, 1995). Planned comparisons were used because the direction of hypotheses was known a priori. For the T3 plus dromedarone experiment, data were analyzed by two-way ANOVA, with T3 or dromedarone treatment being the two between factors; post hoc LSD test comparisons were performed. p < 0.05 (two-tailed) was regarded as significant. Statistical analyses were performed for the two experiments separately and within these experiments for the two genders separately.

Results

Antidepressant-like Effects of Increasing Doses of T3

Effect of Treatments on Weight. Males. Mean ± S.D. weights at the start and end of the treatment regimen were as follows: saline, 25.2 ± 2.7 to 27.7 ± 2.4 g; 20 μg/kg per day T3, 24.73 ± 2.6 to 28.4 ± 2.8 g; 50 μg/kg per day T3, 24.3 ± 2.1 to 28.3 ± 1.7 g; 200 μg/kg per day T3, 25.2 ± 2.2 to 30.1 ± 1.6 g; and 500 μg/kg per day T3, 24.8 ± 2.7 to 29.1 ± 2.5 g [F(4,53) = 2.16, p = 0.08].

Females. Mean ± S.D. weights at the start and end of the treatment regimen were as follows: saline, 20.3 ± 1.2 to 21.6 ± 1.5 g; 20 μg/kg per day T3, 20.3 ± 1.4 to 21.8 ± 1.2 g; 50 μg/kg per day T3, 21.2 ± 1.1 to 23.4 ± 1.9 g; 200 μg/kg per day T3, 21.4 ± 1 to 24.7 ± 1.6 g; and 500 μg/kg per day T3, 21.1 ± 1.3 to 24.2 ± 2.2 g. One-way ANOVA yielded a significant treatment effect [F(4,33) = 3.52, p = 0.016].

Effect of Treatments on Behavioral Tests. Novelty suppressed feeding test. One-way ANOVA on latency to feed for male mice (Fig. 1, left) yielded a significant overall treatment effect [F(4,53) = 3.18, p = 0.02]. Planned comparisons showed that latency to feed was significantly shortened compared with that of controls only in animals treated with 20 μg/kg per day [F(1,53) = 5.47, p = 0.02] or 50 μg/kg per day T3 [F(1,53) = 8.43, p = 0.005], but not in male mice treated with higher doses of T3. There was no overall effect for open field activity (total lines crossed per 5 min) [F(4,53) = 1.43, p = 0.235] or one-way ANOVA for home cage food consumption [F(4,52) = 1.9, p = 0.13] in male mice.

One-way ANCOVA that was conducted on latency to feed in female mice (Fig. 1, right) with change in body weight and open field activity [F(4,33) = 3.04, p = 0.0307] as covariates did not demonstrate a significant treatment effect [F(4,33) = 1.76, p = 0.16]. No significant treatment effect on home cage food consumption was found in one-way ANCOVA (with change in body weight as a covariate) [F(4,32) = 0.21, p = 0.9].

Tail suspension test. One-way ANOVA showed a significant effect of T3 to decrease immobility [F(4,33) = 4.84, p = 0.003] in male mice. Planned comparisons demonstrated that immobility was decreased in mice treated with all T3 doses except 20 μg/kg per day T3 [F(1,33) = 8.8, p = 0.005; F(1,33) = 14.2, p = 0.0006; and F(1,33) = 12.2, p = 0.001 for mice treated with 50, 200, or 500 μg/kg per day T3, respectively].

In female mice (Fig. 2, right) one-way ANCOVA (with change in body weight as a covariate) also showed a significant effect of T3 to decrease immobility overall effect, [F(4,32) = 6.8, p = 0.0004]. Planned comparisons demonstrated that T3 caused a decrease in immobility of all T3

![Fig. 1. Effect of increasing doses of T3 on latency to feed in the novelty suppressed feeding test in male (left) and female (right) BALB/c mice. Bars represent the mean ± S.E.M. *, significant (p ≤ 0.05) difference versus the control group.](image-url)
treatment groups compared with that of control mice \(F(1,32) = 10.2, p = 0.003\); \(F(1,32) = 14.2, p = 0.0006\); \(F(1,32) = 25.4, p = 0.00002\); and \(F(1,32) = 12.26, p = 0.001\) for mice treated with 20, 50, 200, or 500 \(\mu g/kg\) per day T3, respectively).

**Forced swim test.** An overall effect of increasing T3 doses on immobility of male BALB/c mice in the FST was found by one-way ANOVA (Fig. 3, left) \(F(4,33) = 7.69, p = 0.0002\). Planned comparisons showed significant shortening of immobility compared with that of vehicle-treated controls in male mice treated for 21 days with all T3 doses except 20 \(\mu g/kg\) per day T3 versus vehicle, respectively. The immobility period was shortened in mice treated for 21 days with 500 \(\mu g/kg\) per day T3 significantly more than that in the other T3 treatment groups \(F(1,33) = 12.15, p = 0.001\); \(F(1,33) = 6.75, p = 0.01\); and \(F(1,33) = 6.18, p = 0.02\) for 500 \(\mu g/kg\) per day T3 versus 20, 50, or 200 \(\mu g/kg\) per day T3, respectively. One-way ANOVA on the latency period (time elapsing from introduction of the animal to the water tank until first appearance of immobility) data showed a significant overall effect of T3 to increase latency \(F(4,33) = 6.46, p = 0.006\). Planned comparisons showed that latency was prolonged in mice treated with 20, 50, 200, or 500 \(\mu g/kg\) per day T3 compared with that in controls \(F(1,33) = 16.69, p = 0.0002\); \(F(1,33) = 10.76, p = 0.002\); \(F(1,33) = 6.43, p = 0.02\); and \(F(1,33) = 20.23, p = 0.00008\), respectively.

One-way ANCOVA evaluating the effects of increasing T3 doses on immobility in female BALB/c mice (with change in body weight as a covariate) (Fig. 3, right) showed a significant T3 effect \(F(4,32) = 4.11, p = 0.008\). Planned comparisons demonstrated significant effects of T3 to shorten immobility for each T3 treatment group compared with that in female, vehicle-treated mice \(F(1,32) = 4.6, p = 0.03\); \(F(1,32) = 6.72, p = 0.01\); \(F(1,32) = 10.4, p = 0.002\); and \(F(1,32) = 15, p = 0.0005\) for 20, 50, 200, or 500 \(\mu g/kg\) per day T3 versus controls, respectively. One-way ANCOVA showed no overall effect of T3 to prolong latency in female mice \(F(4,32) = 2.06, p = 0.108\).

**8-OH-DPAT-Induced Hypothermia.** Males. Average body basal temperatures for the different treatment groups were as follows: saline, 37.8 ± 0.3°C; 20 \(\mu g/kg\) per day T3, 38.1 ± 0.4°C; 50 \(\mu g/kg\) per day T3, 38 ± 0.3°C; 200 \(\mu g/kg\) per day T3, 38.4 ± 0.3°C; and 500 \(\mu g/kg\) per day T3, 38.3 ± 0.3°C, indicating an overall effect of T3 administered for 21 days to increase basal body temperature in male mice \(F(4,37) = 3.61, p = 0.01\). One-way ANCOVA (with basal body temperature as a covariate) (Fig. 4, left) showed an overall effect of T3 to attenuate 8-OH-DPAT-induced hypothermia in male mice \(F(4,37) = 3.12, p = 0.03\). Planned comparisons showed that mice treated with T3 at doses higher than 20 \(\mu g/kg\) per day demonstrated significant attenuation of 8-OH-DPAT-induced hypothermia \(F(1,37) = 4.91, p = 0.03\); \(F(1,37) = 4.54, p = 0.04\); and \(F(1,37) = 10.97, p = 0.002\) for male mice treated with 50, 200, and 500 \(\mu g/kg\) per day T3 versus control.
mice, respectively]. The 8-OH-DPAT-induced hypothermia of mice treated with 500 \( \mu g/kg \) per day T3 was significantly attenuated compared with that of mice treated with 20 \( \mu g/kg \) per day T3 \( [F(1,37) = 5.29, p = 0.03] \).

**Females.** Average basal body temperature values as measured for the different treatment groups were as follows: saline, 38.1 ± 0.3°C; 20 \( \mu g/kg \) per day T3, 38 ± 0.3°C; 50 \( \mu g/kg \) per day T3, 38.2 ± 0.2°C; 200 \( \mu g/kg \) per day T3, 38.2 ± 0.3°C; and 500 \( \mu g/kg \) per day T3, 38.2 ± 0.2°C, indicating no overall effect of T3 administered for 21 days to increase basal body temperature in female mice \( [F(4,30) = 1.76, p = 0.16] \). Planned comparisons showed that female mice receiving 50 \( \mu g/kg \) per day T3 had enhanced 8-OH-DPAT-induced hypothermia compared with that in control female mice \( [F(1,30) = 5.92, p = 0.02] \) and female mice administered 20 \( \mu g/kg \) per day T3 \( [F(1,30) = 4.24, p = 0.048] \).

**Effects of the Specific TR\( \alpha \) Antagonist, Dronedarone, on T3 Antidepressant-Like Effects**

**Effect of Treatments on Weight.** **Males.** Mean ± S.D. weights at the start and end of the treatment regimen were as follows: saline, 26.4 ± 0.75 to 27.3 ± 0.9 g; 50 \( \mu g/kg \) per day T3, 26.5 ± 0.9 to 27.3 ± 1 g; dronedarone, 26.3 ± 1.1 to 27 ± 0.6 g; and dronedarone plus 50 \( \mu g/kg \) per day T3, 26.1 ± 0.8 to 26.9 ± 0.8 g \( [F(3,28) = 0.12, p = 0.9] \).

**Females.** Mean ± S.D. weights at the start and end of the treatment regimen were as follows: saline, 20.9 ± 1.4 to 21.5 ± 1.4 g; 50 \( \mu g/kg \) per day T3, 20.5 ± 1.7 to 21.2 ± 1.6 g; dronedarone, 20.3 ± 1.8 to 20.9 ± 1.8 g; and dronedarone plus 50 \( \mu g/kg \) per day T3, 20.1 ± 1 to 20.8 ± 1.3 g \( [F(3,34) = 0.14, p = 0.9] \).

**Effect of Treatments on Behavioral Tests.** **Novelty suppressed feeding test.** Because the variances of the treatment groups were not homogeneous, we used a nonparametric test to evaluate the overall treatment effect on latency to feed. The Kruskal-Wallis test for the latency to feed in the NSFT of male BALB/c mice (Fig. 5, left) administered 50 \( \mu g/kg \) per day T3 or 100 \( \mu M/day \) dronedarone, alone or combined, demonstrated an overall significant treatment effect \( [H(3,29) = 13, p = 0.0046] \). Post hoc Mann-Whitney U test comparisons showed that latency to feed of mice treated with 50 \( \mu g/kg \) per day T3 was significantly shorter than that of the vehicle-treated mice \( (Z = 2.5, p = 0.01) \). Complete reversal of the T3 effect on latency to feed was achieved by the addition of dronedarone (T3 plus dronedarone versus T3, \( Z = 2.5, p = 0.01 \); T3 plus dronedarone versus vehicle, \( Z = 0 \),...
p = 1). One-way ANOVA of open field activity and home cage consumption of male mice in the NSFT did not yield significant effects [for open field activity: F(3,24) = 1.43, p = 0.26; for home cage consumption, F(3,25) = 1.04, p = 0.39].

Two-way ANOVA on latency to feed of female BALB/c mice in the NSFT (Fig. 5, right) with T3 and dronedarone treatment as the two factors did not yield any significant effect [F(1,33) = 0.5, p = 0.5; F(1,33) = 1.6, p = 0.2; and F(1,33) = 0.06] for T3, dronedarone, or T3 × dronedarone interaction, respectively. This finding is similar to the results for latency to feed of female mice in the NSFT in the first experimental series of this study. There was no overall treatment effect in open field activity [F(3,34) = 1.72, p = 0.181] or home cage food consumption [F(3,31) = 0.939, p = 0.961] in female mice.

Tail suspension test. Two-way ANOVA on TST data in male BALB/c mice (Fig. 6, left) with T3 and dronedarone as the two factors demonstrated a significant overall T3 treatment effect [F(1,24) = 9.6, p = 0.004], a significant dronedarone treatment effect [F(1,24) = 8.81, p = 0.006], and a significant T3 × dronedarone interaction [F(1,24) = 24.32, p = 0.00009]. Post hoc LSD test comparisons showed a significant shortening of immobility time in male mice administered 50 μg/kg per day T3 versus those administered vehicle (p = 0.000008). There was no effect of dronedarone alone on immobility versus that in control male mice (p = 0.192). The effect of T3 to shorten immobility was completely reversed with the addition of 100 μM/day dronedarone (T3 plus dronedarone versus T3, p = 0.92; T3 plus dronedarone versus vehicle, p = 0.000006).

Two-way ANOVA on TST data of female BALB/c mice (Fig. 6, right) with T3 and dronedarone as two between factors demonstrated a significant overall T3 treatment effect [F(1,35) = 19.02, p = 0.0001] and an almost significant T3 × dronedarone interaction [F(1,35) = 3.88, p = 0.056]. There was no overall dronedarone treatment effect [F(1,35) = 1.76, p = 0.192]. Post hoc LSD test comparisons showed a significant effect of T3 to shorten immobility versus vehicle (p = 0.0001). No effect of 100 μM/day dronedarone alone on immobility was observed in female mice (p = 0.64). The effect of T3 on shortening immobility was partially reversed with the addition of dronedarone (T3 plus dronedarone versus T3, p = 0.03; T3 plus dronedarone versus vehicle, p = 0.04).

Forced swim test. Two-way ANOVA on immobility in male mice (Fig. 7, left) with T3 and dronedarone as the two factors showed an overall effect of 50 μg/kg per day T3 treatment on immobility [F(1,31) = 4.44, p = 0.04] and a significant T3 × dronedarone interaction [F(1,31) = 6.96, p = 0.012]. There was no overall effect of 100 μM/day dronedarone treatment on immobility [F(1,31) = 3.42, p = 0.07]. Post hoc LSD test comparisons showed a significant shortening of immobility compared with that of vehicle-treated controls in male mice treated for 21 days with T3 (p = 0.003). There was no effect of dronedarone alone on immobility compared with that of

Fig. 6. Effects of 50 μg/kg per day T3, 100 μM dronedarone, and their combination on immobility in the tail suspension test in male (left) and female (right) BALB/c mice. Bars represent the mean ± S.E.M. *, significant (p ≤ 0.05) difference versus the control group; †, significant difference versus the T3-administered group.

Fig. 7. Effects of 50 μg/kg per day T3, 100 μM dronedarone, and their combination on various behavioral categories of the forced swim test in male (left) and female (right) BALB/c mice. Bars represent the mean ± S.E.M. *, significant (p ≤ 0.05) difference versus the control group.
vehicle-treated male mice ($p = 0.59$). Complete reversal of the effect of T3 to shorten immobility was demonstrated in mice administered the combination of T3 plus dronedarone ($p = 0.865$ for T3 plus dronedarone versus vehicle; $p = 0.02$ for T3 plus dronedarone versus T3). Two-way ANOVA on the latency period in male mice did not show any significant effect [$F(1,29) = 2.05, p = 0.2; F(1,29) = 3.07, p = 0.09$; and $F(1,29) = 2.41, p = 0.1$] for T3 treatment, dronedarone treatment, and T3 × dronedarone interaction, respectively.

Two-way ANOVA on the immobility time in FST in female mice with T3 and dronedarone as the two factors (Fig. 7, right) demonstrated a significant T3 × dronedarone interaction effect on immobility [$F(1,32) = 11.2, p = 0.002$]. There was an overall effect of dronedarone treatment on immobility [$F(1,32) = 12.21, p = 0.01$], whereas no overall effect of T3 treatment on immobility was found [$F(1,32) = 2.74, p = 0.107$]. Post hoc LSD test comparisons showed a significant effect of T3 (versus vehicle) to shorten immobility time ($p = 0.001$). There was no effect of dronedarone alone on immobility compared with that in vehicle-treated female mice ($p = 0.26$). Complete reversal of the effect of T3 to shorten immobility was demonstrated in mice administered dronedarone plus T3 (T3 plus dronedarone versus vehicle, $p = 0.22$; T3 plus dronedarone versus T3, $p = 0.00005$). Two-way ANOVA on latency data of female BALB/c mice did not yield any significant effect [$F(1,31) = 2.31, p = 0.1; F(1,31) = 0.1, p = 0.7; and F(1,31) = 0.07, p = 0.8$] for T3 treatment, dronedarone treatment, and T3 × dronedarone interaction, respectively.

**8-OH-DPAT-Induced Hypothermia.** *Males.* Average body basal temperature values for the different treatment groups were as follows: saline, $38.5 \pm 0.3^\circ C$; $50 \mu g/kg$ per day T3, $38.6 \pm 0.3^\circ C$; $100 \mu M/day$ dronedarone $38.5 \pm 0.4^\circ C$; and $50 \mu g/kg$ per day T3 plus $100 \mu M/day$ dronedarone, $38.5 \pm 0.3^\circ C$, indicating no overall effect of T3 administered for 21 days to increase basal body temperature in male mice [$F(3,27) = 0.2, p = 0.9$]. Two-way ANOVA with T3 and dronedarone as the two between factors (Fig. 8, left) showed an overall effect of T3 treatment on 8-OH-DPAT-induced hypothermia [$F(1,27) = 5.19, p = 0.016$]. There was also an overall dronedarone treatment effect [$F(1,27) = 2.43, p = 0.04$], whereas the T3 × dronedarone interaction was not significant [$F(1,27) = 2.43, p = 0.139$]. Post hoc LSD test comparisons showed that $50 \mu g/kg$ per day T3 caused a significant reduction of 8-OH-DPAT-induced hypothermia ($p = 0.01$ for male mice treated with T3 versus control mice) and a complete reversal of this effect with the addition to T3 of $100 \mu M/day$ dronedarone ($p = 0.905$ for mice treated with the combination of T3 plus dronedarone versus control mice; $p = 0.013$ for mice treated with the combination versus mice treated with $50 \mu g/kg$ per day T3).

**Females.** Average basal body temperatures were as follows: saline, $38.1 \pm 0.3^\circ C$; $50 \mu g/kg$ per day T3, $38.1 \pm 0.3^\circ C$; $100 \mu M/day$ dronedarone, $38.3 \pm 0.4^\circ C$; and $50 \mu g/kg$ per day T3 plus $100 \mu M/day$ dronedarone, $38.6 \pm 0.3^\circ C$. One-way ANOVA for basal body temperature of female mice demonstrated an overall treatment effect [$F(3,27) = 0.2, p = 0.02$]. Two-way ANCOVA of 8-OH-DPAT-induced hypothermia data of female BALB/c mice (basal body temperature as a covariate) with T3 and dronedarone as two between factors (Fig. 8, right), yielded no significant effect [$F(1,29) = 0.8, p = 0.4; F(1,29) = 0.06, p = 0.8; and F(1,29) = 0.7, p = 0.4$] for T3 treatment, dronedarone treatment, and T3 × dronedarone interaction effects, respectively. This finding is similar to that observed in the T3 dose-response experiments.

**Discussion**

The results of our study show antidepressant-like effects of T3 on behavioral tests that predict antidepressant activity. In both genders of BALB/c mice studied, the effects were dose-dependent, with stronger effects of higher doses in the FST and the TST. In contrast, in the NSFT experiments, T3 showed decreased latency to feed only in male mice and only at lower doses ($20$ and $50 \mu g/kg$ per day). Gender differences were also observed in the 8-OH-DPAT-induced hypothermia experiments. These showed a dose-related effect of T3 to reduce 8-OH-DPAT-induced hypothermia in male mice only. Because experiments with male and female mice were conducted at different time points, direct comparisons between the genders were not possible. In addition, freely cycling female mice were used in the present experiments, and there would be no circadian variation in the female mice during the experiments. Therefore, the results suggest that T3 may have antidepressant-like effects in male mice, but the effect in female mice needs to be investigated further.

![Fig. 8.](https://example.com/fig8.png) **Fig. 8.** Effects of $50 \mu g/kg$ per day T3, $100 \mu M$ dronedarone, and their combination on 8-OH-DPAT-induced hypothermia in male (left) and female (right) BALB/c mice. Bars represent the mean ± S.E.M. Δ (basal – post-8-OH-DPAT) rectal temperature. *, significant ($p \leq 0.05$) difference versus the control group.
is evidence in the literature for an effect of the estrous cycle on performance of female mice on various behavioral tests (Meziane et al., 2007). Therefore, results regarding gender differences should be interpreted with reservation.

In a second set of experiments we sought to determine which thyroid hormone receptor subtype (TRα or TRβ) mediates the antidepressant effect of T3. In the FST, addition of the TRα antagonist, dronedarone (100 μM/day), completely blocked the effect of 50 μg/kg per day T3 to reduce immobility in both genders. Full reversal of the T3 effect on immobility was also found in the TST in males, and a partial reversal was found in females. In the NSFT, dronedarone significantly attenuated the effect of T3 on latency to feed. There was a suggestion that dronedarone may reduce the effect of T3 to attenuate 8-OH-DPAT-induced hypothermia in male mice. In this context, the marked variability in the scale of T3 effects between the dose-response and the T3 plus dronedarone experiments should be noted. This is possibly due to the gap of several months between the two experiments.

Our findings regarding the effect of T3 in the FST and TST are in accordance with previous studies that showed the ability of T3 to induce reversal of escape deficits in the learned helplessness model when administered alone (Martin et al., 1985) or in conjunction with other antidepressants (Brochet et al., 1987). FST and TST results showed sensitivity to T3 at lower doses in female mice, suggesting that T3 may have greater potency in females than in males, subject to the reservation that the genders were tested at different time points. This finding is consistent with clinical evidence for relatively more responders in women receiving T3 augmentation compared with men (Agid and Lerer, 2003).

There was an additional disparity in the behavioral effects of T3 in male and female mice. In the NSFT experiments, a U-shaped T3 dose-response effect to decrease latency to feed was observed in male mice, whereas in females there was no effect of T3 to decrease latency. U-shaped effects of psychoactive compounds on the NSFT have been reported previously (Rex et al., 1998). The lack of effect of T3 in female mice in the NSFT could be related to greater sensitivity of females to the effects of the hormone. Because latency to feed in the NSFT reflects a significant anxiety component apart from the anhedonic component (Bodnoff et al., 1988, 1989; Cryan et al., 2005b; Dulawa and Hen, 2005), it is possible that the anxiogenic effects of lower doses of T3 may be sufficient to cause female mice to abstain from entering the center of the open field arena, whereas this would not be the case for males at the same doses. Again, these considerations are subject to the limitation that male and female animals were tested in different experimental sessions.

A putative mechanism for the antidepressant effects of T3 is down-regulation of inhibitory, presynaptic 5-HT1A and 5-HT1B receptors (Gur et al., 1999, 2002; Lifschytz et al., 2004). In mice, 8-OH-DPAT-induced hypothermia represents a model for the level of activity of somatodendritic, inhibitory, presynaptic raphe 5HT1A receptors (Bill et al., 1991). Chronic administration of selective serotonin reuptake inhibitor antidepressants leads to a reduction in 8-OH-DPAT-induced hypothermia in mice (Maj and Moryl, 1992; Martin et al., 1992). Heal and Smith (1988) showed that administration of 100 μg/kg per day T3 for 10 days to male mice caused a decrease in 8-OH-DPAT-induced hypothermia and in motor responses mediated by 5HT2 receptors. In the present study, we found a dose-dependent effect of chronic T3 administration to reduce the degree of hypothermia induced by 8-OH-DPAT in male mice. Our data suggest that this effect may be attenuated by the TRα antagonist, dronedarone. No significant effect of T3 on 8-OH-DPAT-induced hypothermia was found in female mice. Although subject to the limitation that males and females were tested at different time points, the disparity between the genders suggests that there might be a difference in the neurobiological mediation of the antidepressant effects of T3 between males and females. In both genders, the antidepressant effects of T3 would appear to be mediated by its interaction with TRα receptors (because TST and FST effects were blocked in both genders by dronedarone), but the downstream genes whose transcription is affected may differ (the role of 5-HT1B receptors remains to be studied in this context).

The present findings on the effects of T3 on 8-OH-DPAT-induced hypothermia are in accordance with the results of microdialysis studies previously conducted in our laboratory (Gur et al., 2002, 2004). We found functional desensitization of inhibitory, presynaptic 5HT1A receptors in the raphe, manifesting as attenuation of the reduction in serotonin levels after challenge with the specific 5-HT1A agonist, 8-OH-DPAT, in the cortex of male rats receiving 20, 50, 200, or 500 μg/kg per day T3 for 7 days and in the hypothalamus of male rats receiving 50 μg/kg per day T3 for the same period. Administration of 20 μg/kg per day T3 for 2 weeks to male rats caused a significant desensitization of raphe presynaptic 5-HT1A receptors and of presynaptic 5-HT1B receptors in cortex and hypothalamus (Gur et al., 2002, 2004). However, in vivo microdialysis studies performed in our laboratory on female rats showed no effect of T3 at any of the above doses to desensitize either 5HT1A or 5HT1B receptors in any of the brain areas studied (T. Lifschytz, G. Landshut, M. Newman, and B. Lerer, unpublished data), although behavioral testing did show a stronger delayed effect of T3 on the FST in female rats than in males (Lifschytz et al., 2006). These findings parallel those of the current project in observing no effect of T3 in female mice to desensitize presynaptic 5HT1A receptors as opposed to greater potency of T3 to induce antidepressant-like effects in female mice than in males on the FST and TST. Prior studies on the effects of antidepressant drugs on 8-OH-DPAT-induced hypothermia, although showing an effect in male mice, did not include any female experimental groups (Maj and Moryl, 1992; Martin et al., 1992).

In the current series of experiments, we administered T3 in a chronic (3-week) regimen to model the clinical efficacy of T3 in the treatment of depression, an effect that occurs only after several weeks of treatment (Aronson et al., 1996). Furthermore, we found (Gur et al., 2002; Lifschytz et al., 2004), in the context of in vivo microdialysis experiments, that the effect of T3 on 5HT1A/1B receptor desensitization is maximal only after at least 2 weeks of T3 administration. We chose to comprehensively evaluate the antidepressant-like attributes of T3 as a sole pharmacotherapeutic agent because this has not been done previously and is important in understanding the antidepressant-like mechanism(s) of this agent. Our findings provide preclinical support for an antidepressant-like effect of T3 as indicated by dose-dependent effects on behavioral tests that reflect antidepressant-like activity. Subject to methodological reservations, female mice appeared to be more sensitive to the effects of T3 with possible anxiogenic
consequences. Dose-related attenuation by T3 of 8-OH-DPAT-induced hypothermia was observed in male but not female mice, suggesting a possible gender disparity in the role of presynaptic 5-HTT1A receptors in the antidepressant-like mechanism of T3. Finally, our findings indicate that the antidepressant-like effect of T3 is mediated in both genders by TRβ receptors, suggesting that the antidepressant-like effect of T3 cannot be dissociated from its potentially deleterious effects on heart, bone, and muscle (which are also TRα-mediated) by the use of TRβ-specific agents. As a continuation to our study regarding the role of TRα in the mediation of thyroid hormone antidepressant-like effects, future studies should evaluate the role of TRβ in the same context, using TRβ-specific modulators and thus provide a more comprehensive picture regarding the role of distinct TR populations in mediating the antidepressant-like effect of T3.

Authorship Contributions
Participated in research design: Lifschytz, Zozulinsky, Eitan, Landshut, and Lerer. Conducted experiments: Lifschytz, Zozulinsky, and Ohayon. Performed data analysis: Lifschytz, Zozulinsky, and Lerer. Wrote or contributed to the writing of the manuscript: Lifschytz, Zozulinsky, and Lerer.

References

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