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BRIEF COMMUNICATION

Triiodothyronine (T3) Modifies Cholinergic-Induced Hypothermia and Tremor in Rats

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ALMEIDA, O. M. S. AND R. SANTOS. *Triiodothyronine (T3) modifies cholinergic-induced hypothermia and tremor in rats.* PHARMACOL BIOCHEM BEHAV 46(3) 729–732, 1993.—Hypothermia and tremor responses of oxotremorine and eserine were studied in rats after several T3 treatment regimes. The T3 antagonized oxotremorine-induced hypothermia and failed to antagonize eserine hypothermic effect, but potentiated oxotremorine- and eserine-induced tremors. Acetylcholinesterase activity was not altered in T3 rats. The hypothetical mechanisms to explain changes of central cholinergic responses caused by T3 are discussed.

L-Triiodothyronine Hyperthyroidism Oxotremorine Eserine Tremor Hypothermia Acetylcholinesterase activity

BEHAVIORAL responses to monoamines were changed in altered thyroid states (2,3). It has also been reported that hyperthyroidism decreases and hypothyroidism increases the number of muscarinic binding sites in cardiac membranes (14,16). In contrast, associated with altered thyroid states in rats, it was found that no changes occur in muscarinic binding sites in brain and heart homogenates (6), in submaxillary gland (12), and in ileal or colonic muscle preparations (5). In mice, triidothyroacetic acid, a thyroid analog, did not modify oxotremorine-induced tremor (9) but T3 antagonized oxotremorine-induced hypothermia (8).

Central cholinergic receptor sensitivity, can be evaluated by measuring central responses induced by cholinergic drugs, such as hypothermia (13) and tremor (15).

In the present study, hypothermia and tremor were selected to evaluate central mediated responses of oxotremorine and eserine in T3-treated and control rats. Different levels of experimental hyperthyroidism were obtained by daily administration of several doses of T3 (12.5 to 100 μg/kg) during administration periods ranging from 1 to 20 days. Acetylcholinesterase activity was also measured in total brain preparations of T3-treated rats.

METHOD

Animals

Three- to 4-month-old male Wistar rats (220–300 g) were used. Food and water were continually available and room temperature was 23 ± 2°C. Animals were maintained under a 12L : 12D cycle and all experiments were conducted during the light phase of the cycle (6 a.m. to 6 p.m.).

T3 Treatment

L-Triiodothyronine (Smith Kline e Enila-Brasil) was dissolved in NaOH 0.02 N and administered SC in a volume of 1 ml/kg b.wt. T3 or vehicle (control rats) was injected daily for 1, 5, 10, and 20 days. The following T3 doses were used: 12.5, 25, 50, and 100 μg/kg/day (T3 rats).

All rats were weighed daily. T3 serum levels were measured in animals treated with vehicle (1-day treatment, $n = 9$) and T3 at the doses of 12.5 (10-day treatment, $n = 3$), 25 (5-day treatment, $n = 3$; 10-daystreatment, $n = 6$), 50 (5-day treatment, $n = 6$; 10-day treatment, $n = 6$), and 100 μg/kg/day (1-, 5-, and 10-day treatment, $n = 7$, 8, and 9, respectively). Twenty-four hours after the last injection of T3 or vehicle,

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the rats were anesthetized with ethyl ether, the blood was drawn by cardiac puncture, and the serum was assayed for T3 by radioimmunoassay (1).

Drugs

Oxotremorine and eserine sulphate (Sigma Chemical Co.) were dissolved in distilled water just before use and injected IP in a volume of 1 ml/kg b.wt.

Cholinergic-Induced Hypothermia

Twelve groups of 10 rats were used in oxotremorine experiments. Two groups of animals were injected, respectively, with vehicle and T3 100 μg/kg, for 1-day treatment; five groups received vehicle and 12.5, 25, 50, and 100 μg/kg/day of T3 for 5 consecutive days. This same protocol was also applied to the five remaining groups for 10 consecutive days. All rats studied were injected with 0.5 mg/kg of oxotremorine the day after the last injection of T3. Another set of 12 groups of 10 rats were also injected with vehicle and T3 as described previously, and tested for the effects of 0.5 mg/kg eserine. Temperature was then measured with clinical thermometers before drug treatment, and 20 and 60 min after drug administration. The thermometers were introduced 5 cm into the rectum for at least 1 min. The effect of treatment was assessed by subtracting temperature values from the baselines levels.

Cholinergic-Induced Tremor

Thirty rats were injected with vehicle (control) and other 30 (T3 rats) with T3 100 μg/kg/day, for 10 consecutive days. Twenty-four hours after the last injection, control and T3 rats, divided in six groups (n = 10), were injected with oxotremorine at the doses of 0.25, 0.50, and 1.0 mg/kg b.wt. This same protocol was applied to another set of 60 rats (30 control and 30 T3 rats) for 20 days, before the injections of oxotremorine. For the eserine experiment, 50 rats divided in groups of 10 were treated with vehicle, or T3 12.5, 25, 50, and 100 μg/kg/day, respectively, for 10 consecutive days. Twenty-four hours after the last vehicle/hormone injection, they were treated with 0.5 mg/kg of eserine.

To measure tremor, each rat was individually placed into a wire cage measuring 15 × 20 × 30 cm for observation. The observer was blind to the treatment received by each rat. The intensity of tremor was scored 5–10 min after drug administration, using the following scale [adapted from (15)]: 0—absence of tremor; 1—light tremor; 2—moderated tremor; 3—intense tremor; 4—very intense continuous tremor.

Acetylcholinesterase Activity

Fifty rats, divided in five groups of 10 animals, were treated for 10 consecutive days with: vehicle and 12.5, 25, 50, and 100 μg/kg/day of T3. Twenty-four hours after the last injection, decerebration was effected by cervical section, and acetylcholinesterase activity was assayed in total brain homogenates as described by Ellman et al. (4). Protein content was measured according to Lowry et al. (7).

Statistics

Data from body weight, T3 serum levels, temperature, and acetylcholinesterase activity were analyzed using one-way analysis of variance (ANOVA). Further comparisons between control and T3 rats were performed using the two-tailed Student's unpaired t-test. Hypothermia caused by cholinergic drugs was analyzed using ANOVA on the differences obtained by subtracting temperature readings from the baseline levels in T3 and control rats. Intensity of tremor caused by each dose of cholinergic drugs in control and T3 rats was compared using the nonparametrics Kruskal–Wallis test and, for further comparisons between two groups, the Mann–Whitney U-test.

RESULTS

T3-treated rats (50 and 100 μg/kg/day for 10 days) showed a significant decrease in body weight and a long-lasting and

TABLE 1

HYPOTHERMIA INDUCED BY OXOTREMORINE AND ESERINE IN CONTROL AND T3-TREATED RATS

Treatment Duration (days)	T3 Dose (μg/kg)	Δ Temperature (°C)			
		Oxotremorine		Eserine	
		20′	60′	20′	60′
1	0	−1.59 ± 0.21	−2.43 ± 0.27	−1.54 ± 0.17	−0.94 ± 0.24
	100	−1.26 ± 0.23	−2.41 ± 0.17	−0.81 ± 0.16	−0.73 ± 0.11
5	0	−1.10 ± 0.33	−1.33 ± 0.43	−1.53 ± 0.32	−1.23 ± 0.23
	12.5	−0.66 ± 0.35	−0.93 ± 0.31	−1.79 ± 0.17	−1.47 ± 0.19
	25	−0.62 ± 0.40	−0.98 ± 0.28	−1.48 ± 0.18	−1.35 ± 0.21
	50	−0.35 ± 0.31	−0.73 ± 0.33	−1.55 ± 0.22	−1.49 ± 0.23
	100	−0.22 ± 0.31	−0.48 ± 0.28	−0.98 ± 0.15	−1.11 ± 0.14
10	0	−1.47 ± 0.30	−2.09 ± 0.35	−0.91 ± 0.35	−0.53 ± 0.22
	12.5	−0.68 ± 0.26	−1.16 ± 0.28	−1.06 ± 0.32	−1.02 ± 0.28
	25	−0.73 ± 0.26	−0.88 ± 0.22*	−1.08 ± 0.23	−0.91 ± 0.20
	50	−0.26 ± 0.24*	−0.88 ± 0.23*	−0.80 ± 0.10	−1.02 ± 0.16
	100	−0.42 ± 0.12†	−0.47 ± 0.17‡	−0.18 ± 0.15	−0.45 ± 0.19

Values are mean ± SE.

*†‡Indicate statistically significant differences from corresponding control: *$p \leq 0.01$; †$p \leq 0.05$; ‡$p \leq 0.001$ (Students t-test, two-tailed).

cumulative increase in T3 serum levels 24 h after the last injection of T3 (50 and 100 μg/kg/day for 5 and 10 days). Basal temperature of T3 rats was significantly increased after 5 days of treatment with the doses of 50 or 100 μg/kg/day and after 10 days of treatment with 25, 50, or 100 μg/kg/day of the hormone.

Oxotremorine-induced hypothermia was significantly antagonized by a 10-day treatment period with 25, 50, and 100 μg/kg/day of T3 (Table 1) when compared with respective controls. Eserine-induced hypothermia did not show statistically significant differences between T3 and control rats.

The intensity of oxotremorine-induced tremor was significantly increased for all doses of oxotremorine tested in rats treated with T3 (100 μg/kg/day) for 20 days when compared with respective controls (Table 2). Eserine-induced tremor was also significantly increased after a 10-day treatment period with 100 μg/kg/day of T3 (Table 2). Significant differences of tremor scores were not found between T3 and control rats when testing oxotremorine-induced tremor after a 10-day treatment period.

Acetylcolinesterase activity in total brain homogenates of T3 rats (12.5–100 μg/kg/day; 10-day treatment) did not differ significantly from control rats.

DISCUSSION

Body weight loss, T3 serum accumulation, and hyperthermia in T3 rats clearly demonstrated the efficacy of the method used to obtain different levels of hormone action.

T3 potentiated oxotremorine- and eserine-induced tremor in rats (Table 2). T3 also antagonized oxotremorine-induced hypothermia (Table 1), as previously shown (8), but failed to antagonize eserine-induced hypothermia. Both effects appeared at the more intense levels of experimental hyperthyroidism.

The different influences of T3 on central cholinergic responses should be discussed as hypothetical specific and nonspecific mechanisms. It should be considered that T3-potentiated tremor could result from specific mechanisms. In fact, tremor appears to be a more appropriate response than hypothermia to evaluate specific mechanisms of central cholinergic actions, since T3 by itself caused hyperthermia.

T3-potentiated tremor may be explained by central muscarinic supersensitivity. Binding techniques failed to detected changes in density and affinity of muscarinic receptors in adult brain homogenates of T3-treated rats (6), indicating only an increased equilibrium dissociation constant. In contrast, developing brain (10) showed a significant increase in muscarinic binding sites and acetylcholinesterase activity in T3-treated rats. In adult animals, however, our experiments did not detect significant differences in acetylcholinesterase activity between T3 and control rats.

Indirect specific action of T3 should also be considered. Changes in central dopaminergic responses were observed in hyperthyroid rats (2,11), and binding studies showed that hypothyroidism and iodine-deficient diet increases dopamine receptor sensitivity (3,11). Therefore, a possible reduced dopaminergic sensitivity could lead to an increase of cholinergic responses like the observed T3-potentiated tremor.

However, T3-potentiated tremor could also be explained as a nonspecific mechanism. Our results showed that T3 serum accumulation did not return to control levels 24 h after the last injection of the hormone. Competition between T3 and the drugs tested for plasma protein binding sites could account for the potentiating effect of tremor induced by the drugs used. The potentiating effect of T3 on induced tremor could also be the result of increased brain excitability. It is well known that thyroxine decreases electroconvulsive threshold in rats (17).

The antagonism of oxotremorine-induced hypothermia by T3 could also be explained as a nonspecific mechanism. It should be considered that alterations in metabolic state promoted by T3 caused hyperthermia that could antagonize cholinergic-induced hypothermia.

Further experiments would be necessary to demonstrate that T3-potentiated tremor results from specific changes in muscarinic receptors.

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TABLE 2

INTENSITY OF TREMOR INDUCED BY OXOTREMORINE AND ESERINE IN CONTROL AND T3-TREATED RATS

Drug	Dose (mg/kg)	T3 Treatment Duration (days)	Median Tremor Score After T3 Dose (μg/kg/day)				
			0	12.5	25	50	100
Oxotremorine	0.25	10	0	–	–	–	0
	0.5		1	–	–	–	1
	1.0		2	–	–	–	2
Oxotremorine	0.25	20	0	–	–	–	1*
	0.5		1	–	–	–	2*
	1.0		2	–	–	–	4†
Eserine	0.5	10	1	1	1	2	2.5*

*†Indicate statistically significant differences from corresponding controls: *$p \leq 0.05$; †$p \leq 0.02$ (Kruskal-Wallis test and Mann-Whitney *U*-test for further comparisons between groups).

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