J. Pharm. Pharmacol. 1996, 48: 959–961
Received March 24, 1995
Accepted March 22, 1996

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Inhibitory Effects of (±)-Tetrahydropalmatine on Thyrotropin-stimulating Hormone Concentration in Hyperthyroid Rats

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Abstract

The effects of (±)-tetrahydropalmatine ((±)-THP) on the hypothalamus-pituitary-thyroid system in rats were investigated.

Thyroid function experiments indicated that (±)-THP produces significant decreases in thyroid function in hyperthyroid rats after 14 days of treatment. These effects were the same as those of propylthiouracil. However, propylthiouracil also decreased thyroid function in normal rats. Measurements of thyrotropin-stimulating hormone (TSH) demonstrated that (±)-THP decreased TSH in hyperthyroid rats after 14 days of treatment; however, propylthiouracil increased TSH in hyperthyroid rats. (±)-THP had no influence on TSH, or thyroid and pituitary weight in normal and hyperthyroid rats.

We conclude that (±)-THP has an antithyroid function and the mechanism of action may be related to the inhibition of TSH in the pituitary.

The thyroid is a major endocrine gland for maintaining equilibrium of metabolism. Phenomena such as nervous tension, dietary imbalance, environmental pollution, misuse of drugs and infectious disease may cause an abnormal function of the thyroid (Daughaday et al 1975). Conventional methods for treating hyperthyroidism include surgery, radiation with radioactive isotopes and treatment with thyroid inhibitors.

Hyperthyroidism is often associated with aberrations in the regulation of the hypothalamus-pituitary-thyroid (HPT) system. Typical characteristics include elevated levels of serum thyroxine (T4), serum triiodothyronine (T3), free triiodothyronine (FT3) and free thyroxine (FT4) (Walker et al 1982). The conventional antithyroid drug, propylthiouracil, inhibits the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin; in addition, propylthiouracil also inhibits the coupling of these iodotyrosyl residues to form iodothyronines (Davidson et al 1978; Morley 1981). However, propylthiouracil stimulates thyrotropin-stimulating hormone (TSH) through feedback inhibition to cause an enlargement of follicle cells, and loss of colloid (Taurog 1976). Therefore, an anti-thyroid drug which has a different mode of action from propylthiouracil would be worthwhile.

Based on our previous report, the chloroform B layer extracted from *Corydalis tuber* decreases the serum T3, T4 and TSH levels in L-T4-induced hyperthyroid rats. Furthermore, this extract did not cause thyroid cell enlargement (Hsieh et al 1995). The chloroform B layer extract has been further purified to yield (±)-tetrahydropalmatine ((±)-THP). (±)-THP is a protoberberine type alkaloid. The effect of (±)-THP on thyroid function has not previously been studied. In this study, the T3, T4, FT3, FT4 and TSH levels of normal and L-T4-induced hyperthyroid rats were measured to investigate the effects of (±)-THP on the thyroid function.

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Materials and Methods

Animals

Female Sprague–Dawley rats, 250–280 g, were housed individually and maintained on a reverse 12 h: 12 h dark/light cycle (lights on at 0700 h) with free access to food and water, at a constant environmental temperature (22°C) and relative humidity of 55 ± 5%. Testing was conducted during the light cycle.

Haemodynamic variables in normal rats

Normal rats were intraperitoneally administered (±)-THP (2·5, 5·0 or 10·0 mg kg^{-1}) daily for 14 days or propylthiouracil (5·0 mg kg^{-1}) daily for the same period. On the 15th day, 50 mg kg^{-1} pentobarbital was intraperitoneally injected into the rats which were narcotized. The abdominal cavity was incised to obtain arterial blood, which was allowed to stand for 2 h at room temperature (21°C) before centrifugation at 2500 rev min^{-1} for 10 min to obtain serum. The effectiveness of these treatments was assessed in 6 rats of each group by comparing TSH levels, serum T4, serum T3, FT3, FT4 and the final thyroid, pituitary and brain cortex weights (Taurog 1976) of normal rats. The blood samples from the arterial vessel were taken to determine serum T3, T4, FT3 and FT4 levels, which were determined by specific double antibody radioimmunoassay (Snyder & Utiger 1976; Walker et al 1982). The concentration of TSH in the serum was measured by RIA using TSH kits provided by Abbott Co. Ltd. (Chicago, USA) (Martelo et al 1967; Hershman 1974).

Haemodynamic variables in hyperthyroid rats

The hyperthyroid rats were obtained by subcutaneous injection for 12 days with a daily dose of L-T4 300 μg kg^{-1} and then daily intraperitoneally with (±)-THP (2·5, 5·0, 10·0 mg kg^{-1}) or propylthiouracil for a daily dose of 5·0 mg kg^{-1}. On the 15th day, the experimental procedures were the same as those for normal rats described above.

Effect of (±)-THP on TRH-induced TSH level in normal and hyperthyroid rats

The normal rats and hyperthyroid rats which were induced by daily subcutaneous injection of L-T4 (300 $\mu g\ kg^{-1}$) for 12 days, were given daily intraperitoneal injections of (±)-THP (2·5, 5·0 or 10·0 mg kg^{-1}) for 14 days. On the 15th day, 300 $\mu g\ kg^{-1}$ TRH was intraperitoneally injected. Serum was obtained as above and assayed for TSH.

Drug treatment

(±)-THP was dissolved in distilled water containing 10% phosphoric acid (pH values were adjusted to 4·5 with solid NaOH). Propylthiouracil and L-T4 were dissolved in 0·5 M NaOH isotonic saline. L-T4 300 was dissolved in 0·5 M NaOH isotonic saline, subcutaneously injected daily for 12 days. TRH was dissolved in saline. All drugs were purchased from Sigma Chemical Co. (St Louis, MO).

Statistical analysis

Data were analysed using one-way analysis of variance followed by Duncans multiple range test. $P < 0.05$ was considered to be significant.

Results

The effects of (±)-THP on thyroid function in rats are shown in Tables 1 and 2. The effects of (±)-THP on thyroid, pituitary and cortex weight are shown in Tables 3 and 4. The effect of (±)-THP on TSH level induced by TRH is illustrated in Fig. 1.

Discussion

The hormones secreted by the hypothalamus and the anterior pituitary are peptides or proteins that act by binding to specific hormone receptor sites on their target tissues. The hypothalamic regulatory hormone, TRH, controls the release of TSH from the anterior pituitary. TSH controls the secretion of T3

Table 1. Effect of (±)-THP on thyroid function in normal rats.

Drugs	Dose ($mg\ kg^{-1}$)	TSH (μ units mL^{-1})	T3 ($ng\ dL^{-1}$)	T4 ($\mu g\ dL^{-1}$)	FT3 ($ng\ dL^{-1}$)	FT4 ($ng\ dL^{-1}$)
Control	–	2·3 ± 0·2	54·1 ± 3·4	2·7 ± 0·2	1·2 ± 0·1	2·0 ± 0·2
THP	2·5	1·9 ± 0·1	56·8 ± 3·1	2·3 ± 0·5	1·3 ± 0·1	1·7 ± 0·5
	5·0	2·5 ± 0·4	58·8 ± 3·7	2·5 ± 0·2	1·2 ± 0·2	2·1 ± 0·2
	10·0	1·9 ± 0·1	49·1 ± 2·5	2·3 ± 0·3	1·2 ± 0·1	1·9 ± 1·5
Propylthiouracil	5·0	4·0 ± 0·5***	35·4 ± 3·6**	1·0 ± 0·2***	0·5 ± 0·1***	0·5 ± 0·1**

Mean ± s.e.m. of six experiments. $^{**}P < 0.01$, $P < 0.005$ compared with control group.

Table 2. Effects of (±)-THP on thyroid function in L-T4-induced hyperthyroid rats.

Drugs	Dose ($mg\ kg^{-1}$)	TSH (μ mL^{-1})	T3 units($ng\ dL^{-1}$)	T4 ($\mu g\ dL^{-1}$)	FT3 ($ng\ dL^{-1}$)	FT4 ($ng\ dL^{-1}$)
Control	–	2·3 ± 0·2	54·1 ± 3·4	2·7 ± 0·2	1·2 ± 0·1	2·0 ± 0·2
Hyperthyroid	–	1·0 ± 0·1	72·8 ± 8·9	6·6 ± 0·5	4·1 ± 0·5	9·1 ± 0·9
+ THP	2·5	0·9 ± 0·1	49·9 ± 8·9*	5·1 ± 0·1*	1·8 ± 0·6*	5·7 ± 0·9**
+ THP	5·0	0·8 ± 0·1	42·4 ± 4·3**	4·9 ± 0·2*	1·4 ± 0·6**	5·7 ± 0·9**
+ THP	10·0	0·9 ± 0·1	50·9 ± 1·2*	5·1 ± 0·2*	1·5 ± 0·6**	5·3 ± 1·1**
+ propythiouracil	5·0	3·8 ± 0·7***	37·8 ± 4·0**	1·6 ± 0·3***	1·0 ± 0·1***	3·2 ± 0·3**

Mean ± s.e.m. of six experiments. Hyperthyroidism induced by L-T4 300 $\mu g\ kg^{-1}$, subcutaneously for 12 days. $^{*}P < 0.05$, $^{**}P < 0.01$ $P < 0.005$ compared with hyperthyroid group.

Table 3. Effects of (±)-THP on rat thyroid, pituitary and cortex weight in normal rats.

Drugs	Dose ($mg\ kg^{-1}$)	Thyroid (mg/100 g)	Pituitary (mg/100 g)	Cortex (mg/100 g)
Control	–	8·0 ± 1·2	14·5 ± 2·6	30·4 ± 6·0
THP	2·5	7·3 ± 1·5	15·3 ± 2·5	32·5 ± 5·9
	5·0	7·9 ± 1·0	14·8 ± 2·5	31·3 ± 4·6
	10·0	7·6 ± 0·8	13·6 ± 3·2	28·5 ± 4·6
Propylthiouracil	5·0	16·0 ± 0·8**	15·4 ± 3·2	33·1 ± 2·6

Mean ± s.e.m. of six experiments. $^{**}P < 0.01$ compared with control.

Table 4. Effects of (±)-THP on rat thyroid, pituitary and cortex weight in L-T4-induced hyperthyroid rats.

Drugs	Dose ($mg\ kg^{-1}$)	Thyroid (mg/100 g)	Pituitary (mg/100 g)	Cortex (mg/100 g)
Control	–	7·4 ± 0·9	16·4 ± 1·2	29·6 ± 5·6
Hyperthyroid	–	8·0 ± 1·5	15·6 ± 0·8	30·4 ± 4·9
THP	2·5	7·1 ± 0·6	15·8 ± 0·7	29·9 ± 4·2
	5·0	7·2 ± 0·5	15·8 ± 1·9	30·4 ± 2·7
	10·0	6·7 ± 0·7	16·5 ± 1·3	29·1 ± 1·9
Propylthiouracil	5·0	16·8 ± 0·8**	16·6 ± 1·1	30·5 ± 2·4

Mean ± s.e.m. of six experiments. Hyperthyroidism induced by subcutaneous L-T4 300 $\mu g\ kg^{-1}$, for 12 days. $^{**}P < 0.01$ compared with control.

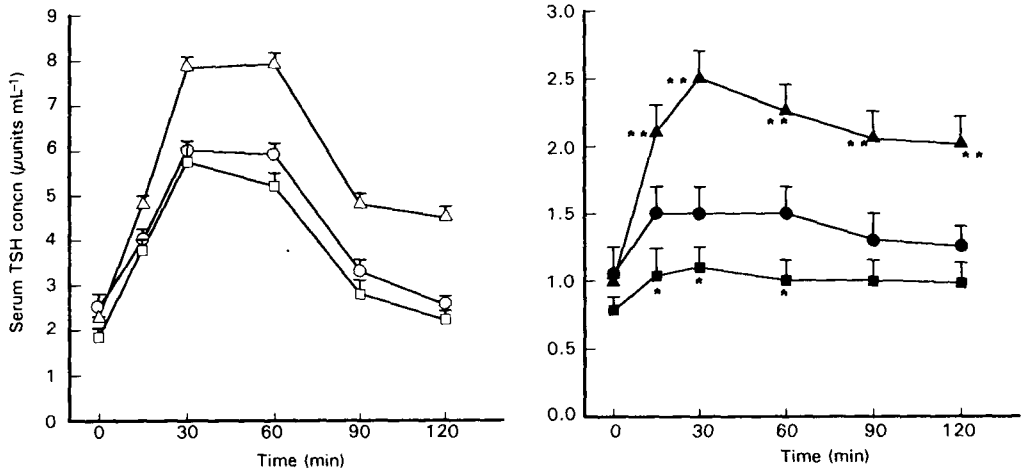

FIG. 1. Effects of 5 mg kg^{-1} propylthiouracil (△), 5 mg kg^{-1} (±)-THP (□), saline (○), 300 μg kg^{-1} L-T4 (●), L-T4- +5 mg kg^{-1} propylthiouracil (▲), or L-T4- +5 mg kg^{-1} (±)-THP (■) for 14 days on serum TSH during TRH stimulation in rats. TSH concentration was measured at time 0, 30, 60, 90, 120 min after TRH injection on the 15th day. Data are presented as the mean ± s.e.m. of 6 rats. $*P < 0.05$, $**P < 0.01$ compared with L-T4 rats.

and T4 from the thyroid cell. Consequently, the secretion of this tropic hormone is partially regulated by a direct inhibitory feedback of high circulating thyroid hormone levels on the pituitary and partially via neural mechanisms operating through the hypothalamus.

Hyperthyroidism is related to the imbalance of thyroid hormone regulation. Thioureylene drugs such as propylthiouracil and methimazole, are extensively employed in the treatment of Graves' hyperthyroidism. They act similarly on the thyroid to block thyroid peroxidase-catalysed iodination and coupling, and increase the TSH level through feedback stimulation to cause the enlargement of the thyroid cell.

(±)-THP exerted no influence on the T3, T4, FT3, FT4, TSH level in the normal rats. (±)-THP showed a dose-dependent response in decreasing serum T3, T4, FT3 and FT4, but had no influence on serum TSH in hyperthyroid rats. However, propylthiouracil decreased serum T3, T4, FT3 and FT4 in normal and hyperthyroid rats. Moreover, propylthiouracil increased the TSH level in the normal and hyperthyroid rats. Low dosages of antithyroid drugs induce hypothyroidism characterized by an increased TSH and a decreased serum T3 or T4 level or both (Mannisto et al 1979). Our results indicate that (±)-THP and propylthiouracil could decrease the thyroid function. However, the mode of action of (±)-THP is different from that of propylthiouracil, which can decrease the thyroxine function and increase the TSH level through feedback stimulation to cause enlargement of the thyroid. We therefore investigated the effects of (±)-THP on the thyroid, pituitary and cortex weights.

(±)-THP exerted no influence on the thyroid, pituitary and cortex weight in normal and hyperthyroid rats. However, propylthiouracil remarkably increased the thyroid weight in normal and hyperthyroid rats. These results indicated that (±)-THP could decrease the thyroxine function, but had no feedback stimulating effect on TSH level.

The inhibition of TSH may be evaluated by determining the serum TSH which responded to thyrotropin-releasing hormone (TRH) in normal subjects (Anderson et al 1971). Neither (±)-THP nor propylthiouracil exerted an influence on the TSH level induced by TRH in normal rats. (±)-THP decreased the TSH level induced by TRH in hyperthyroid rats; however propylthiouracil increased the TRH-induced TSH level in the hyperthyroid rats. These results indicate that the mode of action of (±)-THP on hyperthyroidism may be related to the inhibition of TSH in the pituitary, whereas propylthiouracil inhibits the formation of thyroid hormones in the thyroid gland.

Acknowledgements

This study was supported by grants from the National Sciences Council (Grant NSC-82-0420-B-039-003-M13).

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