The Effect of Nicotine on Thyroid Function in Rats

R. Colzani, S.L. Fang, S. Alex, and L.E. Braverman

Very recently, it has been reported that subclinical hypothyroidism is more severe and peripheral markers of hypothyroidism are more pronounced in women with subclinical or overt hypothyroidism who smoke. Increased concentrations of the known goitrogen thiocyanate, generated from cigarette smoke, have been the major explanation for the decreased thyroid function in these women but do not explain the reported increased peripheral markers of hypothyroidism. There are no data on the effect of the other major product of cigarettes, nicotine, on thyroid function in vivo. The present studies were therefore performed to determine the effects of large doses of nicotine infused for 7 days on thyroid function, outer-ring 5′deiodinase activity (5′D-I), and hepatic malic enzyme activity (a measure of thyroid hormone action) in euthyroid, subclinically hypothyroid (hemithyroidectomized), and L-thyroxine (L-T_4)–treated thyroidectomized rats. Nicotine infusion had no effect on serum T_4, triiodothyronine (T_3), thyrotropin (TSH), and cholesterol concentrations, intrathyroidal metabolism of ^{125}I, liver and kidney 5′D-I activity, and hepatic malic enzyme activity in euthyroid and subclinically hypothyroid rats. Nicotine administration also did not affect serum T_3, TSH, or cholesterol concentrations, liver and kidney 5′D-I activity, and hepatic malic enzyme activity in L-T_4–treated thyroidectomized rats. These studies provide strong evidence that nicotine is not responsible for the observed adverse effects of smoking on the thyroid in humans.

CIGARETTE SMOKING is associated with an increased incidence of goiter,[1-4] autoimmune thyroid disease,[3,5] including Graves' disease with and without ophthalmopathy,[6-8] and variations in thyroid function. It has been reported that cigarette smoking may increase[1,3,9] or decrease[10] thyroid function. Very recently, it has been reported that subclinical hypothyroidism is more severe and peripheral markers of hypothyroidism are more pronounced in women with subclinical and overt hypothyroidism who smoke,[11] and that smoking may increase the risk of hypothyroidism in patients with Hashimoto's thyroiditis.[12] Increased concentrations of the known goitrogen thiocyanate, generated from cigarette smoke, have been the major explanation for the decreased thyroid function[4,13] but do not explain the reported increased peripheral markers of hypothyroidism in these women.

However, there are essentially no data on the effects of the other major product in cigarettes, nicotine, on thyroid function in vivo, although no effect was observed when nicotine was added to cultured porcine thyroid follicles.[14] Thus, we studied the effect of nicotine on thyroid function and thyroid hormone metabolism (hepatic and kidney type I iodothyronine 5′deiodinase [5′D-I] activity) and action (hepatic malic enzyme activity) in euthyroid, subclinically hypothyroid (hemithyroidectomized), and hypothyroid (thyroidectomized) rats.

MATERIALS AND METHODS

Experimental Design

Experiment 1. Adult male CD rats weighing 275 to 300 g were divided into two groups. Beginning on day 1, one group received nicotine continuously through a subcutaneous Alzet pump (Palo Alto, CA) at the rate of 2 mg/kg/d for a total of 7 days. This dose of nicotine results in serum cotinine (a major metabolite of nicotine) levels corresponding to those achieved in humans who smoke approximately 2 packs of cigarettes daily, and is the dose commonly used in other nicotine studies in rats. The control group received the same volume of normal saline through an Alzet pump for 7 days. At the end of the seventh day of infusion, the rats were killed. Another identical experiment was performed to determine the effects of nicotine on intrathyroidal metabolism of ^{125}I.

Experiment 2. Adult male CD rats weighing 275 to 300 g were divided into two groups. Both groups were hemithyroidectomized under ketamine anesthesia. Fifteen days later, the nicotine group received nicotine through a subcutaneous Alzet pump at the rate of 2 mg/kg/d for a total of 7 days. The control group received the same volume of normal saline through an Alzet pump for 7 days. The rats were killed at the end of the seventh day of infusion.

Experiment 3. Adult male CD rats weighing 275 to 300 g were divided into four groups. On day 1, three groups underwent a total thyroidectomy under ketamine anesthesia. On day 14, one of these three groups received nicotine continuously through a subcutaneous Alzet pump at the rate of 2 mg/kg/d for a total of 7 days. Another group received the same volume of normal saline through an Alzet pump for 7 days. On the last 3 days of infusion (days 19, 20, and 21), these two groups received L-thyroxine ([L-T_4] 5 μg/100 g/d intraperitoneally [IP]). The third thyroidectomized group received no treatment and served as thyroidectomized control rats. The fourth group of rats were neither thyroidectomized nor treated and served as intact controls. All rats were killed on day 22, 2 hours after the last dose of L-T_4 in the two groups who received it.

Methods

Animals were killed by decapitation, blood was collected, and serum was obtained and stored at −20°C for thyroid hormone, thyrotropin (TSH), and cotinine measurements. The thyroids were carefully dissected and weighed.

In experiment 1, intrathyroidal metabolism of iodide was determined 2 hours after IP administration of 50 μc ^{125}I. The thyroids were quickly removed, weighed, homogenized in 0.5 mL sodium barbital buffer, pH 8.6, containing 20 mmol/L methimazole and 4 mmol/L KI, and kept on ice. An aliquot from each thyroid homogenate was counted in a gamma counter, and ^{125}I uptake was calculated as the percentage of the administered dose. Homogenate samples (10 to 20 μL) were subjected to electrophoresis on Whatman (Maidstone, UK) No. 1 paper at 200 V for 60 minutes at room temperature in Tris maleate buffer, pH 8.6.

From the Division of Endocrinology and Metabolism, University of Massachusetts Medical Center, Worcester, MA.

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Address reprint requests to L.E. Braverman, MD, Department of Medicine, Division of Endocrinology/Metabolism, University of Massachusetts Medical Center, 55 Lake Ave N, Worcester, MA 01655.

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Table 1. Effects of Nicotine Administration on Various Aspects of Thyroid Function and Thyroid Hormone Metabolism and Action in Euthyroid Rats

Group	Cotinine (ng/mL)	Body Weight (g)	Thyroid Weight (mg/100 g)	T_4 (μg/dL)	T_3 (ng/dL)	TSH (μU/mL)	Liver 5′D-I (pmol/min/mg)	Kidney 5′D-I (pmol/min/mg)	Liver Malic Enzyme (μmol/min/ 100 mg)	Serum Cholesterol (mg/dL)
Control (n = 10)	0.44 ± 0.04	364 ± 2.6	22.2 ± 1.1	4.0 ± 0.3	77.4 ± 3.7	55 ± 8	394 ± 38	335 ± 69	2.2 ± 0.2	60 ± 2.2
Nicotine (n = 10)	116 ± 7.4	348 ± 4.6	20.8 ± 0.8	4.3 ± 0.2	78.2 ± 3.4	49 ± 7	377 ± 30	423 ± 68	2.1 ± 0.2	61 ± 2.9

NOTE. Results are the mean ± SE.

Separation of inorganic and organic ^{125}I was visualized by exposing the paper on x-ray film. Areas corresponding to inorganic and organic ^{125}I were cut and counted in a gamma counter, and the percent organification was calculated. For measurement of monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T_3), and T_4 levels, 0.3 mL of each homogenate was hydrolyzed with 20 mg pancreatin (P-1625; Sigma, St Louis, MO) at 37°C in a water bath with constant shaking for 20 hours. Samples were centrifuged at 14,000 x *g* for 10 minutes in a microfuge. Aliquots of the clear supernatant were then analyzed for MIT, DIT, T_3, and T_4 by HPLC (C-18; Waters division of Millipore, Milford, MA). The percentage of each compound was then calculated from HPLC chromatograms.

Liver and kidney samples were homogenized in 4 vol (wt/vol) 250-mmol/L sucrose, 20 mmol/L HEPES buffer (pH 7.0), 1 mmol/L EDTA, and 1 mmol/L dithiothreitol (DTT), and stored at −20°C for determination of 5′D-I activity. 5′D-I activity was measured by the release of radioiodide from 10 μmol/L (^{125}I) reverse T_3 (rT_3) in the presence of 20 mmol/L DTT. Samples were assayed in duplicate, and the results are expressed as picomoles of iodide per milligram per minute.

Serum T_3 and T_4 concentrations were measured in duplicate in random order and in one assay for each hormone by appropriate radioimmunoassays (RIAs). The serum TSH level was measured by RIA using materials kindly provided by the National Pituitary Agency (National Institutes of Health). Cotinine, a major and measurable metabolite of nicotine, was assayed in duplicate by a RIA kit as described by Langone et al.[15] Before using this assay in rats, the serum cotinine level was measured in smokers and nonsmokers and found to be strikingly elevated in smokers, confirming the efficacy of the assay.

Liver cytosolic malic enzyme activity was measured by the method of Hsu and Lardy[16] as a parameter of thyroid hormone action. The serum cholesterol level was measured by spectrophotometry (Clinitech, Worcester, MA).

Values are reported as the mean ± SE. Statistical analyses were performed by ANOVA followed by the Student-Newman-Keuls multiple-comparisons test, Student *t* test, or Dunnett multiple-comparison test where appropriate.

RESULTS

Experiment 1

Serum cotinine was markedly elevated in the nicotine group (116 ± 7.4 ng/mL) compared with the control rats (0.44 ± 0.04 ng/mL, $P < .001$). Nicotine had no effect on thyroid function, as assessed by serum T_3, T_4, and TSH concentrations and intrathyroidal metabolism of ^{125}I, liver and kidney 5′D-I activity, and liver malic enzyme activity. Nicotine administration did not affect serum cholesterol concentrations (Tables 1 and 2).

Experiment 2

Hemithyroidectomized rats had normal serum T_4 and T_3 concentrations and elevated serum TSH values ($P < .001$) compared with euthyroid rats (Fig 1), which defines subclinical hypothyroidism in man. Serum cotinine concentrations were markedly elevated in nicotine-infused rats (Table 3). Nicotine had no effect on serum thyroid hormone, TSH, and cholesterol concentrations, liver and kidney 5′D-I activity, and thyroid hormone action as assessed by hepatic malic enzyme activity in these subclinically hypothyroid, hemithyroidectomized rats (Table 3).

Experiment 3

Serum cotinine values were markedly elevated in nicotine-infused rats (102 ± 16 *v* 4 ± 2, $P < .001$). Serum T_3 concentrations in L-T_4–treated thyroidectomized rats were significantly higher than in euthyroid and thyroidectomized rats ($P < .001$), and serum T_4 values were markedly elevated in these L-T_4–treated rats ($P < .001$). However, no differences in serum T_3 and T_4 values were found between thyroidectomized, L-T_4–treated rats that received nicotine and those that did not (Table 4).

Serum TSH values were markedly higher in thyroidectomized rats and were significantly lower in rats that received L-T_4 versus rats that did not ($P < .001$). Serum TSH values did not differ between thyroidectomized, L-T_4–treated rats that received nicotine and those that did not (Table 4).

Liver 5′D-I activity was significantly lower in thyroidectomized rats ($P < .01$) and was restored to normal by L-T_4 administration. Nicotine administration did not affect liver 5′D-I activity in L-T_4–treated rats. Similar results were observed for kidney 5′D-I activity (Table 4).

Liver malic enzyme activity was significantly lower in thyroidectomized rats compared with all other groups ($P < .05$ to $P < .01$) and was significantly increased in rats receiving

Table 2. Effects of Nicotine Administration on Intrathyroidal Metabolism of ^{125}I in Euthyroid Rats

Group	^{125}I Uptake (% dose/mL)	Iodide Organification (%)	Organified Iodine (%)			
			MIT	DIT	T_3	T_4
Control (n = 6)	5.5 ± 0.4	98.8 ± 0.1	28.1 ± 1.1	41.3 ± 0.7	4.0 ± 0.3	8.3 ± 0.6
Nicotine (n = 6)	5.9 ± 0.9	97.8 ± 1.1	26.3 ± 1.3	39.4 ± 0.7	4.0 ± 0.5	9.5 ± 1.2

NOTE. Results are the mean ± SE.

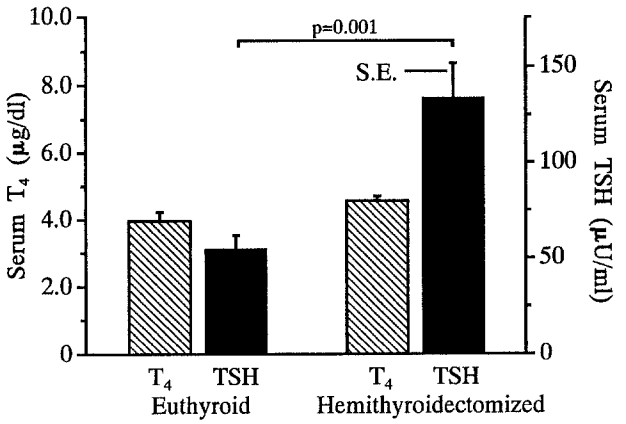

Fig 1. Effect of hemithyroidectomy on serum TSH concentration.

L-T_4. Nicotine administration did not affect malic enzyme activity in L-T_4–treated rats (Fig 2).

Serum cholesterol concentrations were modestly but significantly increased in thyroidectomized rats compared with control ($P < .05$, Dunnett multiple-comparisons test). L-T_4 administration in thyroidectomized rats decreased serum cholesterol, and nicotine administration did not affect this L-T_4–induced decrease of serum cholesterol (Table 4).

DISCUSSION

This study was designed to examine the effects of in vivo administration of nicotine on thyroid function and thyroid hormone metabolism and action in the rat under a variety of clinical situations including euthyroidism, subclinical hypothyroidism, and L-T_4–treated hypothyroidism, in view of data on the adverse effect of cigarette smoking on thyroid function in humans.

In humans, cigarette smoking has resulted in variable effects, either increasing[1,3,9] or decreasing[10,11] thyroid function. In euthyroid rats, we found no effect of large doses of nicotine infused subcutaneously for 7 days on various aspects of thyroid function, including thyroid weight, serum T_4, T_3, and TSH concentrations, intrathyroidal metabolism of ^{125}I, liver and kidney 5′D-I activity, and liver malic enzyme activity. Thus, nicotine does not appear to affect thyroid hormone synthesis, one aspect of thyroid hormone metabolism (5′D-I activity), and one parameter of thyroid hormone action (liver malic enzyme activity). Fukayama et al[14] also found no effect of nicotine on thyroid iodine metabolism and thyroid hormone synthesis in vitro in cultured porcine thyroid follicles, and thus, our negative findings in vivo are not surprising.

The recent report by Müller et al[11] that subclinical hypothyroidism was associated with higher serum concentrations of TSH, cholesterol, and low-density lipoprotein cholesterol in women who smoked versus those who did not suggested that smoking resulted in impaired thyroid hormone synthesis. Increased serum concentrations of cigarette-generated thiocyanate, a known goitrogen, could readily explain the more evident subclinical hypothyroidism observed in the women who smoked. However, it is also possible that an impaired peripheral metabolism and action of thyroid hormones could play a role, and there is no evidence that thiocyanate affects thyroid hormones peripherally. In our rat model of subclinical hypothyroidism, serum cholesterol values were not elevated and large doses of infused nicotine, the other major compound present in cigarettes, had no effect on serum T_4, T_3, TSH, and cholesterol concentrations, peripheral 5′D-I activity, or thyroid hormone action. Thus, nicotine does not appear to be responsible for the changes observed in subclinically hypothyroid women, although our rat model of subclinical hypothyroidism was not associated with elevated serum cholesterol values, a finding consistent with some studies of subclinical hypothyroidism in humans.[17,18]

In women with overt hypothyroidism, smoking had no effect on serum T_4, T_3, and TSH concentrations, but did result in higher clinical scores (evidence of more severe hypothyroidism) and higher serum concentrations of cholesterol and

Table 3. Effects of Nicotine Administration on Various Aspects of Thyroid Function and Action in Subclinically Hypothyroid (hemithyroidectomized) Rats

Group	Cotinine (ng/mL)	Body Weight (g)	Thyroid Weight (mg/100 g)	T_4 (μg/dL)	T_3 (ng/dL)	TSH (μU/mL)	Liver 5′D-I (pmol/min/mg)	Liver Malic Enzyme (μmol/min/100 mg)	Serum Cholesterol (mg/dL)
Control (n = 10)	0.06 ± 0.05	406 ± 10	4 ± 0.26	4.6 ± 0.17	87 ± 5.2	134 ± 19	327 ± 64	1.93 ± 0.2	67 ± 5.5
Nicotine (n = 10)	102 ± 13	396 ± 11	3.8 ± 0.17	4.5 ± 0.24	83 ± 2.4	143 ± 17	288 ± 37	1.69 ± 0.16	65 ± 4.0

NOTE. Results are the mean ± SE.

Table 4. Effects of Nicotine Administration on Various Aspects of Thyroid Function and Thyroid Hormone Metabolism in Hypothyroid (thyroidectomized) Rats

Group	Cotinine (ng/mL)	Body Weight (g)	Serum T_4 (μg/dL)	Serum T_3 (ng/dL)	Serum TSH (μU/mL)	Liver 5′D-I (pmol/min/mg)	Kidney 5′D-I (pmol/min/mg)	Serum Cholesterol (mg/dL)
Control	—	265 ± 8	6.7 ± 0.7	103 ± 9	79 ± 12	228 ± 17	427 ± 61	64 ± 1.1
Thyroidectomized	—	250 ± 21	1.3 ± 0.7	59 ± 2.1	466 ± 112	42 ± 2.9	133 ± 25	84 ± 8.0
Tx + L-T_4	4.0 ± 2.1	243 ± 8	91 ± 11	253 ± 24	35 ± 7	184 ± 29	474 ± 51	54 ± 9.6
Tx + L-T_4 + N	102 ± 16	245 ± 10	95 ± 6.1	222 ± 21	40 ± 9	177 ± 40	392 ± 52	68 ± 7.0

NOTE. Results are the mean ± SE.
Abbreviations: Tx, thyroidectomized; N, nicotine.

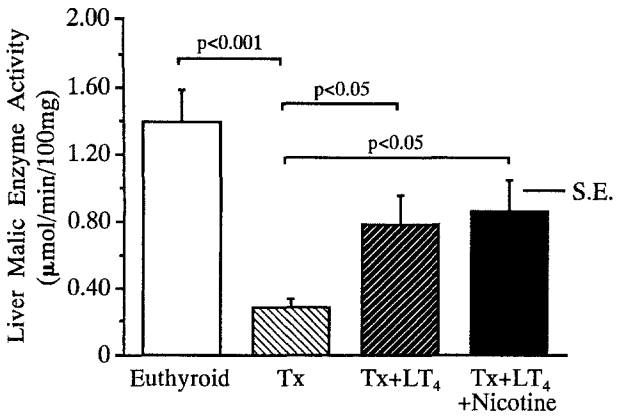

Fig 2. Effect of nicotine infusion on hepatic malic enzyme activity in L-T_4–treated thyroidectomized rats.

creatinine kinase.[11] These findings might be due to the effect of smoking on the peripheral metabolism and action of the thyroid hormones. In the present study, L-T_4 administration for 3 days in hypothyroid rats appropriately decreased serum TSH values and increased both liver and kidney 5′D-I activity and hepatic malic enzyme activity, but nicotine infusion in L-T_4–treated rats did not affect either the serum hormone values or the peripheral effects of L-T_4. In our thyroidectomized hypothyroid rats, serum cholesterol concentrations were significantly elevated, but to a lesser degree than reported by others,[19,20] and were decreased by L-T_4 administration. However, nicotine administration did not affect the L-T_4–induced decrease of serum cholesterol.

The present results strongly suggest that nicotine, a major substance derived from cigarettes, appears to have no effect in the rat on various aspects of thyroid gland function and hormone action as observed in subclinically hypothyroid and hypothyroid women. Although thiocyanate, a known inhibitor of thyroid hormone synthesis, can explain the higher serum TSH concentrations observed in subclinically hypothyroid women, to our knowledge, there have been no studies on the peripheral effects of thiocyanate on thyroid hormone metabolism and action. Such studies would be helpful to further explore whether this major smoking-associated substance, thiocyanate, could explain some of the observed effects of smoking on thyroid function in humans. Finally, other substances are present in tobacco and tobacco smoke such as carbon monoxide and a wide variety of heavy metals and trace elements and, although unlikely, could adversely affect overall thyroid function.[21]

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