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Thyroid Hormone Concentrations in Rats after Chronic Nicotine Metabolite Administration (41965)

D. W. SEPKOVIC,[1] N. J. HALEY, C. M. AXELRAD, AND E. J. LAVOIE

Division of Nutrition and Endocrinology, American Health Foundation, Naylor Dana Institute, Dana Road, Valhalla, New York 10595

Abstract. In an attempt to evaluate the observed relationship of chronic cigarette smoking and reduced thyroid hormone activity, the major urinary metabolites of nicotine were administered to rats for 78 weeks. The animals were divided equally into one control (n = 33) and three treatment groups. Treatment group 1 received 0.1% (w/v) continine, group 2 received 0.02% pure *trans*-nicotine-*N'*-oxide, and group 3 received 0.02% of a *trans/cis* mixture (64/36%) of nicotine-*N'*-oxide. Plasma and urinary nicotine and cotinine concentrations were determined as well as a variety of thyroid hormone parameters. Pure *trans*-nicotine-*N'*-oxide was more extensively metabolized to its cotinine end product, relative to the diasteromeric *N'*-oxide mixture. Serum triiodothyronine (T_3) was markedly reduced in animals receiving nicotine-*N'*-oxides, but was not different in the cotinine treatment group when compared to control values. A reduction in serum thyroxine (T_4) values was noted only among those rats receiving the pure *trans*-nicotine-*N'*-oxide. The T_3/T_4 ratio, free T_3 index, T_3 uptake, and rT_3 were altered in animals receiving nicotine-*N'*-oxides. These findings indicate that specific nicotine metabolites alter thyroid hormone concentrations after chronic low-dose administration and possibly do so through back conversion to the parent compound, nicotine.

Recent studies have shown that cigarette smokers are at increased health risk for certain endocrine related disorders (1, 2). Circulating levels of cortisol, growth hormone, and prolactin are increased in male smokers (3) while serum testosterone is decreased (4). Reduced urinary estrogen excretion, probably reflecting lower estrogen production (5), may play an important role in early menopause experienced by female smokers (6).

Previously, we have measured serum thyroid hormone concentrations in cigarette smokers and found significant reductions in both T_3 and T_4 in heavy users (7). Since the metabolism of the sex steroids is modulated by thyroid hormone, it is reasonable to assume that the observed fluctuations in estrogen and androgen activity may arise from thyroid hormone deficiency (8). Alternatively, nonendocrine related systemic disease states could produce similar changes.

Several constituents of tobacco smoke have been shown to exhibit some antithyroid activity (9, 10), and we have chosen to examine specific primary nicotine metabolites for their relevance to thyroid hormone inhibition. While acute bolus nicotine administration has no demonstrated effect on thyroid hormone metabolism in rats (11), chronic exposure to nicotine or its metabolites has not been investigated with reference to thyroid hormone activity.

Method. Male Fischer-344 rats were equally divided into one control and three experimental groups (n = 33). The animals were subjected to a 12-hr photoperiod and received food and water *ad libitum*. Each treatment group received either 0.02% (w/v) pure *trans*-nicotine-*N'*-oxide or a diasteromeric *N'*-oxide mixture (64% *trans*/36% *cis*) or 0.1% cotinine in drinking water for 78 weeks.

Synthesis of nicotine-*N'*-oxides was carried out according to the method of Craig and Purushothaman (12) which yields a diasteromeric product (64% *trans*/36% *cis*) after passage through a column of basic alumina. Pure *trans*-nicotine-*N'*-oxide was obtained by fractional crystallization with ethyl ether. Purity was assessed by HPLC with a L:Chrosorb (10 μm) Alox T column (10 × 250 mm) using ethyl ether:methanol:water (10:10:1) as previously described (13).

Blood samples were obtained by cardiac

[1] To whom all correspondence should be addressed.

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puncture upon termination of the study. Plasma nicotine and cotinine were quantitated by radioimmunoassay after the method of Langone *et al.* (14). This method uses specific antisera produced by injection into rabbits of *trans*-4-carboxycotinine or *trans*-3-succinyl-methylnicotine bound to albumin. The interassay and intra-assay variations are 6% with a sensitivity of 370 pg/ml for nicotine and cotinine.

Plasma T_3 (15) and T_4 (16) were measured by radioimmunoassay. These assays utilize rabbit anti-T_3 or T_4 serum as primary antibody and 8-anilino-1-naphthalene sulfonic acid to inhibit the binding of thyroid hormone to serum binding proteins. Rat thyroid hormone-free serum (Immunochem. Corp., Carson, Calif.) was used in standard curve preparation. Each sample was assayed in duplicate with a within assay variation of 3%.

The level of saturation of thyroid binding globulin (TBG) was assessed by radioimmunoassay (Immunochem. Corp.) after the method of Coleman *et al.* (17). This method relies on the competition between TBG and immobilized T_3 antibody for unbound radioactive triiodothyronine. The results are compared to those obtained using a calibrated standard of lyophilized human serum of known binding capacity. This procedure has a coefficient of variation of 2.1%.

All samples from the control and experimental groups were treated identically and assayed simultaneously in duplicate to eliminate interassay variation.

The free T_3 index (FT_3I) and free T_4 index (FT_4I) were obtained in the following manner. The values of the *in vitro* uptake test were normalized by expressing them as a fraction of the uptake value in a standard normal serum sample (18). This value was multiplied by the serum T_3 or T_4 concentration to give the normalized index value which has a numerical range similar to the respective thyroid hormone concentrations, but no units.

Serum reverse T_3 (rT_3) was measured according to the method of Chopra (19). This RIA uses anti-rabbit reverse T_3 as primary antibody and polyethylene glycol solution as a precipitating agent (Serono Diagnostics). The assay has a within assay coefficient of variation of 5.0% and a sensitivity of 4.0 pg.

Dunnett's procedure for multiple comparisons with a standard was used to compare treatment groups with control group values. All P values were set at the 0.05 level of significance unless otherwise stated.

Results. Mean serum thyroid hormone concentrations were measured in each treatment and control group (Table I). Significant ($P < 0.005$) reductions in serum T_3 were apparent in both nicotine-*N'*-oxide treatment groups. Rats receiving cotinine had mean serum T_3 values that were similar to control group concentrations.

Mean serum T_4 values were reduced in treatment group 2 (100% *trans*-nicotine-*N'*-oxide), but not in any other group. T_3/T_4 ratios were reduced in both *N'*-oxide treat-

TABLE 1. SERUM THYROID HORMONE CONCENTRATIONS IN RATS ADMINISTERED COTININE AND NICOTINE-*N*-OXIDES

	Serum T_3 (ng/dl)	Serum T_4 (μg/dl)	T_3/T_4 ratio	T_3 uptake (%)	FT_3 index	rT_3 (pg/ml)	FT_4 index
Control ($n = 30$)	165 ± 7.6[a]	3.8 ± 0.2	43.5	59.3 ± 0.8	162 ± 8	67.3 ± 5.8	3.6 ± 0.2
Cotinine ($n = 29$)	152 ± 11	4.1 ± 0.4	37.2	64.1 ± 0.9[b]	133 ± 16	83.3 ± 12.2	4.5 ± 1.5
Nicotine-*N*-Oxide (100% *trans*) ($n = 33$)	99.0 ± 6.4[b]	3.2 ± 0.1[b]	30.9	65.4 ± 0.7[b]	111 ± 8[b]	95.8 ± 8.1[c]	4.8 ± 1.2
Nicotine-*N*-Oxide (64% *trans*-36% *cis*) ($n = 32$)	87.7 ± 6.3[b]	3.9 ± 0.1	21.7	63.1 ± 0.8[b]	93.7 ± 8.9[b]	89.1 ± 7.3	3.9 ± 0.2

[a] Each value is equal to the mean ± SEM.
[b] Significantly different from control group value ($P < 0.005$).
[c] Significantly different from control group value ($P < 0.05$).

TABLE 2. NICOTINE AND COTININE CONCENTRATIONS IN RATS ADMINISTERED COTININE AND NICOTINE-*N*-OXIDES

	Serum levels		Urine levels	
	Nicotine (ng/ml)	Cotinine (ng/ml)	Nicotine (ng/mg creatinine)	Cotinine (μg/mg creatinine)
Cotinine (n = 29)	ND[a]	23,206 ± 1300	76.4 ± 5.8	>650
Nicotine-*N*-oxide (100% *trans*) (n = 33)	119 ± 12	995 ± 125	6150.0 ± 880.1	18.7 ± 2
Nicotine-*N*-oxide (64% *trans*-36% *cis*) (n = 32)	123 ± 17	605 ± 144	3300 ± 269	14.3 ± 1.9

[a] ND = Not detected.

ment groups, but percentage T_3 uptake was significantly above control group concentrations in all experimental groups. Both nicotine-*N'*-oxide treatment groups demonstrated significant ($P < 0.005$) reductions in free T_3 indices when compared to mean control group values. An increase in serum rT_3 was observed in treatment group 3. Serum TSH levels were not quantitated due to lack of a sample for analysis.

Mean serum and urinary nicotine and cotinine concentrations were determined for each group and are presented in Table II. No serum nicotine was observed in the cotinine treatment group, and negligible amounts of nicotine were observed in the urine of these animals. In both nicotine-*N'*-oxide treatment groups, significant concentrations of nicotine were measured in both serum and urine.

Serum and urinary cotinine were also evaluated in all treatment groups (Table II). Approximately one-third more cotinine was detected in the pure *trans*-nicotine-*N'*-oxide treatment group in both serum and urine than was measured in treatment group 3 (64% *trans*-/36% *cis*-nicotine-*N'*-oxide). Random urine collections were normalized by measure of the creatinine content. Excretion of urinary nicotine and cotinine are expressed per milligram creatinine.

Body weights in both nicotine-*N'*-oxide treatment groups were significantly below control group values throughout the time course of the study (Fig. 1). All animals appeared in reasonably good health throughout the duration of the study.

Discussion. Chronic administration of nicotine-*N'*-oxides reduces mean serum T_3 and

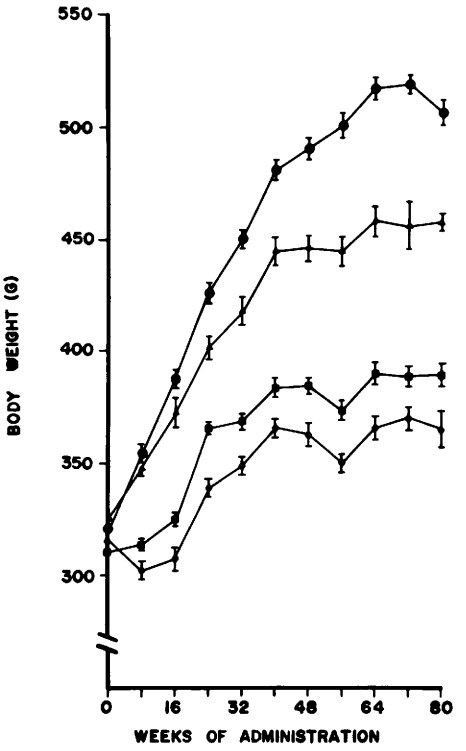

FIG. 1. Mean body weights in rats receiving primary nicotine metabolites for 78 weeks. Each point equals the mean ± SEM.

T_4 and elevates rT_3 concentrations in rats. These findings may be especially relevant since reduced thyroid hormone concentrations in human chronic heavy cigarette smokers have been shown previously (7).

Our overall profile of thyroid hormone parameters in rats (Table I) closely resembles a category of disorders that inhibit T_3 neogenesis in humans. This variety of systemic illness is referred to as "low T_3 syndrome" (20). The observed decreases in serum T_3 concentrations, usually accompanied by increases in serum rT_3, are attributed to a reduction in the 5'-monodeiodination of T_4 and rT_3 so that the formation of T_3 and the degradation of rT_3 are slowed (18). Our observed reductions in FT_3I in the nicotine-*N'*-oxide treatment groups provide further evidence for this conclusion since reductions in free T_3 indices are also sometimes associated with "the low T_3 syndrome" (21). Finally, the values for the *in vitro* T_3 uptake tests are significantly increased in our study, which corresponds to similar observations made by others in the analysis of nonthyroid illnesses (22). The observed reduction of serum T_4 concentrations in the *trans*-nicotine-*N'*-oxide treatment group is not unusual in some forms of severe systemic illness.

We have measured plasma and urinary nicotine and cotinine levels in our various treatment groups (Table II). Substantial concentrations of plasma and urinary nicotine were observed in both nicotine-*N'*-oxide treatment groups which could result from back conversion of these metabolites to nicotine (23). The possibility exists that the observed thyroid hormone alterations may result from chronic nicotine exposure rather than the action of nicotine-*N'*-oxide metabolites. Alternatively, reduced body weight in the nicotine-*N'*-oxide treatment groups raises the possibility that our observed alterations in thyroid hormone levels is a secondary effect due to reduced caloric intake as the result of nonthyroid related illness.

Pure *trans*-nicotine-*N'*-oxide appeared to readily back convert to nicotine. Treatment group 3 (64% *trans*-/36% *cis*-nicotine-*N'*-oxide) revealed approximately two-thirds the amount of back conversion relative to pure *trans*-nicotine-*N'*-oxide. These results indicate that the *cis* isomer of nicotine-*N'*-oxides may be less readily back converted *in vivo* to its nicotine congener.

In summary, chronic administration of primary nicotine-*N'*-oxides results in reduced thyroid hormone levels. This is most probably an indirect effect since the general patterns of change noted closely resemble those observed in the nonthyroid disease state commonly called "the low T_3 syndrome" in humans.

Back conversion of nicotine-*N'*-oxides to nicotine raises the possibility that observed alterations in thyroid status may be caused by nicotine rather than nicotine-*N'*-oxides. The observation of increased back conversion to nicotine from pure *trans*-*N'*-oxide rather than the isomeric mixture is currently being investigated in relation to metabolism of nicotine in heavy cigarette smokers and the metabolic sequelae including thyroidal alterations and lipoprotein responses.

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