Bulbospinal serotonergic activity during changes in thyroid status

William N. Henley, Linda L. Bellush, and Marc Tressler

Abstract: A three-part study explored the basis for an interaction between changes in thyroid status and bulbospinal serotonin (5HT) metabolism. In experiment 1, three well-characterized models of primary hypothyroidism were all accompanied by significant increases in 5HT metabolism. In experiment 2, circulating thyroid hormone levels were experimentally varied from very low methimazole (Meth) treatment to very high (T_3 implants: 2.5, 5.0, or 7.5 mg triiodothyronine). As in experiment 1, Meth led to elevated 5HT. Hyperthyroidism was accompanied by significant reductions in 5HT, while urinary norepinephrine excretion paralleled 5HT. In experiment 3, rats were subjected to Meth either 2 weeks before or after induction of diabetes with streptozotocin (Stz). Meth prevented Stz-associated reductions in 5HT and attenuated development of hyperphagia. Meth could not reverse established Stz-associated reduction in 5HT or hyperphagia, although both were slightly attenuated. Thus, although the first two experiments argue for a simple inverse relationship between circulating thyroid hormone levels and 5HT in the brain, experiment 3 demonstrated that Stz-associated decrements in 5HT could not be reversed by subsequent lowering of circulating thyroid hormone. Nor did accompanying measurements indicate that glycemic status or circulating levels of leptin were important predictors of 5HT. Thus the interaction between thyroid hormones and 5HT is both more subtle and more complex than previously thought.

Key words: hypothyroidism, hyperthyroidism, serotonin, diabetes mellitus.

Résumé : On a effectué une étude comportant trois expériences pour examiner le fondement de l'interaction entre les variations de l'état de la thyroïde et le métabolisme de la sérotonine (5HT) bulbo-spinale. Dans l'expérience 1, trois modèles bien caractérisés d'hypothyroïdie primaire ont été accompagnés d'augmentations significatives du métabolisme de la 5HT. Dans l'expérience 2, on a fait varier les taux d'hormones thyroïdiennes circulantes pour qu'ils soient très faibles, suite à un traitement au méthimazole (Meth), ou très élevés (implants de T_3 : 2,5, 5,0 ou 7,5 mg de triiodothyronine). Comme dans l'expérience 1, Meth a induit une augmentation de la 5HT. L'hyperthyroïdie a été accompagnée de réductions significatives de 5HT, alors qu'une excrétion urinaire de norépinéphrine s'est produite parallèlement à celles-ci. Dans l'expérience 3, les rats ont été soumis à un traitement au Meth, 2 semaines avant ou après l'induction de diabète par streptozotocine (Stz). Meth a prévenu les réductions de 5HT associées à la Stz et a atténué le développement d'une hyperphagie. Meth n'a pu renverser l'hyperphagie ni la réduction de 5HT associée à STZ, bien que l'une et l'autre aient été légèrement atténuées. Ainsi, bien que les deux premières expériences militent en faveur d'une simple relation inverse entre les taux d'hormones thyroïdiennes et la 5HT dans le cerveau, l'expérience 3 a démontré que les diminutions de 5HT associées à Stz n'ont pu être renversées par une autre diminution des taux d'hormones thyroïdiennes circulantes. Les mesures d'accompagnement n'ont pas indiqué non plus que l'état glycémique ou les taux circulants de leptine étaient d'importants prédicteurs de 5HT. Ainsi l'interaction entre les hormones thyroïdiennes est à la fois plus subtile et plus complexe qu'on croyait.

Mots clés : hypothyroïdie, hyperthyroïdie, sérotonine, diabète sucré.

[Traduit par la Rédaction]

Introduction

The abundance of CNS thyroid hormone receptors (Oppenheimer 1979) and hormonal transporters (Dratman et al. 1991), as well as neurochemical and various biochemical responses to hormonal manipulation (Nakamura et al. 1987), argue for functional importance of thyroid hormones in the adult brain. Clinical syndromes such as myxedematous coma and cerebellar ataxia (Crantz et al. 1982), as well as the recognized importance of thyroid hormones as adjunctive therapy for the treatment of depression (Henley and Koehnle 1997), strengthen the hypothesis that thyroid hormones play an important role in the mature mammalian brain.

This laboratory has provided extensive evidence that hypothyroidism is accompanied by robust increments in the activity of bulbospinal serotonergic neurons (Henley et al. 1991; Henley and Bellush 1992). Thus, hypothyroid rats have an increased concentration of brainstem and spinal cord 5-hydroxyindoleacetic acid (5HIAA) and displayed enhanced disappearance of serotonin (5HT) after inhibition of tryptophan hydroxylase with *p*-chlorophenylalanine. These changes are accompanied by increases in indices of

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sympathetic nervous system activity. Furthermore, alterations in cardiovascular responsiveness to the central administration of serotonergic agonists in hypothyroidism provide more direct evidence that functional consequences accompany the neurochemical changes (Henley and Vladic 1997). The importance of these findings is strengthened by the well-described importance of bulbospinal serotonin in cardiovascular regulation (Coote 1990; Feldman and Galiano 1995) and autonomic regulation, in general (Morrison 1993). Thus, it is clear that the neurochemical indices of bulbospinal serotonergic activity are altered during hypothyroidism, and these indices provide an important insight into accompanying pathophysiological changes.

Most investigations of 5HT–thyroid interaction in the adult brain have focused on hypothyroidism. Far less is known about the impact of excessive thyroid hormone on serotonergic activity, particularly concerning bulbospinal serotonergic neurons, which are the focus of this report. Evidence of decrements in 5HT metabolism in brainstem and spinal cord in hyperthyroidism, a pathological state known to be accompanied by suppression of the sympathetic nervous system (Nilsson and Karlberg 1983), would bolster the suggestion that changes in thyroid status have an important impact on sympathetic control via modulation of bulbospinal serotonergic mechanisms.

In trying to clarify the nature of thyroid–5HT interactions, the streptozotocin-diabetic rat provides an interesting contrast to the classically hypothyroid rat. Both diabetic rats and rats with primary hypothyroidism have reduced circulating thyroid hormone levels, but diabetic rats have reductions in 5HT metabolism in brainstem and spinal cord rather than the increases found in primary hypothyroidism (Bellush and Reid 1991; Henley and Bellush 1992). In addition, there are reports of reduced noradrenaline turnover (Yoshida et al. 1985) and reduced urinary epinephrine excretion (Bellush and Rowland 1989) in diabetic rats. Thus, as with 5HT, the impact of diabetes-associated hypothyroidism on the sympathetic nervous system is opposite to that of primary hypothyroidism.

Diabetes-associated hypothyroidism extends beyond reduced circulating thyroid hormones; reduced hypothalamic thyrotropin-releasing hormone (TRH) activity leads to a modest depression of the entire hypothalamus–pituitary–thyroid axis (Sanchez and Jolin 1991). A similar pattern occurs in fasting or starvation (Legradi et al. 1997), and in fact, untreated diabetes resembles fasting in other ways. Leptin is a hormone that is uniquely synthesized in adipocytes and is significantly reduced in both diabetes and fasting (Legradi et al. 1997). Primary hypothyroidism is accompanied by a profound decrease in circulating thyroxine and triiodothyronine, but an increase in TRH due to removal of negative feedback. Unlike diabetes and fasting, primary hypothyroidism is accompanied by a "tendency" for an increase in serum leptin (Escobar-Morreale et al. 1997). Taken together, these findings suggest a functional basis for interactions among the thyroid axis, the sympathetic nervous system, and central serotonergic neurons, specifically, in regulating energy fluxes during changes in substrate availability.

The purpose of the present study was to analyze changes in 5HT as a function of varying levels of circulating thyroid hormone levels, from primary hypothyroidism induced with methimazole (Meth), to hyperthyroidism induced with triiodothyronine (T_3) implants, to diabetes-induced hypothyroidism. Functional consequences of the various thyroid states that might be important covariates of changes in bulbospinal serotonergic activity, including blood glucose levels, urinary catecholamine excretion, plasma leptin levels, and food consumption, were also measured.

In experiment 1, we demonstrated robust increases in serotonergic activity in primary hypothyroidism induced by three different experimental methods. All hypothyroid animals also had impaired glucose tolerance. Findings from experiment 2 indicated that, in contrast with hypothyroidism, hyperthyroidism elicits a reduction in bulbospinal 5HT metabolism, accompanied by a reduction in sympathetic activity and leptin levels and elevations in nonfasting plasma glucose.

In experiment 3, we examined 5HT metabolism in rats given Meth either 2 weeks before or 2 weeks after induction of diabetes with streptozotocin. As we previously showed (Henley and Bellush 1992), prior treatment with Meth prevented diabetes-associated reduction in 5HT metabolism. It also attenuated the development of diabetes-associated hyperphagia. However, Meth could not reverse established 5HT reductions when given subsequent to diabetes induction. Likewise, although there was a reduction in hyperphagia, food consumption remained significantly elevated relative to the group receiving Meth then streptozotocin as well as relative to a group that received only Meth.

Materials and methods

Animals

Adult male Sprague–Dawley rats bred and reared in our colony were used in all experiments. In all experiments, rats were housed in groups of three, unless otherwise indicated. The colony was maintained on an artificial 12 h light : 12 h dark cycle with ambient temperature 23 ± 2°C. Pelleted food and tap water were freely available in experiment 2. Powdered chow was given instead of pelleted chow in experiments 1 and 3.

Surgical induction of hypothyroidism entailed thyroparathyroidectomy under general anesthesia (ketamine, 100 $mg \cdot kg^{-1}$, i.m., plus pentobarbital, 20 $mg \cdot kg^{-1}$, i.m.). In addition, two chemical hypothyroid treatments were used in the experiments. Propylthiouracil (PTU, Sigma Chemical Corp., St. Louis, Mo.) was mixed with powdered chow (0.1% w/w) and fed ad libitum to rats. Methimazole (Meth, Sigma) was mixed in tap water (0.02% w/v) and provided as the only available drinking fluid for the duration of the experimental period. Tap water and powdered rat chow were provided to the rats not receiving chemical hypothyroid agents in experiment 1. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the Institutional Animal Care and Use Committee at Ohio University.

Blood glucose and glucose tolerance determinations

Blood glucose values were routinely obtained from blood taken from a tail nick and analyzed using a One Touch glucometer (Johnson & Johnson, Milpitas, Calif.). In experiment 3, additional glucose concentrations were measured in plasma obtained from heparinized trunk blood following decapitation. Measurements were obtained spectrophotometrically using procedures detailed in a commercially available kit (kit 510, Sigma).

To assess glucose tolerance, baseline blood glucose measurements were obtained, after which a 20% glucose solution in distilled water was injected (5 $mL \cdot kg^{-1}$, i.p.). Blood glucose values were obtained 15, 30, and 60 min postinjection.

Hormonal determinations

Plasma concentrations of total thyroxine (T_4; all three experiments), total triiodothyronine (T_3; experiments 1 and 2), and leptin (experiments 2 and 3) were measured by radioimmunoassay. Commercial kits for the measurement of T_4 (Diagnostic Products, Los Angeles, Calif.; intraassay coefficient of variation, 9%; limit of sensitivity, 4.5 $ng \cdot mL^{-1}$ as determined by 90% of maximum binding), T_3 (Diagnostic Products; intraassay coefficient of variation, 7%; sensitivity, 12.4 $ng \cdot dL^{-1}$), and leptin (Linco Research, St. Charles, Mo.; intraassay coefficient of variation, 5%; interassay coefficient of variation, 7%; and sensitivity, 0.5 $ng \cdot mL^{-1}$) were used. All values for each hormone in a given experiment were obtained from a single assay, with the exception of the leptin assay for experiment 2.

Neurochemical determinations

Tissues were weighed, homogenized in ice-cold 0.1 M perchloric acid containing dihydroxybenzylamine as the internal standard, and centrifuged at 10 000 × *g* at 4°C for 10 min, and the supernatant was filtered through 0.2-μm filters. The filtrate was analyzed using HPLC (BAS, C_{18} stationary phase, isocratic delivery system; flow rate, 0.8 $mL \cdot min^{-1}$) with electrochemical detection. The basic mobile phase used for both urinary and tissue measurements consisted of 0.075 M monochloroacetic acid (pH 3.1), 120 $mg \cdot L^{-1}$ sodium octyl sulphate, 4% acetonitrile, and 2 mM Na_2EDTA. Mobile-phase modifications for experiment 2 (tissue samples only) entailed the use of 240 $mg \cdot L^{-1}$ of sodium octyl sulphate and 9.6% acetonitrile, and an increase in electrochemical detector voltage from 0.7 to 0.88 V to allow for the detection of tryptophan. Concentrations of tryptophan, serotonin (5-hydroxytryptamine; 5HT), and the principal metabolite of 5HT, 5-hydroxyindoleacetic acid (5HIAA), were determined by comparisons with internal and external standards and expressed on the basis of tissue wet weight. In addition, 5HIAA was normalized to its parent amine, 5HT, as an indirect measure of 5HT metabolism.

Urinary norepinephrine was analyzed following extraction as detailed in a commercially available procedure (LCEC application note 15; BAS, West Lafayette, Ind.). Norepinephrine excretion was normalized by two methods. Total excretion was calculated from urine volume and concentration and subsequently normalized to body weight. Concentration was also expressed following normalization to creatinine excretion. Urinary excretion of creatinine was determined from the same urine samples via colorimetric techniques described in a commercially available kit (kit 555; Sigma).

Statistical analyses

When repeated measurements were obtained from the same animal, MANOVA with a within-subjects design provided the primary analysis. Otherwise, one- or two-way analysis of variance (ANOVA) with a between-subjects design was used. When significance was found with primary analyses, Fisher's protected LSD was used for subsequent comparisons of individual means in a between-subjects design; a priori comparisons were considered justifiable for comparisons between hypothyroid and euthyroid rats for which significant data had been obtained previously. In addition, a more extensive within-subjects analysis was used to examine food consumption data from experiment 3. On a daily basis, food consumption was compared with baseline measurements to allow a characterization of changes in food consumption that occurred over the last 2 weeks of experimentation. Finally, linear regression was used to test for correlated findings where indicated. All statistics were performed with the software package Statistica (Statsoft, Tulsa, Okla.).

Experimental protocols

Experiment 1: effects of three hypothyroid treatments on 5HT metabolism

Separate groups of rats (n = 8) were surgically thyroparathyroidectomized (Tx), given PTU in their food (PTU), or given methimazole in their drinking water (Meth). A control group received none of the hypothyroid treatments (C). All rats not subjected to Tx were anesthetized and given sham surgeries. Tx rats were provided 0.4% $CaCl_2$ drinking solution for 4 days following surgery. All rats were subsequently fed ground Purina rat chow supplemented with 0.6% $CaCl_2$ for the remainder of the experimental period.

Body weights were monitored weekly, and 3 weeks after initiating hypothyroid treatments, glucose tolerance tests were conducted. Forty-eight hours later, the rats were killed by decapitation. The brain was removed from the skull and placed on an ice-chilled Petri dish; the cerebellum was removed, and the brainstem (defined by coronal cuts at the caudal border of the inferior colliculi and 0.5 mm caudal to the obex) was dissected. Spinal cord (thoracic vertebral segments, T1–T4) was also removed. Tissues were wrapped in foil and frozen at –80°C for storage until performance of neurochemical analyses (described below). Plasma was harvested from the trunk blood, which was collected in chilled, heparinized tubes, centrifuged at 4°C, and stored at –20°C until hormonal analyses (described below) were performed.

Experiment 2: effects of hyperthyroidism on 5HT metabolism

Rats were assigned to one of five treatment groups (n = 8 per group). The hypothyroid group (Meth) was placed on 0.02% methimazole as its only drinking solution, while all other rats received tap water. Two weeks later, slow-release pellets (Innovative Products, Sarasota, Fla.) containing 0, 2.5, 5, or 7.5 mg of triiodothyronine were implanted subcutaneously in the interscapular region of the back while the rats were under ether anesthesia. Five treatment groups resulted: hypothyroid (Meth + 0 mg), euthyroid control (C; 0 mg), a euthyroid T_3 implant group (2.5 mg), a modestly hyperthyroid T_3 implant (5 mg), and a robust hyperthyroid T_3 implant (7.5 mg). Rats were housed individually in suspended wire cages following implantations. Five days later a 24-h urine collection was obtained in acid and frozen for the subsequent measurement of urinary norepinephrine excretion. Forty-eight hours later, the rats were killed by decapitation 7 days postimplant by a protocol similar to that of experiment 1, except for the separation of brainstem into rostral (defined by transverse cuts at the caudal extent of the inferior colliculus and 2 mm rostral to the obex) and caudal (defined by transverse cuts at 2 mm rostral and 0.5 mm caudal to the obex) regions. In addition, three spinal cord segments, from vertebral segments T1–T5, T6–T10, and T11–L2 were obtained. Spinal cord segments were rapidly removed following decapitation, sectioned, and placed into ice-cold saline prior to removal from the vertebral column. Following removal from the vertebral column, spinal cord segments were handled in a manner described for other tissues.

Experiment 3: modulation of 5HT response to hypothyroidism, diabetes, or both

Rats were housed individually in suspended metal cages, and powdered rat chow and drinking solutions were provided ad libitum. Rats were initially assigned to one of two treatment groups: one made diabetic with streptozotocin (Stz; 65 $mg \cdot kg^{-1}$, i.p.; n = 19), the other made hypothyroid with methimazole (Meth; 0.02% in drinking water; n = 16). Meth rats were injected with

Table 1. Body weight and thyroid hormonal measurements in euthyroid control rats and in rats made hypothyroid by treatment with methimazole or propylthiouracil or by surgical thyroidectomy in experiment 1.

Treatment group	*n*	Initial BW (g)	Final BW (g)	T_4 ($ng \cdot mL^{-1}$)	T_3 ($ng \cdot dL^{-1}$)
C	8	522±12	533±12	57.1±3.9	70.2±4.5
Meth	9	522±14	474±12	—	23.9±1.5
PTU	8	524±15	452±10	—	18.3±1.4
Tx	8	522±16	474±13	—	20.5±1.6
ANOVA	ns	$p<0.05$	—	$p<0.05$	

Note: BW, body weight; C, euthyroid control; Meth, methimazole; PTU, propylthiouracil; Tx, thyroparathyroidectomy; and ANOVA, analysis of variance. Values are means ± SE. ANOVA: ns, nonsignificance; $p < 0.05$, significant differences among groups; —, analysis would be inappropriate. T_3 values for the hypothyroid groups are provided for reference, although individual values fell at or close to the sensitivity limits of the assay.

citrate buffer at the same time that Stz rats were injected. Two days later blood glucose concentrations were checked in Stz rats. All Stz rats were found to have a blood glucose ≥200 $mg \cdot dL^{-1}$.

Twelve days after the start of experimentation, daily 24-h measurements of food consumption were initiated. On day 14, each experimental group was subdivided, such that half the Stz rats were placed on Meth and half the Meth rats were injected with Stz. This resulted in four treatment groups: Stz-C (streptozotocin-injected control, $n = 9$), Stz-Meth ($n = 10$), Meth-C ($n = 8$), and Meth-Stz ($n = 8$). Meth-Stz rats were checked for blood glucose concentrations 2 days after injections, and two rats required a second dose of Stz. Food intakes and body weight measurements initiated 2 days prior to the second phase of experimentation were continued throughout the remaining 2 weeks of experimentation. Blood glucose concentrations were obtained on all rats 5 days after the subdivision into four groups, and the rats were killed 2 weeks after the subdivision. Terminal procedures were similar to those described for experiment 1.

Results

Experiment 1: effects of three hypothyroid treatments on 5HT metabolism

Metabolic data

All hypothyroid groups had levels of circulating T_4 below the sensitivity limits of the assay and circulating T_3 concentrations near the limits of sensitivity, which were significantly lower than C values, but did not differ statistically from each other (Table 1).

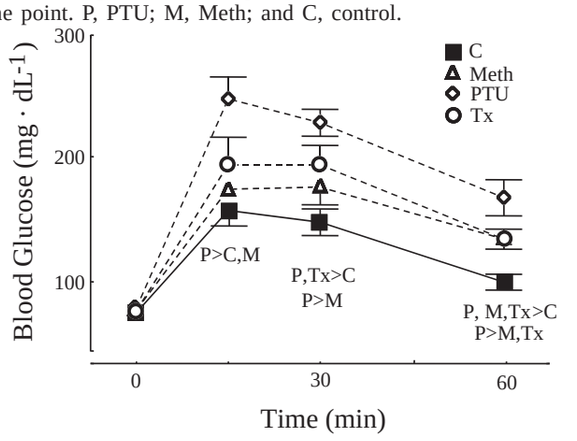
Initially, there were no differences in body weight between the groups. At the end of the 3-week experimental period, all hypothyroid groups had significantly lower body weights than the control group (Table 1). Although the PTU-treated group had a slightly lower mean body weight, there were no significant differences between individual hypothyroid groups when pairwise comparisons were made (LSD; $p > 0.17$).

All three hypothyroid groups had impaired glucose tolerance (Fig. 1; MANOVA, treatment × time, $p < 0.05$). Pairwise comparisons (LSD) indicated that PTU rats were the most glucose intolerant of the three hypothyroid treatment groups, exhibiting higher peak blood glucose concentrations and higher concentrations relative to C at all three time points following glucose challenge. Both Meth and Tx rats showed some impairment, as well, that was statistically significant only at 30 min (Tx only) and 60 min (Meth and Tx).

Neurochemical measurements

One-way ANOVA indicated that the concentration of 5HIAA was significantly higher in both the brainstem and spinal cord of hypothyroid rats, irrespective of treatment, while 5HT concentrations did not differ (Table 2). Pairwise comparisons showed that PTU-treated hypothyroid rats had significantly higher 5HIAA concentrations than the other two hypothyroid groups in both CNS regions assayed. All hypothyroid groups had significantly higher 5HT metabolism than euthyroid controls in both regions, and PTU-induced elevations were again found to be significantly higher than for the other hypothyroid groups (Fig. 2).

Fig. 1. Blood glucose concentrations measured immediately before and 15, 30, and 60 min after intraperitoneal injection of a 20% glucose load in euthyroid control rats and in methimazole-, propylthiouracil-, and surgically induced hypothyroid rats in experiment 1. Data presented are means ± SE. Significant pairwise comparisons (LSD, $p < 0.05$) are indicated below each time point. P, PTU; M, Meth; and C, control.



Experiment 2: effects of hyperthyroidism on 5HT metabolism

Metabolic data

As in experiment 1, Meth-treated rats had plasma T_4 concentrations at or below the limits of sensitivity of the assay, as did the 5- and 7.5-mg implant groups (Table 3). The 2.5-mg implant group also had significantly lower concentrations of T_4 than C. Concentration of T_3 was also significantly decreased in Meth compared with C. In contrast, the 2.5-mg implant group had a small, statistically insignificant increase, while the 5- and 7.5-mg implant groups had significantly

Table 2. Concentration ($\mu g \cdot g^{-1}$) of 5HIAA and 5HT in brainstem and spinal cord of euthyroid control rats and rats made hypothyroid by treatment with methimazole or propylthiouracil or by surgical thyroidectomy in experiment 1.

	Brainstem		Spinal cord	
Treatment group	5HIAA	5HT	5HIAA	5HT
C	0.27±0.01*	0.56±0.01	0.18±0.01*	0.46±0.02
Meth	0.35±0.02	0.53±0.02	0.24±0.01	0.43±0.02
PTU	0.54±0.02†	0.57±0.01	0.31±0.02†	0.45±0.02
Tx	0.36±0.01	0.57±0.02	0.22±0.02	0.42±0.03

Note: C, euthyroid control; Meth, methimazole; PTU, propylthiouracil; and Tx, thyroparathyroidectomy. Values are means ± SE. *Significant difference between C and all three hypothyroid treatment groups in pairwise comparisons (LSD, $p < 0.05$); †significant difference between PTU and both the Meth and Tx groups (LSD, $p < 0.05$).

Table 3. Metabolic measurements in euthyroid control rats, in methimazole-induced hypothyroid rats, and in rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2.

Treatment group	*n*	Initial BW (g)	Implant BW (g)	Final BW (g)	Glucose ($mg \cdot dL^{-1}$)	T_4 ($ng \cdot mL^{-1}$)	T_3 ($ng \cdot dL^{-1}$)	Leptin ($ng \cdot mL^{-1}$)
Meth	8	375±4	376±6*	367±3*	110±8	5±0*	28±1*	3.7±0.3
C	8	376±3	387±4	389±4	118±10	42±1	68±3	3.9±0.5
T_3								
2.5 mg	8	374±4	385±5	386±5	137±5*	20±2*	76±4	3.6±0.2
5.0 mg	8	374±3	383±4	372±4*	130±6	2±1*	125±10*	2.9±0.2*
7.5 mg	8	373±3	385±4	368±4*	140±7*	2±0*	174±12*†	2.4±0.2*

Note: BW, body weight; C, euthyroid control; Meth, methimazole-treated hypothyroid rats. Values are means ± SE. *Significant difference (LSD, $p < 0.05$) relative to C. †Significant difference (LSD, $p < 0.05$) relative to 5.0-mg treatment.

Fig. 2. 5HT metabolism (5HIAA/5HT) in brainstem and spinal cord of euthyroid control rats and methimazole-, propylthiouracil-, and surgically induced hypothyroid rats in experiment 1. Data presented are means ± SE. *Significance (LSD, $p < 0.05$) relative to control values. †Significance (LSD, $p < 0.05$)relative to both Meth and Tx.

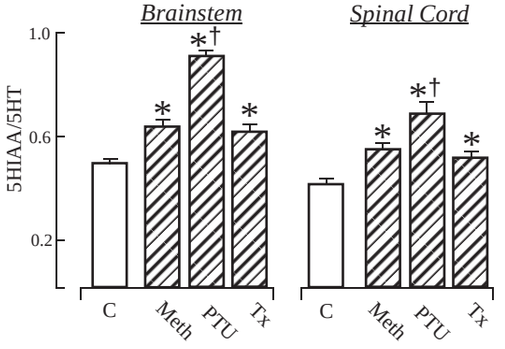
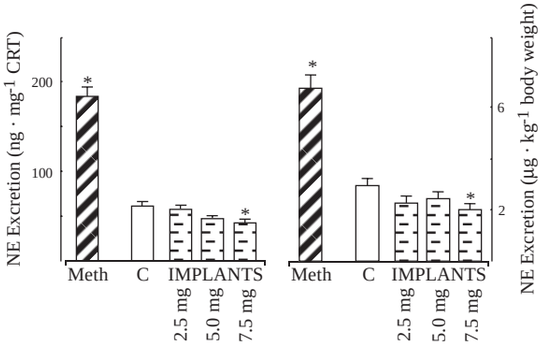


Fig. 3. Urinary norepinephrine excretion of euthyroid control rats, methimazole-treated hypothyroid rats, and rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2. Data presented are means ± SE. *Significance (LSD, $p < 0.05$) relative to control values. CRT, creatinine.



higher circulating T_3 than C. The 7.5-mg group also had significantly higher circulating T_3 than the 5-mg group.

Initial body weights did not differ between groups (Table 3). At the time of T_3 implant 2 weeks later, the Meth group weighed significantly less that the other four groups, which did not differ from each other. At the end of the experiment, 1 week following implants, the 5.0- and 7.5-mg implant groups, as well as the Meth group, had significantly lower body weights than C. Considered together, the body weight and hormonal data characterized the 2.5-mg group as high euthyroid, while the 5- and 7.5-mg groups were clearly hyperthyroid.

Statistically significant differences in urinary norepinephrine excretion were found, whether excretion was normalized to creatinine excretion or body weight (Fig. 3). Subsequent pairwise comparisons indicated an inverse relationship between circulating thyroid hormones and norepinephrine excretion. The Meth group had significantly higher norepinephrine excretion than C, and the 7.5-mg implant group had significantly lower norepinephrine excretion than C.

Significant elevations in nonfasted plasma glucose concentrations, relative to C, were noted in the 2.5- and 7.5-mg implant groups (Table 3). Plasma glucose concentration was also slightly elevated in the 5-mg group, but the difference

Table 4. 5HIAA, 5HT, and tryptophan concentrations in rostral and caudal brainstem of euthyroid control rats, methimazole-induced hypothyroid rats, and in rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2.

	Rostral brainstem			Caudal brainstem		
Treatment group	5HIAA ($ng \cdot g^{-1}$)	5HT ($ng \cdot g^{-1}$)	Tryp ($\mu g \cdot g^{-1}$)	5HIAA ($ng \cdot g^{-1}$)	5HT ($ng \cdot g^{-1}$)	Tryp ($\mu g \cdot g^{-1}$)
Meth	454±17*	635±19	3.2±0.1	414±15*	762±28	3.1±0.1
C	382±19	604±24	3.0±0.1	344±16	723±36	2.9±0.1
T_3						
2.5 mg	386±15	670±31	3.0±0.2	329±18	722±41	2.9±0.1
5 mg	383±39	659±50	3.2±0.2	360±20	756±31	3.2±0.2
7.5 mg	378±19	646±18	3.4±0.1	350±12	764±22	3.2±0.1

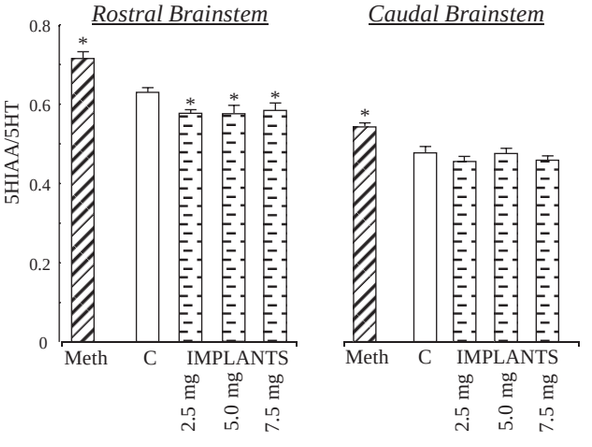
Note: C, euthyroid control; Meth, methimazole; Try, trytophan. Values are means ± SE. *Significant difference between C and the specified group (LSD, $p < 0.05$).

Table 5. 5HIAA, 5HT, and tryptophan concentration in three spinal cord segments of euthyroid control rats, methimazole-induced hypothyroid rats, and rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2.

	T1–T5			T6–T10			T11–L2		
Treatment group	5HIAA ($ng \cdot g^{-1}$)	5HT ($ng \cdot g^{-1}$)	Tryp ($\mu g \cdot g^{-1}$)	5HIAA ($ng \cdot g^{-1}$)	5HT ($ng \cdot g^{-1}$)	Tryp ($\mu g \cdot g^{-1}$)	5HIAA ($ng \cdot g^{-1}$)	5HT ($ng \cdot g^{-1}$)	Tryp ($\mu g \cdot g^{-1}$)
Meth	133±9*	348±11	3.3±0.1	168±5*	450±13	3.3±0.1	347±22*	831±27	2.7±0.2
C	119±3	343±12	3.2±0.2	134±4	409±12	3.0±0.1	299±10	827±31	2.5±0.2
T_3									
2.5 mg	108±5	373±16	3.1±0.1	132±4	438±15	3.1±0.1	277±10	831±30	2.6±0.2
5 mg	111±5	354±12	3.1±0.2	132±6	462±9	3.4±0.2	278±15	834±14	2.7±0.2
7.5 mg	102±2*	343±12	3.2±0.1	121±4	439±16	3.3±0.1	267±8	835±24	2.6±0.1

Note: C, euthyroid control; Meth, hypothyroid control; Tryp, tryptophan. Values are means ± SE. *Significant difference between C and indicated group (LSD, $p < 0.05$).

Fig. 4. 5HT metabolism (5HIAA/5HT) in rostral and caudal brainstem of euthyroid control rats, methimazole-treated hypothyroid rats, and rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2. Data presented are means ± SE. *Significance (LSD, $p < 0.05$) relative to control values.



failed to reach statistical significance. Plasma glucose concentrations of Meth rats also failed to differ statistically from that in C rats.

Plasma leptin levels in the 5- and 7.5-mg implant groups were significantly lower (Table 3), while those in the 2.5-mg and Meth groups did not differ statistically from C. Leptin levels exhibited a negative relationship with glycemic status ($r = -0.43$; $p < 0.05$).

Neurochemical measurements

Neither 5HT nor tryptophan concentration was significantly changed in either brainstem region assayed (Table 4). Similarly, no statistically significant differences in these two variables were noted in any of the three spinal cord segments that were analyzed (Table 5).

In both rostral and caudal brainstem, 5HIAA concentration (Table 4) and 5HT metabolism (Fig. 4) both differed significantly as a function of thyroid status. Subsequent pairwise comparisons indicated that the primary source of significance was due to large increases in Meth relative to C. However, in rostral brainstem, significant decrements in 5HT metabolism occurred in all three implant groups.

In the three spinal cord segments, 5HIAA concentration (Table 5) and 5HT metabolism (Fig. 5) differed significantly as a function of thyroid status. Meth had significantly higher 5HIAA concentration and 5HT metabolism in all spinal cord segments relative to C. Among the implant groups, the concentration of 5HIAA was significantly decreased relative to C only in the 7.5-mg T_3 group and only in segment T1–T5. A significant decrease in 5HT metabolism, relative to C, was noted in segment T1–T5 in the 2.5- and 7.5-mg implant groups; in segment T6–T10 in the 2.5-, 5-, and 7.5-mg implant groups; and in segment T11–L2 in the 7.5-mg implant group. Despite some variability, brainstem and spinal cord neurochemical data reflected a general pattern of increased 5HT metabolism in hypothyroidism and a decrement in

Fig. 5. 5HT metabolism (5HIAA/5HT) in three spinal cord segments of euthyroid control rats, methimazole-treated hypothyroid rats, and rats implanted with T_3 pellets containing 2.5, 5.0, or 7.5 mg of hormone in experiment 2. Data presented are means ± SE. *Significance (LSD, $p < 0.05$) relative to control values.

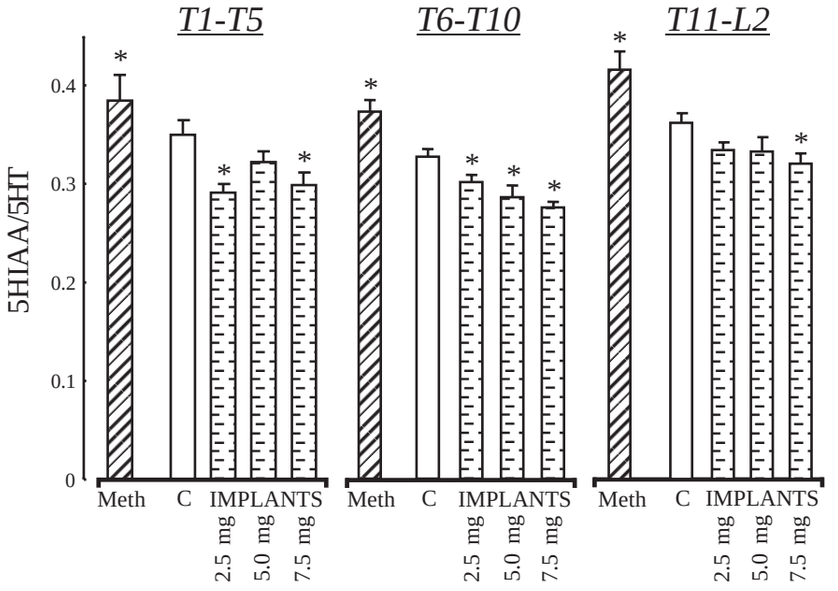


Table 6. Metabolic measurements in rats made diabetic with streptozotocin and then given no further treatment (Stz-C) or given methimazole to induce hypothyroidism as well (Stz-Meth), and in rats first made hypothyroid then given no further treatment (Meth-C) or given streptozotocin (Meth-Stz) in experiment 3.

Treatment group	*n*	Initial BW (g)	Blood glucose, day 20 ($mg \cdot dL^{-1}$)	Plasma glucose, term ($mg \cdot dL^{-1}$)	T_4 ($ng \cdot mL^{-1}$)	T_3 ($ng \cdot dL^{-1}$)	Leptin ($ng \cdot mL^{-1}$)
Stz-C	9	375±4	404±28	526±25	21±3	60±7*	—
Stz-Meth	10	376±3	366±33	496±29	—	40±2	—
Meth-C	8	374±4	78±4*	116±3*	—	38±3	3.0±0.7
Meth-Stz	8	374±3	282±27‡	434±43†	—	34±3	—

Note: BW, body weight; C, control; Meth, methimazole-treated rats; Stz, streptozotocin-treated rats. Values are means ± SE. *Significant differences (LSD, $p < 0.05$) relative to any of the other three groups; †relative to Stz-C, ‡relative to both Stz-C and Stz-Meth. —, values were below the limits of sensitivity of the assay.

hyperthyroidism. It also seemed evident that changes were more robust in hypothyroidism than in hyperthyroidism.

Experiment 3: modulation of 5HT responses to hypothyroidism by diabetes

Metabolic data

T_4 concentrations were above the limits of detection of the assay only in Stz-C. No direct comparisons with non-diabetic euthyroid controls were possible, given the design of this experiment, but the values obtained for Stz-C rats are consistent with a moderately hypothyroid status that we have previously reported (Henley and Bellush 1992). T_3 concentration was higher in Stz-C than in any other treatment group. No significant differences were found among the other three treatment groups.

Blood glucose elevations were evident in Stz rats within 2 days of administration (Stz; blood glucose 326 ± 11 $mg \cdot dL^{-1}$) (Table 6). Streptozotocin was effective in inducing hyperglycemia in rats previously treated with Meth (Meth-Stz; 2 days postinjection blood glucose 240 ± 24 $mg \cdot dL^{-1}$). Subsequent measurements of blood glucose on day 20 of the experiment indicated that the Meth-Stz had significantly lower blood glucose concentrations when compared with either Stz-C or Stz-Meth, obviously because of the different durations of diabetes. By the end of the experiment 8 days later, Meth-Stz rats evidenced further increases in plasma glucose, such that terminal measurements were indistinguishable from those of Stz-Meth, although still significantly lower than Stz-C.

Plasma leptin levels were not detectable in the three streptozotocin-treated groups: Stz-C, Stz-Meth, and Meth-Stz (Table 6). Only in Meth-C were values obtained that were consistently above the limits of sensitivity. The value obtained in this experiment was comparable with that seen for the Meth-treated group in experiment 2. One rat in each of the treatment groups administered streptozotocin had leptin concentrations that were slightly above the limits of detection, although no overlap existed with any individual value obtained for Meth-C rats.

Growth and food consumption

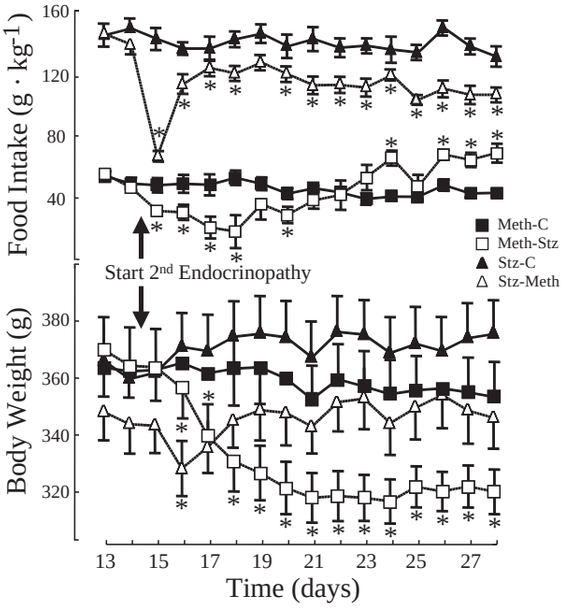
Initial body weights did not differ between groups (Table 6). Daily food consumption and body weights were measured

Table 7. 5HIAA and 5HT concentrations ($ng \cdot g^{-1}$) in brainstem and spinal cord of rats made diabetic with streptozotocin and then given no further treatment (Stz-C) or given methimazole to induce hypothyroidism as well (Stz-Meth), and in rats first made hypothyroid then given no further treatment (Meth-C) or given streptozotocin (Meth-Stz) in experiment 3.

		Brainstem		Spinal cord	
Treatment group	*n*	5HIAA	5HT	5HIAA	5HT
Stz-C	9	194±12	422±16	134±15	421±35
Stz-Meth	10	193±12	399±18	133±11	335±29†
Meth-C	8	329±13‡	450±11	264±12‡	437±18
Meth-Stz	8	266±19*	412±12	226±16‡	407±28

Note: C, control; Meth, methimazole-treated rats; Stz, streptozotocin-treated rats. Values are means ± SE. *Significant differences (LSD, $p < 0.05$) relative to any of the other three groups, †relative to Stz-C, ‡relative to both Stz-C and Stz-Meth.

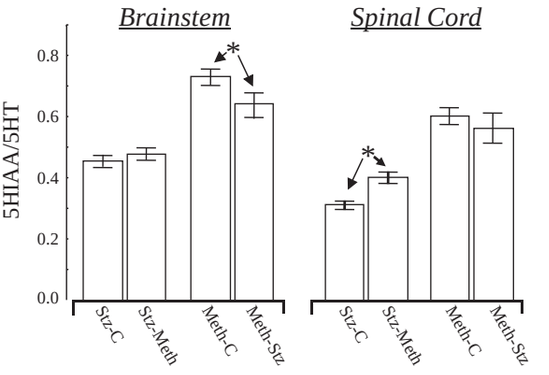
Fig. 6. Daily food intake and body weight of rats made diabetic with streptozotocin and then given no further treatment (Stz-C) or given methimazole to induce hypothyroidism as well (Stz-Meth), and in rats first made hypothyroid then given no further treatment (Meth-C) or given streptozotocin (Meth-Stz) in experiment 3. Data presented are means ± SE. *Significance (MANOVA, within-subjects analysis, $p < 0.05$) when individual values were compared with their baseline values on day 14.



beginning 2 days prior to induction of second endocrinopathies, and continuing until the end of the experiment (Fig. 6). Significant three-way interactions were obtained for both metabolic measurements (MANOVA, $p < 0.05$). Both single endocrinopathy groups exhibited little change in either variable throughout the second half of the study, with Stz-C rats showing consistent hyperphagia in contrast with the low food intake of Meth-C, whereas body weights were similar.

Food intake in Stz-Meth changed significantly during the second experimental period (days 13–28; MANOVA, $p < 0.05$, Stz-Meth analysis in isolation). Food intake dropped precipitously on the first day of Meth, but rebounded almost completely the following day. Nonetheless, a modest but significant reduction in food intake relative to Stz-C persisted for the duration of the experiment, except for day 19

Fig. 7. 5HT metabolism (5HIAA/5HT) in brainstem and spinal cord of rats made diabetic with streptozotocin and then given no further treatment (Stz-C) or given methimazole to induce hypothyroidism as well (Stz-Meth), and in rats first made hypothyroid then given no further treatment (Meth-C) or given streptozotocin (Meth-Stz) in experiment 3. Data presented are means ± SE. *Significant pairwise comparisons (LSD, $p < 0.05$).



(MANOVA, $p < 0.05$, day *x* vs. day 14). In the Meth-Stz group, food consumption dropped from the already low hypothyroid levels on the day of the added endocrinopathy (day 15) and continued to be significantly reduced through day 20 (MANOVA, $p < 0.05$, day 16, 17, 18, 19, or 20 vs. day 14). A gradual increase in food consumption then occurred until it exceeded baseline consumption on days 24, 26, 27, and 28 ($p < 0.05$). By the end of the experiment, an interactive influence of the two endocrinopathies on food consumption was evident (ANOVA, day 28, endocrinopathy 1 × endocrinopathy 2, $p < 0.05$). This finding reflected the reduction in food consumption in Stz-Meth relative to Stz-C (LSD, $p < 0.05$) while Meth-Stz had heightened food consumption relative to Meth-C ($p < 0.05$). In spite of this interaction, the food consumption in Stz-Meth remained markedly higher than that of Meth-Stz.

In contrast with the stable body weights in Meth-C and Stz-C, body weight measurements in Stz-Meth generally reflected the patterns in food intake with an acute drop in weight (days 16 and 17) followed by a subsequent recovery of weight loss. In Meth-Stz, body weight dropped precipitously during the first 5 days of the second experimental period before stabilizing and remaining fairly constant at the new lower level for the duration of the experiment. Between days 14 and 28 of the experiment, changes in body weight

exhibited a strong interactive influence ($p < 0.05$; data not presented), reflecting the large decrement in body weight in Meth-Stz relative to Meth-C (LSD, $p < 0.05$) that was not evident for Stz-Meth relative to Stz-C.

Neurochemical data

In brainstem, concentrations of 5HT did not differ statistically between any of the groups (Table 7). However, 5HIAA concentration was significantly higher in brainstem of rats that initially received Meth. In addition, an interaction was evident resulting from the reduction in 5HIAA concentration in Meth-Stz relative to Meth-C (LSD, $p < 0.05$) which was not seen in Stz-Meth relative to Stz-C. In spite of this reduction in 5HIAA in Meth-Stz, concentrations of 5HIAA remained statistically higher in Meth-Stz than in Stz-C and Stz-Meth. 5HT metabolism in brainstem (Fig. 7) followed a statistically identical pattern.

In spinal cord (T1–T4), the concentration of 5HT was significantly lower in Stz-Meth than in Stz-C (Table 7; ANOVA, $p < 0.05$, LSD). However, Meth-Stz also had lower 5HT than Meth-C, so that there was not a statistically significant interaction, suggesting that this finding was not specific to either primary endocrinopathy. The concentration of 5HIAA was again found to be significantly higher in both groups of rats initially given Meth, relative to their streptozotocin-treated counterparts. 5HT metabolism was significantly higher in rats initially given Meth relative to their streptozotocin-treated counterparts (Fig. 7). In addition, a significant interaction was evident, due to significant elevations in Stz-Meth relative to Stz-C (LSD, $p < 0.05$), with a nonsignificant trend in the opposite direction seen in Meth-Stz versus Meth-C. Despite the significant interaction, 5HT metabolism in both Meth-C and Meth-Stz remained considerably higher than both Stz-C and Stz-Meth (LSD, $p < 0.05$).

Similar patterns between food intake and neurochemical indices of serotonergic activity were apparent. That is, the initial endocrinopathy, either Stz or Meth, was the primary determinant of both food intake and serotonergic activity at the end of the experiment; the second endocrinopathy had little effect on these measured variables. Correlation analysis indicated that significant negative relationships existed between the final day's food consumption and brainstem concentration of 5HIAA ($r = -0.79$), brainstem 5HT metabolism ($r = -0.83$), spinal cord concentration of 5HIAA ($r = -0.84$), and spinal cord 5HT metabolism ($r = -0.77$).

Discussion

Experiment 1 demonstrated significantly increased 5HT metabolism in brainstem and spinal cord of hypothyroid rats, irrespective of the method used to induce the hypothyroid state. Savard et al. (1983) previously showed robust PTU-induced changes in serotonin turnover in various specific brain nuclei of young adult hypothyroid rats. The fact that we replicated this finding, as well as showed the same effect of two other hypothyroid treatments, argues strongly that increased 5HT activity is the result of the hypothyroid state, rather than due to some other effect of the particular treatment used to induce it. Despite the similarity of effects of the three treatments, PTU appeared to produce a more severe hypothyroid state than the other treatments, reflected in body weight reductions and impaired glucose tolerance.

PTU may lead to a greater suppression of thyroid hormones than Meth, because while both Meth and PTU inhibit the activity of thyroid peroxidase activity, thereby reducing thyroid hormone synthesis, PTU also inhibits the activity of type I 5′′-deiodinase in liver and kidney (Silva et al. 1982). Deiodinase inhibition would limit peripheral conversion of T_4 to T_3, the more active thyroid hormone (Oppenheimer 1979). Meth-treated rats might in fact have some residual T_3, whereas PTU-treated rats would not. Surgical thyroidectomy, by totally eliminating the principal source of thyroid hormone, would be expected to suffer at least as profound a hypothyroid state as did the PTU rats. However, the availability of small amounts of T_4 in animal diets (Eales 1997) might offset this deficit in both Meth and surgically thyroidectomized rats, but would be of lesser consequence in PTU rats.

Experiment 2 expanded the examination of thyroid–5HT interactions, looking at the other end of the spectrum, hyperthyroidism. Of paramount interest was determining whether increased circulating thyroid hormones would lead to a decrease in 5HT metabolism. The results, indeed, demonstrated that increasing circulating levels of T_3 led to decreased 5HT metabolism. A methimazole-treated hypothyroid group was also included in the experiment, and this group replicated the finding of hypothyroid-associated increase in 5HT metabolism (experiment 1 and Henley and Bellush 1992). Thus, an inverse relationship between levels of circulating thyroid hormones (from very low to very high) and 5HT metabolism was clearly established. The results in our hyperthyroid rats are consistent with those of Rastogi and Singhal (1976), who demonstrated decreased concentration of 5HIAA in striatum and cerebellum following the administration of high levels of T_3 ($100\ \mu g \cdot kg^{-1} \cdot day^{-1}$) for 30 days. Whether hypothyroid- and hyperthyroid-linked changes in 5HT metabolism reflect altered 5HT release, synaptic availability, and postsynaptic effects has yet to be demonstrated convincingly. However, the central administration of the 5HT agonist 1-(2,5-dimethoxy-4-iodophenyl))-2-amino-propane (DOI) caused a pressor effect in euthyroid rats that was markedly attenuated in hypothyroid rats, suggesting a desensitization of postsynaptic $5HT_2$ receptors in hypothyroid rats (Henley and Vladic 1997).

Reductions in 5HT activity in the hyperthyroid rats were less dramatic than the hypothyroid-associated 5HT increases. The modest response to the T_3 implants is consistent with the finding of nearly full occupancy of thyroid hormone receptors, and therefore the limited number of unbound receptors in the mature euthyroid rat brain (Crantz et al. 1982). If thyroid hormone has an inhibitory effect on 5HT metabolism, and this effect is nearly maximal in euthyroid animals, then increasing the concentration of thyroid hormone to the brain would not be expected to suppress 5HT activity much further. On the other hand, removing thyroid hormone by making animals hypothyroid would produce a dramatic disinhibition of 5HT activity. It should be noted, however, that experiment 3 provides evidence that is potentially inconsistent with speculation that changes in 5HT neurochemical profiles are the direct result of hormonal action.

No changes in tryptophan were observed in the presence of increased or decreased 5HT metabolism. Previously, increased tryptophan concentrations were reported in the central nervous system of the hypothyroid rat (Nozaki 1989). A substrate-driven increase in the activity of tryptophan hydroxylase, the rate-limiting enzyme for 5HT synthesis, would be expected to follow increased tryptophan, given that the enzyme is not fully saturated with substrate (Fernstrom 1983). This in turn would increase the amount of 5HT synthesized, and potentially the amount metabolized. However, in a conflicting study, Mooradian (1990) reported that that tryptophan uptake into the brain was not enhanced in hypothyroid rats. The present findings are consistent with the data of Mooradian (1990).

Changes in urinary norepinephrine excretion paralleled those of 5HT metabolism in both hypothyroid and hyperthyroid rats, extending previous findings of increases in both 5HT activity and sympathetic activity in hypothyroid rats (Henley et al. 1991). The present finding also extended the 5HT measures to a large expanse of the thoracolumbar spinal cord, a well-described location for sympathetic preganglionic neuronal cell bodies. It has long been known that sympathetic neuronal activity is increased in the hypothyroid rat (Landsberg and Axelrod 1968). Also well established is a descending serotonergic pathway from brainstem to sympathetic preganglionic neurons that generally has been characterized as excitatory (McCall 1984; Morrison 1993). Previous findings from our laboratory also indicated that cold-induced sympathoexcitation is accompanied by increases in serotonin synthesis and metabolism (Passerin and Henley 1994). Given the well-characterized synergism between thyroid hormones and the sympathetic nervous system in the face of metabolic challenges (Nilsson and Karlberg 1983), it follows that integration of hormonal and autonomic responses is likely to occur within the CNS. The results of the present study are consistent with previous work indicating that medullary serotonergic neurons projecting to sympathetic preganglionic neurons provide a neuronal link that participates in the coordination of hormonal and autonomic signals (Bowker et al. 1983).

Leptin is a recently discovered hormone that is secreted by white adipocytes in a "well-nourished" state. It is hypothesized to be a hormonal signal that links the "energetic status of adipose tissue to the CNS regulation of energy homeostasis" (Muller et al. 1997), providing a satiety signal that can also increase metabolic rate via hypothalamic receptors. It is notable, therefore, that thyroid hormones have been shown to exert a negative influence on serum leptin concentrations (Escobar-Morreale et al. 1997). Findings in experiment 2 generally conform to this profile, in that the reduced circulating levels were found with hyperthyroidism, a catabolic state that is accompanied by increased food consumption. As such, leptin profiles were lowered in hyperthyroid rats in conjunction with increases in blood glucose levels and reductions in bulbospinal serotonergic metabolism. However, similar measurements in experiment 3 allowed a striking dissociation of both leptin and blood glucose from the serotonergic profile.

Experiment 3 addressed a more complex issue, specifically why experimental diabetes, which leads to a mild hypothyroid state, produces decreased 5HT activity. We previously reported that prior thyroidectomy or methimazole administration interfered with the expected diabetes-associated reductions in 5HT activity (Henley and Bellush 1992). In the present study, methimazole was used to induce primary hypothyroidism either before or after establishing diabetes. We hypothesized that just as prior methimazole treatment prevented diabetes-associated 5HT reductions, methimazole treatment after well-established diabetes might reverse 5HT reductions. This did not occur.

Irrespective of the order in which the endocrinopathies were induced, two groups of rats (Meth-Stz and Stz-Meth) ended up with circulating T_3 and T_4 levels consistent with severe primary hypothyroidism. 5HT activity in the Meth-Stz group resembled the expected pattern of elevation, but the Stz-Meth group continued to have the diabetic pattern of lowered 5HT activity.

Thus, the endocrinopathy initiated first predominated with respect to hormone-dependent changes in 5HT activity. Only small modifications in the initial neurochemical changes occurred when secondary endocrinopathies were superimposed. Parallel findings for food consumption were evident. Diabetes-induced hyperphagia was impacted only transiently and modestly when hypothyroidism was superimposed, while the expected diabetes-induced hyperphagia was largely prevented when the hypothyroidism was established first. Meth-Stz rats continued to evidence extreme hyperglycemia, in spite of its more hypothyroid-like ingestive and neurochemical profiles. Similarly, Stz-Meth rats showed severe hypothyroidism in spite of its more diabetic-like ingestive and neurochemical profiles. Thus, neither glycemic state nor thyroid hormonal status is predictive of 5HT activity in all contexts.

In experiment 3, all Stz-treated had leptin levels too low to detect. This would be expected, since diabetes, like fasting, leads to reductions in adipose tissue (Legradi et al. 1997). Fasting also resembles diabetes in causing central hypothyroidism, i.e., TRH reduction, and leptin administration prevented the fasting-induced suppression of TRH mRNA (Legradi et al.1997). It was suggested that falling leptin, which normally occurs during fasting, resets the set point for feedback inhibition of TRH by thyroid hormones in order to allow adaptation to the fasting state. Leptin was the one measure in this experiment that responded to Stz, irrespective of whether it was given before or after Meth. In both experiments 2 and 3, leptin concentrations were negatively associated with circulating glucose levels rather than food intake, metabolic state, or serotonin metabolism.

The basis for the complex interactive influence that hypothyroidism and diabetes elicit on 5HT activity and food intake cannot be determined from the present study. One metabolic signal likely to be disrupted in the brain of rats with dual endocrinopathies is thyrotropin-releasing hormone (TRH). Thyroid hormone receptors in brain (Sanchez and Jolin 1991), hypothalamic TRH secretion, and thyroid-stimulating hormone secretion (Rondeel et al. 1992) are all decreased in the diabetic rat, but increased in rats with primary experimental hypothyroidism (Bruhn et al. 1991). Therefore, the superimposition of these two endocrinopathies is likely to pose an adaptational dilemma.

TRH is known to be co-localized in medullary serotonergic neurons (Bowker et al. 1983), and medullary TRH mRNA has

been shown to increase during hypothyroidism (Yang et al. 1992). Cold exposure produces increases in TRH expression in both the medulla and the hypothalamus (Arancibia et al. 1996). Thus, a parallel regulation of hypothalamic and medullary TRH may exist, and it has been proposed that parallel changes in hypothalamic and medullary TRH serve to coordinate neuroendocrine and autonomic activity during thermogenesis (Arancibia et al. 1996). The possibility that 5HT activity is coupled with metabolism of co-localized TRH is plausible, although studies of the regulation of medullary TRH in diabetes will be necessary to clarify this point.

In summary, the present studies strengthen and extend findings of the interaction of thyroid status with the activity of serotonergic neurons located in the medullary raphe and with sympathoexcitation. Importantly, a suppression of activity in bulbospinal serotonergic neurons was demonstrated during hyperthyroidism, a finding that was evident in a large expanse of thoracic and lumbar spinal cord, and was shown to co-occur with changes in a global index of sympathetic activity. Results from experiment 3 indicate that thyroid-dependent influences on 5HT neurons cannot be categorized simply by reference to circulating levels of thyroid hormone. Instead, thyroid-dependent changes in 5HT activity are likely to represent a metabolic adaptation that can be influenced by a preexisting metabolic derangement.

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