Hypothyroid-induced changes in autonomic control have a central serotonergic component

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Henley, William N., and Franjo Vladic. Hypothyroid-induced changes in autonomic control have a central serotonergic component. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H894–H903, 1997.—Three experiments were conducted in unanesthetized rats made hypothyroid (Hypo) or maintained as euthyroid controls (Eu) to examine general cardiovascular responsiveness [*experiment I* (*Exp I*)]; responsiveness to a serotonin (5-HT_2) agonist, *dl*-2,5-dimethoxy-4-iodoamphetamine [DOI intracerebroventricularly; *experiment II* (*Exp II*)]; or responsiveness to a 5-HT_{1A} agonist *dl*-8-hydroxydipropyl-aminotetralin hydrobromide [8-OH-DPAT intracerebroventricularly; *experiment III* (*Exp III*)]. In *Exp I*, intravenous infusions of phenylephrine and nitroprusside provided little evidence that findings in *Exp II* and *III* were caused by generalized impairment in cardiovascular responsiveness in Hypo. In *Exp II* and *III*, Eu and Hypo were given either intra-arterial atropine or vehicle. Atropine significantly elevated heart rate (*Exp II* and *III*) and mean arterial pressure (*Exp II*) in Eu only. When compared with Eu, Hypo had a reduced pressor response (5.2 vs. 20.1%), an attenuated pulse pressure response (19.3 vs. 35.4%), and a more robust bradycardia (−17.7 vs. −7.0%) in response to DOI. These differences were atropine sensitive. In *Exp III*, Hypo had larger decrements in mean arterial pressure (−9.0 vs. −5.1%), heart rate (−13.9 vs. −7.7%), and body temperature (−4.5 vs. −2.7%) in response to 8-OH-DPAT in comparison to Eu. Parasympathetic involvement in the differential responses to 8-OH-DPAT was less clear than with DOI. Deranged autonomic control in hypothyroidism may be caused, in part, by changes in central serotonergic activity.

hypothyroidism; serotonin; parasympathetic nervous system; sympathetic nervous system; heart rate

NUMEROUS FINDINGS indicate that various cellular functions in the mature central nervous system may be influenced by the thyroid hormones (7, 10, 26). For instance, 3,5,3′-triiodothyronine (T_3) nuclear receptors in brain were modulated by T_3 concentration in adult rats (26). Most pertinent to the present study is a report that heart rate in hypothyroid rats is more responsive to central rather than peripherally administered T_3 (7).

Evidence exists of an interaction between thyroid hormone and neurochemical activity in the mature brain. For example, reports from various laboratories, including our own, have provided evidence that the thyroid hormones exert an influence on brain stem and spinal cord neurons that utilize serotonin (5-HT) as a neurotransmitter (10, 27). Specifically, hypothyroid rats have increased concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the principal metabolite of 5-HT, in brain stem and spinal cord, and this increase has been shown to be reversible with hormone replacement (10). Enhanced disappearance of 5-HT following synthesis inhibition indicates that the increased concentration of 5-HIAA is accompanied by increased utilization (9).

Although there is ample evidence of altered metabolism of serotonin in brain stem and spinal cord of hypothyroid rats, little is known about the functional significance of these findings. Brain stem serotonergic neurons are believed to play an important role in autonomic control (12, 21). Dense distributions of serotonergic nerve terminals in the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the intermediolateral column of the spinal cord provide an anatomic distribution that would allow a profound influence on the autonomic nervous system (16).

Compromised autonomic control of cardiovascular function is well described with hypothyroidism (22). Despite characteristics more easily associated with a decreased sympathetic state such as bradycardia and decreased contractility, the myocardium of the hypothyroid rat has enhanced turnover of norepinephrine (15). Similarly, hyperthyroid patients are characterized by symptoms characteristic of a hypersympathetic state, although circulating catecholamine levels are low. Deranged parasympathetic control may also play an important role in thyroid-deficient states (13, 17). Recent studies suggest that the serotonergic neurons in the lower brain stem are sympathoexcitatory (21). Because both heightened sympathetic drive and increased serotonergic activity are found in hypothyroidism, we have previously suggested that these findings may be causally linked (10). If true, it seems likely that autonomic responses to a central serotonergic challenge would differ in a hypothyroid subject. In this study we show that there are marked differences in the cardiovascular response to central administration of 5-HT_{1A} and 5-HT_2 agonists in hypothyroid versus euthyroid rats. Surprisingly, a large component of this altered responsiveness appears to be caused by changes in parasympathetic control rather than sympathetic control. Moreover, our findings exclude the possibility that these responses are attributable to a generalized derangement in cardiovascular responsiveness with hypothyroidism.

MATERIALS AND METHODS

Animals. All rats studied were obtained from the Ohio University animal breeding colony and were adult, male rats of the Sprague-Dawley strain. Three weeks before the study, rats were provided either 0.02% methimazole (hypothyroid treatment) or vehicle (tap water) as their only available drinking solution. Fluids and pelleted rat chow were provided ad libitum, and ambient temperature was maintained at 23 ± 2°C. Before the study, rats were anesthetized with ketamine (100 mg/kg im) plus pentobarbital sodium (20 mg/kg im) for necessary catheterizations and instrumentations, were allowed a 24-h recovery period, and subsequently were studied

in an unrestrained, conscious state. At the end of each study, rats were rapidly anesthetized with either an intra-arterial or an intravenous infusion of pentobarbital sodium (70 mg/kg) before decapitation. Blood was collected from the cervical wound for hormonal assays. Drugs were obtained from Sigma Chemical (St. Louis, MO) with the exception of *dl*-2,5-dimethoxy-4-iodoamphetamine (DOI) and *dl*-8-hydroxydipropyl-aminotetralin hydrobromide (8-OH-DPAT), which were obtained from Research Biochemicals International (Natick, MA).

Experiment I: cardiovascular responsiveness in hypothyroidism. Venous (polyethylene; PE-50) and arterial (Teflon, 28 gauge fused to Tygon) catheters were implanted in the external jugular vein and the femoral artery, respectively. After the rats recovered from surgery, we connected catheter extensions, which allowed access to the rats from the exterior of the cage. The arterial catheter was connected to a Statham pressure transducer placed at heart level while the venous catheter was prepared for injections of vasoactive agents. The transducer was connected to a computerized data-acquisition module after amplification, and the pulsatile arterial signal was recorded for subsequent analyses. Evidence of a dicrotic notch was used as documentation of acceptable catheter function.

Care was taken to block the view of the rat and to minimize any external stimuli other than drug injections. A minimum of three resting blood pressures were obtained as baseline measurements before the initiation of drug injections. As a precondition for the start of experimentation, baseline measurements had to be within 10 mmHg mean arterial pressure (MAP) and 15 beats/min heart rate (HR) to be deemed acceptable resting measurements. All rats received both sodium nitroprusside (25 μg/kg, 0.5 ml/kg iv) and phenylephrine (5 μg/kg, 0.5 ml/kg iv), injections separated by at least 20 min. Baseline measurements were reobtained between drug administrations, and the order of administrations was balanced. Continuous recordings of blood pressure were obtained during the drug administrations, which were subsequently used to obtain MAP and HR responses.

Experiment II: cardiovascular responses to central administration of a 5-HT_2 agonist in hypothyroidism. As in *experiment I,* a catheter was placed in the femoral artery. Additionally, a telemetry device (Minimitter, Sunriver, OR) was placed in the abdomen for the measurement of body temperature (T_B) while a 23-gauge stainless steel cannula was implanted in the lateral cerebral ventricle with the aid of a stereotaxic device (0.5 mm caudal and 1 mm lateral to bregma; 4.5 mm deep to the surface of the brain). The cannula was attached with dental cement, which was anchored with stainless steel screws implanted in the skull. Handling of the rats was similar to that of *experiment I,* with the exception that the cerebral cannula was connected to a line containing DOI connected to an infusion pump and a telemetric monitor was placed on the top of the cage to monitor temperature before the start of the experiment. After baseline measurements of MAP, HR, and T_B were obtained, either atropine methyl nitrate (1 mg/kg, 2.5 mg/ml ia) or vehicle (0.1 mg/ml ascorbate in saline) was injected. After new baseline measurements were obtained from resting rats, DOI was infused (20 μg/rat plus dead space consideration; 1 mg/ml at 12 μl/min for 2 min; artificial cerebrospinal fluid as diluent). At the end of the 45-min postinfusion period, rapid anesthesia with intra-arterial pentobarbital, decapitation with blood collection, dye documentation (0.5% trypan blue, 10 μl) of central cannula placement, and brain stem dissections were undertaken. Hemodynamic and T_B measurements were taken at 2, 4, 6, 9, 12, 15, 25, 35, and 45 min after the start of the infusion.

Experiment III: cardiovascular responses to central administration of a 5-HT_{1A} agonist in hypothyroidism. The experimental protocol for *experiment III* was nearly identical to that for *experiment II,* with pertinent differences being the central infusion of 8-OH-DPAT (10 μg/rat; 0.5 mg/ml at 12 μl/min for 2 min) instead of DOI and a slightly different timing for data acquisition. Hemodynamic and T_B measurements were obtained at 2, 5, 8, 11, 14, 17, 27, 37, and 47 min after the start of the infusion.

Neurochemical/hormonal measurements. The brain was removed from the skull and placed on an ice-chilled Petri dish; the cerebellum was removed and the caudal brain stem (defined by coronal cuts 0.5 mm caudal to the obex and the inferior peduncle of the cerebellum) and rostral brain stem (defined by coronal cuts at the inferior peduncle and the most caudal extent of the inferior colliculi) were dissected. Tissues were wrapped in foil and frozen at −70°C until neurochemical analyses were undertaken.

Tissues were weighed, homogenized in ice-cold 0.1 N 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 high-performance liquid chromatography (C_{18} stationary phase, isocratic delivery system, flow rate = 0.8 ml/min) with electrochemical detection (0.7 V). The mobile phase, 0.075 M monochloroacetic acid (pH = 3.1), included 120 mg/l sodium octyl sulfate, 4% acetonitrile, and 2 mM Na_2EDTA. Concentrations of 5-HT and its principal metabolite 5-HIAA were determined by comparisons with internal and external standards.

Plasma for the measurement of total thyroxine (T_4) concentration was obtained from the cervical wound in both experiments, centrifuged at 4°C, and stored at −20°C until assayed by radioimmunoassay (Diagnostic Products; intra-assay coefficient of variation = 9%, limit of sensitivity = 4.5 ng/ml as determined by 90% of maximum binding).

Statistics. Primary statistical analyses included repeated-measures analysis of variance (MANOVA) when multiple measurements were obtained in the same rat and analysis of variance (ANOVA) for independent data sets. Subsequent analyses were initiated when significance ($P < 0.05$) was found with the combined MANOVA; these entailed MANOVA on data from rats from a single treatment group, between-subjects ANOVA at individual time points after DOI administration, and Fisher's protected least significant difference test (LSD) for pairwise analyses between subjects. For purposes of simplifying analyses, complete statistical interpretation (ANOVA at individual time points and LSD comparisons of hemodynamic responses) were limited to the first 15 min after administration of drugs. "Time" is used as a statistical descriptor throughout the results section to define the within-subject changes in response to drug administrations. Drug-induced "changes" are with reference to the final measurements obtained during the second baseline period. Numbers of rats used were as follows: *experiment I,* 15 (Eu, $n = 7$; Hypo, $n = 8$); *experiment II,* 43 (Eu-Sal, $n = 10$; Eu-Atr, $n = 11$; Hypo-Sal, $n = 11$; Hypo-Atr, $n = 11$); and *experiment III,* 47 (Eu-Sal, $n = 12$; Eu-Atr, $n = 13$; Hypo-Sal, $n = 10$; Hypo-Atr, $n = 12$), where Eu is euthyroid, Hyp is hypothyroid, Sal is saline, and Atr is atropine. Small variances in these numbers were evident in some measurements because of technical difficulties. In no cases were data omitted arbitrarily. All analyses were performed with the Complete Statistical System or Statistica (Statsoft, Tulsa, OK).

RESULTS

Experiment I: cardiovascular responses in hypothyroid rats. Methimazole administration was effective in establishing a hypothyroid state. Significant attenuation of growth rate in methimazole-treated rats was accompanied by a suppression in circulating T_4 levels (Table 1). Modest decrements in both blood pressure and HR were noted in hypothyroid rats, although this difference was statistically significant in the latter variable only (MANOVA, baseline data only, $P < 0.05$). No influence of the order of drug administration was detected with reference to the data that follow.

The vasodepressor response to nitroprusside was similar between treatment groups (Fig. 1). Although MANOVA indicated that the blood pressure response of the two groups differed ($P < 0.05$), pairwise comparisons at individual time points did not provide confirmation of this finding. HR response to nitroprusside differed between treatment groups (MANOVA, $P < 0.05$). Pairwise comparisons indicated that this difference primarily reflected an attenuated HR response in hypothyroid rats.

The pressor response to intravenous phenylephrine was significantly attenuated in hypothyroid rats relative to euthyroid rats (MANOVA; $P < 0.05$; Fig. 2). MANOVA uncovered a similar, statistically significant attenuation of the reflex drop in HR with hypothyroidism, although pairwise comparisons were not able to substantiate this finding.

Experiment II: responsiveness to central administration of a 5-HT_2 agonist. As in *experiment I,* methimazole significantly attenuated both growth rate and plasma T_4 concentrations (Table 2). Concentrations of 5-HIAA were elevated in both rostral and caudal brain stems of hypothyroid rats (Table 3). Although modest hypothyroid-dependent decreases in serotonin concentration were evident in caudal brain stem, similar evidence was not obtained in rostral brain stem. Atropine had no significant influence on hormonal or neurochemical measurements.

Before drugs were administered (Bsln 1), a significantly lower HR (Hypo = 318 vs. Eu = 331 beats/min; $P = 0.04$), pulse pressure, and T_B were evident in hypothyroid rats relative to euthyroid rats (Figs. 3–5); MAP did not differ significantly at this time.

Table 1. *Hemodynamic and metabolic measurements in experiment I*

Treatment Group	n	BWT Start, g	BWT End, g	Thyroxine, μg/ml	MAP, mmHg	HR, beats/min
Eu	7	510 ± 9	607 ± 12	21.3 ± 1.5	112 ± 5	318 ± 5
Hypo	8	515 ± 6	476 ± 12*		104 ± 2	304 ± 6*

Values presented are means ± SE. Eu, euthyroid rats; Hypo, hypothyroid rats; MAP, mean arterial pressure; HR, heart rate; BWT, body weight; n, no. of rats. *Significant difference ($P < 0.05$) relative to Eu values. Absence of thyroxine data in hypo indicates that most values were below the limits of sensitivity of the assay. Hemodynamic variables represent mean values obtained from 6 baseline measurements.

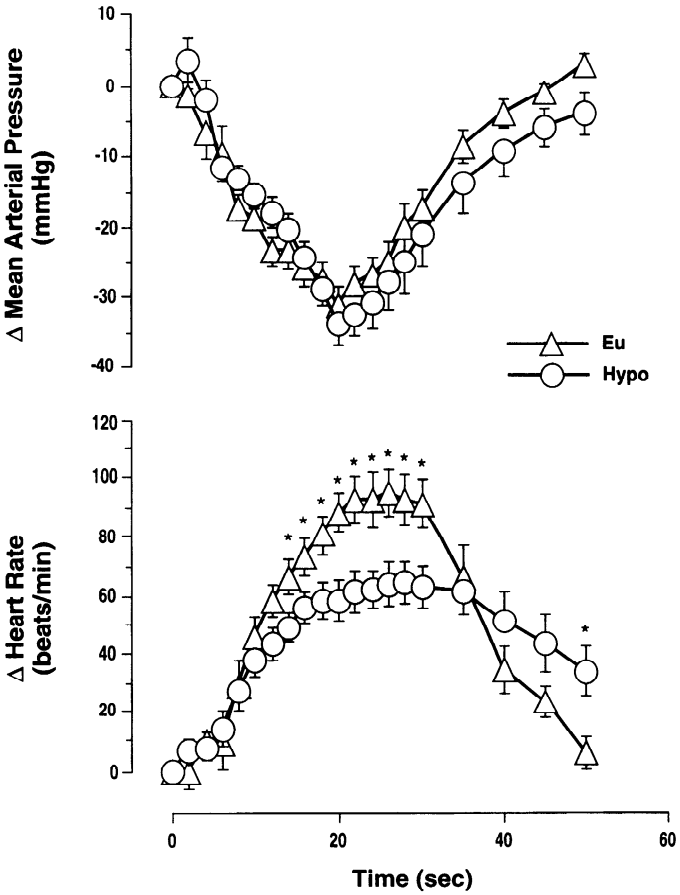

Fig. 1. Hemodynamic responses to intravenous nitroprusside administration in euthyroid (Eu) or hypothyroid rats (Hypo). Values presented are means ± SE. Mean values represent Eu, $n = 7$ and Hypo, $n = 8$. *Significance ($P < 0.05$) between treatment groups at individual time points.

Administration of atropine influenced both MAP and HR during the second baseline (Bsln 2). Significant increases in MAP and HR were evident in euthyroid rats administered atropine, whereas little influence was noted in hypothyroid rats (Fig. 3; MAP, Eu-Atr > Hypo-Atr, LSD, $P < 0.05$, Eu-Sal vs. Hypo-Sal, NS; HR, Eu-Atr > Hypo-Atr or Eu-Sal, LSD, $P < 0.05$). Atropine caused little change in either pulse pressure or T_B during this time period (Figs. 4 and 5). The influence of atropine on HR and MAP in euthyroid rats was evident during the subsequent experimentation with DOI.

Central administration of DOI caused significant pressor responses in all four treatment groups (Fig. 3; MANOVA on each separate treatment group). The magnitude of this response differed markedly [MANOVA, all groups included; thyroid status (T_{status}) × Atr × Time, $P < 0.05$]. For purposes of simplification while uncovering the basis for this interaction, complete statistical interpretation (ANOVA at individual time points and subsequent LSD comparisons) was limited to the first 15 min after administration of DOI. Analysis of changes in blood pressure in response to DOI indicated that an interaction was evident between T_{status} and Atr during the first 12 min of the response to DOI (ANOVA,

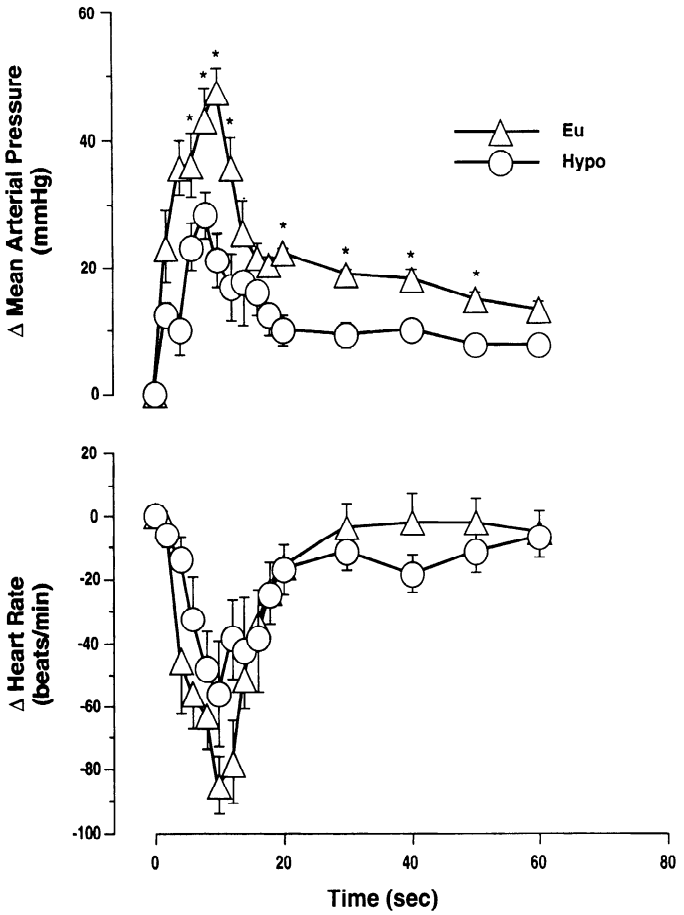

Fig. 2. Hemodynamic responses to intravenous phenylephrine administration in Eu or Hypo. Values presented are means ± SE. Mean values represent Eu, $n = 6$ and Hypo, $n = 8$. *Significance ($P < 0.05$) between treatment groups at individual time points.

$T_{status} \times$ Atr, $P < 0.05$). Subsequent pairwise comparisons indicated that the hypothyroid group not administered atropine had a decreased pressor response to DOI evident 2, 4, 6, and 9 min after the injection (LSD, $P < 0.05$) when compared with any of the other three treatment groups. Statistically significant differences did not exist between either the euthyroid or the hypothyroid group administered atropine.

Table 2. *Metabolic measurements in experiment II*

Treatment Group	n	BWT Start, g	BWT End, g	T_4, μg/ml
Eu				
Sal	10	294 ± 4	444 ± 10	21.6 ± 1.7
Atr	11	296 ± 3	456 ± 10	23.7 ± 1.5
Hypo				
Sal	11	295 ± 5	362 ± 12	
Atr	11	294 ± 5	350 ± 9	
ANOVA				
T_{status}		NS	*	
Atr		NS	NS	
Interaction		NS	NS	

Values presented are means ± SE; n, no. of rats. Sal, saline; Atr, atropine; T_4, thyroxine; T_{status}, thyroid status; NS, not significant. *$P < 0.05$.

Table 3. *Neurochemical indexes of central serotonergic activity in experiment II*

Treatment Group	n	Caudal Brain Stem 5-HIAA, μg/g	Caudal Brain Stem 5-HT, μg/g	Rostral Brain Stem 5-HIAA, μg/g	Rostral Brain Stem 5-HT, μg/g
Eu					
Sal	10	0.44 ± 0.02	0.65 ± 0.03	0.68 ± 0.03	0.76 ± 0.02
Atr	11	0.41 ± 0.02	0.64 ± 0.02	0.62 ± 0.02	0.73 ± 0.01
Hypo					
Sal	11	0.53 ± 0.04	0.60 ± 0.04	0.84 ± 0.04	0.75 ± 0.03
Atr	11	0.50 ± 0.03	0.55 ± 0.04	0.78 ± 0.05	0.75 ± 0.03
ANOVA					
T_{status}		*	*	*	NS
Atropine		NS	NS	NS	NS
Interaction		NS	NS	NS	NS

Values presented are means ± SE; n, no. of rats. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin. *$P < 0.05$.

When HR following DOI administration was analyzed separately for each treatment group (Fig. 3; MANOVA), all groups were found to exhibit a significant drop in HR with the exception of euthyroid rats previously administered atropine. Although this is suggestive of a three-way interaction as found for blood pressure, instead separate significant influences of both T_{status} and atropine were noted (MANOVA; $T_{status} \times$ Time, $P < 0.05$; Atr $\times$ Time, $P < 0.05$). $T_{status} \times$ Time reflected a larger decrease in HR in response to DOI when both groups of hypothyroid rats were compared with both groups of euthyroid rats, whereas the Atr $\times$ Time interaction indicated that atropine-treated rats, irrespective of thyroid status, had a smaller HR response than comparable saline-treated rats. Subsequent analysis (ANOVA) of changes in HR in response to DOI also indicated that atropine attenuated this response at 2, 4, 6, 9, 12, and 15 min after DOI administration, and that the drop in HR was greater in hypothyroid rats at 4, 9, 12, and 15 min. No evidence of an interaction between T_{status} and atropine was obtained with ANOVA.

Central administration of DOI caused significant increases in pulse pressure in all four treatment groups (Fig. 4; MANOVA on each separate treatment group). Initial analyses indicated that the magnitude of this response differed between treatment groups (MANOVA, all groups included; $T_{status} \times$ Atr $\times$ Time, $P < 0.05$). Subsequent analysis of changes in pulse pressure in response to DOI provided statistical confirmation of this interaction at 2 min postadministration only (ANOVA, $T_{status} \times$ Atr interaction, $P < 0.05$), although a similar trend was observed at 4, 6, and 9 min after DOI was administered ($0.05 < P < 0.10$). The interaction was caused by a reduced pulse pressure response to DOI in hypothyroid rats not given atropine. The reduction was reversed by atropine.

T_B dropped gradually in all treatment groups during the 45 min that followed DOI administration (Fig. 5). MANOVA indicated that significantly differential responses to DOI were elicited between treatment groups ($T_{status} \times$ Atr $\times$ Time interaction, $P = 0.02$). Subsequent separate analyses of data from hypothyroid and euthyroid rats indicated that atropine selectively influ-

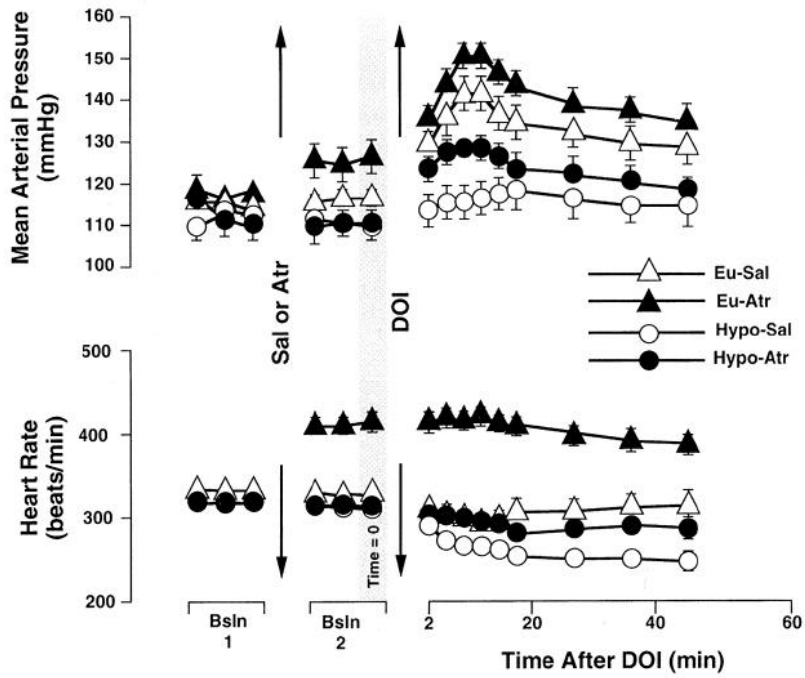

Fig. 3. Hemodynamic responses to intracerebral administration of *dl*-2,5-dimethoxy-4-iodoamphetamine (DOI) following administration of either saline (Sal) or atropine (Atr) in Eu or Hypo. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 10$; Eu-Atr, $n = 10$; Hypo-Sal, $n = 10$; Hypo-Atr, $n = 11$. Statistically significant findings included selective sensitivity to atropine in Eu vs. Hypo (Bsln 2) and uniquely attenuated pressor response to DOI in Hypo-Sal; see text for further statistical details. Bsln, baseline.

enced the DOI response in hypothyroid rats (MANOVA: hypothyroid rats only, Atr × Time, $P < 0.05$; euthyroid rats, Atr × Time, NS). Analysis of changes in T_B revealed significantly larger temperature drops in hypothyroid versus euthyroid rats at two time points, 4 and 45 min post-DOI administration (ANOVA, separate analyses at individual time points). However, no evidence of interaction between T_{status} and atropine could be confirmed by ANOVA at individual time points.

Experiment III: responsiveness to central administration of a 5-HT$_{1A}$ agonist. As in *experiments I* and *II,* methimazole treatment caused significant attenuation of growth rate as well as a suppression of circulating T_4 levels (Table 4). Concentrations of 5-HIAA, but not 5-HT, were significantly elevated in caudal brain stems of hypothyroid rats. As in the previous experiment, atropine appeared to have no influence on hormonal or neurochemical measurements.

Significant differences were not evident for MAP, HR, or T_B with hypothyroidism (Figs. 6 and 7; Bsln 1). In contrast to other measured variables, pulse pressure was significantly lower in hypothyroid rats compared with their euthyroid counterparts during this interval (Fig. 8). No differences in T_B were statistically documented during the first baseline period.

Although no significant changes in MAP occurred in response to atropine (Fig. 6; Bsln 2), significant increases in HR were evident in euthyroid rats administered atropine, whereas little influence was noted in hypothyroid rats treated similarly (MANOVA, Bsln 2, T_{status} × Atr, $P < 0.05$). Differential pulse pressure responses to atropine were evident between euthyroid and hypothyroid rats (Fig. 8; MANOVA, significant three-way interaction, Bsln1 to Bsln2) because of a drop in pulse pressure in euthyroid rats treated with atropine that was not apparent in similarly treated hypothyroid rats. T_B measurements from hypothyroid rats were found to be significantly lower than those obtained from euthyroid rats during the second baseline period. No influence of atropine was detectable in temperature measurements.

Central administration of 8-OH-DPAT caused significant depressor responses in all four treatment groups (Fig. 6; MANOVA, separate treatment groups). A com-

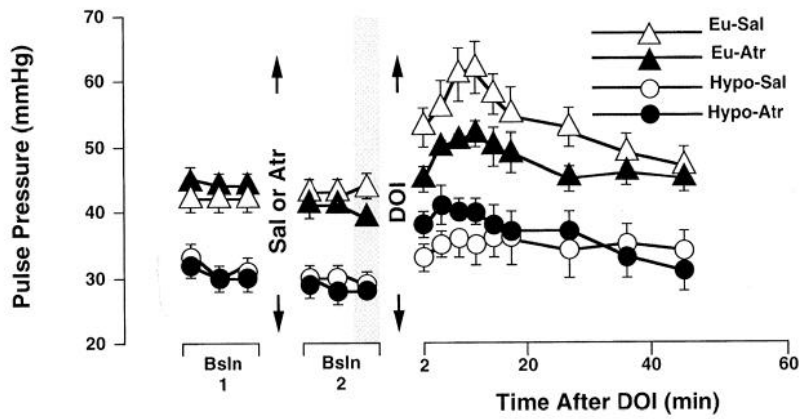

Fig. 4. Pulse pressure response to intracerebral administration of DOI following administration of either Sal or Atr in Eu or Hypo. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 9$; Eu-Atr, $n = 10$; Hypo-Sal, $n = 10$; Hypo-Atr, $n = 10$. Statistically significant findings included an attenuated response to DOI in Hypo-Sal; see text for further statistical details.

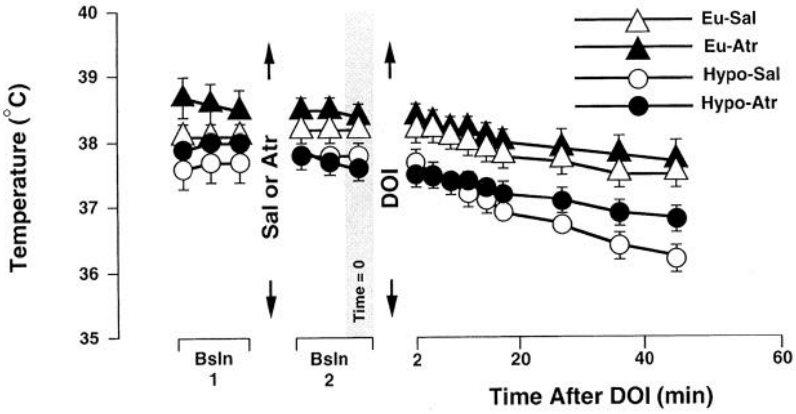

Fig. 5. Temperature response to intracerebral administration of DOI following administration of either Sal or Atr in Eu or Hypo. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 10$; Eu-Atr, $n = 11$; Hypo-Sal, $n = 11$; Hypo-Atr, $n = 11$. Statistically significant findings included greater drop in body temperature in hypothyroid rats following DOI; see text for further statistical details.

bined MANOVA indicated that this response was greater in hypothyroid rats (T_{status} × Time Interaction, $P < 0.05$). Drug-induced hypotension caused hypothyroid rats to have significantly lower blood pressure than their euthyroid counterparts at all of the first six time points after 8-OH-DPAT administration (ANOVA, individual time points). No interaction between T_{status} and atropine was evident.

Administration of 8-OH-DPAT caused a drop in HR in all treatment groups (Fig. 6; MANOVA). These drops were greater in saline- versus atropine-treated rats and in hypothyroid versus euthyroid rats (MANOVA, Atr × Time and T_{status} × Time, $P < 0.05$). No interaction between T_{status} and atropine was evident.

As for HR responses, pulse pressure responses to 8-OH-DPAT were significantly influenced by both T_{status} and atropine (MANOVA, Fig. 8) without evidence of interaction. Changes with T_{status} reflected the increased pulse pressure response in euthyroid rats that was not evident in hypothyroid rats. This increase was especially prominent in saline-treated euthyroid rats relative to their hypothyroid counterparts. Similarly, atropine caused an attenuation of the pulse pressure response that was most prominent in euthyroid rats.

A profound hypothermic response to 8-OH-DPAT was noted in all treatment groups. This response was significantly greater in hypothyroid versus euthyroid rats (MANOVA, T_{status} × Time). No evidence of an atropine influence on temperature control was obtained.

Table 4. *Metabolic measurements in experiment III*

Treatment Group	n	BWT Start, g	BWT End, g	T_4, μg/ml	Caudal Brain Stem 5-HIAA, μg/g	Caudal Brain Stem 5-HT, μg/g
Eu						
Sal	12	330 ± 6	455 ± 13	18.3 ± 1.8	431 ± 39	598 ± 25
Atr	13	330 ± 6	474 ± 10	18.4 ± 1.7	367 ± 19	535 ± 25
Hypo						
Sal	10	332 ± 9	380 ± 10		473 ± 30	581 ± 26
Atr	12	325 ± 7	364 ± 8		514 ± 43	605 ± 22
ANOVA						
T_{status}		NS	*		*	NS
Atropine		NS	NS		NS	NS
Interaction		NS	NS		NS	NS

Values presented are means ± SE; n, no. of rats. *$P < 0.05$.

DISCUSSION

The present study shows that hypothyroid rats have a profoundly altered cardiovascular response to the central administration of serotonergic agonists. These data complement previous findings that demonstrated an aberration in central serotonin metabolism with hypothyroidism (10). The present findings indicate that altered serotonin-mediated parasympathetic control plays an important role in the deranged cardiovascular control that occurs in hypothyroidism.

Plasma T_4 measurements, attenuated growth, decreased pulse pressure, and body temperature measurements documented the effectiveness of methimazole treatment in producing a hypothyroid state. Neurochemical measurements from both *experiments II* and *III* documented the changes in serotonin metabolism previously noted with hypothyroidism. Importantly, the neurochemical profile that provided the basis for experimentation was not altered by the highly invasive character of these studies, nor were neurochemical measurements influenced by atropine methyl nitrate, a muscarinic blocker that does not cross the blood-brain barrier.

Baseline mean arterial pressure (*experiment II*) and heart rate (*experiments II* and *III*) were lower in hypothyroid rats of the present study when compared with euthyroid controls. These differences were small, making it unlikely that they were an important influence on the interpretation of cardiovascular responses to subsequent pharmacological challenges.

Of more concern were the substantial differences in cardiovascular responsiveness to peripheral administration of phenylephrine and nitroprusside documented in *experiment I*. Although similar vasodepressor responses to nitroprusside were noted in euthyroid and hypothyroid rats, the reflex increase in heart rate was reduced in hypothyroid rats. This finding could reflect either reduced parasympathetic withdrawal or diminished sympathetic input at the sinus node of the hypothyroid rat. Because findings in *experiments II* and *III* indicated an absence of parasympathetic tone in resting hypothyroid rats, it is likely that the tachycardiac response to nitroprusside was caused exclusively by heightened sympathetic tone in the hypothyroid rat,

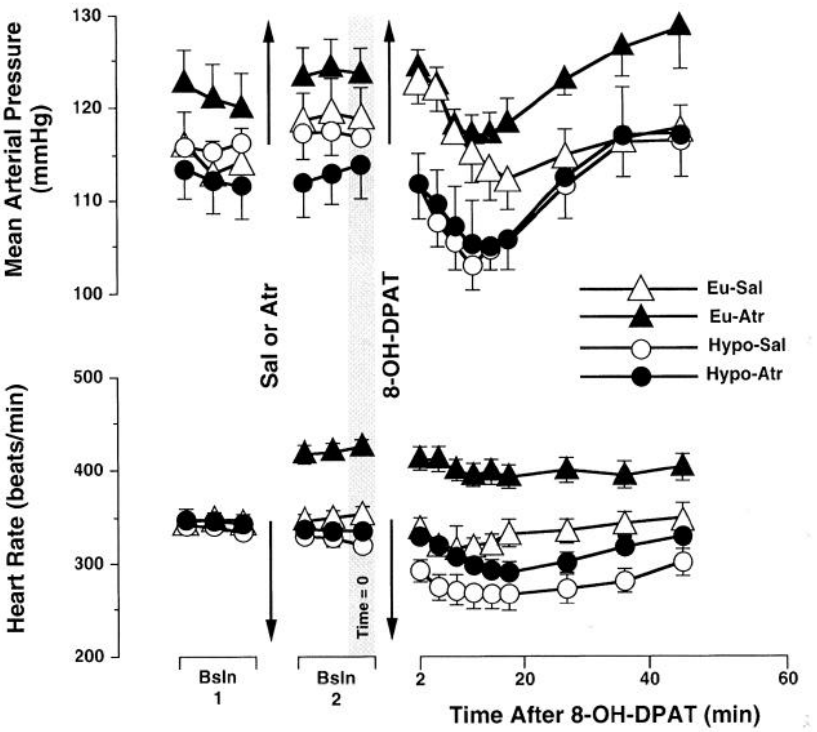

Fig. 6. Hemodynamic responses to intracerebral administration of *dl*-8-hydroxydipropyl-aminotetralin hydrobromide (8-OH-DPAT) following administration of either Sal or Atr in Eu or Hypo rats. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 12$; Eu-Atr, $n = 11$; Hypo-Sal, $n = 11$; Hypo-Atr, $n = 11$. Statistically significant findings included selective sensitivity to atropine in Eu [Bsln 2; HR (heart rate) only], greater HR response to 8-OH-DPAT in Sal vs. Atr, and greater HR response to 8-OH-DPAT in Hypo vs Eu; see text for further statistical details.

whereas a mixed autonomic response was more likely in the euthyroid rats.

Both the pressor and bradycardiac responses to phenylephrine were attenuated in the hypothyroid rat relative to responses in euthyroid rats. The similar magnitude of the attenuation in blood pressure and heart rate indicated that the baroreflex response to a peripherally administered pressor challenge was similar to that found in euthyroid rats.

Despite similar baseline measurements, atropine administration before the second baseline period had a profound and unique influence on baseline heart rate measurements in euthyroid rats of both *experiments II* and *III*. Atropine also selectively increased resting blood pressure in euthyroid rats, although this influence was less robust and was statistically documented in *experiment II* only. These findings indicated that hypothyroid rats were devoid of the basal parasympathetic tone evident in euthyroid rats.

Intravenous phenylephrine challenges of *experiment I* demonstrated, as have previous experiments (23), that hypothyroidism is accompanied by impaired pressor responsiveness to α_1-adrenergic stimulation. This impairment was modest relative to the attenuated response in hypothyroid rats noted with the DOI challenges of *experiment II*. Additionally, the enhanced α_2-adrenergic-mediated vasoconstriction also noted in the hypothyroid rat (23) makes it less likely that attenuated α-adrenergic vasoconstriction contributed significantly to the attenuated pressor response in hypothyroid rats following DOI administration. Reductions in β-adrenergic responsiveness that have been reported in hypothyroidism might provide another basis for impaired pressor responses in hypothyroidism via decreases in chronotropic and inotropic influence on the myocardium. However, the atropine-normalized responses to DOI in hypothyroid rats are not explainable as simple impairment of adrenergic responsiveness in hypothyroidism. Furthermore, the exaggerated drop in heart rate in hypothyroid rats following DOI administration and its normalization with atropine provided compelling evidence that DOI elicited a preferential parasympathetic influence on hypothyroid rats.

The greater drop in body temperature found in hypothyroid rats administered DOI, which was atropine sensitive, is also explainable as an exaggerated parasympathetic influence on the vasculature. The decreased pulse pressure response to DOI in hypothyroid rats, which was atropine sensitive, provides similar evidence. This latter finding is explainable through a parasympathetic-mediated inhibitory influence on sympathetic control of inotropic status in hypothyroidism (28). Together, hemodynamic and temperature data provide a consistent picture of heightened parasympathetic responsiveness to the central administration of DOI with hypothyroidism. A parasympathetic-mediated response to a central serotonergic challenge is provocative given the preponderance of evidence linking central 5-HT_2-mediated control of cardiovascular function via the sympathetic rather than the parasympathetic nervous system (21). The euthyroid rats of the present study, as well, responded to DOI in an atropine-independent manner.

DOI is a 5-HT_2 agonist that has been shown to elicit most of its pressor influence via the central nervous system when administered centrally, as in the present study (4). Application of DOI to the ventral surface of the medulla oblongata has identified a site that activates the sympathetic nervous system in the cat in a

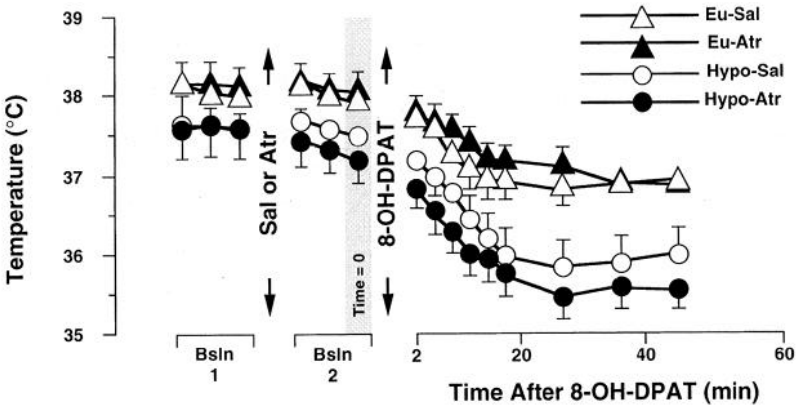

Fig. 7. Temperature response to intracerebral administration of 8-OH-DPAT following administration of either Sal or Atr in Eu or Hypo rats. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 9$; Eu-Atr, $n = 10$; Hypo-Sal, $n = 11$; Hypo-Atr, $n = 9$. Statistically significant findings included greater response to 8-OH-DPAT in Hypo vs. Eu; see text for further statistical details.

viscerotopic manner; anatomic selectivity includes sympathetic influence on the inotropic state of the myocardium without influence on the sinus node (18). However, the characterization of this drug as a sympathoexcitatory agent in the rat is less clear (1, 21). Other studies have provided evidence that a component of the response to central or peripheral administration of DOI includes a parasympathetic component (8). Thus the findings from this lab are consistent with the known profile of DOI-induced autonomic responses, although the hypothyroid rat has a clearly exaggerated parasympathetic component.

Studies with the 5-HT_{1A} agonist 8-OH-DPAT indicate that this drug elicits a profound sympatholytic influence through inhibitory autoreceptors located on serotonergic neurons in the brain stem (21). Because 8-OH-DPAT caused larger responses in hypothyroid rats, these findings are consistent with a central sympathoinhibitory influence impacting on the heightened resting sympathetic tone that is characteristic of hypothyroidism. The larger drop in heart rate in hypothyroid rats is also explainable as a lack of resting parasympathetic tone that could be withdrawn reflexively. Similarly, an increase in pulse pressure after drug administration in euthyroid saline-treated rats is potentially explainable on the basis of reflex activity that was atropine sensitive and selective to euthyroid rats. Nonetheless, primary statistical analyses did not uncover three-way interactions among thyroid status, atropine, and response to 8-OH-DPAT as were found for responses to DOI. Therefore, these data did not provide a clear perspective on which component of the autonomic nervous system was most involved in the exaggerated response to 8-OH-DPAT in hypothyroidism.

The likelihood that the differential response to 8-OH-DPAT was centrally mediated is supported by findings from *experiment I.* In contrast to the attenuated heart rate response to nitroprusside in *experiment I,* the hypothyroid response to the 8-OH-DPAT included both enhanced vasodepressor and bradycardiac responses.

Although results with 8-OH-DPAT are explainable via a sympatholytic influence through inhibitory autoreceptors, postsynaptic 5-HT_{1A} receptors have been described, and serotonin can directly activate cardioinhibitory vagal neurons via 5-HT_{1A} receptors (16). Findings from this study cannot exclude the potential involvement of direct influences of 8-OH-DPAT on the parasympathetic nervous system. Moreover, administration of drug via the lateral ventricle also allows the possibility that elicited effects were initiated or modulated at forebrain sites. Further studies will be required to determine the critical central nervous system site that elicits alterations in cardiovascular control with hypothyroidism.

Clear influences of thyroid status on α- and β-adrenergic receptor number have been described (14), and

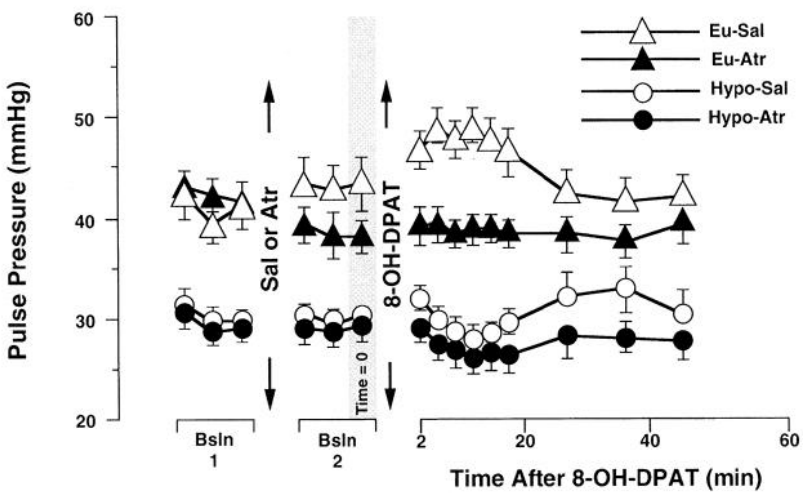

Fig. 8. Pulse pressure response to intracerebral administration of 8-OH-DPAT following administration of either Sal or Atr in Eu or Hypo. Values presented are means ± SE. Mean values minimally represent Eu-Sal, $n = 12$; Eu-Atr, $n = 11$; Hypo-Sal, $n = 11$; Hypo-Atr, $n = 10$. Statistically significant findings included greater pulse pressure response to 8-OH-DPAT in Sal vs. Atr and decreased pulse pressure response to 8-OH-DPAT in Hypo vs. Eu; see text for further statistical details.

these and similar findings have fostered a strong bias regarding the importance of thyroid hormones on sympathetic responsiveness (22). Along similar lines, data indicate that increases in resting sympathetic tone occur in hypothyroidism in both the human and rat (15, 30). Findings include evidence of enhanced norepinephrine turnover in the hypothyroid rat heart (15). Despite these and many other reports of thyroid-dependent alterations in biochemical indexes associated with the sympathetic nervous system, other findings have discounted the importance of thyroid-dependent changes in responsiveness of heart rate (3, 17), inotropic status (19), or airway resistance (29) to sympathetic stimulation. In summary, changes in thyroid status can easily be associated with physiological changes characteristic of alterations in sympathetic status. Nonetheless, mechanistic studies do not provide strong support for the importance of sympathetic involvement in the organismal profile characteristic of changes in thyroid state.

In contrast, several studies in a variety of species have shown marked alterations in parasympathetic responsiveness with changes in thyroid state (11, 17). Thus human hyperthyroid subjects had a greater response to atropine after treatment with the antithyroidal agent propylthiouracil (17), whereas responses to propranolol did not change. Similarly, vagal nerve stimulation has been shown to have an attenuated influence on hyperthyroid rats (6, 11). Tachycardia in hyperthyroid rats could be reversed through the central, atropine-sensitive actions of reserpine, a drug that depletes monoaminergic neurons and stimulates the parasympathetic nervous system (2, 3). In the hypothyroid rat, enhanced responsiveness to vagal or parasympathomimetic stimulation has been described (11), although central involvement has been less well characterized.

Few reports are available that would provide insights relevant to cardiovascular control with thyroid-induced changes in central serotonin metabolism. One report indicated that decreased 5-HT_2 receptors were found in the striatum of hypothyroid rats, although this finding was not also noted in the cortex (20). Additionally, these authors found that high dosages of thyroid hormone increased 5-HT_2 receptor number in striatum, cortex, and hippocampus; brain regions more easily associated with autonomic control were not assessed. Ramalho et al. (25) showed that thyroidectomized rats have a greatly impaired, stress-induced prolactin rise. Furthermore, these authors concluded that this defect was caused by an impaired 5-HT_2 response. Finally, a large body of neuropsychiatric data indicates that the thyroid hormones can potentiate the treatment of depression in patients who are resistant to monotherapy with more traditional medications (5, 24). Importantly, these medications are thought to act, in part, through an influence on serotonin metabolism. Thus receptor data, endocrine responses to stress, and empirical clinical data support our conclusion that central serotonergic mechanisms are modulated by changes in thyroid status.

The present studies provide strong evidence that irregularities in autonomic control of the cardiovascular system in hypothyroidism have a central serotonergic component. The predominant influence appears to be mediated through central parasympathetic drive, although a sympathetic component cannot be ruled out.

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