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Contribution of the autonomic nervous system to blood pressure and heart rate variability changes in early experimental hyperthyroidism

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Abstract

A great deal of uncertainty persists regarding the exact nature of the interaction between autonomic nervous system activity and thyroid hormones in the control of heart rate and blood pressure. We now report on thyrotoxicosis produced by daily intraperitoneal (i.p.) injection of L-thyroxine (0.5 mg/kg body wt. in 1 ml of 5 mM NaOH for 5 days). Control rats received i.p. daily injections of the thyroxine solvent. In order to estimate the degree of autonomic activation in hyperthyroidism, specific blockers were administered intravenously: atropine (0.5 mg/kg), prazosin (1 mg/kg), atenolol (1 mg/kg) or the combination of atenolol and atropine. A jet of air was administered in other animals to induce sympathoactivation. Eight animals were studied in each group. The dose and duration of L-thyroxine treatment was sufficient to induce a significant degree of hyperthyroidism with accompanying tachycardia, systolic blood pressure elevation, increased pulse pressure, cardiac hypertrophy, weight loss, tachypnea and hyperthermia. In addition, the intrinsic heart period observed after double blockade (atenolol + atropine) was markedly decreased after treatment with L-thyroxine (121.5 ± 3.6 ms vs. 141.2 ± 3.7 ms, $P < 0.01$). Of the autonomic indices, vagal tone (difference between heart period obtained after atenolol and intrinsic heart period) was negatively linearly related to intrinsic heart period ($r = 0.71$, $P < 0.05$). Atenolol modified neither the heart period nor blood pressure variability in rats with hyperthyroidism and in these rats the jet of air did not significantly affect the heart period level. The thyrotoxicosis was associated with a reduction of the 0.4 Hz component of blood pressure variability (analyses on 102.4 s segments, modulus 1.10 ± 0.07 vs. 1.41 ± 0.06 mm Hg, $P < 0.01$) and prazosin was without effect on this 0.4 Hz component in these animals. These data show a functional diminution of the vascular and cardiac sympathetic tone in early experimental hyperthyroidism. The marked rise in the intrinsic heart rate could be the main determinant of tachycardia. The blood pressure elevation may reflexly induce vagal activation and sympathetic (vascular and cardiac) inhibition.

Keywords: Hyperthyroidism; Autonomic nervous system; Blood pressure; Heart rate; Variability; Spectral analysis

1. Introduction

Increased resting heart rate is one of the most prominent features of hyperthyroidism (Klein, 1990; Polikar et al., 1993). Rises in the intrinsic heart rate (or diminutions of its reciprocal, heart period) of the sinus node, coupled with an increased β-adrenoceptor activation and reduced muscarinic stimulation of the heart may act in combination to generate this tachycardia. The hemodynamic changes observed in hyperthyroidism, such as elevated cardiac output and increased blood volume, lead, in severe cases, to a rise in systolic blood pressure. However, one of the earliest cardiovascular responses to thyroid hormone administration is a decrease in peripheral vascular resistance. This effect may result in a fall in diastolic blood pressure. Presumably the peripheral vasodilation results from a combined active hyperemia associated with the thyroxine-induced rise in metabolic rate, flow-dependent vasodilation and a direct vasodilator effect of thyroid hormones. It is also plausible that increases in cardiac output and blood volume resulting from thyrotoxicosis determine a baroreflex-mediated arterial vasodilation via vascular sympathetic inhibition. Plasma catecholamine levels are normal or even low in hyperthyroid patients (Coulombe et al., 1977; Esler, 1982). Recently, the study of blood pressure and heart period variability has been considered as a useful

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tool in the detection of autonomic activity in the cardiovascular system (Akselrod, 1988). In the present study, we achieved an evaluation of cardiovascular autonomic function using power spectral analysis of blood pressure and heart rate variations in early hyperthyroidism in rats, before and after autonomic blockade. Furthermore, the blood pressure and heart period responses to air jet stress were quantified to obtain functional indices of sympathetic reactivity in early hyperthyroidism.

2. Materials and methods

2.1. Animals

This study was carried out in normotensive, male Wistar rats weighing approximately 340 g (Janvier, Le Genest-Saint-Isle, France). The rats were given free access to standard laboratory diet chow (A04, UAR, Epinay sur Orge, France) and tap water. The animals were housed individually and maintained under controlled conditions (22°C ± 1°C—lighting 8 a.m. to 8 p.m.).

2.2. Drugs

L-Thyroxine, atenolol, atropine methylnitrate, prazosin, phenylephrine and isoprenaline were obtained from the Sigma (St. Louis, MO, USA). Pentobarbitone was purchased from Sanofi (Libourne, France). Penicillin G was obtained from Diamant (Puteaux, France). L-Thyroxine was dissolved in 5 mM NaOH. The other drugs were dissolved in saline.

2.3. Experiments

Experiments were carried out in the rats' home cages.

Sixty-four rats were randomised in two groups. The first group was pretreated with L-thyroxine 0.5 mg/kg per ml per day intraperitoneally (i.p.) for five consecutive days (Carré et al., 1994; Tse et al., 1980). The second group of rats received an injection of the vehicle for 5 days (1 ml/kg per day i.p. of physiological saline with 5 mM NaOH).

On the third day of the pretreatment period, the animals were surgically prepared under pentobarbitone anaesthesia (60 mg/kg i.p.). An arterial catheter was introduced into the right femoral artery to measure arterial blood pressure and heart period and a venous catheter was inserted into the right jugular vein. The two catheters were tunnelled subcutaneously to exit from the interscapular region. Each animal received penicillin G (100 000 UI i.p.) and was then placed in an individual cage.

After 2 days of recovery, i.e., on the 5th day of the pretreatment period, the exteriorized arterial catheter was connected to a pressure transducer (Spectramed P10EZ, Bilthoven, The Netherlands) to record the pulsatile blood pressure. The transducer was connected to a Gould RS3400 Polygraph (Ballainvilliers, France). The output from the pulsatile blood pressure preamplifier was connected to a PC 486 computer (Dynamit computer, Taipei, Taiwan) with an AS1 acquisition card and Anapres® 3.0 software (Notocord Systems, Croissy-sur-Seine, France) for acquisition, storage and analysis of the data. The blood pressure signal processing and spectrum analysis procedures utilised have been detailed elsewhere (Japundzic et al., 1990). The evenly spaced sampling permitted direct spectral analysis using a fast Fourier transform algorithm of 1024-point stationary periods. Such periods corresponded to 102.4-s segments at the 10-Hz sampling rate. Integrated spectra of the systolic and diastolic blood pressure and heart period were computed in the high (respiratory, centered by the respiratory peak value), mid (0.2–0.6 Hz)- and low (0.02–0.2 Hz)-frequency bands. The center frequency of the high frequency peak corresponded to the respiratory rate. The modulus of each spectral component, having units of milliseconds and mmHg for heart period and blood pressure respectively, were computed. Mean and standard deviations of the distribution of the variables of the 102.4-s files (1024 values) were also computed.

The experiments were commenced approximately 2 h after the last L-thyroxine (or vehicle) injection and after rats had been connected to the pressure transducer and injection syringe. Experiments were performed in conscious and unrestrained animals.

The animals were randomised into four experimental series in each group ($n = 8$ in each series). All animals were weighed daily and throughout the experiments their core temperature was measured with a YSI rectal probe (Yellow Springs, OH, USA). The first 5 min of the blood pressure and heart period recording session were used as baseline in each series of experiments.

The first group of rats was treated with the peripheral muscarinic receptor antagonist, atropine methyl nitrate (0.5 mg/kg per ml i.v.). A second 5-min recording of blood pressure and heart period of 5 min was initiated 10 min after the atropine injection.

The second group of rats received an i.v. injection of the α_1-adrenoceptor antagonist, prazosin (1 mg/kg per ml). Blood pressure and heart period recording were initiated 10 min after the prazosin injection.

The third group of rats received an i.v. injection of the β_1-adrenoceptor antagonist, atenolol (1 mg/kg per ml). Again blood pressure and heart period recording was initiated 10 min after the pharmacological challenge. Following this recording, atropine methyl nitrate was injected (0.5 mg/kg per ml i.v.) to achieve complete (double) blockade of the cardiac autonomic nervous system. Ten minutes subsequent to this a third period of blood pressure and heart period recording was performed.

A fourth group of rats with an intact autonomic nervous system was used to test the cardiovascular reactivity. After

a resting blood pressure and heart period recording was performed, animals were stressed by administering a forceful stream of air into the box (Gaudet et al., 1996). Compressed air was forced into the cage at a constant 1.2 bar pressure (L'Air Liquide, Paris, France). A second 5-min recording of blood pressure and heart period was initiated 10 min after the beginning of the stress.

Doses of antagonists used in this study were determined in a separate series of hyperthyroid ($n = 6$) and euthyroid ($n = 6$) animals (Baudrie et al., 1997). The pressor response to phenylephrine (10 μg/kg, i.v.) was analyzed to validate the α_1-adrenergic blockade achieved with prazosin. The tachycardiac response to isoprenaline (0.3 μg/kg i.v.) was analyzed to validate the β_1-adrenergic blocking effect of atenolol. The vagal bradycardia resulting from phenylephrine (10 μg/kg i.v.) was used to validate the muscarinic blockade induced with atropine. The sequence of pharmacological interventions testing the effectiveness of these antagonists was of approximately 5 h total duration. Initially, phenylephrine was administered. After the blood pressure and heart rate had recovered, an injection of atropine was administered, followed 10 min

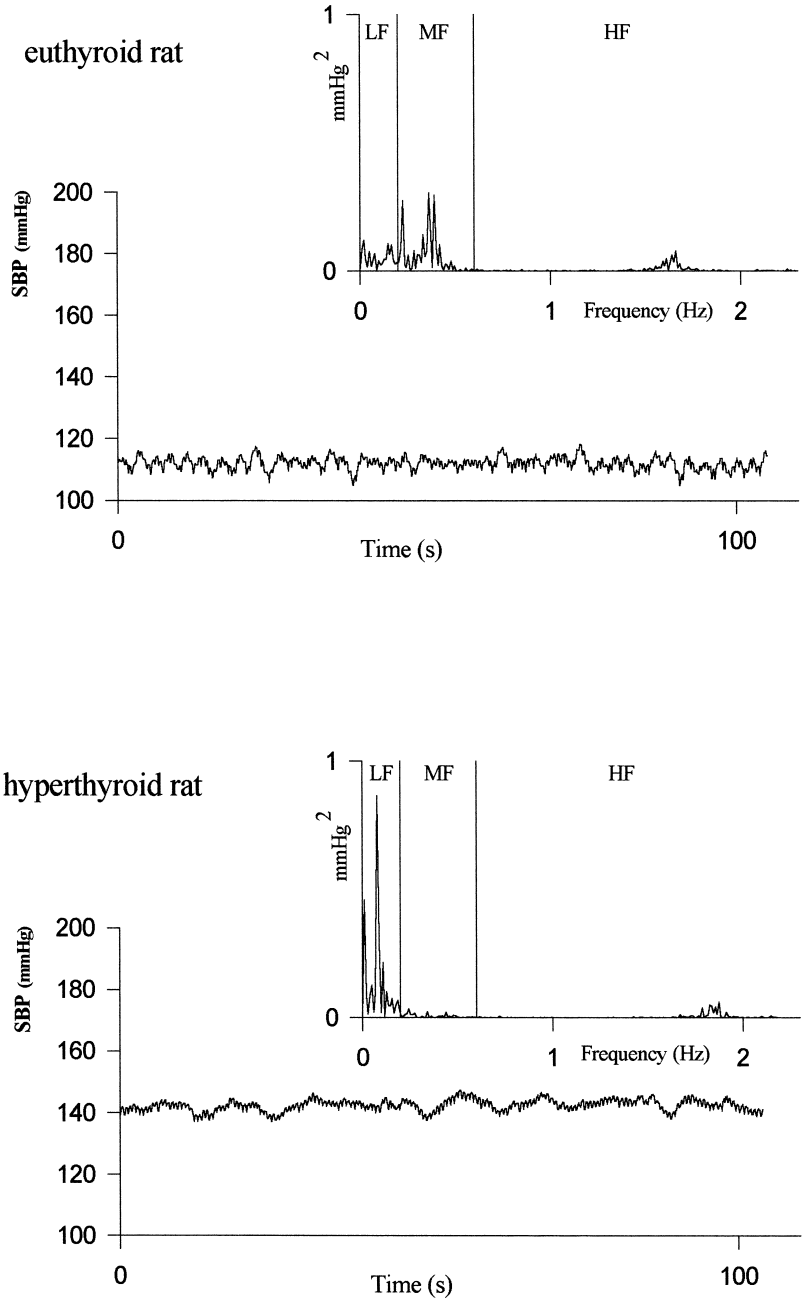

Fig. 1. Examples of digitised 100-s systolic blood pressure recordings, with corresponding spectra (insets), of an euthyroid (above) and a hyperthyroid (below) rat.

later by a second phenylephrine injection. Once the animal's blood pressure and heart rate had returned to their basal values isoprenaline was injected. On recovery from this agent, atenolol was administered followed 10 min later by isoprenaline. On recovery, a third phenylephrine injection was performed and after subsequent hemodynamic recovery, prazosin, was injected followed 10 min later by phenylephrine. The control response to the test drug (phenylephrine or isoprenaline) was used to express the residual effect (in %) to the test drug after autonomic blockade (atropine, atenolol or prazosin).

At the end of the experiments, the animals were overdosed with pentobarbitone and their hearts were removed and weighed.

All experiments conformed to the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals, and our laboratory and personnel are authorized to conduct such investigations according to the Ministry's Executive Order No. 89-02683.

2.4. Statistical analysis

The data are presented as means ± standard error of the mean (S.E.M.). Effects of autonomic blockade and comparisons between euthyroid and hyperthyroid animals were made using a non-paired Student's *t*-test. A logarithmic transformation was performed prior to the comparisons for spectral measures. A paired Student's *t*-test was used to validate the effectiveness of the blockades. The Pearson's test was used to analyze the linear relationship between spectral estimates and vagal tone. Statistical significance was taken as $P < 0.05$.

3. Results

3.1. Effects of L-thyroxine treatment

3.1.1. Effects on blood pressure, heart period, breathing frequency, body weight, heart weight, core temperature

L-Thyroxine administration (0.5 mg/kg per day for 5 days) resulted in profound cardiovascular alterations. These alterations corresponded to a marked decrease in heart period (−27.9 ms, $P < 0.001$) and to an increase in systolic blood pressure (+15 mmHg, $P < 0.001$), without a significant change in diastolic blood pressure. Body weight was decreased (−20 g, $P < 0.001$) yet heart weight was increased (+159 mg, $P < 0.001$) substantially, hence the ratio between heart weight and body weight was increased ($P < 0.001$). Core temperature was 0.8°C higher

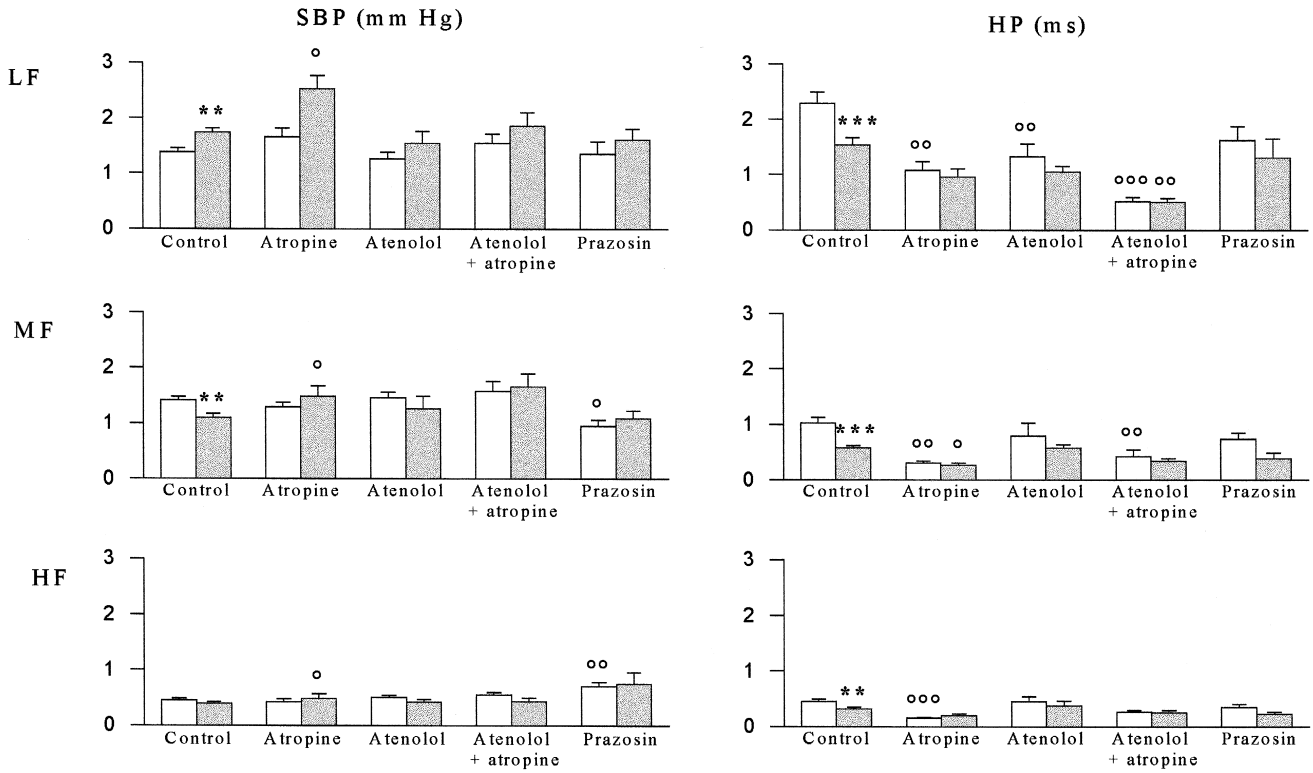

Fig. 2. Area under the curve of the low-(LF), mid-(MF) and high-(HF) frequency components of systolic blood pressure (SBP) and heart period (HP) variabilities before (control; $n = 32$) and after pharmacological blockade ($n = 8$) in euthyroid rats (white bars) and hyperthyroid rats (dark bars). Results are shown as means ± S.E.M. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significant difference between control hyperthyroid levels and control euthyroid levels. $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$, significant difference between control values and values obtained after pharmacological blockade.

in hyperthyroid animals ($P < 0.001$). Tachypnea was observed after L-thyroxine treatment with the average breathing frequency being 2.09 ± 0.04 Hz (corresponding to an average respiratory rate of 125 cycles/min) in the hyperthyroid animals compared to 1.78 ± 0.06 Hz in the control rats (corresponding to an average respiratory rate of 107 cycles/min, $P < 0.001$).

3.1.2. Effects on systolic blood pressure and heart period variations

Examples of digitised recordings of systolic blood pressure are shown in Fig. 1. The short-term variability of systolic blood pressure in euthyroid rats was composed of 2.5-s period oscillations (mid-frequency component) and faster high-frequency (respiratory) fluctuations. The corresponding spectrum exhibited a peak around 1.7 Hz, representing the high-frequency oscillations. The power in the mid-frequency component was concentrated in a peak occurring at approximately 0.4 Hz. The blood pressure of rats treated with L-thyroxine was higher, with slow fluctuations (low-frequency; approximately 20-s period), in addition to a decreased amplitude of the mid-frequency oscillations. The occurrence of these slow fluctuations of blood pressure was illustrated by the peak located below 0.2 Hz in the corresponding spectrum. The tachypnea was illustrated by a faster respiratory oscillation of systolic blood pressure with a right shift of the corresponding high-frequency peak. The average modulus of the three components of systolic blood pressure and heart period variabilities of the two series of rats are shown in Fig. 2. Hyperthyroidism increased the low-frequency component of systolic blood pressure spectra ($P < 0.01$) when compared to the control group. This effect was associated with a decrease in the mid-frequency component of the systolic blood pressure ($P < 0.01$). The high-frequency component of the blood pressure was unaffected by the L-thyroxine pretreatment. The overall variability of the heart period was reduced in hyperthyroid rats, as illustrated by the reduction in the average standard deviation of the heart period time series of these animals compared to that of the euthyroid rats. The three components of the heart period variability were affected to a similar degree.

3.2. Effects of autonomic blockers

Table 1 and Fig. 2 summarize the effects of the autonomic blockers on blood pressure and heart period levels and the effects of these drugs on the three components of blood pressure and heart period variabilities.

3.2.1. Effects of atropine

Atropine induced tachycardia in euthyroid and hyperthyroid rats. The heart period decreases did not differ between the two groups. Atropine did not significantly affect the average systolic blood pressure levels in either of the two groups.

Table 1
Average systolic blood pressure, heart period levels, and standard deviations of systolic blood pressure and heart period at rest and following autonomic blockade

	Euthyroid group		Hyperthyroid group	
	Control	After blockade	Control	After blockade
SBP (mmHg)				
Atropine	123 ± 3	122 ± 3	138 ± 3	138 ± 3
Atenolol	125 ± 3	120 ± 4[a]	136 ± 5	132 ± 5[a]
Atenolol + Atropine	125 ± 3	123 ± 5	136 ± 5	132 ± 5
Prazosin	130 ± 4	120 ± 3	153 ± 7	146 ± 6
SD SBP (mmHg)				
Atropine	2.37 ± 0.20	2.51 ± 0.15	2.47 ± 0.019	3.55 ± 0.25[a]
Atenolol	2.57 ± 0.18	2.39 ± 0.12	2.62 ± 0.22	2.41 ± 0.27
Atenolol + Atropine	2.57 ± 0.18	2.80 ± 0.15	2.62 ± 0.22	3.07 ± 0.28
Prazosin	2.52 ± 0.18	2.49 ± 0.20	2.66 ± 0.21	2.91 ± 0.36
HP (ms)				
Atropine	170.4 ± 3.7	130.8 ± 45.7[c]	143.5 ± 3.6	114.9 ± 1.6[c]
Atenolol	163.6 ± 2.9	180.0 ± 5.2[b]	141.2 ± 3.7	143.9 ± 3.1
Atenolol + Atropine	163.6 ± 2.9	161.8 ± 4.6	141.2 ± 3.7	121.5 ± 3.6[b]
Prazosin	174.0 ± 4.7	148.0 ± 8.1[b]	140.1 ± 5.1	115.0 ± 6.8[b]
SD HP (ms)				
Atropine	3.52 ± 0.66	1.93 ± 0.19[a]	2.37 ± 0.26	1.66 ± 0.17
Atenolol	3.13 ± 0.40	2.42 ± 0.29	2.60 ± 0.26	2.05 ± 0.18[a]
Atenolol + Atropine	3.13 ± 0.4	1.48 ± 0.12[b]	2.60 ± 0.26	1.56 ± 0.11[b]
Prazosin	3.82 ± 0.52	2.62 ± 0.32	2.28 ± 0.45	2.00 ± 0.33

Values are means ± S.E.M. ($n = 8$ rats in each group). Systolic blood pressure (SBP); heart period (HP); standard deviation (S.D.).
[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. control values.

Interestingly, atropine increased the overall variability of systolic blood pressure in hyperthyroid rats, as reflected by an increase in the standard deviation of the systolic blood pressure time series. This change corresponded to an increase in the three components of systolic blood pressure variability, i.e., the low (1.69 ± 0.10 vs. 2.52 ± 0.24 mmHg, control vs. vagally blocked values, $P < 0.05$), mid (0.93 ± 0.12 vs. 1.48 ± 0.19 mmHg, $P < 0.05$) and high (0.31 ± 0.04 vs. 0.48 ± 0.08 mmHg, $P < 0.05$) frequency domains.

Atropine treatment resulted in a diminution of the overall heart period variability in euthyroid animals. This effect was marked in the three frequency domains in these rats (high-frequency domain: 0.38 ± 0.02 vs. 0.15 ± 0.01 ms, control vs. vagally-blocked values, $P < 0.001$; mid-frequency domain: 0.94 ± 0.23 vs. 0.30 ± 0.04 ms, $P < 0.01$ and low-frequency domain: 2.33 ± 0.42 vs. 1.08 ± 0.15 ms, $P < 0.01$). The main effect of atropine in hyperthyroid animals was a decrease in the mid-frequency component of heart period variability (0.80 ± 0.05 vs. 0.54 ± 0.04 bpm, control vs. vagally blocked values, $P < 0.01$).

3.2.2. Effects of atenolol

The β_1-adrenoceptor blockade achieved with atenolol was associated with decreases in systolic blood pressure levels in both euthyroid and hyperthyroid rats. Heart period level was increased in the euthyroid group following atenolol while no significant change was observed in the hyperthyroid group.

Atenolol was without effect on systolic blood pressure variability. The effect of atenolol on the frequency components of heart period variability was limited to a decrease in the low-frequency component in the euthyroid rats (2.29 ± 0.40 vs. 1.33 ± 0.23 ms, control vs. atenolol in the euthyroid group, $P < 0.01$; 1.66 ± 0.20 vs. 1.05 ± 0.10 ms, in the hyperthyroid group, n.s.).

3.2.3. Effects of atropine plus atenolol

The dual blockade with atropine and atenolol induced marked tachycardia in hyperthyroid animals, corresponding to an intrinsic heart period of 121.5 ± 3.6 ms (vs. 141.2 ± 3.7 ms before the blockade, $P < 0.01$). The intrinsic heart period of the euthyroid animals was close to the value recorded at baseline (163.6 ± 2.9 vs. 161.8 ± 1.6 ms after the double blockade). Vagal tone was calculated as the difference between intrinsic heart period and heart period level following β_1-adrenoceptor blockade (Chen et al., 1997). A negative linear relationship was observed between the intrinsic heart period and vagal tone in hyperthyroid rats (slope -0.85, $r = 0.71$, $P < 0.05$).

The standard deviation of heart period was decreased in the two groups. The low-frequency component of the heart period was decreased with the double blockade in the two groups (2.29 ± 0.40 vs. 0.51 ± 0.08 ms following the double blockade in the euthyroid group, $P < 0.001$; 1.66 ± 0.20 vs. 0.50 ± 0.07 ms following the double blockade in the hyperthyroid group, $P < 0.01$). The mid-frequency component of heart period variability was decreased in euthyroid animals (0.91 ± 0.14 vs. 0.42 ± 0.12 ms following the dual blockade, $P < 0.01$).

The combined blockade did not significantly affect either blood pressure levels or blood pressure variability of the two groups of rats.

3.2.4. Effect of prazosin

Blockade of the α_1-adrenoceptor achieved with prazosin induced a non-significant decrease in systolic blood pressure levels with a marked reflex tachycardia in the two groups.

In euthyroid animals, prazosin decreased the mid-frequency component of systolic blood pressure (1.51 ± 0.14 vs. 0.95 ± 0.11 mmHg after prazosin, $P < 0.05$) and increased the high-component of systolic blood pressure (0.39 ± 0.06 vs. 0.70 ± 0.08 mmHg, $P < 0.01$). In hyperthyroid rats, prazosin did not affect the mid-frequency component of systolic blood pressure (1.12 ± 0.13 vs. 1.09 ± 0.14; n.s.).

3.3. Effects of air jet stress

The average values and standard deviations of systolic blood pressure and heart period of the two groups of rats before and during air jet stress are shown in Table 2. The jet of air produced tachycardia associated with systolic hypertension. In both groups, stress was without any effect on the low- and high-frequency components of systolic blood pressure and heart period spectra. A rise in the mid-frequency component of systolic blood pressure was observed in hyperthyroid animals (0.96 ± 0.17 vs. 1.40 ± 0.11 mmHg following the stress, $P < 0.05$). The mid-frequency component of heart period was increased by stress in euthyroid rats (0.82 ± 0.30 vs. 1.20 ± 0.33 ms following stress, $P < 0.01$).

3.4. Validation of autonomic blockades

The control (α_1-adrenoceptor-mediated) pressor responses to phenylephrine were 76.0 ± 4.1 mmHg in euthyroid and 66.6 ± 2.2 mmHg in hyperthyroid rats ($n = 6$). Prazosin (1 mg/kg i.v.) totally prevented the pressor response to phenylephrine in both groups ($-8.0 \pm 2.7\%$ of the control response, $P < 0.001$ in the euthyroid group and $-0.1 \pm 1.2\%$, $P < 0.001$ in the hyperthyroid group, $n = 6$).

The control (vagal) bradycardiac responses to phenylephrine were 93.0 ± 12.8 beats/min in euthyroid and 82.3 ± 8.1 beats/min in hyperthyroid rats ($n = 6$). Atropine (0.5 mg/kg i.v.) totally prevented the bradycardiac response to phenylephrine in both groups and unmasked a (β_1-adrenoceptor-mediated) tachycardiac response to phenylephrine ($-10.8 \pm 8.3\%$ of the control response, $P < 0.001$ in the euthyroid group and $-5.5 \pm 4.3\%$, $P < 0.001$ in the hyperthyroid group, $P < 0.001$, $n = 6$).

The control (β_1-adrenoceptor-mediated) tachycardiac response to isoprenaline was 76.7 ± 10.5 beats/min in euthyroid and 79.3 ± 7.7 beats/min in hyperthyroid rats

Table 2
Average values and standard deviations of the systolic blood pressure and heart period at rest and during air jet stress in the two groups of euthyroid and hyperthyroid rats

	Euthyroid group		Hyperthyroid group	
	Control	During stress	Control	During stress
SBP				
Average (mmHg)	128 ± 4	139 ± 3^b	145 ± 8	155 ± 4
SD (mmHg)	2.25 ± 0.24	2.45 ± 0.17	2.40 ± 0.22	2.64 ± 0.12
HP				
Average (ms)	168.8 ± 4.2	146.7 ± 8.6^a	144.1 ± 3.1	119.2 ± 4.3^b
SD (ms)	3.4 ± 0.9	3.7 ± 0.6	2.9 ± 0.4	2.2 ± 0.1

Standard deviations (S.D.), systolic blood pressure (SBP) and heart period (HP) values are expressed as means ± S.E.M. ($n = 8$ rats in each group). $^aP < 0.05$ and $^bP < 0.01$ significantly different from the control value.

($n = 6$). Atenolol (1 mg/kg i.v.) markedly reduced this response to 12.5 ± 1.4% in the euthyroid group ($P < 0.001$, $n = 6$) and 7.4 ± 6.3% of the control response in hyperthyroid rats ($P < 0.001$, $n = 6$).

4. Discussion

The procedure consisting of L-thyroxine (0.5 mg/kg per day) i.p. administration over 5 consecutive days produced characteristic features of hyperthyroidism including: tachycardia, systolic hypertension, hyperthermia and tachypnea, weight loss and myocardial hypertrophy. It seems that the cardiovascular alterations of hyperthyroidism are easily reproduced with thyroid hormone injections and are observed in rats as early as the third day after initiation of hormonal treatment (Frazer and Hess, 1969; Sanford et al., 1978; Carré et al., 1994). Myocardial hypertrophy depends on three mechanisms, an increase in myocardial workload, a direct action of thyroid hormone on the heart and activation of the cardiac renin–angiotensin system (Klein and Levey, 1984; Weiss and Tse, 1995; Kobori et al., 1997b).

In the present study, a 25% difference in intrinsic heart period was observed between hyperthyroid and euthyroid animals. This result is qualitatively close to that obtained in human hyperthyroidism following atropine plus propanolol administration (Maciel et al., 1987). Valcari et al. (1992) also found, in thyrotoxic subjects, an increased intrinsic activity of the sinus node. The resting tachycardia more likely reflects a direct consequence of thyroid hormone excess, rather than an effect of extrinsic influences exerted by the autonomic nervous system on the sinus node. Meo et al. (1994) studied the effects of in vivo administration of different doses of T_3 to thyroidectomized rats. These authors observed an increased intrinsic frequency proportional to the dose of T_3, indicating a direct action of T_3 on the pacemaker cardiac cells.

In hyperthyroid animals, the autonomic nervous system diminished the chronotropic effect of thyroid hormones since the resting heart period was markedly higher (14%) than the intrinsic heart period. Baroreceptor activation due to the blood pressure elevation may determine vagal activation together with decreased sympathetic activity. Adaptive mechanisms may modify the autonomic imbalance. Increased β-adrenoceptor density has been well documented (Eliades and Weiss, 1989; Crozatier et al., 1991; Moalic et al., 1993) while results published for muscarinic receptor density are conflicting (Carré et al., 1994; Weiss and Tse, 1995).

In this study, we have been able to document possible vagal activation in the early hyperthyroid state. Firstly, vagal tone estimates were dependent upon the corresponding intrinsic heart periods. In hyperthyroid animals, the lower the intrinsic heart period, the higher the vagal tone. In addition, atropine unmasked an increased blood pressure variability in these animals, suggesting an antioscillatory role of the vagus in the hyperthyroid state. This antioscillatory role of the vagus is likely to originate from arterial baroreceptor modulation of vagal cardiac drive (Perlini et al., 1995). Greater sensitivity of hyperthyroid hearts to acetylcholine could also contribute to a state of vagal dominance in thyrotoxicosis (Weiss and Tse, 1995).

Our observations favour a reduced activity of the cardiac sympathetic tone in acute thyrotoxicosis. β_1-Adrenoceptor blockade markedly increased the heart period in control animals while no significant changes in heart rhythm were observed following L-thyroxine treatment. The dose of atenolol was chosen to effectively antagonize the tachycardia in response to isoprenaline in euthyroid and hyperthyroid animals as well. This makes it unlikely that the relative inefficiency of atenolol to exert an effect on heart period in hyperthyroid animals was due to a change in adrenoceptor density in relation with the L-thyroxine treatment. In addition, the heart period variability was unaffected by atenolol. Another argument supporting a cardiac sympathetic deficit in hyperthyroid rats was the lack of response of the mid-frequency component of heart period variability to a jet of air. This index of cardiac sympathetic reactivity was increased with stress in euthyroid rats. Evidence for a reduced cardiac sympathetic tone at this early stage of hyperthyroidism could differ from the state of cardiac sympathetic activation observed at a later stage of the disease. β-Adrenoceptor blockade actually lowers the heart rate in established hyperthyroidism. Although β-adrenoceptor blockade is used therapeutically to treat thyrotoxicosis, most previous investigations have failed to detect evidence of enhanced heart rate sensitivity to β-adrenoceptor antagonists or to catecholamines (Klein, 1990; Polikar et al., 1993). Amos et al. (1994) observed that chronic administration of β-adrenoceptor antagonists in humans produced only limited changes in hyperthyroidism-induced cardiovascular responses such as tachycardia, hyperthermia and increased cell metabolism. Experimental human hyperthyroidism is more relevant to the present observations. Volunteers, treated for 2 weeks with triiodothyronine, exhibited a reduction (15%) in the slope of the regression line representing the sensitivity of the heart to isoproterenol, when calculations were done using the heart period (Martin et al., 1992). The justification for this unit has been discussed elsewhere (Wargon et al., 1998). The diminution of the mid-frequency component of heart period variability also supports a reduced cardiac sympathetic tone and confirms the data published by Carré et al. (1994). However, this component of heart period variability is not a pure index of cardiac sympathetic activity (Japundzic et al., 1990; Cerutti et al., 1991). The effect of the dual blockade shown in Fig. 2 illustrates this mixed origin (vagus and cardiac sympathetic) of the heart period variability, with the lowest modulus values in the three frequency domains. This also translated into a marked decrease of the overall variability index (standard devia-

tion) of heart period variability after dual blockade in the two groups. Although these data tend to indicate a reduced β-adrenoceptor sensitivity in early hyperthyroidism, it is important to consider the variation in thyroid-induced effects on the heart that has been observed in different species of mammals. This makes interspecies extrapolations difficult.

Our data support the concept of a relative vascular sympathetic deficit in early experimental hyperthyroidism. The diminution of the mid-frequency component of systolic blood pressure variability (Mayer waves) illustrated in Figs. 1 and 2 supports this view. Indeed, the mid-frequency oscillations of the systolic blood pressure depend to a large extent on sympathetic activity in the resistance vessels (Pagani et al., 1986; Japundzic et al., 1990; Cerutti et al., 1991; Daffonchio et al., 1991; Persson et al., 1992). Brown et al. (1994) assessed the relationship between sympathetic nervous system activity and spectral profiles of blood pressure and heart rate. These authors reported a close coupling between sympathetic nervous system activity and blood pressure fluctuations at 0.4 Hz, raising the possibility that blood pressure spectral power at 0.4 Hz reliably reflects sympathetic activity. The lack of decrease of these Mayer waves by prazosin could also reflect a decreased vascular sympathetic tone during hyperthyroidism. Although human data are difficult to compare to our observations, it is interesting to mention a report by Matsukawa et al. (1993) of an inverse relationship between thyroid function and muscle sympathetic nerve activity. The degree of the mid-frequency component reduction depends on the contribution of other factors to the genesis of these fluctuations. Similarly, the degree of amplification of the Mayer waves in response to a jet of air depends on other factors, like renin production (Gaudet et al., 1996).

The contribution of the renin–angiotensin system to blood pressure variability is relevant in hyperthyroidism since increased plasma renin activity is associated with thyrotoxicosis. The observation that low-frequency fluctuations were increased after T_4 treatment (Figs. 1 and 2) could well illustrate the importance of this system in the genesis of these fluctuations (Ponchon and Elghozi, 1996, 1997). Kobori et al. (1997a,b) found in rats that thyroid hormone stimulates renin synthesis without involving the sympathetic nervous system. Sernia et al. (1993) have shown that experimental hyperthyroidism in dogs results in activation of the plasma renin–angiotensin system and up regulation of the left ventricular, aortic and liver angiotensin II receptors. This activation could well participate in the increased myocardial hypertrophy observed during hyperthyroidism. The rise in core temperature could also contribute to the alterations in blood pressure variability, as recently shown by Stauss et al. (1997).

In conclusion, evidence for a functional diminution of the vascular and cardiac sympathetic tone was observed in experimental hyperthyroidism at an early stage of the disease. The marked rise in intrinsic heart rate resulting from the direct effect of thyroid hormone on the sinoatrial node could well be the main determinant of tachycardia. The blood pressure elevation could induce, via baroreceptor reflexes, reciprocal alterations in vagal and sympathetic (vascular and cardiac) activities. The role of renin secretion in the amplified low-frequency fluctuations in systolic blood pressure warrants further investigation.

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