Species Differences in Myocardial 
\textbf{\textbeta-Adrenergic Receptor Regulation in Response to Hyperthyroidism}

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The coupling between myocardial \textbeta-adrenergic receptors, adenylate cyclase activity, and the in vivo cardiac response to catecholamines is controversial in hyperthyroidism. The possibility of species differences in \textbeta-adrenoceptor regulation after thyroxine treatment was studied in dogs and in rats. In dogs instrumented with a left ventricular (LV) pressure micromanometer, hyperthyroidism was induced by L-thyroxine (0.5 mg/kg/day i.v. for 10 days). After hyperthyroidism, heart rate was increased to 167±10 beats/min (control, 107±8 beats/min; \(p<0.005\)) with an increase of peak LV dP/dt from 4,243±471 to 6,105±862 mm Hg/sec (\(p<0.01\)). LV response to injection of increasing doses of isoproterenol and dobutamine was not significantly different before and after induction of the hyperthyroid state, as shown by the unchanged slopes of the LV peak dP/dt versus the log of the dose of isoproterenol and dobutamine. B_{max} of \textbeta-receptors measured in crude membranes using \(^3\text{H}-\text{CGP 12177}\) and in homogenates using \(^{125}\text{I}-\text{cyanopindolol}\) was not increased in hyperthyroid animals as compared with a control group. Basal adenylate cyclase activity was not different in control and hyperthyroid dogs (32±3 versus 29±3 pmol/mg/min), and maximal adenylate cyclase activity response to isoproterenol was similar in control and hyperthyroid dogs. In contrast, in rats subjected to hyperthyroidism (0.5 mg/kg/day i.p. L-thyroxine for 10 days), B_{max} of adrenoceptors measured using the same methods was significantly increased as compared with control (+72.5% using \(^3\text{H}-\text{CGP 12177}\) and +41% using \(^{125}\text{I}-\text{cyanopindolol}, but adenylate cyclase activity was not increased in hyperthyroid rats. We conclude that both adenylate cyclase activity and LV response to catecholamines are not increased by thyroxine-induced hyperthyroidism in dogs and that, in contrast with rats, \textbeta-adrenergic density is not increased in hyperthyroid dogs. This indicates a species difference in myocardial \textbeta-adrenoceptor regulation in response to hyperthyroidism. 

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The role of the sympathetic nervous system in the regulation of cardiac function in response to hyperthyroidism has been the subject of considerable works with conflicting results, using a number of different preparations: isolated papillary muscles,\(^1-^4\) isolated hearts,\(^5-^7\) open-chest animals,\(^4,^8,^9\) closed-chest anesthetized dogs,\(^1^0\) closed-chest conscious animals,\(^1^1,^1^2\) and patients,\(^1^3-^1^8\) The effect of exogenous sympathetic stimulation on the heart of hyperthyroid subjects has been described as increased,\(^4,^6,^7,^1^0\) normal,\(^5,^8,^9,^1^1,^1^4,^1^8\) or decreased,\(^1,^2\) and it has been proposed that cardiac effects of hyperthyroidism are mediated by the sympathetic nervous system entirely,\(^1^0\) only partially,\(^4,^1^1,^1^2,^1^6,^1^7\) or not at all.\(^1,^1^3,^1^5,^1^8\)

In contrast with these studies of cardiac response to catecholamines, in vitro \textbeta-adrenergic studies of isolated cardiac membranes are less controversial. There is an almost universal agreement\(^1^9-^2^5\) that the number of cardiac \textbeta-adrenergic receptors is increased in hyperthyroidism, with an increased adenylate cyclase activity (ACA). However, most of these studies were performed in rats, and some studies showed an impaired ACA in cats\(^5,^2^6\) and in rats\(^2^7\) subjected to hyperthyroidism.

To elucidate some of the bases of the discrepancies between previously published results, and particularly the possibility of species differences in response to hyperthyroidism, we designed a study in which 1) basal left ventricular (LV) performance and LV response to catecholamines were studied in vivo in dogs before and after induction of hyperthyroidism, 2) \textbeta-adrenergic receptors and ACA on cardiac mem-

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branes obtained from these animals were studied in vitro in comparison with those of euthyroid animals, and 3) $\beta$-adrenergic receptors and ACA were also studied in hyperthyroid and euthyroid rats.

**Materials and Methods**

Twenty-two (10 control and 12 hyperthyroid) rats of either sex and 26 beagle dogs weighing 11.3–22.4 kg (mean, 16.5 kg) were used for this study. A first series of 18 dogs was divided into three groups: group 1 (six dogs) did not receive any thyroid treatment; group 2 and group 3 (six dogs in each group) were given 0.5 mg/kg/day i.v. $\alpha$-thyroxine (T4) for 3 and 10 days, respectively. These dogs underwent both physiological and biochemical studies. A second series of eight dogs were subjected only to biochemical studies. They were divided into four control and four dogs treated with 0.5 mg/kg i.v. T4 for 10 days. 3,5,3'-Triiodothyronine and T4 levels were measured in control dogs by use of an enzymoimmunological assay (T3 E.I.A. and T4 E.I.A., respectively, Institut Bioremie, France). The chosen dose produced a fivefold increase of the plasma T4 level 24 hours after the last T4 injection to 28.7±5.2 ng/l from a control of 5.1±0.4 ng/l ($p<0.02$); the 3,5,3'-triiodothyronine level increased to 2.28±0.7 μg/l from a control of 0.72±0.1 μg/l ($p<0.05$).

**Surgery and Instrumentation of Dogs**

A fifth left intercostal space thoracotomy was performed in four dogs of groups 1 and 2 and three dogs of group 3 under sterile conditions after anesthesia (25 mg/kg i.v. thiopental sodium, with additional doses injected when necessary). Dogs were ventilated with room air delivered by a respirator (Harvard Apparatus, South Natick, Mass.) through an endotracheal tube.

The pericardium was opened, and a high fidelity micromanometer (model P7, Konigsberg Instruments, Inc., Pasadena, Calif.) was inserted into the LV cavity at the cardiac apex. One pair of ultrasonic crystals was positioned in the plane of the minor-axis diameter for measurement of the anteroposterior diameter, according to techniques described in detail elsewhere. A fluid-filled catheter was introduced into the left atrium through the left atrial appendage.

The chest was then closed, and tubes and wires were brought under the skin and exteriorized in the interscapular area.

Dogs were trained to lie quietly on the right side, and experiments were performed at least 15 days after surgery, when they had fully recovered and were apparently healthy.

**Physiological Studies in Dogs**

All physiological studies were performed in the conscious state, with the dogs lying quietly on the table; in the first series, the dogs were studied before T4 treatment (the six dogs of each group), 6 hours after the second T4 injection (groups 2 and 3), and 6 hours after the ninth T4 injection (group 3). In the two dogs of groups 1 and 2 and in the three dogs of group 3 that were not thoracotomized, physiological studies were performed after introduction of a probe (Millar Instruments, Houston, Tex.) into the LV by retrograde catheterization of a femoral artery.

After signal recording in the control resting state, increasing bolus doses of isoproterenol were injected intravenously (0.01–0.50 μg/kg). The delay between injections was the time necessary for return to control of monitored variables. After the last injected dose of isoproterenol, when monitored parameters had returned to control, dobutamine was perfused at increasing rates (from 2.25 to 15 μg/kg/min i.v.) during 5 minutes for each dose.

Data (LV pressure, dP/dt, and diameter measurements) were monitored on an eight-channel graphic recorder and stored on a magnetic tape for subsequent analysis.

**Biochemical Studies in Dogs**

Dogs of groups 2 and 3 were killed 3 and 10 days, respectively, after the first T4 injection (24 hours after the last physiological study). Dogs of group 1 were killed in the euthyroid state. After anesthetization of dogs of all three groups with thiopental sodium, the hearts were immediately excised and placed into iced saline solution. A thin layer of epicardium and endocardium (~1 mm) was removed, and the LV was frozen with liquid nitrogen and stored at −71°C until membrane preparation and assay.

**Membrane preparations.** Crude membranes were prepared, according to the techniques of Vatner et al., from the six hearts of groups 1, 2, and 3 (four dogs of groups 1 and 2 and three dogs of group 3 had been previously instrumented, and two dogs of groups 1 and 2 and three dogs of group 3 had only been subjected to LV retrograde catheterization). LV (10 g) was ground under liquid nitrogen, and all subsequent procedures were carried out in 4°C buffer (0.25 M sucrose, 1 mM MgCl2, and 1 mM KHC03). Tissue was homogenized with a PT-10ST Polytron tissue disruptor for three 30-second periods at half-maximal speed and for a 30-second period at maximal speed. The homogenate was then filtered through silk with a 112-μm mesh and centrifuged at 1,000g for 15 minutes. The supernatant was respun at 45,000g for 15 minutes, and the pellet was resuspended in buffer (100 mM Tris, 5 mM MgCl2, and 1 mM EDTA, pH 7.2) using a Teflon pestle in a Potter-Elvehjem homogenizer. The homogenization and 45,000g centrifugation were repeated twice. The pellet was resuspended to a protein concentration of 3–6 mg/ml and stored in liquid nitrogen until assayed.

To test the hypothesis that a selection of tissue was obtained by centrifugation that could have led to a loss of receptors in the discarded pellets, homogenates were prepared according to the technique of Bristow et al. in an additional series of eight dogs that had not been instrumented and had not been subjected to physiological study (four control and
four hyperthyroid dogs). Briefly, 1 g LV was ground under liquid nitrogen, and all subsequent procedures were carried out in 4°C buffer (10 mM Tris, pH 8, and 1 mM EGTA). Tissue was homogenized with the PT-10ST Polytron for three 10-second periods at maximal speed. A crude membrane fraction was made by extracting the contractile proteins in 0.5 M KCl and washing a 50,000 g pellet two times. The pellet was rehomogenized after each centrifugation. The final pellet was resuspended in a buffer (50 mM Tris, pH 7.5, 1 mM EGTA, and 250 mM sucrose), frozen in liquid nitrogen, and stored at −80°C until assayed. In the following text, membranes obtained using the first procedure are called “crude membranes,” and those obtained using the second procedure are called “homogenates.”

**Binding studies.** At the time of assay, the membranes were again washed in the Tris buffer and centrifuged at 45,000 g for 15 minutes. All studies were performed in triplicate in the Tris buffer.

Radioligand binding assays were performed in two series of experiments. In a first series of experiments, crude membrane preparations (100 µl, 3–6 mg protein/ml) were incubated according to the technique of Vatner et al. at 37°C for 20 minutes with increasing concentrations (from 0.15 to 15 nM) of 3H-CGP 12177 (New England Nuclear, Boston) with or without unlabeled dl-propranolol (2 µM) in a final reaction volume of 500 µl buffer containing (mM) Tris 100, pH 7.2, MgCl2 5, and EDTA 1. In a second series of experiments, homogenates (100 µl, 0.2–0.5 mg protein/ml) were incubated according to the technique of Bristow et al. at 30°C for 2 hours with increasing concentrations (from 6 to 255 pM) of 125I-cyanoindolol (125I-CYP) (Amersham Corp., Arlington Heights, Ill.) with or without unlabeled l-isoproterenol (10−6 M) in a final reaction volume of 500 µl buffer containing (mM) Tris 20, pH 7.5, NaCl 135, MgCl2 10, and ascorbate 10. After incubation, 3 ml iced buffer was added to the 500 µl reaction mixture, which was rapidly filtered under vacuum onto GF/C glass fiber filters (Whatman Inc., Clifton, N.J.). This operation was repeated three times, and the filters were again quickly washed four times with 3 ml iced buffer. The filters were counted for 10 minutes in 10 ml Lumagel (Lumac, Landgraaf, The Netherlands) in an SL40 liquid scintillation counter (Intertechne, Velizy, France) with a 40% efficiency (3H-CGP 12177) or in a gamma well counter (Intertechne, Velizy, France) with an 80% efficiency (125I-CYP).

ACA was assayed in crude membranes according to the method of Salomon modified by Hanoune et al.31 Maximal ACA was assessed by measuring cAMP production in the presence of 10 mM NaF or 0.1 mM Gpp(NH)p. Isoproterenol stimulation of cAMP production was measured for increasing doses of isoproterenol (from 10−8 to 10−4 M) in the presence of 0.1 mM GTP. Protein concentrations for each membrane assay were determined by the method of Lowry et al.32

5′-Nucleotidase activity of homogenates was measured according to the technique of Gentry and Olsson.33

Plasma epinephrine and norepinephrine levels were determined according to the technique of Peuler and Johnson.34 Plasma samples were obtained in four dogs at the same time in the morning after a quiet resting period of at least 10 minutes before the first T4 injection, before the third T4 injection, and 24 hours after the ninth T4 injection.

**Rat Experiments**

The hyperthyroid state was induced in 17 rats by T4 injections (0.5 mg/kg/day i.p. for 10 days). Rats were killed on the day after the last T4 injection. Hearts were rapidly removed, frozen in liquid nitrogen, and stored at −71°C. Another group of 14 rats was used as controls.

Membranes were prepared in both rat groups by the same method used in dogs, and binding studies were performed with 3H-CGP 12177 (crude membranes, n = 14) or 125I-CYP (homogenates, n = 17), as used in dogs. Linearity with protein content, competition curves with dl-propranolol (3H-CGP 12177) and with l-isoproterenol (125I-CYP), and experiments for search of optimal time for equilibrium had previously been performed in membranes obtained from both species with similar results. To test the possibility of an uncoupling of β-receptors in the hyperthyroid state, competition binding experiments were performed in control (n = 4) and in hyperthyroid (n = 5) rats using fixed concentrations of 125I-CYP (50 pM) in homogenate and varying concentrations of l-isoproterenol (from 10−9 to 10−3 M).

ACA and 5′-nucleotidase activity were performed in crude membranes and homogenates, respectively, by the same methods used in dogs.

**Data Analysis**

**Physiological data.** Hemodynamic parameters stored on magnetic tapes were digitized using a MINC 11-23 computer as described in detail elsewhere.28 Analyzed parameters included heart rate, LV end-diastolic and end-systolic pressure and diameter, LV peak dP/dt, and the percentage of diameter shortening. Beats obtained during a 5-second period of time were averaged either during stable conditions (control period and dobutamine infusions) or during the peak response to isoproterenol injection. Response to catecholamine was analyzed by calculating the slope of the linear relation between changes from control values of heart rate or LV dP/dt and log of the dose of injected isoproterenol or dobutamine.

**Biochemical studies.** Adrenergic Bmax and Kd of membrane preparations were calculated by nonlinear curve fitting using the LIGAND program of Munson and Rodbard.35

**Statistical analysis.** Data are expressed as mean ± 1 SEM. Comparisons of specific measurements obtained with and without T4 treatment were made using Student’s t test. Statistical significance was set
Hyperthyroidism

Followed for

Log

Regression analyses

Slopes

RESULTS

No clinical signs of heart failure were present in
dogs and in rats when they were killed 10 days after
T₄ treatment. LV weight was slightly but not significa-
tically increased in dogs after T₄ treatment
(67.0±2.9 g) as compared with control dogs
(60.5±7.2 g, p=NS), and the LV weight/body
weight ratio was also slightly increased
(4.93±0.30 g/kg) as compared with control dogs
(4.32±0.51 g/kg, p=NS). In contrast, heart weight was signifi-
cantly increased in T₄-treated rats
(1.28±0.03 g) as compared with control rats
(1.07±0.03 g, p<0.001), with a 39% increase of the heart weight/body weight
ratio (2.87±0.06 g/kg compared with 2.06±0.05
g/kg in control rats, p<0.001).

Physiological Studies

In vivo ventricular function and response to catecholamines. Basal ventricular function data of six
dogs that were followed serially during 9 days after
T₄ administration are given in Figure 1. There was
a progressive increase of heart rate, which was
significantly higher 2 days after the onset of T₄
treatment than during the control period before
injections and was further increased 7 days later.
LV end-diastolic pressure was slightly but not sig-
ificantly decreased, and end-systolic pressure was
slightly increased after induction of hyperthy-
roidism. Peak positive dP/dt increases were parallel
to those of heart rate.

Changes in LV end-diastolic diameter were similar
to those of end-diastolic pressure, with a decrease
from 32.1±3.9 mm during control to 30.7±2.1 mm 2
days after T₄ treatment and an increase to 31.5±2.1
mm 9 days after T₄ treatment.

End-systolic diameter dimensions decreased from
23.4±2.6 to 22.3±1.8 mm 2 days after T₄ treatment, in
spite of an end-systolic pressure increase, and
returned to control 9 days after T₄ treatment
(23.8±1.5 mm). The percentage of diameter shortening tended
to decrease during the evolution of hyperthyroidism,
but the changes were not statistically significant (from

TABLE 1. Heart Rate and Peak dP/dt Responses to Catecholamines Expressed as the Slope of the Relation Between
the Variable and Log (Catecholamines)

<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Before T₄</th>
<th>2-Day T₄</th>
<th>9-Day T₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min/log [isoproterenol])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=5</td>
<td>27.5±2.4</td>
<td>23.7±3.0</td>
<td>20.2±2.5</td>
</tr>
<tr>
<td>n=9*</td>
<td>25.8±1.8</td>
<td>25.7±2.0</td>
<td>...</td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec/log [isoproterenol])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=5</td>
<td>1,820±156</td>
<td>1,780±201</td>
<td>1,267±308</td>
</tr>
<tr>
<td>n=9*</td>
<td>1,673±132</td>
<td>1,668±141</td>
<td>...</td>
</tr>
<tr>
<td>Dobutamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec/log [dobutamine])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=6</td>
<td>827±121</td>
<td>1,235±176</td>
<td>961±215</td>
</tr>
<tr>
<td>n=10*</td>
<td>754±88</td>
<td>1,041±165</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. T₄, 1-thyroxine treatment; 2-Day T₄ and 9-Day T₄, 2 and 9 days, respectively, after
initiation of T₄ treatment; n, number of dogs. There was no significant difference of slopes when the euthyroid and the
hyperthyroid states were compared.

*Dogs followed for only 2 days after T₄ treatment.
26.9±1.1% during control before T<sub>4</sub> administration to 25.5±1.0% and 23.4±1.7% 2 and 9 days after treatment, respectively).

**LV response to catecholamines.** Isoproterenol injections produced dose-dependent increases of heart rate before and after T<sub>4</sub> treatment. On a semilogarithmic plot, the relation between log (isoproterenol dose) and heart rate was highly linear (for all correlation coefficients: \( r>0.84, \) mean=0.956±0.011), and the slope of this relation was not significantly different before, 2 days after, and 9 days after T<sub>4</sub> treatment (Table 1).

Increasing doses of isoproterenol also produced dose-dependent increases of LV peak dP/dt (Figure 2). The relation between the injected dose of isoproterenol and the LV peak dP/dt was highly linear on a semilogarithmic plot (for all correlation coefficients: \( r>0.88, \) mean=0.958±0.08). The slope was not different before and 2 days after T<sub>4</sub> treatment and tended to be smaller 9 days after the beginning of the injections, although this change was not statistically significant (Figure 2, Table 1).

In contrast with isoproterenol injections, dobutamine infusions did not significantly modify either heart rates or end-systolic pressures (Table 2). In three of these dogs, LV peak dP/dt response to dobutamine under different heart rates was studied in the euthyroid state. During atrial pacing, which increased the heart rate from 87±9 beats/min during control to 183±9 beats/min, the slope of the relation between log (dobutamine dose) and LV peak dP/dt was increased to 1,071±298 mm Hg/sec/log (dose) from 734±274 mm Hg/sec/log (dose) during control, with an increase in the intercept from 3,120±367 mm Hg/sec during control to 3,562±282 mm Hg/sec (Figure 3).

Dobutamine infusions produced dose-dependent increases of LV peak dP/dt that were similar before and after T<sub>4</sub> treatment (Figure 4). When six different doses of dobutamine infusion were considered, there was a linear correlation between log (dobutamine infusion) and LV peak dP/dt with \( r>0.81 \) in all but one infusion (mean \( r=0.881±0.019 \)). The slopes of these relations were not significantly increased after induction of hyperthyroidism (Table 1), in spite of a possible underestimation of the slope in the euthyroid state due to a slower heart rate.

### Table 2. Heart Rate and Systolic Pressure Before and During Dobutamine Infusions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3 µg/kg/min</th>
<th>6 µg/kg/min</th>
<th>11 µg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>113.0±11.0</td>
<td>103.3±6.1</td>
<td>101.3±9.1</td>
<td>101.2±7.0</td>
</tr>
<tr>
<td>2-Day T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>132.0±14.8*</td>
<td>128.2±12.9</td>
<td>132.8±17.7</td>
<td>135.0±15.6</td>
</tr>
<tr>
<td>9-Day T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>179.7±7.8*</td>
<td>181.2±8.0</td>
<td>193.3±10.2</td>
<td>208.3±11.4</td>
</tr>
<tr>
<td>End-systolic pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>128.8±14.7</td>
<td>138.3±14.4</td>
<td>139.7±14.0</td>
<td>143.3±11.6</td>
</tr>
<tr>
<td>2-Day T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>137.2±4.9*</td>
<td>139.2±5.9</td>
<td>142.5±6.4</td>
<td>145.2±6.6</td>
</tr>
<tr>
<td>9-Day T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>138.5±6.7</td>
<td>140.0±5.8</td>
<td>146.0±8.4</td>
<td>154.3±10.9</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. Pre-T<sub>4</sub>, control period before thyroxine (T<sub>4</sub>) treatment; 2-Day T<sub>4</sub>, 2 days after initiation of T<sub>4</sub> treatment; 9-Day T<sub>4</sub>, 9 days after initiation of T<sub>4</sub> treatment. None of the changes produced by dobutamine infusion were statistically significant, but control heart rates were significantly larger 2 days and 9 days after T<sub>4</sub> treatment compared with pre-T<sub>4</sub> value.

*\( p<0.05 \) and \( \text{7}p<0.005 \) compared with corresponding pre-T<sub>4</sub> value.
Biochemical Studies in Dogs

Membrane receptors. There was a saturable and high affinity binding of radiolabeled ligand $^3$H-CGP 12177 in both euthyroid and hyperthyroid animals. Nonspecific binding evaluated by the ligand program was only 6.6% and 5.2% of specific binding for ligand concentrations close to $K_d$ values in control and hyperthyroid dogs, respectively.

$K_d$ values were not affected by the induction of the hyperthyroid state, and there was no significant increase of $B_{\max}$ after induction of the hyperthyroid state (Table 3, Figure 5).

In homogenates in which the whole tissue content was considered, $B_{\max}$ measured with $^{125}$I-CYP was not increased in hyperthyroid dogs as compared with euthyroid dogs. $K_d$ values were not different in the two groups of dogs (Table 3). Nonspecific binding was 22.4% and 20.2% of specific binding in control and hyperthyroid dogs, respectively.

Adenylate cyclase activity. Basal ACA was not different in control and hyperthyroid dogs treated for 3 and 10 days (Figure 6). Maximal stimulation of ACA by NaF (10 mM) resulted in a 6.0-fold increase in basal activity in normal dogs and a 6.4 and 5.6 increase in dogs treated with $T_4$ for 3 and 10 days, respectively (Figure 6). Results of stimulation of ACA with Gpp(NH)p and isoproterenol were also similar in normal and hyperthyroid dogs. The dose-response curve of ACA versus isoproterenol was not different in euthyroid and hyperthyroid dogs (Figure 7).

$5'$-Nucleotidase activity. The activity of another membrane marker ($5'$-nucleotidase) in homogenates was not different in euthyroid and hyperthyroid dogs (Table 3).

Plasma epinephrine and norepinephrine levels. Plasma catecholamines levels were not significantly different in normal and hyperthyroid states. Norepinephrine level was $330\pm127$ pg/ml before treatment, $348\pm183$ pg/ml 48 hours after the first $T_4$ injections, and $237\pm30$ pg/ml 7 days later, with epinephrine levels of $190\pm57, 277\pm179$, and $156\pm64$ pg/ml, respectively.

Rat Experiments

In contrast with dogs, $B_{\max}$ measured using $^3$H-CGP 12177 or $^{125}$I-CYP in crude membranes and homogenates was significantly increased after induction of hyperthyroidism in rats, without significant changes of $K_d$ values (Table 3, Figure 5). Nonspecific binding was 8.9% and 6.3% of specific binding in control and hyperthyroid rats, respectively, with $^3$H-CGP 12177; it was 9.7% and 4.1% in control and hyperthyroid rats, respectively, with $^{125}$I-CYP.

In competition binding experiments using 1-isoproterenol, the ligand program showed that all fits were significantly improved with a two-sites model. The percentage of high affinity binding sites was similar in control (71.0±4.8%) and in hyperthyroid (80.0±0.8%) rats, with a similar $K_d$ of high affinity sites (1.9±0.5 versus 1.5±0.4 nM, respectively).

Basal adenylate cyclase and cAMP production in response to NaF and isoproterenol were not different in euthyroid and hyperthyroid rats (Table 4).

The activity of another membrane marker, $5'$-nucleotidase, was also not significantly different in euthyroid and hyperthyroid rats (Table 3).
Discussion

LV Function and Response to Catecholamines

The induction of the hyperthyroid state was associated with a progressive increase of resting LV function, but LV response to catecholamines was not increased in hyperthyroid dogs.

LV function increase after T₄ treatment was demonstrated by the increase in LV dP/dt, although the percentage of systolic diameter shortening was not significantly modified by the development of hyperthyroidism. These results are in agreement with those previously published in conscious dogs¹¹ and also in conscious calves¹² in which hyperthyroid animals showed significant increases of heart rate, cardiac output, and LV dP/dt without significant changes of LV pressure and percentage of systolic diameter shortening. LV dP/dt increase could have been attributed to the associated heart rate increase, but previous studies in dogs¹¹ showed that positive inotropic action of T₄ is independent of tachycardia.

The absence of further changes in heart rate and LV function response to catecholamines in hyperthyroid animals as compared with euthyroid animals was demonstrated by the similar dose–response curves of heart rate and LV peak dP/dt to isoproterenol injections. Since heart rate changes produced by isoproterenol injections in the hyperthyroid state were not different from those in the control state before T₄ injections, LV dP/dt changes are probably not affected by heart rate changes produced by isoproterenol. Our results are in close agreement with those of Rutherford et al.,¹¹ who showed, in conscious dogs with an instrumentation similar to that of our study, an absence of increased LV response to isoproterenol injections or infusions with and without atrial pacing; this lack of response was demonstrated by similar modifications of LV pressure, peak dP/dt, and dimensions before and after induction of hyperthyroidism despite different baselines. However, isoproterenol induced changes of other hemodynamic variables, such as end-diastolic pressure and volume and systolic pressure, which could have affected LV dP/dt variations. This was the reason for the study of LV response to dobutamine infusions, a β-effector agent that did not significantly modify heart rate and systolic pressure (Table 2). Basal heart rates were different in the euthyroid and hyperthyroid states, but preliminary studies (Figure 3) showed that the slope of peak LV dP/dt versus log (dobutamine dose) was slightly increased by heart rate increase. This could have only underestimated the response to dobutamine in the euthyroid state. Results obtained with dobutamine infusions were similar to those obtained during isoproterenol injection, showing an absence of significant change of the slope of the dose–response of LV dP/dt in the development of hyperthyroidism, particularly 9 days after the onset of T₄ injections (Table 1).

The sensitivity of the heart to sympathomimetic amines during hyperthyroidism is controversial, with studies showing an increased,⁶,⁷,¹⁰,²³ no change,¹,³,⁸,⁹,¹¹ and even a decreased sensitivity.¹,² Methods used to test LV sensitivity to catecholamines are quite differ-

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Table 3. In Vitro Receptor Binding Studies in Normal and Thyrotoxic Dogs and Rats

<table>
<thead>
<tr>
<th></th>
<th>Dogs</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>³H-CGP 12177</td>
<td>³H-CGP 12177</td>
</tr>
<tr>
<td>Group</td>
<td>Bmax (fmol/mg)</td>
<td>Bmax (fmol/mg)</td>
</tr>
<tr>
<td></td>
<td>Kd (nM)</td>
<td>Kd (nM)</td>
</tr>
<tr>
<td>Control</td>
<td>87.0±7.1</td>
<td>87.0±7.1</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>T4 treatment</td>
<td>99.0±9.3</td>
<td>99.0±9.3</td>
</tr>
<tr>
<td>for 10 days</td>
<td>(n=6)</td>
<td>(n=6)</td>
</tr>
<tr>
<td></td>
<td>5'-Nucleotidase activity (nmol/mg/min)</td>
<td>5'-Nucleotidase activity (nmol/mg/min)</td>
</tr>
<tr>
<td></td>
<td>1.22±0.3</td>
<td>1.22±0.3</td>
</tr>
<tr>
<td></td>
<td>(n=4)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>T4 treatment</td>
<td>22.6±1.5*</td>
<td>22.6±1.5*</td>
</tr>
<tr>
<td>for 10 days</td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. Bmax and Kd were measured in crude membranes using ³H-CGP 12177 and in homogenates using ¹²⁵I-cyanopindolol (¹²⁵I-CYP) as ligands. In hyperthyroid dogs, both Bmax and Kd were not significantly different from control values (after 3 days of thyroxine [T₄] treatment, Bmax and Kd measured using ³H-CGP 12177 were 92.8±7.1 fmol/mg and 1.9±0.2 nM, respectively; n=6). In contrast, Bmax was significantly increased in T₄-treated rats as compared with control rats with both ligands.

*p<0.005 and tšt<0.02 compared with corresponding value for control rats.

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Figure 5. Scatchard representation of typical left ventricular myocardial β-adrenoceptor binding studies using ³H-CGP 12177 in dogs and rats with and without hyperthyroidism. Bmax was markedly increased in rats with hyperthyroidism as compared with control rats without affinity changes. In contrast, Bmax was not different in control and hyperthyroid dogs.
ent. Responses might not be similar in conscious animals and in open-chest anesthetized animals because of modifications of sympathetic activity and responsiveness induced by anesthetic agents. However, even when conscious animal or human studies alone are considered, results are conflicting. Most studies showed an unchanged cardiac sensitivity to catecholamines. In contrast, using techniques and analyses similar to those of our study, Hammond et al showed in conscious pigs an increased chronotropic sensitivity to isoproterenol. There are probably species differences in cardiac responses to catecholamines, but our results indicating an absence of enhancement of cardiac response to sympathomimetic amines in hyperthyroidism are in agreement with most currently available data in unanesthetized animals or humans. Thus, the basal increase of heart rate and ventricular function observed in the resting state in hyperthyroid dogs does not appear to be due to an increased sensitivity to catecholamines but is probably due to the expression of different isoforms of proteins and/or to a direct effect of thyroid hormones on the canine heart.

In Vitro Cardiac β-Adrenergic Receptor Binding and Adenylate Cyclase Activity

The absence of increased β-adrenergic receptor number in hyperthyroid dogs is in agreement with the absence of increased in vivo response to catecholamines in these animals. These results are different from presently published studies on the effects of thyroid hormones on cardiac β-adrenergic receptors that showed an increased receptor density. Nevertheless, β-adrenergic receptor density was increased in our study by 72.5% or 41% when it was measured in hyperthyroid rats with 3H-CGP 12177 or 125I-CYP, respectively, which is in agreement with previously reported data in rat hyperthyroidism. Another species difference between dogs and rats is shown by the anatomic data. Heart weight was significantly increased after T4 treatment in rats but not in dogs. Similar results had been published by Taylor et al, who showed an unchanged LV weight in dogs after subcutaneous injections of 1 mg/kg T4 for 10–20 days. The absence of β-adrenoceptor increase in dog hyperthyroidism does not appear to be due to the techniques used, such as those used for membrane preparations and ligand studies, since they were the same in both species. Furthermore, similar results were found using different techniques (homogenates versus more purified membranes, 125I-CYP versus 3H-CGP 12177). This eliminates a possible role of the chosen ligand or a selection of cellular subfractions by centrifugation such as that found by Wolff et al, who showed that purification of membrane preparations could mask receptor variations due to ischemia. Both in dogs and in rats, Bmax obtained from homogenates, which do not discard any cellular fraction, were similar to those found with more purified

Figure 6. Bar graph showing left ventricular myocardial basal adenylate cyclase activity and responses to NaF, Gpp(NH)p, and isoproterenol in the euthyroid state and 3 days (3D) and 10 days (10D) after the onset of thyroxine (T4) treatment.

Figure 7. Dose–response curve of left ventricular myocardial adenylate cyclase activity vs. isoproterenol concentration. Adenylate cyclase activity was expressed as the percentage of the maximal response, with the zero value corresponding to the value obtained without isoproterenol. Points represent the means of each group, which are not different in the euthyroid state and 3 days (3D) and 10 days (10D) after thyroxine (T4) treatment.
Table 4. Adenylate Cyclase Activity in Cardiac Membranes of Euthyroid and Hyperthyroid Rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>Control</th>
<th>NaF (10^-4 M)</th>
<th>Isoproterenol (10^-4 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>11.4±2.0</td>
<td>77.8±7.0</td>
<td>30.8±3.4</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>10.0±1.1</td>
<td>72.2±3.1</td>
<td>29.5±2.4</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. Adenylate cyclase activity was not different in euthyroid and hyperthyroid rats in basal conditions or after stimulation by NaF or isoproterenol.

preparations. Another membrane marker, 5'-nucleotidase, did not show significant differences of activities in normal and hyperthyroid animals (Table 3), confirming the similar plasma membrane composition of the preparations in euthyroid and hyperthyroid animals.

These physiological and receptor results are corroborated by ACA data, which were not different in cardiac membranes obtained from euthyroid and hyperthyroid dogs: there were no detectable differences in basal activity and maximal stimulation by NaF and isoproterenol (Figure 6), and there were no changes in the dose–response curve of isoproterenol (Figure 7), suggesting an absence of change of β-adrenoceptor affinity for isoproterenol. In rats, basal and stimulated ACAs with NaF or isoproterenol were not increased in the hyperthyroid state, in spite of the increased β-adrenergic receptor number (Table 4). Previously published results of ACA are as conflicting as those concerning ventricular sensitivity to catecholamines in vivo. Most studies showed an increased ACA in rats. In contrast, two studies showed a normal basal and isoproterenol-stimulated ACA in hyperthyroid cats, which might suggest again species differences, but other studies in rats also failed to detect changes of ACA in hyperthyroidism. In the recent study by Levine et al., basal ACA was decreased in hyperthyroid animals but, similar to our results, the increase in cAMP production produced by isoproterenol, Gpp(NH)p, or NaF was not different in the hyperthyroid and euthyroid state. The mechanism for an absence of increased ACA in this species is unknown. Changes of signal transduction might exist. However, we did not find any decrease in the percentage of high affinity binding sites for isoproterenol in hyperthyroid rats, and Levine et al. did not show any change in the amounts of Gα and Gβ/γ proteins in the hyperthyroid state. These authors suggested that Gα or Gβ/γ reassociation or both could be modulated by thyroid status, but further studies need to be done to clarify the absence of increase of isoproterenol-induced ACA in the hyperthyroid rat. In contrast, the absence of increase of ACA after stimulation by isoproterenol in hyperthyroid dogs is explained by the absence of increased β-adrenoceptors in this species, which is associated with an absence of changes in receptor affinity for isoproterenol as shown by the dose–response curve of cAMP production versus isoproterenol doses (Figure 7).

In conclusion, it appears that cardiac β-adrenergic receptor density may have different evolutions in response to hyperthyroidism induced by T4 injections in different species and that an increased β-adrenergic receptor number is not a universal finding in this model. One possibility is a different metabolism of T4 in dogs and in rats, since, with the same treatment, ventricular hypertrophy was present in rats but not in dogs, in spite of an observed direct effect of T4 on basal ventricular function. Another possibility is a species difference in the effect of thyroid hormones on β-adrenoceptor homeostasis. For instance, tissue specificity is already known with a decrease of β-adrenergic receptor number in the liver and an absence of change in the lungs or the lymphocytes. Responses to thyroid hormones could be similarly different in different species. Physiological responses to a number of interventions are different in rats compared with other mammals; for instance, there are differences in myosin isoenzyme distribution in normal conditions and in response to ventricular overloads. The reason for an absence of increased ACA with an increased β-adrenoceptor density in rats is unknown, but our study does indicate that the absence of β-receptor increase in canine hyperthyroidism is associated with an absence of changes in both ACA and the dose–response curve of cAMP production with isoproterenol stimulation, leading to an absence of increased cardiac response to catecholamine injection in the hyperthyroid state in this species. Similar results can be expected in humans, in whom a number of studies have shown an absence of mediation of the sympathetic nervous system in the cardiac response to hyperthyroidism.

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References


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B Crozatier, J B Su, A Corsin and Bouanani N el-H

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