Hypothyroidism Delays Ischemia-Induced Contracture and Adenine Nucleotide Depletion in Rat Myocardium


Isolated perfused paced hearts from rats rendered hypothyroid by chronic administration of propylthiouracil have a delayed onset of ischemia-induced myocardial contracture in contrast to hearts from control rats. In addition, the time to reach maximum contracture is delayed, and the magnitude of the contracture pressure is reduced. Preischemia myocardial adenosine triphosphate (ATP) values in the hypothyroid rat hearts are similar to those of control, but the rate of decrease in ATP is slower in the hearts of hypothyroid rats. Thus, it appears that in the hypothyroid state the development of ischemic contracture is associated with a slower fall of ATP. (Circulation Research 1987;60:649–652)

Protection or functional preservation of the ischemic myocardium has been achieved by numerous interventions that are generally directed toward conserving energy stores by manipulating the frequency of depolarization or the force of contraction. Experimental interventions, such as hyperkalemia, β-blockade, hypothermia, or calcium channel blockade, have all been shown to delay the onset or diminish the severity of ischemia-induced cardiac contracture.1-3 Theoretically, a decreased basal metabolic rate as in hypothyroidism should have a similar capacity. It is interesting that in the past, the induction of the hypothyroid state was found to be therapeutically useful in treating the symptoms of angina pectoris.4 The present studies sought to examine the effect of propylthiouracil-induced hypothyroidism on the time course and metabolic basis of ischemic myocardial contracture in the rat.

Materials and Methods

Sprague-Dawley rats (350–500 g, from Charles River Breeding Co., Waltham, Mass.), were given 0.06% propylthiouracil (Sigma Chemical Co., St. Louis, Mo.) in their drinking water (hypothyroid group) or water (controls) for at least 4 weeks prior to contracture assessment. This concentration and route of propylthiouracil (PTU) administration have been shown by others to be reliable in producing hypothyroidism.5,6 At the time of killing, a blood sample was removed from the thorax for measurement of thyroid-stimulating hormone by the use of kits supplied by the National Institute of Arthritis, Diabetes, and Metabolic Diseases. After administration of 500 U heparin i.p. (Elkins-Sinn, Inc., Cherry Hill, N.J.) 10 minutes before loss of consciousness by ether exposure, the heart and great vessels were rapidly excised and placed in ice-cold 0.9% sodium chloride solution; the aorta was attached to a Langendorff perfusion apparatus. The isolated hearts were then perfused at constant pressure (85 mm Hg) with a Krebs-Henseleit solution (KHS) consisting of (mmol liter−1) sodium chloride 118.5, sodium bicarbonate 25.0, dextrose 5.5, potassium chloride 4.4, calcium chloride 1.3, potassium monobasic phosphate 1.2, and magnesium sulfate 1.2.

The KHS was equilibrated with 95% O2–5% CO2 (Sunox) at room temperature and warmed to 37° C in a water-jacketed column before perfusion through the heart (20.6 ± 0.7 ml/min control, 18.3 ± 0.8 (p < 0.05) hypothyroid). The pH was 7.39 ± 0.01, Paco2 40.9 ± 0.7 mm Hg, and Paco2 590 ± 1.6 mm Hg. A small compliant balloon-tipped catheter was then placed in the left ventricle via the mitral valve and filled with saline to produce a left ventricular end diastolic pressure of 6 mm Hg. The peripheral end of the catheter was attached to a Gould transducer and recorder (model 440) to monitor heart rate, left ventricular systolic pressure (LVSP), and left ventricular diastolic pressure (LVEDP) throughout the experiment.7 Coronary flow was measured with a graduated cylinder and stopwatch as it exited the perfusion chamber, which encased the heart and kept the temperature of the nonperfused hearts at 37° ± 0.2° C.

Since preliminary studies revealed a significantly slower heart rate in rats rendered hypothyroid by PTU administration or thyroidectomy, hearts were paced at a rate of 320 min−1 by means of a Grass SD9 stimulator (7 volts; 2 milliseconds). After 25 minutes of stabilization, during which LVSP and LVEDP were measured, ischemia was induced by halting perfusion.

In a separate series of experiments using the same protocol, the adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) content of whole hearts was measured either before or at one of two points during the time course of

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ischemia. At each time point, a pair of Wollenberger tongs, cooled in liquid nitrogen, was applied to the heart. The frozen samples were kept at -80°C for several days until analyzed by high pressure liquid chromatography. After solubilization in 1 N NaOH, myocardial protein content was measured in perchloric acid insoluble tissue pellets by the method of Lowry et al. Differences between groups were measured using Student's t test for unpaired observations. Values are expressed as mean ± SEM.

Results

Preischemic Effects of Hypothyroidism

Chronic exposure to PTU produced less increase in body weight (392 ± 14 g) relative to controls fed ad libitum (512 ± 14 g, p < 0.001). The heart/body wt ratio was also significantly lower (p < 0.001) in the PTU-treated group (control 5.56 ± 0.08 × 10⁻⁴; PTU 4.73 ± 0.13 × 10⁻⁴, p < 0.001). The plasma TSH activity was normal at 774 ng/ml in control rats and greater than 5,000 ng/ml in PTU-treated rats. All of these values suggest successful induction of hypothyroidism. After establishing perfusion but before pacing the heart, the heart rate of the hypothyroid rats was 225 ± 5 min⁻¹, whereas that of the controls was 280 ± 5 min⁻¹ (p < 0.01). While paced at 320 min⁻¹, the LVSP in hypothyroid hearts (76.2 ± 1.9 mm Hg) was only 85% that of control hearts (88.6 ± 2.1 mm Hg, p < 0.001). Calculation of coronary flow per gram dry heart weight was significantly less in the PTU group (78.8 ± 3.5 ml/g min) than in control rats (66.9 ml/g min, p < 0.5).

Time Course of Ischemic Contracture

With the cessation of perfusion, pacing was continued for 3 minutes, by which time contractions had ceased in both groups. Following the induction of ischemia, the control group appeared to lose contractility more rapidly. Representative tracings of the different patterns of ischemia-induced contracture in the two groups are shown in Figure 1. When compared with controls (9.4 ± 0.4 minutes), the time of onset of ischemic contracture was delayed by a factor of about 2 in the PTU-treated rat hearts (20.0 ± 0.6 minutes, p < 0.01). The time to peak contracture tension in controls (15.3 ± 0.6 minutes) was also delayed in PTU-treated rats (25.8 ± 0.6 minutes), and the maximal degree of contracture produced was less (control 74.2 ± 4.4 mm Hg; PTU 43.5 ± 4.2 mm Hg, p < 0.01). In 3 animals surgically thyroidectomized 3 weeks previously, a similar time course of delayed and blunted ischemic contracture was also observed.

Alteration in Cardiac Adenine Nucleotides During Ischemia

Prior to the onset of ischemia, the myocardial adenine nucleotide content of the PTU-treated group was not significantly different (p > 0.05) from that of controls (Table 1). During ischemia, however, the ATP declined much more rapidly in this preparation. At 6 and 15 minutes after cessation of perfusion, both total adenine nucleotides and the ATP/ADP ratio were reduced to a smaller degree in PTU hearts than in controls. Conversely, the AMP present in PTU-treated hearts 15 minutes after onset of ischemia was less than half that present in the control heart group (Table 1).

Discussion

The isolated perfused rat heart model has been used in numerous studies of myocardial contracture, function, and metabolic processes. Most studies of ischemia in this preparation have focused on the effects of changes in perfusate substrates, ions, and drugs on the
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Figure 2. Rate of decline in cardiac ATP during ischemia. Hearts of control and PTU-treated rats were prepared as in Figure 1 with freezing 25 minutes after initiating control period perfusion (0 time) or at 6 or 15 minutes after cessation of perfusion.

time course of ischemic contracture. Relatively few experimental studies have been done with pathologic cardiac states and the coincident changes in the ischemic contracture process.

Our initial studies in isolated heart preparations from surgically thyroidectomized rats revealed a slower spontaneous heart rate compared to controls. Since Hearse et al have shown that a faster heart rate before induction of ischemia can alter the development of ischemic contracture, we deliberately paced both control and experimental hearts at a rate slightly above that of the fastest spontaneously beating control hearts. Hearse also showed that ischemic contracture is dependent in part on a critical depletion of ATP. The delay in onset of contracture that we observed in the hypothyroid rats could be a result of either increased cellular stores of ATP at the onset of ischemia or a decreased rate of consumption after the start of ischemia. However, in this experiment, cardiac ATP, ATP/ADP ratio, and total adenine nucleotides immediately prior to ischemia, after 25 minutes of Krebs-Henseleit perfusion, were comparable in the control and the PTU-treated hearts. Buccino et al observed that in hypothyroid anesthetized cats, cardiac ATP content was slightly but significantly lower than in controls. However, in that experiment the tissue samples were only frozen after isolation with a room temperature

Table 1. Effects of Global Ischemia on Adenine Nucleotides (µmol/g protein)

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>Total</th>
<th>ATP/ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischencia</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PTU</td>
<td>38.02±1.50</td>
<td>13.4±0.2</td>
<td>4.6±0.1</td>
<td>55.85±1.62</td>
<td>2.81±0.08</td>
</tr>
<tr>
<td>Control</td>
<td>37.0±0.3</td>
<td>13.4±0.8</td>
<td>4.3±0.04</td>
<td>54.56±0.97</td>
<td>2.78±0.20</td>
</tr>
<tr>
<td>6 minutes of ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>26.6±1.35*</td>
<td>16.1±0.9</td>
<td>6.9±0.2</td>
<td>49.62±1.79</td>
<td>1.66±0.9</td>
</tr>
<tr>
<td>Control</td>
<td>21.5±0.9</td>
<td>16.3±1.6</td>
<td>8.6±1.5</td>
<td>46.31±2.16</td>
<td>1.36±0.18</td>
</tr>
<tr>
<td>15 minutes of ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>19.5±1.3†</td>
<td>13.3±0.5†</td>
<td>12.2±0.8†</td>
<td>44.96±0.88†</td>
<td>1.51±0.15*</td>
</tr>
<tr>
<td>Control</td>
<td>8.3±0.4</td>
<td>7.3±0.9</td>
<td>26.2±0.6</td>
<td>41.29±0.73</td>
<td>1.15±0.8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of 8 observations. *p<0.05, †p<0.001 relative to corresponding control values.

Hypothyroid rats could be a result of either increased pendent in part on a critical depletion of ATP. The only frozen after isolation with a room temperature However, in that experiment the tissue samples were slightly but significantly lower than in controls. Hypothyroid anesthetized cats, cardiac ATP content were comparable in the control and the experimental hearts at a rate slightly above the fastest spontaneously beating control hearts. Since Hearse et al have shown that ischemic contracture is dependent in part on a critical depletion of ATP. The delay in onset of contracture that we observed in the hypothyroid rats could be a result of either increased cellular stores of ATP at the onset of ischemia or a decreased rate of consumption after the start of ischemia. However, in this experiment, cardiac ATP, ATP/ADP ratio, and total adenine nucleotides immediately prior to ischemia, after 25 minutes of Krebs-Henseleit perfusion, were comparable in the control and the PTU-treated hearts. Buccino et al observed that in hypothyroid anesthetized cats, cardiac ATP content was slightly but significantly lower than in controls. However, in that experiment the tissue samples were only frozen after isolation with a room temperature

rongoeurs or sampling forceps, making comparisons difficult.

Various investigations have demonstrated the effect of thyroid hormones in modulating enzymes that control the rate of ATP hydrolysis and release of energy for cellular metabolic consumption. It has also been reported that the sarcoplasmic reticulum calcium-stimulated ATPase varies as a function of the thyroid state; a reduction in catalytic activity of this enzyme by the absence of thyroid hormone would serve to conserve ATP. The hypothyroid rat shows decreased cardiac myosin Ca-ATPase activity. Perhaps an increased level of the V, low activity form of the myosin ATPase isoenzyme, would also serve to conserve ATP stores. The decreased amount of ATP consumed in the above processes in hearts from hypothyroid rats may serve to reduce more gradually the depletion of myocardial ATP stores relative to control rat hearts.

Many years ago, it was demonstrated that the hypothyroid rat heart has an accelerated turnover of norepinephrine. This is accompanied by a decrease in β-receptor numbers. Since a portion of the release of adenosine (derived from ATP) is due to catecholamine release during ischemia, a decrease in the amount of norepinephrine within the hypothyroid heart, together with a decrease in number of β-receptors, would reduce the rate of adenosine base loss from the PTU-treated hearts relative to controls. Thus, ATP levels would remain higher for a longer period of time.

Hearse has suggested that contracture begins when myocardial ATP reaches about 60% of basal preischemic values. Present observations show that the rate of ATP loss with ischemia is slower in hypothyroid hearts, which, along with a delayed onset of contracture, would support this suggestion. Moreover, the concentrations of ATP/mg protein (or dry weight) is roughly the same at durations of ischemia chosen to precede the onset of contracture. It would thus appear that the delay in onset of ischemic contracture in the hearts of hypothyroid rats is associated with a slower fall in the high energy phosphate supply.

It is interesting that the most recent forms of therapy directed at ischemic heart disease have modulated the metabolic activity of the heart. Examples include β-
blockade and the increasingly popular calcium channel blockers. The hypothyroid state may achieve the same result by controlling cellular metabolic activity within the cell and therefore improving supply-demand relations. Obviously, further studies are required to evaluate the ability of hearts from hypothyroid rats to recover functionally from ischemia. Since Fleckenstein and Jennings and Ganote noted a correlation between cell damage and ATP exhaustion, it is possible that the maintenance of myocardial ATP in hypothyroid ischemic hearts may presage an enhanced chance of recovery.

The relevance of the present observations to clinical management of hypothyroid patients is unclear. However, the presence of mild or moderate hypothyroidism does not appear to represent inordinate risk to patients in danger of hypoxic episodes. Thus, Weinberg et al retrospectively assessed the outcome of anesthesia and surgery in 59 hypothyroid patients and in 59 paired euthyroid controls matched for age, sex, surgery, and surgeon. They found that in patients with mild or moderate hypothyroidism, no evidence of surgery-enhanced perioperative morbidity was present. It is conceivable that part of the reason for the resilience during the perioperative period is related to resistance of the heart to ischemic damage.

Conclusions

Hearts of rats made hypothyroid by propylthiouracil administration develop normothermic ischemic contracture more slowly than do hearts of control rats. Since ATP stores fall more slowly in hearts of hypothyroid rats, the delay in onset and the magnitude of ischemic contracture may be functions of delayed exhaustion of adenine nucleotide stores.

References

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Key Words • hypothyroidism • myocardial contracture • adenine nucleotides • ischemia • propylthiouracil
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