Reduction of 5′-Nucleotidase Activity in Rat Thyroid and Adenohypophysis following Methylthiouracil Treatment

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Synopsis

5′-Nucleotidase activity was determined in rat thyroid and some other organs employing a specific assay method. During the course of methylthiouracil (MTU) treatment, thyroid 5′-nucleotidase activity decreased significantly. This decrease was specific for this enzyme since the activity of neutral phosphatase did not change and the activity of alkaline phosphatase and Mg2+-activated adenosine triphosphatase increased markedly. The 5′-nucleotidase activity of the adenohypophysis also decreased following MTU treatment. This enzyme activity of the liver, heart and whole brain remained unchanged after the treatment. The role of this enzyme was discussed in relation to tissue growth and increased contents of RNA and DNA in the thyroid and adenohypophysis.

It is well known that thyrotropin (TSH) regulates the biosynthesis of nucleic acids in the thyroid. The incorporation of labelled precursors into nucleic acids is increased by TSH administered in vivo or in vitro (Fiala et al., 1957; Hall, 1963; Begg and Munro, 1965; Creek, 1965; Shimada and Yasumasu, 1966; Kerkof and Tata, 1967). In TSH-stimulated thyroids, the increases are observed in both concentrations and total amounts of ribonucleotides (Lindsay and Cohen, 1965) and of RNA (Matovinovic and Vickery, 1959; Ekholm and Pantić, 1963). Although such higher contents have been ascribed mainly to increased synthesis, these might also be partly due to a decrease in the catabolic activity.

TSH does not affect thyroid alkaline ribonuclease activity but increases a specific inhibitor for this enzyme (Greif and Eich, 1972; Murthy and McKenzie, 1974). As Kraft and Shortman (1970) suggested, increased ribonuclease inhibitor would bring about reduced RNA degradation and raise RNA level. 5′-Nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) has also been shown to play a key role in catabolism of nucleic acids by lysosomes (Arsenis and Touster, 1968). The present paper reports a decreased activity of 5′-nucleotidase and increased contents of RNA and DNA in the thyroid and adenohypophysis from rats treated with methylthiouracil (MTU).

Materials and Methods

Male rats of Wistar strain weighing approximately 200 g were used. A group of rats were given 0.03% MTU and another group 1% sodium perchlorate in drinking water so as to induce TSH secretion by blocking the synthesis of thyroid hormone. Beef thyroids were obtained from a local abattoir. Homogenization of thyroids and other organs and differential fractionation of the beef thyroid homo-
genase were performed as described previously (Matsuzaki et al., 1973).

5'-Nucleotidase was assayed with adenosine 5'-monophosphate (5'-AMP) as substrate after the principle described by Ipata (1967). Unless otherwise stated, the standard reaction mixture contained, in a final volume of 3.0 ml, 60 mM Tris-HCl buffer, pH 7.4, 0.5 μg (0.1 unit) adenosine deaminase (Boehringer Mannheim), 10 mM MgCl₂, 0.1 mM 5'-AMP and 20 mM sodium β-glycerophosphate. The excess amount of β-glycerophosphate was added to inhibit competitively the hydrolysis of 5'-AMP by nonspecific phosphatase(s) (Belfield and Golberg, 1968). Adenosine deaminase was added in excess to secure complete conversion of adenosine, liberated by the action of 5'-nucleotidase, into inosine. The decrease in absorbance was followed at 262.5 nm at 37°C by a Cary Spectrophotometer Model 17.

Neutral (pH 7.4) and alkaline (pH 10.5) phosphatases and Mg²⁺-adenosine triphosphatase (Mg²⁺-ATPase) were assayed as described previously (Matsuzaki et al., 1973). Enzymatic dephosphorylation of 5'-AMP, 3'-AMP and adenosine triphosphate (ATP) was assayed by determining the released inorganic phosphate after the method of Chen et al. (1956). All the enzyme activities of rat tissues were determined using whole homogenates as enzyme source.

RNA, DNA and protein were measured using the orcinol method (Schneider, 1957), the diphenylamine reaction (Burton, 1957) and the method of Lowry et al. (1951), respectively.

**Results**

*Some properties of beef thyroid 5'-nucleotidase*

5'-Nucleotidase activity of beef thyroid was mainly found in 165,000 × g ("microsomal") pellet and the supernatant (Matsuzaki et al., 1973). β-Glycerophosphate (1–10 mM) inhibited the activity of nuclear and mitochondrial 5'-nucleotidases in a dose-related manner (10–25%) but only slightly (0–2%) that of the microsomal and soluble enzymes, indicating a greater contamination of nonspecific phosphatase(s) in heavier particles.

Both microsomal and soluble 5'-nucleotidases had a maximal activity at around pH 7.4 (Tris-HCl buffer) in the presence of 10 mM MgCl₂. Although a double pH optimum has been reported for 5'-nucleotidases in some animal tissues (Center and Behal, 1966; Levin and Bodansky, 1966; Song and Bodansky, 1966), the second optimum at alkaline pH was not detectable in the beef thyroid by the present assay method, since adenosine deaminase lost its activity at pH 9 or higher. However, when 5'-AMP hydrolysis was determined on the basis of released inorganic phosphate, the second pH optimum was found at around pH 9.2 (glycine-NaOH buffer). These data show that the pH-activity curves for thyroid 5'-nucleotidases closely resemble those of 5'-nucleotidases purified from bull seminal plasma (Bodansky and Schwartz, 1963; Levin and Bodansky, 1966) and human liver (Song and Bodansky, 1966). Magnesium ion is essential for optimal 5'-nucleotidase activity of the thyroid, as in most other tissues (Heppel and Hilmoe, 1951; Ahmed and Reis, 1958). The stimulatory effect of 10 mM Mg²⁺ was noted only at pH 8.0–8.5 with glycine-NaOH buffer. At around pH 7.4 (Tris-HCl buffer) the effect was only slight, and at a lower pH range (acetate buffer) the effect was almost negligible. On the basis of these findings, 5'-nucleotidase from rat thyroid and some other organs was assayed at pH 7.4 in the presence of Mg²⁺ for the further study.

*Changes in rat thyroid weight, RNA and DNA contents, and RNA/DNA ratio during MTU treatment*

Following MTU administration, serum protein-bound iodine (PBI) decreased markedly, circulating TSH levels rose several fold over the control level within a few days, and, thyroid enlargement occurred (Matsuzaki and Suzuki, 1974, 1975). MTU at 0.03% in drinking water was more potent than perchlorate at 1% in inducing both TSH secretion and thyroid enlargement. The adenohypophysis weight increased slightly but significantly after 15 days of MTU treatment.

Fig. 1 shows that RNA and DNA
accumulated in the thyroid during goiter formation induced by MTU. Total amount of thyroidal RNA and RNA/DNA ratio increased markedly between the 2nd and the 7th day of the treatment. The increase in RNA content was greater than that of thyroid weight, and the increase in RNA content started prior to that in DNA content. It appears that the increase in nucleic acids is an important aspect of TSH regulation of thyroid size.

**Effect of MTU treatment on 5'-nucleotidase activity in several organs of the rat**

Fifteen days after MTU treatment, thyroid 5'-nucleotidase activity per mg thyroid protein decreased slightly but significantly (Table 1). Pituitary 5'-nucleotidase was the most active among the organs studied, and the enzyme activity reduced to below 50% of the normal level. No significant change was observed in the liver, heart or whole brain, although heart weight decreased significantly.

**Table 1. Effect of methylthiouracil (MTU) treatment on 5'-nucleotidase activity of various organs**

<table>
<thead>
<tr>
<th>Organ</th>
<th>5'-Nucleotidase (nmole/min/mg protein)</th>
<th>MTU (5)a</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (5)a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>26.7±1.30b</td>
<td>22.0±1.21b</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>Adenohypophysis</td>
<td>65.9±4.23</td>
<td>28.7±1.29</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Liver</td>
<td>15.2±0.74</td>
<td>13.3±0.92</td>
<td>NS</td>
</tr>
<tr>
<td>Heart</td>
<td>4.6±0.37</td>
<td>5.6±0.46</td>
<td>NS</td>
</tr>
<tr>
<td>Whole brain</td>
<td>8.8±0.16</td>
<td>8.1±0.35</td>
<td>NS</td>
</tr>
</tbody>
</table>

MTU (0.03%) in drinking water was given to rats for 15 days. a, number of rats; b, mean±standard error; NS, statistically non-significant.

**Effect of goitrogen treatment on the activity of 5'-nucleotidase and some other phosphatases**

Thyroid 5'-nucleotidase activity per mg DNA decreased significantly 10 days after MTU treatment but not after sodium perchlorate treatment (Table 2). Pituitary 5'-nucleotidase activity also reduced significantly.

**Table 2. Effect of MTU and sodium perchlorate treatment on the activity of some thyroid enzymes**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Enzyme activity (nmole/min/mg DNA)</th>
<th>Control (5)a</th>
<th>MTU (5)a</th>
<th>Perchlorate (6)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-Nucleotidase</td>
<td>1.02±0.022</td>
<td>0.83±0.048c</td>
<td>0.96±0.115</td>
<td></td>
</tr>
<tr>
<td>(pH 7.4)</td>
<td></td>
<td></td>
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<tr>
<td>Neutral phosphatase</td>
<td>0.78±0.087</td>
<td>0.88±0.018</td>
<td>0.83±0.057</td>
<td></td>
</tr>
<tr>
<td>(pH 7.4)</td>
<td></td>
<td></td>
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<tr>
<td>Alkaline phosphatase</td>
<td>0.18±0.022</td>
<td>0.80±0.068d</td>
<td>0.63±0.070d</td>
<td></td>
</tr>
<tr>
<td>(pH 10.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg²⁺-ATPase</td>
<td>3.59±0.32</td>
<td>5.63±0.19d</td>
<td>6.06±0.19d</td>
<td></td>
</tr>
</tbody>
</table>

MTU (0.03%) and sodium perchlorate (1%) in drinking water were given to rats for 10 days. a, number of determinations; c, p<0.01; d, p<0.001.
significantly to 49% and 73% of the control level after MTU and perchlorate, respectively. In contrast, nonspecific neutral phosphatase activity of the thyroid, when measured with p-nitrophenyl phosphate as substrate, remained unchanged after either MTU or perchlorate treatment, while alkaline phosphatase activity increased about threefold after the goitrogen treatments. Mg$^{2+}$-activated ATPase activity also increased significantly. Dephosphorylation of 3'-AMP by thyroid and pituitary homogenates was only 21 and 9% that of 5'-AMP, respectively. 3'-AMP dephosphorylation activity in the thyroid increased significantly after 20 days of MTU treatment.

Changes in 5'-nucleotidase activity of the thyroid and adenohypophysis during MTU treatment

Fig. 2 shows the time course of MTU effect on 5'-nucleotidase activity. Thyroid 5'-nucleotidase activity decreased to 65% of the control level within 5 days after MTU treatment and appeared to remain decreased until the 15th day. Pituitary 5'-nucleotidase activity did not change until the 5th day of the treatment, decreased to 50% of the control level on the 10th day and reached 40% of the control on the 15th day. Pituitary 5'-nucleotidase was also found to decrease to 25% of the control 6 weeks after thyroidectomy.

Discussion

The present study has shown that thyroid and pituitary 5'-nucleotidase activity is reduced by the treatment of rats with MTU within 10 days. The thyroid enzyme activity, expressed whether in terms of unit per mg protein or per mg DNA, decreases slightly but significantly. Since calculated mean cell volume of the follicular cell increases by 50% or more (Philp et al., 1969) and the colloid content in the thyroids decreases after MTU treatment, the decrease in the intracellular concentration of 5'-nucleotidase is expected to be much greater than the present data.

Bastomsky et al. (1971) reported that the activity of thyroid 5'-nucleotidase from propylthiouracil-treated or low iodine diet-fed rats increased significantly after 30 to 35 days. The possibility, however, cannot be excluded that some part of the enzyme activity measured in their study is due to nonspecific phosphatase(s), which increase after goitrogen treatment. Another alternative is that thyroid 5'-nucleotidase activity shows a biphasic variation after goitrogen treatment, i.e. an initial decrease and an increase after a prolonged treatment. Because of a high activity of nonspecific phosphatase(s) as shown in Table 2, a specific assay method is required in order to appropriately measure 5'-nucleotidase activity. The technique used in this report is a specific method which enables separation of 5'-nucleotidase from nonspecific phosphatase(s).

A number of enzymes have been shown
to increase their activity after a single or repeated injections of TSH or following goitrogen treatment. Among them are well known oxidative enzymes (DeGroot and Dunn, 1966), polyamine-synthesizing enzymes (Matsuzaki and Suzuki, 1974, 1975), uridine kinase and uridine phosphorylase (Lindsay and Cohen, 1965). In this study, alkaline phosphatase and Mg\(^{2+}\)-ATPase showed increased activities. Thyroid 5'-nucleotidase is an exceptional enzyme which decreases in activity during the course of thyroid enlargement caused by goitrogen treatment. The direct effect of MTU or perchlorate on 5'-nucleotidase activity could be excluded since the enzyme activity in tissues other than thyroid and adenohypophysis was unaffected (Table 1). It is likely, therefore, that the chronic high level of TSH, but not the goitrogens, caused the decrease in the enzyme activity.

In regenerating liver, it was reported that the activity of 5'-nucleotidase (Fritzson, 1967) and uracil-catabolizing enzymes (Fritzson, 1962) show decreases. A reciprocal relationship was observed between nucleoside kinases and 5'-nucleotidase in regenerating liver (Arima et al., 1972), and between alkaline phosphatase and 5'-nucleotidase in human bone (Goldberg and Belfield, 1974). This might also be the case in the thyroid.

The physiological role of 5'-nucleotidase is not fully understood, but it may play a key role in nucleic acid catabolism (Arsenis and Touster, 1968). 5'-Nucleotidase is different from alkaline phosphatase in that the former is capable of hydrolyzing 5'-nucleotides much more rapidly than other phosphoric acid esters, such as 3'-AMP or \(\beta\)-glycerophosphate (Heppel and Hilmoe, 1951; Song and Bodansky, 1966). It is, therefore, highly probable that 5'-nucleotidase participates in regulation of the level of precursors available for the polymerization reaction leading to nucleic acid synthesis. The present data strongly suggest that one possible mechanism accounting for increased ribonucleotide concentrations in goitrous thyroid is a decreased activity of 5'-nucleotidase. It has been shown that the 5'-nucleotidase activity decreases in proliferating mammary gland (Wang, 1962) and regenerating liver (Fritzson, 1967; Arima et al., 1972) of the rat, as in the case of goitrous thyroid.

MTU administration causes an increase in weight of rat adenohypophysis. In this tissue, RNA synthesis is primarily very active, and activated further in hypothyroid state of the rat (Tonoue and Yamamoto, 1968; Lee et al., 1968; Matsuzaki, 1969). The stimulated RNA synthesis would be supported by a high level of nucleotides due to decreased 5'-nucleotidase.

In general, during accelerated growth of tissues, including the thyroid and adenohypophysis, reduced degradation of nucleic acids or nucleoside monophosphates is probable for several reasons: 1) concentrations and total amounts of nucleic acids and ribonucleotides are increased (Lindsay and Cohen, 1965), 2) alkaline ribonuclease inhibitor is increased (Greif and Eich, 1972; Murthy and McKenzie, 1974), 3) levels of polyamines are elevated (Matsuzaki and Suzuki, 1975) which would lead to an inhibition of ribonucleases as well as an increase in nucleic acid synthesis (Stevens, 1970), and 4) 5'-nucleotidase activity is decreased.

Although Fritzson (1967) suggested that the decreased 5'-nucleotidase activity of regenerating liver is explained by blocked synthesis of the enzyme, further studies are needed to elucidate whether the decreased 5'-nucleotidase activity in the thyroid and adenohypophysis is due to decreased synthesis, increased degradation of the enzyme or increased concentration of nucleotidase inhibitor.
Acknowledgments

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References

Lindsay, R.H. and P.P. Cohen (1965). Endocrinology 76, 737.