EFFECTS OF VARIOUS THYROID STATES ON THE METABOLISM OF ADENINE NUCLEOTIDES AND OF GLYCOGEN IN RAT LIVER

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1. Introduction

It is well known that thyroid hormone administration causes a reduction of the glycogen content of rat liver [1, 2], and in hypothyroid state, the glycogen content is usually normal [2]. The glycogen content seems to be mainly regulated through two enzyme activities, glycogen synthetase (UDPglucose : glycogen α-4-glucosyltransferase, EC 2.4.1.11) and phosphorylase (α-1,4-glucan : orthophosphate glucosyltransferase, EC 2.4.1.1). However, these two enzyme activities are modulated by many metabolic effectors which are supposed to compose the regulatory mechanism of the liver glycogen metabolism. Among them, it is now recognized that adenine nucleotides play important roles in metabolic processes, not only as substrates, but also as powerful modulators of enzymatic reactions [3,4]. Recent studies have shown how activities of enzymes, such as glycogen synthetase and phosphorylase can be affected by slight changes in concentrations of AMP, ATP and glucose-6-phosphate (G-6-P) [5–7].

Therefore, thyroid hormone may act to decrease the glycogen content either by altering the quantities of the enzymes or by changing the concentrations of modulators such as AMP, ATP and (G-6-P). Considering a rather rapid rate of the reduction of the glycogen concentration [2], the latter assumption would be probable.

In this experiment, the concentration of glycogen, the activities of glycogen synthetase and phosphorylase and the concentrations of adenylates and G-6-P in rat liver were measured under various states of thyroid function. The results show that though the glycogen content decreases clearly after thyroxine (T_4) injection, glycogen synthetase and phosphorylase activities do not change significantly in either state if assayed *in vitro* in systems containing sufficient coenzymes and cofactors. On the other hand, the concentrations of ATP and G-6-P increase markedly after thyroidectomy and are quickly restored to the normal level by T_4 injection. An inverse phenomenon to the above finding on the concentration of AMP is also observed.

Our present findings may suggest that the change in the glycogen content in various thyroid states is caused by the rapid changes in the concentrations of ATP, AMP and G-6-P.

2. Materials and methods

Male Wistar rats, weighing 180–250 g at the time of sacrifice, were used. Thyroidectomy was performed 18 days before sacrifice. The normal control group received a sham operation. A single injection of T_4 (20 μg/100 g body weight) was given subcutaneously on the neck of thyroidectomized rats, and both normal and thyroidectomized control groups were injected with the same dose of the solvent. The both control rats were given the solvent 12 hr before sacrifice. No change in the food intake was observed after T_4 injection.

Rats were decapitated between 10 and 11 am and the liver was rapidly removed, pressed and frozen between metal clamps previously cooled in liquid N_2 [8]. The time between the decapitation and freezing was less than 90 sec. The frozen tissue was pulverized in a morter, and approximately 2 g of the tissue were homogenized with 3 vol. of ice-cold 6% (w/v) $HClO_4$ in a glass homogenizer with a Teflon pestle for about 30 sec. Protein was removed by centrifugation in the

Table 1
Effects of thyroidectomy and T_4 supplement on the glycogen concentration of the rat liver.

Condition	No. of animals	Glycogen (mg/per g wet liver wt.)
Normal control	4	58.03 ± 4.13
Thyroidectomized	5	67.96 ± 3.14
T_4 12 hr	5	64.96 ± 1.83
T_4 24 hr	5	52.88 ± 2.25*
T_4 48 hr	5	44.80 ± 2.38**
T_4 72 hr	5	55.96 ± 2.51*

The thyroidectomized rats were killed 12, 24, 48 and 72 hr after the single injection of T_4 (20 μg/100 g body weight). Results shown are mean ± S.E.

*, **: Differences between the thyroidectomized control and the others are statistically significant at the level of 0.05 and 0.01, respectively.

cold at 3,000 *g* for 20 min. The supernatant fluid was adjusted to pH 6–7 with a small amount of 70% (w/v) K_2CO_3 solution and, after standing for 30 min in the cold, the precipitate of $KClO_4$ was removed after centrifugation. The supernatant was analysed for ATP, ADP, AMP and G-6-P.

ATP was measured with luciferine and luciferase enzyme extracted from fire fly lantern in a liquid scintillation spectrometer [9], Beckman Model LS-200B. ADP and AMP were determined by the method of Adam [10] and G-6-P by the method of Hohorst [11] at 30°.

Glycogen was determined by the anthrone method [12] after approximately 0.5 g of the frozen liver was digested in 1.5 ml of hot 30% (w/v) KOH.

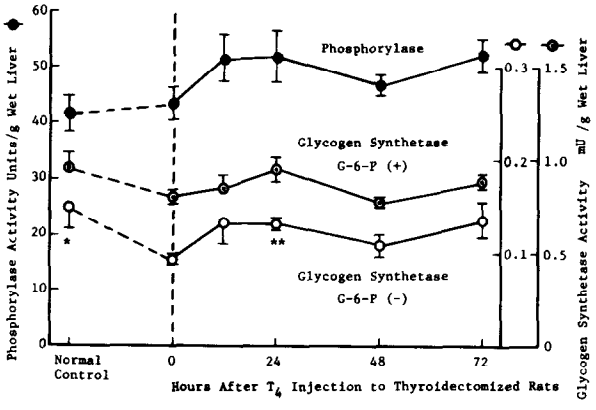

Fig. 1. Activities of phosphorylase and glycogen synthetase. One unit of phosphorylase represents 1 μmole inorganic phosphate liberated/min/g wet liver, and that of glycogen synthetase 1 μmole glucose of UDPG incorporated into glycogen/min/g wet liver. Experimental conditions and symbols are the same as in table 1.

Phosphorylase was assayed at 37° by the micromethod described by Hers and van Hoff [13]. A 1% liver homogenate in cold 0.1 M NaF was made with a tightly fitting Teflon homogenizer and used for the assay.

Glycogen synthetase was assayed by the method of Villar-Palasi et al. [14]. The liver was homogenized with 9 vol of cold 0.01 M glycylglycine buffer (pH 7.8) and the homogenate was centrifuged in the cold at 8,000 *g* for 10 min. The supernatant, kept in ice, was used for assay within 1 hr after preparation to avoid the time-dependent increase of the enzyme activity reported by Gold and Segal [15].

Adenylate kinase (EC 2.7.4.3) was assayed spectrophotometrically [16] at 25°. The fresh liver was homogenized with 4 vol. of cold 0.25 M sucrose with the Teflon-homogenizer and the homogenate was centrifuged in the cold at 105,000 *g* for 60 min. The supernatant was used for the assay.

3. Results and discussion

The concentration of liver glycogen tended to increase, but not significantly after thyroidectomy (table 1). T_4 administration decreased the concentration of glycogen significantly 24 hr after injection and the decrease was observed to remain 72 hr after injection.

As shown in fig. 1, glycogen synthetase and phosphorylase activities were not changed greatly by thyroidectomy or by T_4 administration, though the form-

Table 2
Effect of thyroidectomy and T_4 supplement on "energy charge" ratio* in the rat livers.

Condition	No. of animals	"Energy charge" ratio	p***
Normal control	4	0.52 ± 0.04**	<0.02
Thyroidectomized	5	0.64 ± 0.02	—
T_4 12 hr	5	0.55 ± 0.01	<0.01
T_4 24 hr	5	0.54 ± 0.02	<0.01
T_4 48 hr	5	0.54 ± 0.03	<0.05
T_4 72 hr	5	0.47 ± 0.05	<0.02

Experimental conditions are the same in table 1.

* (ATP + ½ ADP)/(ATP + ADP + AMP)
** Mean ± S.E.
*** Compared with thyroidectomized rats.

Table 3
Effect of thyroidectomy and T_4 supplement on adenylate kinase activity of the rat liver.

Condition	No. of animals	Activity (μmoles NADH oxidized/min/g wet liver)
Normal control	4	18.40 ± 1.17
Thyroidectomized	5	15.26 ± 1,47
T_4 12 hr	5	15.78 ± 2.42
T_4 24 hr	5	17.43 ± 0.96
T_4 48 hr	5	20.85 ± 0.64*
T_4 72 hr	5	18.19 ± 0.64

Conditions was the same as table 1.

* Difference between the thyroidectomized control and T_4 48 hr is statistically significant at the level of 0.01.

er enzyme activity in the absence of G-6-P was decreased by thyroidectomy and increased by T_4 injection after 24 hr. Glycogen synthetase activity was as low as 1/10 of that measured in the presence of G-6-P. Therefore, it would be conceivable that a slight change in G-6-P concentration affects the enzyme activity as mentioned previously.

Recent studies have suggested that G-6-P protects and stimulates active glycogen synthetase *a* [5]. On the contrary, ATP is reported to interfere with the interaction between G-6-P and the enzyme, thus depressing the activity of this enzyme [6]. On the other hand, phosphorylase *b* is activated by AMP and inhibited by ATP [7, 17, 18].

We, then attempted to measure the concentrations of adenylates and G-6-P in various thyroid conditions. ATP accumulated in the livers of thyroidectomized

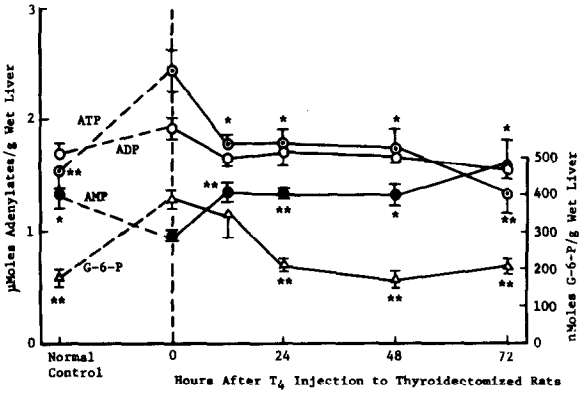

Fig. 2. Concentrations of liver ATP, ADP, AMP and G-6-P. Experimental conditions are the same as in table 1. Mean values with standard errors are shown. Asterisks indicate significant differences from 0-time values; *, $p<0.05$; **, $p<0.01$.

rats. Twelve hours after the administration of a low dose of T_4, the ATP level had decreased almost to normal (fig. 2). Conversely the AMP concentration was descreased in thyroidectomized rats and it increased after T_4 administration nearly to the normal level within 12 hr. However, the ADP concentration was not changed significantly either by thyroidectomy or T_4 administration. In order to represent more clearly the metabolic state of the high energy bonds of adenylates, an index of "energy charge" ratio [19] was calculated (table 2). The ratio was increased by thyroidectomy and normalized within 12 hr of T_4 administration, indicating that the charge of energy is higher in the thyroidectomized than in the normal and T_4 treated animals.

Tata et al. [2] have shown that it cannot be assumed that oxidative phosphorylation is stimulated in the thyroidectomized rats and decreased in the thyroidectomized and T_4 supplemented rats. It is probable, therefore, that the increased level of ATP in the livers of thyroidectomized rats is caused by a decreased consumption of ATP, and that this is restored by T_4 treatment. Since the fall in ATP level after T_4 treatment ceases within 12 hr and thereafter the concentration remains in the normal range, T_4 may also stimulate the formation of ATP in thyroidectomized and T_4 supplemented rats to balance the increased consumption of ATP, which may be followed by the increased glycogenolysis and glycolysis.

The activity of liver adenylate kinase, which catalizes the transphosphorylytic reaction between ATP and AMP forming 2 moles of ADP, did not change significantly under various thyroid states, except that the activity increased 48 hr after T_4 injection to the thyroidectomized rats (table 3). This might suggest that the change in the adenylate concentrations is not mediated by the change of adenylate kinase activity at least 12 and 24 hr after the injection.

In thyroidectomized rats, the G-6-P concentration increased significantly (fig. 2). This increased G-6-P concentration is supposed to stimulate effectively glycogen synthetase activity. However, since ATP, an inhibitor to the enzyme, increased simultaneously (fig. 2), the enzyme activity may not change much. The activity of phosphorylase may be decreased both by the increased ATP and the decreased AMP levels.

If we observed our present findings from the point of view of a time course experiment, it would be of interest that the fastest reaction to T_4 administration is the decreased ATP and the increased AMP, 12 hr after the injection, followed by the decreased levels of G-6-P and glycogen. Probably, T_4 may stimulate the consumption of ATP through some unknown mechanism, which leads to the alterations in the two enzyme activities and then to the decrease of glycogen level.

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