THYROID HORMONE CONTROL OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES AND THE REGULATION OF THE SENSITIVITY OF THE LIVER TO HORMONES

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1. Introduction

One of the more striking, but least studied, aspects of thyroid action is its effect on the hormone responsiveness of tissues. The action of lipolytic hormones such as glucagon, catecholamines and ACTH is markedly reduced in adipose tissue following thyroidectomy, while the action of insulin is potentiated after administration of thyroid hormone [1]. Insulin and glucagon both modulate the cyclic nucleotide content of liver and adipose tissue, insulin causing a rapid and transient rise in cGMP [2,3] and glucagon a marked rise of cAMP [4,5]. The reports that there is an increase of cAMP- and cGMP-phosphodiesterases in adipose tissue following thyroidectomy [6–9] may well be particularly relevant to these tissue responses to hormones.

The similarities in the response to thyroidectomy of the enzymes of carbohydrate metabolism and of fatty acid synthesis in rat liver and adipose tissue [10] prompted the present study of the enzymes regulating the tissue level of the cyclic nucleotides in rat liver. Changes in the balance of the enzymes adjusting the tissue content of these nucleotides could provide a common locus for thyroid action in a number of tissues responsive to insulin and glucagon and thus act as a unifying factor in the coordination of liver and adipose tissue metabolism.

2. Materials and methods

2.1. *Animals*

Adult male albino rats of the Wistar strain were thyroidectomized or sham-operated: thyroidectomized rats were given 1% calcium gluconate in the drinking water: both groups were fed a standard laboratory cube diet (MRC 41b) and were used two weeks after operation.

2.2. *Enzyme and cyclic nucleotide assays*

The preparation of liver extracts and membrane fractions was as described by Sapag-Hagar and Greenbaum [11]. cAMP- and cGMP-phosphodiesterases were estimated by the two-step procedure of Thompson et al. [12] using 10 μM or 100 μM cAMP or cGMP for the low- and high-K_m phosphodiesterases [11]. Adenylate cyclase activity was estimated by the method of Davis and Lazarus [13] as modified by Sapag-Hagar and Greenbaum [11]. Enzyme activities are expressed as milliunits, 1 mU being equivalent to 1 nmol of cAMP or cGMP hydrolysed or produced/min at 37°C.

cAMP and cGMP were estimated by radioisotope dilution procedures; cAMP by the method of Tovey et al. [14] using a binding protein prepared from beef muscle and cGMP by a radioimmunoassay procedure using a commercial kit from the Radiochemical Centre, Amersham, Bucks, England.

3. Results

The most striking observation was the contrasting responses of the two phosphodiesterases in both the soluble and particlebound fractions. Thyroidectomy significantly increased the activity of both the low-

Table 1
Activity of low- and high-K_m cAMP- and cGMP-phosphodiesterases, and of adenylate cyclase of liver from thyroidectomized and control rats together with tissue content of cyclic nucleotides

	Control	Thyroidectomized	% $\frac{Tx}{C}$	P
No. of observations	7	12		
Body weight (g)	338 ± 18	255 ± 7	75	***
Liver weight (g)	14.5 ± 0.4	10.2 ± 0.4	70	***
Liver weight/100 g body wt.	4.31 ± 0.14	4.01 ± 0.09	93	
Cyclic nucleotide phosphodiesterase activity (mU/g)				
4000 × *g* membrane fraction				
Low-K_m cAMP-PDE	1.85 ± 0.10	2.31 ± 0.08	129	***
High-K_m cAMP-PDE	18.0 ± 0.48	20.4 ± 0.68	113	*
Low-K_m cGMP-PDE	5.11 ± 0.39	5.66 ± 0.15	110	
High-K_m cGMP-PDE	18.9 ± 1.44	20.9 ± 1.14	110	
105 000 × *g* pellet fraction				
Low-K_m cAMP-PDE	1.87 ± 0.03	2.41 ± 0.12	130	***
High-K_m cAMP-PDE	13.9 ± 0.74	14.3 ± 0.45	103	
Low-K_m cGMP-PDE	4.47 ± 0.31	5.25 ± 0.19	117	
High-K_m cGMP-PDE	12.6 ± 0.9	12.6 ± 0.7	100	
105 000 × *g* supernatant fraction				
Low-K_m cAMP-PDE	20.9 ± 1.8	20.4 ± 1.1	98	
High-K_m cAMP-PDE	166 ± 12	193 ± 8	116	
Low-K_m cGMP-PDE	52.9 ± 4.1	69.0 ± 3.7	130	**
High-K_m cGMP-PDE	150 ± 17	200 ± 16	133	*
Adenylate cyclase activity (mU/g)				
4000 × *g* membrane fraction	1.18 ± 0.14	1.70 ± 0.25	144	
Total pellet fraction	1.91 ± 0.33	1.90 ± 0.33	100	
Cyclic nucleotide content (pmol/g)				
cAMP	546 ± 28	528 ± 22	97	
cGMP	23.6 ± 2.3	23.9 ± 2.2	101	

Values are given as means ± SEM. Fisher's P values are shown by asterisks;
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$
Enzymes (CBN Recommendations 1972) cAMP-phosphodiesterase also 3':5'-cyclic nucleotidase (EC 3.1.4.17), abbreviated in table 1 to cAMP-PDE and cGMP-PDE; adenylate cyclase (EC 4.6.1.1)

and high-K_m cAMP phosphodiesterases (the former to an appreciably greater extent) of the particle- and membrane-bound forms of the enzyme while having no effect on the two soluble forms. On the other hand, thyroidectomy significantly increased the activity of the two cGMP-phosphodiesterases of the soluble fraction while leaving the particulatebound forms unaffected (table 1). Thus, the contrasting intracellular sites for the regulation of cAMP and cGMP hydrolysis by thyroid hormone is apparent.

The adenylate cyclase activity of the membrane fraction was increased by some 40% following thyroidectomy (Fishers $P = 0.08$) but no alteration was observed in the activity of the total particulate fraction. The tissue content of cAMP and of cGMP in freeze-clamped liver was also unchanged.

4. Discussion

The changes in cAMP- and cGMP-phosphodiesterases in the liver of thyroidectomized rats reported here, parallel those found in adipose tissue in this condition as reported [6–9,15]. The similarity between these results on different tissues perhaps points to a unifying hypothesis for the action of thyroid hormone on two major insulin- and glucagon-responsive tissues, liver and adipose tissue.

In liver, thyroidectomy results in a marked decrease in both major routes of glucose oxidation, of lipogenesis and gluconeogenesis [10,16–18]. The hepatic enzyme profile of thyroidectomized rats shows many marked similarities to that of insulin-deficient rats, particularly in respect to the decrease in activity of glucokinase, pyruvate kinase, the oxidative enzymes of the pentose phosphate pathway and the enzymes related to lipid synthesis (citrate lyase, fatty acid synthetase and 'malic enzyme') [10]. The remarkable parallelism between changes in hepatic and adipose tissue enzymes related to glucose oxidation and lipid synthesis was also emphasized by Bacquer et al. [10]. Further, there is substantial evidence that both liver and adipose tissue from thyroidectomized rats exhibit changes which can be attributed to a glucagon-insensitive state, namely, in liver, a decreased rate of gluconeogenesis, as shown by studies with perfused liver presented with lactate as substrate [19] and by changes in key gluconeogenic enzymes, pyruvate carboxylase and phosphoenolpyruvate carboxykinase [16] and, in adipose tissue, by a decreased lipolytic response to glucagon and epinephrine [6–9,15].

Thyroidectomy, and the accompanying rise in the potential rate of hydrolysis of cAMP and cGMP, could effectively counteract the signals received at the plasma cell membrane from insulin (with the associated rise in cGMP) and glucagon (with the associated rise in cAMP) in both liver and adipose tissue and, in this sense, thyroid hormone could exert a 'permissive' role in insulin and glucagon action. The observations by Correze et al. [9] that insulin raises still further the cAMP- and cGMP-phosphodiesterases of adipose tissue from thyroidectomized animals, but is without effect on the cGMP-phosphodiesterase of intact animals, points to the possibility of an augmentation of the modulating activity of thyroidectomy of the response of the cells to external hormones. It is interesting to note that Nunez et al. [20] have also used the word 'permissive' in their discussion of the action of thyroid hormones. Alterations in the level of circulating thyroid hormone would thus reset the response of tissues to rapidly changing hormones such as insulin, glucagon and catecholamines by regulation of the effective cyclic nucleotide level via changes in the tissue cAMP- and cGMP-phosphodiesterase activity. Thyroxine and tri-iodothyronine may occupy a unique position among hormones in that they probably affect an exceptionally wide range of cell types and in that their blood levels do not normally undergo large fluctuations [21], characteristics emphasizing the possibility of their exerting a generalized 'permissive' role.

The importance of compartmentation of cyclic nucleotides, discussed by many previous authors [22–24], is again illustrated by the present results; both by the observation that no 'steady-state' change in the cyclic nucleotides of freeze-clamped liver is apparent after thyroidectomy, despite the rise in cAMP- and cGMP-phosphodiesterase activity, and by the differing intracellular locations of the hormone-sensitive cyclic nucleotide phosphodiesterases.

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