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Thyroid hormone increases glyceraldehyde 3-phosphate dehydrogenase gene expression in rat liver

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Livers from hypophysectomized rats had low levels of glyceraldehyde 3-phosphate dehydrogenase mRNA. Administration of L-triiodothyronine increased these levels over 20-fold. The peak response was seen 72 h after hormone administration. A half-maximal response was obtained with 5 μg of T_3 per 100 g of body weight. Thus the expression of hepatic glyceraldehyde 3-phosphate dehydrogenase appears to be regulated by thyroid hormone.

Thyroid hormone; Glyceraldehyde 3-phosphate dehydrogenase; Northern blotting control; Rat liver

1. INTRODUCTION

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme which exists as a tetramer of identical subunits each with a molecular weight of 37 000. The structure of GAPDH is known at 3 Å resolution [1]. The enzyme has a dinucleotide fold. Full length cDNAs for GAPDH have been isolated [2]. Since the reaction catalyzed by GAPDH is not considered a major site of regulation of the glycolytic pathway, relatively few studies of the regulation of expression of this gene have been reported. However, it has been demonstrated that insulin acts to increase GAPDH mRNA levels in adipocytes and hepatoma cells [3,4]. This increase in mRNA occurs very rapidly (within 1 to 2 h) and is due to increased transcription of the GAPDH gene mediated via *cis*-acting sequences in the 5′ flanking region [4]. In thyroid cells TSH acts to increase GAPDH mRNA levels by stabilizing the mRNA [5].

The cDNA for GAPDH has been, perhaps, most extensively used as an internal control in Northern blotting and run-on transcription analysis, see for example [6–11]. In this regard, GAPDH was likely selected because it is believed to be constitutively expressed at high levels in all tissues. In our studies [12] on the hormonal regulation of key proteins involved in cholesterol homeostasis, we selected GAPDH as an internal standard particularly for tissue comparison studies. Much to our surprise GAPDH mRNA levels were markedly increased in liver by thyroid hormone administration.

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Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; T_3, triiodothyronine

2. MATERIALS AND METHODS

Male hypophysectomized Sprague–Dawley rats weighing 125–150 g were purchased from Harlan Industries of Madison, WI. The rats were injected with T_3 in doses ranging from 5 to 100 μg per 100 g of body weight and killed at the indicated times after administration of T_3. Some hypophysectomized rats were placed on diets containing 0.5% desiccated thyroid powder for 8 days. All animals were killed at the midpoint of the dark cycle [12]. Poly A^+ RNA was prepared from 1 g portions of their livers [12]. The cDNA probes for human catalase and GAPDH, pCAT10 and pHc GAP respectively, were obtained from American Type Culture Collection. These probes were labeled with ^{32}P by nick translation and hybridized as previously described [12]. The resulting autoradiograms were scanned with a laser densitometer to determine relative mRNA levels. The values for GAPDH were corrected using catalase as the internal control.

3. RESULTS

As shown in Fig. 1, hepatic GAPDH mRNA levels were quite low in hypophysectomized rats. Administration of T_3 resulted in over a 20-fold increase in GAPDH mRNA levels. This increase occurred slowly with the maximal response reached 72 h after T_3 administration. When hypophysectomized rats were rendered thyrotoxic by feeding desiccated thyroid powder (TP lanes of Fig. 1), no further increase in GADPH mRNA levels was seen. In terms of dose, it was found that 5 μg per 100 g of body weight was sufficient to obtain half-maximal response (Fig. 1).

4. DISCUSSION

The data presented here suggest that the expression of glyceraldehyde 3-phosphate dehydrogenase is regulated by thyroid hormone in addition to the

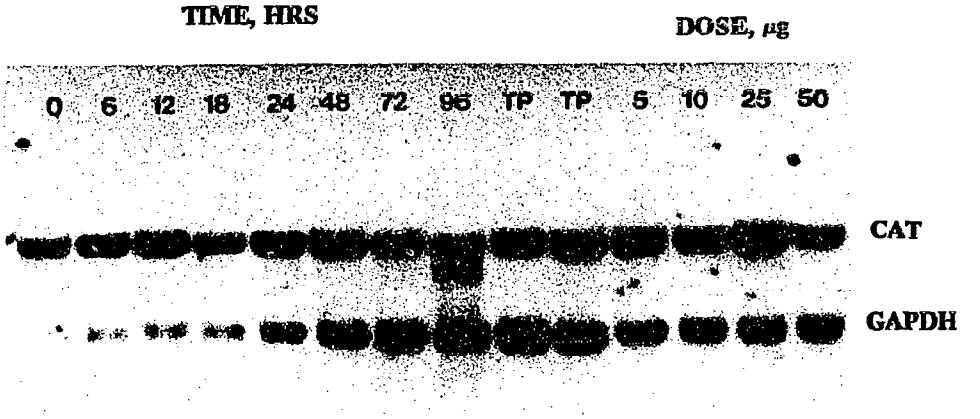

Fig. 1. The effect of thyroid hormone treatment of hypophysectomized rats on hepatic GAPDH mRNA levels. Hypophysectomized rats were injected with 100 μg per 100 g of body weight of T_3 and killed 6–96 h later, or with 5–50 μg per 100 g of body weight and killed 72 h later. Some hypophysectomized rats were fed 0.5% desiccated thyroid powder for 8 days (TP). Ten μg of poly A^+ RNA were applied to each lane and separated on a 1% agarose gel [12] as described. The migration positions for catalase (CAT) and GAPDH mRNAs are labeled. The relative levels for GAPDH mRNA determined by laser densitometry were: hypophysectomized control (0 h), 0.66; 6 h, 1.09; 12 h, 1.63; 18 h, 3.38; 24 h, 3.29; 48 h, 7.00; 72 h, 14.88; 96 h, 10.12; TP, 14.39; TP, 11.98; 5 μg, 6.07; 10 μg, 6.11; 25 μg, 4.05 and 50 μg, 10.83.

previously reported [3,4] regulation by insulin. In contrast with the rapid (within 1 h) increase seen in response to insulin, the thyroid hormone response required 72 h. This would be consistent with T_3 acting post-transcriptionally to stabilize GAPDH mRNA rather than at the transcriptional level as in the case of insulin [3,4]. Stabilization of several other mRNAs by thyroid hormone has been reported [13–15]. It was also reported that GADPH mRNA was stabilized by the action of TSH in thyroid cells [5].

GAPDH catalyzes a branchpoint reaction in glycolysis and therefore can be considered a logical site for regulation. Glyceraldehyde 3-phosphate can enter the hexose monophosphate shunt through the actions of transaldolase or transketolase or it can be converted to dihydroxyacetone phosphate by the action of triose phosphate isomerase and supply the glycerol backbone for triglyceride and phospholipid synthesis in addition to being converted to 1,3-diphosphoglycerate by GAPDH which is the first step in the production of lactate from trioses. GAPDH activity has been shown to be regulated by several glycolytic intermediates [16].

It appears that the increase in hepatic GAPDH mRNA levels caused by T_3 may be a physiological response to the hormone. A half-maximal response was obtained with 5 μg T_3 per 100 g of body weight which is less than the 15.6 μg per 100 g of body weight required to achieve 95% saturation of nuclear T_3 receptors for 4 h [17]. Doses of 200 μg are required to maintain 95% saturation for 54 h [17]. Also, we have noted that GAPDH mRNA levels in normal euthyroid rats were about 20-fold greater than those in hypophysectomized rats (data not shown). Perhaps, the T_3-promoted increase in glycolysis is due, in part, to increased GAPDH gene expression. Also it would seem that selection of GAPDH as an internal control in Northern blotting studies [6–11] may not be appropriate in all cases, particularly in studies of regulation by insulin or thyroid hormone.

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