The Effect of Thyroid Hormones and 5-Azacytidine on Transcription of Malic Enzyme and 6-Phosphogluconate Dehydrogenase Genes

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Great attention is now being paid to elucidating the correlation between the nature of DNA methylation and the functional status of the cell, since DNA methylation is thought to be one of the factors responsible for the regulation of gene activity [1].

Earlier, we studied the effect of thyroid hormones on DNA methylation in rat liver *in vivo* and *in vitro* using the following approaches: determination of m^5C content in total nuclear DNA from rat liver at various levels of thyroid hormones; comparative study of DNA methylase activity in cell nuclei and investigation of acceptor properties of DNA, chromatin, and intact nuclei in the methylation reaction performed *in vitro* in the presence of bacterial DNA methylases. As was shown, DNA is relatively hypomethylated after injection of triiodothyronine (T_3) in both thyroidectomized and intact rats, and simultaneously we recorded a decrease in DNA methylase activity. These experiments led us to conclude that thyroid hormones block the system of DNA methylation [2]. The aim of the present study was to analyze how the blocking of DNA methylation impacts the functional activity of thyroid hormone-responsive genes.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 100-150 g. Thyroidectomy was carried out under ether anesthesia. Thyroidectomized animals were kept on a low iodine diet with the addition of 0.9% $CaCl_2$ to the drinking water and were used for the experiments 4-5 weeks after the operation. Triiodothyronine (T_3) in a dose of 30 μg per 100 g body weight was administered in two injections with a 24-h interval. The total RNA from the rat liver homogenate was isolated using guanidine isothiocyanate. Poly(A)+RNA was purified by chromatography on poly(U) Sepharose 4B equilibrated with the following buffer: 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.25% SDS. Poly(A)+RNA was eluted with 0.2% SDS at 45o. RTZ-18R plasmid containing the EcoR I fragment (1.3 kb) of malic enzyme gene was a generous gift of Dr. J. Oppenheimer, USA. PBR 322 plasmid carrying the Pst I fragment (880 b) of 6-phosphogluconate dehydrogenase (6-PGD) was kindly provided by Dr. H. C. Towle, USA. For transformation we used *E.coli* strain TG 1. Bacteria were grown in liquid or on agarized Hottinger medium (produced at the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Moscow). Propagation of plasmid DNAs, electrophoretic separation of restriction fragments, and isolation of the inserted fragment were carried out as described by Maniatis *et al.* [3]. Nick-translation of DNA was performed according to the Nick translation kit (Amersham) manual. The necessary reagents were from Amersham. DNA-RNA hybridization was carried out after immobilization of

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poly(A)+RNA on nitrocellulose filters after Thomas [4].

RESULTS

Like triiodothyronine, the methylation inhibitor 5-azacytidine (5-azaC), is known to affect gene activity [5]. In view of this, we studied the level of expression of thyroid hormone-responsive genes in animals treated with these reagents. Figure 1 demonstrates the results of DNA-RNA hybridization of poly(A)+RNA isolated from the liver of different experimental animals with the ^{3}H-labeled fragment of malic enzyme gene. Injection of T_3 into thyroidectomized animals led to a 3-4-fold stimulation of malic enzyme mRNA synthesis. These results are in good agreement with earlier data attesting to chromatin activation after T3 injection. In particular, the share of nucleotides coding for malic enzyme per DNA unit in the chromatin fraction with an enhanced sensitivity to micrococcal nuclease and, to a greater extent, in the fraction of nuclear matrix-associated DNA was substantially increased in comparison with that in thyroidectomized rats [6].

6-Phosphogluconate dehydrogenase (6-phospho-D-gluconic: NaDP+2-oxidoreductase (decarboxylating), EC 1.1.1.44) is one of the first enzymes of the hexomonophosphate shunt, whose activity is also regulated by the thyroid hormones. Hormonal regulation of the hepatic 6-PGD activity proceeds at the level of enzyme synthesis following changes in the intracellular concentration of 6-PGD mRNA [7]. Specific mRNA coding for 6-PGD is approximately 2400 bases long. The cloned DNA contained 880 bases, i.e., 35% of all mRNA. Figure 2 presents the results of DNA-RNA hybridization of poly(A)+RNA from the liver of different experimental animals with the ^{3}H-labeled fragment of the 6-PGD gene. The data of hybridization analysis indicate that expression of the 6-PGD gene is suppressed in thyroidectomized rats, while after hormone administration a tendency toward restoration of the normal mRNA level can be traced. These results correlate with the data of Miksicek and Towle [7], who demonstrated that administration of T_3 led to a rise in the liver 6-PGD mRNA level to 0.1% and even higher compared to the normal level of 0.015%.

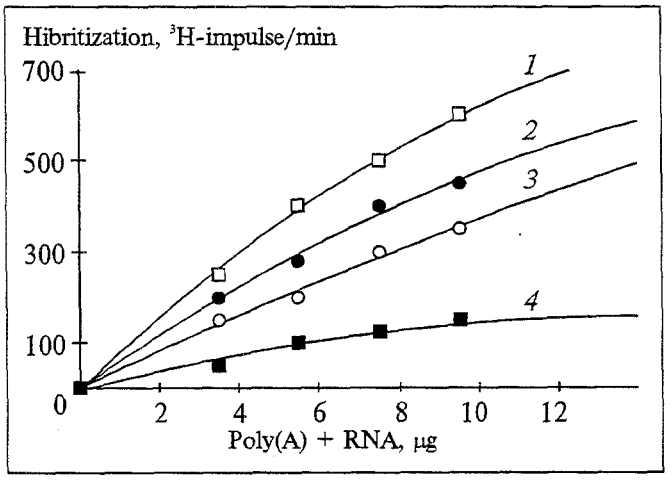

Fig. 1. DNA–RNA hybridization of ^{3}H–labeled fragment of malic enzyme gene with poly(A)+RNA from different experimental models of rats. Here and in Fig. 2.: *1*) normal; *2*) thyroidectomy + T_3; *3*) thyroidectomy + 5–azaC; *4*) thyroidectomy.

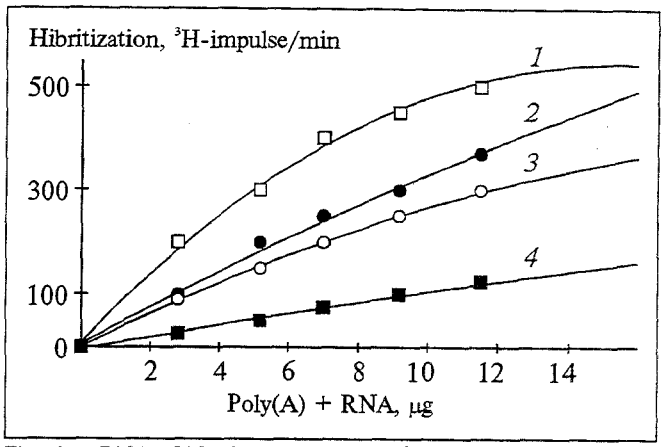

Fig. 2. DNA–RNA hybridization of ^{3}H–labeled fragment of 6–phosphogluconate dehydrogenase gene with poly(A)+RNA from different experimental models of rats.

The 5-azaC-induced blocking of DNA methylation enhances the expression of both thyroid hormone-responsive genes to an extent comparable to the effect of the exogenous hormone. Unfortunately, we did not have the probes including the regulatory regions of the genes, and changes in the methylation level in this region supposedly influence the gene transcriptional activity [8]. Nevertheless, on the basis of the concept that DNA hypo- or demethylation is a factor permitting gene expression, we are inclined to believe that, as in the case of T_3 [2], a drop of the total level of methylated DNA induced by the methylation inhibitor 5-azacytidine involves the regulatory elements of these genes and leads, as a result, to activation of their transcription.

Thus, the data obtained for two thyroid hormone-responsive genes (those of malic enzyme and 6-phosphogluconate dehydrogenase) have revealed the existence of an inverse correlation between the level of DNA methylation and gene expression. The phenomenon of DNA demethylation is now regarded as a parameter characterizing the competent-for-transcription state of genes [9-11]. The similar effects of 5-azaC and thyroid hormones on

the system of DNA methylation allow us to assume that regulation of gene activity by thyroid hormones may proceed via the blocking of DNA methylation. DNA demethylation may be among the structural changes necessary for the binding of thyroid hormones with DNA elements recognized by the thyroid hormone receptors and for further induction of the synthesis of specific mRNAs.

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Effect of the ω6/ω3 Ratio of Polyunsaturated Fatty Acids in the Rat Diet on Eicosanoid Content in the Blood Plasma and in the Liver

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The inclusion of fatty products rich in ω3 polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic and docosahexaenoic, in the diet has recently been established to be therapeutic in the treatment of a number of diseases, in particular, cardiovascular diseases, diabetes mellitus, hypercholesterolemia, and allergic and skin disorders [3,6, 11]. The positive effects associated with an increased intake of highly unsaturated ω3 fatty acids are realized at the level of structural and functional alterations in the biomembranes, as well as at the level of biosynthesis of various eicosanoids, which direct the cell metabolism. The ω3 family of fatty acids has been shown to be just as essential as the acids of the linoleic group (ω6), which are regarded as vital in nutrition. The ω3 and w6 fatty acids supplied with food are precursors of diverse groups of eicosanoids modulating oppositely directed reactions. In a number of studies, a direct relationship has been shown to be absent between the content of PUFA of one of the groups in the diet and the synthesis of the corresponding group of prostaglandins (PG) in the organism [5,9]. One of the reasons for this lies in the competitive metabolic relationships between the two groups of fatty acids [12]. Therefore, it is of prime importance to choose the optimal ratio between the es-

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