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ORIGINAL ARTICLE

Pyrimidine and purine salvage deoxyribonucleoside metabolism in hepatic and renal homogenates from rats pretreated with propylthiouracil or L-thyroxine

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Abstract OBJECTIVES: *In vitro* activities of thymidine kinase (TK, EC 2.7.1.21), adenosine kinase (AK, EC 2.7.1.20) and deoxycytidine kinase (dCK, EC 2.7.1.74) enzymes involved in the salvage pathway of DNA precursor synthesis, in homogenates of the rat liver and kidney, were examined. Type I iodothyronine-5'-deiodinase (5'D-I) is the main enzyme responsible for peripheral metabolism of thyroid hormones. This occurs especially in the liver, kidney and muscle. The activity of 5'D-I is inhibited by propylthiouracil (PTU), an antithyroid drug. METHODS: The liver and kidney were collected from rats pretreated *in vivo* with either a 0.1% solution of PTU in drinking water for 2 weeks or injected with levothyroxine (L-T_4, 50 μg/kg BW, daily) for 2 weeks. The enzyme activities were measured by ascending chromatography and expressed as the amounts of radioactive reaction products of the phosphorylation of dThd (for TK), of dAdo (for AK and dCK) and of dGuo (for dCK). RESULTS: In liver homogenates, PTU-pretreatment decreased the activities of the three enzymes when compared to control values and those of L-T_4-treated animals; also L-T_4 injections decreased the AK and dCK activities in the liver homogenates. PTU-pretreatment increased TK activity and the rate of dGuo phosphorylation in kidney homogenates, when compared to controls and to the L-T_4-pretreated animals. Conversely, both PTU- and L-T_4-pretreatment reduced the rate of dAdo phosphorylation in kidney homogenates. CONCLUSION: Changes in the activities of examined enzymes which participate in pyrimidine or purine metabolism of the salvage pathway of DNA synthesis in the liver after PTU-pretreatment (as shown herein) are similar to the changes of the 5'D-I activity after PTU-treatment (as reported by others). Thus, the observations suggest a role of the salvage pathway of DNA synthesis in the peripheral metabolism of thyroid hormones.

Introduction

Several enzymes are involved in the pyrimidine salvage pathway of DNA synthesis. Included in this group are deoxythymidine kinase (thymidine kinase, TK, EC 2.7.1.21), adenosine kinase (AK, EC 2.7.1.20) and deoxycytidine kinase (dCK, EC 2.7.1.74).

Thymidine kinase is an enzyme responsible for catalyzing the phosphorylation of thymidine (dThd), which functions as a part of the pyrimidine salvage pathway involved in DNA synthesis [1]. In eucaryotic cells, two TKs are known: TK1 (usually identified as TK) which is localized in cytoplasm and TK2 which is predominantly found in mitochondria. The activity of TK is cell cycle-regulated and increases during the S phase. The enzyme in question is thought to be an index of liver regeneration [1, 2, 3]. In studies of human thyroid diseases it was shown that an increase in DNA synthesis proceeded parallel with an increased activity of cytoplasmic TK [4]. It has been suggested that thyroid hormones can influence DNA synthesis during liver regeneration and regulate the activity of enzymes such as TK. This indicates the enzymes are important for DNA synthesis and enhanced cell division [5].

Adenosine kinase phosphorylates deoxyadenosine (dAdo) to deoxyadenosine monophosphate (dAMP). In humans, dAdo phosphorylation is catalyzed especially by dCK. However, in mouse, AK is responsible for the larger proportion of dAdo phosphorylation [6].

Deoxycytidine kinase, an enzyme which is responsible for the phosphorylation of deoxycytidine (dCyd), deoxyguanosine (dGuo) and dAdo, is not cell cycle-regulated [7]. The nucleosides are, therefore, phosphorylated to the same extent in the S phase as in G_1 or G_2 phase. The phosphorylation of dGuo is catalyzed almost exclusively by dCK. Deoxyguanosine kinase (dGK) phosphorylates dGuo to its monophosphate form; however, in most organisms, the existence of dGK activity and its physiological functions are unknown.

The main enzyme responsible for the peripheral metabolism of thyroid hormones is type I iodothyronine-5'-deiodinase (5'D-I). This enzyme is especially prominent in the liver and kidneys [for review see: 8]. The enzyme is extremely sensitive to inhibition by the antithyroid drug, 6-propyl-2-thiouracil (PTU) [9].

In the present study, we examined the activities of TK, AK and dCK in homogenates of livers and kidneys, collected from rats pretreated with either PTU or L-T_4.

Materials and Methods

Twenty-seven male Wistar rats, weighing 120±10 g, were used as tissue donors. Nine rats were given a 0.1% solution of propylthiouracil (PTU; Sigma Chemical Co.) in drinking water for 2 weeks, and 10 animals were injected daily with levothyroxine (L-T_4; Sigma Chemical Co.) (50 μg/kg b.w, i.p..), also for 2 weeks. Eight rats served as controls and received daily injections of 0.9% NaCl, i.p., for 2 weeks. After the 2-week treatment period the animals were sacrificed by decapitation, the livers and kidneys were immediately collected and placed into vials at 0°C.

Enzymes assays

Thymidine kinase (TK) activity

Thymidine kinase was measured as described by Cheng and Prusoff [10] and as modified by Greger and Draminski [11]. The liver and kidneys were homogenized in the recommended medium (25 mM Tris-HCl buffer, 25 mM KCl and 5 mM $MgCl_2$ - pH 7.4, temperature 0°C). Following centrifugation (10,000 g for 20 min), the postmitochondrial fraction (approximately 100 μg protein) was incubated for 30 min (37°C) in a medium consisting of 50 mM Tris-HCl buffer (pH 7.4), 10 mM ATP, 10 mM $MgCl_2$ and, additionally, with 0.2 μCi [2-^{14}C]dThd per sample (200 μl). The reaction was stopped by immersion in boiling water bath (100°C, 2 min). After deproteinization (by centrifugation for 3 min), aliquots of the supernatant were placed on Whatman DE81 chromatography paper. The reaction products (dTMP, dTDP, dTTP and dThd) were separated by ascending chromatography at room temperature in a solvent of 5 mM ammonium formate (pH 5.6).

Phosphorylation of dAdo

Phosphorylation of dAdo (catalyzed by both dCK and AK) was measured using the method of Cheng and Prusoff [10] as modified by Greger and Draminski [11]. The liver and kidneys were homogenized in the same medium as above. The samples were also centrifuged as above to produce postmitochondrial fractions (approximately 60-100 μg protein). These fractions were incubated for 30 min (37°C) in a medium consisting of 50 mM Tris-HCl buffer (pH 7.4), 10 mM ATP, 5 mM $MgCl_2$, 0.1 mM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, adenosine deaminase inhibitor) and, additionally, with 0.05 mM [8-^{14}C]dAdo [0.1 μCi per sample (200 μl)]. The reaction was stopped by immersion in boiling water bath (100°C, 2 min). After deproteinization (by centrifugation for 3 min), aliquots of the supernatant

were placed on Whatman No. 1 chromatography paper. The reaction products (dAMP, dADP, dATP and dAdo) were separated by ascending chromatography at room temperature in a solvent of 1 mM ammonium acetate : 95% ethanol, 3:7 (pH 7.5).

Phosphorylation of dGuo

Phosphorylation of dGuo (catalyzed only by dCK) was also measured using the method described by Cheng and Prusoff [10] as modified by Greger and Draminski [11]. The liver and kidneys were homogenized and centrifuged as above. The obtained post-mitochondrial fractions (approximately 100 μg protein) were incubated for 30 min (37°C) in a medium consisting of 50 mM Tris-HCl buffer (pH 8.0), 10 mM ATP, 10 mM $MgCl_2$ and, additionally, with 0.1 mM [U-^{14}C]dGuo [0.2 μCi per sample (200 μl)]. The reaction was stopped by immersion in boiling water bath (100°C, 2 min). After deproteinization (by centrifugation for 3 min), aliquots of the supernatant were placed on Whatman DE81 chromatography paper. The reaction products (dGMP, dGDP, dGTP and dGuo) were separated by ascending chromatography at room temperature in a solvent of 10 mM of ammonium formate (pH 5.6).

Three parallel chromatographic separations were conducted for each animal, for both liver and kidney, and for each enzyme. The chromatograms were dried and the radioactive spots, corresponding to all reaction products, was cut out and placed into counting vials. Radioactivity was measured in an LKB Wallac liquid scintillation counter. The protein content was determined using the method of Lowry et al. [12]. Enzyme activity was expressed in cpm/100 μg protein, as the amounts of radioactive products of the phosphorylation of dThd (catalyzed by TK), of dAdo (catalyzed by AK and dCK) and of dGuo (catalyzed by dCK).

Statistical analysis

The data were statistically analyzed using a one-way analysis of variance (ANOVA). The statistical significance of differences among particular groups was evaluated with the Student-Neuman-Keuls' test.

Results

In liver homogenates, PTU-pretreatment decreased the activities of the three enzymes when compared to levels in controls and those in L-T_4-treated animals (Fig. 1a,b,c). In liver homogenates, L-T_4 injections reduced the rate of dAdo phosphorylation (catalyzed by AK or dCK) (Fig. 1b) and the rate of dGuo phosphorylation (catalyzed by dCK) (Fig. 1c).

In kidney homogenates, PTU-pretreatment increased TK activity (Fig. 2a) and the rate of phosphorylation of dGuo (Fig. 2c), when compared to control levels and those in the L-T_4-treated group. Both PTU-pretreatment and L-T4-injections decreased the rate of dAdo phosphorylation (Fig. 2b).

Discussion

The reduced activities of the examined enzymes, especially TK, in rat liver are consistent with the observation that, in hepatectomized hypothyroid rats, DNA synthesis is lower than in euthyroid rats [5]. Moreover, when examining the kinetic parameters of TK, these authors observed an increased Michaelis- Menten constant for thymidine in livers from hypothyroid hepatectomized and sham-operated animals, which signifies a decreased affinity of the enzyme for thymidine. The affinity of the enzyme increased significantly after triiodothyronine treatment. In the present study, PTU but not L-T_4-pretreatment, decreased TK activity in liver homogenates. Previous it was shown that PTU-pretreatment reduced the rate of the phosphorylation of histone H2A, which is a major phosphorylated protein in interphase cells, as well as decreased the content of protein and RNA in the nuclei of rat liver, due to a lower growth rate and a reduced transcription rate [13].

In an earlier report we found that TK activity was lower in the homogenates of thyroid tissue collected from hypothyroid animals, while it was higher in thyroid tissue of hyperthyroid animals when compared to tissues from euthyroid controls [14]. Earlier an increased TK activity was demonstrated in thyrocytes of autonomous nodules [15], in thyroids of patients with Graves' disease [4] and in human toxic adenomas [16]. However, this phenomenon is not exclusively related to hyperthyroidism since an increased TK was also observed in cancerous thyroid tissue [4]. In this latter report, TK activity was also increased in non-toxic nodular goiter and thyroid adenoma, a finding not confirmed by Brzezinski et al. [16].

The inhibitory effect of PTU-pretreatment on TK, dAK and dCK activities in rat liver is similar to the well-known inhibitory influence of this antithyroid drug on 5'D-I activity in the liver [9]. Consistent with this is that thyroidectomy exerted an inhibitory effect on both DNA synthesis [17] and 5'D-I activity [18] in the rat liver. It is possible that both the decreased

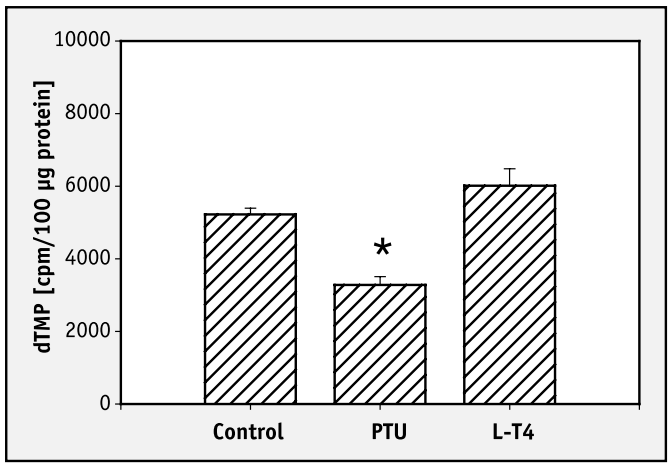

Fig. 1a

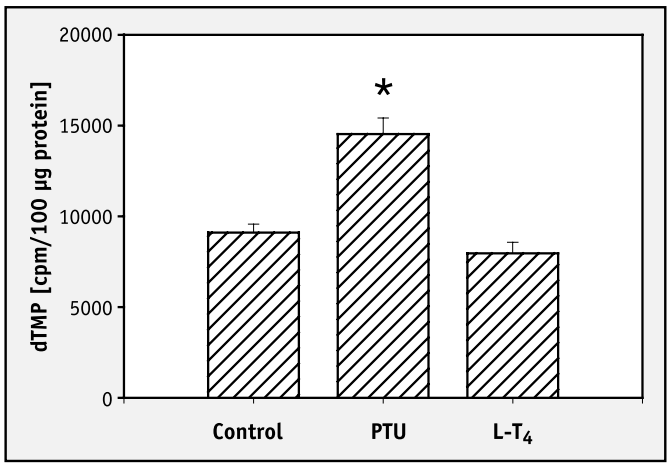

Fig. 2a

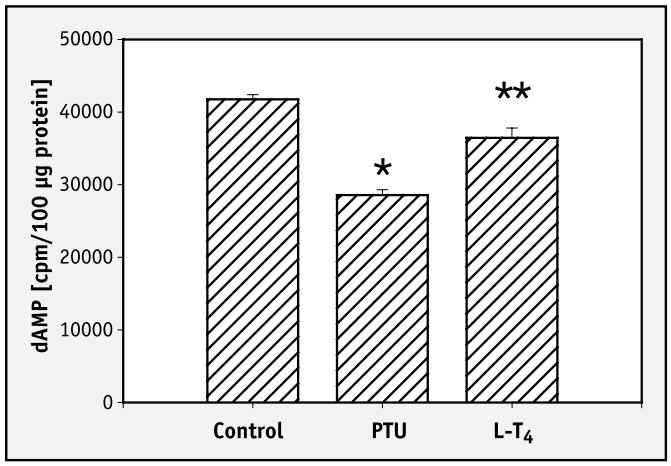

Fig. 1b

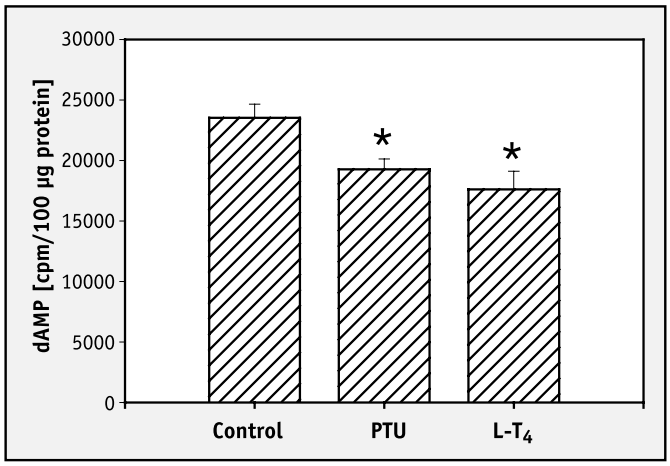

Fig. 2b

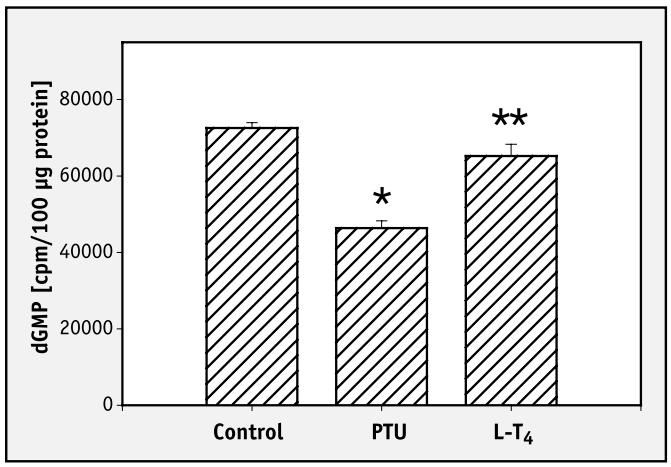

Fig. 1c

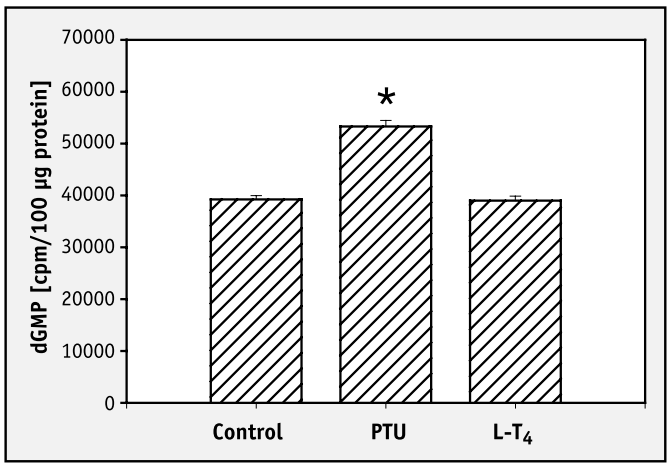

Fig. 2c

Fig. 1. Enzyme activities in the homogenates of livers collected from rats which did not receive any treatment (Control) or were pretreated with propylthiouracil (PTU) or levothyroxine (L-T_4); **a** - TK activity measured as the amount of reaction product, deoxythymidine monophosphate (dTMP), * $p<0.001$ vs. Control and vs. L-T_4; **b** - The rate of the phosphorylation of dAdo measured as the amount of reaction product, deoxyadenosine monophosphate (dAMP), * $p<0.001$ vs. Control and vs. L-T_4, ** $p<0.05$ vs. Control;
c - The rate of the phosphorylation of dGuo measured as the amount of reaction product, deoxyguanosine monophosphate (dGMP), * $p<0.001$ vs. Control and vs. L-T_4, ** $p<0.05$ vs. Control. Product amounts are expressed in cpm/100 µg protein/30 min. Bars represent means±SEM.

Fig. 2. Enzyme activities in the homogenates of kidneys collected from rats which did not receive any treatment (Control) or were pretreated with propylthiouracil (PTU) or levothyroxine (L-T_4); **a** - TK activity measured as the amount of reaction product, deoxythymidine monophosphate (dTMP), * $p<0.001$ vs. Control and vs. L-T_4; **b** - The rate of the phosphorylation of dAdo measured as the amount of reaction product, deoxyadenosine monophosphate (dAMP), * $p<0.05$ vs. Control; **c** - The rate of the phosphorylation of dGuo measured as the amount of reaction product, deoxyguanosine monophosphate (dGMP), *$p<0.001$ vs. Control and vs. L-T_4. The amounts of products are expressed in cpm/100 µg protein/30 min. Bars represent means±SEM.

activities of TK, AK and dCK and the reduced 5'D-I activity in the liver during hypothyroidism may simply be the result of the suppressive influence of hypothyroidism on liver growth and on the basal metabolic rate. Similar changes in the activities of enzymes, participating in the salvage pathway of DNA synthesis and in the activity of 5'D-I after PTU-treatment, suggest a role for this pathway in the peripheral metabolism of thyroid hormones.

Contrary to results observed in the liver homogenates, in kidneys collected from PTU-pretreated rats TK activity was higher than in controls. This suggests that hypothyroidism does not decrease DNA synthesis in the kidney or, if it does, DNA synthesis and TK activity did not change in a parallel manner after PTU-pretreatment.

Interestingly, in the present study, both PTU and L-T_4-pretreatment decreased the rate of dAdo phosphorylation, both in liver and kidney homogenates. These results indicate that dysfunction of the thyroid gland may lead to an accumulation of dAdo, which can be directly toxic, or which can activate the catabolic pathway of purine and pyrimidine metabolism. The lethal and harmful effects of dAdo are well known, e.g., dAdo exerts toxic actions in chromaffin cells of rat adrenal gland [19].

The changes of the dGuo phosphorylation were dependent on the treatment (L-T_4 vs. PTU) and on the organ examined. The decreased rate of dGuo phosphorylation after PTU and L-T_4-treatment in liver homogenates suggests that changes of thyroid status may lead to suppressive effects on dCK and, possibly, on physiological processes within the cell cycle since dCK is not cell cycle-regulated [7]. On the other hand, the increased rate of dGuo in kidney from hypothyroid rat is not consistent with this conclusion.

In summary, the pyrimidine and purine salvage pathways of DNA synthesis may play a role in the peripheral metabolism of thyroid hormones. Both hypo- and hyperthyroidism can lead to an intracellular accumulation of dAdo in the liver. Further studies are required to define the significance of the changes in the pyrimidine and purine salvage DNA synthesis in liver and kidney tissues in cases of thyroid dysfunction.

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