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ORIGINAL ARTICLE

Thymidine kinase and adenosine kinase activities in homogenates of thyroid lobes in hemithyroidectomized rats; effects of melatonin *in vitro*

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Abstract

OBJECTIVES: Thymidine kinase (TK, EC 2.7.1.21) is a part of the pyrimidine salvage pathway, involved in DNA synthesis. In turn, adenosine kinase (AK, EC 2.7.1.20) functions as a part of the purine metabolic pathway, involved in DNA synthesis. Melatonin (Mel) is an indoleamine which is known to inhibit growth processes in the thyroid gland and also in other endocrine and non-endocrine tissues. The aim of our study was to examine TK and AK activities in homogenates of the rat thyroid lobes remaining after contralateral hemithyroidectomy (hemiTx); additionally, incubations with Mel (10^{-6}, 10^{-9}, and 10^{-12} M) were performed. METHODS: The experiment was performed on young male Wistar rats (6-week old). The enzyme activities were measured by ascending chromatography and expressed as the amounts of radioactive reaction products of the phosphorylation of dThd (for TK) and of dAdo (for AK). RESULTS: 1. HemiTx increased TK activity in homogenates of the remaining thyroid lobe; 2. Mel increased TK activity in all the groups (intact, sham-operated- and hemiTx-rats), except for the concentrations of 10^{-9} and 10^{-12} M in the hemiTx-rats, in which the increasing effects of Mel on TK activity reached the borderline statistical significance only; 3. Mel increased the AK activity in intact and in shamTx animals; 4. No statistically significant changes were found in AK activity following Mel *in vitro* in the incubated remaining thyroid lobes, collected from hemiTx-rats. CONCLUSION: The obtained results suggest that in young rats Mel may affect the *de novo* synthesis of DNA, i.e., the pathway in which TK is not involved. Our results suggest also a role of AK in the regulation of (patho)-physiological processes in the thyroid gland after hemiTx. Melatonin can putatively be involved in the thyroid blood flow regulation, through an influence of that hormone on AK activity.

Abbreviations

TK	thymidine kinase
dThd	deoxythymidine
Thd	thymidine
dTMP	deoxythymidine monophosphate
AK	adenosine kinase
dAdo	deoxyadenosine
Ado	adenosine
dAMP	deoxyadenosine monophosphate
Mel	melatonin
hemiTx	hemithyroidectomy
shamTx	sham operation
FCS	fetal calf serum
incub. fl.	incubation fluid
RL	right lobe
LL	left lobe
KCl	kalium chloride
$MgCl_2$	magnesium chloride
ATP	adenosine triphosphate
EHNA	erythro-9-[2-hydroxy-3-nonyl] adenine
cpm	counts per minute
TS	thymidylate synthetase
dUMP	deoxyuridinemonophosphate
PD-ECGF	platelet-derived endothelial cell growth factor

Introduction

Thymidine kinase (TK; EC 2.7.1.21) is an enzyme catalyzing the phosphorylation of thymidine (deoxythymidine) – dThd to thymidine monophosphate (deoxythymidine monophosphate) – dTMP, functioning as a part of the pyrimidine salvage pathway involved in DNA synthesis [1]. The enzyme activity increases in proliferating tissues [2, 3]. A correlation was shown between TK activity and the intensification of cellular proliferation in various organs, e.g., in the small intestine [4], pancreatic islets [5] and in bone marrow [6] in rats. An increased TK activity was observed in thyrocytes of thyroid autonomous nodules [7], and also in thyroids of patients with Graves' disease, with non-toxic nodular goiter, in adenomas and, especially, in cancers of the thyroid gland [8]. An increased TK activity has recently been shown in human thyroid tissue obtained from toxic adenoma [9].

Adenosine kinase (AK; EC 2.7.1.20) is an enzyme catalyzing the phosphorylation of adenosine (Ado) and deoxyadenosine (dAdo) to adenosine monophosphate (AMP) and deoxyadenosine monophosphate (dAMP), respectively. Adenosine kinase functions as a part of the purine metabolic pathway, involved in DNA synthesis, and is the key enzyme regulating the intracellular content of Ado. Adenosine is known to be one of the most important and strongest vasodilators, especially in coronary circulation [10].

Melatonin (Mel) is an indole hormone, produced mainly in the pineal gland, described, for the first time, in 1958 [11]. Numerous reports from the past strongly suggest that Mel is a substance inhibiting growth processes in the thyroid gland [12], and in other endocrine glands [13, 14], as well as in non-endocrine tissues [15, 16].

It is worth recalling that the stimulatory effect of hemithyroidectomy (hemiTx) on the growth processes in the remaining thyroid lobe is a well-known phenomenon [17]. Therefore, the surgical procedure of hemiTx can be a good way to assess the morphological, histological and biochemical changes which accompany the hypertrophy and/or proliferation processes.

The aim of our study was to examine TK and AK activities in the remaining thyroid lobe after contralateral hemiTx; additionally, incubations with Mel were performed. Such a joint assessment of TK and AK activities seems to be useful because TK activity provides some information about changes in growth processes and AK activity can be regarded as an indicator of the content of adenosine – a strong vasodilator. It is also known that the observed compensatory growth of thyroid lobe after hemiTx is related to an increased blood flow in the thyroid [18], the process in which Ado is involved.

Materials and methods

The experiment was performed on 320 young male Wistar rats (6-week old), weighing 95±10 g each. The rats were divided into 16 groups (20 animals in each). After decapitation, both thyroid lobes – collected in time "0", both lobes – from sham-operated (shamTx)-animals and the lobe which remained after contralateral hemiTx – in respective groups collected 2 weeks after the surgery, were weighed and placed into an incubation fluid. Then, the lobes were incubated for four hours in the temperature of 37°C (atmosphere: 95% O_2 and 5% CO_2) in RPMI 1640 medium (Gibco BRL, UK), containing 25 mM HEPES buffer, 15% fetal calf serum – FCS (Biochrom, D), gentamicin (Polfa, PL) and Mel (Sigma, USA), the last substance used in three concentrations: 10^{-12} M (thought to be the "physiological" concentration in rats [19]), 10^{-9} M, and 10^{-6} M ("supraphysiological" concentrations). The thyroid lobes, collected from the intact and from the shamTx-rats, served as controls.

The following groups were considered:

Groups I–IV: intact animals – assessment of TK and AK activities in time "0"; **Groups V–VIII**: sham-operation – assessment of TK and AK activities 2 weeks after the surgery; **Groups IX–XVI**: hemithyroidectomy – assessment of TK and AK activities 2

weeks after the surgery. In the subsequent groups, the following thyroid lobes were examined: **Groups I** and **V** [incubation fluid (incub. fl.) + right lobe (RL) and left lobe (LL), incubated separately], **Groups II** and **VI** [Mel 10^{-6} M + RL and LL], **Groups III** and **VII** [Mel 10^{-9} M + RL and LL], **Groups IV** and **VIII** [Mel 10^{-12} M + RL and LL], **Group IX** [incub. fl. + RL], **Group X** [incub. fl. + LL], **Group XI** [Mel 10^{-6} M + RL], **Group XII** [Mel 10^{-6} M + LL], **Group XIII** [Mel 10^{-9} M + RL], **Group XIV** [Mel 10^{-9} M + LL], **Group XV** [Mel 10^{-12} M + RL], **Group XVI** [Mel 10^{-12} M + LL]. A solvent for Mel (absolute alcohol : 0.9% NaCl – 1:10, V:V) was added to the incubation fluid in **Groups I**, **V**, **IX** and **X**.

After termination of incubation, the thyroid lobes were cooled to the temperature of 0°C.

Thymidine kinase activity was assayed according to the method described by Cheng and Prusoff [20], using the modification of Greger and Draminski [21]. The thyroid lobes were homogenized in the medium: 25 mM Tris-HCl buffer (pH 7.4), 25 mM KCl (kalium chloride) and 5 mM $MgCl_2$ (magnesium chloride) at 0°C. Following centrifugation (10000 x g for 20 min), the obtained postmitochondrial fraction (70 μl) was incubated for 30 min (37°C) in a medium consisting of 50 mM Tris-HCl buffer (pH 7.4), 10 mM ATP (adenosine triphosphate), 10 mM $MgCl_2$ and, additionally, with 35 μl of [2-^{14}C]Thd (Amersham, UK). The reaction was stopped by immersion in a boiling water bath (100°C, 2 min). After deproteinization (by centrifugation for 3 min), aliquots of the supernatant were placed on Whatman DE 81 chromatography paper.

The reaction products and substrates were separated by ascending chromatography at room temperature in a solvent of 5 mM ammonium formate (pH 5.7). Five parallel chromatographic separations for each group were conducted. The chromatograms were dried and the radioactive spots, corresponding to dTMP and dThd, were cut out and placed in counting vials. Radioactivity was measured in an LKB Wallac liquid scintillation counter. The protein content was determined, according to the method described by Bradford [22].

Adenosine kinase activity was assayed, using the method based on the description by Muraoka et al. [23], with our modifications. The thyroid lobes were homogenized in the same medium as that used for TK activity assay. Following centrifugation (10000 x g for 20 min), the obtained postmitochondrial fraction was incubated for 45 min (37°C) in a medium (240 μl) consisting of 50 mM Tris-HCl buffer (pH 7.4), 10 mM ATP, 5 mM $MgCl_2$, 60 nM EHNA (erythro-9-[2-hydroxy-3-nonyl] adenine; adenosine deaminase inhibitor) and, additionally, with 0.05 mM [8-^{14}C]dAdo. The reaction was stopped as above. After deproteinization (by centrifugation for 3 min), aliquots of the supernatant were placed on Whatman I chromatography paper.

The reaction products and substrates were separated by ascending chromatography at room temperature in a solvent of 1 M ammonium acetate (ammonium acetate: 95% ethanol–3:7, V:V) (pH 7.5). The subsequent steps of procedure (chromatographic separations, measurement of radioactivity and of the protein content) were the same as those for TK.

Thymidine kinase and AK activities were expressed as cpm/100 μg protein/45 min; cpm-counts per minute. The data were statistically evaluated by the one-way analysis of variance (ANOVA). The statistical significance of differences among the individual groups was evaluated by the Neuman-Keuls' test [24]. The results, obtained for the right and left lobes in each experimental group, have been jointly shown in Figures 1–4 and expressed as means ± SEM.

Results

Hemithyroidectomy increased TK activity in homogenates of the remaining thyroid lobes (Fig. 2). Melatonin increased TK activity in the thyroid glands of intact rats (Fig. 1). Melatonin – in the concentration of 10^{-6} M – increased TK activity in homogenates of the thyroid lobes, obtained from shamTx-animals and from hemiTx-rats (Fig. 2). Melatonin (10^{-9} and 10^{-12} M) caused an increase of TK activity in homogenates of the thyroids obtained from shamTx-rats (Fig. 2). The increasing effects of Mel (10^{-9} and 10^{-12} M) on TK activity in the hemiTx-rats reached the borderline statistical significance only (Fig. 2).

Melatonin increased AK activity in homogenates of the thyroid lobes in intact rats (Fig. 3). Melatonin augmented also AK activity in homogenates of the thyroid lobes in shamTx-animals (that effect was less pronounced than in intact rats) (Fig. 4). No statistically significant changes were found in the activity of the enzyme in question in homogenates of the remaining thyroid lobes (incubated with or without Mel) collected from hemiTx-rats (Fig. 4). Hemithyroidectomy decreased AK activity when compared to the activity of that enzyme in intact rats.

Discussion

In our experiment, hemiTx increased TK activity in the remaining thyroid lobes. Considering the stimulatory effect of hemiTx on growth processes in the remaining thyroid lobe, the above effect is consistent with the data from earlier reports, demonstrating an increased TK activity in rapidly prolif-

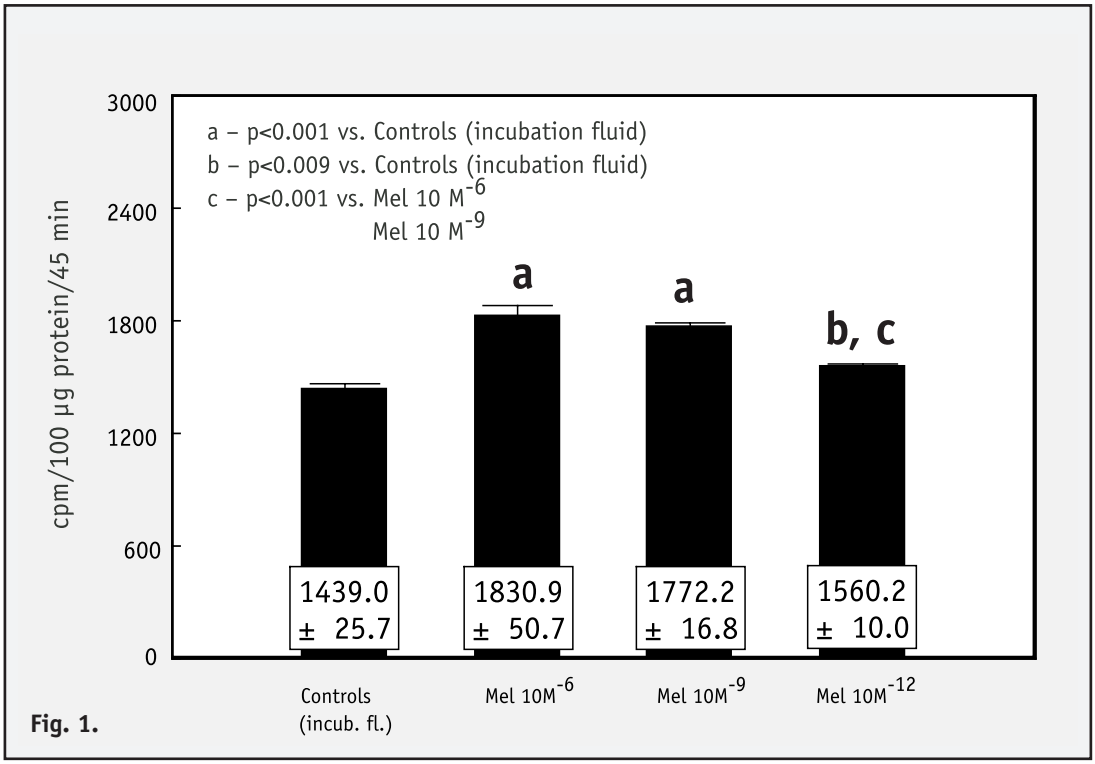

Fig. 1.

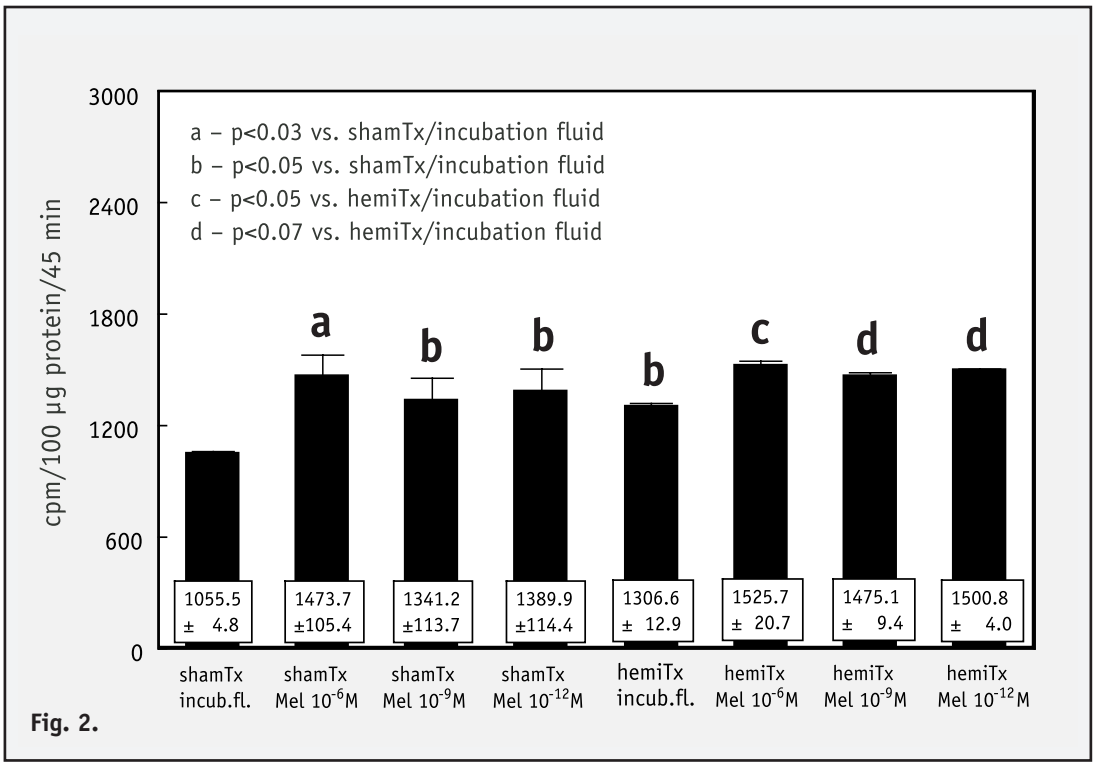

Fig. 2.

Fig. 1. Thymidine kinase activity in the homogenates of intact rat thyroid lobes, incubated *in vitro* in the presence of Mel. Incub. fl. – incubation fluid. Each value represents the mean ± SEM.
Fig. 2. Thymidine kinase activity in the homogenates of shamTx- or hemiTx-rat thyroid lobes, incubated *in vitro* in the presence of Mel. Incub. fl. – incubation fluid. Each value is the mean ± SEM.

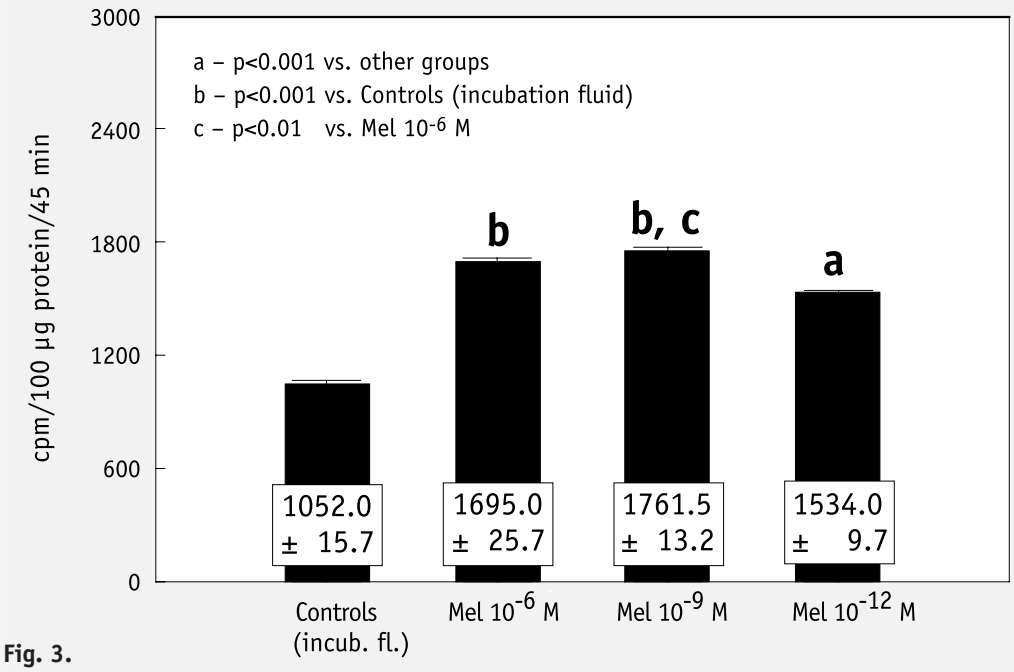

Fig. 3.

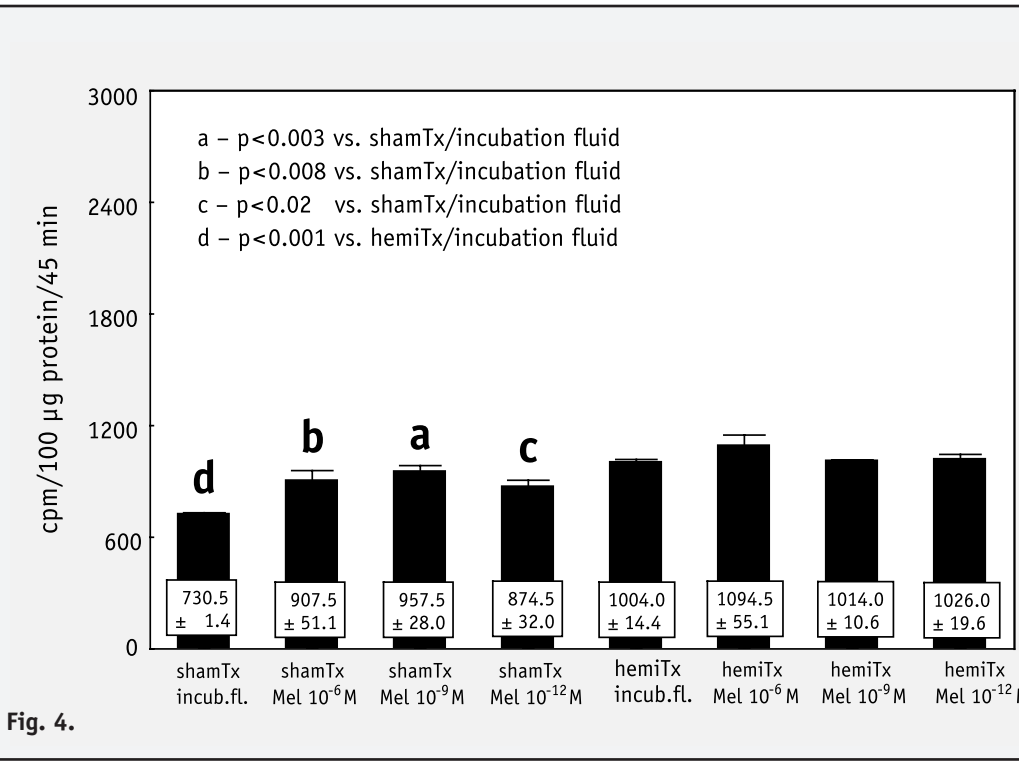

Fig. 4.

Fig. 3. Adenosine kinase activity in the homogenates of intact rat thyroid lobes, incubated *in vitro* in the presence of Mel. Incub. fl. – incubation fluid. Each value illustrates the mean ± SEM.
Fig. 4. Adenosine kinase activity in the homogenates of shamTx- or hemiTx-rats thyroid lobes, incubated *in vitro* in the presence of Mel. Incub. fl. – incubation fluid. The results are expressed as means ± SEM.

erating tissues [2, 3]. Generally, Mel increased TK activity in the thyroids of all the groups (collected from intact, sham-operated- and hemiTx-rats). The obtained results appear to be in contrast with the inhibitory effects of Mel on growth processes in various tissues and organs, e.g., on TK activity in the thyroids of Wistar rats, as earlier observed in our laboratory [19]. One of possible explanations of the observed differences between our present results and the previous ones, cited above report, could be the young age of rats, used in the present study, and/or their low body weight (95±10 g), when compared to the earlier investigation (150±20 g).

The effects of Mel in young rats can hypothetically be different from those in older animals. Thiéblot et al. [25] have shown that the administration of Mel to young prepubertal rats produces a marked hyperactivity of the thyroid, as shown by histological changes in the gland. In another study, it has been shown that administration of Mel in Sprague-Dawley rats (50 g BW) results in an increased radioactive thyroidal iodine uptake, a higher total thyroxine (T_4) content and an increased thyroxine:triiodothyronine ratio (T_4:T_3) [26].

It is worth stressing that not only are the inhibitory effects of Mel known which are exerted on thyroid growth and function. The dose of 25 μg daily reduced the circulating levels of thyroid hormones, but a high dose of Mel (2.5 mg) significantly increased T_4 level in female hamsters [27]. The late-afternoon (16.00–18.00) subcutaneous Mel injections (25 μg/daily) increased T_3 concentration in rats after a 5-day administration [28]. In other experiment, an inhibitory effect of Mel *ex vivo in vitro* was observed on ^{3}H-thymidine incorporation into DNA of thyroid lobes only for the dose of 25 μg/daily [29]. Melatonin, in the dose of 50 μg/daily, produced no effect, but in the dose of 100 μg/daily, an increase of ^{3}H-thymidine incorporation was observed [29]. These – not only inhibitory – effects of Mel on growth processes were also showed in other cells and tissues [30, 31, 32].

Another possible explanation, which should be considered, is the fact that – apart from TK – thymidylate synthetase (TS) is also responsible for dTMP synthesis in the pyrimidine pathway. This second enzyme catalyzes the methylation of dUMP for the *de novo* synthesis of dTMP, whereas TK is involved in the salvage synthesis of dTMP. High activities of TK and TS were observed in rapidly proliferating tissues. A two-fold increase of both TK and TS activities was reported in human thyroid carcinoma, in comparison with a normal thyroid tissue [33]. Takeda et al. [34] have shown that a high potential for proliferation in cultured human malignant cells may mainly depend on the *de novo* pyrimidine pathway of DNA biosynthesis. In another investigation, the authors have indicated that in the proliferation of bone marrow cells, the DNA *de novo* synthesis rises first, and the DNA salvage synthesis is second [6]. On the basis of the above cited results, one can hypothesize that in our experiment Mel exerted its inhibitory effect on DNA *de novo* synthesis, the process in which TK is not involved. We also consider a presumption that the effects of Mel in young rats can be different from those observed in older animals.

It is known that a high AK activity decreases the Ado content. In our study, Mel increased AK activity in the thyroid lobes collected for incubation from intact rats. Melatonin also augmented AK activity in the thyroid lobes of shamTx-animals. Such effects of Mel (the increase of AK activity and, presumably, the decrease of the Ado content which may result in a diminution of vasodilating effects) could be expected, while regarding the growth inhibitory actions of Mel [12], e.g., Mel inhibitory effects on angiogenesis, as shown by the decrease of PD-ECGF/thymidine phosphorylase activity in thyroid homogenates from hemithyroidectomized rats [35]. The lack of changes in AK activity under the influence of Mel, found in lobes collected from hemiTx-rats, can putatively be balanced by compensatory growth processes which occur after hemiTx and which are accompanied by an increased thyroid blood flow [18], the process in which Ado is certainly involved. Hemithyroidectomy decreased AK activity when compared to the activity of that enzyme in intact rats, and – presumably as a consequence of that fact – increased the content of Ado. Keeping in mind the vasodilating effects of Ado and taking into consideration that an increased release of TSH from pituitary and a compensatory growth of thyroid lobe left after hemiTx are related to an increased thyroid blood flow [18], the obtained decreased activity of AK in the thyroid tissue after hemiTx is not surprising and seems to be a logical sequel.

The obtained results suggest a role of AK and, assumingly, of Ado in the regulation of (patho)physiological processes in the thyroid gland after hemiTx. Melatonin, besides its well-known mechanisms of action, may be involved in thyroid growth and function control, through its influence on AK activity.

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