THYMIDINE KINASE ACTIVITY IN HOMOGENATES OF RAT THYROID LOBES COLLECTED FROM EUTHYROID, HYPOTHYROID AND/OR HYPERTHYROID RATS AND INCUBATED WITH EGF: STUDIES *EX VIVO IN VITRO*

M. Karbownik[1], H. Modrzejewska[2], A. Gesing[1], J. Greger[2], A. Lewinski[1]

[1]Department of Thyroidology, Institute of Endocrinology and [2]Department of Biochemistry, Institute of Physiology and Biochemistry, Medical University of Lodz, Poland

Objective. To examine thymidine kinase (TK – ATP:thymidine 5'-phosphotransferase, EC 2.7.1.21) activity in homogenates of rat thyroid lobes incubated *in vitro* with epidermal growth factor (EGF).

Methods. The thyroid lobes were collected from euthyroid, hypothyroid and/or hyperthyroid animals. Hypothyroidism was developed in the experimental rats by an administration of 0.1 % solution of propylthiouracil (PTU) in drinking water for 2 weeks, while hyperthyroidism was obtained by daily i.p. injections of L-thyroxine (50 μg/kg, B.W.), also for 2 weeks. After collecting, the thyroids were incubated for 4 hours in RPMI 1640 medium with an addition of 20 mM of Hepes buffer, 15% FCS, penicillin (200 U/ml), streptomycin (10 μg/ml) and with EGF (Sigma) (0.1 ng/ml, 10 ng/ml, 1000 ng/ml). The control lobes were incubated without any addition of EGF to the medium. TK activity was expressed as the amount of reaction products, measured by ascending chromatography.

Results. 1. in the absence of EGF, TK activity in the homogenates of thyroid lobes from hypothyroid rats was lower, while it was higher in the lobes from hyperthyroid animals, when compared to these obtained from euthyroid controls; 2. EGF in the concentration of 0.1 ng/ml or 1000 ng/ml decreased, while that in the concentration of 10 ng/ml increased TK activity in lobes collected from euthyroid or hyperthyroid rats; 3. in the tissue collected from hypothyroid rats, the addition of EGF (0.1 ng/ml or 10 ng/ml) caused a slight increase in TK activity versus hypothyroid controls – a tendency towards diminishing TK activity could be observed as parallel to increasing EGF concentration.

Conclusions. TK activity in the homogenates of rat thyroid lobes depends on the functional thyroid status and on applied EGF concentration *in vitro*.

Key words: Hypothyroidism – Hyperthyroidism – Rat thyroid – Thymidine kinase – EGF

Deoxythymidine kinase [thymidine kinase (TK) – ATP : thymidine 5'-phosphotransferase, EC 2.7.1.21] is an enzyme responsible for catalyzing the phosphorylation of thymidine, which functions as a part of the pyrimidine salvage pathway involved in DNA synthesis (Kahn et al. 1980). Several authors observed parallel changes of TK activity and ^{3}H-thymidine incorporation into DNA of various tissues or with cell divisions (Kahn et al. 1980; Zieve et al. 1985; Lambotte et al. 1997). An increased TK activity was demonstrated in thyrocytes of autonomous nodules (Mueller-Gaertner et al. 1989) and in thyroids of patients with Graves' disease, non-toxic nodular goiters, adenomas and, especially, with thyroid cancers (Murakami 1988). It was shown that an increase in DNA synthesis proceeded in parallel with

the increased activity of cytoplasmic TK (Murakami 1988). In our laboratory, we found an increased TK activity in the homogenates of thyroid tissue collected from the thyroids of patients with toxic adenoma, when compared to the activity in non-toxic adenoma and in macroscopically unchanged tissue; EGF increased TK activity in all the above thyroid specimens *in vitro* (Brzezinski et al. 1998).

The epidermal growth factor (EGF) is one of the best known mitogenic agents for thyroid follicular cells (TFC) (Lewinski et al. 1993). That growth factor was demonstrated to stimulate TFC proliferation in different experimental conditions, e.g., in cell cultures prepared from porcine thyrocytes (Westermark et al. 1983), in a suspension culture of porcine thyroid follicles (Gaertner et al. 1985) and in an organ culture prepared from rat thyroid lobes (Zerek-Melen et al. 1990). On the other hand, some authors failed to observe the proliferogenic effect of EGF on rat thyrocytes (Ambesi-Impiombato et al. 1980; Smith et al. 1986), especially on FRTL-5 cell line (Veneziani et al. 1986; Pang and Hershman 1990).

In the *in vivo* experiments, EGF has been shown to stimulate thyroid growth processes after xenotransplantations of rat (Ozawa and Spaulding 1990) or human thyroid tissue (Hoelting et al. 1994; Paschke et al. 1995) into nude mice. On the other hand, EGF – injected directly into rat thyroid lobes (Brzezinski et al. 1996b) – did not significantly affect the rate of ^{3}H-thymidine incorporation into DNA of rat thyroid lobes incubated with that labelled nucleoside.

However, the influence of EGF on the activity of enzymes participating in DNA synthesis has not yet been defined. The inhibitory effect of EGF on TK activity was observed in the homogenates of rat thyroid lobes (Brzezinski et al. 1997a).

In the present study, we have attempted to examine the *in vitro* effects of EGF on TK activity in the homogenates of rat thyroid lobes, collected from euthyroid, hypothyroid [after propylthiouracil (PTU) administration] and hyperthyroid [after L-thyroxine (L-T_4) treatment] rats.

Materials and Methods

Animals. Two hundred and sixty (260) male Wistar rats, weighing 80±10 g each, were used as donors of thyroid lobes. The rats were made hypothyroid by an administration of 0.1 % solution of propylthiouracil (PTU; Sigma Chemical Co.) in drinking water for 2 weeks (Groups VI-IX) or were made hyperthyroid by daily i.p. injections of L-thyroxine (L-T_4; Sigma Chemical Co.) (50 µg/kg b.w.), also for 2 weeks (Groups X-XIII). The rats of Group I served as controls for Groups VI-IX and did not receive any treatment, while the rats of Group II were injected i.p. with 0.9% NaCl daily for 2 weeks (controls for Groups X-XIII). After 2 weeks of treatment, the animals (their weight raising to 120±20 g each) were sacrificed by decapitation, the right thyroid lobes were immediately collected, weighed on a torsion balance (m=total mass of thyroid lobes of each group) and placed into the vessels containing an incubation fluid.

Incubation. The thyroid lobes were incubated for 4 hours in RPMI 1640 medium with an addition of 20 mM of Hepes buffer, 15% FCS, penicillin (200 U/ml), streptomycin (10 µg/ml) and, additionally, with EGF (Sigma Chemical Co.) applied in three different concentrations: 0.1 ng/ml (Groups III, VII, XI), 10 ng/ml (Groups IV, VIII, XII) or 1000 ng/ml (Groups V, IX, XIII); thyroid lobes of Groups I, II, VI and X were incubated without any addition of EGF to the medium.

The individual groups, the applied treatment and the mass (m) of the collected thyroid lobes are presented in Table 1.

Subsequent steps of the procedure were carried out according to Cheng and Prusoff (1974) in a modification of Greger and Draminski (1989). After the incubation, the thyroid lobes of particular groups were homogenized in the medium (25 mM Tris-HCl buffer, 25 mM KCl and 5 mM $MgCl_2$ – pH 7.4, temp. 0 °C). Following centrifugation (10000 g for 20 min), the obtained postmitochondrial fraction (70 ml) was incubated for 30 min (37 °C) in the medium consisting of: 50 mM Tris-HCl buffer (pH 7.4), 10 mM ATP, 10 $MgCl_2$ and, additionally, with 35 µl [2-^{14}C]dThd. The reaction was terminated by an immersion in a boiling water bath (100 °C, 2 min). After deproteinization (by centrifugation – 3 min), the aliquots of the supernatant were placed on a Whatman DE81 chromatography paper. The reaction products – dTMP and dThd – were separated by ascending chroma-

T a b l e 1
Thyroid mass in individual experimental groups

Number of group	Substances applied *in vivo* and/or *in vitro*	Mass of the thyroid lobes
Group I	Controls	m=107.4 mg
Group II	Controls, 0.9% NaCl	m=119.0 mg
Group III	0.9% NaCl, EGF 0.1 ng/ml	m=124.4 mg
Group IV	0.9% NaCl, EGF 10 ng/ml	m=114.1 mg
Group V	0.9% NaCl, EGF 1000 ng/ml	m= 98.6 mg
Group VI	0.1% PTU	m=357.0 mg
Group VII	0.1% PTU, EGF 0.1 ng/ml	m=262.2 mg
Group VIII	0.1% PTU, EGF 10 ng/ml	m=313.3 mg
Group IX	0.1% PTU, EGF 1000 ng/ml	m=241.5 mg
Group X	L-thyroxine	m=100.2 mg
Group XI	L-thyroxine, EGF 0.1 ng/ml	m=115.4 mg
Group XII	L-thyroxine, EGF 10 ng/ml	m= 89.3 mg
Group XIII	L-thyroxine, EGF 1000 ng/ml	m=112.2 mg

tography at room temperature in a solvent of 5 mM of ammonium formate (pH 5.6). Six (6) parallel chromatographic separations were conducted for each group. 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 by the method of Lowry et al. (1951). The enzyme activity was expressed in cpm/min/mg protein.

Statistical evaluation. 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 use of Neuman-Keuls' test (Hinkle et al. 1979).

Results

The repeated injections of L-T_4 *in vivo* increased, while the *in vivo* administration of PTU decreased TK activity in the homogenates of rat thyroid lobes *in vitro* (Fig. 1a).

When the thyroid lobes of control (0.9 % NaCl-injected) animals were subjected to incubation with EGF *in vitro*, the stimulatory effect of that growth factor could be observed only for the concentration of 10 ng/ml, while the higher (1000 ng/ml) and the lower (0.1 ng/ml) concentrations brought about a slight decrease of TK activity (Fig. 1b). The similar "bell-shape response" to EGF could be observed for hyperthyroid animals (Fig. 1d).

When the animals were pretreated with PTU and the collected lobes were subsequently incubated in the presence of EGF, TK activities – for all the three examined EGF concentrations – were significantly lower than those in controls (1c) and also than those in the EGF-exposed lobes from 0.9 % NaCl-injected animals (2a, 2b, 2c). At the same time, a tendency towards decreasing TK activity parallel to the increase of EGF concentration, was noticed (Fig. 1c).

Either PTU– or L-T_4-pretreatment of rats brought about a decrease in TK activity in the homogenates of thyroid lobes exposed to EGF in the concentration of 0.1 ng/ml; the lowest TK activity was observed in the PTU-pretreated animals (Fig. 2a).

After exposure of thyroid lobes to EGF in the concentration of 10 ng/ml, the increase in TK activity was observed in lobes collected from 0.9 % NaCl and L-T_4-pretreated rats, whereas a decrease in TK activity appeared in thyroids from PTU-pretreated rats (Fig. 2b).

The TK activities in the thyroids collected from all the examined groups and exposed to EGF in the concentration of 1000 ng/ml, were lower than in con-

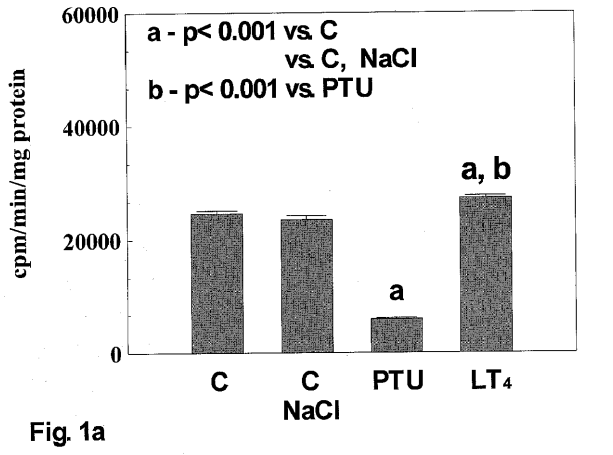

Fig. 1a

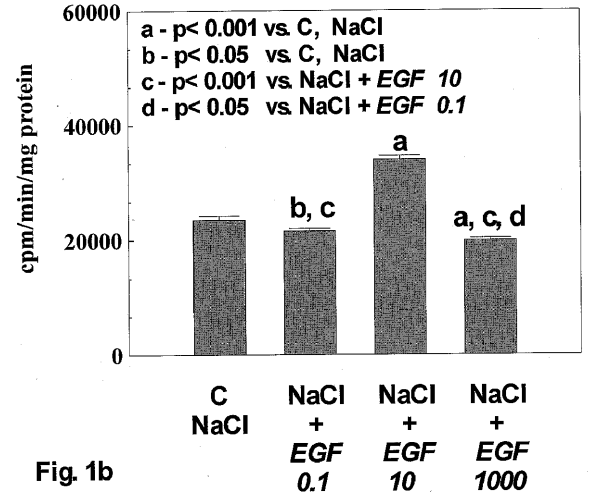

Fig. 1b

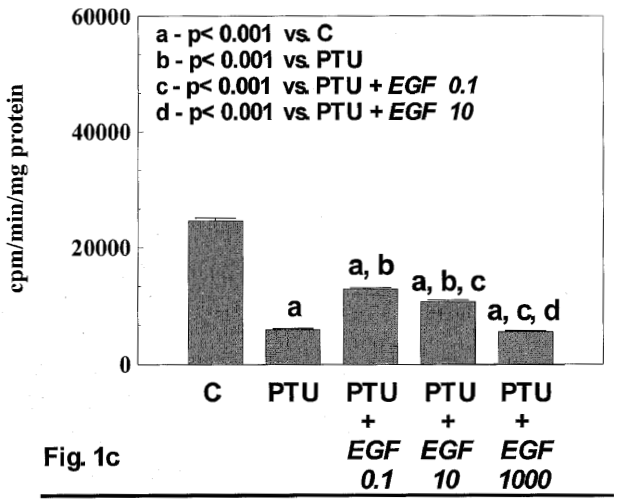

Fig. 1c

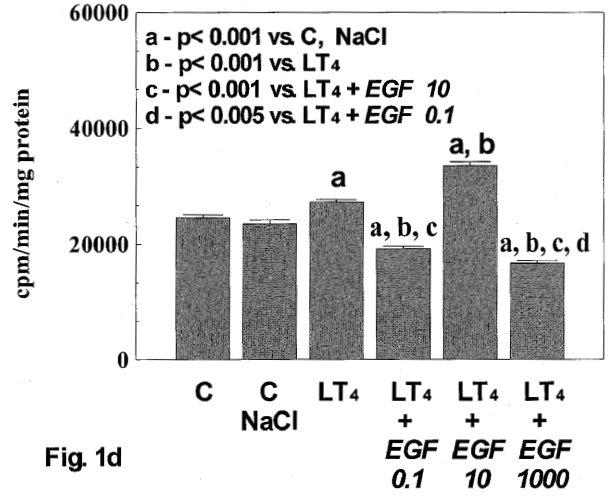

Fig. 1d

Fig. 1
Thymidine kinase activity in the homogenates of rat thyroid lobes collected from:
1a. euthyroid [Controls (C) or Controls NaCl (C, NaCl)], hypothyroid (after PTU administration) and hyperthyroid (after L-T4 injections) rats;
1b. euthyroid [Controls NaCl (C, NaCl)] rats and additionally exposed to EGF in vitro in three different concentrations [0.1 ng/ml (EGF 0.1), 10 ng/ml (EGF 10) and 1000 ng/ml (EGF 1000)];
1c. euthyroid [Controls (C)] and hypothyroid (after PTU administration) rats, three groups additionally exposed to EGF in vitro [0.1 ng/ml (EGF 0.1), 10 ng/ml (EGF 10) and 1000 ng/ml (EGF 1000)];
1d. euthyroid [Controls (C) or Controls NaCl (C, NaCl)] and hyperthyroid (after L-T4 injections) rats, three groups additionally exposed to EGF in vitro [0.1 ng/ml (EGF 0.1), 10 ng/ml (EGF 10) and 1000 ng/ml (EGF 1000)].
Bars represent means ± SEM, p – level of significance.

trols; the lowest activity of the enzyme in question was observed in the PTU-pretreated rats (Fig. 2c).

Discussion

In earlier studies performed in our laboratory, we demonstrated that the inhibitory effect of the vaso-active intestinal polypeptide (VIP) on TK activity in the homogenates of rat thyroid tissue (Karbownik et al. 1994) and on ^{3}H-thymidine incorporation into the DNA of rat thyroid lobes (Lewinski et al. 1992), occurred in a similar range of concentrations. However, not always did the changes of TK activity and ^{3}H-thymidine incorporation in the thyroid gland run

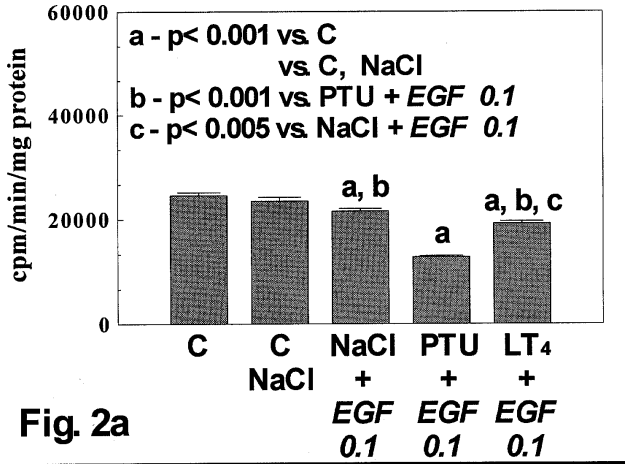

Fig. 2a

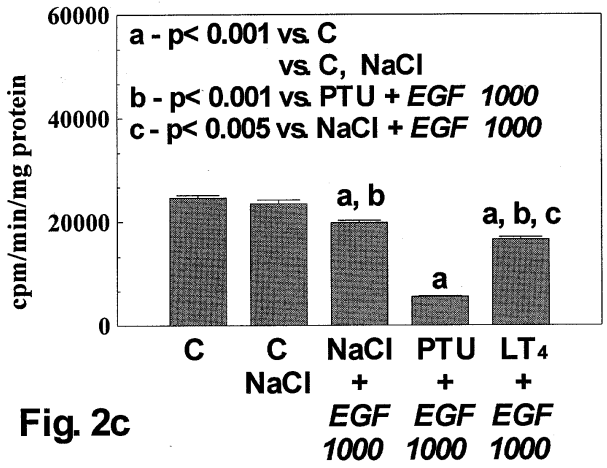

Fig. 2c

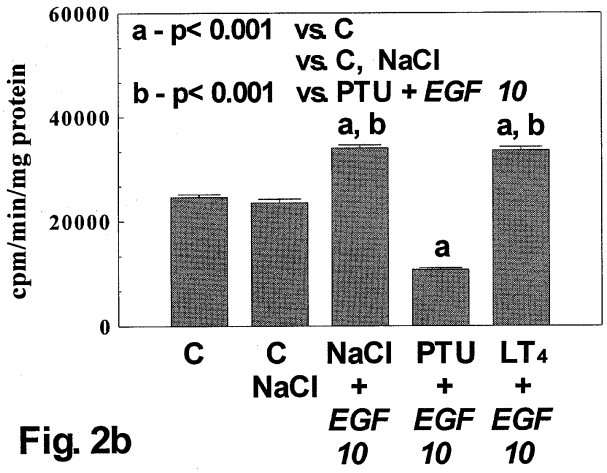

Fig. 2b

Fig. 2
Thymidine kinase activity in the homogenates of rat thyroid lobes collected from euthyroid [Controls (C) or Controls NaCl (C, NaCl)] rats incubated without EGF exposure in vitro, and euthyroid (C, NaCl) hypothyroid (after PTU administration) and hyperthyroid (after L-T4 injections) rats, exposed to EGF in vitro in the concentration of:
2a. 0.1 ng/ml (EGF 0.1);
2b. 10 ng/ml (EGF 10);
2c. 1000 ng/ml (EGF 1000).
Bars represent means ± SEM, p – level of significance.

parallel. It is obvious that the amount of synthesized DNA results not only from the activity of one but from that of several different enzymes involved in DNA synthesis [e.g., deoxythymidine monophosphate kinase (dTMP kinase, EC 2.7.4.9), polymerase DNA (DNAp, EC 2.7.7.7) or thymidylate synthetase (TS), the last enzyme catalyzing the methylation of deoxyuridine monophosphate for *de novo* synthesis of deoxythymidine monophosphate]. It is worth recalling that the high activities of TK and TS were observed in rapidly proliferating tissues; SAKAMOTO et al. (1991) reported on a 2-fold increase of both TS and TK activities in human thyroid carcinoma, in comparison with normal thyroid tissue.

In the present experiment we observed that TK activity was lower in the homogenates of thyroid lobes collected from hypothyroid rats, while it was higher in the lobes from hyperthyroid animals, when compared to the euthyroid controls. The above results are in agreement with the majority of previous observations from other laboratories. Accordingly, TK activity of autonomously functioning thyroid adenomas was shown to be higher than that in normal thyroid tissue (MUELLER-GAERTNER et al. 1989). The average TK activities in Graves' disease, non-toxic nodular goitre, thyroid adenoma and carcinoma were higher than the activity in question in normal thyroid tissue (MURAKAMI 1988). We also found an increase in TK activity in the homogenates of thyroid tissue collected from the thyroids of patients with toxic adenoma (BRZEZINSKI et al. 1998).

We have previously shown that the rate of ^{3}H-thymidine incorporation into DNA of rat thyroid lobes, collected from hypothyroid rats, was higher, while in the lobes from hyperthyroid animals, it was lower, when compared to the euthyroid controls (KARBOWNIK et al. 1996). Those entirely opposite direction of changes in TK activity and in ^{3}H-thymidine incorporation, depending on the functional status of the thyroids, should remind that TK is only one of the enzymes participating in DNA synthesis.

In our previous experiment, EGF – used in a wide range of concentrations – decreased the activity of the enzyme in question in rat thyroid lobes (BRZEZINSKI et al. 1997a). In the present experiment, EGF decreased TK activity in the homogenates of thyroid lobes only in the concentrations of 0.1 ng/ml and of 1000 ng/ml. The above differences in TK activity in rat thyroid tissue, following EGF exposure, result from age differences of the used animals. Nevertheless, one can conclude that EGF reveals a tendency towards decreasing TK activity in the homogenates of rat thyroids.

In the present experiment, EGF brought about an increase of TK activity in the thyroid lobes collected from hypothyroid rats, however, all the recorded TK activities in those lobes, even after the stimulation by EGF, were significantly lower than those in the lobes collected from hyperthyroid or euthyroid animals. A tendency towards diminishing TK activity could be observed parallel to increasing EGF concentration.

SALLER et al. (1993) observed an increased EGF binding capacity in autonomously functioning thyroid adenomas. A stronger EGF-receptor staining was found in hyperfunctioning thyroid gland when compared to normal thyroid tissue and even papillary carcinomas; the immunostaining was stronger in follicles with columnar cells compared to those covered by cuboidal cells (WESTERMARK et al. 1996). One can speculate that the decrease in TK activity in thyroids collected from hyperthyroid rats and exposed to EGF is the direct effect of the stronger bound of EGF to its receptors and, in consequence, of its stronger inhibitory influence on the activity of intracellular enzymes.

Summing up, TK activity in homogenates of rat thyroid lobes *in vitro* depends on both the functional thyroid status and the applied EGF concentration; pretreatment of rat thyroid-donors with L-thyroxine increased, while the administration of PTU decreased TK activity.

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Corresponding author: Prof. A. Lewinski, M.D., Ph.D.
Department of Thyroidology
Institute of Endocrinology
Medical University of Lodz
Dr. Sterling St. No. 5
91-425 Lodz, Poland
Phone/fax: +48 42 6322594
E-mail: alewin@psk2.am.lodz.pl

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