THE INFLUENCE OF THYROXINE ON INTENSITY OF ENERGY METABOLISM IN BONE MARROW MYELOID CELLS AND NEUTROPHILIC POLYMORPHONUCLEAR LEUKOCYTES OF NEONATAL PIG

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Objectives. To investigate the participation of thyroxine in the regulation of energy metabolism in neutrophilic polymorphonuclear leukocytes and their bone marrow precursors.

Materials and Methods. The influence of L-thyroxine (T_4; 4 mg/kg every 12 hr from day 2 to 10 of age) was estimated on the activity of hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), NADP-dependent isocitrate dehydrogenase (ICDH) and cytochrome C-oxidase in bone marrow myeloid cells and circulating neutrophils of 3, 5 and 10 day (d) old piglets. Serum T_4 and 3,5,3'-triiodothyronine (T_3) concentrations were estimated at every stage of experiment by radioimmunoassay. Bone marrow cells of myeloid lineage and blood neutrophilic polymorphonuclear leukocytes were separated by differential centrifugation of haematopoietic cell suspension using Ficoll-Hypaque gradients.

Results. The hyperthyroid status resulted in significant increase in PFK and LDH activity in myelocaryocytes of 3 and 3-5 d piglets, while the activity of HK and PK in the cells of 3-10 d animals remained unchanged. Moreover, ICDH activity in myelocaryocytes increased on day 10 and that of cytochrome C oxidase in bone marrow cells at all intervals. Marked increase in HK and LDH activity on day 3-5 was found also in blood polymorphonuclear granulocytes, while PFK and PK activity was increased during the whole period. At the same time even the increase in ICDH and cytochrome C-oxidase activity was observed, respectively, in 3 and 5-10 d old piglet neutrophils. Besides that, T_4 inhibited G-6-PDH activity in myeloid cells on day 3 to 10 and did not influence the enzyme activity in circulating leucocytes.

Conclusions. The administration of T_4 resulted in preferential stimulation of oxidative stages of carbohydrate catabolism in myelocaryocytes, while the activity of glycolytic enzymes in these cells was less affected. On the contrary, the enzymes of glycolysis in blood neutrophils showed higher sensitivity to T_4 action as compared to catalysts of oxidative reactions. The intensity of pentose phosphate pathway seems to be inhibited in bone marrow myelocaryocytes of T_4 treated animals, while that in blood leukocytes remained unaffected.

Key words: Thyroid hormones – Thyroxine – Haematopoiesis – Myelopoiesis – Myeloid cells – Leukocytes – Neutrophils – Energy metabolism

Neonatal organism is unusualy vulnerable to several infections due to the impairment in functioning of immune system during the early postnatal period (Yang and Hill 1994). Such immune deficiency in newborn organism is mainly caused by abnormal functions of neutrophilic polymorphonuclear leukocytes that further results in impaired hemotaxis, adherence, aggregation, phagocytosis

and intracellular killing (Hill 1987). It is known that important role in maintaining functional activity of circulating neutrophils in mammalian organism is being played by energy generating system (Styrt 1989; Thelem et al. 1993). Appropriate intensity of energy reactions is essential to provide the adequate rate of proliferation processes in haematopoietic tissue. On the other hand, several stages of energy metabolism (such as pentose phosphate pathway) in mature leukocytes play significant role in maintaining appropriate levels of NADPH – components of NADPH-oxidase and glutathione reductase (Hill 1987, Christensen 1990). In this view the processes of energy metabolism in polymorphonuclear neutrophils and their bone marrow precursors seem to be of great importance in the mechanism of host defense and reactions of cellular immunity in animal organism.

Despite of numerous experimental studies the mechanisms underlying the impairment of neutrophilic polymorphonuclear leukocyte functions in early postnatal period as well as regulation of energy metabolism in marrow myeloid cells and blood granulocytes have not yet been completely investigated. It is known, however, that the cells of myeloid lineage remain under the influence of growth factors, hormones and other bioregulators (Bokoch 1995; Ferrante et al. 1996; Price at al. 1996; Akmayev 1997). Thyroid hormones are important regulators of metabolic reactions in animal and human organism (De Groot et al 1989; Harper et al. 1993; Mc Nabb 1995) and participate in the regulatory mechanisms of cell differentiation in several tissues (Koehrle et al. 1995; Klaushofer et al. 1995; Bernal and Nunez 1995; El Hadri et al. 1996; Perrin et al. 1997). Iodothyronine receptors have been found in both blood leukocytes and bone marrow myeloid cells (Chomienne et al. 1995; Meier 1997). However, there is little evidence of thyroxine involvement in the regulation of energy metabolism in these cells. Nevertheless, such investigations could elucidate the effect of increased levels of iodothyronines in mammalian blood in the period of neonate (Girard and Ferre 1982).

The aim of this study was to evaluate the effects of thyroxine on the activity of glycolysis rate limiting enzymes, Krebs cycle and hexose monophosphate pathway dehydrogenases as well as on cytochrome C oxidase activity in bone marrow myelocaryocytes and blood neutrophils in neonatal pigs.

Materials and Methods

Animals. The suckling piglets 1-10 days of age were maintained at 23 ^{o}C under 12:12 h light:dark cycle.

Reagents and enzymes. All reagents and enzymes were obtained from Sigma Chemical Co.(St. Louis, MO), except for Ficoll and Hypaque which were from Pharmacia Fine Chemicals (Piscataway, NJ). All measurements were performed using Specord M-40.

Induction of hyperthyroidism. Disodium L-thyroxine was dissolved in a small amount of NaOH (0.1 mol/l) and diluted with water. Each animal of studied group (n=15) received i.p. injection of this hormone in a dose of 4mg/kg every 12 hours from the 2nd to 10th day of age. Animals of control group (n=15) were treated with an equivalent volume of 0.85 % NaCl, by the same route, for the 9 days.

Separation of bone marrow cells. Piglets of control and thyroxine treated groups were sacrificed by rapid decapitation at 3, 5 and 10 days of age (5 animals at every stage of investigation). Cells of myeloid lineage were separated by differential centrifugation of haematopoietic cell suspension using Ficoll-Hypaque gradients (Harrison et al. 1981). Briefly, bone marrow was removed from animal long bones immediately after sacrifice and gently disagregated in 10 ml of LMES medium (0.3 M lactose, 14 mM 2-thioethanol, 2mM EDTA (pH 8.0), 150 mM NaCl) to form a single cell suspension, by using pipettes of 4 mm and then of 2 mm diameter aperture sequentially. The cells were pelleted at 150 x g for 5 min, washed with further 10 ml of LMES medium and pelleted again. The cells were then resuspensed in approximately 1 ml of PBS medium and filtered through nylon. Cell concentration in this suspension was determined by counting a diluted sample in haemocytometer.

A three-step Ficoll-Hypaque gradient was produced by sequentially layering each gradient fraction (1.10 g/ml, 1.09 g/ml, 1.03 g/ml) into the bottom of a 50 ml centrifuge tube from a 10 ml syringe under gravity flow. Bone marrow cells ($1x10^9$) were loaded, after thorough resuspension, into the top of the gradient in 2 ml of PBS medium and the gradient was centrifugated at 3000 g for 10 min. Frac-

tions (2 ml) were collected by hand from the top of gradient by using a fired Pasteur pipette and the cell distribution was determined by counting each fraction. To remove Ficoll-Hypaque, the combined fractions were diluted 20-fold with PBS medium and the cells were pelleted at 300 g for 5 min.

Separation of blood neutrophiles. Neutrophil polymorphonuclear leukocytes were obtained according to Boyum (1968). The heparinized blood was treated with gelatine (10 %) for 15-30 min. Plasma with the suspended leukocytes was removed by aspiration and resuspended in medium 199. A two-step Ficoll-Hypaque gradient was produced by sequentially layering each gradient fraction (1.119 g/ml and 1.077 g/ml) into the bottom of a 50 ml centrifuge tube from 10 ml syringe under gravity flow. The cell suspension was layered into the top of gradient and centrifugated at 3000xg for 15 min. The fraction of granulocytes was collected from the interface of gradient and treated with 0.83 % NH_4Cl in a ratio of 1 part cell suspension per 8 parts NH_4Cl and centrifugated at 120 g for 3 min. The resulting pellet was washed twice with 0.83 % NH_4Cl to ensure elimination of contaminating erythrocytes. This procedure resulted in a cell suspension composed primarily of neutrophils. The cells were suspended in salt-Tris buffer (pH 7.4, composed of 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 0.5 % dextrose) supplemented with 2 % fetal bovine serum. Cell viability was determined using Trypan blue exclusion.

Enzyme assays. The activity of hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), glucose-6-phosphate dehydrogenase (EC 1.1.1.42), NADP-isocitrate dehydrogenase (EC 1.1.1.42) were measured according to the methods, described by Kraaijenhagen et al. (1980), Vora (1981), Lestas et al. (1982), Fujii and Miwa (1983), Deutsch (1983), Goldberg and Ellis (1983).

The extraction medium for enzyme assays contained 25 mmol Tris-HCl/l, 1 mmol EDTA/l at pH 8.0. For the assay of hexokinase the following medium was used: 75 mmol Tris-HCl/l, 0.5 mmol D-glucose/l, 1 mmol ATP/l, 0.5 mmol $MgCl_2$/l, 0.5 mmol $NADP^+$/l, 0.3 U glucose-6-phosphate dehydrogenase, pH 7.5. The assay medium for pyruvate kinase consisted of Tris-HCl (75 mmol/l), 1 mmol phosphoenolpyruvate (1 mmol/l), KCl (10 mml/l), $MgCl_2$ (5 mmol/l), (1 mmol/l), NADH (0.05 mmol/l), 0.3 U lactate dehydrogenase, pH 7.5. The assay medium for glucose-6-phosphate dehydrogenase consisted of Tris-HCl (75 mmol/l), glucose-6-phosphate (1 mmol/l), $NADP^+$ (0.5 mmol/l), $MgSO_4$ (5 mmol/l), pH 7.5. The assay medium for lactate dehydrogenase consisted of Tris-HCl (75 mmol/l), pyruvate Na (1 mmol/l), NADH (0.05 mmol/l), $MgCl_2$ (3 mmol/l), pH 7.5. The medium for NADP-isocitrate dehydrogenase assay consisted of Tris-HCl (75 mmol/l), $NADP^+$ (0.5 mmol/l), $MgSO_4$ (1.7 mmol/l), DL-isocitrate (1.5 mmol/l), pH 7.5.

Cytochrome c-oxidase (EC 1.9.3.1) activity was assayed as described by Wharton and Tzagoloff (1967).

The isoenzyme pattern of lactate dehydrogenase was determined by disk-electrophoresis on 7.5 % polyacrylamide gel according to Davis (1964) at pH 7.5. Staining for the enzyme activity was performed as described by Setchenska and Arnstein (1978).

Determination of energy substrates. The determination of glucose, lactate and pyruvate levels were made by enzymatic methods using glucose oxidase and peroxidase for glucose measurements (Hanson 1981) and lactate dehydrogenase for both lactate and pyruvate measurements (Hanson 1981; Czok and Lemprecht 1974).

Protein estimation. Protein content in the cells was measured by the method of Lowry et al. (1951).

Statistical analysis. Results are presented as means±S.E.M. The results were initially analyzed by two-way analysis of variance (ANOVA). When the ANOVA detected differences among the groups, Student's test was used to determine the significance level which was assumed to be different when the comparison showed a significance level of at least $P<0.01$.

Results

Repeated T_4 administration resulted in 3.7- and 8.5-fold increase in serum T_4 level and 4.2- and 3.4-fold increase in serum T_3 concentration in 3- and 10-day old piglets, respectively (data not shown) which showed the hyperthyroid state. Such hormonal changes were accompanied by the alterations in white blood cell and bone marrow myelocaryocyte metabolism.

TABLE 1
Activities of enzymes of energy metabolism in bone marrow myeloid cells of piglets treated with thyroxine. Values are means ± S.E.M. of five piglets in each group

Enzyme	3-day piglets control	3-day piglets T_4	5-day piglets control	5-day piglets T_4	10-day piglets control	10-day piglets T_4
Hexokinase (nmol NADP· min^{-1}· mg protein^{-1})	20.50±1.16	23.44±1.48	19.78±1.05	12.49±1.40	16.50±1.20	11.39±0.42
Phospho-fructokinase (nmol NADH· min^{-1} · mg protein^{-1})	93.2±12.3	376.2±22.3*	128.4±17.2	151.5±12.0	154.4±21.7	180.8±14.7
Pyruvate kinase (nmol NADH· min^{-1} · mg protein^{-1})	65.4±5.0	62.6±5.8	48.2±4.6	67.20±7.32	39.8±2.4	45.37±5.74
Lactate Dehydrogenase (nmol NADH· min^{-1} · mg protein^{-1})	157.0±8.0	184.5±7.8*	82.8±2.8	125.3±5.9*	78.5±6.8	76.3±7.1
Isocitrate Dehydrogenase (nmol NADP· min^{-1} · mg protein^{-1})	4.60±0.18	3.87±0.11	4.46±0.27	5.67±0.42	4.78±0.21	8.56±0.79*
Cytochrome						
c-oxidase (nmol· min^{-1} · mg protein^{-1})	2.94±0.15	12.56±0.75*	3.21±0.25	7.39±0.87*	3.58±0.32	8.03±0.79*
Glucose-6- phosphate-dehydrogenase (nmol NADP· min^{-1} · mg protein^{-1})	46.6±4.2	28.4±2.5*	45.3±6.0	23.0±1.5*	64.9±4.6	31.5±3.1*

*$P < 0.05$ compared with the control animals
T_4 – group of thyroxine injected animals

Tab. 1 shows the stability in the rates of hexokinase and pyruvate kinase reactions in myeloid cells of 3- and 10-day old animals treated by thyroxine. In spite of this, myelocaryocytes of 3-day old piglets exerted the 4-fold increase in phosphofructokinase activity. These data may suggest the intense formation of glucose-6-phosphate (precursor of phosphofructokinase substrate) in phosphoglucomutase reaction due to the enhancement of glycogenolysis in the cells under the influence of thyroxine (Fowden and Silvel 1995).

The glycolytic enzymes in circulating granulocytes also demonstrated significant increase in catalytic activity after hormone administration (Tab. 2). In particular, 2-fold stimulation of hexokinase activity within 24 hours of T_4 treatment was observed in these cells. This was presumably conditioned by significant increase of glucose concentration in white blood cells after hormone treatment (Tab. 3). The reaction rate remained increased during the further period of investigation. Phosphofructokinase and pyruvate kinase activities in blood neutrophils were similarly stimulated in hyperthyroid conditions (Tab. 2).

In 3- and 5-day old T_4 treated animals the activity of lactate dehydrogenase in myelocaryocytes was increased (Tab. 1), while such hormonal effect was diminished in the cells of 10-day piglets. Simultaneously, the significant stimulatory influence of T_4 on lactate dehydrogenase activity was observed in blood neutrophils. Tab. 2 shows more then 4-fold increase in lactate dehydrogenase reaction rate in polymorphonuclear granulocytes of 3- and 5-day old animals.

At the same time significant decrease in lactate concentration was found in both leukocytes and myelocaryocytes of 10-day old T_4 treated pigs simultaneously with the increase in pyruvate level in the cells of 5- and 10-day old animals (Tab. 3). Thus, in view of the data represented in Tab. 4, it can be suggested that T_4 provides enhancement of pyruvate formation in lactate dehydrogenase reaction (Everse and Kaplan 1975) by means of increase in H_3M_1 and H_2M_2

TABLE 2
Activities of enzymes of energy metabolism in neutrophilic polymorphonuclear leukocytes of piglets treated with thyroxine. Values are means ± S.E.M. of five piglets in each group.

Enzyme	3-day piglets control	T_4	5-day piglets control	T_4	10-day piglets control	T_4
Hexokinase (nmol NADP· min^{-1}· mg protein^{-1})	3.53±0.41	7.15 ±0.59*	5.70 ±0.51	17.89±1.35*	7.74 ±0.41	7.98 ±0.53
Phospho-fructokinase (nmol NADH· min^{-1} · mg protein^{-1})	89.9 ±4.5	117.4 ±7.9*	112.3 ±8.0	396.3±31.7*	133.2 ±5.2	170.2 ±9.5*
Pyruvate kinase (nmol NADH· min^{-1} · mg protein^{-1})	30.5±1.60	126.8 ±5.4*	23.8 ±0.95	59.3 ±6.4*	25.4 ±1.3	64.02 ±3.53*
Lactate Dehydrogenase (nmol NADH· min^{-1} · mg protein^{-1})	37.6 ±2.1	175.3 ±7.9*	54.1 ±2.3	241.8 ±9.5*	166.5 ±9.9	135.2 ±9.2
Isocitrate Dehydrogenase (nmol NADP· min^{-1} · mg protein^{-1})	2.91±0.21	4.83 ±0.37*	4.58 ±0.53	6.03 ±0.45	3.80 ±0.46	4.52 ±0.25
Cytochrome c-oxidase (nmol· min^{-1}· mg protein^{-1})	3.28±0.14	3.76 ±0.40	4.04 ±0.17	10.84±0.62*	4.35 ±0.51	5.83 ±0.27*
Glucose-6- phosphate-dehydrogenase (nmol NADP· min^{-1} · mg protein^{-1})	9.26±0.35	8.54 ±0.35	8.90 ±0.48	9.08 ±0.63	8.15 ±0.68	7.73 ±0.55

*P < 0.05 compared with the control animals
T_4 – group of thyroxine injected animals

TABLE 3
Levels of glucose[a], lactate[a] and pyruvate[b] in bone marrow myeloid cells and blood neutrophilic polymorphonuclear leukocytes of piglets, treated by thyroxine. Values are means ± S.E.M. of five piglets in each group.

	3-day piglets control	T_4	5-day piglets control	T_4	10-day piglets control	T_4
Bone marrow myeloid cells						
Glucose	1.25±0.07	1.79±0.08	1.37±0.08	1.46±0.09	1.98±0.11	1.60±0.09
Lactate	0.40±0.02	0.34±0.02	0.43±0.03	0.35±0.02	0.53±0.02	0.37±0.01*
Pyruvate	46.8±1.8	57.0±2.5*	50.6±1.7	59.3±2.2*	62.7±2.3	75.2±3.8*
Neutrophilic polymorphonuclear leukocytes						
Glucose	0.85±0.04	1.15±0.09*	0.94±0.05	1.18±0.06*	0.81±0.05	1.03±0.07
Lactate	0.35±0.02	0.20±0.03*	0.22±0.01	0.10±0.03*	0.29±0.02	0.10±0.01*
Pyruvate	37.1±2.2	54.0±3.7*	44.5±4.1	57.8±2.9*	42.3±3.1	61.2±3.5*

Levels are expressed as: [a]1mol ·10^6 cells^{-1}, [b] nmol ·10^6 cells^{-1} measured after 1 h of incubation.
*P < 0.05-0.001 compared with the control animals
T_4 – group of thyroxine injected animals

lactate dehydrogenase isozymes contents. On the other hand, reducing in lactate formation rate may be due to the preferential intensification of NADH utilization by glycerophosphate dehydrogenase which is known to be positively regulated by thyroid hormones (Muller and Seitz 1994).

To estimate the T_4 provoked changes in aerobic reaction rates in bone marrow and blood cells the

TABLE 4
The relative activities of lactate dehydrogenase isoenzymes in bone marrow myeloid cells and blood neutrophilic polymorphonuclear leukocytes of 5-day old piglets. Values are means ± S.E.M. of five animals in each group.

Lactate dehydrogenase isoenzymes	Relative activities of isoenzymes in the cells of 5-day old piglets %) Control	T_4
Bone marrow myeloid cells		
H_4	54.5±2.9	47.2±3.8
H_3M_1	20.1±1.3	26.0±1.7*
H_2M_2	11.5±0.7	15.3±1.1*
H_1M_3	8.1±0.6	6.5±0.4
M_4	5.8±0.3	5.0±0.3
Neutrophilic polymorphonuclear leukocytes		
H_4	23.6±2.0	22.0±1.6
H_3M_1	8.8±0.5	12.2±0.7*
H_2M_2	20.4±1.2	23.8±1.5
H_1M_3	34.0±2.3	32.0±2.5
M_4	12.2±0.9	13.0±1.1

*$P < 0.05$ compared with the control animals
T_4 – group of thyroxine injected animals

analysis of alterations in NADP-dependent isocitrate dehydrogenase activity has been performed. The obtained data suggested the stimulation of enzyme catalytic activity in neutrophils during the initial period of experiment, while the stimulatory effect of T_4 on the reaction rate in bone marrow myelocaryocytes was seen in 10-day old piglets (Tab. 1 and 2).

The results suggest that catalytic activity of cytochrome C-oxidase in investigated cell populations is also susceptible to the influence of T_4. Thus, significant increase in enzyme reaction rate was established in myelocaryocytes within 48 hours after the first injection of hormone (Tab. 1). The stimulation of cytochrome C-oxidase reaction was observed in myeloid cells of 5- and 10-day old animals as well. However, the activity of cytochrome C-oxidase in blood granulocytes demonstrated less sensitivity to hormone influence and reached increased levels in the cells of 5-and 10-day old piglets (Tab. 2).

In order to estimate the changes in intensity of hexose monophosphate shunt reactions under T_4 action we investigated the functional activity of glucose-6-phosphate dehydrogenase in bone marrow myeloid cells and blood leukocytes. During the period of thyroxine treatment significant inhibition of enzyme catalytic action was established in myelocaryocytes, while no detectable effect on basal glucose-6-phosphate dehydrogenase activity was observed in blood neutrophils of hormone injected animals (Tab. 1, 2).

Discussion

Thyroid hormones are well known important metabolic regulators in many cell types, including erythroid cells and the cells of lymphopoietic system, where iodothyronines are involved in the regulation of both energy processes and synthesis of organic compounds, and participate in the regulatory mechanisms of cell proliferation and differentiation (De Groot et al. 1989; Harper et al. 1993; Klaushofer et al. 1995; Bernal and Nunez 1995; El Hadri et al. 1996; Perrin et al. 1997). The results of present investigations provided the evidence that the energy metabolism also in the cells of myeloid lineage of mammalian bone marrow and blood is influenced by thyroxine. As found, significant changes in the activity of enzymes of energy metabolism occurred simultaneously with the alterations in glucose, lactate and pyruvate contents and took place in bone marrow myelocaryocytes and blood neutrophilic polymorphonuclear leukocytes of neonatal pig after repeated injections of T_4 (4 mg/kg). In previous studies we have established that the treatment with above mentioned dose of T_4 provides the development of hyperthyroid state in animal organism and causes significant changes in erythroid cell metabolism in both adult rats and neonatal pigs (Sukhomlinov et al. 1986; Antonyak 1999).

It was found that the increase of glycogenolysis in the cells of several tissues plays important role in the mechanism of T_4 action on the metabolism in animal organism (Fowden and Silvel 1995). On the one hand, the enhancement of this process causes the increase in plasma glucose concentration, and respectively, activation of the mechanism that provides intensification of carbohydrate transmembrane transport into the myeloid cell. On the other hand, such effect is associated with the enhancement of intracellular glycogen utilization in myelocaryocytes and blood granulocytes. The high activity of phosphofructokinase as observed in the cells of myeloid lineage after T_4 treatment suggests that glycogen

seems to be the main energy substrate in both bone marrow myeloid cells and polymorphonuclear granulocytes in the conditions of hyperthyroidism.

However, the data shows different responses of myelocaryocyte enzyme systems to the influence of T_4 as compared to mature neutrophilic leukocyte enzymes. In our experiment T_4 administration resulted in preferential stimulation of oxidative stages of carbohydrate catabolism in neonatal pig myelocaryocytes, while the activity of glycolytic enzymes in these cells was less effected. Such effect seems to be similar to thyroid hormone action on the metabolism in several other tissues, where iodothyronines stimulate the processes of oxidative phosphorylation (Nelson 1990). On the contrary, oxidative stages of energy metabolism in piglet blood neutrophils are less sensitive to the influence of T_4 in comparison to that in bone marrow cells. In particular, the activity of cytochrome C-oxidase increased significantly in leukocytes of 5- and 10-day old T_4 treated piglets, while significant increase in activity of myelocaryocytes enzyme was observed within 48 hours after the first injection of hormone. This may suggest that bone marrow precursors are the primary target for T_4 action, while mature leukocytes with increased enzyme activity appear in circulation at later stages of experiment. At the same time the glycolytic enzymes in polymorphonuclear granulocytes demonstrated higher sensitivity to thyroid hormone as compared to the catalysts of oxidative reactions.

Significant activation of glycolytic enzymes observed in polymorphonuclear neutrophils after T_4 injections suggests the participation of iodothyronines in the regulation of leukocyte activity, since glycolysis in these cells represents the main source of ATP for energy support of chemotaxis and phagocytosis (Strauss and Mauer 1978).

On the contrary to the activation of glycolytic enzymes, the activity of glucose-6-phosphate dehydrogenase in blood neutrophils did not change significantly in the cells of T_4 treated pigs. However, the marked decrease in the enzyme activity was observed in bone marrow myelocaryocytes under the influence of T_4. In view of these data the intensity of pentose phosphate pathway seems to be inhibited in bone marrow myelocaryocytes of T_4 treated animals. It can be suggested that such effect leads to limited formation of ribose-5-phosphate – a product of this pathway, and, respectively, to the inhibition of nucleotide synthesis in myelocaryocytes. In particular, impairment in proliferative activity of myeloid cells of T_4 treated piglets was found in previous studies (Babych et al. 1998; Antonyak 1999).

It is necessary to note that the most visible effects of T_4 action in the investigated cells were observed during the 3rd and especially 5th day of animal life, while the influence of hormone seemed to be diminished in the cells of 10-day piglets. This may be due to the involvement of iodotyronines in the regulation of enzyme molecules synthesis. The regulatory influence of hormone consisted in the stimulation of this process during the first days of experiment followed by inhibition of enzyme activities with rising concentrations of products of reactions. On the other hand, the increase in several reaction rates in granulocytes of 5-day old animals could be explained by the appearance of young cells that are more responsive to hormone action, in animal blood.

Taken together, the obtained data provide evidence of thyroid hormone involvement in the regulation of energy metabolism in bone marrow myeloid cells and blood polymorphonuclear leukocytes. The presented results suggest that the cells of myeloid lineage are highly sensitive to thyroxine influence in the neonatal period of animal development. As regards the mechanism of thyroxine participation in the regulation of haemopoiesis, it should be noted the functioning of thyroxine to triiodothyronine conversion system in the pig bone marrow (Antonyak et al. 1997; Babych et al. 1998, 1999). Beside that, the influence of thyroxine on both glucocorticosteroid and insulin secretion can play significant role in the metabolic effects of this iodothyronine in the cells of haematopoietic tissue and blood in the conditions of hyperthyroidism (Lenzen and Bailey 1984).

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NEW BOOKS

NEUROSTEROIDS: A NEW REGULATORY FUNCTION IN THE NERVOUS SYSTERM

EDITED BY ETIENNE-EMILE BAULIEU, PAUL ROBEL, MICHAEL SCHUMACHER
(COLLEGE DE FRANCE, PARIS)

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"Steroids are remarkable molecules: basically they look almost alike, being derivatives of cholesterol, but the few slight chemical differences suffice to give them the extraordinary diverse biological specificities that are important in animal physiology and medical therapeutics" – these are words of the Editors of this comprehensive and delighting monograph.

It has been known for a long time that the brain is a target organ for peripheral steroid hormones. However, in 1981 E.E. Baulieu proposed a new term "neurosteroid" which applies to such steroids which accumulate in the central and peripheral nervous system independently of the supply of peripheral endocrine glands and which can be synthesized de novo in the nervous system.

This monographs brings up to date comprehensive review on the present state of art in this new and rapidly developing field. Twenty chapters written by selected experts and carefully edited cover most of major fields of actual interest from molecular biology, biosynthesis and metabolism, mechanisms of receptor transmission and interactions with the receptors of several other neurotransmitters. The distribution of neurosteroidogenic enzymes in the central and peripheral nervous system suggests that neurosteroidogensis appears to be developmentally regulated and that the initial steps of biosynthesis are common to all steroidogenic structures. Special chapters are dealing with cytochrome P450 in CNS, with the key role of steroidogenic factor 1 in adrenal and gonadal development and in endocrine function. Of special interest are several chapters on the distribution and function of individual steroid receptors in CNS, on the neurosteroid binding sites and modulations of the action of benzodiazepine, GABA-ergic, acetylcholine, glutamate and opioid receptors by neurosteroids including either their potentiation or inhibition. Similar modulatory effects of neurosteroids were found also on neuronal voltage-gated calcium channels. From these basal findings further generate the studies on the role of neurosteroids in brain functions starting with their effects on the synaptic plasticity in the brain which is closely related to the presence and distribution of steroid receptors in the brain. Finaly, there are several observations on the effects of steroids on the developing brain, on their memory-enhancing effects and even on several pathways to the future perspectives of promising psychopharmacological profile of neurosteroids and their analogues including the considerations on their membrane and genomic effects.

This monograph will be of great to endocrinologists, biochemists, pharmacologists, neurologists, psychiatrists and all those dealing with any functions of nervous system and its integrative role.

Pavel Langer