Changes in Intra- and Extracellular Ca^{2+} Concentration and Prostaglandin E_2 Synthesis in Osteoblasts of the Femoral Bone in Experimental Hyper- and Hypothyroidism

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Subclinical form of hypothyroidism was not associated with considerable changes in Ca^{2+} content in osteoblasts and blood plasma and in the content of ATP and prostaglandin E_2. Activation of prostaglandin E_2 synthesis in response to binding of extracellular Ca^{2+} in osteoblasts in the absence of ATP was less pronounced (by 11%) compared to the control. Progression of hypothyroidism and development of clinical signs of the disease were accompanied by a decrease in Ca^{2+} content in osteoblasts and plasma by 45 and 12%, respectively, and ATP content in osteoblasts by 30%, and by activation of prostaglandin E_2 synthesis by 117%. Moreover, the synthesis of prostaglandins in response to binding of extra- and intracellular Ca^{2+} also considerably changed. Hyperthyroidism (2 months) was characterized by a moderate decrease in plasma content of Ca^{2+} by 15% and ATP by 25%, together with an increase in prostaglandin E_2 level by 55.5%. The release of prostaglandin E_2 in response to chelation of extracellular Ca^{2+} increased even more markedly, but somewhat decreased in response to addition of 5 mM ATP due to compensation of metabolic acidosis.

Key Words: *hyperthyroidism; hypothyroidism; osteoblasts; prostaglandin E_2; extracellular and intracellular Ca^{2+}*

Optimum homeostasis of minerals and mechanical integrity are maintained in the bone tissue [3]. However, the mechanisms of cell response and signal pathways responsible for bone endurance and remodeling are not quite clear. It was demonstrated that rapid flows pass through the interstitial space of the bone with a shear stress ~8-10 dyn/cm^2 along the cortical axis [9]. It is assumed that homeostasis of intracellular fluid mediates both local and systemic homeostasis by stimulating autoparacrine factors maintaining the dynamics balance between osteoblast (OB) and osteoclast activities. Imbalance between bone resorption and formation induced by various factors can led to a decrease in bone mass and to the development of osteoporosis [2,7]. An important risk factor of osteoporosis is endocrine pathology: hyperparathyroidism, Recklinghausen disease, pseudohypoparathyroidism, inherited and acquired hypoparathyroidism, thyrotoxicosis, hypothyroidism, hypogonadism, hyperprolactinemic syndrome, Itsenko—Cushing syndrome and disease, acromegaly, hypopituitary, type 1 diabetes mellitus, Addison disease, pheochromocytoma, *etc.* [8].

Here we studied changes in Ca^{2+} homeostasis, state of the energy supply system, and release of prostaglandin E_2 (PGE_2) in OB of the femoral bone during experimental hypo- and hyperthyroid states.

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MATERIALS AND METHODS

The experiments were carried out on 32 male and female albino rats maintained in a vivarium under standard conditions. The animals were randomized into control and experimental groups (8 rats per group). Experimental groups comprised rats with 10-14-day-long and 3-month-long hypothyroidism and L-thyroxin-induced toxicosis. Hyperthyroidism was reproduced by daily intraperitoneal injections of 300 μg/kg thyroxin (T_4) dissolved in 100 μl 0.5 M NaCl. Hypothyroidism was induced by long-term administration of 0.06% propylthiouracil with drinking water.

After euthanasia, the femoral bone was isolated and crushed to 1-3 mm^2 fragments in RPMI-1640. Bone fragments were placed in a round-bottom 15-ml tube and precipitated at 0-4°C in RPMI-1640. OB were perfused with a medium M199 (Sigma) supplemented with 10% FCS (Hyclone Laboratories), 1% penicillin/streptomycin (Sigma) and 1% glutamine (Sigma). The cells were resuspended and transferred into cultural flasks (5 ml per flask). The medium was replaced after 24 h.

The number of viable cells evaluated by trypan blue exclusion test was >98%. The content of free intracellular Ca^{2+} was measured using Fura-2AM fluorescent probe (Calbiochem). For this analysis, the cell monolayer was treated with trypsin and cell suspension (5×10^4/ml) was transferred into wells of a 96-well plate. The content of intracellular ATP was determined using the luciferin-luciferase method (5-2500 nM linearity range at absorption wavelength of 259 nm and extinction coefficient of 15,400). PGE_2 was assayed using commercial RIA kit in a medium containing 100 mM NaCl, 10 mM NaH_2PO_4, 0.5 mM EDTA, and 0.15% BSA. Rabbit polyclonal antibodies to PGE_2 were purchased from Chemicon (Temecula). The reaction was stopped by adding 4 mM EDTA, total protein in OB was measured by the method of Lowry (Sigma, kit N5656).

The data were processed statistically using Student *t* test.

RESULTS

Treatment with T_4 and propylthiouracil led to gradual body weight loss, which was most pronounced in 3-month-long hypothyroidism (Table 1). Mean blood pressure, pulse pressure, HR, weight of the kidneys and heart ventricles, and levels of triiodothyronine (T_3) and T_4 increased in hyperthyroidism and decreased in hypothyroidism, which attests to the development of clinically manifest forms of hyper- and hypothyroidism after 2 and 3 months and less pronounced (subclinical) forms in 14-day-long hypothyroidism (Table 1).

Subclinical form of hypothyroidism was not associated with considerable changes in Ca^{2+}-content in OB and blood plasma and in the content of ATP and PGE_2 (Table 2); activation of prostaglandin E_2 synthesis in response to binding of extracellular Ca^{2+} in osteoblasts in the absence of ATP was less pronounced (11%) compared to the control, while in response to binding of intracellular Ca^{2+} in OB it was inhibited to a greater extent than in the control (Table 3). In normal OB, binding of extracellular Ca^{2+} after addition of 2 mM EGTA into culture medium induced considerable ATP-independent release of PGE_2 (Table 3). It can be hypothesized that disturbed response of the PG system to changes in extra- and intracellular Ca^{2+}

TABLE 1. Changes in Body Weight, Weights of Heart Ventricles and Thyroid Gland, Blood Pressure, HR, and Levels of T_3 and T_4 in Animals with Experimental Hypothyroidism and L-Thyroxin Toxicosis (*M*±*m*)

Parameter	Control	L-thyroxin toxicosis	Hypothyroidism	
			14 days	3 months
Body weight, g	423±13	334±12**	292±12***++	212±16***++o
Weight of heart ventricles, mg	945±21	1149±34**	789±30**+++	603±21***++o
Weight of kidneys, mg	1224±43	1685±45*	1006±23**++	856±32***++o
Weight of thyroid gland, mg	35.0±2.8	24.5±1.8**	98±12***+++	113±14***+++
Mean blood pressure, mm Hg	121±5	152±17*	113±4+	107±6+
HR, bpm	356±16	423±23*	392±10+	312±12+o
T_3, ng/dl	52±4	290±34***	23±3***+++	6±1***+++oo
T_4, μg/dl	5.6±0.6	40±4***	3.8±0.2**+++	0.8±0.2***+++oo

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control; +p<0.05, ++p<0.01, +++p<0.001 compared to hyperthyroidism; op<0.05, oo$p$<0.01 compared to 14-day hypothyroidism.

TABLE 2. Levels of PGE_2 and Ca^{2+} in OB of Animals with Experimental Hypothyroidism and L-Thyroxin Toxicosis of Different Duration (*M*±*m*)

Group	Ca^{2+}, mmol/liter		ATP, μmol/mg protein	PGE_2, ng/mg protein/h
	extracellular	plasma		
Control	60±6	2.90±0.14	8.0±0.4	1.8±0.2
Hypothyroidism, 14 days	54±6	2.72±0.13	7.4±0.2	2.4±0.2
Hypothyroidism, 3 months	33±4	2.56±0.10	5.6±0.2	3.9±0.2
Hyperthyroidism, 2 months	66±4	2.45±0.09	6.0±0.2	2.85±0.18

TABLE 3. Release of PGE_2 in Response to Binding of Extra- and Intracellular Ca^{2+} in Hypo- and Hyperthyroidism (*M*±*m*)

Parameter	Percent of inhibition/activation of PGE_2 synthesis after 6 h			
	control	hypothyroidism, 14 days	hypothyroidism, 3 months	hyperthyroidism, 2 months
Binding of extracellular Ca^{2+}				
without ATP	+89±6	+78±4	+76±4	+53±4
with ATP	+84±4	+86±8	+72±5	+70±6
Binding of intracellular Ca^{2+}				
without ATP	-63±4	-86±7	-86±10	-56±4
with ATP	-60±4	-75±6	-67±9	-65±5

levels is an early risk factor of pathological changes in the bone tissue induced by thyroid gland pathologies.

PGE_2 is an integral messenger transducing mechanochemical signals and a regulator of bone formation *in vivo* and *in vitro* [5]. Changes in intracellular Ca^{2+} concentration in OB are determined by the level of parathyroid hormone, vitamin D metabolites, PGE_2, and stimulation of endothelin-1 [4]. Ca^{2+} can be released from intracellular stores or from extracellular space. We showed that both Ca^{2+} pools are necessary for the maintenance of normal production of PGE_2. It can be hypothesized that Quin 2 AM also chelates extracellular Ca^{2+} distributed in OB. Chelation of extra- and intracellular Ca^{2+} pools considerably suppressed PGE_2 release.

Progression of hypothyroidism and appearance of clinical signs of the disease (increase in the weight of the thyroid gland by 323%, decrease in T_3 and T_4 content by 8.7 and 7 times, body weight loss by 50%, and decrease in the weight of heart ventricles and kidneys by 36 and 30%, respectively) were accompanied by a decrease in Ca^{2+} concentration in OB and blood plasma by 45 and 12%, ATP content in OB by 30%, and activation of PGE_2 synthesis by 117% (Table 2). Moreover, synthesis of PG in response to binding of extra- and intracellular Ca^{2+} also considerably changed (Table 3).

Hyperthyroidism of the same duration also led to body weight loss and decrease in the weight of the kidneys (less pronounced than in hypothyroidism); the weight of heart ventricles increased and the weight of the thyroid gland decreased by 30%, T_3 and T_4 concentrations increased by 5.5. and 7 times, respectively. Under these conditions, Ca^{2+} concentration in the plasma decreased by 15% and ATP concentration by 25%, while PGE_2 level increased by 55.5% (Table 2). It should be emphasized that under conditions of severe hyperthyroidism, the release of PG in response to chelation of extracellular Ca^{2+} considerably decreased, but to a certain extent recovered in response to addition of 5 mM ATP to the medium (correction of metabolic acidosis).

Progression of chronic metabolic acidosis and energy deficiency in the bone tissue in hypothyroidism and L-thyroxin toxicosis probably leads to enhanced urinary excretion of Ca^{2+} and stimulation of Ca^{2+} absorption in the interstitium resulting in Ca^{2+} loss in the bone tissue and OB. Cell signaling mechanisms responsible for the maintenance of Ca^{2+} homeostasis under conditions of metabolic acidosis remain little studied [1]. However, we can conclude that modulation of PGE_2 level is one of these adaptation responses. Ca^{2+} level in OB can be a co-factor of activation of phospholipase A_2 and diacylglycerol lipase [7,8].

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