EXPERIMENTAL BIOLOGY

Thyrocyte Calcium Response to Changes in Thyroid Function

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 125, No. 1, pp. 101-103, January, 1998
Original article submitted October 25, 1996

Calcium regulation in thyrocytes of rats with thyroid hyper- and hypofunction is studied. A single, but not prolonged, administration of thyrotropin increases the cytoplasmic calcium content. A course of triiodothyronine injections increases calcium level and suppresses the thyroid hormone function. Study of *in vitro* calcium response of thyrocytes revealed decreased sensitivity of these cells to carbacholine, thyrotropin, and ATP and decreased functional activity of the gland.

Key Words: *intracellular calcium; thyrocytes; thyrotropin; thyroid; regulation*

Intracellular calcium as a second messenger is involved in transfer of signal from thyrotropic hormone (TTH), growth factors, and other agonists affecting proliferation, differentiation, and functional activity of thyrocytes, to these cells. Some mechanisms of calcium regulation in thyrocytes of intact animals and cell cultures are known in sufficient detail [4,5, 8]. Changes in basal concentration of cytoplasmic calcium ($[Ca^{2+}]_i$) and calcium response of thyrocytes to different inductors during prolonged effects thereof on the gland in vivo are unknown. We investigated calcium regulation in thyrocytes in hyper- and hypofunction of the organ.

MATERIALS AND METHODS

Experiments were carried out on 60 outbred male albino rats. Thyroid hyperfunction was induced by a single or repeated (at 12-h intervals for 5 days) injections of bovine TTH (Sigma) in a dose of 0.5 U/kg [7] (series I and II). Hypofunction was induced by subcutaneous injections of triiodothyronine (T_3, Reanal) in a daily dose of 10 μg/kg for 7 days (III series). Control rats were injected with the solvent. Animals of the I and II series were sacrificed after 4 h, of the III series 24 h after the last injection. For assessing thyroid function, T_3, thyroxin (T_4), and TTH in the serum were radioimmunoassayed using Orion Diagnostica kits on the day of sacrifice. Basal levels of Ca^{2+} in thyrocytes and its changes induced by carbacholine, ATP, and TTH were measured using Fura 2-AM fluorescent probe (Sigma). For preparing a suspension of isolated cells, fragments of the thyroid gland were incubated at 37°C for 1 h in HEPES buffer [3] with 1 mg/ml collagenase/dispase (Sigma) [2]. The resultant suspension was filtered and washed from enzymes by centrifugation at 1500g for 10 min. Cell concentration was adjusted to $8\text{-}10\times10^6$/ml and incubated with Fura 2-AM (3 μM) for 30 min at 37°C. Then the probe was removed by centrifugation, and measurements were carried out in a Jobin Yvon spectrofluorimeter as described previously [3].

RESULTS

Basal Ca^{2+} level in control rats was 116±13 nM (Fig. 1). A single injection of TTH induced a secretory

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response of the thyroid gland (Table 1) and a drastic increase in basal $[Ca^{2+}]_i$. Inhibition of TTH secretion and suppressed hormone production, as evidenced by a drop in serum T_4, during the course of T_3 injections also elevated $[Ca^{2+}]_i$ in thyrocytes. Prolonged administration of TTH induced opposite changes: the concentration of free Ca^{2+} in the cytoplasm dropped more than 2-fold in comparison with the control. Different changes in $[Ca^{2+}]_i$ after a single and repeated injection of TTH correlate with gradual decrease in the intensity of thyrocyte proliferation during prolonged stimulation [9]. Increase of $[Ca^{2+}]_i$ in thyrocytes of animals in the first series of experiments is apparently caused by TTH effect on phosphoinositide metabolism, and the effect observed in the second series may be due to increased production of cAMP [4]. Accumulation of cAMP promotes sequestration of Ca^{2+} in intracellular depots and decreases cytosolic Ca^{2+} content [8]. An increase in $[Ca^{2+}]_i$ upon T_3-induced suppression of hormone production can be caused by degenerative changes in the endoplasmatic reticulum and mitochondria in thyrocytes [7] and release of this ion into the cytoplasm.

Study of Ca response of thyrocyte suspension to TTH, carbacholine, and ATP showed a relationship between $[Ca^{2+}]_i$ and the agonist dose. Carbacholine in a dose of 1 μM did not modify the basal level of thyrocyte Ca in intact rats and induced a moderate calcium response in doses 10 and 100 μM (Fig. 2, *a*), which is in line with published reports [4,5]. A similar effect was observed in experiments on animals exposed to long stimulation of the thyroid. No Ca response to carbacholine was observed in rats with hypofunction of the organ (third series). $[Ca^{2+}]_i$ increase in response to all agonists after a single injection of TTH was negligible (data not shown). The effect of ATP was observed at doses of 100 and 500 μM. The sensitivity to agonist is decreased in animals with T_3-induced thyroid hypofunction: increase in $[Ca^{2+}]_i$ is less expressed and observed only at a dose of 500 μM (Fig. 2, *b*). In control animals, calcium

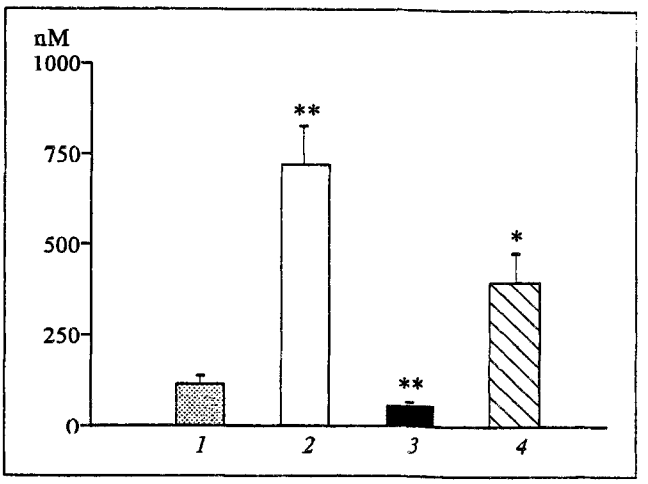

Fig. 1. Basal level of calcium in thyrocytes cytoplasm of rats with different thyroid function. *1*) control (n=11) animals injected isotonic NaCl (control for series injected thyrotropin) and 0.25 M NaOH (control for series injected triiodothyronine), and several intact males; 2) single injection of thyrotropin, 0.5 U/kg (n=7); 3) thyrotropin, 0.5 U/kg at 12-h intervals for 5 days (n=8); 4) triiodothyronine, 10 mg/kg daily for 7 days, n=8. *p<0.05, **p<0.01: significant changes in basal calcium in experimental vs. control animals.

response to TTH in thyrocytes is observed at hormone concentrations of 100 and 500 U/ml. Calcium level did not increase in response to TTH in T_3-treated rats (Fig. 2, *c*). It is noteworthy that a single injection of TTH caused a greater increase in $[Ca^{2+}]_i$ compared with that in vitro. Presumably, the intensity of Ca response to TTH depends on thyrocyte microenvironment and presence of growth factors. The absence of changes in $[Ca^{2+}]_i$ during exposure to agonists in rats with thyroid hypofunction may be due to reduced expression of the receptors on the plasma membrane (the probability of down- regulation has been shown for TTH and α_1-adrenoreceptors of thyrocytes [1,6]).

Thus, calcium level in thyrocytes depends on the receptor system and is determined by the initial status of these cells.

The study was supported by the Russian Foundation for Basic Research (project No. 96-04-48423).

TABLE 1. Changes of Thyroid Status of Rats Injected TTH and T_3 (*M±m*)

Series	Serum concentration on the day of sacrifice		
	T_3, nmole/l	T_4, nmole/l	TTH, U/l
Control (n=24)	1.473±0.152	28.916±3.536	1.639±0.478
TTH, 0.5 U/kg once (n=10)	2.268±0.225**	51.400±6.617**	3.700_0.472*
TTH, 1 U/kg daily for 5 days (n=9)	3.450±0.314**	60.012±7.188**	3.210_0.321*
T_3, 10 mg/kg daily for 7 days (n=12)	1.256н0.433	2.063н0.574***	0.055н0.018**

Note. Controls for TTH and T_3 series are united in one group due to absence of changes in hormone status. *p<0.05, **p<0.01, ***p<0.001 *vs.* the control.

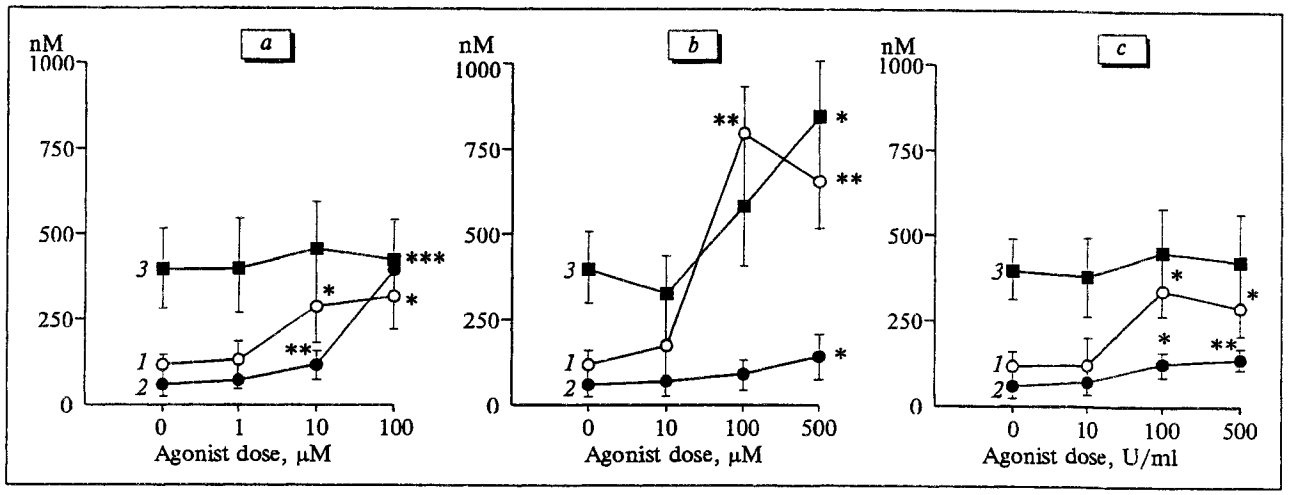

Fig. 2. Calcium response of thyrocytes to carbacholine (*a*), ATP (*b*), and thyrotropin (*c*) in rats with different thyroid status. *1*) thyrocytes of controls; 2) thyrocytes of animals with thyroid hyperfunction (prolonged administration of thyrotropin); *3*) thyrocytes of animals with thyroid hypofunction (triiodothyronine). *$p<0.05$, **$p<0.01$, ***$p<0.001$: significant changes of Ca level under the effect of agonist against relevant basal level.

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