Effect of Thyrotropin on the Activity of Calpains in the Thyroid Gland. The Role of Second Messengers

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Specific features of calpain regulation were studied in rat thyrocytes. Stimulation with physiological concentrations of thyrotropin induced a considerable activation of studied proteinases. Activation developed over a few minutes and lasted for more than 30 min. Studies of the effects of a BAPTA chelator, ionomycin, dibutyryl cAMP, and phorbol ester showed that the process described did not depend on the main systems of second messengers in the thyroid gland.

Key Words: *calpains; thyroid gland; thyrotropin; calcium; secondary messengers; regulation*

Our previous studies showed that single and prolonged stimulation of the thyroid gland with thyrotropic hormone (thyroid-stimulating hormone, TSH) is accompanied by a significant increase in the activity of Ca^{2+}-dependent neutral proteinases (calpains, CP) in several subcellular fractions of the thyrocyte [1]. However, the mechanism of this effect and its significance remain unclear. Here we attempted to study the mechanism of physiological activation of CPs in detail. We used the method for a continuous registration of proteinase activities in live thyrocytes by the hydrolysis of a specific fluorescent substrate that passes through the plasma membrane.

MATERIALS AND METHODS

Experiment were performed on euthyroid male albino rats. A suspension of thyrocytes was obtained by treatment of freshly isolated thyroid glands with collagenase/dispase (Sigma) for 1 h. We used HEPES buffer containing 10 mM HEPES (Sigma), 140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $NaHPO_4$, 5 mM glucose, and 1 mM $CaCl_2$ (pH 7.4). The substrate, N-Succinyl-Leu-Leu-Val-Tyr 7-amido-4-methylcoumarin (Sigma) at a final concentration of 20 μM was added to the cell suspension (2×10^6/ml) in the HEPES buffer to assay the CP activity. An increase in the fluorescence of 7-amino-4-methylcoumarin formed by enzymatic reaction (37°C, λ_{ex}=360 nm, and λ_{em}=440 nm) was registered in an OTD System 3 fluorimeter at 10-min intervals [2,6]. The method specificity was controlled by the synthetic CP inhibitors, N-Acetyl-Leu-Leu-norleucinal (CP-I inhibitor, 10 μM) and N-Acetyl-Leu-Leu-methioninal (CP-II inhibitor, 10 μM) [2]. The medium containing 3 mM BAPTA (Sigma) instead of $CaCl_2$ was used to measure the activity in the absence of extracellular calcium [1].

Basal activity of CPs was measured during the initial 30-min period. The corresponding agonist was then added to the suspension. The affects of bovine TSH, ionomycin, N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (Bu_2cAMP), and tetradecanoylphorbol 12-myristate-13-acetate (PMA, Sigma) were studied.

The concentration of free calcium ions ($[Ca^{2+}]_i$) in the thyrocyte cytosol was measured FURA 2-AM (Calbiochem) [7].

RESULTS

TSH at concentrations of 10 mU/ml and 100 mU/ml (and not 1 mU/ml) induced a long-lasting (more than 30 min) and dose-dependent increase in CP activity,

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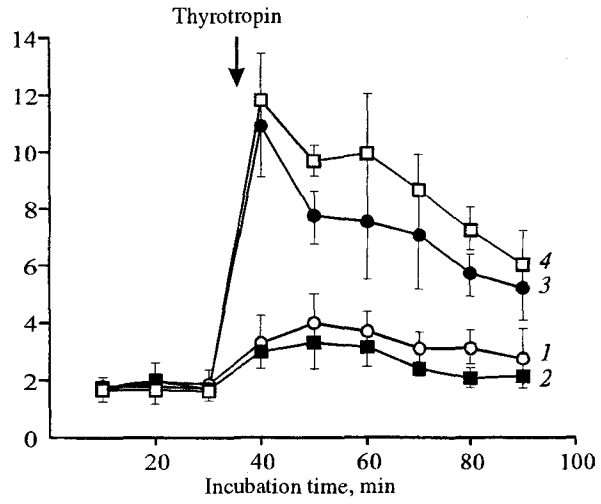

Fig. 1. Time and dose dependences of thyrotropin effects on the activity of calpains in rat thyrocytes. Ordinate: activity of calpains, nM 7-amino-4-methylcoumarin/10^6 cellxmin (here and in Figs 2 and 3). Thyrotropin (10 mU/ml) in 1) medium containing 1 mM Ca^{2+} and 2) Ca^{2+}-free medium.

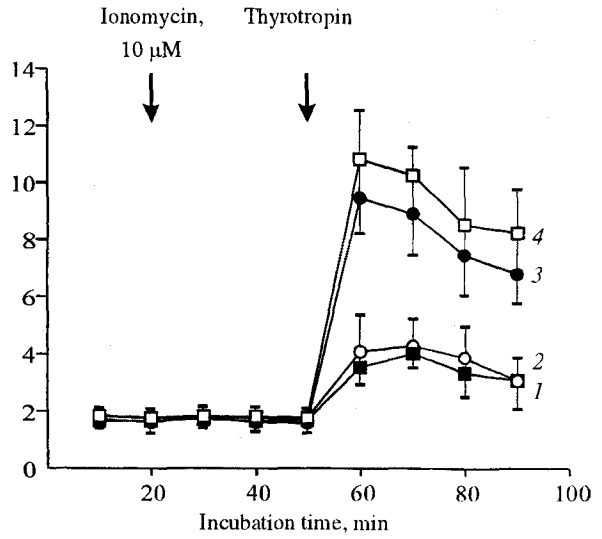

Fig. 2. Activity of calpains in rat thyrocytes under consecutive stimulation with ionomycin and thyrotropin.

which did not depend on the presence of extracellular calcium (Fig. 1). The hormone effect on $[Ca^{2+}]_i$ was relatively insignificant. TSH at the concentration of 100 mU/ml induced $\Delta[Ca^{2+}]_i$ responses of 223.42± 32.18 nM and 90.44±8.15 nM in Ca^{2+}-containing and Ca^{2+}-free media, respectively. TSH applied at concentrations of 10 mU/ml and 1 mU/ml did not lead to any significant increase in Ca^{2+} in the medium. The period of the increase in the CP activity was several times longer then period of the cellular Ca^{2+} response which lasted 4-6 min (in the presence of external Ca^{2+}) or only a few seconds (in the presence of BAPTA). The inhibitors of CP-I and CP-II decreased considerably basal activities of proteinases and blocked TSH effects (Table 1).

The Ca^{2+} ionophore ionomycin (10 μM) [4] induced no changes in CP activity and nearly did not affect the TSH effect (Fig. 2). The hormone effect was not associated with changes in $[Ca^{2+}]_i$. Obviously, Ca^{2+} which is necessary for the proteolytic activity of CPs [8] does not trigger the proteinase activation cascade in thyrocytes. This is in agreement with the results of experiments on purified CPs. The concentration of Ca^{2+} necessary for the activation (more than 2 μM for the CP-I activation *in vitro* [3,8]) is much greater than the content of Ca^{2+} in alive cells.

Bu_2cAMP, which simulates TSH effects mediated through the activation of adenylate cyclase [5,12], as well as PMA (0.01-1 μM), which activates protein kinase C in thyrocytes [10], did not increase the activity of Ca^{2+}-dependent proteinase (independent of the

TABLE 1. Effects of Synthetic Inhibitors on Basal and Stimulated Activities of CPs in Rat Thyrocytes (*M*±*m*)

Index		Without inhibitor	CP-I inhibitor	CP-II inhibitor
		nM 7-amino-4-methylcoumarin/10^6 cellxmin		
Basal activity:				
in the medium with Ca^{2+}		1.86±0.23	0.43±0.12	0.34±0.11
in the Ca^{2+}-free medium		1.91±0.24	0.31±0.10	0.38±0.12
The maximum increase in the activity during stimulation with TSH, mU/ml:				
in the medium with Ca^{2+}	10	2.31±0.45*	—	0.07±0.03
	100	9.30±1.64**	0.09±0.03	0.08±0.04
in the Ca^{2+}-free medium	10	1.87±0.44*	—	—
	100	10.20±1.12**	0.07±0.03	0.09±0.04

Note: *$p<0.05$ and **$p<0.001$, respectively, compared with basal activity. The activity in the presence of the inhibitor was measured after a 30-min incubation with the corresponding compound. Dashes indicate no positive changes in the activity.

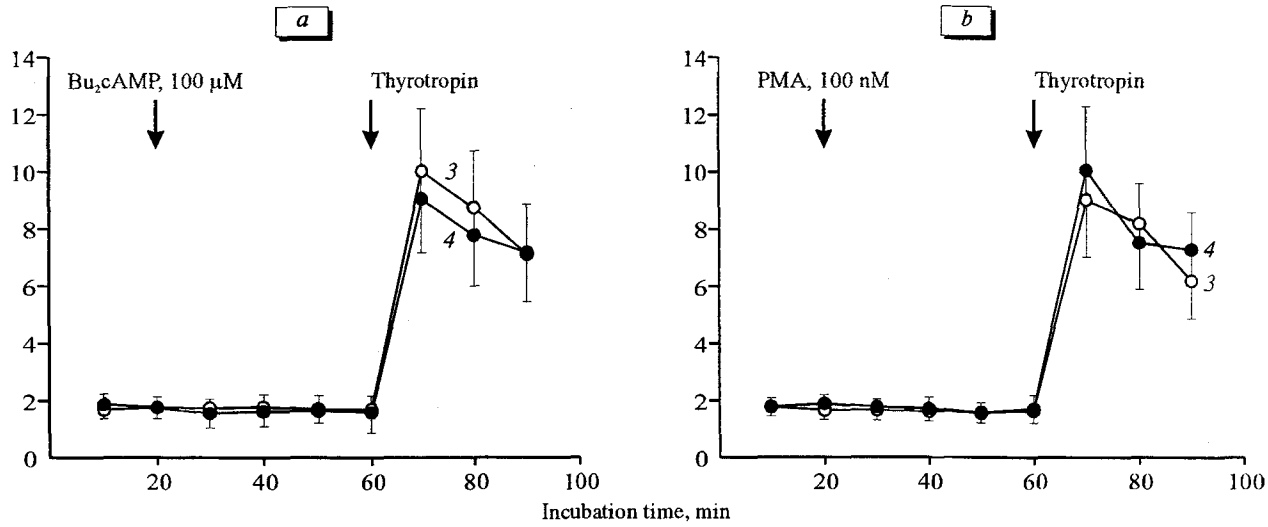

Fig. 3. Effect of thyrotropin on the activity of calpains in the thyroid gland in the presence of *a*) Bu_2cAMP and *b*) PMA.

presence of extrtacellular Ca^{2+}) and did not inhibit the stimulatory action of TSH (Fig. 3).

Thus, TSH induced rapid activation of CPs in thyrocytes which reached the maximum after a few minutes. This effect of TSH and its physiological importance require further investigations. The independence of the TSH effect on second messengers allows us to suggest the presence of a rigid bond between the hormonal receptor and protein kinase. The nature of this bond remains to be studied.

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