Effect of Disaggregation on Calpain Activity in Explants from Rat Thyroid Glands

E. A. Stroev, V. G. Makarova, E. A. Ryazanova, and I. V. Plyakhin

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We studied the regulation of calpains in explants from rat thyroid glands. Collagenase disaggregation decreased proteinase activity in thyrocytes. It was hypothesized that this effect is mediated by heterotrimeric G proteins, protein kinase C, and tyrosine kinase, but not via Ca^{2+} and cAMP-dependent signal pathway.

Key Words: *calpains; thyroid gland; collagenase; second messengers; regulation*

Regulation of cell functions by exogenous factors is mediated by changes in activity of Ca^{2+}-dependent neutral proteinases (calpains). These enzymes produce various effects via activation of key metabolic enzymes or receptors and disaggregation of proteins (including myofibrillar, membrane, and cytoskeletal proteins) [4]. The effects and subcellular localization of these proteinases in cell-matrix contacts [4,6] attests to close interaction between the calpain-calpastatin system and extracellular matrix components. However, calpain activation was established only for post-traumatic platelet aggregation [3].

Here we studied calpain activity (CA) in thyroid gland (TG) explants with impaired cell-cell interactions.

MATERIALS AND METHODS

Experiments were performed on outbred male albino rats. Freshly isolated TG were cut into 20-mg fragments and thoroughly minced (final volume 1 mm^3). The samples were placed in Eppendorf tubes with 4 ml HEPES buffer (pH 7.4) [6] containing various effectors, including dibutyryladenosine-3':5'-monophosphate (Bu_2cAMP), tetradecanoylphorbol-12-myristate-13-acetate, cholera toxin, and genistein (Sigma). BAPTA (1 mM) and BAPTA-AM (0.1 mM, Sigma) replacing $CaCl_2$ were used as chelators of extra- and intracellular Ca^{2+}. For evaluation of reaction specificity N-acetyl-Leu-Leu-norleucinal (20 μM, Sigma), a synthetic calpain I inhibitor, was added to HEPES buffer. After preincubation at 37°C for 10 min, collagenase III (1 mg/ml, Sigma) wad added to test samples and incubated at 37°C for 60 min. The samples were centrifuged at 1500 rpm for 10 min, and the precipitate was resuspended in the same buffer. CA was estimated by hydrolysis of the substrate N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Sigma) [1] on an OTD System 3 spectrofluorometer at excitation and emission wavelengths of 360 and 440 nm, respectively. CA was expressed in nmol 7-amino-4-methylcoumarin/10^6 cells/min after additional treatment with 1 mg/ml collagenase for 1 h and calculation of cells on a hemocytometer.

The results were analyzed by Student's *t* test.

RESULTS

Disaggregation of TG cells was accompanied by a decrease in CA, which did not depend on the duration of collagenase treatment. In samples incubated with collagenase and calpain I and II inhibitors for 20 min CA decreased to 4.48±0.41 and 4.77±0.48 nmol/10^6 cells/min, respectively, and after 60-min incubation CA decreased to 2.79±0.18, and 2.8±0.2 nmol/10^6 cells/min, respectively (*vs.* 6.21±0.81 nmol/10^6 cells/

Department of Biochemistry, I. P. Pavlov Ryazan State Medical University. ***Address for correspondence:*** jabko@rmi.ryazan.su. Ryazanova E. A.

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TABLE 1. Effects of Various Effectors on CA (nmol 7-Amino-4-Methylcoumarin/10^6 cells/min) during Disaggregation of Rat Thyrocytes (*M*±*m*)

Effector	Control (*n*=6-9)	Effector (*n*=4-6)	Effector+collagenase (*n*=4-6)
BAPTA+BAPTA-AM	6.21±0.81	5.74±0.12	4.18±0.24$^+$
Bu_2cAMP	6.21±0.81	6.36±0.48	4.08±0.30$^+$
Cholera toxin	6.12±0.62	9.03±0.90*	10.01±0.59
Tetradecanoylphorbol-12-myristate-13-acetate	5.66±0.11	6.60±0.10*	6.62±0.12
Genistein	7.03±0.57	4.89±0.20*	5.77±0.16

Note. $p<0.01$: *compared to the control; $^+$compared to CA without collagenase.

min in the control). Chelation of extra- and intracellular Ca^{2+} also reduced proteinase activity (Table 1). The specificity of these reactions was confirmed in the presence of calpain inhibitors.

Treatment with 1 mM collagenase in the presence of Bu_2cAMP exhibiting no calpain-regulating effects inhibited CA (Table 1). Cholera toxin (0.1 μM) produced a 47.5% increase in CA, which persisted in the presence of collagenase. The protein kinase C activator tetradecanoylphorbol-12-myristate-13-acetate (1 μM) blocked calpain inhibition during disaggregation of TG cells and slightly increased proteinase activity in explants. The tyrosine kinase inhibitor genistein (50 μM) decreased CA by 30.4% in the absence of collagenase, but slightly increased this activity after disaggregation of thyrocytes.

Our results confirm the existence of a collagen-sensitive mechanism underlying the regulation of CA in TG and not depending on Ca^{2+} (similarly to thyrotropin-dependent activation of calpains) [7]. Experiments with cholera toxin, tetradecanoylphorbol-12-myristate-13-acetate, and genistein suggest that heterotrimeric G proteins, protein kinase C, and tyrosine kinase (but not cAMP) are involved in these mechanisms.

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