Trypsin increases the production of cAMP in isolated bovine thyroid cells

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1. INTRODUCTION

Adenylate cyclase activity of rat liver plasma membranes, which catalyzes cyclic 3′,5′-adenosine monophosphate (cAMP) production, can be stimulated by several proteases with the exception of trypsin [1,2]. A similar increase of cAMP activity after treatment with diverse proteases, was observed [3,4] in membranes from cultured fibroblasts, and in a particulate fraction of rat ovary [6]. Other investigators found a trypsin-sensitive site on the pathway of receptor-mediated inhibition of adenylate cyclase in human platelet membranes [7]. Here, we show that the adenylate cyclase system is activated by trypsin in isolated bovine thyroid cells.

2. MATERIALS AND METHODS

Bovine thyroid cells were isolated by the trypsinization procedure in [8]. A portion of 0.1 ml (3×10^6) cells was incubated in 100 vol. Eagle's medium [9] with 10% calf serum. The incubations were generally done in 25 ml Ehrlenmeyer flasks, in an atmosphere of 95% O_2–5% CO_2 with shaking at 80–100 oscillations/min. The procedure for the determination of [^{131}I]C/M (intracellular to medium) ratios was exactly as in [10]. The production of cAMP in the cells was measured as in [11,12]. The statistical significance was calculated by the Student's *t*-test.

3. RESULTS AND DISCUSSION

When bovine thyroid cells are incubated with TSH, iodide transport to the cells is altered in a biphasic manner [10]. The transport of iodide into cells was measured with ^{131}I as tracer and the [^{131}I]C/M ratio had become stabilized during 30 min incubation. Following the TSH addition to the cells [^{131}I]C/M ratios declined over 30 min. After 1 h incubation the concave form of the curve becomes a convex one and reaches the maximal value of the [^{131}I]C/M ratio after 5–6 h incubation with TSH. In these time intervals the [^{131}I]C/M ratio in cells was determined when TSH was preincubated in Eagle's medium with various concentrations of trypsin for 15 min (Worthington, crystallized), then 0.1 ml fresh cells was added to the medium. Trypsin at 0.05% completely digested TSH in the medium (fig.1) so that the [^{131}I]C/M ratio was abolished in both the 30 and 360 min incubations. Fig.2 demonstrates the effect of TSH and trypsin on cAMP production in cells. When TSH was added to the cells first for 20 min incubation, the cAMP production significantly increased ($p < 0.001$). Following treatment of these cells with 0.06% trypsin during 15 min, the effect of TSH on cAMP was deleted. The subsequent readdition of TSH to the trypsin-treated cells and incubation for 5 min caused a significant rise of the cAMP production in comparison to cells stimulated with TSH, untreated with trypsin ($p < 0.02$). A similar course was found when cells

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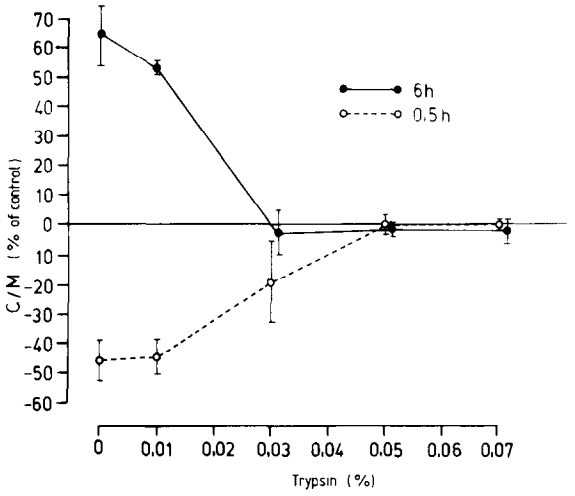

Fig.1. Effect of various concentrations of trypsin added to the incubation medium at the beginning of incubation on [^{131}I]C/M ratios in bovine thyroid cells. TSH (100 munits/ml) was incubated in Eagle's medium containing 1 μmol.l^{-1} ^{131}I with 10% calf serum and trypsin for 15 min. Then 0.1 ml thyroid cells was added to the incubation mixture. The incubation continued and [^{131}I]C/M ratios were determined after 30 and 360 min incubation. Each value is the mean of duplicate results in this experiment ± SEM.

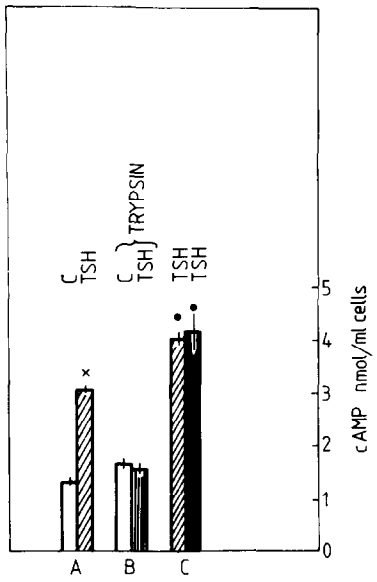

Fig.2. Effect of TSH on cAMP content in thyroid cells: (A) thyroid cells were incubated for 20 min at 37°C in Eagle's medium with 10 mmol.l^{-1} aminophyline and 100 munits TSH/ml; (B) as in (A), but cells were washed and then incubated for 15 min with 0.06% trypsin; (C) same as in (B), but cells were resuspended in fresh medium with 100 munits TSH/ml and incubated for 5 min. Values are means of triplicate determinations ± SEM from 3 expt: (×) $p < 0.001$ (K *vs* TSH); (●) $p < 0.02$ (TSH *vs* C group).

were preincubated with TSH for 2 h, then exposed to trypsin and incubated for 5 min with TSH (fig.3).

In the other series of experiments the bovine thyroid cells were initially preincubated with or without trypsin for 15 min, then divided and incubated for 20 min with or without TSH. Cells treated with trypsin produced significantly higher amounts of cAMP than untreated cells in both control and TSH-stimulated cells (fig.4).

These results show that trypsin increases cAMP production via adenylate cyclase activity not only in human platelet membranes [7] but also in whole cells. The observed effect of trypsin is reversible because readdition of TSH to trypsin-treated cells fails to modify the response in a negative manner. These data support findings [13] that refractoriness in relation to cAMP production is not permanent and development of refractoriness does not depend upon new protein synthesis, since trypsin treatment of cells first preincubated with TSH restored normal responsiveness to TSH readdition.

To the contrary, in rat liver plasma membranes trypsin strongly and irreversibly inhibits both adenylate cyclase activity and cAMP production [2]. It was clearly demonstrated that in platelet membranes the effect of trypsin is selective because the catalytic moiety of adenylate cyclase was unaltered [7]. Recently, such a conclusion was drawn from experiments in which guanylate cyclase activity was stimulated by trypsin and other proteases in purified rat liver plasma membranes, but remained unchanged in soluble enzyme preparations. It was suggested that trypsin in human platelet membranes produced a very localized lesion probably in the GTP-dependent inhibitory pathway of the adenylate cyclase system, since inhibitory pathways were more sensitive to the effects of trypsin than the stimulatory pathway [7]. Our results obtained on bovine thyroid cells support these findings and show that trypsin increases both basal and TSH-stimulated cAMP production although an effect of trypsin on receptor site cannot be excluded. From the results published to now, sensitivity of adenylate cyclase to pro-

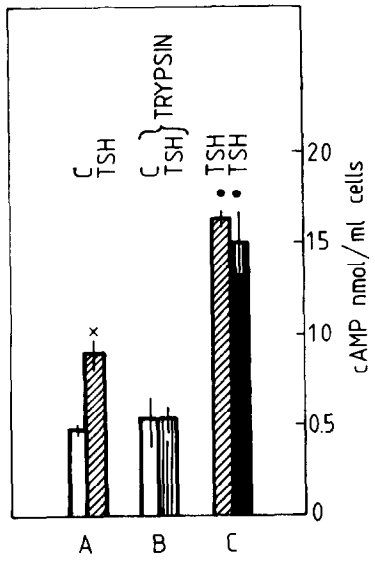

Fig.3. Effect of TSH on cAMP content in thyroid cells: (A) thyroid cells were incubated for 2 h at 37°C in Eagle's medium with 10 mmol.l^{-1} aminophyline and 100 munits TSH/ml media; (B) as in (A), but cells were washed, then incubated for 15 min with 0.06% trypsin; (C) as in (B), but cells were resuspended in fresh medium with 100 munits TSH/ml and incubated for 5 min. Values are means of triplicate determinations ± SEM from 2 expt. (×) $p < 0.01$ (C *vs* TSH) and (TSH in A group *vs* TSH in C group).

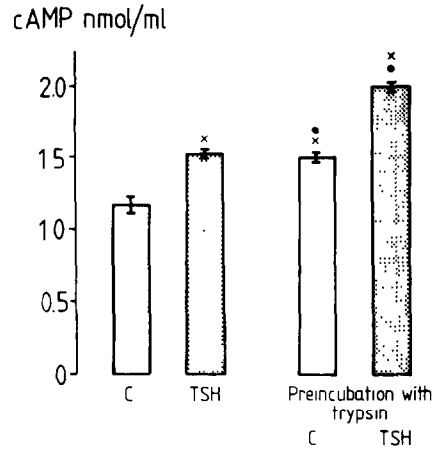

Fig.4. Effect of trypsin and TSH upon cAMP content of bovine thyroid cells. Thyroid cells (0.1 ml) were preincubated with or without 0.06% trypsin for 15 min in Eagle's medium containing 10 mmol.l^{-1} aminophyline, then cells were divided between treated and untreated, and incubated without (C, control) or with 100 munits TSH/ml medium for 20 min at 37°C. Values are means of triplicate determinations ± SEM from 2 expt: (×) $p < 0.02$ (C *vs* TSH); $p < 0.02$ (C *vs* C trypsin, TSH *vs* TSH trypsin).

teolysis may be different according to the tissue chosen for investigation. Whether or not the effect of proteolysis might be important in regulating physiological processes in vivo remains to be elucidated.

ACKNOWLEDGEMENT

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