# REGULATION OF HORMONAL SECRETION AND DNA SYNTHESIS IN LACTOTROPHS OF THE RAT ADENOHYPOPHYSIS IN PRIMARY CELL CULTURE IN VITRO

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Primary monolayer cultures of rat adenohypophyseal cells have found widespread application in the study of regulation of the functions of this important endocrine organ. Cells in culture can not only spontaneously produce trophic hormones [6], but they can also enter the mitotic cycle [1]. Some regulators of hormonal secretion have been shown to affect multiplication of adenohypophyseal cells [2, 4, 7]. However, autoradiographic or biochemical investigations of incorporation of $^3$H-thymidine into DNA of the total cell population, so far undertaken, have not answered the question of the character of changes in DNA synthesis in lactotrophs, somatotrophs, and other cell types composing the adenohypophysis, for primary cultures, like the native organ, contain all representatives of the parenchyma, with the inherent specific features of regulation of their function.

In the investigation described below a combined immunoperoxidase and autoradiographic technique was used for the first time to attempt a quantitative analysis of changes in DNA synthesis in prolactin- (PRL-) secreting cells of primary cultures of the adenohypophysis.

## EXPERIMENTAL METHODS

Experiments were carried out on adult female DFY rats. The procedure of isolation of the cell culture was described previously [5]. The cells were grown in the form of a monolayer in 24-well plastic macropanels (Flow Laboratories, England), containing coverslips, in medium 199 containing 10% fetal calf serum, at 37°C in an atmosphere of air with 5% $CO_2$. Starting with the 4th day of culture, thyrotrophic releasing hormone (TRH), bromocriptine (CB154), or somatostatin (SST) was added to the incubation medium daily for 3 days. Samples of medium were taken 24 h after the last addition of the preparations for subsequent radioimmunoassay of PRL. Control and experimental cultures were treated with $^3$H-thymidine (1 μCi/ml, 19.5 Ci/mmole, Czechoslovakia). After fixation of the cells (picric acid and paraformaldehyde) the immunocytochemical test for PRL was carried out [7], followed by autoradiography of the same preparations [1]. By ordinary and phase-contrast light microscopy the total percentage of labeled cells of the cultures and the percentage of labeled lactotrophs separately, were determined in preparations stained with diaminobenzidine. The present writers have suggested and developed a method of assessing changes in DNA synthesis in lactotrophs by analysis of the distribution of labeled cells of this type among the total population of $^3$H-thymidine-incorporating cells. The method has high sensitivity and can be used to study changes in DNA synthesis of individual trophic cells in mixed primary cell cultures of the adenohypophysis.

## EXPERIMENTAL RESULTS

Table 1 gives data on the effect of TRH (10 ng/ml), CB154 (0.75 μM), and SST (20 ng/ml) on the labeling index of the total pool of cell cultures and on the percentage of labeled

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TABLE 1. Effect of TRH, CB154, and SST on DNA Synthesis in Total Population (A) and in Lactotrophs (B) of Primary Cultures of Rat Adenohypophyseal Cells

| Series of expts. and parameter tested | No. of expt. | Control | TRH | CB154 | CB154 + TRH | SST |
|---|---|---|---|---|---|---|
| A. Percentage of cells with grains of silver above nucleus | 1 | 4,9±0,07 (3) | 8,5±0,24 (3)** | 3,7±0,53 (3) | — | 6,7±0,95 (3) |
| | 2 | 5,3±0,36 (4) | 4,9±0,30 (4) | 5,1±0,56 (4) | 2,4±0,18 (4)** | — |
| | 3 | 7,8±0,35 (3) | 11,9±0,63 (3)** | 5,8±0,57 (3)* | — | 10,2±0.44 (3)* |
| | 4 | 7,4±0,27 (4) | 10,2±0,41 (4)** | 4,5±0,18 (4)** | 5,2±0,43 (4)** | — |
| B. Fraction of labelled lactotrophs (in %) in total cell population incorporating $^3$H-thymidine into DNA | 1 | 5,3±0,33 (3) | 4,8±0,48 (3) | 2,0±0,28 (3)** | — | 2,0±0.38 (3)** |
| | 2 | 4,6±0,49 (4) | 5,0±0,54 (4) | 1,2±0,20 (4)** | 2,0±0,25 (4)**,*** | — |
| | 3 | 5,2±0,50 (3) | 8,1±0,73 (3)* | 1,9±0,57 (3)* | — | 3,2±0.36 (3)* |

Legend. From 250 to 500 cells were counted in each preparation. *p < 0.05, **p < 0.01 compared with control; ***p < 0.05 compared with CB154. Here and in Table 2, number of observations given in parentheses.

TABLE 2. Effect of TRH, CB154, and SST on Prolactin Production (in μg/ml/day) in Cell Cultures of Rat Adenohypophysis

| No. of expt. | Control | TRH | CB154 | CB154 + TRH | SST |
|---|---|---|---|---|---|
| 1 | 3,48±0,22 (4) | 2,80±0,11 (4) | 1,56±0,76 (4)** | — | 1,39±0,72 (4)** |
| 2 | 5,81±0,15 (6) | 4,69±0,27 (6)* | 2,42±0,12 (6)** | 4,33±0,24 (6)**,*** | — |
| 3 | 6,08±0,36 (6) | 3,99±0,24 (6)** | 1,23±0,84 (6)** | — | 3,03±0,15 (6)** |
| 4 | 7,68±0,38 (6) | 7,92±0,17 (6) | 2,34±0,14 (6)** | 4,67±0,22 (6)**,*** | — |

Legend. *p < 0.05, **p < 0.01 compared with control; ***p < 0.01 compared with CB154.

lactotrophs among DNA-synthesizing cells. TRH significantly increased the percentage of labeled cells of the cultures in three of four experiments but had no selective action on DNA synthesis of the lactotrophs, for a small stimulating effect took place in only one of three experiments.

CB154 caused mild inhibition of DNA synthesis in the whole population; a significant action was discovered in two of the four experiments. Meanwhile the dopamine agonist increased by many times the percentage of labeled lactotrophs. Incidentally, TRH weakened the inhibitory action of CB154. SST, which is an inhibitor of secretion not only of STH, but also of PRL and TSH [3, 8], did not reduce the number of DNA-synthesizing cells in the culture. In one experiment, moreover, SST induced cell multiplication. However, the use of an immunocytochemical technique did reveal a selective inhibitory action of SSR on DNA synthesis by lactotrophs. Since the fraction of PRL-secreting cells in the S period of the cycle is very small (Table 1), changes in the lactotrophs induced by SST did not change the general character of cell proliferation in the culture. The results suggest that the same regulator, in particular, SST, may exert opposite effects on different populations of hormone-secreting cells of the adenohypophysis.

Determination of the PRL concentration in the medium (Table 2) during the last 24 h of incubation confirmed the known facts about the inhibitory action of CB154 and SST on the lactotrophic function of the adenohypophysis [8]. By contrast with the short-term effect of TRH, exposure of the cells to the tripeptide for several days did not cause any increase in PRL production. In two of the four experiments TRH actually inhibited hormonal secretion, although the intensity of this effect was much weaker than that due to the action of CB154 or SST. It must be pointed out that TRH weakened the intensity of the inhibitory action of CB154.

The results of these investigations demonstrate clearly that hypothalamic neuropeptides with hypophyseotrophic action and certain other biologically active substances exert control over individual functions of the adenohypophysis not only to changes in the rate of biosynthesis and secretion of trophic hormones, but also by their direct or indirect influences on DNA synthesis and on proliferation of certain types of hormone-secreting cells. Changes

in the numerical composition of the individual cell populations in the adenohypophysis evidently arise after long-term exposure to regulators. For example, as a result of the long-term hormonal restructuring taking place during pregnancy, profound changes in adenohypophyseal function occur with redistribution of individual types of cells.

The methods of quantitative estimation of changes in DNA synthesis in hormone-secreting cells developed by the present authors may find application in the study of the genesis of hormone-secreting tumors of the adenohypophysis and of other glands of internal secretion, in the search for new preparations and in the designing of rational schedules of hormone therapy.

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# ACTIVATION OF CYTOTOXIC FACTOR PRODUCTION OF MOUSE SPLEEN CELLS THROUGH IN-VITRO STIMULATION BY LIPOPOLYSACCHARIDE AND MURAMYL DIPEPTIDE


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Lipopolysaccharides (LPS) and muramyl dipeptide (MDP) are bacterial products which stimulate antitumor immunity in vitro [9, 14] and in vivo [11, 13]. There is evidence of synergism in the action of these substances on activation of the tumoricidal function of macrophages in vitro [9] and on regression of syngeneic tumors in mice [1, 2]. A tumor necrosis factor (TNF), or cytokine, to which an important role in antitumor immunity is ascribed, appears in the serum of animals sensitized by bacteria containing MDP, or by MDP itself [6], in response to injection of LPS.

The aim of this investigation was to determine the conditions of synergic action of LPS and MDP on cytotoxin production by mouse splenocytes in vitro, revealed by lysis of L-929 target cells. This property is known to be a feature of TNF produced mainly by activated macrophages [15] and, to a lesser degree, by natural killer cells [12] and T lymphocytes [7], and also of lymphotoxins (LT), secreted by activated T and B lymphocytes [5]. For convenience these factors will be described by the general name of "cytotoxic factors" (CTF).

## EXPERIMENTAL METHODS

DBA/2 ($H\text{-}2^d$) and C57Bl/6 ($H\text{-}2^b$) mice of both sexes, aged 2-3 months, were used. Spleen cells were suspended ($5.5 \times 10^6$/ml) in medium RPMI-1640 (Flow Laboratories, England), containing


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