EFFECT OF SOME REGULATORS ON RAT ADENOHYPOPHYSEAL CELL FUNCTION IN PRIMARY MONOLAYER CULTURE

V. P. Fedotov, I. S. Komolov, and G. N. Pluzhnikova

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Some features of secretion of labeled somatotrophic hormone (STH) and prolactin in a 5-day monolayer culture of rat adenohypophyses were studied. Hormones were isolated from the culture medium by electrophoresis in polyacrylamide gel. Sodium-dibutyryl cyclic AMP and theophylline stimulated STH and prolactin secretion. Thyrotrophin releasing hormone (TRH) increased the rate of incorporation of L-leucine-^{14}C into the cell proteins, stimulated prolactin secretion, but did not affect STH liberation. Somatostatin completely inhibited theophylline-induced STH secretion but did not affect prolactin secretion. The characteristics of formation of the labeled STH and prolactin pool in the cells and of the secretion of these hormones into the culture medium are discussed.

KEY WORDS: adenohypophyseal cultures; labeled somatotrophic hormone and prolactin; secretion regulators.

Isolation of adenohypophyseal cells and their growth as primary monolayer cultures have played an important role in the study of hypothalamic-hypophyseal regulation. By means of monolayer cultures it is possible to study the mechanism of the direct effect of various compounds on hormone secretion by the cells [12, 13]. The advantage of cell culture over incubation of whole pituitary glands or their slices or primary suspensions is that the quantity of hormones secreted by the culture is much greater. Furthermore, with culture it is possible to study the characteristics of the secretion process over a long period of time [1, 5, 14]. Compared with the incubated pituitary, cells in primary culture are more accessible to regulators or labeled precursors added to the medium, so that the observed effects may be more demonstrative.

In the investigation described below the functional activity of adenohypophyseal cells in primary culture and the character of secretion of hormonal proteins in response to the action of certain stimulators and inhibitors were studied.

EXPERIMENTAL METHOD

The methods of isolation of adenohypophyseal cells from adult rats (150-200 g) and their growth in primary monolayer culture were described previously [1]. Experiments were carried out on 5-day cultures. After a change of medium, L-leucine-^{14}C (Czechoslovakia, specific activity 40 μCi/mmole) was added to the Carrel flasks in which the cells were grown for 24 h. The medium was then removed and the flasks were washed six times with two volumes of Hanks' solution containing excess of unlabeled leucine (50 mg%) in order to remove all the serum and isotope from the medium. The flasks were filled with serum-free medium No. 199 and the stimulator or inhibitor of secretion of the trophic hormones added for 3 h. Theophylline, sodium-dibutyryl cyclic AMP (db-cyclic AMP; from Sigma, USA), thyrotrophin releasing hormone (TRH; from Hoechst, West Germany), and somatostatin (synthesized in the laboratory of protein hormone chemistry, Head Yu. P. Shvachkin) were used in doses of 10 mmoles, 2.5 mmoles, and 1 and 2 μg/ml respectively. The effect of prolonged exposure to TRH on protein synthesis also was studied. Cells were incubated for 24 h with TRH (1 μg/ml), and 1 h before the end of the experiment 0.5 μCi/ml L-leucine-^{14}C was added to the medium. After removal of the excess of isotope the cells were lysed with 0.5 N NaOH and the radioactivity determined. Total proteins were determined by Lowry's method. Secretion of hormones were studied by a method developed

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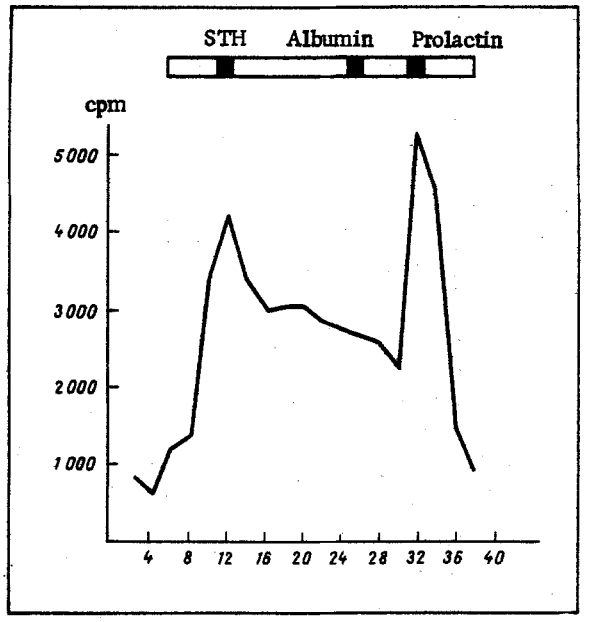

Fig. 1. Distribution of labeled proteins in culture medium along length of electrophoretic columns. Peaks of radioactivity represent STH and prolactin and correspond to position of stained gel strips of these hormones, illustrated schematically at top of figure. Abscissa, length of columns starting from separating gel (in mm); ordinate, incorporation of L-leucine-^{14}C into culture medium proteins, in cpm.

on a model of short-term incubation of adenohypophyses, by electrophoresis in polyacrylamide gel [10]. This method was modified for use with cultures of adenohypophyseal cells. Since the content of labeled hormones in the medium was negligible, in order to detect them by electrophoresis, electrophoresis of the samples was carried out simultaneously with extract of adenohypophyses of intact rats. The latter thus served as "internal marker" of somatotrophic hormone (STH) and prolactin.

To obtain a distribution curve of labeled native proteins along the whole length of the gel, the latter was cut into disks 2 mm thick by means of a semiautomatic instrument consisting of a set of rigidly fixed razor blades. The disks were placed in scintillation jars. Protein was extracted with 0.5 ml 0.3% sodium dodecyl sulfate (SDS) for 24 h at 37°C. Bray's fluid was then poured into the jars and radioactivity determined on an Intertechnique counter. Proteins in some of the gel columns were denatured with 5% TCA for 20 min and the gel was stained with Amido black. Three clearly stained bands were discovered (Fig. 1) and, as was shown previously [3, 9], they represent STH, albumin, and prolactin. The corresponding areas of the gel were cut out, homogenized in 0.5 ml of 0.3% SDS solutions, and their radioactivity determined.

EXPERIMENTAL RESULTS

A characteristic feature of primary adenohypophyseal cultures is that the cells retain their functional activity for a long time (30 days or more) although the relative proportion of the individual "trophs" changes in the course of growth [5]. In the present investigation, experiments were carried out on cultures aged 5-6 days. Observations showed that this is the optimal time for the study of hormonal secretion. By this time the cells have become adapted to the conditions of growth *in vitro*, the colonies of hormone-secreting cells are fairly numerous, and the basal secretion of STH and prolactin can be satisfactorily determined by the methods indicated above.

To investigate the concentration of STH and prolactin by electrophoresis it was first necessary to prove the hormonal specificity of fractions isolated from extract of intact adenohypophyses. The biological activity of STH was confirmed by the tibia-test on hypophysectomized rats [4], and prolactin activity by a micromethod on the pigeon thymus [8].

TABLE 1. Secretion of Labeled STH and Prolactin (in CPM/ml medium) during Action of db-Cyclic AMP, Theophylline, Somatostatin, and TRH

Treatment	No. of experiment	STH	P	Prolactin	P
Control	1	3700±630		5050±850	<0,01
db-Cyclic AMP		8110±1250	<0,01	12870±1800	
Control	2	1980±180	<0,01	3920±580	<0,01
Theophylline		3610±170		8340±260	
Theophylline plus somatostatin		2030±120	>0,05	7500±1200	<0,05
Control		1960±330		2030±620	
	3		>0,05		<0,05
TRH		2480±340		5520±830	

Analysis of the curve of distribution of labeled proteins along the length of the gel column showed two distinct peaks of radioactivity (Fig. 1). Comparison of the curve with the stained electrophoretic gels showed that these peaks corresponded to the position of STH and prolactin. The presence of peaks of radioactivity in the region of these hormones was explained by the fact that the number of somatotrophs and lactotrophs in the culture was much greater than the number of other types of hormone-secreting cells [5, 7].

The results of the action of various regulators of STH and prolactin secretion are given in Table 1.

They show that db-cyclic AMP, a cyclic AMP analog, and theophylline stimulated the liberation of both STH and prolactin considerably. This is in agreement with data in the literature [13] and confirms once again the role of cyclic nucleotides in the mechanism of secretion of these hormones. A different pattern was found when hypothalamic releasing factors were added to the medium. TRH also stimulated prolactin secretion but did not affect the rate of liberation of growth hormone. Another hypothalamic regulator of pituitary function — somatostatin — completely abolished the stimulating effect of theophylline on STH secretion but did not change the action of theophylline on prolactin secretion.

The results of these experiments are evidence that adenohypophyseal cells *in vitro*, in 5-day cultures, preserve their selective sensitivity to physiological regulating factors.

In another series of experiment the stimulating effect of TRH on total protein synthesis in culture was demonstrated. Preincubation of the cells for 24 h with a large dose of TRH (1 μg/ml) followed by radioactive labeling (for 1 h) with L-leucine-^{14}C led to a twofold increase in the incorporation of this precursor into proteins of the culture. Whereas the radioactivity of the control samples was 2200 ± 140 cpm/mg protein, under the influence of TRH it rose to 4100 ± 450 cpm/mg protein $P < 0.01$). This strong effect during long-term incubation must be explained, on the one hand, by stimulation of STH and prolactin synthesis de novo [6], and on the other hand, by activation of proliferation processes in culture and a corresponding increase in the weight of total protein [2].

The experiments with L-leucine-^{14}C showed that in all cases the specific radioactivity of prolactin in the medium (cpm/mg hormone) was higher than that of STH. In addition, the secretory response of the lactotrophs to the action of the stimulators was more marked than that of the somatotrophs (Table 1). These results can evidently be explained by the relative proportions of the two types of cells in the culture and also by the characteristics of synthesis and secretion of these hormones. Experiments on incubated rat pituitary glands have shown that the STH content is higher than the prolactin content in the gland but lower in the medium [10]. Only 19-23% of the intracellular STH is liberated into the medium. Meanwhile, practically all the synthesized prolactin is secreted fairly quickly [10, 11]. It is also known that lactotrophs predominate in monolayer cultures of adenohypophyses, especially of female rats, and are constantly found during long periods of culture, whereas the number of somatotrophs decreases gradually [5]. This may evidently explain the higher peak of radioactivity in the region of prolactin than of STH (Fig. 1).

Adenohypophyseal cells in culture under the experimental conditions used thus not only maintain the basal secretion of STH and prolactin, but also preserve their selective sensitivity to different stimulators and inhibitors of pituitary functions.

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MIGRATION OF HEMATOPOIETIC STEM CELLS AFTER BURNS

G. I. Bezin, B. B. Moroz, O. O. Romashko, and R. V. Petrov*

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Experiments on (CBA × C57BL)F_1 mice showed that during the period of a sharp rise in the blood endogenous glucocorticoid level 30 min-6 h after burns the number of circulating colony-forming units (CFU) falls by 50-60%. At the same time migration of CFU from an area of bone marrow screened during irradiation (850 R) was inhibited. On the 3rd-4th day after burns, migration of CFU was intensified.

KEY WORDS: burns; hematopoietic stem cells; endogenous glucocorticoids; migration of CFU.

Migration and circulation of hematopoietic stem cells *in vivo* are essential conditions for the normal functioning of the hematopoietic and immune systems. The writers showed previously that when the blood endogenous glucocorticoid level is considerably elevated, migration of colony-forming units (CFU) from the bone marrow is inhibited. After burns, periods of a sharp rise in the blood corticosteroid concentration are observed [5]. There is no information in the literature on the state of migration of hematopoietic stem cells after thermal injury. The only data given relate to a fall in the CFU content in the bone marrow of burned mice [3].

The object of the present investigation was to study the migration and circulation of CFU after burns.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 mice were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Third degree burns were inflicted on an epilated area of the animals' back (about 10% of the total body surface) by exposure for 35 sec to an IK-500 electric lamp. The temperature under the skin was 55-60°C. At different times after infliction of the burns the mice were decapitated and the number of CFU in their whole blood determined by the exogenous colony formation method. For this purpose, at each time blood was taken from 5-7 burned donor mice and injected intravenously in a volume of 0.2 ml into lethally irradiated syngeneic recipients. The latter were killed eight days after transplantation and the number

*Corresponding Member of the Academy of Medical Sciences of the USSR.

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