EFFECT OF TACTIVIN ON CORTICOSTERONE PRODUCTION BY A MOUSE ADRENOCORTICAL CELL SUSPENSION

E. V. Ignat'eva, V. M. Chesnokova, and L. N. Ivanova

UDC 612.453.018:577.175.536].014.46:615.362.438.017:615.275.4].085.23

KEY WORDS: tactivin; thymus factors; adrenals

Differentiation and maturation of T lymphocytes take place in the thymus under the influence of its polypeptide hormonal factors. Many such factors have now been isolated from the thymus (families of polypeptides and individual proteins), with varied molecular weight [8]. The thymus hormone thymosin, fraction 5, obtained by Goldstein's method (mol. wt. 1000-15,000) plays an important role in interaction between the immune and endocrine system [9]. This thymic factor has been shown to take part in the formation of the pituitary-adrenal system in mice [6]. The Soviet hormonal preparation tactivin (a family of peptides with mol. wt. of 1500-6000), isolated from the thymus, is now being extensively used in clinical practice [1]. However, there are no data yet available on the action of this factor on the endocrine system. The aim of this investigation was to study the action of tactivin on corticosterone production by a suspension of mouse adrenal cells in vitro. Cells in suspension are extremely sensitive to the action of various kinds of modulators on their function [10].

EXPERIMENTAL METHOD

The cell suspensions was prepared from the adrenals of female BALB/c mice by Sayer's method [11], using trypsin ("Spofa," Czechoslovakia) and soy trypsin inhibitor ("Reanal," Hungary). The cells were incubated for 1.5 h at 37°C in Krebs–Ringer medium with trypsin inhibitor and bovine serum albumin (0.5%), with the addition either of tactivin alone, in

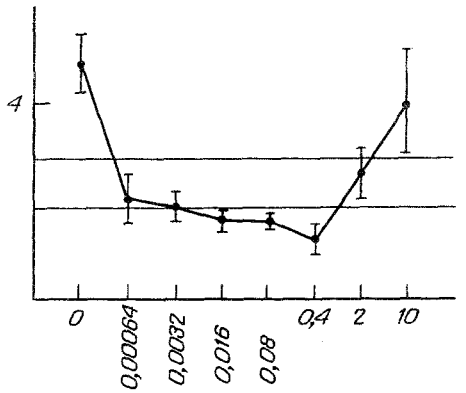

Fig. 1. Corticosterone production by adrenal cell suspension without treatment with ACTH. Here and in Figs. 2 and 3: abscissa, tactivin concentration (in μg/ml); ordinate, corticosterone production by suspension (in ng/100 μl suspension in 1.5 H). Concentration of initial suspension 1.2×10^6 cells/ml. *$p < 0.05$: differences significant compared with control.

Laboratory of Physiologial Genetics, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 53-55, January, 1990. Original article submitted May 29, 1989.

0007-4888/90/0001-0069$12.50

Table 1. Corticosterone production by Adrenal Cell Suspension After Addition of ACTH to Medium.

ACTH concentration, μIU/ml	Corticosterone production, ng/100 μl of suspension in 1.5*
0	2,5
1,6	11,0
16	13,1
160	17,4
1600	22,0
16 000	22,0
160 000	21,3

Legend. Values given represent average production for 2 samples.

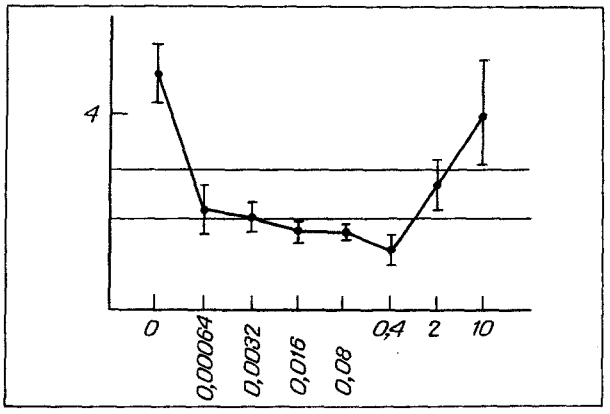

Fig. 2. Corticosterone production by suspension of adrenal cells in the presence of ACTH (1.6 μ IU/ml). Here and in Fig. 3, horizontal lines indicate boundaries of "mean basal corticosterone production ± error of the mean." Here and in Fig. 3, concentration of initial susepnsion was 0.8×10^6 cells/ml.

concentrations of 0.00064, 0.0032, 0.16, 0.4, 2, and 10 μg/ml or of tactivin (the same concentrations) together with ACTH ("Serva") in concentrations of between 1.6 and 160,000 μIU/ml. The volume of each sample was 250 μl of suspension with a concentration of 0.8×10^6 or 1.2×10^6 cells/ml, with the addition of ACTH, tactivin alone, of tactivin preceded by ACTH, diluted in 50 μl of physiological saline. The action of ACTH on the cells was tested in duplicate, and the effect of activin alone and preceded by ACTH was tested in duplicate, and the effect of activin alone and preceded by ACTH was tested in quadruplicate. After incubation the medium was separated from the cells by centrifugation for 10 min at 4000 rpm and the corticosterone concentration in it was determined by the competitive protein binding method [5]. The results were subjected to statistical analysis, by correlation analysis and Student's t test.

EXPERIMENTAL RESULTS

Experiments with the addition of tactivin to the medium in which the cells were incubated showed that, in concentrations of 0.08 and 2 μg/ml, it significantly inhibits corticosterone production (Fig. 1). In three other groups (0.016, 0.4, and 10 μg tactivin/ml) a tendency also was noted for this parameter to decrease. On the whole the results of correlation analysis revealed significant ($p < 0.05$) negative correlation between the concentration of thymus factor and corticosterone production ($r = -0.388$; $N = 32$). Tactivin thus reduces basal corticosterone production by adrenal cells.

Adrenocortical function is known to depend primarily on the ACTH level. We studied the effect of tactivin on corticosterone production by adrenal cells under stimulation by ACTH. Preliminary experiments showed that ACTH, over a wide range of concentrations (from

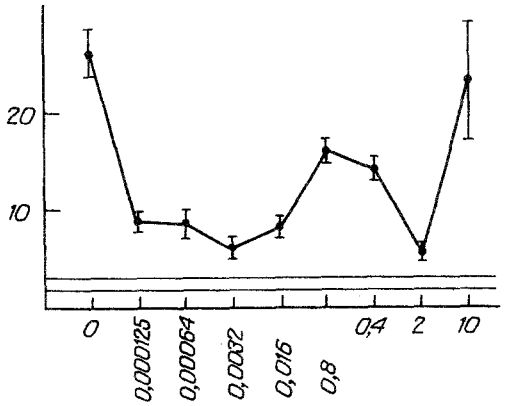

Fig. 3. Corticosterone production by suspension of adrenal cells in the presence of ACTH (1600 μIU/ml).

1.6 to 160,000 μIU/ml) induces a marked increase in steroid production (Table 1). We used two ACTH concentrations in the experiments: low (1.6 μIU/ml) and high (1600 μIU/ml). Tactivin was found to prevent the increase in corticosterone production caused by CATH, and it acted more strongly on stimulated than on intact cells. After addition to ACTH to the suspension in a concentration of 1.6μIU/ml, for instance, a significant increase was observed in corticosterone production by the cells (Fig. 2). Tactivin in doses of 0.00064 to 0.04 μg/ml completely abolished the stimulating effect of ACTH on the cells, and in this case production was the same as the basal level. The inhibitory action of tactivin on steroid production also was observed when a high ACTH concentration was used (1600 μIU/ml; Fig. 3). The greatest decrease took place when tactivin was used in doses of between 0.000125 and 0.016 μg/ml and 2 μg/ml; when it was used in a dose of 0.08 and 0.04 μg/ml its inhibitory effect was weaker. It was difficult at present to suggest any reason for this irregular response of the cells to different doeses of tactivin. The dose of ACTH which we used (1600 μIU/ml) evidently has an excessively strong stimulating action, as is shown by the fact that even when depressed by tactivin, corticosterone production was still significantly higher than the basal level. In both experiments with ACTH, incidentally, the highest of the concentrations of tactivin used had no inhibitory action on steroid production by the stimulated adrenal cells. It can be tentatively suggested that tactivin concentrations between 0.00064 and 2 μg/ml are close to physiological. Mikhna and co-workers [4], for instance, found that tactivin in concentrations of 0.1-1 μg/ml potentiates the proliferative response of mature T cells to phytohemagglutinin. Higher doses, as in our own experiments, were ineffective. Disappearance of the effect with an increase in concentration is evidently characteristic also of two other thymus factors. Miroshnichenko and co-workers [3] showed that pentapeptides of three thymus factors, namely α_1-thymosin, thymus serum factor, and thymoptin, affect maturation of T cells, but with an increase in their dose, these peptides have no such effect. In their opinion, this phenomenon is associated with the pharmacological action of high concentrations of peptides on cells membranes, leading to insensitivity or so-called "freezing" of the membranes.

The results thus show that tactivin affects steroid production in the adrenals. However, whereas this hormone reduces basal corticosterone production by intact cells only very slightly, on its addition within a certain concentration range to stimulated cells, corticosterone production is inhibited much more strongly. It is important to emphasize that in this case, if small, probably near-physiolgoial, concentrations of tactivin are added to adrenal cells, stimulated by a small, physiological dose of ACTH, the action of ACTH on the cells is completely abolished. The action of tactivin on the adrenals which we found is evidently one manifestation of interaction between the immune and endocrine systems. Another thymus factor, namely α_1-thymosin, increases the sensitivity of adrenal cells to ACTH [12]. As our experiments showed, tactivin has an action opposite to that of thymosin, preventing the stimulating effect of ACTH on the adrenals. The presence of different thymus fctors, with opposite action on adrenal function, evidently reflects the ability ofthe body to modulate glucocorticoid levels in accordance with the functional state of the immune system. Lowering the corticosterone level evidently favors proliferation of T lymphocytes in the early stages of differentiation, when they are most sensitive

to hormones [7]. Elevation of the glucocorticoid level up to certain limits may lead to recirculation and the outflow of mature T cells to the periphery [2]. Our results are evidence that the thymus, acting through peptides in the composition of tactivin, can modulate both the functional activity of the adrenals and the response of these glads to ACTH; in turn, this is an additional and by no means unimportant factor in the regulation of the immune status.

LITERATURE CITED

1. V. Ya. Arion, Progress in Science and Technology. Series; Immunology [in Russian], Vol. 10, Moscow (1982), pp. 44-53.
2. A. Yu. Letyagin and A. V. Shurlygina, Functional Morphology of the Immune System [in Russian], ed. by V. V. Vinogradov, Novosibirsk (1987), pp. 179-229.
3. I. V. Miroshnichenko, A. A. Yarilin, I. D. Ryanina, et al., Immunologiya, No. 2, 85 (1986).
4. M. G. Mikhna, I. V. Miroshnichenko, M. F. Nikonova, et al., Byull. Éksp. Biol. Med., No. 2, 189 (1988).
5. A. G. Reznikov, Methods of Determination of Hormones [in Russian], Kiev (1980).
6. V. M. Chesnokova, E. V. Ignat'eva, L. N. Ivanova, et al., Probl. Éndokrinol., No. 1, 55 (1987).
7. H. Besedovsky and E. Sorkin, Clin. Exp. Immunol., 27, 1 (1977).
8. R. W. Carsia, G. J. Macdonald, and S. Malamed., Steroids 41, 3120 (1983).
9. A. L. Goldstein, T. L. K. Low, G. B. Thurman, et al., Recent Progress in Hormone Research, Vol. 37, New York (1981), pp. 369-415.
10. N. R. Hall, J. P. McGillis, B. L. Spangelo, et al., Springer Semin. Immunopathol., 8, 153 (1985).
11. G. Sayers, R. L. Swallow, and N. D. Giordano, Endrocrinology, 88, 1063 (1971).
12. L. Torres-Aleman, M. T. Rejas, I. Barasonan, et al., Life Sci., 40, 929 (1987).

EFFECT OF HIGH- AND LOW-MOLECULAR-WEIGHT SOLUBLE BONE MARROW FACTORS ON ANTIBODY FORMATION AND PAIN SENSITIVITY IN ANIMALS

O. G. Yanoskii, L. A. Zakharova, and A. M. Vasilenko

UDC 612.419.017.1.019.08

KEY WORDS: bone marrow factors; immune response; pain sensitivity

Low-molecular-weight peptides of bone marrow origin – myelopeptides (MP) – have a wide spectrum of immunoreulatory action. They are known to stimulate antibody formation [5], to affect the functional activity of T cells [1] and macrophages [2], and to influence cell differentiation [6]. Meanwhile MP can induce a naloxone-dependent hypoalgesic action [3, 4]. The writers showed recently that low doses of MP lead to the development of hyperalgesia in animals. These facts raise the fundamentally important question of interconnection between antibody-stimulating activity of MP and their activiites affecting pain sensitivity.

The aim of this investigation was to compare the effects of three different fractions of supernatant of a bone marrow cell culture on antibody formation and pain sensitivity in mice.

Laboratory of Mediators of the Immune System, Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 55-57, January, 1990. Original article submitted April 30, 1989.

0007-4888/90/0001-0072$12.50