EFFECT OF STEROID HORMONES ON β-GALACTOSIDASE ACTIVITY IN E. coli K-12 STRAINS WITH AN INDUCED CONSTITUTIVE AND SUPERREPRESSED STATE OF THE lac-OPERON

N. N. Zhukov-Verezhnikov, N. I. Rybakov, R. D. Seifulla, A. I. Maiskii, Z. P. Kozlovskaya, M. Yu. Klimova, E. M. Khalilov, and M. N. Boichenko

UDC 576.851.48.098.31.095.18: 577.174.53

The action of steroid hormones – hydrocortisone, cortisone, estradiol, and 21-hydroxypregnane-3,20-dione sodium succinate (viadril) – on biosynthesis of the β-galactosidase of the lactose operon of Escherichia coli was studied. The experiments showed that the glucocorticoids and viadril act in these molecular systems as accelerators, and not as depressors, of the transcription process. Estradiol did not affect the enzymic activity of β-galactosidase.

KEY WORDS: Escherichia coli; β-galactosidase; steroid hormones; transcription.

Steroids are the most widely distributed class of natural compounds that control the functional state of animal and plant cells [7]. This effect can be attributed either to their direct action on the template activity of the chromatin or to their indirect action through changes in the physicochemical properties of biological membranes [4, 5]. Since the details of the regulation of genome function in eukaryote cells have received little study, it would serve a useful purpose to clarify the possible effects of steroids by a study of simple biological systems. Microorganisms whose genes have been mapped provide a very promising model from this point of view for the fine analysis of the biologically active substances on the various parts of the genome [3, 4]. The basic principles of the regulation of transcription in bacteria were, of course, established by Jacob and Monod with respect to the synthesis of the enzyme β-galactosidase of the lactose operon of Escherichia coli [9].

The object of the present investigation was to study the character of function of lactose operon of E. coli with an induced, constitutive, and superrepressed type of synthesis of the enzyme, under the influence of cortisone, hydrocortisone, and estradiol in various concentrations, used alone and also in conjunction with acrinomycin D.

EXPERIMENTAL METHOD

Various strains of E. coli obtained from Jacob and differing from each other in the functional state of their lac-operon were studied. The first strain, E. coli 200 PS/F lac, was characterized by the fact that derepression of the appropriate operon took place on the addition of the inducer isopropyl-thio-β,D-galactoside (IPTG) to the medium. The second strain, E. coli 2000 i^S was distinguished by permanent blocking of the operator of the lactose operon in both the presence and absence of the inducer. The third strain, E. coli 3300, unlike the first two, synthesized β-galactosidase constitutively.

Unlabeled steroids (N. V. Organon-Oss) hydrocortisone, cortisone, estradiol, and 21-hydroxypregnane-3,20-dione sodium succinate (viadril) used in the investigation were added to the incubation medium in different concentrations. A 4-h culture in medium M-9 was incubated with steroids for 30 min. This time interval was chosen because a separate series of experiments showed that maximal accumulation of

Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 2, pp. 104–106, February, 1975. Original article submitted December 28, 1973.

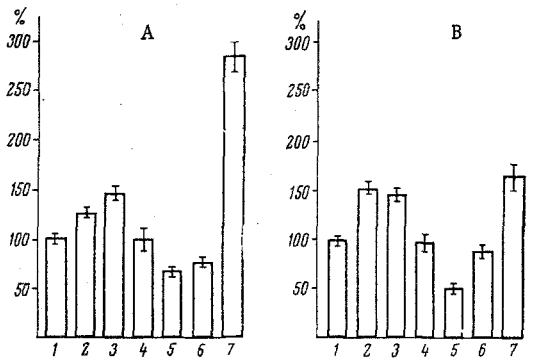

Fig. 1. Effect of steroid hormones on β-galactosidase synthesis by strains of E. coli: A) E. coli 200 PS/F lac: 1) culture plus IPTG (control); 2) culture plus IPTG plus cortisone, 1000 μg/ml; 3) culture plus IPTG plus hydrocortisone, 1000 μg/ml; 4) culture + IPTG + estradiol (concentrations from 0.1 to 10,000 μg/ml); 5) culture + IPTG + actinomycin D, 3 μg/ml; 6) culture + IPTG + actinomycin D, 3 μg/ml, + cortisone, 1000 μg/ml; 7) culture + IPTG + viadril 1000 μg/ml; B) E. coli 3300; 1) culture (control); 2) culture + cortisone, 500 μg/ml; 3) culture + hydrocortisone, 100 μg/ml; 4) culture + estradiol (from 0.1 to 10,000 μg/ml); 5) culture + actinomycin D, 3 μg/ml; 6) culture + actinomycin D, 3 μg/ml, + cortisone, 1000 μg/ml; 7) culture + viadril, 1000 μg/ml.

cortisone-H^3 takes place in the constitutive and induced strains after 30 min. At 4°C the strains accumulated practically no label.

EXPERIMENTAL RESULTS

Determination of the activity of β-galactosidase [11] revealed the following pattern: on the addition of the steroids to a culture of strain E. coli 200 PS/F lac in the absence of inducer no changes in enzyme activity were found. Addition of the inducer led to a statistically significant increase in β-galactosidase activity (Fig. 1). With lower concentrations of cortisone, hydrocortisone, and viadril, but not of estradiol, an increase was found, but in higher concentrations, there was a decrease in the activity of the enzymes.

None of the steroids effective derepressed the E. coli operon in strain 2000 i^S. Analysis of the action of the steroids in the constitutive strain E. coli 3300 showed a marked increase in β-galactosidase activity depending on the chemical structure and concentration of the steroids (Fig. 1). With lower concentrations of cortisone, hydrocortisone, and viadril, but not of estradiol, an increase in enzyme activity was found, but in higher concentrations the activity was reduced.

Although labeled steroids accumulated in the strains of E. coli their action on α-galactosidase differed considerably depending on the functional state of the lactose operon. Incidentally, all the steroids investigated, except estradiol, showed common features in their effect on β-galactosidase synthesis. Since the steroids investigated did not derepress the lac-operon in strain 200 PS/F lac without the inducer, and since β-galactosidase activity was unchanged in the superrepressed strain, it can be concluded that they did not possess direct derepressive action as such, but, most probably, they were accelerators of enzyme synthesis.

To clarify this situation a series of experiments was carried out with the use of actinomycin D and the steroids. The results showed (Fig. 1) that preliminary addition of actinomycin D (3 μg/ml) to the culture of the induced or constitutive strains reduced both the inducing effect of the inducer and the action of the steroids. These results show indirectly that the mechanism of action of the steroid hormones on α-galactosidase includes an increase in RNA-polymerase activity and in the synthesis of the corresponding types of RNA. A similar action in mammalian and insect cells has been described and is not disputed [6, 10].

Analysis of the results described above, and of data in the literature suggests that the nonspecific action of steroids in the E. coli system can be explained from the point of view of the change in perme-

ability of the membranes of the cells and subcellular structures [2, 8], for these steroid hormones and viadril have been shown to be active in regulating the permeability not only of mitochondrial membranes, but also of bimolecular phospholipid membranes and liposomes. These and also other effects of these compounds are connected with their physicochemical properties and their hydrophobic types of interaction with the cell and its biologically important molecules. The difference found in the action of the estradiol can evidently be explained by the chemical structure of this hormone, which has three double bonds in the A rings containing the hydroxyl group at C_3. Estradiol also has no side chain at C_{17}.

LITERATURE CITED

1. Yu. A. Vladimirov, P. V. Sergeev, R. D. Seifulla, et al., Molekul. Biol., No. 2, 248 (1973).
2. Yu. P. Denisov and R. D. Seifulla, in: The Biophysics of Membranes [in Russian], Kaunas (1973), p. 228.
3. N. N. Zhukov-Verezhnikov, The Theory of Genetic Information [in Russian], Moscow (1966).
4. N. N. Zhukov-Verezhnikov, P. V. Sergeev, M. Yu. Klimova, et al., Zh. Mikrobiol., No. 11, 4 (1971).
5. R. I. Salganik, in: The Cell Nucleus [in Russian], Moscow (1972), p. 112.
6. P. V. Sergeev, Yu. P. Denisov, R. D. Seifulla, et al., in: The Biophysics of Membranes [in Russian], Kaunas (1973), p. 568.
7. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
8. P. V. Sergeev, R. D. Seifulla, and I. A. Sultanov, Farmakol. i Toksikol., No. 4, 452 (1973).
9. F. Jacob and J. Monod, J. Molec. Biol., 3, 318 (1961).
10. P. Karlson, J. Cell. Physiol., Suppl. 1, 66, 69 (1965).
11. A. B. Pardee, F. Jacob, and J. Monod, J. Molec. Biol., 1, 165 (1959).