QUANTITATIVE DETERMINATION OF EPIDERMAL G_2-CHALONE-LIKE FACTOR BY RADIAL IMMUNODIFFUSION

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A homogeneous high-molecular-weight glycoprotein possessing tissue-specific, but not species-specific antimitotic activity relative to epithelium of dermal type (epidermal G_2-chalone) was isolated electrophoretically and immunochemically from rat skin. A method of quantitative immunodiffusion determination of this substance by means of monospecific antiserum in certain tissues is described. Clear correlation between the mitotic index and concentration of G_2-chalone in the mucous membrane at various stages of the estrous cycle was demonstrated in the vaginal epithelium.

KEY WORDS: chalone; proliferative activity; mitosis; epidermis; estrous cycle.

The only method at present available for identifying endogenous tissue-specific inhibitors of proliferation (chalones) is testing aqueous or alcoholic tissue extracts containing them for biological activity by histological methods. However, since these methods are so time-consuming and laborious, new methods more adequate to the problems of chalone research are required.

Immunologic methods would appear to be most suitable for these purposes, for in addition to their high qualitative and quantitative resolving power, they can be used to identify a particular substrate in crude tissue extracts. Previous experiments [2-4] have shown the suitability of the immunologic approach to the study of rat epidermal G_2-chalone. This glycoprotein, with a molecular weight of 34,000 [5], is similar in its basic biochemical parameters to the epidermal G_2-chalone of pigs [15], and is a powerful antigen which induces the formation of monospecific antibodies in a heterologous system. With its aid the presence of epidermal G_2-chalone has been demonstrated in all tissues capable of forming squamous-cell keratinizing structures, irrespective of their histogenetic origin [13]. It is on these tissues that epidermal G_2-chalone also exerts its biological action [12].

A quantitative evaluation of the G_2-chalone content in certain tissues frequently used as test objects in cancer research is described below.

EXPERIMENTAL METHOD

Altogether 35 male and 20 female albino rats aged 3-8 months, from the Rappolovo Nursery, Academy of Medical Sciences of the USSR, were used. Blood serum, dorsal skin, esophagus, forestomach, lung, and bladder were removed from the males and the vaginal mucous membrane and blood serum from the females for investigation. By cytological investigation of vaginal smears the stage of the estrous cycle in the females was determined, after which the animals were divided into four corresponding groups with five animals in each group. The skin was shaved and the dermis separated as completely as possible mechanically. Residual epidermal tissues were freed from mucus with 0.9% NaCl and the mucous membrane was separated and homogenized with crushed glass in distilled water (100 mg/ml). The protein concentration in the extracts was determined by the method of Lowry et al. [9] and G_2-chalone was determined by the method of Mancini et al. [10].

Monospecific antiserum against G_2-chalone [3] was diluted 1:30 in a 1% solution of liquified agarose in 0.9% NaCl, made up in 0.05 M phosphate buffer (pH 7.2) with the addition

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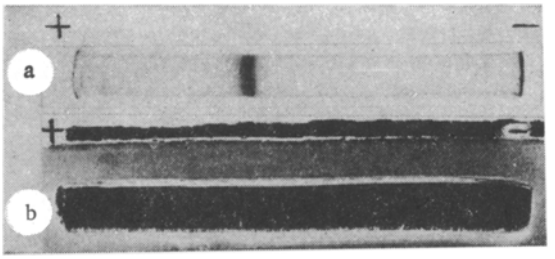

Fig. 1. Electrophoresis (a) and immunoelectrophoresis (b) of purified epidermal G_2-chalone from rat skin. 5% polyacrylamide gel, pH 8.3. For immunoelectrophoretic analysis one of the gels (b, below) was incorporated in 1% agar; b (above) trough filled with antiserum. Location of antigen matched agains mobility of bromphenol blue (in a, on left). For details, see [5].

of 0.25% bovine serum albumin. The solution in a volume of 16 ml was applied to plates measuring 9×12 cm (thickness of the layer of cell 1.5 mm). Wells 4 mm in diameter were filled with standard or test specimens. Double dilutions of a solution of purified G_2-chalone [5], applied to each plate (initial concentration 20 μg/ml) were used as standards for plotting the calibration curve. The results of the reaction were read after incubation of the plates for 48 h in a humid chamber at room temperature and were expressed in μg G_2-chalone/mg total protein of the extract.

To detect G_2-chalone in blood serum, mucous membrane of the bladder, and lung the test system was additionally diluted 24-32 times and used for indicrect autoradiography. The ^{125}I-antibodies against rabbit γ-globulins used were from the Medpreparat factory, Moscow (specific activity 1 μg/mg). The dried gels were exposed for 7-12 days with RM-1 x-ray film. The stages of the method, as used for double immunodiffusion in gel are described in detail in [6].

EXPERIMENTAL RESULTS

The electrophoretically and immunochemically homogeneous preparations illustrated in Fig. 1 possessed biological activity of epidermal G_2-chalone [4, 5]. Under the conditions of immunodiffusion mentioned above, the most reproducible results were obtained by the reaction with an antigen concentration of 3-9 μg to 1 ml of the standard solution or test extract, when the error of the method was ±0.4 μg/ml. All determinations were accordingly carried out within this zone.

Epidermal G_2-chalone was found to be present in the corresponding tissues in relatively large amounts. Its content (in μg/mg protein), for instance, was 25.1±4.3 (limits of varia-

TABLE 1. Content of G_2-Chalone in Vaginal Mucosa of Rats at Different Stages of Estrous Cycle Compared with Mitotic Activity (M±m)

Stage of estrous cycle	Chalone concentration, μg/mg protein	Mitotic activity, %*
Diestrus	4,3±1,3	5,1±2,2
Proestrus	3,0±0,6	3,2±1,2
Estrus	8,6±0,6†	10,1±1,4†
Metestrus	4,6±0,2	5,3±2,4

*Pooled data from [7].
†Difference significant compared with other values in the same column ($P<0.01$).

tions 10.2-33.4) in the epidermis, 8.3±1.7 (3.9-18.0) in the esophageal mucosa, and 31.0±5.8 (13.7-49.6) in the mucous membrane of the forestomach. The relatively wide zone of variations in the G_2-chalone level in the tissues can evidently be explained by the fact that animals of different ages were used for the experiments and the tests were carried out at different times of day and year. These factors could influence the proliferative activity of the tissues and, consequently, the level of one of the regulators of this process.

The above conclusion was confirmed by the results of determination of G_2-chalone in the vaginal mucosa of rats in different stages of the estrous cycle (Table 1), during which the mitotic activity of the vaginal epithelium fluctuates substantially [7].

Considerable variations in the concentration of G_2-chalone in the vaginal mucosa during the estrous cycle, the individual phases of which in rats last only a few hours [7], indicate that synthesis and degradation of epidermal G_2-chalone take place within relatively short periods of time. For instance, the content of this antimitotic factor may be reduced by 60-67% in the course of 12 h after treatment of castrated rats with estradiol propionate, a time which corresponds approximately to the state of proestrus in rats with a normal cycle and leads to a subsequent increase in mitotic activity of the vaginal epithelium [1].

No G_2-chalone was detected in the tissues of the lung, mucous membrane of the bladder, and blood serum of normal rats by immunoautoradiography (sensitivity of the method 60-80 μg antigen/ml). Meanwhile, it is found in a relative high concentration in squamous-cell keratinizing tumors of the rat bladder [13], which suggests that the antigenic properties of epidermal G_2-chalone can be used as an immunologic marker of squamous-cell tumors whatever their origin. The only point to be emphasized in this connection is that in the present case it was a question of using the immunochemical specificity of the chalone, and no attention was paid to its biological activity. This may be considerably reduced or absent altogether in tumor tissues [8], but under these circumstances the G_2-chalone still retains its antigenic properties. These and other data on the effects of various factors on biological [11, 15] and immunochemical activity are evidence that combining sites are localized on different parts of the chalone molecule. Moreover, there are solid grounds for considering that the substrate actually determined in the present experiments is a natural complex of chalone itself with a tissue-specific protein carrier, and that the latter is the major component.

In experiments in vivo purified G_2-chalone in a dose of 30-100 μg has reliably detectable biological activity on mice [11]. The same quantity of the compound is present, as shown by the results of immunologic analysis, in 1.5-5 mg of lyophilized alcoholic extract of skin, i.e., in the dose used in experiments such as these [2, 4, 5, 11]. This correlation between the biologically active dose and the results of immunodiffusion indicates that it would be possible to make a comparative study of the biological activity of extracts containing epidermal G_2-chalone in immunologically equivalent quantities. This is a particularly interesting topic from the oncologic and age aspects [8, 14].

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LITERATURE CITED

1. V. N. Anisimov, M. N. Ivanov, and V. B. Okulov, Byull. Éksp. Biol. Med. (1979).
2. V. B. Okulov and S. A. Ketlinskii, Tsitologiya, 17, 1294 (1975).
3. V. B. Okulov and S. A. Ketlinskii, Arkh. Anat., No. 2, 84 (1977).
4. V. B. Olulov and S. A. Ketlinskii, Arkh. Anat., No. 4, 98 (1977).
5. V. B. Okulov, S. A. Ketlinskii, É. A. Ratovitskii, et al., Biokhimiya, 43, 971 (1978).
6. D. A. Él'gort and G. I. Abelev, Byull. Éksp. Biol. Med., No. 2, 118 (1971).
7. F. D. Bartalanffy and C. Lan, Acta Anat. (Basel), 54, 39 (1963).
8. W. S. Bullough, Life Sci., 16, 323 (1975).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. G. Mancini, A. O. Carbonara, and J. E. Heremans, Immunochemistry, 2, 235 (1965).
11. F. Marks, in: Chalones, J. C. Houck (ed.), Amsterdam (1976), pp. 173-227.
12. O. Nome, Arch. Path. Anat., B. Zellpath., 19, 1 (1975).
13. V. B. Okulow and S. A. Ketlinski (V. B. Okulov and S. A. Ketlinskii), Cancer Lett., 3, 215 (1978).
14. L. Ollson and P. Ebbesen, Exp. Geront., 12, 59 (1977).
15. A. L. Thornley and E. B. Laurence, Int. J. Biochem., 6, 313 (1975).