SEROLOGIC ASSESSMENT OF MOLECULAR FORMS OF CHORIONIC GONADOTROPIN IN THE COURSE OF PREGNANCY

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Data on the existence of isohormones — hormones differing in molecular parameters and certain biological properties — have recently been published. Three associations of insulin molecules, free from zinc ions, have been found in solution [4]. Unpurified preparations of ACTH contained several isohormones, and the biological activity of the high-molecular-weight fraction is less than one-hundredth of the expected value [5]. High- and low-molecular-weight forms of ACTH have been isolated from the sheep pituitary gland [6]. Forms of chorionic gonadotropin (CG) differing in molecular parameters have been found in extracts of human chorionic tissue [7]. The identification and study of isohormones, procedures of great importance, require the use of laborious immunochemical methods. It was shown previously that simpler serologic methods can be used to assess molecular parameters not only of microbial antigens, but also of CG [1, 2]. Integral antigenic *in vitro* (antibody-binding) activity of CG is determined by its activity in the antibody neutralization test (ANT) with antigenic erythrocytic diagnostic serum. It has recently been shown that the activity of antigens in the erythrocyte disaggregation test (EDT) depends not only on antibody-binding properties, but also on the dimensions of antigen molecules [2]. Although the primary mechanism of this reaction (dissociation of the antigen-antibody complex or simply "disagglutination" of the agglutinated cells) is not yet known, the link between the molecular parameters of the antigen and their disaggregating activity, which has been described for various antigens including CG, enables the component of antigenic activity dependent on the dimensions of CG molecules to be assessed from the results of EDT [2].

The object of this investigation was to assess the dynamics of changes in serologic activity of CG in the urine during pregnancy with the aid of the above tests.

EXPERIMENTAL METHOD

The ANT was set up with erythrocytic antigenic diagnostic serum prepared by the writers from tanninized formalinized erythrocytes and with CG obtained from "Gedeon Richter" (Hungary). Material for testing for CG activity was diluted consecutively in a volume of 0.25 ml with buffered (pH 7.2) 0.85% NaCl solution. The first dilution of urine was always made up in phosphate buffer, pH 7.2. To the resulting dilutions 0.25 ml of immune serum against CG, containing two minimal agglutinating doses of specific antibodies against the erythrocytic antigenic CG diagnostic serum, was added. The mixtures were exposed at 20°C for 30 min. To all the mixtures 0.05 ml of a 2.5% suspension of erythrocytic antigenic CG diagnostic serum was added. The ANT was read after 1.5-2 h. The EDT was set up with commercial erythrocytic pregnancy-diagnostic serum, manufactured by the Leningrad Research Institute of Toxins and Sera, which is an agglutinate of antigenic CG erythrocytic diagnostic serum with anti-CG rabbit serum. The reaction was carried out in a volume of 0.55 ml: To 0.5 ml of serial dilutions of urine, prepared in the same way as for the ANT, 0.05 ml of a commercial pregnancy-diagnostic serum was added. The mixtures were shaken. The result was read after exposure for 2 h at 20°C.

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TABLE 1. Dynamics of Serologic Indices of CG Activity during Pregnancy

Index	Time of pregnancy, weeks							
	5	6—10	11—15	16—20	21—25	26—30	31—35	36—39
Titer of:								
ANT	1:62	1:92	1:3870	1:5580	1:6630	1:3380	1:4920	1:2330
EDT	1:2,8	1:4,2	1:46,0	1:55,0	1:54,0	1:16,0	1:17,0	1:12,0
CRDA	17	19	70	115	125	251	371	191

TABLE 2. Assessment of Divergence of Dependence of Serologic Indices of CG Activity in the Urine on Time of Pregnancy

Curves of dependence on time of pregnancy compared	Statistical indices					
	overall differ. bet. curves			divergence of curves		
	K_1 and K_2	F	P	K_1 and K_2	F	P
ANT titer and EDT titer	8 and 126	16,27	<0,001	7 and 126	3,15	0,01>P>0,001
ANT titer and CRDA	8 and 126	14,69	<0,001	7 and 126	2,68	0,05>P>0,01

Antigenic activity of CG in the urine was determined by the ANT titer. The distribution of CG molecules by size was judged from their coefficient of relative disaggregating activity (CRDA), calculated by the equation:

$$\text{CRDA} = \frac{\text{disaggregating activity}}{\text{neutralizing activity}}$$

where activity is expressed by the titer of the corresponding test. Divergence of the two curves was assessed by the F criterion [3].

Altogether 75 samples of urine from women between the 5th and 39th weeks of pregnancy were tested. The urine was diluted with phosphate buffer, pH 7.2, and the final active dilution was determined in each test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that CG activity during pregnancy changed, as reflected in the titers both of the ANT and the EDT. The increase in the ANT and EDT titers continued until the 25th week, after which both titers fell. The sensitivity of the EDT was significantly lower than that of the ANT. Calculation of the values of CRDA showed that this coefficient, from which the dimensions of the CG molecules can be judged, changes rather differently during pregnancy: It rises until the 35th week and does not fall until the last 5 weeks.

Having obtained these results it was decided to test the significance of the differences discovered in the dynamics of the serologic indices by the method of determination of divergence of the two curves. Curves for dependence of the titer of the two tests on the time of pregnancy were compared in pairs; the titer of the ANT with the titer of the EDT and the titers of the ANT and CRDA (Table 2). In neither case were the curves found to be parallel.

The results indicate that not only antigenic activity of CG in the urine (corresponding to its antibody-binding activity in ANT), but also the disaggregating activity of CG changes during the course of pregnancy. Taking previous observations [2] into account, this last fact can be explained by a change in the molecular parameters of CG in the urine in the course of pregnancy. In addition, the dynamics of changes in the molecular parameters of CG was found not to coincide with the dynamics of the change in antibody-binding activity of CG. The concrete factors responsible for the unequal molecular parameters of CG in the course of pregnancy are not yet clear and require special investigation. The biological role of the change in molecular parameters of CG under normal conditions (pregnancy) and, perhaps, in pathological states (chorionepithelioma) needs to be evaluated. It can be tentatively suggested that the development of appropriate systems of serologic tests for other hormones

would make possible the introduction of convenient methods for assessment of differences in molecular parameters of several hormones.

LITERATURE CITED

1. L. Ts. Doroshkevich, B. V. Karal'nik, T. G. Vasil'eva, et al., Zh. Mikrobiol. No. 9, 90 (1974).
2. B. V. Karal'nik, S. V. Prokhorova, and Yu. P. Tsarevskii, Zh. Mikrobiol., No. 10, 109 (1978).
3. A. M. Dlin, Mathematical Statistics in Engineering [in Russian], Moscow (1958).
4. L. A. Holladay, M. Ascoli, and D. Puett, Biochim. Biophys. Acta, 494, 245 (1977).
5. F. Kawakami, K. Moriwaki, and Y. Itoh, Folia Endocrinol. Jpn., 53, 615 (1977).
6. H. Lee Teh and S. Lee Mang, Biochemistry (Washington), 16, 2824 (1977).
7. S. Tojo, Y. Ashitaka, T. Maruo, et al., Endocrinol. Jpn., 24, 351 (1977).

EFFECT OF ALKYLATING DERIVATIVES OF CYCLIC AMP ON PROLIFERATION OF MOUSE BONE MARROW STEM CELLS

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It has been frequently demonstrated that cyclic AMP can restore normal differentiation of many tumor cells, can inhibit their proliferation, and can exert a cytostatic action [1]. It was accordingly decided to study the antileukemic action of various new alkylating derivatives of cyclic AMP synthesized in Professor E. S. Severin's laboratory. Nearly all of the alkylating derivatives of cyclic AMP studied inhibited proliferation of P-388A mouse leukemic cells in culture [2]. However, the antileukemic action of a compound largely depends on its side effects on normal hematopoiesis and, in particular, on proliferation of bone marrow stem cells (colony-forming units — CFU_c). If alkylating derivatives of cyclic AMP, such as dibutyryl-cyclic AMP, stimulate proliferation of CFU_c, this gives them an undoubted advantage over all antitumor preparations so far known, which inhibit hematopoiesis or even bring about its aplasia.

EXPERIMENTAL METHOD

The effect of cyclic AMP analogs of alkylating type — 1-(N-chloroacetylaminoethoxy)-cyclic AMP, 8-(N-chloroacetylaminoethylamino)-cyclic AMP, and 1-[N-(p-fluorosulfonyl)-benzoyl-aminoethoxy]-cyclic AMP — generously provided by Professor E. S. Severin, on proliferation of stem cells was studied. The action of cyclic AMP (from FERAK, Berlin) was studied as the control. Hydroxyurea (from Serva) was used to determine the number of CFU_c.

Tests were carried out on female (CBA × C57BL)F_1 mice weighing 18-20 g obtained from "Stolbovaya" Nursery, Academy of Sciences of the USSR. The number of CFU_c was determined by the method of Till and McCulloch [5]. Bone marrow was flushed from the femora with medium No. 199 with the addition of Hepes (10 mM), penicillin (50 units/ml), and streptomycin (50 μg/ml), and was forced through a needle to produce disaggregation of the cells. The medullary cells were washed and resuspended in medium No. 199. A cell suspension with a density of 4 million 400 thousand cells/ml was poured into flasks, allowing for the volume of solution of the reagents, each of which was added in a volume of 0.1 ml, and of cyclic AMP and its analogs (final concentration of the sample 10^{-8} M) and of hydroxyurea (10^{-3} M). The volume of the mixture thus prepared did not exceed 1 ml. The incubation time of the samples

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