circulatory anoxia and accumulation of iron in the brain parenchyma evidently leads to a further increase in concentrations of LPO products in the brain and a decrease in ARA of lipids.

The results of the present investigation thus demonstrate progressive activation of LPO in brain tissue in the acute period of intracerebral hemorrhage, and is the justification for the early use of antioxidant therapy.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medico-Biological Research [in Russian], Leningrad (1973).
3. A. I. Zhuravlev, Bioantioxidants [in Russian], Moscow (1975), p. 15.
4. G. A. Kochetkov, Textbook of Practical Enzymology, 2nd ed. [in Russian], Moscow (1980).
5. M. B. Plotnikov, Farmakol. Toksikol., No. 5, 568 (1981).
6. A. S. Saratikov and M. B. Plotnikov, Vestn. Akad. Med. Nauk SSSR, No. 11, 68 (1984).
7. J. Folch, M. Lees, and G. H. Sloane Stanley, J. Biol. Chem., 226, No. 1, 497 (1957).
8. J. Graving, Acta Chem. Scand., 17, No. 6, 1665 (1963).
9. W. K. Hass, Neurol. Clin., 1, No. 1, 345 (1983).
10. H. Payan, M. Toda, and M. Berard-Badier, Epilepsia, 11, No. 1, 81 (1970).
11. L. Plazer and L. Kuzela, Acta Biol. Med. Germ., 21, No. 1, 121 (1968).
12. W. J. Triggs and L. J. Willmore, J. Neurochem., 42, No. 4, 976 (1984).

INTERACTION OF PENTACYCLIC PROGESTERONE DERIVATIVES WITH PROGESTIN-BINDING SITES OF UTERINE CYTOSOL

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Introduction of an additional D' carbon ring, condensed with the steroid skeleton in the 16α,17α-positions, into the progesterone molecule led to the creation of a new class of highly active progestogens [5], known as pregna-D'-pentarans. The activity of these compounds, tested in vivo on rabbits in the Clauberg–McPhail pregnancy supporting tests, was 3-9 times greater than the activity of progesterone itself [1]. X-ray structural analysis of compounds of the pentaran series showed virtual coincidence of the geometry of the A, B, and C rings with that of progesterone, and the existence of differences only in the conformation of the D ring and the side chain [2]. Meanwhile the pentarans which have been tested revealed completely unexpected extremely low relative affinity for progesterone receptors in the cytosol of the guinea pig uterus, between limits of 1 and 15% [7].

In the investigation described below interaction of progesterone and some of its derivatives with an additional D' carbon ring (I-X) with progestogen-binding systems of the uterine cytosol of the rabbit, rat, guinea pig, and man, was studied.

EXPERIMENTAL METHODS

A 10 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM EDTA and 10% glycerol was used; ^{3}H-progesterone was obtained from Amersham International (England) and ^{3}H-promegestone – synthe-

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TABLE 1. Affinity of Pregna-D'-pentarans for Gestogen-Binding Systems of Uterine Cytosol of Animals of Different Species Compared with That of Progesterone (100%)

Compound	Guinea pig	Rat	Rabbit
I. 16 α,17α-Cyclohexane-progesterone	1.7	15,3	5,2
II. Δ^6-16 α,17 α-Cyclo-hexane-progesterone	1.3	—	—
III. 6 α-Methyl-16 α,17 α-cyclohexane-progesterone	0.9	—	—
IV. 16 α,17 α-Cyclohexene-progesterone	1.5	4.5	1,6
V. Δ^6-6-Methyl-16α ,17α-cyclohexane-progesterone	1.2	2,3	0,8
VI. 16 α,17 α-Cyclopropane-progesterone	7.6	20,1	—
VII. 16 α,17 α-Cyclobutene-progesterone	7.6	20,4	—
VIII. 16 α,17 α-Cyclopentene-progesterone	<0.04	<0,01	—
IX. 16 α,17 α-Cyclopentene-progesterone	5.2	13,0	—
X. 16 α,17 α-Cyclopentane-progesterone	5.3	14,6	—

Legend. Mean values of five measurements are shown; standard error of means did not exceed 20%.

tic progestogen R-5020, with high affinity for progesterone receptors and not interacting with other progesterone-binding proteins – from New England Nuclear (USA); ^{3}H-16α,17α-cyclohexane-progesterone (D_6^1-pentaran; I) with specific activity of 87, 94, and 75 Ci/mmole, respectively; unlabeled compound – progesterone (from Calbiochem, USA), promegestone (New England Nuclear); pregna-D'-pentarans (Table 1) were synthesized in the N. L. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR; 0.5% and 5% suspensions of activated charcoal Norit A (USA) with the addition of 0.1% gelatin, and scintillation fluid consisting of 0.5% PPO + 0.05% POPOP in toluene. Cytosol from the uterus of weaned Wistar rats and a weaned guinea pig, of sexually immature intact and estrogenized rabbits, and also cytosol from the uterus of postmenopausal women, both untreated and receiving estrogens therapeutically, were used. Human uteri were obtained during hysterectomy on medical grounds. Preparation of the cytosols was described previously [3, 6]. The concentration of progestogen-binding sites was determined by Scatchard's method and ligand exchange [4, 8]. The equilibrium constant (K_{eq}) of the reaction between the labeled compounds and cytoplasmic binding sites was calculated by Scatchard plot analysis. The method of determination of the relative affinity of unlabeled compounds for binding sites in cytosols was described previously [7]. All the experiments were carried out in the presence of a 100-fold excess (relative to the concentration of the labeled hormone) of unlabeled cortisol to prevent binding of progestogens with blood transcortin. The linearity of the Scatchard plots was estimted by Fisher's test at a level of significance $\alpha = 0.05$.

EXPERIMENTAL RESULTS

The results of measurement of the relative affinity of the pentarans for the progestogen-binding system of uterine cytosol from intact animals of different species (Table 1) indicate definite interspecific differences in ligand specificity. In every case, however, affinity measured under conditions of competitive interaction was extremely low and did not correspond in principle to the high progestogen activity of the preparations determined in vivo. The greatest affinity of the test compounds for the gestogen-receptor system was found in cytosol of rat uterus. This may perhaps indicate that the specificity of gestogen receptors in the rat uterus is lower than that of the guinea pig and rabbit, and as a result, the usefulness of rats for biological testing of preparations with gestogen or antigestogen activity is limited.

Meanwhile, after estrogenization of the animal the relative affinity of one of the pregna-D'-pentarans (I) for the gestogen-binding system of the cytosol of rabbit uterus increased from 5 to 70%. To sum up the results it can be concluded that the reason for this phenomenon is a change in the gestogen binding parameters in the uterus of estrogenized animals.

TABLE 2. Concentration of Binding Sites and K_{eq} for Interaction of Labeled Progesterone, Promegestone, and Pentran with Gestogen-Binding System of Rabbit Uterine Cytosol

Parameter	Rabbits					
	nonestrogenized			estrogenized		
	progesterone	promegestone	pentaran	progesterone	promegestone	pentaran
$K_{eq} \cdot 10^9$, M^{-1}	0,41±0,10	0,39±0,14	1,10±0,40 (I) 0,21±0,09 (II)	0,11±0,01	,012±0,02	0,10±0,001 (I) 0,009±0,001 (II)
Concentration of binding sites by Scatchard's method, nM	0,32±0,08	0,30±0,10	0,20±0,03 (I) 0,58±0,11 (II)	1,91±0,31	2,10±0,40	2,12±0,51 (I) 40±21 (II)
Concentration of binding sites by ligand exchange method, nM	0,26±0,06	0,22±0,10	0,74±0,20	1,44±0,51	1,60±0,42	35±14

Legend. Type of binding sites shown in parentheses.

In the experiments with ^{3}H-D'-pentaran (I) Scatchard plot analysis showed that this compound (unlike progesterone and promegestone), interacts with two different types of binding sites in the uterine cytosol of intact and estrogenized rabbits. One subpopulation of binding sites corresponded in its parameters of interaction with this particular compound to progesterone receptor sites (type I), whereas the second differed from them in the value of K_{eq} and binding capacity (type II; Table 2). The results given in Table 2 are evidence that after estrogenization of rabbits the concentration of receptor sites for pentaran (I) was increased approximately tenfold and did not differ from that for progesterone and promegestone, whereas the concentration of type II pentaran binding sites was 20 times greater than the concentration of specific binding sites. It must be emphasized that according to results obtained by the ligand exchange method and by analysis of Scatchard plots, progesterone and promegestone virtually do not interact with binding sites other than those of type I. The concentration of binding sites for pentaran, measured by the ligand exchange method, corresponded to the total concentrations of binding sites of types I and II, measured by a Scatchard plot in cytosols of both estrogenized and intact sexually immature rabbits. Activity of interaction of labeled pentarans with binding sites of types I and II in the cytosol of nonestrogenized animals differed by a factor of 5, as shown by the values of K_{eq}. The capacity of the system of type II binding sites in this case was 3 times greater than that of type I.

This ratio between the values of effective K_{eq} and capacities of the type I and type II binding systems determines the weak ability of this compound to compete with labeled progesterone and promegestone for binding with gestogen receptors.

Meanwhile the change in the binding parameters after estrogenization of rabbits may perhaps affect the results of measurement of the relative affinity of this pentaran for the gestogen-binding system of the uterine cytosol.

The Scatchard plots for this pentaran (I) for cytosols of the guinea pig and human uterus had a slope which did not differ significantly from zero. This may be explained by the nonspecific interaction of this compound with progesterone receptors in the cytosols tested.

These experiments show, first, that the ability of pentaran (I) to form complexes with protein components of the cytosol is greater than that of progesterone, possibly on account of an increase in the hydrophobic component of binding, and second, that interspecific differences are found in the interaction of compounds with progesterone-like activity with the receptor systems of uterine cytosol.

LITERATURE CITED

1. V. V. Korkhov, G. V. Nikitina, A. V. Kamernitskii, et al., Farmakol. Toksikol., No. 1, 95 (1981).
2. V. I. Simonov, V. M. Tseikinskii, A. V. Kamernitskii, et al., Bioorg. Khim., 7, 920 (1981).
3. N. D. Fanchenko, R. N. Shchedrina, and S. V. Sturchak, Byull. Éksp. Biol. Med., No. 4, 467 (1978).
4. J. Anderson, J. H. Clark, and E. J. Peck, Biochem. J., 126, 561 (1972).
5. A. V. Kamernitskii (A. V. Kamernitzky), I. S. Levina, L. E. Kulikova, et al., J. Steroid Biochem., 16, 61 (1982).

6. J. A. Katzenellenbogen, H. J. Johnson, and K. E. Carlson, Biochemistry (Washington), 12, 4092 (1973).
7. I. S. Levina, A. V. Kamernitskii (A. V. Kamernitzky), N. D. Fanchenko, and V. I. Simonov (V. J. Simonov), Endocrinologie, 80, 266 (1982).
8. G. Scatchard, Ann. N. Y. Acad. Sci., 51, 660 (1949).

SOLID-PHASE ENZYME IMMUNOASSAY (ELISA) OF HUMAN PLACENTAL ALKALINE PHOSPHATASE

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Human placental alkaline phosphatase (HPAP) is a membrane protein found in the microvilli of the syncytiotrophoblast [5]. During the development of certain neoplastic diseases, an abnormal increase is found in the HPAP content in the cells of several human tissues, and for that reason HPAP has been regarded as a biochemical marker of tumor growth [4, 6, 8]. Determination and characterization of HPAP are difficult because of the presence of other tissue-specific alkaline phosphatases in biological test preparations, whose amount and enzyme activity may be considerably greater than those of HPAP. Enzyme immunoassay (EIA), which is distinguished by its specificity, its high sensitivity, and the speed of its performance, is therefore a promising method of HPAP detection.

In this paper several versions of solid-phase EIA are suggested for the determination of HPAP, based on the use of the enzyme activity of HPAP itself (endogenous test), and with the use of horseradish peroxidase as marker enzyme (competitive and "sandwich" test). By means of these tests it is possible to determine the presence of HPAP in a test sample, to obtain information about its enzyme activity (in U/liter, where U is the international unit of enzyme activity of alkaline phosphatase) and its concentration (in ng/ml) and, on the basis of these data, to determine the specific enzyme activity of HPAP (in U/mg).

EXPERIMENTAL METHODS

To obtain a hyperimmune serum rabbits were immunized with an electrophoretically homogeneous preparation of thermostable HPAP (pI 4.6, activity 100,000 U/liter, specific enzyme activity 100 U/mg). The antigen was injected into the popliteal lymph nodes of rabbits in a dose of 250 μg per node, in a volume of 200 μl, in the ratio of 1:1 with Freund's complete adjuvant. After 30 days the rabbits were reimmunized by intravenous injections of 300-600 μg HPAP in physiological saline. Blood was taken 7-10 days after reimmunization. The gamma-globulin fraction was obtained from blood serum by triple precipitation with 50% $(NH_4)_2SO_4$ followed by dialysis against 0.01 M K-phosphate buffer, containing 0.15 M NaCl, pH 7.4 (phosphate buffered saline – PBS) by the method in [1].

Specific antibodies to HPAP were isolated by affinity chromatography on protein A sepharose CL-4B (Pharmacia, Sweden) and CNBr-activated sepharose 4B (Pharmacia), with immobilized HPAP. To obtain the IgG fraction of rabbit antiserum, 3 mg of the gamma-globulin fraction of the antiserum was applied to a column containing 5 ml of protein A sepharose, equilibrated beforehand with PBS. The column was then washed with 20 volumes of PBS and the IgG was eluted with 0.1 M glycine-HCl buffer (pH 2.5) at the rate of 25 ml/h. The IgG fractions collected were quickly neutralized with 0.1 M Na-carbonate buffer (pH 11.0), lyophilized, dissolved in 1 ml of PBS, and dialyzed against PBS overnight at 4°C. The resulting IgG preparation was kept in the frozen state at −20°C.

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