REGULATORY PROPERTIES OF PEPTIDES OF THE THYMUS AND FABRICIUS' BURSA DURING IMMUNODEPRESSION IN BIRDS

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UDC 615.361.438+615.361.476].03
[616-092:612.017.1.064]-092.9:598.2/.9

KEY WORDS: thymus; bursa of Fabricius; immunodepression; thymalin; bursalin

Functions of the lymphoepithelial tissue of the thymus and bursa of Fabricius are clearly demarcated in birds. Cells of these organs are known to produce factors involved in the regulation of immunologic reactions [2, 7, 11]. It has been shown that on injection of peptides isolated from the bursa of Fabricius into bursectomized chicks, reactions of the humoral immune response are restored, whereas no such effect is found after injection of thymus peptides [2].

Certain immunodepressive substances are known to selectively inhibit function of cells of the thymus and bursa in birds. Glucocorticoids have a marked action on thymocytes, characterized by reduction of mitotic activity and DNA and RNA synthesis, and also by loss of viability of some of the cells [8]. Injection of cyclophosphamide into chicks is one way of suppressing the B system of immunity, linked with marked atrophy of the bursa of Fabricius [10, 13]. Inhibition of the normal function of this organ is known to lead to a disturbance of humoral immunity [12, 15], and cyclophosphamide causes only very slight changes in structure of the thymus in birds [9]. Investigation of the immunoregulatory action of these factors of the thymus and bursa of Fabricius under these conditions is of great interest.

The aim of this investigation was to compare the effects of peptide bioregulators of the thymus and bursa of Fabricius on parameters of nucleic acid and protein metabolism in the lymphoepithelial organs in chicks subjected to selective immunodepression.

EXPERIMENTAL METHOD

Experiments were carried out on chicks of the "Broiler-6" cross, 1 day old, and kept under identical conditions of maintenance and feeding. At the age of 3 days, 45 chicks were given an intraperitoneal injection of cyclophosphamide in a dose of 60 mg/kg daily for 3 days [9]. Chicks of another group, consisting of 45 birds, received a single intraperitoneal injection of hydrocortisone in a dose of 50 mg/kg [4].

Peptide bioregulators (cytomedins) with a molecular weight of 1000-10,000 Daltons [3], isolated from the thymus (thymalin) and bursa of Fabricius (bursalin), were used. These preparations were injected twice into the birds (15 chicks in each group) in optimal doses (100 μg/kg for thymalin, 50 μg/kg for bursalin), starting with the 2nd day after injection of the immunodepressants. The control chicks [15] received 0.9% NaCl solution by the same scheme.

The composition of the subpopulations of lymphocytes and values of the parameters of nucleic acid and protein metabolism in cells of the thymus and bursa of the birds was studied on the 1st, 5th, and 15th days after the end of injection of the preparations. The number of T- and B-lymphocytes in the organs was determined by indirect immunofluorescence, using antisera to chick T and B lymphocytes. Antisera were obtained by immunizing rabbits with lymphocytes from the thymus and bursa of Fabricius of chicks. As secondary antigens donkey antiserum against rabbit γ-globulins, labeled with fluorescein isothiocyanate, was used. The intensity of nucleic acid synthesis was estimated as incorporation of ^{3}H-thymidine or ^{3}H-uridine, which were injected intraperitoneally into the chicks (in 0.9% NaCl solution) 3 h before sacrifice in a dose of 10 μCi/100 g body weight. The intensity

S. M. Kirov Military Medical Academy. All-Union Veterinary Research Institute of Aviculture, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 169-172, February, 1990. Original article submitted May 10, 1989.

 0007-4888/90/0002-0210$12.50

TABLE 1. Effect of Thymalin and Bursalin on Number of T- and B-Lymphocytes in Thymus and Bursa of Fabricius on 5th Day after Treatment with Immunodepressants ($M \pm m$)

Experimental conditions	Thymus			Bursa of Fabricius		
	weight, mg	number of T lymphocytes	number of B lymphocytes	weight, mg	number of T lympho-	number of B lymphocytes
Control	197,0±19,1	610,1±78,2	1,51±0,17	135,0±15,3	46,1±5,3	330,2±47,1
Injection of hydrocortisone	62,0±8,5*	370,3±42,3*	1,03±0,11	118,4±11,9	40,2±4,9	290,4±33,6
Injection of cyclophosphamide	167,4±14,3	510,9±67,2	1,12±0,14	53,9±6,1*	41,4±5,1	190,2±22,3*
Injection of cyclophosphamide and bursalin	180,1±17,2**	530,4±61,3**	1,13±0,11	124,0±15,1	44,3±6,7	309,4±41,6
Injection of cyclophosphamide and bursalin	188,4±21,4	545,2±69,0	1,21±0,13	128,4±14,3**	43,3±5,1	280,0± ±37,4**

Legend. *$p < 0.05$ compared with control, **$p < 0.05$ compared with group of chicks receiving cyclophosphamide or hydrocortisone.

TABLE 2. Effect of Thymalin and Bursalin on Nucleic Acid Synthesis in Chicks after Exposure to Immunodepressants ($M \pm m$)

Experimental conditions	Time of investigation, days	Incorporation of ^{3}H-thymidine into cell DNA		Incorporation of ^{3}H-uridine into cell RNA	
		thymus	bursa Fab.	thymus	bursa Fab.
Control	1	2270±30	5599±108	4291+116	12 500±204
	5	2280±26	5833±95	4248±97	12 295±108
	15	2340±45	5853±106	4450±126	12 803±213
Injection of cyclophosphamide	1	1122±30*	1474±44*	3622±108*	3641±145*
	5	998±37*	813±27*	3480±110*	1454±113*
	15	1142±37*	1695±30*	3790±106*	2986±123*
Injection of cyclophosphamide and thymalin	1	2206±34**	1535±58	4020±97**	3825±88
	5	2265±75**	1346±36**	3876±69**	2665±152**
	15	2334±89**	2023±53**	4255±91**	3066±128
Injection of cyclophosphamide and bursalin	5	1093±56	3033±131**	3699±102	4810±93**
	1	1165±54	3856±95**	3805±108	5343±169**
	15	1207±55	4057±180**	3895±83	5415±143**
Injection of hydrocortisone	1	473±12*	3042±102*	1725±90*	5243±109*
	5	311±10*	3209±88*	1049±37*	4221±74*
	15	241±15*	4122±75*	1514±47*	5358±149*
Injection of hydrocortisone and thymalin	1	1219±14*	4147±87**	2813±91**	6497±202**
	5	1120±15**	3647±103**	3047±146**	5745±170**
	15	1836±28**	5248±104**	3945±104**	6013±181**
Injection of hydrocortisone and bursalin	1	521±15**	5415±60**	1932±65**	6787±151**
	5	426±19**	5486±97**	1477±117**	6755±129**
	15	346±19**	5537±105**	2795±88**	7495±172**

of protein synthesis was determined as incorporation of ^{14}C-glycine, which also was injected intraperitoneally in a dose of 10 μCi/100 g body weight 2 h before sacrifice. All the radioisotopes (from "Izotop," USSR) were used in a final concentration of 37 MBq/ml. The test tissues, immediately after removal, were homogenized in liquid nitrogen. The nucleic acid content in the tissues was determined by a spectrophotometric method [6]. For more complete separation of the nucleic acids, preparatory treatment of the material was carried out by the method in [14] in the modification in [1]. The protein concentration was determined spectrophotometrically after addition of Benedict's solution to the hydrolyzate. The γ-globulins also were isolated from the birds' blood serum by salting out with ammonium sulfate, and they were determined quantitatively by the microbiuret method, and the intensity of their synthesis was estimated on the basis of incorporation of ^{14}C-glycine. A hydrolysate of the nucleic acids or protein, in a volume of 0.5 ml, together with 9.5 ml of scintillation fluid were introduced into vials for determination of the radioactivity of the samples. Radioactivity was determined in a "Mark III" liquid scintillation counter (USA). The experimental results were subjected to statistical analysis by parametric and nonparametric methods [5].

TABLE 3. Effect of Thymalin and Bursalin on Protein Synthesis in Chicks after Exposure to Immunodepressants ($M \pm m$

Experimenteal conditions	Time of investigation, days	Incorporation of ^{14}C-glycine into proteins, cpm/mg protein		
		thymus	bursa of Fabricius	blood serum γ-globulins
Control	1	792±23	1499±38	820±28
	5	842±19	1532±51	864±24
	15	804±22	1513±49	874±41
Injection of cyclophosphamide	1	632±16*	429±19*	325±15*
	5	624±11*	245±21*	172±22*
	15	675±11*	694±27*	385±31*
Injection of cyclophosphamide and thymalin	1	706±15**	461±18	491±36**
	5	776±18**	423±28**	396±29**
	15	784±12**	828±12**	670±28**
Injection of cyclophosphamide and bursalin	1	679±23	824±55**	591±21**
	5	674±16**	899±23**	670±27**
	15	728±19**	1253±54**	793±23**
Injection of hydrocortisone	1	277±12*	1228±31*	478±16*
	5	190±18*	1227±32*	495±28*
	15	406±13*	1178±32*	545±19*
Injection of hydrocortisone and thymalin	1	348±12**	1299±27	614±17**
	5	405±15**	1356±30**	641±26**
	15	630±19**	1365±41**	796±26**
Injection of hydrocortisone and bursalin	1	286±8	1340±22**	779±29**
	5	203±14	1511±45**	807±25*
	15	472±12**	1492±62**	855±19**

EXPERIMENTAL RESULTS

Immunodepression induced by injection of cyclophosphamide and hydrocortisone, was accompanied by a decrease in the cell content and by changes in the composition of the lymphocyte subpopulations of the thymus and bursa of Fabricius (Table 1). However, whereas cyclophosphamide depressed the number of B lymphocytes appreciably in the bursa of Fabricius but had virtually no effect on thymic lymphocytes, injection of hydrocortisone, on the other hand, was accompanied by a predominant decrease in the number of T lymphocytes in the thymus, and there was no change in the numbers or ratio between T and B lymphocytes in the bursa of Fabricius. Injection of cyclophosphamide and of hydrocortisone into the chicks caused a decrease in the intensity of incorporation of ^{3}H-thymidine and ^{3}H-uridine into nucleic acids of the thymus and bursa of Fabricius, and also inhibited incorporation of ^{14}C-glycine into proteins of these organs and into γ-globulins of the birds' blood serum (Tables 2 and 3). Under these circumstances cyclophosphamide had a stronger inhibitory action on DNA, RNA, and protein synthesis in the bursa and on α-globulin synthesis. Hydrocortisone had a stronger effect than cyclophosphamide on the chick thymus. Hence it follows that lymphocytes of the test organs of the birds differ in their sensitivity to the immunodepressive action of cyclophosphamide and hydrocortisone.

During the study of cyclophosphamide as immunodepressant, injection of bursalin into the chicks restored the number of B lymphocytes and nucleic acid and protein synthesis mainly in the bursa of Fabricius. Thymus peptides under these conditions restored the cell composition and activity of synthesis of the biopolymers under these conditions only partially in the thymus and bursa; their action on the bursa, moreover, was weaker than that of bursalin.

When hydrocortisone was used as immunodepressant, injection of thymalin into the chicks led to normalization of the number of T lymphocytes in the thymus and to more effective restoration of the intensity of DNA, RNA, and protein synthesis in that organ compared with the bursa of Fabricius. In turn, bursalin had a stronger stimulating action on the bursa than thymalin.

The results are evidence that thymus cells react more strongly to hydrocortisone and thymus peptides, whereas lymphocytes of the bursa of Fabricius have increased sensitivity to cyclophosphamide and to peptides of the bursa. Considering the significant differences in the cell composition of the thymus and bursa of Fabricius (predominance of T lymphocytes in the thymus and of B lymphocytes in the bursa at different stages of their maturation), it can be postulated that peptides of the thymus restore nucleic acid and protein synthesis predominantly in T lymphocytes whereas peptides of the bursa do so predominantly in B cells and their precursors. This is shown by normalization of the number of T lymphocytes in the thymus and of B lymphocytes in the bursa of Fabricius after injection of thymalin and bursalin into chicks against the background of selective immunodepression caused by injection of hydrocortisone or cyclophosphamide (Table 1).

The stronger depression of γ-globulin synthesis in the chicks after injection of cyclophosphamide (Table 2) can be associated with the predominant action of this immunodepressant on the bursa of Fabricius and, in particular, on the B cells maturing in it. Injection of bursalin and thymalin led to restoration of the intensity of γ-globulin synthesis in the birds. However, peptides of the bursa caused a more significant increase in the γ-globulin level in the chicks' blood serum.

During combined administration of thymalin and bursalin the end result of injection of the preparations was determined chiefly by the initial state of immunodepression. In chicks receiving hydrocortisone the effect typical of thymalin was observed: restoration of the number of T lymphocytes and of nucleic acid and protein synthesis mainly in the thymus. Meanwhile, in birds receiving cyclophosphamide, normalization of the number of B lymphocytes and of RNA and DNA synthesis was observed mainly in the bursa of Fabricius, which is a characteristic effect of bursalin. In some cases the effects of the two preparations appeared to undergo summation, but this was not significant.

Thus thymalin and bursalin can regulate nucleic acid and protein synthesis in cells of the thymus and bursa of Fabricius. Differences in the biological action of the preparations are evidently linked with their effect on different cell populations: thymalin on T lymphocytes, bursalin on B lymphocytes and their precursors. In the presence of selective immunodepression, peptides of the thymus and bursa act mainly on the organs producing them. Thymalin and bursalin can be used as substances for targeted correction of the functions of lymphoid cells and for increasing their resistance under conditions of immunodepression.

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