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ENDOCRINE AND METABOLIC MECHANISMS OF THE PATHOLOGICAL SYNDROME INDUCED BY HOMOLOGOUS GLIAL TISSUE ANTIGENS IN MONKEYS

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Exposure to immunoneurophysiological factors (using antigens of brain origin of different kinds or antisera against them) may lead to significant changes in functions of the CNS. An original model has been developed at the Institute of Pathological Physiology, Medical Faculty, Belgrade University, in which antigens of glial tissue of homologous monkey brain are used as the antigenic stimulus [11]. Immunization with these antigens led to the development of a pathological syndrome including disturbances of CNS functions (changes in the animals' behavior, ability to form new and reproduce old conditioned reflexes, and so on), as well as endocrine disturbances, expressed as depression of thyroid function [4-6, 13].

The aim of the present investigation was to continue the study of some endocrine and metabolic mechanisms of development of this syndrome, including parameters of function of the hypothalamo-hypophyseo-adrenocortical and hypothalamo-hypophyseo-thyroid systems (HHACS and HHTS respectively), and also parameters of serum protein metabolism, investigated in immunized and unimmunized animals at rest and exposed to stress.

EXPERIMENTAL METHOD

Experiments were carried out on six monkeys (*Macaca mulatta*) of both sexes, divided into two equal groups. The animals of group 1 were immunized with complex glial antigen in the form of an emulsion prepared from 0.5 ml of glial tissue homogenate from an animal of the same blood group. 0.25 ml of Freund's complete adjuvant (from "Difco," USA), and 0.21 ml of "Areacel A" mineral oil (from "Serva," West Germany), which was injected intramuscularly in four separate doses with intervals of 1 week (total dose of protein injected about 50 mg).

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TABLE 1. Changes in Serum 11-HCS Concentration (in μg/100 ml) in Response to Stress at Different Times After Completion of Immunization with Glial Antigens

Group of animals	№	After 2nd injection				After 3rd injection				Seven months after completion of immunization			
		time after application of stress, h											
		0	1	2	3	0	1	2	3	0	1/2	1	3
Exptl.	1	40,6	61,4	61,4	59,6*	63,2	82,6*	82,6	104,0	54,4	62,7	58,6	73,2
	2	46,9	85,8	72,2	75,8*	55,5	104,0*	84,1	—	31,4	48,1	46,0	60,6
	3	53,3	90,3	88,5	80,4*	86,7	96,4*	72,8	69,4	52,3	62,7	64,8	73,2
M		46,9	79,1	74,0	71,9	68,4*	94,0*	79,8	86,7	46,03	57,8	56,5	69,0
%		100	168,6	157,8	153,3	100	137,4	116,7	126,7	100	125,6	122,7	149,02
Control	4	51,3	75,3	97,0	74,2	50,3	73,8	73,7	78,0	—	—	—	—
	5	36,1	87,6	63,2	65,0	40,7	78,0	79,8	58,1	—	—	—	—
	6	41,5	56,0	55,1	59,6	53,7	65,9	69,4	78,0	31,4	37,6	48,1	50,3
	7	—	—	—	—	—	—	—	—	52,3	56,5	48,1	62,7
	8	—	—	—	—	—	—	—	—	39,7	46,0	56,5	64,8
M		42,9	73,0	71,8	66,2	48,2	72,2	74,3	71,3	41,1	46,7	50,9	55,9
%		100	170,2	167,4	154,3	100	149,8	154,1	147,9	100	113,6	123,8	136,0

Legend. *P < 0.05 compared with control (unimmunized animals). Here and in Table 2, data analyzed by Wilcoxon–Mann–Whitney nonparametric U test [1].

TABLE 2. Changes in TT_4 (in μg/100 ml), FT_4 (in ng/100 ml), T_3 (in ng/100 ml), and TSH (in micromoles/ml) Concentrations in Response to Stress in Control and Immunized Monkeys

Group of animals	№	Before immunization		After 2nd injection		Seven months after completion of immunization															
						time after application of stress, h															
						0				1/2				1				3			
		TT_4	T_3	TT_4	T_3	TT_4	FT_4	T_3	TSH	TT_4	FT_4	T_3	TSH	TT_4	FT_4	T_3	TSH	TT_4	FT_4	T_3	TSH
Exptl.	1	6,2	165	4,9*	170	3,8*	1,0	219,4	6,7**	4,2	100	188,3	8,7	3,7	1,14	189,7	0,5	4,4	0,9	109,6	2,8
	2	6,3	340	5,4*	310	4,5*	0,83	323,2	4,5**	4,2	0,83	299,6	2,6	4,0	0,75	279,9	4,4	3,7	0,77	236,5	3,6
	3	5,7	237	5,6*	155	4,8*	1,25	222,6	4,6**	4,6	1,40	198,1	0,8	4,4	1,03	211,7	4,4	4,1	1,0	166,2	3,2
M		6,07	247	5,3*	212	4,4*	1,03	255,1	5,3**	4,3	1,08	228,7	4,03	4,0	0,97	227,1	3,1	4,1	0,9	170,8	3,2
Control	6	—	—	—	—	6,9	1,0	329,9	0,7	6,2	0,8	261,1	3,8	6,5	0,95	274,2	0,3	5,8	0,8	173,3	3,1
	7	—	—	—	—	4,5	0,65	357,8	0,3	4,2	0,52	318,3	1,5	4,2	0,79	298,1	0,0	4,2	1,0	314,0	2,7
	8	—	—	—	—	5,3	1,12	258,1	0,2	5,5	1,12	258,6	6,7	4,6	1,25	242,9	6,8	—	—	—	—
M						5,6	0,92	315,2	0,4	5,3	0,81	279,3	4,0	5,1	1,0	271,7	2,4	5,0	0,9	243,7	2,9

Legend. *P < 0.05 compared with corresponding values in same animals before immunization; **P < 0.05 compared with control (unimmunized control).

One month later an intravenous injection of the reacting dose of the antigen was given (5 ml of sterile physiological saline containing glial homogenate in a concentration corresponding to 2.5-5 mg protein, 0.165 ml of a 1% solution of $Alk(O_4)$, and 0.1N NaOH is sufficient quantity to adjust the pH to 6.0), and this was repeated seven times at intervals of 1 month. Animals of group 2 (control) were injected with the corresponding volumes of physiological saline instead of the antigen. Five days after the 2nd injection of the reacting doses of antigen, animals of both groups were subjected to stress by electrical stimulation of the skin in the inguinal region (the voltage at the 1st application was 10 V, during the next four applications 5 V, duration of stimulation 30 sec, intervals between stimuli 30 sec). The experiment was repeated 5 days after the 3rd reacting injection and 7 months after completion of immunization. Blood for determination of 11-hydroxycorticosteroids (11-OHCS), thyroxine, and proteins in the serum was collected from the femoral vein 0, 0.5, 1, 2, and 3 h after stimulation. The 11-OHCS concentration was measured fluorometrically [2]. Concentrations of total thyroxine (TT_4) and free thyroxine (FT_4) were determined by a competitive

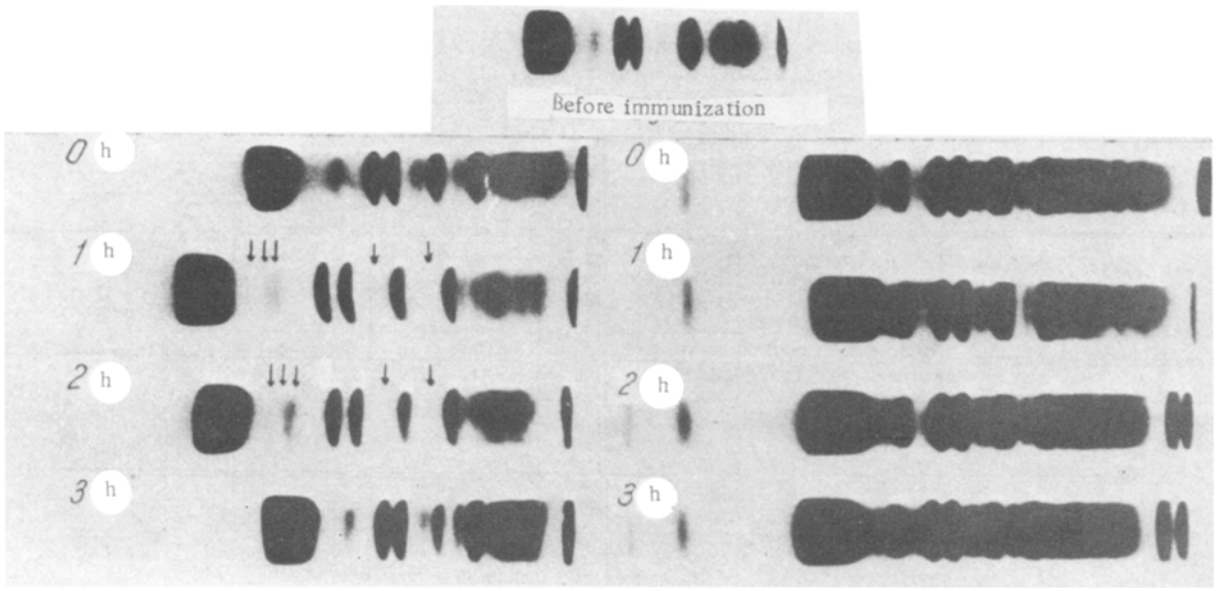

Fig. 1. Disk electrophoresis of serum proteins of monkeys immunized with glial antigens: a) before and after 1st exposure to stress (5 days after 2nd injection of reacting dose of antigen); b) before and after 2nd exposure to stress (5 days after 3rd injection of reacting dose of antigen).

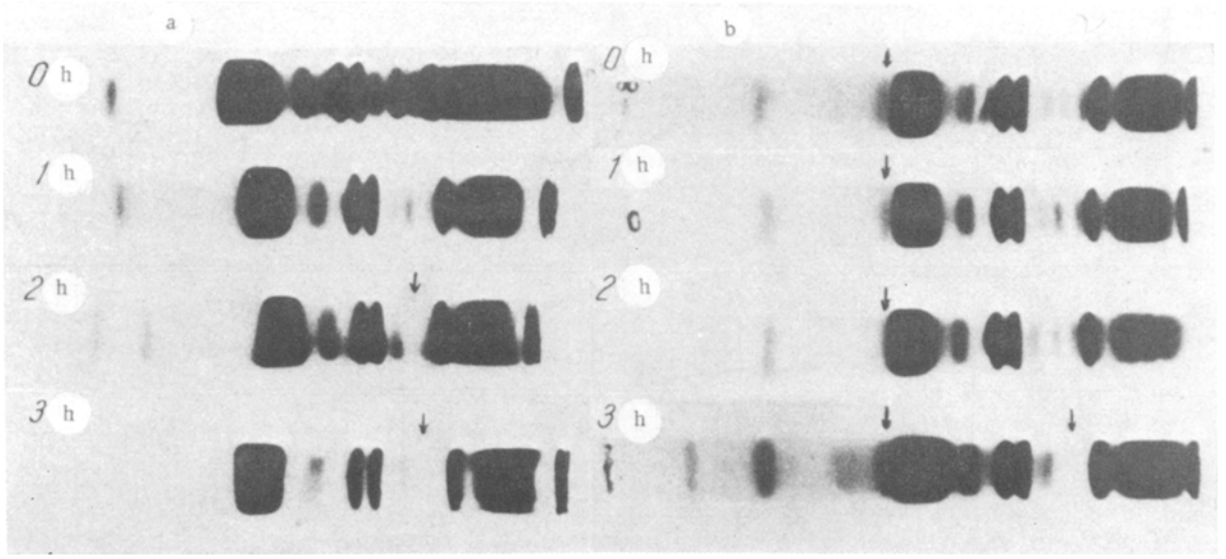

Fig. 2. Disk electrophoresis of serum proteins of unimmunized monkeys: a) before and after 1st exposure to stress; b) after 2nd exposure to stress.

radioreceptor method [7, 12], and the concentrations of tri-iodothyronine (T_3) and throid-stimulating hormone (TSH) were determined with the aid of standard kits of reagents from "Behringwerke AG" and "Byk-Mallinckrodt" (West Germany). Serum proteins were fractionated by disk electrophoresis on 11% polyacrylamide gel by a modified Davis' method [8].

EXPERIMENTAL RESULTS

Investigation of the 11-HCS levels at rest in the control and immunized monkeys showed a significant increase 5 days after injection of the 3rd reacting dose of antigen. Five days after injection of the 2nd reacting dose of antigen, just as 7 months after the end of immunization, this effect was absent (Table 1). Stress regularly led to an increase in the 11-HCS concentration in the control and experimental animals by 36-70% with peak response

after 1-3 h. After the 3rd injection of the reacting dose of antigen the intensity of the hormonal response as a percentage of the initial level was a little lower in monkeys of the experimental group (Table 1).

The serum TT_4 concentration was appreciably lowered after the 2nd injection (Table 2). The lower concentration of this hormone than in the same animals before immunization also was observed 7 months after completion of immunization (Table 2). Seven months after completion of immunization significant differences were not found in the FT_4 concentration between control and experiment, but the TSH level was appreciably higher as the immunized animals. Exposure to stress caused no regular changes in the TT_4, FT_4, T_3, and TSH levels in animals of the two groups.

Immunization of the monkeys with glial antigens led to the appearance of a new prealbumin and an α-globulin serum fraction and also an increase in the intensity of the γ-globulin fraction after injection of the 2nd reacting does of antigen (Fig. 1a).

Stress caused a reversible fall in the content of the serum β-globulin fractions and a decrease in the intensity of the α-fractions in nonimmunized monkeys. On the 2nd exposure to stress 5 days after injection of the 3rd reacting dose of antigen the appearance of a new prealbumin factor was observed in the original sample of serum, and the number of β-globulins was reduced after exposure to stress for 3 h (Fig. 2a, b).

In the immunized monkeys a decrease in the number or intensity of the prealbumin fraction and component of the β-fractions and also the presence of new components of α-globulins were observed during stress (Fig. 1a). After the 2rd injection of antigen an increase in the intensity of all fractions of serum proteins was found, with no change in the number of fractions (Fig. 1b).

Thus significant disturbances of the hormonal functions and parameters of protein metabolism studied were found in animals immunized with glial antigens. The most lasting changes were those in function of HHTS, by contrast with changes in the function of HHACS, which were temporary in character. Parallel with lowering of the total thyroxine level in the blood changes were observed in the spectrum and intensity of the serum protein fractions, especially prealbumin and P-globulin fractions, which include thyroxine-binding proteins [9, 10]. The mechanism of appearance of these disturbances requires further analysis. The permanent character of disturbances of thyroid function may be evidence of the immunologic nature of the phenomena observed. Changes in thyroid function in turn may significantly disturb processes of protein synthesis and metabolism [3]. Changes in the blood serum protein spectrum evidently reflects both purely immunologic processes and those induced by hormonal changes.

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