

KINETICS AND PROPERTIES OF THE CORTISOL-RESISTANT LYMPHOCYTE POPULATION FROM LYMPH NODES OF GUINEA PIGS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Lymphocytes from the cervical lymph nodes of guinea pigs were incubated in medium No. 199 for 24 h in the presence of cortisol in a concentration of 20 or 100 $\mu\text{g}\%$. The survival rate of the lymphocytes and their cortisol metabolism were determined and the nucleic acid content estimated cytophotometrically. A considerable decrease was found in cortisol metabolism by the lymphocytes from the 6th day after addition of an encephalitogenic mixture and there was a marked increase in the cortisol-resistant population of lymphocytes in guinea pigs with encephalomyelitis on the 17th-30th days after injection of the complete adjuvant. Cortisol in a concentration of 100 $\mu\text{g}\%$ lowered the nucleic acid content of the lymphocytes of the intact animals but had no effect on lymphocytes of guinea pigs of the two experimental groups during the period of a considerable increase in the cortisol-resistant population. Progesterone depressed the lympholytic action of cortisol and the metabolism of this hormone by lymphocytes of intact guinea pigs. The ability of progesterone to reduce the lympholytic action of cortisol was weakened in guinea pigs with encephalomyelitis and guinea pigs receiving the adjuvant.

KEY WORDS: *cortisol; cortisol-resistant lymphocyte population; progesterone; nucleic acids; experimental allergic encephalomyelitis.*

The object of this investigation, which is a continuation of a series started earlier [1, 3] was to study the kinetics of the cortisol-resistant lymphocyte population of the lymph nodes of guinea pigs during the development of experimental allergic encephalomyelitis (EAE) and also some properties of this cell population.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male guinea pigs weighing 250-300 g. EAE was induced by injecting 0.2 ml of an encephalitogenic mixture (EM), consisting of a homogenate of rabbit spinal cord and complete adjuvant (2 g spinal cord to 3 ml of adjuvant) into all four footpads. Pareses and paralyzes developed on the 14th-25th day. The control animals received an injection of complete adjuvant or physiological saline. The guinea pigs were killed on the sixth day after the injection of EM or on the second to third day after the appearance of pareses and paralyzes. Lymph nodes were removed from all the guinea pigs and the lymphocytes were isolated and cultured by the method described previously [3] with the addition of cortisol (20 and 100 $\mu\text{g}\%$) and progesterone (10^{-5} M). The survival rate of the lymphocytes, their cortisol metabolism, and their nucleic acid content were determined [3].

EXPERIMENTAL RESULTS

The effects of cortisol on the survival rate of the lymphocytes and the metabolism of the hormones are shown in Table 1. Clearly on the sixth day after injection of the EM the survival rate of the cells was the same as in the control although the intensity of metabolism was significantly lower. During the period of development of pareses and paralyzes the cortisol-resistant population of lymphocytes was considerably increased, and this also was ac-

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TABLE 1. Effect of Various Concentrations of Cortisol on Survival Rate of Lymphocytes and on Cortisol Metabolism by Lymphocytes of Control Guinea Pigs and of Guinea Pigs with EAE

Group of animals	Number of animals	Survival rate of lymphocytes, %		Metabolism of cortisol, $\mu\text{g}/10^7$ lymphocytes	
		20 $\mu\text{g}\%$	100 $\mu\text{g}\%$	20 $\mu\text{g}\%$	100 $\mu\text{g}\%$
Intact	32	73,2 \pm 1,60	57,3 \pm 1,74	0,401 \pm 0,017	0,931 \pm 0,040
On 6th day after injection of EM	6	76,13 \pm 2,7	54,38 \pm 3,71	0,145 \pm 0,020	0,439 \pm 0,041
P		>0,05	>0,05	<0,001	<0,001
On 2nd-3rd day of appearance of pareses and paralyses	11	95,5 \pm 1,22	86,3 \pm 1,56	0,244 \pm 0,049	0,515 \pm 0,03
P		<0,001	<0,001	<0,01	<0,001
On 17th-30th day after injection of complete adjuvant	7	92,5 \pm 1,43	78,3 \pm 1,26	0,115 \pm 0,007	0,601 \pm 0,08
P		<0,001	<0,001	<0,001	<0,001

TABLE 2. Changes in RNA Content during Incubation of Lymphocytes from Lymph Nodes of Intact Guinea Pigs and of Guinea Pigs with EAE

Group of animals	Number of animals	RNA content per lymphocyte, in conventional units			
		before incubation	after incubation for 24 h		P
			without cortisol	with cortisol (100 μg%)	
Intact	5	14,35±0,115	14,04±0,127	12,22±0,168	<0,001
EAE	5	14,96±0,45	14,96±0,33*	14,44±0,43	>0,1
On 17th-30th day after injection of complete adjuvant	5	15,01±0,18*	15,26±0,26*	15,34±0,25	>0,1

*Result differs significantly from corresponding result in intact guinea pigs.

accompanied by a significant decrease in the intensity of cortisol metabolism. According to Pytskii [2], the concentration of 11-hydroxycorticosteroids in the blood plasma of guinea pigs with EAE is increased and, for that reason, the increase in the percentage of surviving lymphocytes could have been due to the lytic action of cortisol in vivo on the lymphocytes. However, in guinea pigs receiving the complete adjuvant only, the cortisol-resistant population of lymphocytes also was increased and the cortisol metabolism was depressed, although the concentration of cortisol in the blood plasma of these animals did not increase after injection of the adjuvant. It can accordingly be concluded that the increase in the cortisol-resistant population was an independent process connected with the action of immune mechanism.

Glucocorticoids, whether in vivo [4, 6] or in experiments in vitro [7], are known to reduce the RNA content in lymphocytes, partly on account of a decrease in the activity of RNA polymerase [5, 8]. However, it is not yet clear whether nucleic acid synthesis is depressed in all lymphocytes or whether there are subpopulations of lymphocytes in which this synthesis undergoes little change. Accordingly it was decided to investigate the effect of cortisol on the nucleic acid content in the lymphocytes of guinea pigs with EAE.

The experiments showed (Table 2) that under the influence of cortisol the nucleic acid content in the lymphocytes of intact animals decreased significantly, whereas in the lymphocytes of guinea pigs with EAE and guinea pigs receiving complete adjuvant, their content was unchanged during the period when the cortisol-resistant population of lymphocytes was increased (Table 1). It is interesting to note that under the same conditions, the nucleic acid content in the lymphocytes of guinea pigs sensitized with protein decreased during the period of increase of the cortisol-resistant lymphocyte population [3]. The nucleic acid content is thus changed differently by cortisol in different lymphocyte populations. The cortisol-resistant lymphocyte population also is heterogeneous with respect to this feature. This state of affairs must be taken into account when the effect of glucocorticoids on nucleic acid synthesis in the total lymphocyte pool obtained from lymphoid organs is estimated. It must also be pointed out that during incubation of lymphocytes from guinea pigs with EAE and receiving the adjuvant but without cortisol, although there was no increase in the nucleic acid content, their content was nevertheless higher than in the lymphocytes of intact animals (Table 2).

TABLE 3. Effect of Progesterone on Survival Rate of Lymphocytes and Cortisol Metabolism by Lymphocytes of Intact Guinea Pigs and Guinea Pigs with EAE ($M \pm m$)

Group of animals	Number of animals	Survival rate of lymphocytes, %			Metabolism of cortisol, $\mu\text{g}/10^7$ lymphocytes		
		cortisol (100 $\mu\text{g}\%$)	cortisol + progesterone	P	cortisol (100 $\mu\text{g}\%$)	cortisol + progesterone	P
Intact	11	52,9 \pm 2,72	78,4 \pm 2,30	<0,001	1,023 \pm 0,07	0,641 \pm 0,12	<0,01
EAE	11	86,3 \pm 1,56	92,5 \pm 2,80	>0,05	0,463 \pm 0,04	0,211 \pm 0,064	<0,01
On 17th-30th day after injection of complete adjuvant	7	78,3 \pm 1,26	91,5 \pm 1,50	<0,001	—	—	—

In this investigation, just as in a previous one with guinea pigs sensitized with protein [3], the action of progesterone, which competes with cortisol for receptors in the cytoplasm of the target cells [9], was tested. The ability of progesterone, in a concentration of 10^{-5} M, to inhibit the destruction of lymphocytes and its effect on cortisol metabolism were investigated. The results showed (Table 3) that although progesterone reduced the lympholytic action of cortisol in guinea pigs with EAE by 6.2%, the degree of this decrease was much smaller (by 25.5%) than in intact animals. Meanwhile the degree of inhibition of cortisol metabolism was not reduced in the guinea pigs of the experimental group.

It is considered [10] that the lympholytic action of cortisol is effected only after this hormone, after binding with the cytoplasmic receptor, has passed into the nucleus. The protective action of progesterone is absent in guinea pigs with EAE, which suggests that some change in the receptors binding cortisol plays a role in the mechanism of the resistance of the lymphocytes of cortisol in EAE. The resistance of lymphocytes to cortisol in guinea pigs sensitized with protein [3] is based on a different mechanism, for in that case the protective action of progesterone is not reduced. In turn, the inhibition of cortisol metabolism which develops as an early feature of various types of allergic processes has its own special mechanism, although its initial stage is also the binding of cortisol to the cytoplasmic receptor.

LITERATURE CITED

1. D. S. Donadze, "Activity of cortisol in guinea pigs with experimental allergic encephalomyelitis," Candidate's Dissertation, Moscow (1972).
2. V. I. Pytskii, Vestn. Akad. Med. Nauk SSSR, No. 2, 86 (1967).
3. V. I. Pytskii and E. É. Arutyunova, Byull. Éksp. Biol. Med., No. 1, 68 (1977).
4. J. Drews, Europ. J. Biochem., 7, 200 (1969).
5. K. E. Fox and J. D. Gablurel, Mol. Pharmacol., 3, 479 (1967).
6. M. H. Makman, B. Dvorkin and A. B. White, J. Biol. Chem., 243, 1485 (1968).
7. M. H. Makman, S. Nakagawa, and A. B. White, Recent Prog. Horm. Res., 23, 195 (1967).
8. S. Nakagawa and A. B. White, J. Biol. Chem., 245, 1448 (1970).
9. H. H. Samuels and G. M. Tomkins, J. Mol. Biol., 52, 57 (1970).
10. E. B. Thompson and M. E. Lippman, Metabolism, 23, 159 (1974).