EFFECT OF ETHIMIZOLE ON THE FORMATION OF POSTVACCINAL IMMUNITY TO TUBERCULOSIS

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The neurotropic drug ethimizole, which excites hypothalamic neurons, weakened the intensity of tuberculin allergy in mice immunized with BCG and neutralized the immunizing action of the vaccine in immunized mice infected with Mycobacterium tuberculosis. These results demonstrate the importance of the functional state of the hypothalamus in the formation of postvaccinal immunity to tuberculosis.

Functional inhibition of certain structures in the posterior (mamillary region) and central (medial nuclei) zones of the hypothalamus is observed in animals infected with tuberculosis and also in animals immunized with BCG vaccine [4-6]. This evidently is an important factor in the formation of immunity to tuberculosis. This inhibition is abolished by ethimizole (ethylnorantiphein; an imidazole alkylamide) [7]. This compound is one of a group of antipheins synthesized in the Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, which excites neurons of subcortical (especially hypothalamic) brain-stem structures [2, 3, 8].

In this investigation the effect of ethimizole was studied on a number of indices of immunity to tuberculosis.

EXPERIMENTAL METHOD

Of a total of 116 mice weighing about 20 g 18 were unimmunized and 98 immunized by subcutaneous injection of BCG vaccine in a dose of 0.05 mg per mouse. Of the experimental animals, 51 received ethimizole in therapeutic doses (20 mg/kg daily) while 47 did not receive the drug (control). The compound was injected subcutaneously from the 1st to the 10th and 21st to the 30th days after immunization. On the 40th day after immunization the intensity of tuberculin allergy was tested quantitatively by the authors' method. An injection of 0.1 ml tuberculin PPD-L (1:10) was given into the skin of the plantar surface of the right hind limb by Grey's method, and 0.1 ml physiological saline was injected into the left limb. The intensity of the action was estimated after 48 h by means of the MK-0-25 mm micrometer.

The middle third of the foot was placed with its plantar surface on the fixed stage of the micrometer. The rotor of the instrument was turned until smooth engagement of the ratchet was obtained, and in this way the thickness of the paw was measured in the region examined. The difference between the mean (of three measurements) dimensions of the right and left paws, expressed in 100ths of a millimeter, gave the reaction of the particular animal to tuberculin in quantitative terms. Some of the animals (21 experimental and 21 control) were sacrificed and the weight of the lungs and spleen determined. The remaining mice, as well as 18 intact unvaccinated animals, were infected intravenously with a virulent culture of Mycobacterium tuberculosis (strain Bov. 109) in a dose of 0.1 mg and sacrificed on the 7th and 40th days after infection. The state of immunity to tuberculosis was judged from the extent of the lesions in the lungs and spleen, allowing for the weight of the organs. These organs were investigated histologically in material stained with hematoxylineosin and for RNA by Brachet's method.

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TABLE 1. Effect of Ethimizole on Indices of Immunobiological Response in Mice Vaccinated with BCG

Index	Before infection		7 Days after infection			40 Days after infection		
	BCG (control)	BCG (ethimizole)	intact animals	BCG (control	BCG (ethimizole)	intact animals	BCG (control)	BCG (ethimizole)
Intensity of tuberculin allergy (in 100ths of a millimeter)								
$M \pm m$	32±2,6	21±2,0	—	—	—	—	—	—
P	—	<0,01						
Weight of lungs (in mg)								
$M \pm m$	280±14	210±7	220±20	211±9,3	204±6,8	400±21	320±10	380±16
P	—	<0,001	>0,1	—	>0,05	<0,01	—	<0,01
P_1					>0,05	—		>0,1
Weight of spleen (in mg)								
$M \pm m$	250±12	170±9	204±36	248±19,4	190±11,3	350±25	320±32	190±12
P	—	<0,001	<0,05	—	<0,05	>0,1	—	<0,01
P_1			—		>0,1	—		<0,001

Legend: P denotes significance of difference relative to immunized control mice, P_1 the same relative to intact mice.

EXPERIMENTAL RESULTS

In immunized mice ethimizole reduced the intensity of tuberculin allergy and also the weight of the spleen (Table 1), indicating that the drug depresses the immunobiological response.

On the 7th day after infection the lungs of the unvaccinated animals as a rule were unchanged. Only in solitary cases were small clusters of lymphocytes and histiocytes found around the blood vessels and bronchi, and foci of pneumonia occupied individual alveoli, from which the exudate contained leukocytes, lymphocytes, and macrophages carrying out phagocytosis of tubercle bacilli. Moderate hyperplasia was observed in the spleen. In sections stained by Brachet's method RNA-containing cells were observed mainly in the red pulp.

In the animals infected after immunization, large areas of peribronchial and perivascular infiltration, containing histiocytes, lymphocytes, and plasma cells, giving a positive reaction for RNA, and also clusters of RNA-containing macrophages and lymphocytes in the alveoli, were demonstrated in the lungs. The spleen was enlarged (Table 1) and exhibited marked hyperplasia of the follicles and proliferation of the red pulp, with numerous pyroninophilic cells in the latter and in the marginal zones of the follicles. Some follicles contained tiny specific epithelioid granulomas.

In the immunized mice receiving ethimizole the changes in the lungs and spleen on the 7th day after infection were not significantly different from those in the unimmunized animals.

Ethimizole thus strongly inhibits the development of immunomorphological reactions observed in immunized mice during the first days after infection.

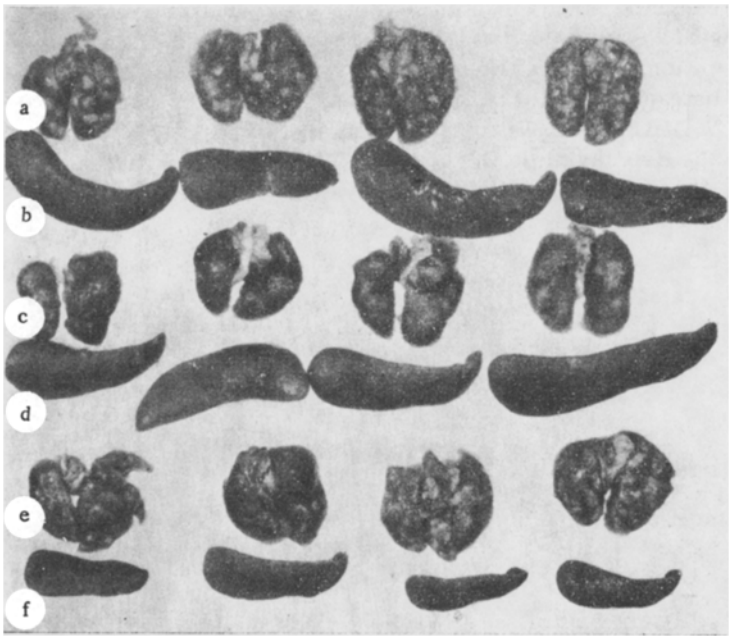

Fig. 1. Lungs and spleen of mice on 40th day after infection with 0.1 mg of a culture of M. tuberculosis. Multiple large tubercles in lungs of mice immunized with BCG and receiving ethimizole before infection (e) and in control unimmunized animals (a); single tubercules in lungs of control immunized mice (c). Marked hyperplasia of spleen in control animals (intact and immunized – b and d) and absence of hyperplasia in mice receiving ethimizole (f).

By the 40th day after infection the process had progressed both in the mice receiving ethimizole and in the unimmunized animals, as could be seen even with the unaided eye (Fig. 1). Histological investigation revealed multiple large, partially confluent foci of tuberculous pneumonia in the lungs. Only a few small foci were found in the immunized mice not receiving ethimizole.

The morphological picture of the spleen at this period after infection varied significantly in the animals of the different groups. Multiple epithelioid granulomas had developed in the unimmunized mice, mainly in the follicles, while in the immunized mice paraspecific changes were predominant. In the mice receiving ethimizole, both specific and paraspecific morphological reactions were inhibited and the weight of the spleen was reduced.

Ethimizole thus largely neutralized the immunizing effect of BCG vaccine. This is in agreement with the authors' views regarding the role of inhibition of the mamillary region and medial structures of the central hypothalamus in the formation of immunity to tuberculosis.

When the mechanism of the immunodepressive action of ethimizole is analyzed, it is essential to take into account the evidence for the relationship between these hypothalamic structures and the regulation of ACTH secretion [9-11] and for the marked stimulant action of ethimizole on the secretion of ACTH and glucocorticoids [8], which evidently have an inhibitory action also on immunogenesis.

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