ROLE OF CERTAIN HYPOTHALAMIC STRUCTURES IN REGULATION OF IMMUNOBIOLOGICAL PROCESSES DURING IMMUNIZATION WITH BCG VACCINE

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Destruction of the posterior hypothalamic nucleus in rabbits immunized with BCG vaccine lowered their sensitivity to tuberculosis. After immunization with a large dose of BCG vaccine, the number of neurons with spontaneous activity in the posterior hypothalamic nucleus was increased, but in the region of the mamillary bodies it was reduced.

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Recent investigations have demonstrated the important role of the posterior hypothalamus in the regulation of immunogenesis [3, 6-8]. A factor of definite importance for this regulation is hypophyseal ACTH, the production of which, according to some observations [11-16], is regulated by basal structures of the posterior hypothalamus (in the region of the mamillary bodies).

In the present investigation the effect of stereotaxic injury to the posterior hypothalamic nucleus on some immunobiological processes was studied in rabbits immunized with BCG vaccine (reactivity of the peripheral nervous structures to tuberculin, dynamics of the primary vaccinal focus, Mantoux test). The functional state of the posterior hypothalamic structures was also investigated in rabbits immunized with a large dose of BCG vaccine.

EXPERIMENTAL METHOD

The experiments of the first stage of the investigation were carried out on 23 rabbits weighing 2.5-3 kg. Destruction of the posterior hypothalamic nucleus was carried out on ten animals by means of a stereotaxic apparatus, and in two rabbits the brain damage was localized to the region of the thalamus. Seven days after the operation the experimental animals, and also six control rabbits, were immunized with 1 mg BCG vaccine, injected into the skin of the hind limb. Five rabbits remained intact and acted as an additional control. The vaccinated animals were used in the experiment between 15 and 40 days after injection of the vaccine.

In an acute experiment under urethane anesthesia (0.9 g/kg intramuscularly) afferent activity was recorded from the genicular branches of the saphenous nerve in both hind limbs. The potentials were amplified by means of a type UBP1-02 ac amplifier and recorded on a type MPO-2 loop oscillograph.

After the background afferent activity in an area of skin supplied by the genicular branch of the saphenous nerve had been recorded for 30 sec, 0.2 ml of a solution of purified whole tuberculin was injected intradermally. Impulses evoked by injection of tuberculin were recorded during the first 10 sec and 30 sec and 1, 2, 3, 5, and 10 min after injection of tuberculin.

The results were read by visual counting of the number of spikes, and statistical analysis was carried out by Student's method. Altogether 59 injections of tuberculin were studied.

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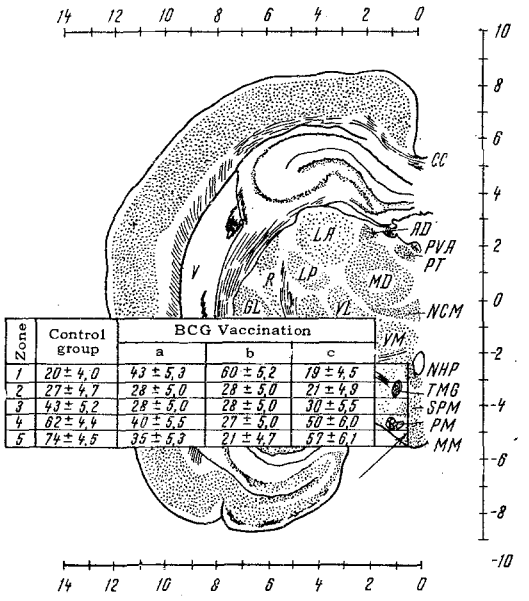

Zone	Control group	BCG Vaccination a	b	c
1	20 ± 4,0	43 ± 5,3	60 ± 5,2	19 ± 4,5
2	27 ± 4,7	28 ± 5,0	28 ± 5,0	21 ± 4,9
3	43 ± 5,2	28 ± 5,0	28 ± 5,0	30 ± 5,5
4	62 ± 4,4	40 ± 5,5	27 ± 5,0	50 ± 6,0
5	74 ± 4,5	35 ± 5,3	21 ± 4,7	57 ± 6,1

Fig. 1. Frontal plane AP 3 (from Fifkova and Marsala's atlas of the rabbit's brain). Electrode track (indicated by arrow) and its subdivision into zones of 600 μ illustrated together with functional state of each zone in one of the control groups and at various times after immunization with BCG. a) 7-10th day; b) 25-30th day; c) 60-70th day.

The experiments of the second stage of the investigation were carried out under acute conditions on 22 unanesthetized rabbits weighing 2.5-3 kg, immobilized with listhenon, 7-10, 25-30, and 60-70 days after intradermal injection of 10 mg BCG vaccine. At each of these times four rabbits took part in the experiment. Control tests were carried out on intact animals (two groups, five rabbits in each).

Using stereotaxic coordinates, a tungsten microelectrode (tip diameter 4-10 μ) was inserted into the region of the posterior hypothalamic nucleus. Next, in steps of 60 μ, the electrode was moved by means of a micromanipulator from above downward along a track 3000 μ in length in the posterior hypothalamus. At each step the presence or absence of unit activity was determined in recordings made by means of an electrophysiological apparatus manufactured at the experimental workshops of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. The track studied (3000 μ) was divided conventionally into five zones, each 600 μ long (Fig. 1). The index of the functional state of each zone was the ratio (in %) between the number of points at which spike activity was detected and the total number of points tested in that zone in all animals of the group. The experimental and control results were compared and the statistical significance of the observed differences determined. Altogether 2083 points were tested in the posterior hypothalamus.

At the end of the experiments the location of the brain damage and the correctness of positioning of the electrode track in the hypothalamus were verified histologically (Fig. 2).

EXPERIMENTAL RESULTS

The results of the experiments of the first stage of the investigation showed (Fig. 3) that injection of tuberculin into intact animals had virtually no effect on the frequency of afferent impulses recorded from the nerve. Injection of tuberculin into vaccinated rabbits caused a definite, statistically significant increase in the flow of afferent impulses which persisted up to 3 min of observation inclusive. The same results were obtained after injection of tuberculin into rabbits with brain damage outside the posterior hypothalamic nucleus, the reaction to tuberculin in this case being indistinguishable from the reactions of intact, vaccinated animals. In rabbits with destruction of the posterior hypothalamic nucleus there was a statistically significant weakening of the reaction to tuberculin compared with the reaction of intact vaccinated rabbits and of animals with brain damage located outside the posterior hypothalamic nucleus. This difference in the intensity of electrical activity was observed for 3 min, after which it disappeared.

Macroscopic investigation of the primary vaccinal focus revealed some differences between its course in rabbits with a destroyed posterior hypothalamic nucleus compared with immunized animals of the other groups. The focus was larger in size, and areas of necrosis appeared in it sooner. The intensity of the Mantoux reaction (1 : 10) in the animals with destruction of the posterior hypothalamic nucleus was basically the same as in the intact rabbits, but in the former a positive reaction appeared on the average 7 days later.

During the experiments to study the functional state of posterior hypothalamic structures in immunized animals, the basic assumption was that in the case of excitation of these structures the number of active points on the electrode track would be increased, and during inhibition it would be reduced [5]. The experimental results showed (Fig. 1) that a statistically significant increase in the number of spontaneously active neurons took place in the vaccinated rabbits compared with the intact animals. The difference was most marked on the 25th-30th day after immunization, and by the 60th-70th day it was no longer statis-

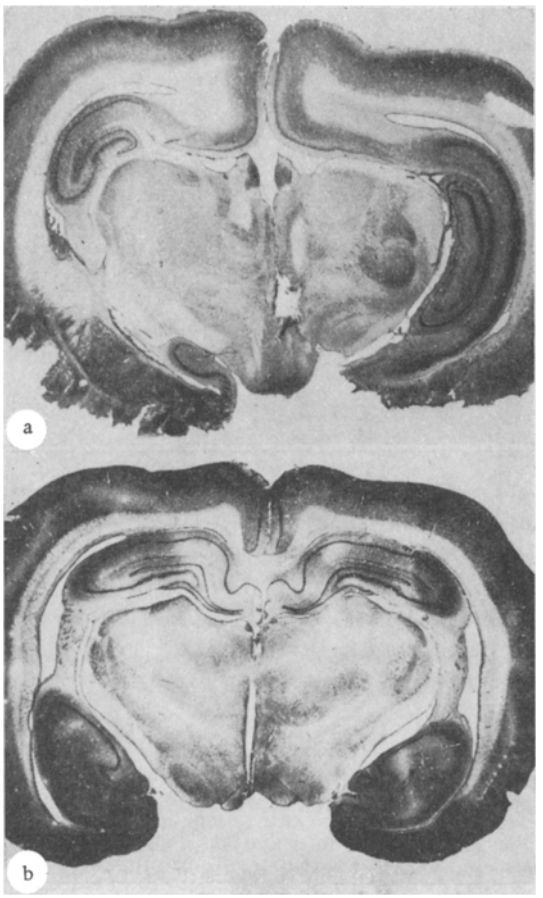

Fig. 2. Frontal sections through brain at level of posterior hypothalamus: a) Brain damage in region of posterior hypothalamic nucleus; b) track of microelectrode through posterior hypothalamic nucleus, supramamillary region, and region of mamillary bodies.

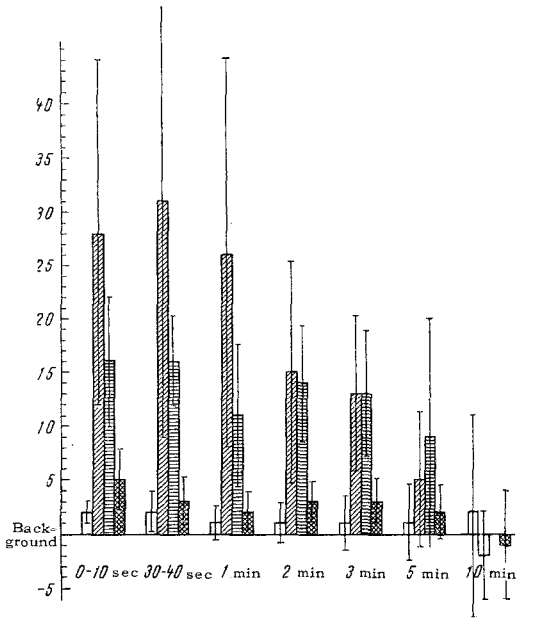

Fig. 3. Effect of tuberculin on afferent impulse flow in regional nerve. Abscissa, time after injection of tuberculin; ordinate, increase in afferent activity (in spikes/sec). Unshaded columns represent intact rabbits; obliquely shaded columns vaccinated rabbits; cross-hatched columns vaccinated rabbits with destruction of posterior hypothalamic nucleus; horizontally shaded columns vaccinated rabbits with destruction of other brain regions.

tically significant. In zones 2 and 3 (supermamillary region) more significant differences were observed between the activity of the vaccinated and intact rabbits. In the mamillary region (zones 4 and 5) a definite and statistically significant decrease in the number of active points was found in the vaccinated rabbits compared with the corresponding control values. The decrease in activity of these zones appeared on the 7th-10th day and was most marked on the 25th-30th day after injection of the vaccine. By the 60th-70th day after immunization the differences were no longer statistically significant except in zone 5, where, although much reduced, they still remained statistically significant.

The results of these experiments showed that during immunization by BCG vaccine the sensitivity of peripheral nervous structures to tuberculin is increased, in agreement with many observation by Soviet investigators [1, 3, 4].

The diminished reaction of peripheral nervous structures to tuberculin, the change in the dynamics of the primary vaccinal focus, and some decrease in the intensity of the Mantoux reaction observed in rabbits with destruction of the posterior hypothalamic nucleus, and also the increase in activity of this nucleus in rabbits immunized with BCG vaccine suggest that the posterior hypothalamic nucleus plays a role in the development of immunity in response to BCG vaccination. This conclusion is in agreement with results obtained by Korneva and Khai [6-8], who demonstrated the importance of the posterior hypothalamic nucleus in the regulation of immunogenesis.

When discussing the results of investigation of the functional state of the mamillary region, the relationship of this structure to stress mechanisms and to the regulation of ACTH secretion must be remembered.

There can be no doubt that the procedures to which the animals were subjected in the course of the experiment (operation, fixation in the stereotaxic apparatus, muscle relaxants, and so on) produced a marked state of stress. This evidently must account for the high activity of the mamillary region in the intact animals. This is in agreement with the observations of Porter [15], who found an increase in the electrical activity of the mamillary region during procedures causing stress.

The decrease in number of spontaneously active neurons in the mamillary region of animals immunized with a large dose of BCG vaccine is evidence of lowered reactivity of this structure relative to stress-producing procedures and supports the view that hypothalamic mechanisms of stimulation of ACTH secretion are inhibited during immunogenesis evoked by BCG vaccination.

The validity of this conclusion is confirmed by experimental and clinical data indicating depression of the function of the pituitary – adrenal cortex system in tuberculosis and in certain other infectious and allergic diseases [2, 9, 10, 17].

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