ANTIBODY-FORMING CELLS OF THE RAT SPLEEN AFTER INJURY TO THE MIDBRAIN

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The number of plaque-forming cells (PFC) in the spleen of rats immunized with sheep's red blood cells (SRBC) after injury to the anterior or posterior part of the medial hypothalamus and also of the thalamus did not differ significantly from the number of PFC in the spleen of intact animals. The titers of hemolyzing and hemagglutinating antibodies in the animals with injuries to the midbrain were a little lower than in intact rats. The decrease in the quantity of circulating antibodies was not connected with the location of the foci of injury but was evidently a consequence of the craniocerebral trauma.

KEY WORDS: hypothalamus; thalamus; antibody-forming cells; antibodies.

Evidence is continually being obtained that the hypothalamus contains no structures specially regulating the magnitude of the immune response and whose inactivation would significantly reduce the intensity of immunogenesis [1, 8-11], as some workers have suggested. Nevertheless, for a final solution to the problem of the role of the hypothalamus in the regulation of immunogenesis, it was necessary to study whether injury to individual parts of the hypothalamus affects the function of the antibody-forming cells directly, for the quantity of circulating antibodies, which was determined in most investigations of this problem [3, 4, 7-12], depends on several factors not directly related to immunogenesis.

In this investigation the production of antibody-forming (plaque-forming) cells (PFC) was studied in the rat spleen after injury to certain structures of the midbrain.

EXPERIMENTAL METHOD

Experiments were carried out on 140 Wistar rats weighing 180-200 g. Structures of the midbrain were injured electrolytically, symmetrically on both sides, in animals of three groups using an electrode 0.4 mm in diameter (dc, 1.5 mA, duration of action 10 sec). In the animals of group 1 the anterior part of the hypothalamus in plane AP-0 [5] was injured at a depth of 7 mm from the brain surface and 0.8 mm laterally to the midline, in the region of the paraventricular and supraoptic nuclei. In the rats of group 2 the posterior part of the hypothalamus in plane AP-3 [5] was injured at a depth of 7 mm from the brain surface and 0.8 mm laterally to the midline in the region of the posterior hypothalamic nucleus. In the animals of group 3 the thalamus was injured in plane AP-3 [5] at a depth of 5 mm from the brain surface and 0.8 mm laterally to the midline in the region of the thalamus. The animals were immunized 11-12 days after the operation by intraperitoneal injection of sheep's red blood cells (SRBC) in a dose of 10^9 cells. The animals were killed 3, 4, 5, 7, and 14 days after immunization by exsanguination and the number of PFC in the spleen was determined [6]. The quantity of hemolyzing and hemagglutinating antibodies against SRBC in the blood serum also was determined by the usual method. Intact animals were immunized and investigated by the scheme described above. All the rats were weighed before the operation and the intact rats 11-12 days before immunization and at the end of the experiment. On the basis of these findings the mean daily increase in weight during the period of the experiments was calculated in per cent for the animals of the four groups, which were kept on a standard diet. The brain of the animals undergoing the operation was investigated histologically to verify the location of the injuries.

EXPERIMENTAL RESULTS

In the group of intact animals the number of PFC 3 days after immunization was $119 \pm 22/10^6$ nucleated spleen cells (Table 1), and later it increased to reach a maximum (755 ± 48) 5 days after immunization, when

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TABLE 1. Number of PFC in Spleen per 10^6 Nucleated Cells (M ± m)

Group of animals	Number of animals	Time after immunization, days				
		3	4	5	7	14
Intact	35	119±22	665±51	755±48	97±19	35±8
Injury to anterior hypothalamus	35	103±29	641±72	710±65	73±21	26±11
Injury to posterior hypothalamus	35	127±32	680±62	788±59	86±15	42±19
Injury to thalamus	35	98±19	710±55	806±68	105±18	29±12

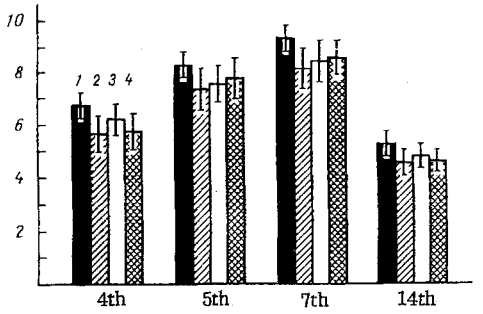

Fig. 1

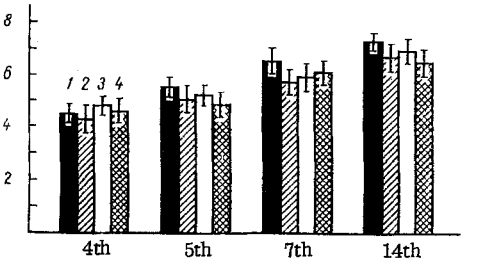

Fig. 2

Fig. 1. Titers of hemolyzing antibodies (M ± m). Abscissa, time after immunization (in days); ordinate, titers of hemolysins (in $\log_2$). Here and in Fig. 2: 1) intact animals; 2) injury to anterior hypothalamus; 3) injury to posterior hypothalamus; 4) injury to thalamus.

Fig. 2. Titers of hemagglutinating antibodies (M ± m). Abscissa, days after immunization; ordinate, titers of hemagglutinins (in $\log_2$).

it began to fall quickly. The curve of the rise and fall in the number of PFC in the three groups of animals undergoing the operation was similar in shape to that of the intact animals. The maximal number of PFC 5 days after immunization in the animals with injuries to the anterior hypothalamus was only a little lower, and in animals with injury to the posterior hypothalamus and thalamus only a little higher than in the intact animals. The difference between the number of PFC in the groups of injured and intact animals at all times of determination was not statistically significant ($P > 0.05$).

In most animals, whether undergoing the operation or not, hemolysins appeared 4 days after immunization. Their titers were maximal 7 days after immunization, and after 14 days they had decreased (Fig. 1). The hemolysin titers in all groups of animals undergoing the operation, regardless of the position of the foci of injury, were a little lower than in the intact animals, by not more than 1.1 $\log_2$ unit. The difference was not statistically significant because of differences in the individual immunological reactivity of the animals.

Hemagglutinating antibodies were detected in the animals of all groups 4 days after immunization, but later their titers increased gradually (Fig. 2). The hemagglutinin titers in the rats with injury to the midbrain were lower, but not significantly, than in the intact animals.

The rate of increase in weight of the intact animals during the period of observation was considerably greater than in all groups of animals undergoing the operation. The mean daily increase in weight of the intact rats was 0.95 ± 0.08%, in the group with injury to the anterior hypothalamus it was 0.38 ± 0.04%, in animals with injury to the posterior hypothalamus 0.49 ± 0.05%, and in animals with injury to the thalamus 0.44 ± 0.03%. The difference between the mean daily increase in weight in all animals undergoing and not undergoing the operation was significant ($P < 0.05$).

These results do not confirm the substantial decrease in the number of PFC in the spleen of rats immunized after injury of their posterior hypothalamic nucleus described previously [2]. On the contrary, they are evidence that localized injuries both to the hypothalamus and to the thalamus have no significant effect on the production of splenic antibody-forming cells in vitro. The small decrease in the antibody titers in all groups of animals undergoing the operation was not connected with injury to the components of a hypothetical midbrain system regulating immunogenesis, but with a consequence of the craniocerebral trauma which, bearing in mind the considerable reduction in the gain in weight, must have caused a disturbance of metabolism, with the result that the conditions for antibody biosynthesis in vivo were impaired.

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EFFECT OF ENZYMES OF THE CONTACT PHASE OF BLOOD CLOTTING ON PHAGOCYTIC ACTIVITY OF THE NEUTROPHILS

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The effect of the product of the contact phase of blood clotting (CAP), i.e., factor XII and its activated form on the reaction of reduction of nitro-BT by human neutrophils was studied. In all cases CAP caused marked stimulation of the neutrophils. The response of the neutrophils to factor XII was observed irregularly. It was more regular and stronger after activation of this factor. The indirect effect of CAP on neutrophils is postulated, through activation of interconnected enzyme systems of the blood plasma.

KEY WORDS: neutrophils; phagocytosis; contact activation product.

Various humoral factors of the blood plasma participate in the regulation of metabolic processes maintaining the phagocytic function of the neutrophils. Activation products of the complement system [2, 3], the α_2-glycoprotein fraction [9, 11, 13], C-reactive protein [4], fetuin [13], and the kallikreins [5] are all stimulators of neutrophils. The functional state of the neutrophils has been observed to change during incubation of blood in glassware not treated with silicone. Accordingly, the investigation described below was undertaken to study the mechanism of this phenomenon, namely the possibility of its mediation through factors participating in the contact phase of blood clotting.

EXPERIMENTAL METHOD

Contact activation product (CAP) was obtained from human blood plasma by the method of Schoenmakers et al. [12]. The eluate from the glass was concentrated with dry Sephadex G-50. The glycine buffer was changed for isotonic 0.15 M phosphate buffer, pH 7.2, on a column with Sephadex G-25. The preparation had the property of restoring the defective clotting of plasma not containing contact factors, obtained by Nossel's method [8].

Partially purified factor XII (F-XII) – fraction IVS – also was obtained from bovine plasma [12]. During electrophoresis in polyacrylamide gel three protein zones were detected in it. The protein content in the zone capable of correcting the defective clotting of plasma deprived of contact factors was about 80% of the total pro-

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