EFFECT OF ELECTROLYTIC INJURY TO THE POSTERIOR HYPOTHALAMUS ON PRODUCTION OF ANTIBODIES AGAINST DNA

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Experiments on rats, in which the posterior hypothalamus was damaged electrolytically by means of a stereotaxic apparatus, revealed an increase in production of normal antibodies against DNA.

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Tissue proliferation is accompanied by death of some cells, and this is characteristic not only of tumor tissue, but also of normal tissue [4]. In the living organisms it is thus possible for nucleoprotein material both from dying normal cells and from mutant cells to enter the blood stream. On this basis, the existence of regulatory processes in the body directed toward maintenance of genetic constancy of the tissues can therefore be postulated. Immunologic reactions may be related to this process, because the presence of antibodies against DNA has recently been demonstrated in the sera of healthy persons and patients [5-7, 9].

Because the posterior hypothalamic structures are predominantly concerned with the regulation of immunogenesis [3], in the present investigation changes in production of antibodies against DNA were studied after injury to the posterior hypothalamus.

EXPERIMENTAL METHOD

The titer of antibodies against DNA and their character were determined by the passive hemagglutination reaction (PHR), and the antibody neutralization reaction (ANR) of Poverennyi and Levi [5]. All reactions were carried out with erythrocytes of series I sensitized by hen erythrocyte DNA. Sera were heated to 56°, absorbed with formalinized sheeps' erythrocytes and investigated in the PHR with DNA-sensitized and control erythrocytes. The ANR was performed with native and denatured DNA.

TABLE 1. Antibodies Against DNA in Serum of Albino Rats after Injury to Posterior Hypothalamus

Time after injury (in days)	No. of animals	No. of time detected and titer of antibodies against DNA
14	10	2 – 0 4 – 1 : 80 4 – 1 : 40
28	7	1 – 1 : 80 1 – 1 : 160 1 – 1 : 640 2 – 1 : 320 1 – 1 : 1280 1 – 1 : 2560

Bilateral electrolytic injury to the posterior hypothalamus in the region of the mammillary bodies was produced by means of an ophthalmologic diathermy coagulator using ultrahigh-frequency current (7 W for 3 sec). The current was applied through a steel needle insulated throughout its length with glass except for 0.5 mm at the end. Electrical coagulation was performed on a stereotaxic apparatus, permitting the place of injury in the posterior hypothalamus to be localized in accordance with predetermined coordinates. To develop the method of electrical coagulation, the stereotaxic coordinates given in the atlas of Fifková and Marsala [6] were used.

The experiments of series I were performed on 10 noninbred albino rats weighing 200 g. Animals with a titer of less than 1 : 10 in the PHR were placed in this series. The second investigation was carried out two weeks, the third one month, and the fourth 1.5 months after electrical coagulation of the posterior hypothalamus. Ten intact

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animals with an antibody titer less than 1 : 10 were used as controls. In the same series 5 guinea pigs were investigated before and after electrical coagulation of the posterior hypothalamus. The experiments of series II were performed on 10 Wistar albino rats. The titer of these animals in the PHR was investigated 8 months after electrical coagulation of the hypothalamus. Ten Wistar rats of the same age and weight acted as controls.

In the animals of a special control series, the titer of antibodies against DNA was investigated after bilateral electrical coagulation of the temporal cortex. Ten Wistar rats were used in these experiments. The titer of antibodies against DNA was investigated at the same periods after the operation as in the animals of series I.

EXPERIMENTAL RESULTS

In all the animals of series I the initial titer in the PHR was less than 1 : 10. Two weeks after electrical coagulation of the hypothalamus the antibody titer in the experimental animals rose, and a further increase was observed after one month (see Table 1).

In the control animals with an antibody titer of less than 1 : 10 when taken for the experiment, a higher titer was not determined in the course of the experiment. The ANR was performed with sera taken one month after injury to the hypothalamus, revealing antibodies against single-helical DNA. The minimal neutralizing dose for single-helical DNA was 0.10-0.15 μg/ml. The antibody titer fell to its initial value 1.5 months after injury to the hypothalamus.

An increase in antibody titer was also found in guinea pigs two weeks after electrolytic destruction of the posterior hypothalamus (agglutination of the erythrocytes did not commence in a dilution of 1 : 20, as before coagulation, but in dilutions of 1 : 80 and 1 : 160). The results of investigation of 10 Wistar animals eight months after injury to the hypothalamus (experiments of series II) showed that in two sera, the antibody titer was less than 1 : 10, in one it was 1 : 20, in two 1 : 40, in two 1 : 160, and in one 1 : 320. In the intact rats of the same line and the same age, no differences in antibody titer were found: it was not less than 1 : 40 and not more than 1 : 160.

Hence, the maximal rise of antibody production occurred 28 days after coagulation of the nuclei of the posterior hypothalamus.

In the animals of the special control group, in which the cortex was coagulated, the antibody titer remained unchanged throughout the month (less than 1 : 10).

In an electrophoretic study of the serum of the animals with injury to the hypothalamus, an increase in the γ-globulin fraction was observed: 0.95% before the operation and 1.44% after the operation ($t = 3.06$; ($P < 0.01$).

The discovery of antibodies against DNA suggests immunization of the animal and, consequently, the presence of DNA in the serum in the period before appearance of antibodies. In fact, when several sera, some containing antibodies against DNA, others not (obtained the first time serum was taken from these same rats), were mixed, the combined serum showed no activity in the PHR. If the simple dilution of the serum taking place under these conditions is allowed for, the cooled serum ought to have contained antibodies against DNA. Since no antibodies were found, it may be supposed that neutralization of the antibodies were found, it may be supposed that neutralization of the antibodies had taken place by the DNA present in the serum which was inactive in the PHR.

The reaction with sensitized erythrocytes is highly specific not only for detection of antibodies against DNA (in the PHR), but also during inhibition of this reaction (in the ANR) by DNA preparations themselves [5]. It may thus be concluded that DNA was present temporarily in the serum of the experimental animals, and the body reacted to it by antibody formation.

The results of this investigation confirm that areas of the posterior hypothalamus are directly concerned with the regulation of immunologic reactions. Marked inhibition of antibody formation (as shown by the complement fixation reaction), and considerable retention of antigen in the blood of rabbits after electrolytic destruction of the posterior hypothalamus have been demonstrated previously [3]. From a comparison of these results with those obtained in the present investigation it may be postulated that there is a difference between the regulation of complement-fixing and of DNA-neutralizing antibodies.

It is generally agreed that the active form of DNA during replication possesses a single-helical structure. It may, therefore, be postulated that in the present experiments a temporary increased synthesis of DNA took place, accompanied by the entry of part of the single-helical DNA into the blood stream (in the form of a hapten bound with protein). This could lead to the activation of antibody formation. This hypothesis is also possible because in similar experimental conditions, following electrolytic injury to the hypothalamus of albino rats [1], we were unable to detect activation of protein metabolism in the tissue but, on the contrary, observed its inhibition. This is in good agreement with the fact that protein synthesis and DNA replication are mutually exclusive [7], and it suggests that antibodies against DNA may be one of the repressors in the DNA – RNA – protein system.

Another possibility is that hormonal changes and changes in tissue permeability, which were undoubtedly present after disturbance of hypothalamic activity, play an important role in the mechanism of stimulation of antibody production. The possibility likewise is not ruled out that the antibodies were not directed against the DNA of the body, but were virus-neutralizing antibodies. This calls for special investigations to obtain evidence of activation of latent viruses in the body of the experimental animals.

The results obtained support the view that the hypothalamus participates in the regulation of immunologic mechanisms controlling the genetic constancy of the organism.

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