ROLE OF THE POSTERIOR HYPOTHALAMUS IN THE REGULATION OF MACROPHAGE FUNCTION

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On the 10th to 15th day after bilateral coagulation of the posterior and dorsal hypothalamic areas in rabbits there was a distinct decrease in cathepsin activity in the lysosomal fraction of the macrophages of the peritoneal exudate and stabilization of the lysosomal membranes by comparison with control rabbits. At these same times labeled typhoid vaccine was found in significantly larger amounts in the phagolysosomal fraction of the macrophages from rabbits with destruction of the posterior hypothalamus than in control animals. Ingestion of antigen by the macrophages was unchanged. Coagulation of the posterior hypothalamus thus had a significant effect on the function of the macrophages by disturbing their ability to digest antigen.

The wide variety of functions of the hypothalamus and its direct participation in the regulation of vitally important bodily functions have led to the intensive study of its role in biological processes of defense. Investigations have shown that the hypothalamus, particularly its posterior portion, plays the leading role in the regulation of antibody formation [1, 2, 5, 8].

Other investigations have established an important role of the hypothalamus and the whole hypothalamo-adrenal system in the regulation of immunogenesis, although it has not yet been discovered which phases of immunogenesis are controlled by the posterior hypothalamus. It is particularly important to study the role of the hypothalamus in the regulation of functions of the macrophages and their lysosomal apparatus, which has an essential role in immunogenesis [3, 4, 7].

The object of this investigation was to study the effect of coagulation of the posterior hypothalamus on cathepsin activity in spleen and peritoneal exudate cells, the ingestion of labeled antigen by the macrophages, and its distribution along the individual subcellular fractions.

EXPERIMENTAL METHOD

Thirty-eight chinchilla rabbits weighing 2.5-3 kg were used.

The posterior hypothalamus was coagulated bilaterally in the region of the posterior and dorsal hypothalamic areas by electrolysis with a direct current of 2 mA for 1 min. Coordinates were taken from the stereotaxic atlas of Sawyer et al. [9]. At the end of the experiments the size and location of the brain lesions were verified histologically.

The rabbits were sacrificed on the 10th, 15th, and 21st day after coagulation of the hypothalamus and the spleen and cells of the peritoneal exudate were taken for investigation.

In all the experiments the animals were divided into three groups: 1) experimental rabbits (after coagulation of the posterior hypothalamus); 2) animals undergoing a mock operation, i.e., a control group of rabbits with coagulation in another part of the brain; 3) intact rabbits, on which no operation was performed. Three rabbits from group 1, two from group 2 and three from group 3 were used at each time of investigation.

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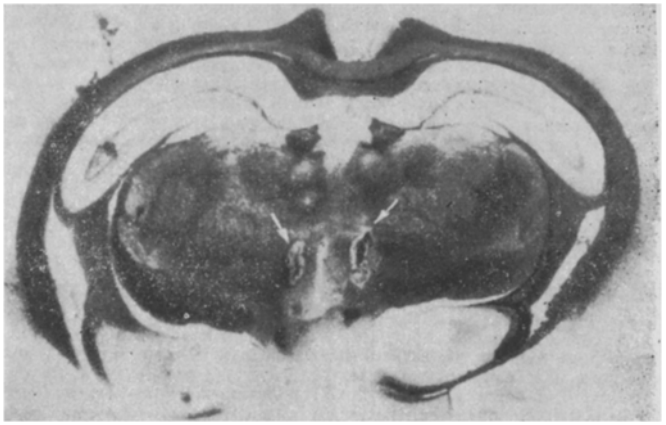

Fig. 1. Focus of destruction in region of posterior and dorsal hypothalamic areas (arrows).

In the experiments of series I the activity of cathepsin, a proteolytic lysosomal enzyme, was determined, while in the experiments of series II the phagocytosis of labeled antigen by cells of the reticulo-endothelial system was studied.

Macrophages were obtained by irrigating the peritoneal cavity of the rabbits after preliminary (three days beforehand) stimulation with nutrient broth and Hanks's solution containing heparin. Lysosomal fractions were obtained from the spleen cells and macrophages by differential centrifugation by the method of Weissman and Thomas [10].

Cathepsin activity was determined by Anson's method [6] in the cytoplasmic fraction of the cells (fraction I) and in the lysosomes themselves after their lysis by distilled water (1 : 10) for 30 min and removal of the fragments of membranes by centrifugation (fraction II). A 2.5% solution of hemoglobin in acid medium was used as the substrate. The results were expressed in micrograms tyrosine per milligram protein.

The strength of the lysosomal membranes was judged from the ratio between the enzyme activity in the cytoplasmic fraction and in the lysosomes (I/II).

To study phagocytosis by the spleen cells and macrophages of the peritoneal exudate, heated typhoid vaccine (Ty = 4446), labeled with sodium carbonate-1-C^{14}, was used as the labeled antigen.

On the ninth day after coagulation of the posterior hypothalamus the rabbits of all groups received 1 ml typhoid vaccine (2 billion cells), containing 300,000 pulses/min per ml, intraperitoneally.

Radioactivity was determined 30 min and 72 h after injection of the labeled antigen. Macrophages from the peritoneal exudate, spleen cells, blood serum, and peritoneal exudate itself (after sedimentation of the macrophages) were used for the determination.

Spleen cells and macrophages were fractionated and the radioactivity determined in the insoluble residue of the cells (membranes, nuclei, mitochondria, and so on), the cytoplasmic fraction (I), and the lysosomes, without producing their lysis with distilled water (II).

The protein content in 1 ml solution was first determined in all the samples by Lowry's method. Protein was then precipitated from 1 ml of each sample by the addition of an equal volume of 10% TCA and the proteins applied to No. 5 membrane filters. The filters with the samples were placed in scintillation fluid and their radioactivity determined with the SL-40 counter.

The results were expressed as the number of pulses in 1 min/mg protein. All the numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The picture of coagulation of the posterior hypothalamus in the experimental animals is shown in Fig. 1. During final analysis of the experimental results only those animals in which histological examina-

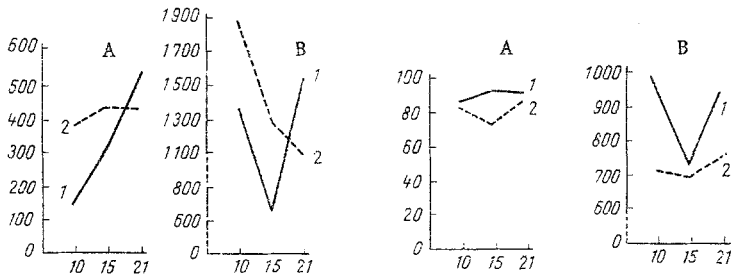

Fig. 2. Cathepsin activity in macrophages of peritoneal exudate (I) and spleen cells (II) of rabbits. A) fraction I; B) fraction II; 1) rabbits with coagulation of posterior hypothalamus; 2) intact rabbits. Abscissa, day of investigation; ordinate, cathepsin activity (in μg tyrosine/mg protein).

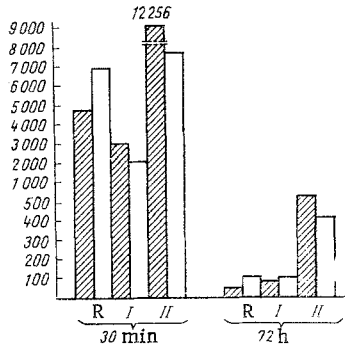

Fig. 3. Phagocytosis of labeled typhoid vaccine by macrophages of peritoneal exudate of rabbits. Shaded columns represent rabbits with coagulation of posterior hypothalamus; unshaded columns represent intact rabbits; R) insoluble residue of cells; I) cytoplasmic fraction; II) lysosomes. Abscissa, time of determination; ordinate, radioactivity (in pulses/min/mg).

tion confirmed that the focus of injury corresponded precisely to the posterior hypothalamus were taken into consideration. Rabbits in which the size and localization of the lesion did not correspond to the posterior hypothalamus were disregarded.

The results of experiments to study changes in the cathepsin activity in the spleen cells and macrophages at different times after coagulation of the posterior hypothalamus are illustrated in Fig. 2. On the 10th day after the operation cathepsin activity was sharply reduced in the experimental animals in the cytoplasmic fraction of the macrophages and reduced to a rather lesser degree in the lysosomes, while in the animals undergoing the operation stabilization of the lysosomal membranes was well-marked. On the 15th day cathepsin activity (in fractions I and II) in the animals undergoing the operation was still considerably lower than in the control. By the 21st day cathepsin activity in fractions I and II of the macrophages was increased and was actually a little higher than in the control.

Cathepsin activity in the spleen on the 10th day after the operation showed a slight tendency toward an increase, and this continued in the cytoplasmic fraction until the 15th day. On the 21st day a small increase in cathepsin activity could be seen in the lysosomal fraction. The permeability of the lysosomal membranes was unchanged at all periods of the investigation.

The results of experiments to study phagocytosis of labeled typhoid vaccine by the macrophages on the ninth day after coagulation of the posterior hypothalamus are shown in Fig. 3. It is clear that the antigen was very quickly (within 30 min) almost completely ingested by macrophages of both the experimental and the control animals, for only a low level of radioactivity remained in the exudate. High activity was determined in the insoluble residue of the cells, rather lower in the cytoplasm, although the level was almost the same. In the lysosomes of the experimental rabbits, however, the level of radioactivity was nearly twice as high as in the control animals. After 72 h, when the label had begun to be excreted, the level of radioactivity in the lysosomes of the experimental animals still remained high.

No label could be detected in the blood serum at all times of the investigation.

Radioactivity was detected in very small amounts in the spleen (in all fractions), almost the same in all groups of animals.

The group of rabbits undergoing the mock operation occupied an intermediate position between the groups of control and experimental animals in the experiments to determine cathepsin activity and also in the experiments to study phagocytosis of labeled antigen.

The considerably higher level of radioactivity in the phagolysosomal fraction of the macrophages in the animals with a coagulated posterior hypothalamus is noteworthy. If these results are compared with the decrease in cathepsin and stabilization of the lysosomal membrane observed in these experiments, it can be postulated that the accumulation of antigen 30 min after its injection did not happen by chance in the animals undergoing the operation, but reflected changes in the function of the lysosomal apparatus of the macrophages.

These experiments thus showed that coagulation of the posterior hypothalamus has a marked influence on macrophage function; it is not the actual ingestion of antigen by the macrophages which is affected, but the ability of the macrophages to digest it.

Presumably the depression of antibody formation observed by some workers [1] after coagulation of the posterior hypothalamus is connected with changes in the function of the lysosomal apparatus of cells of the reticulo-endothelial system. However, this problem requires further study.

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