PHYSIOLOGICAL EFFECT OF ELECTROCOAGULATION OF MAGNOCELLULAR NEURONS OF THE HYPOTHALAMUS

E. M. Parshkov and O. V. Molotkov

UDC 612.826.4-06:615.832.74

Electrocoagulation of the supraoptic or paraventricular nuclei of the hypothalamus in male Wistar rats did not give rise to a significant increase in the 24 h excretion of urine or water intake. Combined destruction of both these nuclei led to the development of polyuria and polydipsia, most marked in the first week after operation and disappearing by the 8th-10th day. Compensatory hypertrophy of undestroyed neurons of the magnocellular nuclei of the anterior hypothalamus reached its maximum in the 3rd-4th week.

* * *

Experimental and clinical evidence has now been obtained to indicate the development of a symptom-complex of diabetes insipidus as a result of injury, not so much to the pituitary, as to certain zones of the diencephalon or to the tracts joining them to the pituitary [1, 2, 4, 9, 11]. Antidiuretic hormone (ADH), a deficiency of which causes the development of diabetes insipidus, is synthesized by the magnocellular neurons of the hypothalamus, most probably in the supraoptic and paraventricular nuclei [10, 12].

It was therefore decided to investigate whether polyuria and polydipsia can develop after the destruction of the supraoptic and paraventricular nuclei separately and together, and to examine the course of diabetes insipidus in relation to the severity of injury to the magnocellular nuclei of the hypothalamus and repair processes in the zone of electrocoagulation.

EXPERIMENTAL METHOD

Experiments were carried out on 88 male Wistar albino rats weighing 180-200 g. Under nembutal anesthesia, and by means of a stereotaxic apparatus, electrocoagulation of the supraoptic nuclei (15 animals), the paraventricular nuclei (10 animals), and the supraoptic and paraventricular nuclei together (35 animals) was carried out on the experimental animals. When identifying the site of destruction, bearings were taken from De Groot's maps [6]. To obtain more complete blocking of the neurons of the supraoptic nucleus and, at the same time, with minimal destruction of the surrounding tissue, the supraoptic nucleus was coagulated with a current of 0.5 mA for 5 sec at two points in plane A: 6.8 and 7.4, located 2 and 1.6 mm laterally to the sagittal sinus respectively. Two hours after the operation the animals were placed in separate cages so that the volume of urine excreted and fluid consumed every 24 h could be measured. The rats were decapitated 2, 7, 10, 14, 20, and 30 days after the operation; 13 animals acted as controls.

The hypothalamus and pituitary were fixed in Bouin's fluid. Serial paraffin sections of the hypothalamus were stained with paraldehyde-fuchsin by Gomori's method as modified by V. F. Maiorova, and the pituitary was stained with paraldehyde-fuchsin without counterstaining for photometry of the sections with the MF-4 instrument. Having regard to the existing opinion on the identity of ADH and vasopressin, the pressor activity of acetic acid extracts of the posterior lobe of the pituitary was determined for each rat undergoing operation by Dekanski's method [5] with slight modifications. Pituitrin P was used as the standard.

EXPERIMENTAL RESULTS

Separate destruction of the supraoptic or paraventricular nucleus led to a slight and transient increase in diuresis and to an increase in the volume of water drunk compared with that in the control anesthetized animals. On the 2nd day after operation these indices returned to normal, remaining at that level until the end of the experiment. Histological investigation of the hypothalamus showed that foci of electrocoagulation

Laboratory of Radiation Neuroendocrinology, Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk (Presented by Academician of the AMN SSSR G. A. Zedgenidze). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 5, pp. 3-7, May, 1969. Original article submitted July 5, 1968.

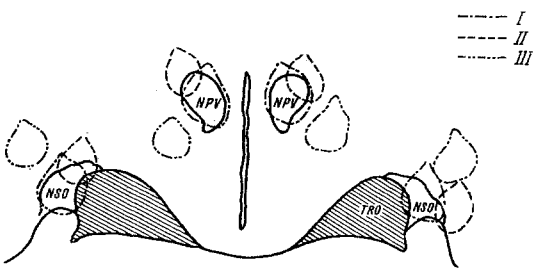

Fig. 1. Localization of zones of electrocoagulation of supraoptic (NSO) and paraventricular (NPV) nuclei respectively. I) considerable destruction of nuclei; II) partial destruction; III) outside nuclei.

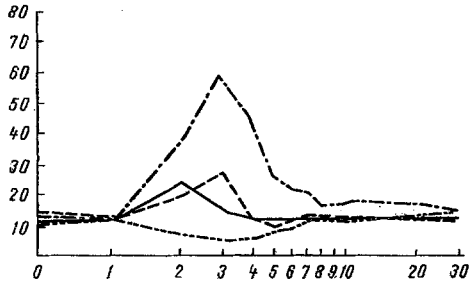

Fig. 2. Volume of urine excreted after electrocoagulation of supraoptic and paraventricular nuclei. Abscissa, days after operation (on logarithmic scale); ordinate, volume of urine excreted (in ml). Continuous line, control; remainder of legend as in Fig. 1.

were present in the zones of the corresponding nuclei. As a rule neurons of the paraventricular nucleus were completely destroyed, and only in rare cases were single cells left intact in the zone of the burn or in the central part of the reactive inflammation. So far as the supraoptic nucleus is concerned, after application of a current of 0.5 mA for 5 sec, not all the neurons were destroyed, and for this reason a current of 1 mA was applied to 5 rats for 10 sec. Under these conditions histological examination of the zone of electrocoagulation revealed massive destruction of the supraoptic nucleus, involving surrounding tissue. However, even in these animals the permanent development of manifestations of diabetes insipidus was not observed. The vasopressor activity of acetic acid extracts of the posterior lobe of the pituitary of these rats as a rule was reduced by 30-40% on the 2nd-5th day after operation, returning to normal by the 7th day.

Following simultaneous destruction of the supraoptic and paraventricular nuclei in the rats a marked disturbance of water balance was observed. Some animals developed polyuria and polydipsia, others oliguria, and only in 8 rats was there no significant disturbance of diuresis. Histological examination of the hypothalamus of these animals revealed three conventional zones of predominant distribution of the foci of injury (Fig. 1).

1. The focus of electrocoagulation covers the whole area of the paraventricular nucleus and a large part of the supraoptic nucleus. In this case the residual neurons of the supraoptic nucleus were shrunken and hyperchromatic, with a pycnotic nucleus. Occasional cells among them were very little different from the control, they contained a moderate amount of Nissl's substance at the periphery of the cytoplasm and a narrow border of neurosecretory granules in the perinuclear zone. The volume of urine excreted and of fluid drunk by these animals was sharply increased, reaching a maximum on the 2nd day after operation (Fig. 2). With such marked polyuria and polydipsia, absence of vasopressor activity (or of ADH, which amounts to the same thing in the opinion of most workers) would be expected in the posterior lobe of the pituitary, but the vasopressor activity of extracts of the neurohypophysis was in fact lowered only by 50-60%. This discrepancy can apparently be attributed either to the presence of two different hormones — vacopressin and ADH [3], or to disturbance of the connection between the hypothalamus and posterior lobe of the pituitary as a result of the destruction and subsequent development of reactive inflammation. This evidently stopped the arrival of information in the posterior lobe of the pituitary about the actual requirements of ADH of the body, and as a result of this an insufficient amount of it was liberated into the blood stream. The second hypothesis appears more possible, more especially because the development of diabetes insipidus, sometimes of a more severe degree, is observed after destruction of the median eminence and the pituitary stalk [7-9]. Further evidence in support of this view is the fact that, despite the marked polyuria and polydipsia, in no case was the complete absence of neurosecretion observed in the proximal part of the neurohypophysis, although the amount of secretion, determined by photometry, was reduced by 60-70% below the control level.

On the 8th-10th day after operation the volume of urine excreted fell to normal, evidently because of the adequate arrival of ADH in the blood stream with restoration of communication between the magnocellular neurons of the hypothalamus and the neurohypophysis (Fig. 2). Histological examination showed that the residual neurons of the supraoptic and paraventricular nuclei were moderately hypertrophied (9030 μ^3), while in the later period (14th-20th day) their volume reached 26,099 μ^3 compared with 5686 μ^3 in the control. The large quantity of Nissl's substance and of neurosecretory material in the cytoplasm of the cells and axons, together with the large nucleus and nucleolus are all evidence of hyperfunction of these neurons. The median

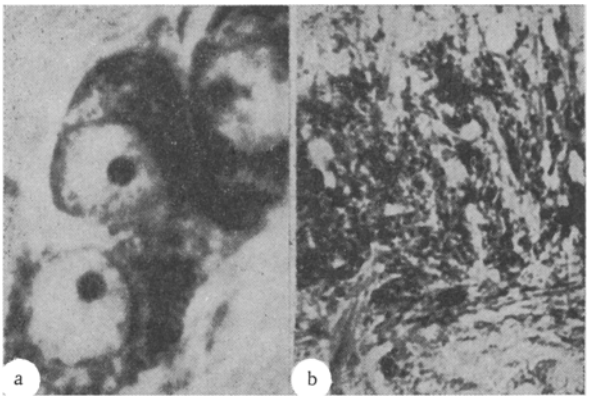

Fig. 3. Neurons of the diffuse supraoptic nucleus. Hypertrophy of nucleus and nucleolus 8-10 days after destruction of supraoptic and paraventricular nuclei (a). Accumulation of Gomori-positive neurosecretion in median eminence after partial destruction of supraoptic and paraventricular nuclei (b). Stained by Gomori's method as modified by Maiorova. 1800 ×.

eminence and neurohypophysis contained large quantities of Gomori-positive substance. An extract of the posterior lobe of the pituitary possessed vasopressor activity, which as a rule was indistinguishable from that of the control.

After injury to the supraoptic and paraventricular nuclei, evidence of marked activation of the function of some other nuclear structures of the hypothalamus was observed: the diffuse supraoptic nucleus and magnocellular neurons of the retrochiasmal region (Fig. 3a), which, together with the residual neurons of the supraoptic and paraventricular nuclei can evidently take over to a considerable extent the function of the destroyed neurons under these circumstances, leading in the course of time to the adequate satisfaction of the ADH requirements of the body. The validity of this hypothesis is confirmed by the fact that in these experiments no stable or prolonged disturbance of water metabolism was observed even after total inactivation of the supraoptic and paraventricular nuclei. Other workers have obtained similar results [12].

2. The zone of electrocoagulation covers peripheral parts of the nuclei, with certain variations (Fig. 1). In this case the focus of injury evidently acted as a special type of stimulus for neurons of the supraoptic and paraventricular nuclei, resulting in marked activation of the liberation of neurosecretion into the axons. The median eminence and posterior lobe of the pituitary were packed with neurosecretion (Fig. 3b). The vasopressor activity of extracts of the neurohypophysis in some cases was actually somewhat higher than in the control. As a rule, the excretion of urine by these rats was slightly reduced, subsequently returning to normal on the 5th-6th day after operation (Fig. 2).

3. In 8 rats, because of cranial anomalies or technical errors, the focus of destruction lay at a considerable distance from these nuclei (Fig. 1), so that these animals could be regarded as having undergone mock operations. No significant disturbances of water balance were observed in them. Histological examination of the supraoptic and paraventricular nuclei of the hypothalamus and neurohypophysis likewise revealed no differences from the control. The vasopressor activity of extracts of the neurohypophysis was indistinguishable from normal.

These results thus support the suggestion that the single system of magnocellular neurons of the hypothalamus is responsible for ADH production. Development of polyuria and polydipsia after destruction of the supraoptic and paraventricular nuclei or media eminence is evidently the result of disturbance of the connection between the hypothalamus and neurohypophysis, while liberation of ADH from the posterior lobe of the pituitary is an active process under the control of neurons of the magnocellular nuclei of the hypothalamus.

LITERATURE CITED

1. L. N. Karlik, in: Higher Nervous Activity [in Russian], No. 1, Moscow (1929), p. 361.
2. A. I. Lakomkin, Abstracts of Proceedings of the 10th Conference of Physiologist, Biochemists, and Pharmacologists of the South of the RSFSR [in Russian], No. 1, Simferopol' (1950), p. 87.
3. A. V. Tonkikh, The Hypothalamo-Hypophyseal Region and Regulation of Physiological Functions of the Organism [in Russian], Moscow–Leningrad (1965).
4. J. Camus and G. Roussy, J. Physiol. Path. Gen., 20, 509 (1922).
5. J. Dekanski, Brit. J. Pharmacol., 7, 567 (1952).
6. J. de Groot, J. Comp. Neurol., 113, 389 (1959).
7. D. de Wied, P. G. Smelik, J. Moll, et al., in: Major Problems in Neuroendocrinology, Baltimore (1964), p. 156.
8. M. Fusco, L. Malvin, and P. Churchill, Endocrinology, 79, 301 (1966).
9. C. C. Gale, S. Paleisnik, and S. M. McCann, Am. J. Physiol., 201, 811 (1961).

10. W. Ingram, C. Fischer, and S. M. Kanson, Arch. Intern. Med., 17, 1067 (1936).
11. F. A. Laszlo and D. de Wied, J. Endocrinol., 36, 125 (1966).
12. H. Olivecrona, Acta Physiol. Scand, 40, Suppl. 136, 1 (1957).