DEVELOPMENT OF FETAL HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY NEURONS TRANSPLANTED INTO THE THIRD VENTRICLE OF THE ADULT RAT BRAIN

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The formation of features of structural and functional specificity of the neurosecretory cells (NSC) of the hypothalamic nuclei is determined by the complex spatiotemporal organization of neuro-ontogeny: proliferation of precursor cell in matrix zones, postmitotic migration with the formation of neurosecretory nuclei (NSN), morphological and neurochemical differentiation, the formation of specific connections, and the formation of neurosecretory function. The above stages of neuro-ontogeny are, on the one hand, under genetic control and, on the other hand, under the influence of the specific environment of NSC development. It can be tentatively suggested that the relative role of genetic and environmental factors is not the same for different components of NSC ontogeny. The goal-directed study of this problem has become possible due to the creation of a new experimental model, namely transplantation of embryonic anlagen of different parts of the brain into the brain of adult animals [1, 2, 8, 9]. The importance of this method for the study of neuro-ontogeny is due to the fact that the development of neurocytes of the grafted tissue takes place under extremely unusual microenvironmental conditions. It must be postulated that those stages of neuronogenesis which are under the control mainly of epigenetic factors are selectively disturbed in the grafts. The aim of this investigation was to analyze features of similarity and difference in the ontogeny of NSC of intact rats and of magnocellular neurocytes of fetal hypothalamic tissue transplanted into the third ventricle of the adult rat brain.

EXPERIMENTAL METHOD

The recipients were 15 female Wistar rats weighing 180-200 g and the donors were rat fetuses at the 12th and 14th days of intrauterine development. The time of taking the tissue for transplantation was determined by the fact that proliferation of the matrix cells of NSN in the anterior hypothalamus is complete by the 13th-15th days of embryogenesis [6]. In females with a strictly dated time of pregnancy, fetuses were removed from the uterine cornua under hexobarbital anesthesia, and under a binocular loupe, the meninges were removed from the brain and the hypothalamic region was excised. The excised tissue, measuring 5-6 mm^3, was introduced into a syringe containing 0.1-0.2 ml of physiological saline. Transplantation was carried out under sterile conditions and under hexobarbital anesthesia. The implant was introduced into the third ventricle of the recipient by means of a stereotaxic apparatus, corresponding to coordinates AP 1.6, L 0, H 8, relative to the bregma [12].

To study the times of completion of proliferation by the grafted tissue cells, incorporation of ^{3}H-thymidine into the magnocellular neurons of the grafts was assessed. The method of double injection of isotope with an interval of 24 h between injection, in a total dose of 10 μCi/g, was used [6]. The age of the donated tissue at the time of injection of ^{3}H-thymidine into the recipients corresponded to the 13th-18th days of embryogenesis in animals of the different experimental groups. To study the response of NSC of the transplanted tissue to water deprivation, a group of animals (seven recipient rats) kept on a

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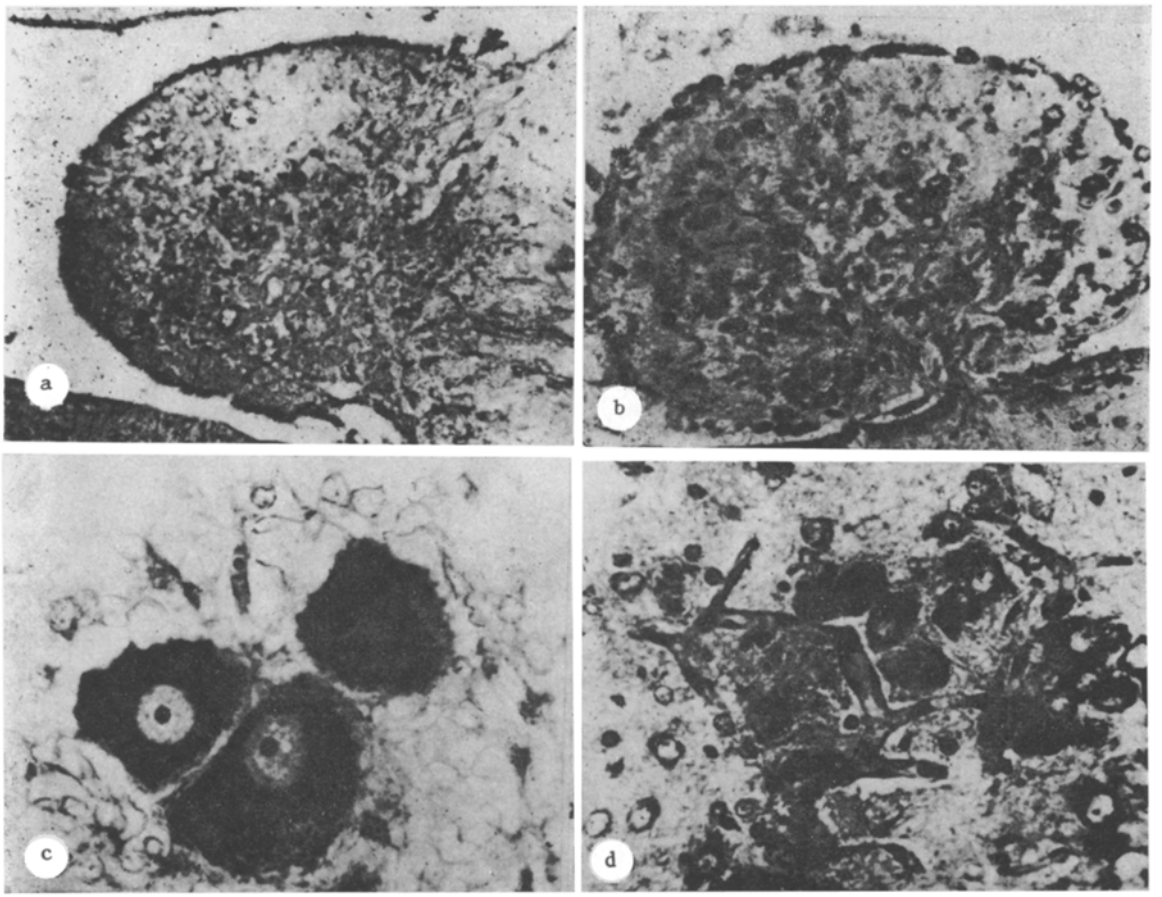

Fig. 1. Translated fetal hypothalamic tissue in third ventricle of recipient rat (40 days after transplantation). a) Attachment of graft to lateral wall of third ventricle; b) attachment of graft to recipient's median eminence, 140 ×; c) large perikarya of graft cells, abundantly packed with Gomori-positive granules, 900 ××; d) vascularization of graft by vessels arising from the host's brain, 280 ×. Stained with paraldehyde-fuchsin by Gomori Gabe method.

dry diet for 5 days before decapitation, was segregated. After the end of the experiment (40th day after transplantation) the animal's brain was fixed in Bouin's fluid; frontal paraffin sections were stained with paraldehyde-fuchsine by the Gomori-Gabe method and coated with photographic emulsion, then exposed for 4 weeks at 4°C. The intensity of incorporation of ^{3}H-thymidine by cells of the transplanted tissue was estimated by counting grains of silver in the region above the nucleus. The relative percentage of neurocytes with a high (40 granules or more) and average (about 20 granules) intensity of labeling was calculated. The degree of morphological differentiation of the magnocellular neurocytes of the transplanted tissue was estimated by visual analysis of the preparations and also by morphometry of nuclei and nucleoli [3, 4, 7] and counting cells with different numbers of neurosecretory granules in their perikarya [5]. Neurons of the supraoptic nucleus (SON) of month-old rats, i.e., a cell population isochronous with neurocytes of the transplants, served as the control. The response of cells of the transplanted tissue to water deprivation was assessed by comparing these morphometric parameters in transplants of recipient rats kept on a normal water intake and those subjected to water deprivation, and also in SON of dehydrated month-old rats.

EXPERIMENTAL RESULTS

Transplants were found in the third ventricle of all 15 rats undergoing the operation and exhibited close contact with the lateral wall and floor of the recipients' their ventricle (Fig. 1), abundant vascularization of the transplanted tissue by vessels arising from adjacent areas of the recipient's brain, and the presence of magnocellular and parvocellular neurocytes and glial cells.

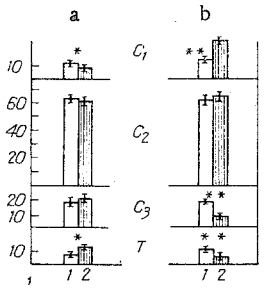

Fig. 2

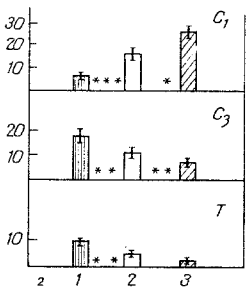

Fig. 3

Fig. 2. Changes in relative percentages of NSC of different types in grafted tissue and SON of month-old rats in response to water deprivation for 5 days. Ordinate, relative percentages of NSC of different types (C_1, C_2, C_3, T). a) Graft tissue; b) SON of month-old rats. 1) Normal water intake; 2) water deprivation. Vertical lines indicate 99% confidence interval. *$p < 0.05$, **$p < 0.01$.

Fig. 3. Number of NSC of different types in grafts in contact with median eminence and lateral wall of third ventricle, and in SON of month-old intact rats (water deprivation for 5 days). Ordinate, relative percentage of NSC of different types (C_1, C_3, T). 1) Attachment of graft to lateral wall of third ventricle; 2) grafts in contact with median eminence; 3) SON of month-old rats. Vertical lines indicate 99% confidence interval. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Incorporation of ^{3}H-thymidine into magnocellular neurocytes of the grafts was observed when the isotope was injected at times corresponding to the 13th-15th days of ontogeny of the transplanted tissue. No ^{3}H-thymidine was incorporated into the cells studied, if at the time of its injection into the recipient, the age of the donated tissue corresponded to the 16th-18th days of embryogenesis. The times of appearance of magnocellular neurons in the grafts and SON of intact animals [6] coincided. Consequently, in the transplanted embryonic tissue the time schedule of generation of magnocellular neurons was reproduced in a manner characteristic of normal ontogeny. Magnocellular neurons of the transplants had well developed perikarya, in which granules of neurosecretion were discovered. The mean volumes of the nuclei and nucleoli of these neurocytes coincided with the corresponding values for cells of SON of month-old animals: the corresponding values for volumes of nuclei of NSC were 385.10 ± 21.08 and 356.30 ± 6.50 μ^3 ($p < 0.1$), and for nucleoli 12.27 ± 1.74 and 9.23 ± 1.06 μ^3 ($p < 0.1$). The ratio of cells containing different quantities of neurosecretion in their perikarya also was similar in the experimental and control animals. Consequently, despite removal of the transplanted tissue from the hypothalamic region of the fetal brain, neurocytes of the transplants attained a high degree of differentiation and reproduced the specific features of the NSC cytophenotype. These facts are in good agreement with the results of an immunocytochemical analysis of the cell composition of transplanted anterior hypothalamic tissue [10, 11], demonstrating a high content of vasopressin and of the corresponding neurophysin in neurons of the grafts.

The results of the study of the spatial organization of magnocellular neurons of the transplanted tissue were fundamentally different. Cells of the grafts were arranged diffusely or in a mosaic pattern and were not combined into structures similar to NSN. Their distribution in the transplanted tissue was not determined by the times of proliferation of the precursor cells: neurons incorporating the isotope were found in many areas of the grafts simultaneously, regardless of the time of injection of ^{3}H-thymidine. The limited opportunities for migration of the transplanted tissue cells also are demonstrated by the fact that all perikarya which incorporated the label remained within the grafts. Meanwhile distinct accumulations of NSC of the transplanted tissue were observed at places of contact of the grafts with the receipient's median eminence.

Under conditions of water deprivation the morphometric parameters of synthetic activity of NSC of the transplanted tissue were significantly higher than those for recipients maintained on a normal water intake: the corresponding volumes of the nuclei were 500.00 ± 31.26 and 385.10 ± 21.08 μ^3 ($p < 0.05$), and of the nucleoli 18.70 ± 1.65 and 12.27 ± 1.74 μ^3

($p < 0.05$). The content of cells deprived of neurosecretion (type C_1 according to A. L. Polenov's classification, 1968) in graft tissue of the recipient rats subjected to water deprivation was significantly reduced, whereas the number of neurons which were densely packed with neurosecretory granules (type T) was increased ($p < 0.05$ for these cell types; Fig. 2a). Such a redistribution of the NSC of the different types indicates accumulation and retention of neurosecretion in the perikarya of the neurocytes of the grafted tissue. Changes of the opposite character were observed (Fig. 2b) in SON of month-old rats subjected to water deprivation, indicating intensive release of neurosecretion from the perikarya. Thus the increased synthetic activity of NSC of the grafts in response to water deprivation was not accompanied by adequate activation of transport and removal of neurosecretion from the cytoplasm. Analysis of the histograms in Fig. 3 shows that if the grafts were located on the median eminence, the number of the type C_1 NSC in their tissue was significantly greater, and the number of C_3 and T type significantly smaller than in grafts in contact with the lateral wall of the third ventricle. Meanwhile in SON of month-old animals these parameters differed significantly from those obtained in rats with grafts on the median eminence. Consequently, activity of release of neurosecretion by cells of the grafted tissue increased on contact of the graft with the median eminence of the recipient, without, however, reaching the characteristic level for SON neurons of intact animals. Similar patterns were observed in a study of vasopressin-containing neurons of fetal hypothalamic tissue, transplanted into the third ventricle of adult Brattleboro rats, with genetically determined diabetes insipidus [13]: the presence of fenestrated capillaries of the portal system of the median eminence evidently creates the local conditions required for establishment of neurohemal junctions with transplanted NSC.

To sum up it can be concluded that genetic control is of predominant importance for components of ontogeny of NSC such as proliferation and differentiation of precursor cells. It can be tentatively suggested that the biological role of the strict genetic determination of these processes lies in reproduction of the specific morphological and functional organization of NSC in a wide spectrum of microenvironmental conditions. Other principles evidently lie at the basis of formation of NSN and their connections. These processes are determined by the complex spatiotemporal organization of postmitotic migration of neuroblasts with amalgamation of neurocytes into the nucleus and establishment of specific connections. Transplantation disturbs these stages of neuroontogeny. This is evidence of the decisive role of epigenetic factors (microenvironmental conditions) for the formation of NSN and their connections and, in particular, pathways of transport and secretion of neurohormones.

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