Study of Mitotic Activity and Degeneration of Cells in the Dorsolateral Wall of the Anterior Cerebral Vesicle in Rat Embryos on the Model of Ectopic Neurotransplantation

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Ectopic neurotransplantation can be used as an *in vivo* culture method for evaluation of the effect of various environmental factors (neurotransmitters, cytokines, and other bioactive substances) on histogenesis of the developing brain. The study was performed 1 day after allotransplantation of neocortical primordia from embryos of rats receiving intraperitoneal injection of *p*-chlorophenylalanine on day 11 of pregnancy. Under these experimental conditions the number of degenerating cells increased, while the count of mitotic neuroepithelial cells was 2.5-fold lower compared to neurotransplants of intact embryos at the same stage of development. Incubation of neocortical primordia in serotonin-containing medium before transplantation prevented cell death and promoted division of transplanted cells. Serotonin plays a role in the regulation of neuroepithelial cell proliferation and prevents cell death in the developing neocortex.

Key Words: *neocortex; neurotransplantation; p-chlorophenylalanine; proliferation; serotonin*

Studying of the effect of growth factors on embryogenesis of the brain and development of hereditary diseases under adverse environmental conditions is now of particular importance [1]. Changes in histogenetic processes of the developing brain are studied on various experimental models, including prenatal hypoxia, irradiation of pregnant animals, and administration of neurotoxins during various stages of embryogenesis [3]. In the present work, abnormalities of brain development in the modified microenvironment were studied by the method of ectopic neurotransplantation [6]. We studied mitotic activity (MA) and degeneration of transplanted cells, the processes which, together with cell migration and differentiation, determine histogenesis of the nerve tissue. The regulation of cell death and division of precursor cells in the developing brain are poorly studied.

Here we studied histogenesis of the developing rat neocortex after injury and exposure to environmental factors.

MATERIALS AND METHODS

Experiments were performed on 45 Wistar rats weighing 200-250 g. In series I, fragments of the dorsolateral wall of the anterior cerebral vesicle were isolated from rat embryos on days 14, 15, and 17 of embryogenesis. These fragments were transplanted into the sciatic nerve of adult animals [6]. In series II, neocortical primordia were taken on day 15 of embryogenesis and transplanted into the neocortex and nerve of adult rats to perform a comparative study. In series III, female rats received intraperitoneal injection of *p*-chlorophenylalanine (PCPA, Serva) in a dose of 400 mg/kg on day 11

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of pregnancy. Fragments of the dorsolateral wall of the anterior cerebral vesicle were isolated on day 14 of prenatal development, incubated in medium 199 for 1 h, and transplanted into the nerve. In series IV, neocortical primordia obtained from rat embryos in series III were incubated in medium 199 with serotonin-creatinine sulfate (Reanal) in a concentration of 3 μg/ml and transplanted into the nerve. The sciatic nerves of the recipient rats with transplants were fixed in Bouin's fluid and alcohol-formalin to perform histological study and immunohistochemical detection of fragmented DNA by the TUNEL method [2,10]. Experiments were performed in various periods after surgery (from 3 h to 7 days). Electron microscopic examination was performed 1 day after transplantation to study apoptosis in transplanted cells. The number of mitotic and degenerating cells per 1000-3000 cells was determined.

The results were analyzed by Student's *t* test.

RESULTS

In series I, MA of neuroepithelial cells (NEC) was studied in transplanted embryonic primordia. On days 14 and 15 of embryogenesis the wall of the anterior cerebral vesicle in rats is mainly presented by low differentiated cells of the ventricular and subventricular zones. It also contains a small number of migrating neuroblasts. Apart from low differentiated NEC and migrating neuroblasts, 17-day-old embryos have cells of the cortical plate. The ventricular and subventricular zones are mosaic structures consisting of committed neuronal precursors, astrocytes, oligodendrocytes, ependymal cells, bipotent precursor cells, and multipotent precursor cells. Published data show that the number of multipotent precursor cells is maximum in primordia of the earlier embryonic stage [13]. NEC number in the dorsolateral wall of the anterior cerebral vesicle in rats progressively decreases during the prenatal period [7]. After 3-24 h the transplants are located in the central or peripheral zone of the nerve trunk and consist of the same cells as the source material. MA of NEC sharply decreases after 3 h. The number of mitotic cells increases 1 and 3 days after transplantation. The count of these cells in 14- and 15-day-old embryos is higher than in 17-day-old embryos (Fig. 1, *a*). Proliferative activity of NEC can differ under conditions of the same damage to primordia (isolation from the brain, transfer into the nutrient medium, and transplantation in the ectopic site). The younger is the primordium, the higher is the number of proliferating cells. Similar features are observed during normal ontogeny.

In series II, we studied the effect of microenvironmental conditions on MA of transplanted cells. A comparative study of transplants in the nerve and brain showed that 5-9% cells die after 3-24 h, which does not depend on the site of transplantation. Histological and immunohistochemical studies confirmed cell death via apoptosis (the presence of apoptotic bodies and DNA fragments in NEC nuclei, Fig. 2, *a*). The results of electron microscopy confirmed our findings. Several cells are diffusely localized and have apoptotic structural changes in the nucleus. Condensed chromatin is located in the peripheral zone of the nucleus and forms

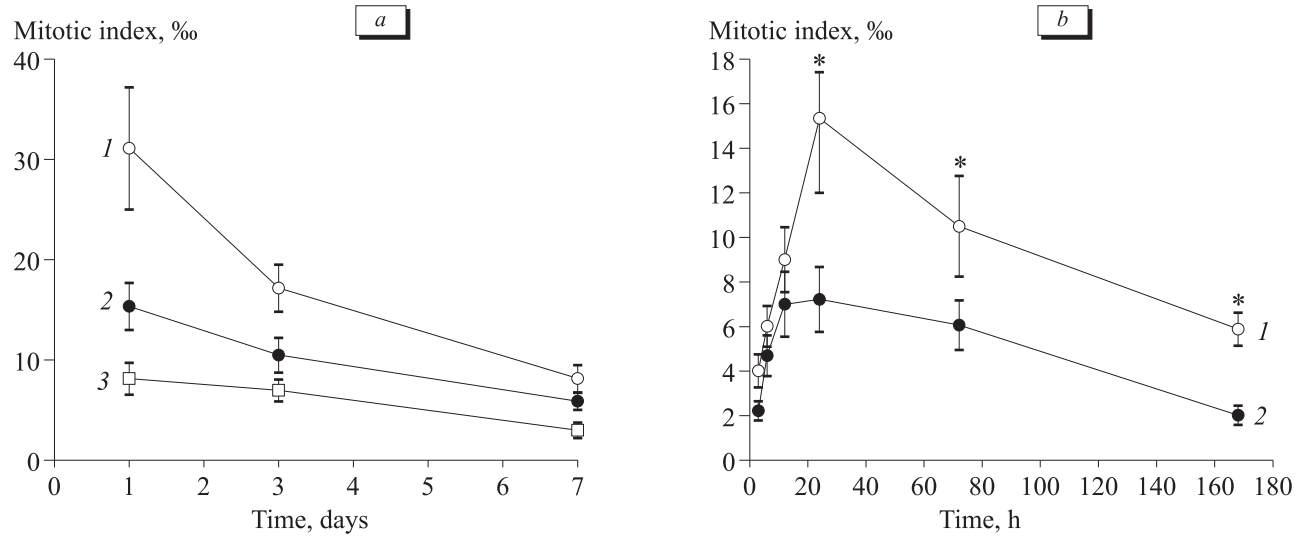

Fig. 1. MA of NEC from rat embryonic neocortex after transplantation into the nerve (dependence on embryonic age, *a*); and brain and nerve (dependence on the site of transplantation, *b*). *a*) Embryonic neocortex transplants; days 14 (*1*), 15 (*2*), and 17 of development (*3*). *b*) Embryonic neocortex transplants; day 15 of development in the nerve (*1*) and brain (*2*). *$p<0.05$ compared to transplants in the brain.

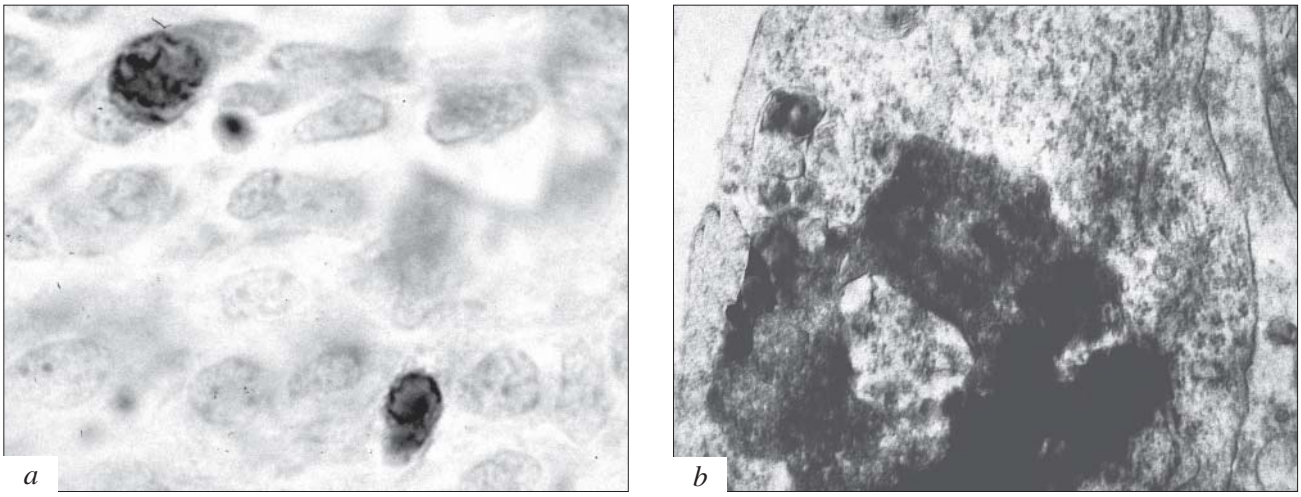

Fig. 2. Cell apoptosis in transplants of rat embryonic neocortex 1 day after transplantation into the nerve: immunohistochemical detection of nuclei with fragmented DNA by the TUNEL method, staining with methylene green, ×900 (*a*); ultrastructure of degenerating NEC in the transplant, ×7000 (*b*).

spherical structures (Fig. 2, *b*). Apoptotic cells are phagocytosed by adjacent cells and leukocytes. Published data on neurotransplantation of embryonic brain cells show that a considerable number of transplanted cells die by the mechanism of apoptosis (from 5-20 [8] to 80-95% cells [9]). Death of considerable number of transplanted cells is probably associated with the procedure of cell isolation and dissociation [9]. Since the goal of our study was to evaluate specific features of brain development, the test transplants contained fragments of the wall of the anterior cerebral vesicle (not dissociated cells). The number of dead cells does not exceed 5-9%. MA of transplanted cells is lowest after 3 h. MA of cells progressively increases after 6 h and reaches maximum 12 and 24 h after surgery. It should be emphasized that after transplantation the number of mitotic cells in the nerve is higher than in the brain (Fig. 1, *b*). These data indicate that the microenvironment modulates proliferative activity of transplanted cells. Our previous studies showed that these features are related to the presence of the glial layer at the boundary between the transplant and nerve tissue of the recipient [4]. Environmental factors in the regenerating nerve probably modulate differentiation of several multipotent precursor cells into the glia.

Series III was performed on the model of prenatal blockade of serotonin synthesis induced by treatment of pregnant females with PCPA [5]. One

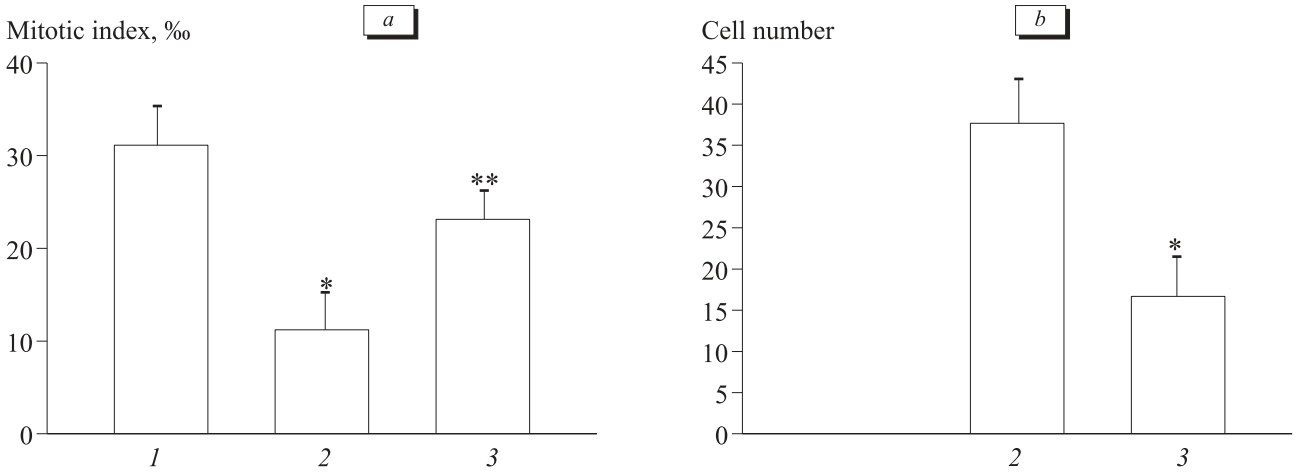

Fig. 3. Effect of serotonin on MA (*a*) and degeneration (*b*) of cells in embryonic neocortex transplants (day 14 of development) into the nerve; 1 day after transplantation. Control transplants from embryos on day 14 of development (*1*); transplants from females receiving PCPA on day 11 of pregnancy (*2*); transplants after incubation of the embryonic material in a serotonin-containing medium (*3*). $p<0.05$: *compared to the control; **compared to PCPA.

day after surgery, MA of cells in the neurotransplants from embryos of rats receiving intraperitoneal injection of PCPA on day 11 of pregnancy decreased by 2.5 times compared to the control (Fig. 3, *a*). PCPA is an inhibitor of serotonin synthesis in the brain. Serotonin deficiency in the developing brain probably decreases MA. Series IV was performed to test this hypothesis and evaluate the role of serotonin in division of cells from the developing neocortex. Neocortical primordia were isolated from embryos of rats receiving intraperitoneal injection of PCPA on day 11 of pregnancy. Before transplantation into the nerve they were incubated in a medium containing serotonin-creatinine sulfate. After 1 day, the number of mitotic cells in the transplants significantly increased (Fig. 3, *a*), while the number of degenerating cells decreased (Fig. 3, *b*). The mechanisms for action of serotonin on proliferation and survival of transplanted cells should be estimated in further studies of graft development.

Our results are consistent with published data that serotonin improves viability and development of nerve cells. *In vitro* and *in vivo* studies showed that serotonin increases cell survival, accelerates cell differentiation, and regulates migration of precursor cells in the neocortex [11,12].

Ectopic neurotransplantation can be used as an *in vivo* culture method to study the effect of various environmental factors (neurotransmitters, cytokines, and other biologically active substances) on histogenesis of the developing brain. Proliferative activity of transplanted NEC is realized with differentiation of the embryonic primordium. Comparative study of transplants under various microenvironmental conditions (peripheral nerve and neocortex of adult rats) showed that environmental factors modulate division of precursor cells. Serotonin plays a role in the regulation of NEC proliferation and prevents cell death in the developing neocortex.

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REFERENCES

1. N. N. Volodin, M. I. Medvedev, and S. O. Rogatkin, *Zh. Nevropatol.*, **101**, No. 7, 4-8 (2001).
2. D. E. Korzhevskii, *Brief Statement of Bases of Histological Technique for Physicians and Laboratory Assistants in Histology* [in Russian], St. Petersburg (2005).
3. V. A. Otellin, L. I. Khozhai, D. E. Korzhevskii, *et al.*, *Vestn. Ros. Akad. Med. Nauk*, No. 12, 32-35 (2002).
4. E. S. Petrova and V. A. Otellin, *Tsitologiya*, **42**, No. 8, 750-757 (2000).
5. L. I. Khozhai, *Morfologiya*, **127**, No. 1, 17-21 (2005).
6. E. I. Chumasov and E. S. Petrova, *Byull. Eksp. Biol. Med.*, **108**, No. 8, 198-201 (1990).
7. J. Altman and S. A. Bayer, *Exp. Neurol.*, **107**, No. 1, 36-47 (1990).
8. P. Brundin, J. Karlsson, M. Emgard, *et al.*, *Cell Transplant*, **9**, No. 2, 179-195 (2000).
9. M. Emgard, J. Karlsson, O. Hansson, and P. Brundin, *Exp. Neurol.*, **160**, No. 1, 279-288 (1999).
10. Y. Gavrieli, Y. Sherman, and S. A. Ben-Sasson, *J. Cell. Biol.*, **119**, No. 3, 493-501 (1992).
11. A. A. Lavdas, M. E. Blue, J. Lincoln, and J. G. Parnavelas, *J. Neurosci.*, **17**, No. 20, 7872-7880 (1997).
12. T. Vitalis and J. G. Parnavelas, *Dev. Neurosci.*, **25**, Nos. 2-4, 245-256 (2003).
13. B. P. Williams and J. Price, *Neuron*, **14**, No. 1, 1181-1188 (1995).