MORPHOLOGY AND PATHOMORPHOLOGY

Serotonin Is Involved in the Regulation of Histogenetic Processes in Rat Embryonic Neocortex

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We compared the dynamics of the development of ectopic transplants of embryonic (day 14) primordial neocortex from rats injected with serotonin inhibitor (para-chlorophenylalanine; 400 mg/kg) on day 11 of pregnancy and transplants of similar primordial neocortex incubated before transplantation in a medium with serotonin (3 μg/ml). The study of mitotic activity and differentiation of transplanted cells showed that serotonin promoted survival of the transplanted neuroepithelial cells and their differentiation into nerve cells, and is involved in the regulation of their proliferation. We hypothesized that serotonin accelerated the cell cycle of transplanted cells, thus accelerating the neuron differentiation.

Key Words: *serotonin; para-chlorophenylalanine; histogenesis; neocortex*

Study of the consequencies of prenatal injuries to the brain in mammals under conditions of unfavorable environment is an important problem [2,4]. Study of the mechanisms regulating histogenetic processes in the developing brain and their disorders caused by various factors will help to detect the causes of pathological states of the brain. Various models are used for the study of delayed effects of disorders in the CNS precursor cell proliferation, migration, and differentiation. Models of prenatal serotonin (5-HT) depletion induced by parachlorophenylalanine (pCPA) [9] and ectopic neurotransplantation of rat embryonic primordial brain cells into the nerve [8] are developed at Department of Morphology of Institute of Experimental Medicine. The model of prenatal 5-HT depletion is used for investigating the neocortical histogenesis under conditions of deficiency of 5-HT synthesis (5-HT is a multifunctional compound acting as a neurotransmitter, hormone, and morphogene at different stages of ontogeny) [1]. Isolation of the embryonic primordium during certain periods of development, incubation of primordial cells in a medium with 5-HT, and transplantation help to evaluate the histogenesis of embryonic neocortex after exposure to pCPA and 5-HT. The cells were transplanted into adult rat peripheral nerve. Ectopic transplantation of embryonic primordial cells of the CNS as a method of *in vivo* culturing is a convenient model for the study of the early histogenetic processes in transplanted primordial cells [8].

We studied the impact of 5-HT for the division and differentiation of the embryonic neocortical cells developing after transplantation in adult rat nerve.

MATERIALS AND METHODS

The study was carried out on 35 Wistar rats (200-250 g). Females were intraperitoneally injected with pCPA (Acros organics) in a dose of 400 mg/kg on day 11 of pregnancy [9]. Fragments of the dorsolateral wall of the anterior cerebral vesicle containing primordial neocortex were isolated from 14-day

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embryos and placed in medium 199 for 1 h (37°C). We chose the period of rat prenatal development, when the studied primordium contained the greatest number of serotonin receptors [14]. Half of the isolated primordial preparations were incubated under the same conditions in a medium containing 3 μg/ml 5-HT creatinine sulfate (Reanal). This concentration was selected after comparison of concentrations of 3, 10, and 100 μg/ml and detection of the cytotoxic effects of the two higher concentrations for the transplanted cells 24 h after the operation. The 5-HT concentration selected for incubation of the primordial cells was similar to the concentrations used for *in vitro* studies of cells [7]. Fragments of the dorsolateral wall of the anterior cerebral vesicle were then transplanted into the sciatic nerve of adult rats under ether narcosis [8]. After 1, 3, 7, and 10 days postoperation the sciatic nerves of the recipient rats with the transplants were fixed in Bouin's solution for histological studies and in ethanol-formalin for immunohistochemical reaction for detection of NeuN neuronal nuclear protein [3]. Paraffin sections (5 μ) were stained with hematoxylin and eosin. Dividing cells were counted in the initial material and in the transplants. The mitotic index was calculated from the data on 1000-3000 cells.

The data were statistically processed using Student's *t* test.

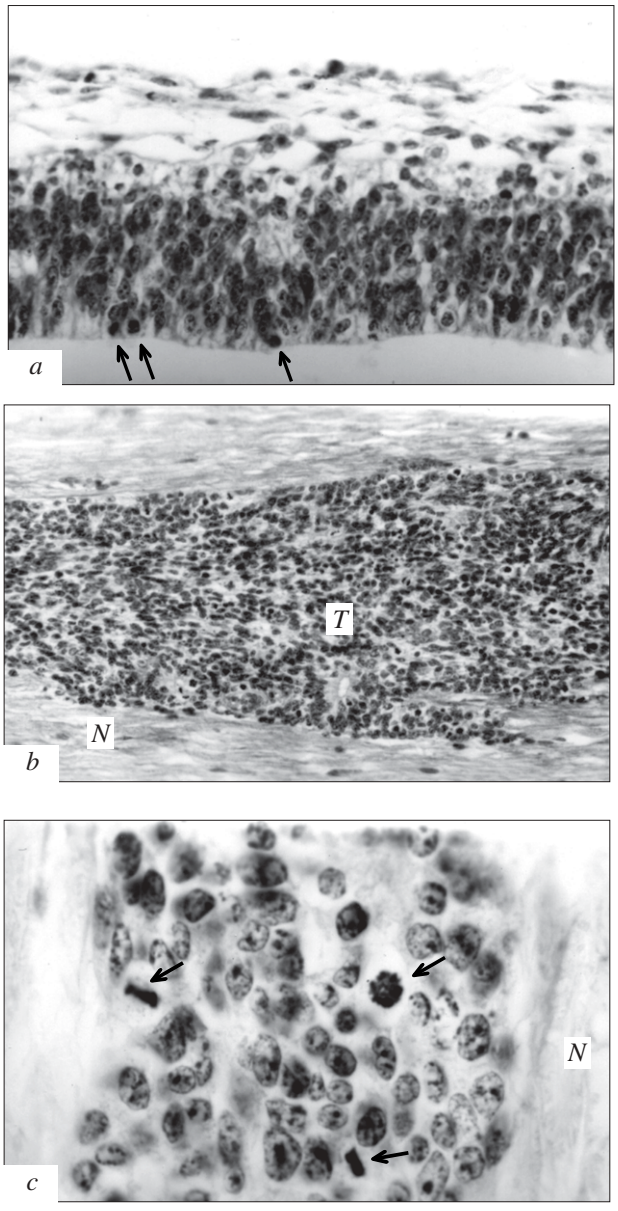

Fig. 1. Fragments of dorsolateral wall of the anterior cerebral vesicle of a 14-day rat embryo in the initial material (*a*) and 3 days after transplantation into the nerve (*b*, *c*). Arrows show mitotically dividing cells. *T*: transplant; *N*: recipient nerve tissues. Hematoxylin and eosin staining, ×400 (*a*), ×200 (*b*), ×1000 (*c*).

RESULTS

The dorsolateral wall of the anterior cerebral vesicle of the rat embryos was presented by neuroepithelial cells (NEC) and was a mosaic structure consisting of nerve and glial cell precursors [15]. NEC reached 6-8 μ in size, had cylindrical shape, chromatin-rich oval nuclei, and narrow cytoplasm rims. Some NEC underwent mitotic division (Fig. 1, *a*). Mitotic activity in the wall of the anterior cerebral vesicle of rat embryos treated with pCPA on day 11 of pregnancy was 22.50±0.95.

After 1-3 days, the transplants were detected in the center or at the periphery of the nerve trunk; they consisted of the same cell elements as the initial material (Fig. 1, *b*, *c*). One day after trans-

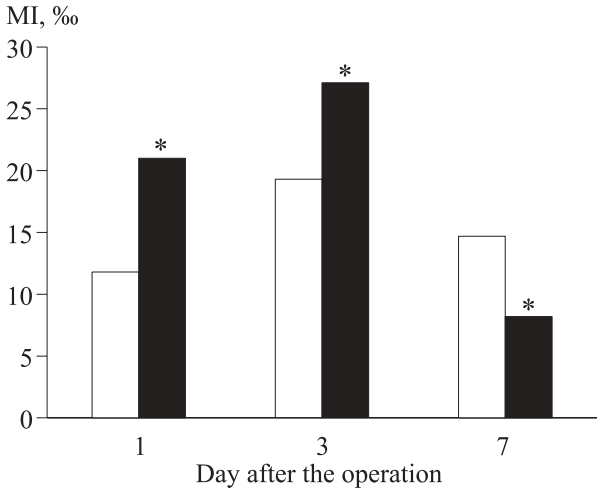

Fig. 2. Changes in mitotic activity in transplants of the rat embryonic neocortex (E14) after treatment with pCPA and 5-HT. Light bars: transplants from rat embryos treated with pCPA on day 11 of pregnancy (control); dark bars: transplants of similar primordial cells preincubated in a medium with 5-HT before transplantation. *$p<0.05$ compared to the control.

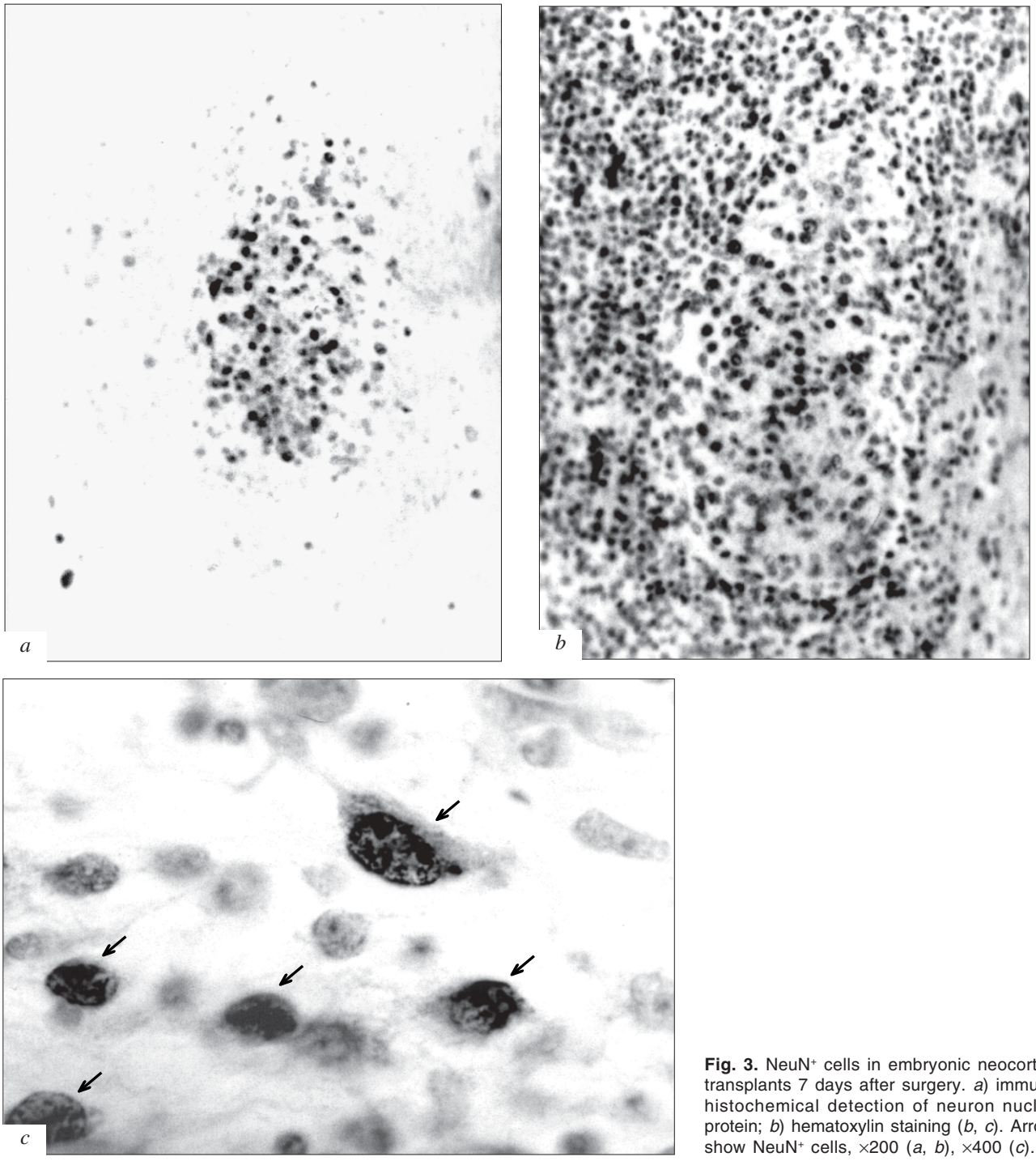

Fig. 3. NeuN$^+$ cells in embryonic neocortical transplants 7 days after surgery. *a*) immunohistochemical detection of neuron nuclear protein; *b*) hematoxylin staining (*b*, *c*). Arrows show NeuN$^+$ cells, ×200 (*a*, *b*), ×400 (*c*).

plantation, mitotic activity of transplanted NEC was lower than in the initial material. Degenerating cells were seen in the thickness of the transplants. The presence of apoptotic bodies and immunohistochemical detection of fragmented DNA by the TUNEL method in the previous study showed that transplanted cells died mainly via apoptosis [5]. Incubation of embryonic primordial brain cells in medium with 5-HT before transplantation reduced the mortality of precursor cells [5]. The number of mitotic cells in the transplants of primordial cells incubated in medium with 5-HT before transplantation was significantly higher than in the transplants of primordial cells incubated in medium without 5-HT (Fig. 2).

After 3 days the number of mitotic cells in the transplants increased in comparison with the previous term. This can be explained by a compen-

satory reaction to the death of some transplanted cells during the previous period and reduction of mitoses 1 day after surgery. The compensatory peak in embryonic primordial transplants incubated in medium with 5-HT was less pronounced in comparison with the previous term, because the decrease in the number of mitoses was less pronounced 1 day after surgery.

After 7 days the transplants grew in size due to the increase in the number of cell elements as a result of NEC proliferation and emergence of differentiating neuroblasts in the transplants. The neuroblasts in embryonic transplants from rats treated with pCPA were small (up to 7-8 μ) with a narrow cytoplasmic rim. The majority of these cells did not differ from NEC by their morphology. By their maturity they did not reach the level of the neural elements in intact embryonic neocortical transplant (E14) of the corresponding developmental period [6,8]. They could be detected only by immunohistochemical reaction to NeuN (Fig. 3, *a*, *b*). The $NeuN^+$ cells in the embryonic primordial transplants pre-incubated in medium with 5-HT reached a higher degree of maturity after 7 days. Many of them were 9-10 μ in size, had large clear nuclei with finely grained chromatin and clearly discernible 1-2 nucleoli, a wide cytoplasmic rim, with a neuropil forming between the cells. Hence, the delay of neuron differentiation in the embryonic primordial neocortical transplant after prenatal treatment with pCPA was prevented by 5-HT. This phenomenon was even more pronouned 10 days after surgery. By this term the primordial transplants incubated in medium with 5-HT consisted of numerous nerve and glial elements. Nerve cells of different shape reached 10-12 μ in size and formed processes; pyramidal cells were seen among neurons. Nerve cells were intensely stained for NeuN (Fig. 3, *c*). Transplants not incubated in medium with 5-HT contained less differentiated nerve cells. The majority of $NeuN^+$ elements still did not differ from NEC by their morphology. The detected favorable effect of 5-HT on differentiation of neocortical cell is in line with the data of other authors reporting that 5-HT promotes *in vitro* differentiation of glutamatergic neurons [13,14] and stimulates the growth of dendrites and synaptogenesis in nerve cell cultures [12].

The results demonstrate the involvement of 5-HT in the regulation of histogenetic processes in the developing neocortex. A delay of neuron differentiation in embryonic primordial transplants from rats treated with 5-HT inhibitor (pCPA) was detected. Delayed differentiation was presumably a result of modification of precursor cell proliferation and division processes. Mitotic activity of NEC increased after incubation of embryonic primordium in a medium with serotonin. The mechanism of the increase in the number of mitoses remains not quite clear. It seems that mitotic division is blocked in the embryonic neocortex under the effect of pCPA and 5-HT deficiency at the beginning of neuronogenesis and the cell cycle duration in precursor cells is changed. Incubation of the primordium in a medium with 5-HT before transplantation promotes division of transplanted cells 24 h after surgery. This results in earlier differentiation of neurons.

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