PROLIFERATION AND DIFFERENTIATION OF NERVE CELLS TRANSPLANTED FROM HUMAN SUPRAOPTIC AND PARAVENTRICULAR NUCLEI INTO ADULT RAT BRAIN

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KEY WORDS: hypothalamus; xenografting; paraventricular nucleus; transplantation of embryonic brain tissue; supraoptic nucleus

An attempt is now being made in several countries to utilize the method of neurotransplantation in the treatment of nervous and mental diseases [8, 9]. While accepting that the method has a certain promise, there is an urgent need for preliminary experimental investigations of anlagen from the embryonic human brain when subjected to transplantation. The reason why investigations of this kind are possible is the positive results which have been obtained by xenografting embryonic nerve tissue into the brain of adult animals [7].

The aim of this investigation was to analyze the survival and also the pattern of proliferation and course of differentiation of precursors of primordial nerve cells of the supraoptic (NSO) and paraventricular (NPV) nuclei of the human brain, taken at different stages of embryogenesis and transplanted into the cavity of the third ventricle of the adult rat brain.

EXPERIMENTAL METHOD

The recipients were 30 noninbred female albino rats weighing 180-200 g; the donors were 7-9-week human embryos. The time when material was taken for transplantation was the time when the rudimentary NSO and NPV had appeared and could be identified separately, and when mitotic activity of the cells of the matrix was high. Embryos were obtained from healthy pregnant women who had undergone medical examination before abortion. The meninges were removed from the brain under a binocular loupe and parts of the hypothalamic region containing neuroblasts of NSO and NPV were excised [4]. The excised tissue, measuring 3-5 mm, was placed in a syringe containing 0.1-0.2 ml of physiological saline. The implant was injected into the third ventricle by means of a stereotaxic apparatus, at coordinates AP 1.6, L 0, H 8 relative to the bregma [10]. Transplantation was carried out under sterile conditions and under pentobarbital anesthesia. To determine the pattern of proliferation of cells in the graft depending on the stage of embryogenesis, ^{3}H-thymidine was given to the recipient animals in two injections, with an interval of 6 h between them, intraperitoneally in a total dose of 10 μCi/g [3]. Rats of experimental group 1 received ^{3}H-thymidine injections on the 2nd day after transplantation. Animals of group 2 received the isotope 4 weeks after transplantation, when the age of the graft corresponded to the 12th week of embryogenesis, rats of group 3 received it 8 weeks after transplantation (16 weeks of embryogenesis), those of group 4 16 weeks after transplantation (24 weeks of embryogenesis). At the end of the experiment (2nd day, 4th, 8th, and 16th weeks after transplantation) the animals were killed. The brain was fixed in Bouin's fluid and embedded in paraffin wax by the standard method. Frontal serial sections were stained with paraldehyde-fuchsine by the Gomori–Gabe method [2], covered with type M photographic emulsion, and exposed for 4 weeks at −4°C. The intensity of incorporation of ^{3}H-thymidine by cells of

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TABLE 1. Distribution of Percentage of Surviving Grafts in Groups of Animals

No. of group of experimental animals	Duration of survival of graft	Age of donor's tissue (weeks)	No. of animals undergoing operation	Number of grafts found	Survival rate (in %)
1	2 days	8	7	2	28,6
2	4 weeks	12	7	3	42,9
3	8 weeks	16	7	3	42,9
4	16 weeks	24	8	3	37,5

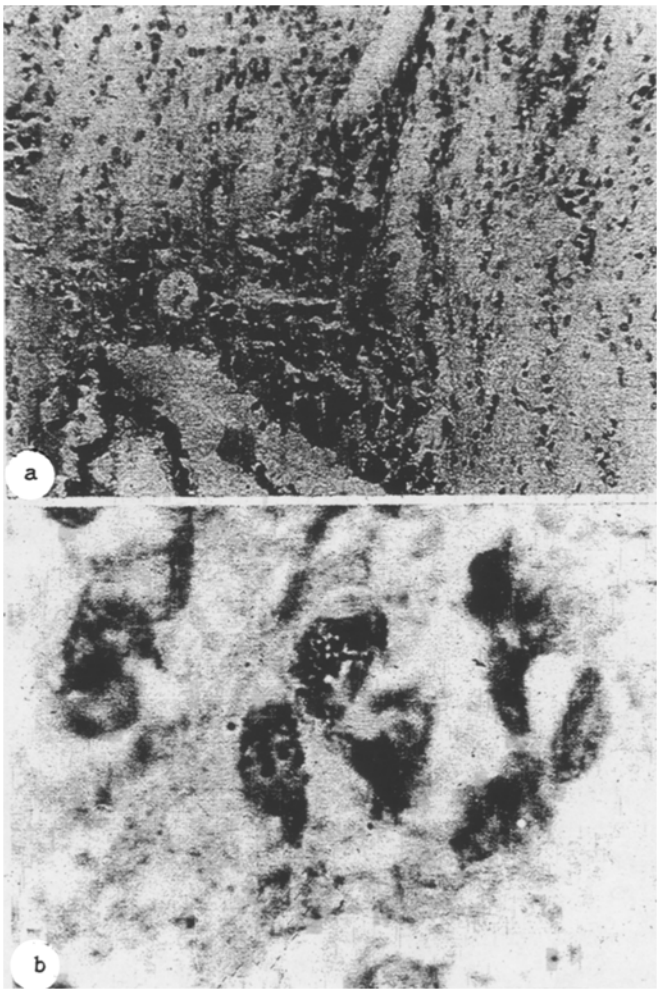

Fig. 1. Transplanted tissue of human embryonic hypothalamus into cavity of third ventricle of an adult recipient rat (8 weeks after transplantation): a) attachment of graft to lateral wall of third ventricle. 40×; b) incorporation of ^{3}H-thymidine by neurons of transplanted tissue. Age of grafted tissue at time of injection of isotope corresponded to 16 weeks of normal embryogenesis. Methylene blue. 400×.

the graft was estimated by counting grains of silver in the supranuclear zone. The labeling index was calculated from the number of labeled cells in the graft. The course of cytodifferentiation of the transplanted nerve cells was assessed from the presence of perikarya with neurosecretory granules.

TABLE 2. Distribution of Labeling Index of Graft Cells Among Groups of Animals

No. of group of experimental animals	Labeling index (in %)
1	89,0±0,471
2	78,7±1,905
3	78,3±1,190
4	21,3±1,190

EXPERIMENTAL RESULTS

Grafts were found in the third ventricle of 11 of the 30 rats undergoing the operation, representing a survival rate of 36.7%. The percentage of surviving grafts for different groups of experimental animals is shown in Table 1.

Surviving grafts were characterized by close contact with the lateral wall or floor of the third ventricle (Fig. 1a). The grafted tissue was invaded by blood vessels, arising from adjacent areas of the recipients' brain. Depending on the survival time of the grafted tissue, neuroblasts, neurons, and glial cells were found in it. The perikarya of the nerve cells of the grafts differed from neurons from adjacent regions of the recipient's brain by having a relatively large round or oval nucleus with one or two nucleoli and a narrow rim of cytoplasm. The picture described above was observed at all times of survival of the graft: from 2 days to 16 weeks after the operation.

Active incorporation of ^{3}H-thymidine into the nuclei of the large nerve cells of the graft was observed when the isotope was injected at times corresponding to 12 and 16 weeks of normal embryogenesis of grafted tissue. The labeling index was high also in one experimental group (8 weeks of embryogenesis), although magnocellular neurons could not be differentiated in the graft. No ^{3}H-thymidine was incorporated into the cells studied if, at the time of its injection into the recipient, the age of the donor's tissue had reached 24 weeks of embryogenesis. The index of labeled cells of the grafts at different times after transplantation is shown in Table 2.

Single granules of neurosecretion, according to our observations, appeared for the first time in large neurons of the graft at a time corresponding to 16 weeks of embryogenesis. In material corresponding to a period of 24 weeks, both the number of secretion-positive cells and the number of secretory granules per cell were increased.

Thus, despite removal of the grafted tissue from the embryonic brain, cells of the grafts proliferated at the usual times and, on reaching a high degree of differentiation, reproduced the specific features of the cytophenotype of the neurosecretory neurons. The distribution of the perikarya of the large neurons in the volume of the grafted tissue was diffuse or mosaiclike. They did not join into typical structures of neurosecretory nuclei observed during normal embryogenesis. Similarly, neurons labeled with ^{3}H-thymidine were found in different parts of the grafts, irrespective of the time of injection of the precursor. This indicates limited ability of cells of the grafted tissue to migrate. Meanwhile definite accumulations of neurosecretory cells were observed in parts of the grafts adjacent to the recipient's median eminence.

This experimental model of xenografting of human embryonic nerve tissue into the adult rat brain can be used to characterize proliferation and differentiation of nerve cells in order to obtain data of interest to clinical neurotransplantology. The temporal organization of proliferation of precursors of neurosecretory cells under transplantation conditions is in harmony with that for the corresponding cells during normal human ontogeny.

Human neurosecretory cells begin their specific function at the same times of embryogenesis, whether they develop under conditions of normal embryogenesis or are grafted into the brain of another organism.

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EFFECT OF MALE SEX HORMONES ON SPECIFIC UPTAKE AND RELEASE OF ^{3}H-SEROTONIN BY THE RAT HYPOTHALAMUS IN VITRO

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In the perinatal ontogeny of the rat sex hormones play the role of inducers of cell differentiation in target cells. The main target region in the brain for these hormones is the hypothalamus [9], and possibly its serotoninergic system. Indirect proof of this may be given by data on sexual dimorphism in the serotonin (5-HT) content and the distribution of serotoninergic fibers in the sexual dimorphic nucleus of the medial preoptic region of the hypothalamus [12]. We showed previously that blocking the action of male sex hormones from the 1st day of postnatal life leads to an increase in the 5-HT content in the hypothalamus of sexually mature males to the level observed in females [1]. Sexual dimorphism in 5-HT metabolism begins to appear, evidently, during the "critical period" of sexual differentiation of the brain.

The aim of this investigation was to study the role of male sex hormones in differentiation of the serotoninergic system of the brain. Using a model with exclusion of the action of sex hormones in the period of sexual differentiation of the brain by castrating males from the 1st day of life, we studied the basic characteristics of functional activity of the serotoninergic elements of the hypothalamus, namely specific uptake and Ca^{2+}-dependent release of 5-HT.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of Wistar rats aged 4-5 months: 1) intact males, 2) intact females in the diestrus stage (D1 and D2), 3) males castrated on the 1st day of life. Material was taken at 2-3 p.m. in the cold. The part of the brain removed included the medial preoptic region and the anterior hypothalamus. Specific uptake of 5-HT, spontaneous release of ^{3}H-5-HT and release evoked by K^+-depolarization, from fragments of the hypothalamus were studied by an "isotopic" method, which was described in detail by the writers previously [15]. To detect specific uptake in the control, unlabeled 5-HT was added to the incubation medium in a concentration of 10^{-5}

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