Complex Analysis of Efficiency of Transplantation of Embryonic Nerve Tissue to Rats with Hemiparkinsonism

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Effect of transplantation of embryonic ventral mesencephalon preparation containing dopaminergic neurons on repair of the dopaminergic nigrostriatal system was studied in rats with hemiparkinsonism induced by 6-hydroxydopamine. Transplantation of embryonic ventral mesencephalon into denervated striatum led to a more than 50% decrease in apomorphine-induced rotation, recovery of dopamine and DOPAC levels in the brain, and to an increase in DOPAC excretion and the DOPAC-dopamine ratio in daily urine of rats with hemiparkinsonism. Dopaminergic neurons of the transplant survived, forming a network of tyrosine hydroxylase-positive processes growing beyond the transplant and reinnervating the adjacent compartments of the striatum. A positive correlation between urinary excretion of DOPAC and brain concentration of dopamine was revealed in denervated rats after transplantation of ventral mesencephalon. Intrastriatal transplantation of cell preparations of embryonic striatum containing no dopaminergic neurons and isolated local injury to the striatum did not affect regeneration of the denervated nigrostratal system.

Key Words: *6-OHDA-induced hemiparkinsonism; embryonic nerve tissue transplantation; dopamine*

Neurodegenerative diseases now attract special attention as regards clinical application of embryonic nervous tissue transplantation, *e. g.* Parkinson's disease, with pathogenesis in which selective death of monoaminergic neurons of the nigrostratal dopaminergic system (DNS) plays the key role [1,13]. According to modern concepts, embryonic dopaminergic neurons transplanted into denervated striatum differentiate, express tyrosine hydroxylase (TG) and dopa-decarboxylase genes, form synaptic contacts with host neurons, synthesize levodopa and dopamine (DA), and catabolize exogenous levodopa thus compensating for critical dysfunction of the substantia nigra [4,6-8,10,11].

On the other hand, local injury to the brain or transplantation of other than dopaminergic tissues can stimulate regeneration of own dopaminergic neurons and thus promoting repair of the DNS function [5]. This viewpoint is confirmed by the results of *in vivo* experiments, which demonstrated the positive effect of glial neurotrophic factors, actively produced by respective embryonic cells, on denervated DNS [14,15]. It was therefore interesting to investigate the contribution of such factors as secretory activity of dopaminergic neurons, activating effect of local cerebral injury, and nonspecific trophic effect of embryonic nervous tissue to the compensation of deficiency of DNS function in transplantation.

We investigated the efficiency of transplantation of embryonic nervous tissue containing dopaminergic neurons in rats with experimental hemiparkinsonism and the factors underlying this effect.

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MATERIALS AND METHODS

Adult male Wistar rats (250-300 g) were used in the study. Hemiparkinsonism was induced by stereotactic injection of 6-hydroxydopamine (6-OHDA, Sigma) into the right ascending mesostriatal dopaminergic tract under Ketamine narcosis (80 mg/kg) with a Narishige stereotactic device [3]. Apomorphine-induced rotation asymmetry (APO test) was used as the criterion for quantitative evaluation of motor deficit.

Thirty rats (3 groups, 10 animals each) with pronounced damage to the DNS were used; their APO values were at least 7.5 rpm (DA concentration in the striatum decreased by 90-95%) [12]. Ventral mesencephalon (VM) containing dopaminergic neurons was transplanted to experimental rats. Controls (groups 1 and 2) were transplanted embryonic nervous tissue containing no dopaminergic neurons (1) and an equivalent volume of Hanks' medium (2). Transplantation was carried out on day 21 after injection of 6-OHDA under Ketamine narcosis into the dorsolateral compartments of the striatum (coordinates AP 1, L 3.0, V 4.5-4.1).

VM containing dopaminergic neurons was isolated from the brain of 13-day rat embryos by the method of P. Brundin *et al.* [7], suspended in Hanks' medium, and kept on ice until transplantation into the brain. Embryonic striatum was used as the transplant containing no dopaminergic neurons. Both transplants were prepared by the same method and contained 10^6 cells per 7 μl medium.

Directly before transplantation and 2 weeks, 1.2 and 3 months after it the animals were subcutaneously injected with 0.05 mg/kg apomorphine and their rotation in an automated rotometer was evaluated for 40 min.

At the end of the experiment 5 rats from each group were used for morphological analysis. The brain was perfused with 4% neutral paraformaldehyde and serial sections of the striatum and substantia nigra (40 μ) were made. Dopaminergic neurons were visualized by immunohistochemical staining for TG [6] using appropriate monoclonal antibodies (Sigma) and Vectstain ABC kit of biotinylated murine anti-IgG antibodies and avidin conjugated with horse radish peroxidase (Vector lab.). Morphological analysis was carried out on parallel sections stained with cresyl violet.

Cerebral catecholamines were examined in 5 experimental rats and 5 animals from control group 2. After the last testing the animals were decapitated under hexenal narcosis and the zone containing the striatum was isolated from the brain. Daily urine was collected before and 8 weeks after transplantation for evaluating catecholamine excretion in experimental rats and control group 1 animals. Urine and brain samples were frozen and stored at -70°C. The concentrations of catecholamines and their metabolites were measured by high performance liquid chromatography with electrochemical detection as described previously [12]. The concentrations of DA, 3,4-DOPAC, and homovanillic acid (HVA), norepinephrine and its metabolite 3,4-dioxyphenylethylene glycol (DOPEG) were measured.

Quantitative values were compared by pairs using Student's *t* test. Correlations between the values were analyzed using Pierson's correlation analysis. Estimations were made using statistical analysis software.

RESULTS

The mean values ($M\pm m$) of apomorphine-induced rotation in the experimental group before transplantation was 8.9±0.5 rpm *vs.* 8.2±0.3 and 8.8±0.7 rpm in control groups 1 and 2, respectively, which indicates high level of denervation-induced hypersensitivity of DA receptors resulting from critical decrease of DA concentration in the damaged striatum [7]. The rotation parameters progressively increased during the 4th week and decreased during the 8th and 12th weeks in animals treated with cell-free medium (Fig. 1), though the final value 9.8±0.9 rpm did not reach the initial level. This indicates stability of the pathological process and the absence of spontaneous recovery in denervated rats.

Induced rotation in control group 1 during the first 4 weeks after transplantation varied (Fig. 1), but the fluctuations were negligible in comparison with the control group 2 (p=0.06). Presumably, the decrease of APO test values during the 4th week can be due to trophic effects of embryonic stratum. This phenomenon was transitory and did not appreciably modify the

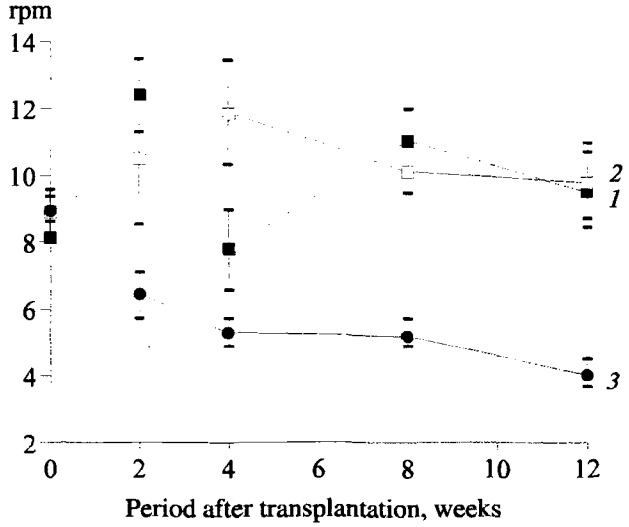

Fig. 1. Time course of apomorphine-induced rotation in rats with hemiparkinsonism after transplantation of embryonic nervous tissue (ENT). *1)* ENT without dopaminergic neurons; 2) Hanks medium; 3) ventral mesencephalon containing dopaminergic neurons.

DNS function. Starting from week 8 after transplantation, motor deficiency in this group did not differ from that in control group 2.

By contrast, transplantation of VM markedly reduced rotation asymmetry after 1 month ($p<0.05$); at the end of the experiment this parameter was 4.03± 0.49 rpm ($p<0.01$ *vs.* both control groups), *i.e.* decreased by more than 50% compared to the initial level. A significant decrease of denervation-induced hypersensitivity of DA receptors (judging from APO test) indirectly indicated repair of DNS function after VM transplantation.

Histological analysis of the substantia nigra in experimental and control rats 3 months after transplantation showed pronounced gliosis at the site of injection of 6-OHDA (Fig. 2, *b*) and the absence of TG-positive neurons in pars compacta in all specimens (Fig. 2, *d*), which attests to incompetence of regeneratory processes in the midbrain.

Examination of cresyl violet-stained sections of the striatum from experimental and control (group 1) rats showed transplants containing viable neurons. Both homo- and heterotopic intrastriatal transplants were well consolidated with the host striatum, which

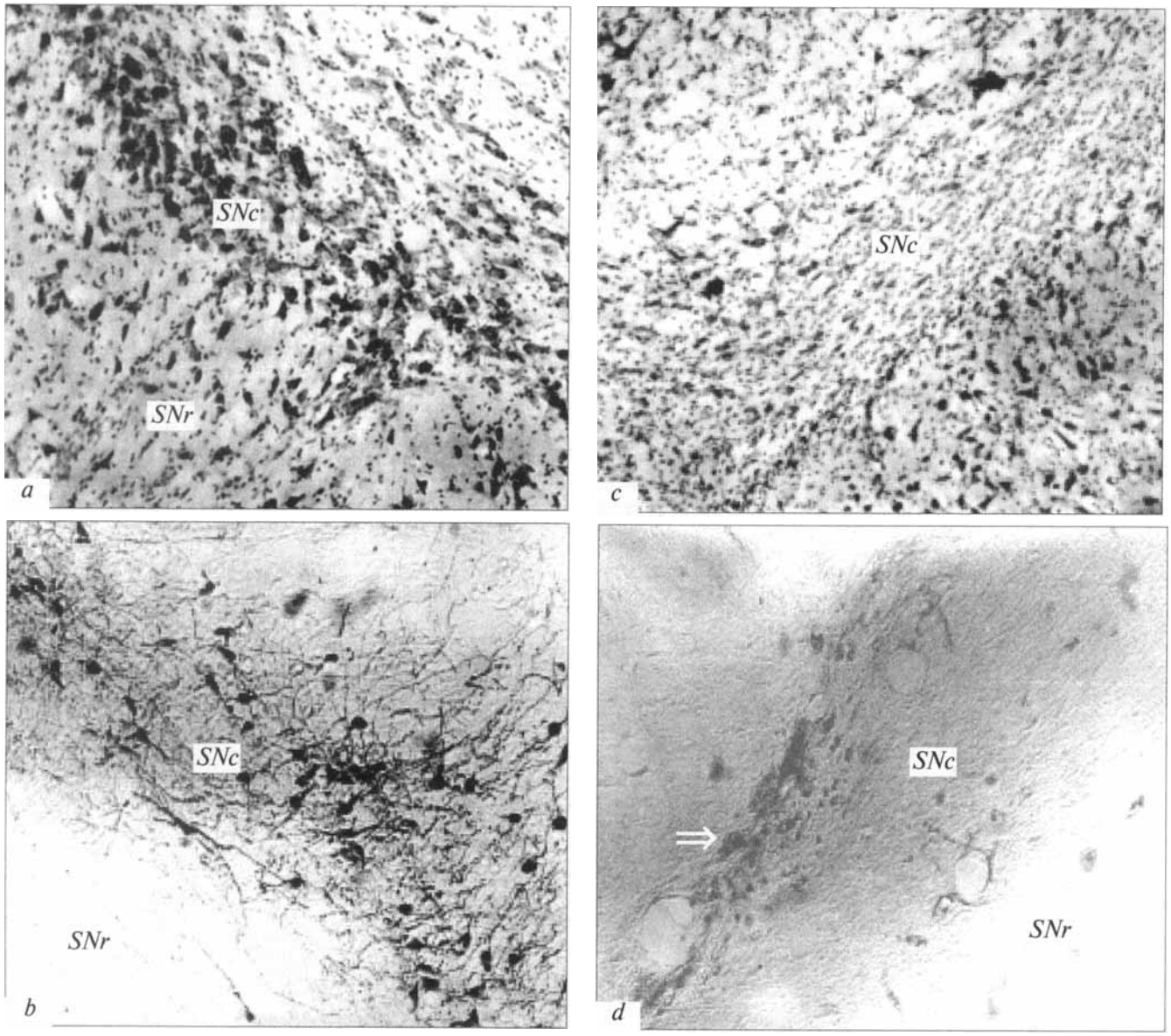

Fig. 2. Pathomorphological changes in substantia nigra pars compacta (SNc) in rats with hemiparkinsonism. Cresyl violet staining (*a, b*) and staining with antibodies for tyrosine hydroxylase (TG) (*c, d*), ×100. *a*) intact left-hemispheric SNc; *b*) dopaminergic neurons of intact SNc; *c*) right-sided SNc after denervation by 6-OHDA. Pronounced gliosis, neuronal nuclei are not seen; *d*) absence of specific TG immunoreactivity in substantia nigra 4 months after denervation. Arrow shows pathological changes at the site of injection of 6-OHDA. SNr: substantia nigra pars reticulata.

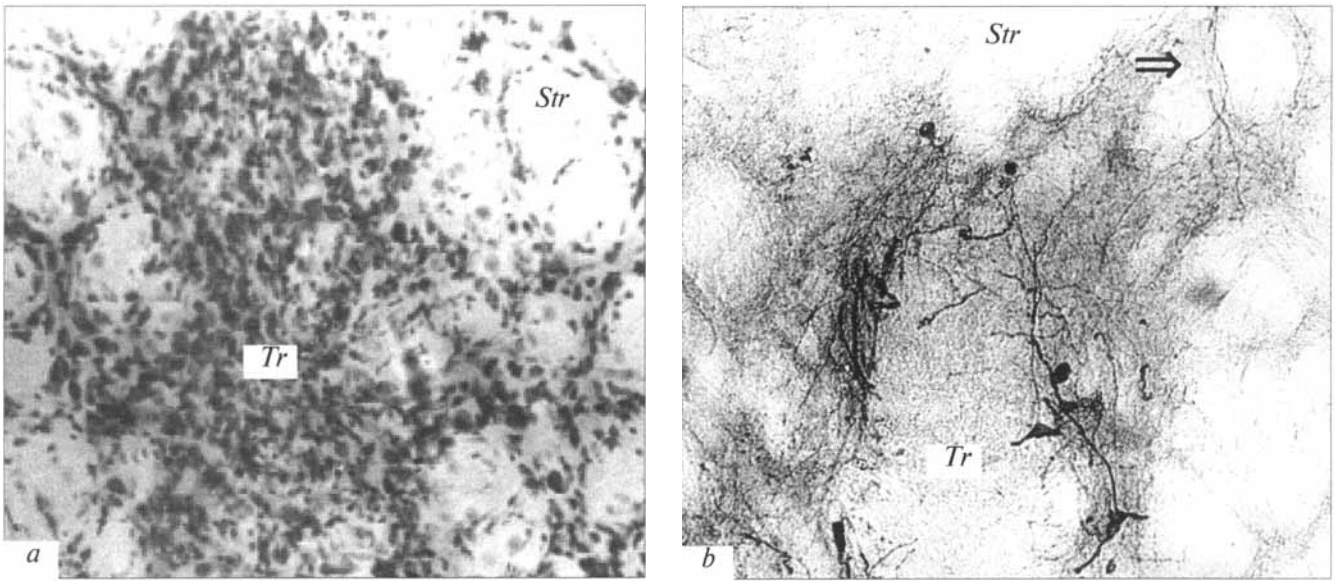

Fig. 3. Morphological characteristics of intrastriatal ventral mesencephalon (VM) cell transplant. Staining with cresyl violet (*a*) and for tyrosine hydroxylase (TG) (*b*), ×100. *a*) intrastriatal embryonic transplant; *b*) dopaminergic neurons of the transplant. Arrow shows long TG-positive neuronal processes growing into adjacent parts of the striatum (Str).

was characterized by the absence of glial cicatrix, and contained polymorphous neurons in the entire transplant (Fig. 3, *a*).

TG-specific staining showed no specific immunoreactivity in the striatum of control animals on the damaged side neither in the transplant, nor in the adjacent tissue. On the contrary, VM transplants contained TG-positive neurons. Intensive staining was also seen in adjacent tissue. In some cases TG-positive processes of the transplant neurons were seen, which grew beyond the transplant into the host striatum (Fig. 3, *b*). Striatal sites distant from the transplant were not stained.

Morphological analysis showed that homo- and heterotopic intrastriatal transplants survived in the host brain for 3 months and consolidated with the host striatum. After transplantation of VM, TG-positive processes of embryonic dopaminergic neurons grow beyond the transplant and presumably formed synaptic contacts. Decreased values of APO test attest to functional activity of dopaminergic neurons in the transplant. The key role of transplanted dopaminergic neurons in regression of rotation asymmetry is confirmed by the absence of recovery of DNS function in animals with healed embryonic striatum transplants.

The content of catecholamines (DA and DOPAC) in denervated striatum after transplantation of embryonic VM cell suspension was partially restored. Changes in the concentrations of norepinephrine, epinephrine, DOPEG, and HVA after transplantation were negligible (Table 1).

We previously showed changes in urinary catecholamine excretion (low concentration of DOPAC) in rats with hemiparkinsonism [3]. In this study we eva-

TABLE 1. Concentrations of Catecholamines (ng/g Dry Substance) in the Brain of Rats with Hemiparkinsonism after Transplantation of Embryonic Nervous Tissue (*M*±*m*)

Catecholamines	Hanks' medium (*n*=5)		VM (*n*=5)	
	S	D	S	D
DA	2364.7±618.4	154.8±57.6*	1641.5±492.2	1056.8±292.5*
DOPAC	215.0±82.2	22.3±8.8**	227.9±91.9	54.5±12.2**
Norepinephrine	486.2±126.8	426.7±116.9	458.0±101.3	349.9±46.7
Epinephrine	8.6±4.9	10.8±6.1	9.1±4.4	5.7±3.5
DOPEG	9.2±6.6	15.0±7.1	14.5±8.8	8.7±1.6
HVA	96.5±44.3	20.4±9.9	35.1±19.9	18.9±10.7

Note. S: left (intact), D: right (denervated and subjected to transplantation) brain hemispheres. *$p<0.01$, **$p<0.05$ *vs.* D hemisphere of control rats.

TABLE 2. Daily Excretion of Catecholamines (ng/day) in Rats with Hemiparkinsonism after Transplantation of Embryonic Nervous Tissue (*M*±*m*)

Catecholamines	Hanks' medium (*n*=10)		VM (*n*=10)	
	before injection	after injection	before transplantation	after transplantation
DA	2746.5±487.0	2486.4±271.5	2767.1±186.3	2164.7±287.0
DOPAC	1849.0±79.9	1874.8 ±253.4	1741.0±189.2	2329.6±218.6**
DOPAC/DA	0.8±0.2	0.8±0.2	0.7± 0.1	1.2±0.1*

Note. *$p<0.01$, **$p<0.05$ *vs.* pretransplantation values.

luated excretion after transplantation of embryonic nervous tissue.

Study of catecholamine excretion showed a significant increase in the level of DOPAC in daily urine and of DOPAC/DA ratio in experimental rats (Table 2). We carried out a correlation analysis of urinary concentrations of DOPAC and cerebral (right hemisphere) concentration of DA in experimental and control group 2 (n=10). A strict positive correlation was revealed (r=0.60, $p<0.05$).

Positive correlation between the level of DOPAC excretion and DA concentration in the brain and increased urinary content of this metabolite after transplantation confirmed our previous hypothesis that DOPAC excretion reflects to a certain measure catecholamine metabolism in brain tissue [3].

Increased DOPAC/DA ratio in VM transplant (measured by microdialysis) was reported previously [7]. This ratio reflects the rate of DA deamination and indicates the intensity of DA metabolism. In this study we observed increased concentrations of striatal DA after transplantation, on the one hand, and a decrease in urinary DA excretion paralleled by a significant increase in urinary DOPAC content, on the other, which resulted in an increased DOPAC/DA ratio in the urine. This probably indicates a stimulatory effect of embryonic VM transplants on DA metabolism at the organism level.

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