Effects of Neurotransplantation and Electrostimulation of Subthalamic Nucleus on the State of the Catecholaminergic System of Nucleus Caudatus in Rats with Nigrostriatal Failure

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Abstract. The purpose of the research was to study the effects of neurotransplantation of embryonic nervous tissues (ENT) and electrostimulation of subthalamic nucleus (STN) on catecholamine contents in nucleus caudatus in rats with electrolytic lesion of substantia nigra (SNc). The following methods were used: the model of nigrostriatal failure created by electrolytic lesion of SNc in rats; transplantation of rat brain ENT into nucleus caudatus; electrostimulation of STN with low intensity current. Transplantation of ENT into nucleus caudatus contributes to the recovery of movement disorders and dopamine levels in nucleus caudatus disturbed as a result of SNc lesion. Electrostimulation of STN significantly enhances the effect of neurotransplantation and is accompanied with the increase of content and metabolism of dopamine in nucleus caudatus. Electrostimulation of STN with low intensity current creates additional conditions for compensation of dopamine deficiency in nucleus caudatus after neurotransplantation in rats with nigrostriatal failure.

Keywords: Movement disorders, neurotransplantation of embryonic tissue, electrostimulation of STN, nucleus caudatus, catecholamines.

1 Introduction

The development of extrapyramidal pathology under nigrostriatal failure is a consequence of neurotransmitter metabolism disturbances, which results from the neurodegenerative processes in the substance nigra which manifested themselves by muscle rigidity, akinesia and tremor at rest. The key role of these events belongs to neurodegeneration of dopaminergic nigrostriatal pathways [1, 2]. Current treatment is directed towards replenishment of dopamine deficiency, but not to the recovery of dopamine synthesizing neurons activity. It was shown that partial recovery of amount and function of dopaminergic neurons is possible by using the transplantation of embryonic nervous tissues which synthesize the dopamine [3, 4, 5]. The positive clinic effect of compensation of extrapyramidal pathology was obtained by deep brain stimulation of subthalamic nucleus (STN) in patients with advanced Parkinson Disease [6, 7]. However the life-time estimation of the state of neurotransmitter systems which are involved in movement disorders is not possible in clinic research. That is why we set the goal to study the changes in dopamine metabolism in striatum under experimental correction of movement disturbances by the neurotransplantation of embryonic nervous tissues and electrostimulation of STN in rats with extrapyramidal pathology.

2 Materials and Methods

Investigations were carried out in 40 nonlinear white male rats 5 – 6 months of age weighting from 280 to 340 g. Keeping animals and manipulations with them were in accordance with the “European Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Strasbourg, 1986). Experimental animals were divided into four groups: group 1 – the intact rats (control group); group 2 – the rats with unilateral electrolytic lesion of the substantia nigra pars compacta (SNc); group 3 – the rats with unilateral electrolytic lesion of the SNc and transplantation of embryonic nervous tissues into the caudate nucleus; group 4 – the rats with unilateral lesion of the SNc, transplantation of embryonic nervous tissues into the caudate nucleus and electric stimulation of STN.

Stereotactic operations were conducted under general anesthesia of rats with sodium thiopental, 50 mg/kg of body weight intraperitoneally. We used the model of SNc electrolytic lesion by current with intensity ranged from 3 to 4 mA for 15 seconds (on the left side of the brain). Stereotactic coordinates for SNc were determined with the maps of the rat's brain by E. Fifkova and D. Marshall [8]. These coordinates corresponded to the distance from cross point of sagittal suture and bregma (zero point): in the rear direction – 4.0 mm, laterally – 2.0 mm, in depth – 8.1 mm. For neurotransplantation the embryonic tissue of SN of 20 – 21day gestation period with weight ranged from 8 to 10 mg, which was administrated via a cannula into the caudate nucleus on the left side of the brain was used. Stereotactic coordinates for the caudate nucleus were: in the front direction – 2.0 mm, laterally – 2.0 mm, in depth – 3.5 mm. Unilateral stimulation of STN on the left side of the brain was produced by low current intensity ranged from 5 to 10 mcA and frequency 100 Hz for 60 seconds daily during 15 days. Stereotactic coordinates for the STN were: in the rear direction – 3.0 mm, laterally – 2.0 mm, in depth – 7.5 mm.

The content of catecholamines in the head of caudate nucleus on the left side of the brain was determined in 30 days after electrolytic lesion of SNc (group 2), in 30 days after electrolytic lesion of SNc and in 28 days after following neurotransplantation (group 3), in 30 days after electrolytic lesion of SNc and in 28 days after following neurotransplantation with daily stimulation of STN (group 4). The brains were isolated from decapitated rats and caudate nuclei were quickly separated from the brains under cold conditions. The brain tissues were homogenized in the chilled double-distilled water with a glass minihomogenizators and diluted in proportion 1:60. After centrifugation at 4000 g under t = 4ºC during 10 minutes the supernatants were used for determination of concentrations of catecholamines (dopamine, noradrenaline, adrenaline) with the test system 3-CAT ELISA ("IBL International", Germany). Optical density was measured by microplate analyzer GBG Stat FAX 2100 (USA) at a wavelength of 405 nm. Obtained results were processed on PC with mathematical and statistical analysis Microsoft Excel software.

3 Results and Discussion

The observations on rats after SNc electrolytic lesion were conducted during 30 days. It was shown that unilateral destruction of SNc resulted in disturbances of statokinetic reflexes in 85 % rats: postural asymmetry, inclination of the head to the side, the elevated tail reaction. The animals weconre inhibited, indecisive in starting movement acts. They made circular movements, which were interrupted by frequent pauses. Tremor of head, tail and the whole body was observed in 27 % of rats. The intensification of movement disturbances and postural asymmetry towards 30 day after SNc lesion were noted. In 14 % cases muscle rigidity was developed. The functional test with amphetamine in a dose of 5 mg/kg of body weight initiated rotating movements from right to left up to 20 per 60 seconds. It was the evidence of lesion location in compact zone of SN on the left side of the brain. Behavioral studies in rats with following neurotrasplantation revealed a positive dynamics of recovery of statokinetic reflexes, intentional movement acts and orientate-searching activity, the improvement of movement functions. The significant increase of positive dynamics started at 14 days after implantation. The amphetamine treatment didn't evoke rotating movements. Sometimes nonsignificant inclinations of the body or the head to the left were observed. Daily unilateral stimulation of STN started 7 days later after neurotrasplantation. Already at the first impacts of STN stimulation by low intensity current were noted an activation of orientate-searching behavior, more frequent breath and appearance of grooming reactions. The changes of STN functional activity during 15 days of stimulation led to more intensive regress of movement disturbances.

The loss of dopaminergic neurons in nigrostriatal pathways as a result of electrolytic distraction of SNc is the reason for dopamine concentration decrease in the nucleus caudatus accompanied by development of extrapyramidal pathology. The dopamine concentration in the nucleus caudatus of rats on the 30th day after SNc lesion was about 56 % of that one in control group (intact animals). The level of adrenaline was also lower and counted 72 % comparing with control group (table. 1). These data suggested the loss of normal level of dopamine and diminishing of its metabolism in the nucleus caudatus on the side of brain impair and attenuating of dopamine functions in the striatum.

Table1. Concentration of catecholamines in nucleus caudatus on the side of SNc lesion after transplantation of embryonic nervous tissue (tENT) and electrostimulation of STN (sSTN).

Variable	Intact rats	Lesion of SNc	Lesion of SNc + tENT	Lesion of SNc + tENT + sSTN
Dopamine, ng/g of tissue	4338±70	2412±605[1)]	11199±2127[1,2)]	22234±11141[1,2)]
Noradrenaline, ng/g of tissue	340±26	327±77	509±108	454±99
Adrenaline, ng/g of tissue	115±4	83±10[1)]	177±18[1,2)]	230±84[1,2)]

Abbreviations: SNc – substantia nigra pars compacta; STN – subthalamic nucleus; [1)] - $p \leqslant 0.05$ comparing with intact rats; [2)] - $p \leqslant 0.05$ comparing with rats with lesion of SNc.

Intracerebral implantation of dopamine synthesizing embryonic tissues into caudate nucleus of rats with SNc lesion caused positive effect not only on movement and orientate-searching behavior, but also on the dopamine metabolism as well. The study carried out on the 28th - 30th days after neurotransplantation has shown the enlargement of dopamine, noradrenaline and adrenaline contents in nucleus caudatus. Appearance of source of neurotransmitters supply enhanced the levels of catecholamines: dopamine − by 158 %, noradrenaline − by 50 % and adrenaline − by 54 %. Existence of different growth factors in the transplants contributes to their surviving and prolongs the time of their acting.

Repeated stimulations of STN by low intensity current were implicated to enhance the effect of neurotransplantation. This procedure increased the dopamine production in caudate nucleus and as a result − intensification of its metabolism. The level of dopamine has grown up almost five-fold, noradrenaline − by 34 %, adrenaline − by 100 %. It’s of great interest, that there was one rat with overmuch increase of dopamine concentration − up to 55137 ng/g of tissue. It was accompanied by growing movement activity and playing forms of behavior. These data allow us to suggest, that STN stimulation involves the additional dopaminergic mechanisms in caudate nucleus activation, including dopaminergic neurons of ventral tegmental area.

The investigations of recent years give a lot of evidences of STN as a key structure of indirect pathway from striatum to internal globus pallidum in circuits of thalamocortical regulation [9, 10, 11]. In our view the results presented indicate that safety of STN and its wide spread regulatory pathways is one of the key moments in mechanisms of compensation of nigrostriatal disorders. Possibly that is why the symptoms of nigrostriatal failure are manifestated in very late period of disease development.

4 Conclusion

The research conducted shows that stimulation of STN in rats with behavioral markers of nigrostriatal failure amplifies positive effects of neurotransplantation already at early terms of engraftment, when specific dopamine compensatory properties of a transplant do not fully manifest themselves yet. Electrostimulation of STN with low intensity current creates conditions for additional dopamine influx into nucleus caudatus by activation of other unimpaired dopaminergic pathways for regulation of its activity.

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