Transplantation of Cultured Astrocytes Attenuates Degenerative Changes in Rats with Kainic Acid-Induced Brain Damage

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Viability of astrocyte grafts introduced into CA1 pyramidal layer of the left dorsal hippocampus after injection of kainic acid into this brain region and the effects of these grafts on the hippocampus and amygdala were studied on Wistar rats. In rats with astrocyte grafts the degree of destruction in fields CA1-CA2 of the dorsal and ventral hippocampus, fields CA3-CA4 of the ventral hippocampus, and central and basolateral amygdala was lower compared to animals with kainic acid-induced hippocampal damage and control rats; destructions in the dentate fascia were absent. Our results suggest that astrocyte grafts stimulate neurogenesis in the mature brain of recipient rats with kainic acid-induced brain damage.

Key Words: *kainic acid; seizures; transplantation; astrocytes; hippocampus*

Intracerebral implants, including tissue fragments of the immature brain, stem cells, and cultured neurons or glial cells, play a role in the recovery of recipient brain structures after neurotoxin-induced damage. Our previous studies showed that transplantation of fetal cells from the hippocampal CA1 field or multipotent stem cells into damaged hippocampal CA1 field in rats and monkeys led to partial recovery of the damaged brain tissue [9,11,15].

Intracerebral injection of kainic acid (KA, structural analogue of glutamate) disturbs the balance between inhibitory (taurine and GABA) and excitatory (glutamate and aspartate) neurotransmitters, the content of excitatory neurotransmitters being significantly increased at the site of injection. Administration of KA into the hippocampus induces limbic seizures and "wet dog shaking" phenomenon [5]. Severe destruction of cells is observed not only at the site of injection, but also in adjacent and distant brain structures. Cell destruction in various brain structures is associated with the development and spread of seizures, but not with diffusion or intraaxonal transport of KA [2,14]. A correlation was found between seizure activity and cell destruction in the hippocampus, amygdala, and other brain structures [14].

The hippocampus enriched with kainate and glutamate receptors plays a key role in the development of kainate-induced seizures. The number of these receptors is maximum in field CA3 and somewhat lower in field CA1 and dentate fascia [2]. Published data show that astrocytes can accumulate excess of excitatory neurotransmitters (*e.g.*, glutamate and aspartate) [1]. It can be hypothesized that transplantation of astrocytes can remove excess of excitatory neurotransmitters resulting from KA treatment and restore the balance between excitatory and inhibitory neurotransmitters. It will prevent the spread of seizures and attenuate degenerative changes in the recipient brain.

Here we studied the effect of cultured hippocampal astrocytes transplanted into KA-damaged

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hippocampal CA1 field on histological changes in two epileptogenic structures (hippocampus and amygdala).

MATERIALS AND METHODS

Experiments were performed on 34 male Wistar rats weighing 250-280 g. The animals were kept in cages at 21°C and artificial light/dark regimen (14:10-h light/dark cycle).

Histological analysis of the brain was performed. The animals were divided into 4 groups. In group 1 rats (n=5, controls) the dura mater was damaged with a needle, no injections and transplantations were performed. Group 2 consisted of rats with KA-induced damage to the hippocampal CA1 field (n=9); group 3 (n=9) included sham-operated rats receiving culture solution of HBSS into the damaged zone; in group 4 rats (n=11) HBSS and suspension of cultured astrocytes were transplanted into the damaged hippocampal CA1 field.

Before injection of KA the animals was anesthetized with 3.0 ml/kg equithesin and fixed in a stereotaxis. KA (2 μg in 1 μl phosphate buffer) was administered through a needle using a Hamilton syringe with a Hamilton pump. Infusion was performed over 2 min. A cannula was inserted into the CA1 pyramidal layer of the left dorsal hippocampus (AP=-4.0, L=3.0, V=2.8-3.0; atlas of G. Paxinos and C. Watson). The needle was left in the application zone for 1 min after injection of KA. Then it was slowly removed from the brain (over 2-3 min).

Astrocyte culture was prepared as described elsewhere [4]. Astrocyte suspension was transplanted 7 days after injection of KA into field CA1 of the left hippocampus [3]. Narcotized rats were fixed in a stereotaxis. Donor tissue (30,000 cells) and 2 μl HBSS solution were administered using a glass cannula (inner diameter 0.6 mm) connected to a micromanipulator and inserted through a small trephine opening (diameter 1.5 mm) into the damaged hippocampal CA1 field. After treatment the cannula was slowly removed and examined for the presence of residual suspension. Sham-operated rats received an equivalent volume of HBSS.

Three months after surgery the rats were perfused intracardially with 100 ml 10% formaldehyde (pH 7.2-7.4) in 0.1 M phosphate buffer under deep sagital anesthesia (1 mg/kg intraperitoneally). The brain was removed, postfixed in the same solution for 1 week, and treated with 20% sucrose in 0.1 M phosphate buffer at 4°C for 2 days. Frontal brain sections (20 μ) were prepared using a Shandon freezing microtome (Bright Instruments). Every 10th (groups 1-4) and 11th sections (group 4) starting from the initial part of the dorsal hippocampus to the end of the ventral hippocampus were mounted on slides covered with 1% gelatin. Every 10th section was stained with cresyl violet by the method of Nissl to estimate the degree of rat brain destruction, as well as the site of location and state of cell grafts. Every 11th section from group 4 rats was treated with monoclonal antibodies against gliofibrillar acid protein (GFAP) to identify astrocyte grafts. Five stained brain sections at different levels (from 2.12 mm above bregma to 5.8 mm below bregma). The area of recipient brain regions (pyramidal layer of fields CA1-CA2 and CA3-CA4; dorsal and ventral hippocampus; dentate fascia; and basolateral, corticomedial, and central amygdala) not damaged with KA was estimated morphometrically. The measurements were performed using an Axioplan light microscope and automatic Optimas image analysis system. The data for each brain structure from rats of each group were averaged. The results were analyzed by Student's t test for independent variables (Statistika). The sections were selected and photographed using an Opton light microscope.

RESULTS

Rats exhibiting typical hippocampal seizures ("wet dog shaking", myoclonus, tremor, twitching of the forelimbs, *etc*.) in response to intrahippocampal injection of KA during the postanesthetic recovery period were selected for morphological examination.

Large GFAP-positive astrocytic cells were found in the CA1 transplantation site in 6 rats of group 4 (228.6±20.3 μ; Fig. 1, *a*). These cells were larger than astrocytes in the hippocampal CA1 field of the contralateral hemisphere in recipient rats (6.32± 0.40 μ; Fig. 1, *b*) and astrocytes in the hippocampal CA1 field of the left hemisphere in control animals (21.4±1.6 μ; Fig. 1, *c*). These cells had small processes (Fig. 1, *a*). Thick fibrous astrocytes with deformed processes were found in the CA1 field of group 3 rats (Fig. 1, *d*). Similar changes were revealed in group 2 animals. In group 4 astrocyte grafts were not found in 3 rats, and in 2 animals the transplants were necrotic. Thus, analysis of brain sections stained with antibodies against GFAP allowed us to divide group 4 rats into 2 subgroups: animals with viable grafts (n=6, subgroup 1) and animals in whom the transplants underwent necrosis or were absent (n=5, subgroup 2).

Degenerative changes in brain structures ipsilateral to the site of damage in the left hemisphere were found in rats of groups 2 and 3 (p<0.01-0.0001). The protective effect was observed in various fields of the dorsal and ventral hippocampus,

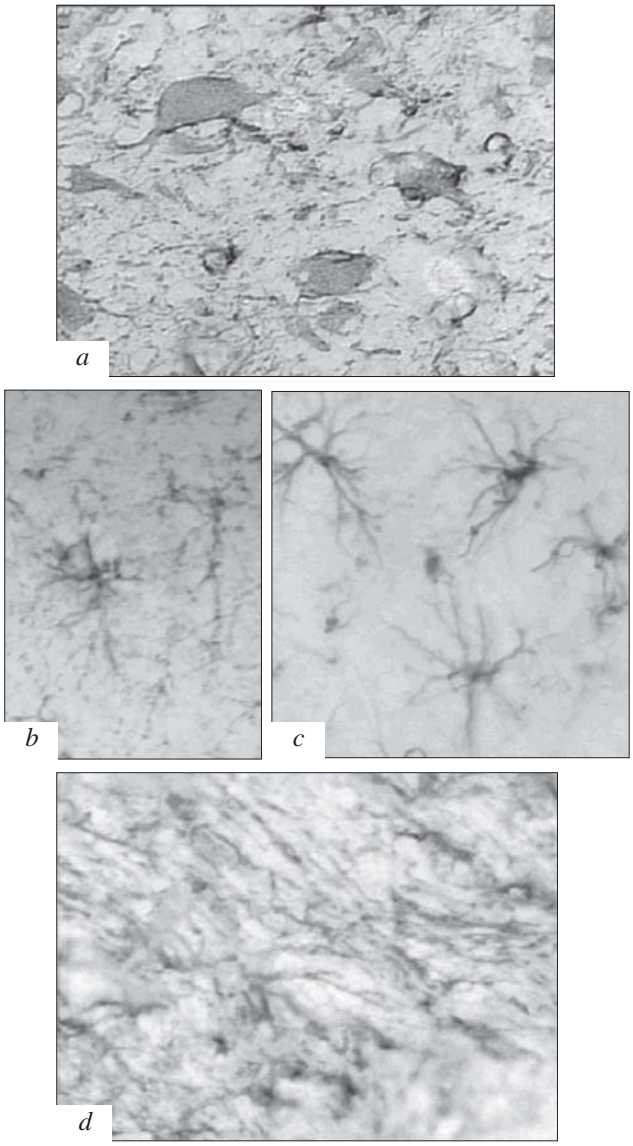

Fig. 1. Astrocytes stained with monoclonal antibodies against gliofibrillar acid protein and present in the implant (*a*), CA1 field of the contralateral hemisphere in recipient rats (*b*), CA1 field in control rats (*c*), and CA1 field in group 3 rats (*d*); ×1000.

dentate fascia of the dorsal hippocampus, and central and basolateral amygdala in subgroup 1 rats of group 4 (Table 1, Figs. 2 and 3). This effect was not detected only in the corticomedial region of these rats. In animals of subgroup 2 the protective effect was not revealed in various fields of the dorsal hippocampus, dentate fascia, and amygdala. The area of preserved brain tissue in these animals (dentate fascia, dorsal hippocampus, and corticomedial amygdala) was lower compared to rats of groups 2 and 3. Therefore, resorption of implants had a negative effect on the recipient brain. Under these conditions the protective effect was observed only in the ventral hippocampus (Table 1).

Our results show that transplantation of cultured astrocytes into the KA-damaged hippocampal CA1 field had a protective effect and decreased the degree of destruction in various brain structures of recipient rats (compared to animals of groups 2 and 3). This was mainly observed in rats with preserved grafts. The dentate fascia playing a major role in neurogenesis of the mature brain was completely preserved in animals with viable grafts (Fig. 2).

The protective effect of astrocyte grafts on the brain could be mediated by various mechanisms. First, this effect can be associated with the influence of trophic factors secreted by astrocytes. It was hypothesized that neurotrophic factors of astrocyte grafts play a role in the recovery of cognitive function in the brain of recipient rats with damage to the cholinergic gigantocellular nucleus [4,6]. Published data show that neurotrophic factors protect neurons from the neurotoxic effect of glutamate and KA and increases the number of survived cells [8,10]. Second, the protective effect can be related to the ability of astrocytes to accumulate excess of excitatory neurotransmitters and restore the balance between neurotransmitters [1]. Third, the protective effect can be associated with stimulation of neurogenesis in the recipient brain due to the influence of the astrocyte grafts. Similarly to the subventricular zone, the hippocampus plays a role in neurogenesis in adult mammals [7]. *In vitro* experiments demonstrated stimulation of neurogenesis after coculturing of hippocampal stem cells from adult animals with neonatal hippocampal astrocytes [12]. Astrocytes not only increase neuronal survival rate, but also play a regulatory role [12,13]. These data explain our findings that the degree of

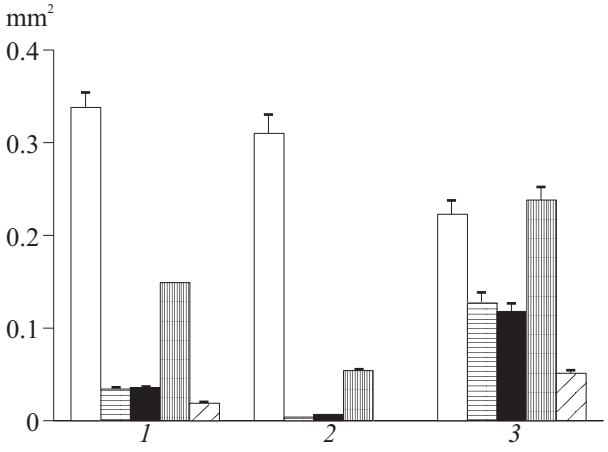

Fig. 2. Area of preserved pyramidal layer in hippocampal fields CA1-CA2 (*1*) and CA3-CA4 (*2*) and granular layer of rat dentate fascia (*3*). Light bars: control group 1; horizontal shading, group 2; dark bars, group 3; vertical shading, subgroup 1 of group 4; and slant shading, subgroup 2 of group 4.

hippocampal tissue destruction in rats with viable astrocyte grafts is lower compared to other brain structures in these animals, as well as compared to the hippocampus in rats of groups 2 and 3. It can be hypothesized that astroglial grafts stimulate neurogenesis in the KA-damaged hippocampus. This

TABLE 1. Area of Preserved Brain Regions in Rats of Various Groups (mm^2)

Structure, area		Level	Group	Mean±standard error (*M*±*SEM*)	Significance level, *p*
Dorsal hippocampus	CA1-2	1-3	1 (control)	0.338±0.031	
			2	0.034±0.008	
			3	0.036±0.009	
			4 subgroup 1	0.149±0.022	0.000001*; 0.000003$^+$
			subgroup 2	0.018±0.008	
	CA3-4	1-3	1 (control)	0.310±0.019	
			2	0.004±0.002	
			3	0.007±0.003	
			4 subgroup 1	0.053±0.022	0.009*; 0.015$^+$
			subgroup 2	<0.0001	
	dentate fascia	1-2	1 (control)	0.223±0.026	
			2	0.127±0.028	
			3	0.118±0.026	
			4 subgroup 1	0.238±0.027	0.012*; 0.005$^+$
			subgroup 2	0.051±0.024	
Ventral hippocampus	CA1-2	4-5	1 (control)	1.137±0.034	
			2	0.501±0.069	
			3	0.649±0.060	
			4 subgroup 1	0.933±0.059	0.0001*; 0.03$^+$
			subgroup 2	1.081±0.099	0.00004*; 0.0005$^+$
	CA3-4	4-5	1 (control)	0.627±0.070	
			2	0.181±0.038	
			3	0.145±0.021	
			4 subgroup 1	0.277±0.051	0.01$^+$
			subgroup 2	0.309±0.047	0.05*; 0.001$^+$
Amygdala	basolateral	2	1 (control)	1.903±0.131	
			2	1.472±0.245	
			3	0.985±0.192	
			4 subgroup 1	1.709±0.177	0.021$^+$
			subgroup 2	0.997±0.439	
	central	1	1 (control)	1.133±0.048	
			2	0.761±0.035	
			3	0.831±0.042	
			4 subgroup 1	1.151±0.046	0.00001*; 0.0002$^+$
			subgroup 2	0.672±0.276	
	corticomedial	1-2	1 (control)	1.931±0.153	
			2	1.451±0.098	
			3	1.228±0.105	
			4 subgroup 1	1.438±0.126	
			subgroup 2	0.854±0.260	0.017*

Note. Significant neuroprotective effect (sometimes negative effect) of astrocyte graft: *compared to group 1; $^+$compared to group 3.

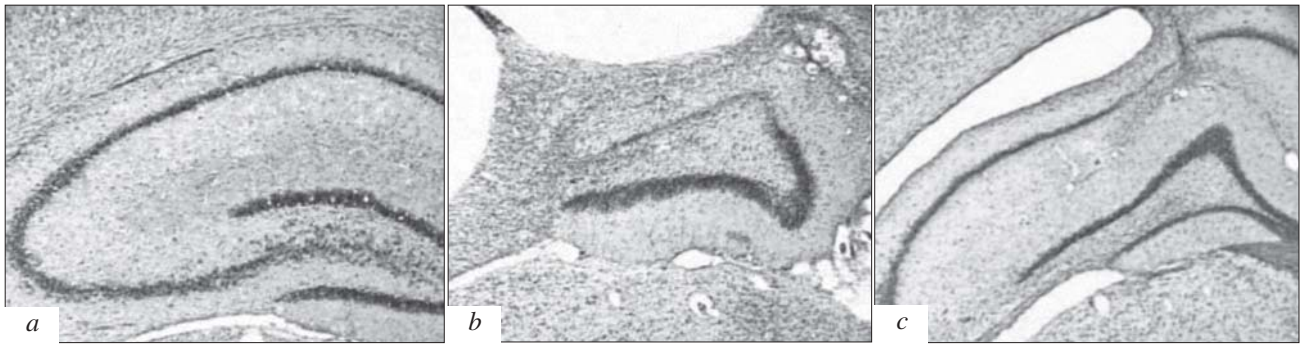

Fig. 3. CA1 field of the dorsal hippocampus in rats of the control group (*a*) and groups 3 (*b*) and 4 (*c*). Cresyl violet staining, ×400.

hypothesis is confirmed by the data that hippocampal dentate fascia (major zone of neurogenesis in the mature brain) remains preserved in rats with viable grafts (Fig. 2). It cannot be excluded that activity of astrocyte grafts is associated with multipotent effects of trophic factors, accumulation of excess of excitatory neurotransmitters, and stimulation of neurogenesis in the hippocampus.

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