Effect of Fetal Striatal and Astrocyte Transplants into Unilateral Excitotoxin-Lesioned Striatum

Sunny Y. Lu, Sarah K. Pixley, Dwaine F. Emerich, Michael N. Lehman and Andrew B. Norman

Division of Neuroscience, Departments of Psychiatry, Anatomy, and Physiology
University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA

*Present address: CytoTherapeutics, Inc., Four Richmond Square, Providence, RI 02906

ABSTRACT

Studies have suggested that neurotrophic mechanisms may underlie transplant-induced functional recovery. Astrocytes have been reported to be a source of neurotrophic factors. The present study examined the possible role of cultured astrocytes in promoting recovery of apomorphine-induced rotation behavior in rats with unilateral kainic acid (KA) lesions of the striatum. Five weeks after the lesions, one group of rats received fetal striatal tissue (E17) transplants, another group received transplants of cultured astrocyte suspension, and the remaining rats received sham transplants and served as controls. Apomorphine-induced rotation behavior was tested 4 weeks after the KA lesions, and 5 and 10 weeks following the transplantation. The KA-induced rotation behavior was reduced by the striatal transplants but not by the cultured astrocyte transplants 5 and 10 weeks following the transplantation. Histochemical analysis indicated that the striatal transplants had survived and grown and contained neurons and glia with similar morphology to those in the host brain. Immunocytochemical analysis of the astrocyte transplant sites revealed heavy glial fibrillary acidic protein and OX-42 staining in the transplant areas, suggesting that the transplanted astrocytes may have survived in the host brain. Although fetal striatal transplants can ameliorate apomorphine-induced rotation behavior, transplants of astrocytes alone may not be sufficient to reverse the functional deficits produced by KA lesions.

KEY WORDS

neural transplants, glia, apomorphine, rotation behavior, dopamine receptors, kainic acid

INTRODUCTION

It has been demonstrated that neural transplants can promote functional recovery of lesion-induced behavioral deficits in animal models of Parkinson's disease, Huntington's disease, and Alzheimer's disease. Despite the clinical potential of neural transplants, the mechanisms by which transplanted tissue exerts its beneficial effects are largely unknown. Recent studies have indicated that transplanted astrocytes may play an important role in transplant-induced recovery. Kesslak et al. reported that transplants of cultured fetal rat cortical astrocytes were able to reverse the behavioral deficits induced by lesions of the frontal cortex. In addition, Klion et al. indicated that transplants of millipore membranes coated with embryonic astrocytes facilitated the regeneration of crushed dorsal root fibers into the spinal cord.

Glial cells have been reported to synthesize and secrete neurotrophic and neurotropic factors, to increase neuronal survival and to facilitate axonal growth in vivo and in vitro. Furthermore, glial cells are important to maintain...
homeostasis of the neuronal environment and remove neurotoxic materials such as glutamate. Glial cells within transplanted striatal tissues may play a positive role in the ability of transplants to exert their beneficial effects. However, it is not clear whether transplants of astrocytes alone are sufficient to reverse striatal excitotoxic lesion-induced deficits. The present study further investigated the possible role of transplanted astrocytes in promoting recovery of lesion-induced behavioral deficits in rats with excitotoxic lesions of the striatum.

MATERIALS AND METHODS

Subjects

Three groups of adult male rats (Sprague-Dawley, 200 g, n=28) received unilateral kainic acid (5 nmol) lesions of striatum. The coordinates were AP=0.3 mm, ML=2.6 mm from bregma and DV=5.6 mm from dura. Five weeks after the lesions, one group of rats (n=9) received fetal rat striatal tissue transplants (E17), another group (n=11) received transplants of a suspension of astrocytes cultured from newborn (0-3 days) rat striatum. The remaining group (n=8) received sham (equivalent volume of saline) transplants and served as the control group.

Transplantation surgery

1. Striatal transplants

The techniques for tissue preparation and transplantation have been previously described in detail. Briefly, fetal striatal tissue was obtained from commercially-purchased timed-pregnant female Sprague-Dawley rats (Zivic Miller). Under sodium pentobarbital (40 mg/kg i.p.) anesthesia, a laparotomy was performed in order to expose the uterus, and embryos were then extracted individually. After the removal of the cranium and overlying integument, the fetal brain was removed and submerged in sterile lactated Ringer's solution. Under a dissecting microscope, the cortex was peeled laterally and the half-moon-shaped fetal striatal tissue was removed. The dissected fetal striatum was then aspirated into a glass capillary needle (25 5/8 G) connected to a Hamilton syringe (50 μl) and undissociated cells were then stereotaxically delivered into the lesioned host striatum. Tissue was injected at the rate of one μl per min, the needle was left in place for two additional min and then the needle was raised 0.8 mm. The procedure was repeated until 4 μl was delivered (approximately 1-1.5 mm³ of fetal striatal tissue). The coordinates were AP=0.3 mm, ML=3.0 mm from bregma and the initial DV coordinate was 6.0 mm from dura.

2. Cultured astrocyte transplants

Astrocytes were prepared by a modification of the procedure of McCarthy and de Vellis and Morrison and de Vellis. Briefly, rat striatum was dissected away from the brain tissue of the newborn rat pups, meninges were removed and the tissue was minced with fine scissors. The tissue was treated for 30 min with 0.025% trypsin and 0.02% EDTA (trypsin/EDTA) and then dissociated by passage through a plastic 10 ml pipet. The resulting cell suspension was plated into 75 cm² culture flasks (Falcon) at a density of 2x10⁵ cells per flask. The culture medium (changed every 2-3 days) was Ham's F-10/Dulbecco's modified Eagle's medium, 1:1 (vol/vol), with 1.2 g/l NaHCO₃, 15 mM HEPES and 10% (vol/vol) fetal calf serum (FCS). After 10-14 days in culture, the flasks were capped tightly and shaken overnight at 37°C on a rotary shaker. The medium was removed and replaced with fresh medium and the astrocytes were maintained as before until use. After approximately 3 weeks the astrocytes were removed from the dish by treating with trypsin/EDTA, then medium with 10% FCS to inactivate the trypsin. Cells were then rinsed twice in Hank's balanced salt solution (HBSS) (Ca²⁺ and Mg²⁺ free) by centrifugation (7 min at 1000 g), viable cell counts were determined by trypan blue treatment and a hemocytometer and the cells were resuspended at 3.2x10⁵ viable cells/4 μl HBSS. The same multiple injection transplant procedure was used as described for the fetal striatal tissue. The cell suspension was stereotaxically injected into the lesioned striatum at the coordinates AP=0.3 mm, ML=3.0 mm from bregma and the initial DV coordinate was 6.0 mm from dura.
Behavioral testing

Behavioral testing was performed 4 weeks after the KA lesion, and repeated at 5 and 10 weeks following transplantation.

Animals were individually placed in an open field environment consisting of a Plexiglas box (dimensions: 40 x 40 cm) and left to habituate for 20-30 min. Animals were then injected with apomorphine (1 mg/kg s.c., Sigma Chemical Co.) dissolved in normal saline containing 0.07% ascorbate. Sensitization of apomorphine-induced rotation behavior occurs in unilateral KA lesioned rats /28/. Therefore, rats received two priming injections of apomorphine (1 mg/kg s.c.) and rotations were measured following the third injection. A three day period separated each injection. Rotations were continuously counted visually in five min periods until all rotation behavior ceased. Rotations were defined as complete 360° turns and were reported as the net difference between the two directions.

Statistical analysis of rotational behavior used two-way analysis of variance (ANOVA). Preplanned comparisons were used to compare the transplant groups with the KA lesion group.

Immunocytochemistry

Glia fibrillary acidic protein (GFAP) and OX-42 immunocytochemistry was used to visualize astrocytes and microglia respectively.

The rats for these studies were intracardially perfused with ice-cold saline. Brains were removed and immediately buried in powdered dry ice, then frozen at -70°C until use. Brains were mounted in a cryostat at -20°C and 20 μm thick sections were cut. Alternate brain sections were thaw mounted on gelatinized microscope slides, and four sets of slides were obtained. One set was stained with cresyl violet and the remainder of the sets were used to determine autoradiography of D1 and D2 dopamine and muscarinic cholinergic receptors, respectively.

For receptor autoradiography, the slide-mounted sections were dried at 4°C in a sealed container with desiccant overnight. For D1 and D2 dopamine receptors, the slides were immersed in a buffered solution containing either [3H]SCH23390 or [3H]spiperone, respectively. The buffer consisted of 50 mM Tris-HCl (pH=7.7, 25°C) containing 125 mM NaCl and 5 mM MgCl2. Ketanserin (40 nM) was also added to prevent binding of [3H]SCH23390 and [3H]spiperone to 5-HT2 receptors. The final concentration of [3H]SCH23390 or [3H]spiperone was 1 nM or 0.5 nM, respectively. Some of the slides were incubated with the radioligand and 1 μM (+)butaclamol for determination of non-specific binding. For the labeling of muscarinic cholinergic receptors, the slides were immersed in Tris-HCl (pH=7.7, 25°C) containing 130 mM NaCl and 0.5 nM [3H]QNB for one hour. Some slides were incubated with the radioligand and 1 μM atropine for determination of non-specific binding.

Slides were incubated in the appropriate solution for one hour, rinsed twice in ice-cold buffer without staining. After a rinse in PBS, sections were incubated for 1 h in biotinylated secondary antibodies (goat anti-rabbit IgG for GFAP, and horse anti-mouse IgG for OX-42). After a rinse in PBS, sections were incubated 1 h in avidin-biotin-HRP conjugate (Vetor Laboratories). A five min incubation in 3,3'-diaminobenzidine tetrachloride (DAB) and hydrogen peroxide was done to complete the reaction.
radiolabel for five min each, rapidly dipped into distilled water and then dried rapidly using cold air from a blow dryer. When the sections were fully dried, the slides were placed in an X-ray cassette and in a photographic dark room, the slides were juxtaposed to a sheet of LKB ultrafilm and the X-ray cassettes sealed. The X-ray cassettes were stored at 2°C for 10-16 days for the [3H]SCH23390 and [3H]QNB labeled sections, 25 to 35 days for the [3H]spiperone labeled sections.

RESULTS

Rotational behavior

There was no spontaneous rotation behavior following the placement of the rats into the open field. Four weeks after the KA lesion, the rats displayed rotation ipsilateral to the lesion following the injection of apomorphine, consistent with a lesion in the more posterior aspect of the striatum /29/. As shown in Table 1, no significant difference in the number of rotations was found between the lesion and transplant groups [F(2,25)=0.72, p>0.5]. Five weeks after the transplantation the number of rotations in the striatal transplant group was marginally reduced compared to the sham transplant group although the difference failed to reach significance (p=0.056). The astrocyte transplant group did not differ from the sham transplant group. Ten weeks after the transplantation, the striatal transplant group showed a significant reduction in the number of rotations compared to the sham transplant group (p<0.002). There was no difference between the astrocyte transplant group and the sham transplant group.

Histochemistry

As shown in Fig. 1A, Nissl staining revealed that the striatal transplants had survived and grown in the lesioned host striatum and contained many neurons of similar morphology to those in the host brain. Glial cells were more dense surrounding the transplants (Fig. 1A). Transplant survival and a similar morphological appearance of transplant cells was observed in all of the animals.

The KA lesioned striatum is often characterized by depletion of neurons with dense but evenly distributed glial cells. In all of the animals, in areas of striatum where the astrocytes had been transplanted, a greater density of glial cells was observed compared to the KA lesioned striatum and to the striatum in sham transplanted rats.

Immunocytotoxic staining

A high density of GFAP stained astrocytes or OX-42 stained microglia was observed in the areas containing striatal and astrocyte transplants compared to that in the host. There was also an increase in the density of GFAP and OX-42 staining in the KA lesioned striatum relative to the unlesioned striatum. However, a higher density of GFAP and OX-42 staining were often observed in astrocyte transplant areas (Fig. 2).

| TABLE 1 |
| Effect of astrocyte and striatal transplants on apomorphine-induced rotation behavior in rats with unilateral kainic acid lesions of the striatum |

|                        | Pretransplant | 5 weeks post | 10 weeks post |
|------------------------|---------------|--------------|---------------|
| Lesion (n=8)           | 428 ± 63      | 658 ± 72     | 721 ± 104     |
| Lesion and astrocyte transplant (n=11) | 371 ± 46     | 685 ± 92     | 651 ± 71      |
| Lesion and striatal transplant (n=9)   | 463 ± 63      | 424 ± 89     | 361 ± 49*     |

Rotation behavior in response to apomorphine (1 mg/kg s.c.) was measured at four weeks following unilateral KA lesion (pretransplant) and at five and at 10 weeks posttransplant. Values represent the mean ± SEM number of ipsilateral rotations from the number of animals shown in parentheses. Significantly different from lesion values *p<0.002, ANOVA.
Fig. 1: Receptor autoradiograms from the striatal transplant in lesioned host striatum. The transplant (14 weeks post-transplantation) indicated by the arrows, can be seen in the cresyl violet-stained section (A). The distribution of $[^3H]SCH23390$ binding to $D_1$ dopamine receptors (B), $[^3H]spiperone$ binding to $D_2$ dopamine receptors (C) and $[^3H]QNB$ binding to muscarinic receptors (D). Note the high degree of correspondence between the high-density patches of dopamine $D_1$ and $D_2$ and muscarinic receptors. T=transplant.
Fig. 2: Photomicrographs of Nissl (A), GFAP (B) and OX-42 (C) stained striatal areas which received a transplant of cultured astrocytes. Scale bar = 200 μm.

T = transplant
Receptor autoradiography

Autoradiography for striatal transplants revealed that dopamine D_1 (Fig. 1B) and D_2 (Fig. 1C) receptors and muscarinic cholinergic receptors (Fig. 1D) were present in the transplants with a patchy distribution. However, these receptors were absent in the astrocyte transplants (data not shown).

DISCUSSION

Neurotrophic mechanisms have been suggested to play an important role in transplant-induced functional recovery /1,5,18,19/. Since glial cells have been reported to synthesize and secrete trophic and tropic factors which improve neuronal survival and axon regeneration following lesions /10,26,32,34,37/, one possible source of neurotrophic factors is transplanted glial cells. In addition, glial cells have important functions in maintaining ionic and pH balance, and absorb endogenous excitotoxins.

Our results indicated that striatal but not cultured astrocyte transplants ameliorated the rotational behavior induced by apomorphine in rats with unilateral KA lesions. This result is consistent with the finding of Kesslak et al. /19/ that transplants of fetal hippocampal tissue into KA lesioned hippocampus facilitated the behavioral recovery measured in an alternation task, whereas astrocyte transplants did not. A previous study by Kesslak et al. /18/ reported that transplants of cultured astrocytes were effective in accelerating the rate of spontaneous behavioral recovery after frontal cortex ablation. The lack of effect of transplants of purified astrocytes in promoting KA-induced deficits may be due to insufficient amounts of trophic factors released from the transplants. However, most importantly, the depletion of neurons and circuitry by KA produces a long-term functional deficit, which cannot be reversed by astrocytes or trophic factors alone. Repletion of neurons and reconstruction of neural circuitry by neural tissue transplants may be essential for functional recovery.

Whether the presence of D_1 and D_2 dopamine or muscarinic receptor binding sites in the striatal transplant contributes to the behavioral recovery observed in the rats from the striatal transplant group is uncertain. Norman et al. /30/ reported a similar behavioral recovery produced by striatal transplants in which few receptors were found. The lack of D_1, D_2 and muscarinic receptors in the astrocyte transplanted area indicated that the depletion of intrinsic striatal neurons due to KA lesion is not affected by astrocyte transplants. This lack of neuronal elements may be partially responsible for the poor effect of astrocyte transplants in ameliorating KA-induced deficits.

Recent studies have indicated that there are differences in the ability of immature and mature astrocytes to facilitate plastic changes in adult brain. Immature astrocytes can synthesize trophic factors to support neuronal survival, produce a permissive environment for neurite extension and reduce scar formation /10,35/. In contrast, mature astrocytes produce a non-permissive environment for axon growth and increase scar formation /36/. Mature astrocytes may not promote functional recovery. In the present studies, it is possible that the astrocytes which were grown from newborn striatum and were in culture for approximately three weeks may have developed characteristics of mature astrocytes.

Cultured astrocytes have been reported to survive and migrate following transplantation /2,13, 14/. In the present study, histochemical and immunocytochemical staining indicated the presence of a high density of glial cells in the areas containing astrocyte transplants and suggested the survival of the transplanted astrocytes. However, it is difficult to distinguish the transplanted astrocytes from any host reactive astrocytes using the present techniques, since either migration of transplanted astrocytes out of the transplant site and/or migration of host reactive astrocytes into the transplant site has been reported /8,9,38/. Labeling of astrocytes prior to transplantation is necessary to determine the survival and migration of transplanted astrocytes.

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