Afferent and efferent connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adult rats

M. Pritzel*, O. Isacson, P. Brundin, L. Wiklund, and A. Björklund

Department of Histology, University of Lund, Biskopsgatan 5, S-223 62 Lund, Sweden

Summary. The afferent and efferent connections of grafts of fetal caudate-putamen, implanted into the ibotenic acid (IA)-lesioned striatum of adult rats, have been studied with wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) as a combined retrograde and anterograde tracer, and with aldehyde fluorescence histochemistry for the visualisation of dopamine-containing nigrostriatal afferents from the host. The WGA-HRP was deposited in crystalline form (within a capillary tip) either into the depth of the graft tissue, or into the IA lesioned host striatum as a control. Labelling was only evaluated in specimens where the WGA-HRP deposit was entirely confined within the graft. Retrogradely labelled neurons were most consistently found in the ipsilateral host substantia nigra and the spared portions of the host CP, and in one case also in the midline and intralaminar thalamic nuclei normally projecting to the striatum. Some neurons, although weakly labelled, occurred in the deep layers of the frontal cortex in all grafted rats. Signs of anterograde WGA-HRP labelling in the host were found in one of the five animals in the ipsilateral globus pallidus and substantia nigra, pars reticulata. Fluorescence histochemistry revealed extensive ingrowth of dopamine-containing fibres from the host striatum into the grafted striatal tissue. The ingrowing fibres formed distinct and partly interconnected patches, most prominently in the peripheral regions of the grafts. The results provide evidence that intrastriatal grafts of fetal striatal tissue receive extensive dopaminergic afferents from the host substantia nigra, and that they may be capable of establishing connections also with thalamus, neocortex and globus pallidus of the host, as well as with the spared portions of the host caudate-putamen. The afferent connections from the thalamus and neocortex were notably more variable and sparse. However, since the control WGA-HRP deposits (into the lesioned host striatum) labelled the cortical and thalamic afferent neurons only poorly, it appears that the cortico-striatal and thalamo-striatal afferents (in contrast to the nigro-striatal ones) had undergone substantial degenerative changes (atrophy and/or cell death) in the long-term (6–11 months) IA-lesioned rats. The sparse thalamic and cortical afferent connections to the grafts may thus reflect an inability of the grafted striatal tissue to prevent the course of degenerative changes in these striatal input systems.

Key words: Neural transplantation – Striatum – Ibotenic acid – Wheat germ agglutinin-horseradish peroxidase tracing – Afferent and efferent connections – Dopamine

Introduction

The present experiment was designed to investigate possible sources of innervation of grafts of fetal caudate-putamen (CP) implanted into the striatum of adult rats subjected to an extensive ibotenic acid (IA) lesion. IA, and other so-called excitotoxic amino acids, are known to cause axon-sparing lesions in the central nervous system (for review, see Coyle and Schwarcz 1983). Previous studies have shown that grafts of fetal striatal tissue can survive well in the excitotoxin-lesioned neostriatum (Schmidt et al. 1981; Deckel et al. 1983, 1986; Isacson et al. 1984, 1985, 1986; McGeer et al. 1984; McAllister et al. 1985). Moreover, the striatal grafts have been shown to be capable of normalizing the locomotor hyperac-
tivity characteristically seen following excitotoxic CP lesions (Deckel et al. 1983, 1986; Isacson et al. 1984, 1986) and to partly compensate for the increased metabolic activity induced by the IA lesion in parts of the extrapyramidal system (Isacson et al. 1984). Recently, we have found that also cognitive impairments, as seen in the low performance rate of the IA-lesioned animals in so-called delayed alternation tasks (Divac 1974; Divac et al. 1967, 1978; Dunnett and Iversen 1981), can be markedly influenced by the intra striatal striatal grafts (Isacson et al. 1986).

Based on the assumption that at least part of the observed graft-induced behavioral recovery may be due to transmitter-mediated interactions with the host brain, one may expect to find indications for an anatomical integration of the graft into those striatal neuronal circuitries – severely disrupted by the striatal IA lesion – which are known to mediate or control striatum-dependent behaviors in the intact animal. In the present study we have made an initial attempt to clarify this issue, using, first, small crystal-line deposits of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP), a sensitive combined retrograde and anterograde tracer, into the intra striatal CP grafts, and secondly aldehyde fluorescence histochemistry for the visualisation of dopamine-containing afferents from the host nigrostriatal pathway.

Methods

Subjects

Adult female Sprague-Dawley rats (ALAB, Stockholm, Sweden; 190–200 g at the time of surgery) were used. All surgery was performed in a Kopf stereotaxic apparatus under deep anaesthesia with a mixture of ketamine (Ketalar, Parke-Davis; 10 mg/kg) and xylazine (Rompun, Hoechst; 5 mg/kg).

Ibotenic-acid (IA) lesion

IA (Sigma; 20 μg/μ1 in phosphate buffer, with pH adjusted to approximately 7) was injected at 4 sites in the head of CP; (i) A: 0.4; L: 3.9; V: 5.3; (ii) A: 0.4; L: 3.1; V: 4.0; (iii) A: 1.3; L: 3.1; V: 4.4; (iv) A: 1.3; L: 2.0; V: 4.3. A = anterior to bregma, L = lateral from midline, V = ventral from dura, with the tooth-bar set at the level of the inter-aural line. At each site 0.25 μ1 equivalent to 5 μg IA was injected by a fine needle attached to a 2 μ1 Hamilton syringe. Each injection was performed over 10 min, followed by a slow retraction of the needle to prevent back-leakage. Some of the animals received unilateral IA-lesions, while others were lesioned bilaterally (serially, with one week's interval between each operated hemisphere). After recovering from anaesthesia the rats were housed in groups under a 12 : 12 h light: dark cycle with food and water ad libitum.

Transplantation

Four to six days after the IA lesion the rats received striatal implants into the lesioned part of CP (coordinates as above) using the cell suspension technique (Schmidt et al. 1981; Björklund et al. 1983; Isacson et al. 1985). Tissue was obtained from the striatal primordia dissected from fetuses of 14–15 day gestational age (crown-to-rump length 12–14 mm, from the same breeder as stock of Sprague-Dawley rats as the recipient animals). The striatal primordia, protruding into the lateral ventricle, were retracted via an incision through the overlying cortex. They were carefully cut out from both hemispheres and stored in the medium (0.6% sterile glucose-saline) for the duration of the dissection. The striata collected from one litter were incubated in 0.1% trypsin (Sigma, crude type II) dissolved in the glucose-saline medium, for 20 min at 37° C. After repeated washing the tissue was dissociated into a milky cell suspension by sucking through the opening of a fire-polished Pasteur pipette (about 15–20 strokes). The final suspension contained approximately 2 striatal primordia per 10 μ1 of medium. One to 1.25 μ1 of the cell suspension was injected over 3–5 min at each of the 4 coordinates of the IA lesion sites.

WGA-HRP tracing

Six–11 months after the lesion and transplantation surgery, WGA-HRP (wheat germ agglutinin conjugated to horseradish peroxi-dase) (Sigma) was deposited via a glass micropipette (tip diameter 30–60 μm) into the presumed site of grafted CP tissue in 8 lesioned and transplanted rats (rats LT1–LT8) and into the lesioned CP in one lesioned but non-grafted rat (rat L1). The pipette was filled by paraffin, except for its tip and a shaft with a length from 50 to 80 μm (volume: 200–300 pl), and the space at the tip was packed with WGA-HRP crystals (see Mori et al. 1980). The pipette was fixed to the skull by dental acrylic and left in place, for slow dissolving of the WGA-HRP crystals, until transcardial perfusion with a modified Karnovsky solution 24 h later (Mesulam 1978). Blood was rinsed out with 100 ml of saline prior to 30 min of fixation with 500 ml of a solution containing 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). This was followed by 500 ml of 10% sucrose in the same buffer over 20 min. The brains were dissected out, and serial frontal sections (30 μm) were cut on a freezing microtome. All sections were processed histochemically according to the tetra-methyl benzidine method of Mesulam (1976). They were mounted on gelatinized slides, and half of them were counterstained with neutral red or cresyl violet. A fluorescence microscope, the distribution of labelled cell bodies was mapped by the use of a X–Y plotter attached to photomicrographs on the microscope stage.

Fluorescence histochemistry

Three months after surgery 3 unilaterally lesioned and transplanted rats (LT9–LT11) and 1 unilaterally lesioned control rat (L2) were processed for visualisation of dopamine-containing cells and fibres according to the aluminium-catalysed formaldehyde (ALFA) method of Lorén et al. (1980). In brief, the rats were perfused transcardially by, first, 150 ml of ice-cold Tyrode's buffer, pH 7.4, followed by 300 ml of the ice-cold ALFA solution under high pressure. The dissected brains were freeze-dried, reacted with formaldehyde vapours at + 80° for 1 h, and embedded in paraffin in vacuo. Fifteen μm sections were mounted in Entellan (Merck, GFR) for fluorescence microscopy, and alternate sections were stained with cresyl violet.