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Björklund, A., Stenevi, U., Schmidt, R.H., Dunnett, S.B., Gage, F.H.: Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cells implanted in different brain sites. Acta Physiol. Scand., Suppl. 522, 9-18, 1983.

Schmidt, R.H., Björklund, A., Stenevi, U., Dunnett, S.B., Gage, F.H.: Intracerebral grafting of neuronal cell suspensions. III. Activaty of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. Acta Physiol.Scand., Suppl. 522, 19-28, 1983

Dunnett, S.B., Björklund, A., Schmidt, R.H., Stenevi, U., Iversen, S.D.: Intracerebral grafting of neuronal cell suspensions. IV. Behavioural recovery in rats with unilateral implants of nigral cell suspensions in different forebrain sites. Acta Physiol.Scand., Suppl. 522, 29-38, 1983.

Dunnett, S.B., Björklund, A., Schmidt, R.H., Stenevi, U., Iversen, S.D.: Intracerebral grafting of neuronal cell suspensions. V. Behavioural recovery in rats with bilateral 6-OHDA lesions following implantation of nigral cell suspensions. Acta Physiol.Scand., Supp. 522, 39-48, 1983.

Björklund, A., Gage, F.H., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VI. Survival and growth of intrahippocampal implants of septal cell suspensions. Acta Physiol.Scand. 522, 49-58, 1983

Björklund, A., Gage, F.H., Schmidt, R.H., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippccampus reinnervated by septal suspension implants. Acta Physiol.Scand., Suppl. 522, 59-66, 1983.

Gage, F.H., Björklund, A., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VIII. Cell survival and axonal outgrcwth of dcpaminergic and cholinergic cells in the aged brain. Acta Physiol.Scand., Suppl. 522, 67-75, 1983.

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Intracerebral Grafting of Neuronal Cell Suspensions III. Activity of Intrastriatal Nigral Suspension Implants as Assessed by Measurements of Dopamine Synthesis and Metabolism

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The activity of intrastriatal grafts of nigral cell suspensions has been monitored biochemically, using radioenzymatic assays of dopamine, its major acidic metabolite, DOPAC, and DOPA accumulation after DOPA-decarboxylase inhibition. Implants of 4-9 µl of nigral cell suspension restored striatal DA levels by an average of 13-18%, with the highest individual values reaching about 50% of control. DOPAC was restored from about 5% in the lesioned controls to about 20% of normal in the grafted animals. The DOPAC: DA ratios and the DOPA accumulation measures indicated that the grafted DA neurons were spontaneously active and that the transmitter turnover rate was on the average some 50-100% higher than the intact intrinsic nigrostriatal DA neurones. These results thus provide evidence that the intrastriatal nigral suspension grafts are capable of restoring dopaminergic neurotransmission in the previously denervated striatum.

INTRODUCTION

The function of monoaminergic systems in the CNS can be explored and monitored by biochemical analytical techniques. In the present study we have studied three neurochemical parameters in order to characterize the transmitter function of nigral dopamine (DA) neurones implanted as a dissociated cell suspension into the depth of the neostriatum in adult recipient rats. (i) Striatal DA levels have been used as a quantitative measure of the degree of dopaminergic reinnervation obtained from the intrastriatal suspension grafts. (ii) The DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) has been measured as an index of the rate of release and metabolism of DA in the grafted DA neurones. The DOPAC:DA ratio, in particular, under physiological conditions appears to accurately reflect the activity level of dopaminergic neurones (8). (iii) The transmitter synthesis rate has been measured with the DOPA accumulation method of Carlsson et al. (4). Indeed, the rate of DOPA accumulation after DOPA decarboxylase inhibition, related to tissue DA concentration, appears to provide an accurate estimation of the transmitter turnover rate in dopaminergic neurons (6, 14).

Similar to our previous findings in grafts of solid pieces of substantia nigra, the present results show that the intrastriatal nigral suspension grafts are spontaneously functional and that they can partly reinstate dopaminergic neurotransmission in the previously denervated neostriatum.

METHODS

Fifty-five young adult female Sprague-Dawley rats (180-200g at the time of surgery) were used. Thirty-seven of these were subjected to a unilateral 6-hydroxydopamine (6-OHDA) lesion of the meso-telencephalic DA system 2-3 weeks before grafting. The injections consisted of 8 µg 6-OHDA-HCl (AB Hässle, Goteborg, Sweden) dissolved in 4 µl of ascorbate-saline (0.2 mg/ml), and the injection coordinates were identical to those used previously (Chapter II; see also 3, 10). In the amphetamine-induced rotation test, administered 7-10 days after lesion, all rats included in the present study exhibited a rotation rate of at least 7 turns per min over 90 min.

Transplantation surgery

Nigral cell suspensions were prepared from 14 to 16 day old rat embryos (crown-rump length 11-15 mm) as described in Chapter I. Twenty six of the 6-OHDA lesioned animals were grafted in two groups. The remaining 11 lesioned rats served as lesioned controls.

Group I consisted of 12 rats that had received a single 4 µl implant in the dorsal part of the neostriatum, ipsilateral to the 6-OHDA lesion. The suspension was delivered as two 2-µl deposits (one 1.7 mm above the other along the same needle-track) over 4 min, and the needle was left in place for a further 3 min before the needle was withdrawn. The injection

coordinates were: 1 mm rostral to bregma, 2.5 mm lateral, and 4.0 and 5.7 mm below dura. The incisor bar was set at the level of the inter-aural line. Six of the rats in this group were taken from a group of rats (a total of 12) comprising the "dorsal striatum (Do) group" of the behavioural study reported in Chapter IV. These rats received suspensions prepared from CRL 11.5 mm donors (c. day 14 of gestation), and they were all compensated on the amphetamine-induced rotational measure at the time of sacrifice. These rats formed the "Compensated 4 µl Graft" group. The 6 remaining rats in Group I received suspension grafts prepared from CRL 15 mm donors (c. day 16 of gestation). All these rats were uncompensated on the rotational measure, and the subsequent biochemical assay showed poor graft survival. These rats, which were not included in the behavioural analyses of Chapter IV, formed the "Uncompensated 4 µl Graft" group in the present biochemical study.

Group II consisted of 14 rats that had received a total of 9 µl of suspension in the dorsal striatum, split into three 3 µl deposits injected over 3 min each. The needle was left in situ 2 min for each injection, before it was removed. The injection coordinates were: (a) ant. +0.5 (rostral to bregma), lat. 2.0, ventr. 4.8; (b) ant. +0.5, lat. 3.0, ventr. 4.8; (c) ant. +1.5, lat. 2.5, ventr. 4.8, with the incisor bar set at the level of the inter-aural line. The suspensions in this group were made from CRL 11-13 mm donors (c. day 14-15 of gestation). At the time of sacrifice, all 14 rats were compensated on the rotational measure. Six of them were used for DA and DOPAC analysis and 8 for the DOPA accumulation test.

Rotation tests

The turning behaviour in response to a single injection of methamphetamine (5 mg/kg, i.p.) was assessed according to Ungerstedt and Arbuthnott (13). The tests were conducted in hemispheric perspex bowls, with on-line registration of 180° half-turns in either direction via an ABC 80 microprocessor for 90 min following injection. All rats received an initial amphetamine test 7-10 days after the 6-OHDA lesion, in order to establish the completeness of the lesion, and then further tests at 3-6 weeks intervals. The rotation data reported here are based on the last test, performed 4-5 months after transplantation.

Biochemical analyses

The rats were killed by decapitation under ether anaesthesia 4 months (Group II) or 5 months (Group I) after transplantation. The caudate-putamen was trimmed free of overlying cortex. Ventrally, the caudate-putamen sample was limited by a cut made at the level of the anterior commissure, and caudally by a cut made at the level of the rostral globus pallidus. The forebrain sample consisted of the rest of the telencephalon separated from the rest of the brain by a vertical cut running between thalamus and septum down to the rostral aspect of the optic chiasm. This sample thus comprised the entire neocortex, hippocampus, amygdaloid-piriform lobe, septum, basal forebrain, olfactory tubercle and olfactory bulb on one side of the brain.

DA, NA and DOPAC were assayed according to a modification (11) of the radioenzymatic method of Da Prada and Zürcher (5), Argiolas et al. (1) and Saller and Zigmond (9). DOPA was assayed by a slight modification (11) of the radioenzymatic procedure of Zürcher and Da Prada (15). The animals used in the DOPA accumulation experiment received an injection of the DOPA-decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD 1015, 100 mg/kg, s.c.; Sandev Ltd., England) 30 min before sacrifice.

Statistical analysis

The data were analysed with a one-way analysis of variance, followed by application of Duncan's multiple comparison procedures with $p < 0.05$ as the criterion for significance. Following the guidelines of Steel and Torrie (12), logarithmic transforms were utilized when appropriate to equalize variances between the different groups.

RESULTS

Rotation tests

The nigral suspension grafts used in the present study were very effective in compensating for the amphetamine-induced turning behaviour induced by the unilateral 6-OHDA lesion. The time-course of this effect is illustrated for the entire group of 4 µl grafts in Fig. 2A in Chapter IV (Group "Do"). The rats showed an average turning response prior to grafting of about 10 turns/min in the direction towards the lesion (ipsilateral turning). In the rats not receiving suspension grafts this remained unchanged throughout the 4 or 5-month observation periods, whereas in the grafted animals the ipsilateral turning was abolished within 3 weeks after grafting. At later postoperative times the rats exhibited an overall overcompensation of the turning response (mean of about 2 turns/min in the contralateral direction in the Do group).

The 6 rats in the "Compensated 4 µl Graft" group (selected from the larger "Do" group in Chapter IV) showed a mean rotational score of 7.7 in the contralateral direction, the rats in the "Compensated 9 µl Graft" group a mean score of 3.5 in the contralateral direction, and the rats in the "Uncompensated 4 µl Graft" group showed a mean score of 15.1 in the ipsilateral direction.

Catecholamine concentrations

Table I shows the DA content in caudate-

putamen and rest of forebrain expressed as ng total per sample, and Tables II-V show the DA and NA levels expressed as ng/mg wet weight. The 6-OHDA lesion caused an average reduction of about 99% in the caudate-putamen, and of about 95% in the rest of forebrain (i.e. in the limbic, ventral striatal and cortical target areas of the meso-telencephalic DA system). The NA levels were reduced by 95-99% in the caudate-putamen and by 80-85% in the rest of forebrain.

The nigral suspension grafts restored the DA concentration levels in the caudate-putamen to 13.2%, 13.9% and 18.4% of normal in the three different graft groups analysed. As illustrated in Figs. 2 and 3 the values obtained from individual rats ranged from 4% to 18% in the 4 µl groups, and from 4% to 50% in the 9 µl group. In those animals in which the amphetamine-induced rotation remained unaffected by the graft (Uncompensated 4 µl group) the catecholamine levels were no different from those of the non-grafted lesioned control rats (Tables II and III), indicating that the DA cells in the implants had not survived in the uncompensated rats. The striatal NA levels were only marginally affected by the implants. In fact, from the data in Tables II and IV one can estimate that the NA content of the grafts constituted only between 0.5 and 1% of the DA content.

Table I shows that the average total forebrain DA content (ipsilateral to the lesion) was reduced by 94-97% by the 6-OHDA lesion, i.e. from about 640 ng in the normal rats to between 15 and 40 ng in the lesioned controls. The nigral suspension grafts restored this figure to an average of 11-16% of normal, with the highest individual values reaching about 30% of normal. From the values in Table I one can estimate the average total DA content of a 9 µl graft to be between 35 and 80 ng, which represents some 5-13% of the total forebrain DA content on that side. The implications of these latter figures will be further dealt with in the Discussion, below.

Correlation between DA levels and rotational scores

In Figure 1 the individual DA concentration values of the animals in the DOPAC experiment

TABLE I

TOTAL DOPAMINE CONTENT IN WHOLE FOREBRAIN IPSILATERAL TO THE 6-OHDA LESION (mean ± S.E.M.)

	EXPERIMENT I (DOPAC-group)		EXPERIMENT II (DOPA-group)	
	ng (per hemisphere)	% of normal	ng (per hemisphere)	% of normal
NORMAL				
CAUDATE-PUTAMEN	270.0 ± 13.8	100.0 ± 5.1	256.8 ± 20.8	100.0 ± 8.1
REST OF FOREBRAIN	404.4 ± 27.2	100.0 ± 6.7	347.6 ± 72.2	100.0 ± 20.8
WHOLE FOREBRAIN	674.4 ± 12.9	100.0 ± 1.9	604.4 ± 57.6	100.0 ± 9.5
LESIONED CONTROLS (lesioned side)				
CAUDATE-PUTAMEN	2.6 ± 1.2	1.0 ± 0.4	2.0 ± 0.7	0.7 ± 0.3
REST OF FOREBRAIN	37.3 ± 9.8	9.2 ± 2.4	13.4 ± 4.6	3.9 ± 1.3
WHOLE FOREBRAIN	39.9 ± 10.0	5.9 ± 1.5	15.4 ± 4.8	2.5 ± 0.8
COMPENSATED 9 µl GRAFTS (transplanted side)				
CAUDATE-PUTAMEN	30.9 ± 7.5*	11.4 ± 2.8	50.6 ± 15.2*	19.7 ± 5.9
REST OF FOREBRAIN	45.2 ± 8.3^{ns}	11.2 ± 2.1	44.2 ± 12.4*	12.7 ± 3.6
WHOLE FOREBRAIN	76.1 ± 11.9*	11.3 ± 1.8	94.8 ± 18.7*	15.7 ± 3.1

* $p < 0.05$, as compared with the lesioned control values.

TABLE II

CATECHOLAMINE AND DOPAC CONCENTRATIONS IN CAUDATE-PUTAMEN
(mean ± S.E.M.)

GROUP	n	DOPAMINE ng/mg	DOPAC ng/mg	DOPAC/DA	NORADRENALINE ng/mg
N. NORMAL CONTROLS	12	13.11 ± 0.48	1.187 ± 0.062	0.091 ± 0.004	0.194 ± 0.007
L. LESIONED CONTROLS	6				
intact side		13.56 ± 0.82	1.165 ± 0.068	0.087 ± 0.007	0.224 ± 0.009
lesioned side		0.14 ± 0.06*N,C_1,C_2	0.052 ± 0.019*N,C_1,C_2	1.109 ± 0.507*N,U,C_1,C_2	0.010 ± 0.004*N,C_1,C_2
U. UNCOMPENSATED 4 µl GRAFTS	6				
intact side		14.61 ± 0.86	1.487 ± 0.116	0.104 ± 0.010	0.229 ± 0.010
lesioned side		0.17 ± 0.09*N,C_1,C_2	0.035 ± 0.014*N,C_1,C_2	0.286 ± 0.054*N,L,C_1,C_2	0.007 ± 0.001*N,C_1,C_2
C_1 COMPENSATED 4 µl GRAFTS	6				
intact side		14.69 ± 0.80	1.314 ± 0.113	0.089 ± 0.008	0.243 ± 0.013
lesioned side		1.73 ± 0.26*N,L,U	0.231 ± 0.018*N,L,U	0.150 ± 0.021*N,L,U	0.023 ± 0.004*N,L,U
C_2 COMPENSATED 9 µl GRAFTS	6				
intact side		14.54 ± 0.82	1.111 ± 0.072	0.078 ± 0.006	0.218 ± 0.008
lesioned side		1.83 ± 0.43*N,L,U	0.244 ± 0.040*N,L,U	0.158 ± 0.031*N,L,U	0.025 ± 0.004*N,L,U

Significant differences, $p<0.05$:*, from contralateral (intact) side; N, from normal controls, L, from lesioned controls (lesioned side); U, from uncompensated grafts (lesioned side); C_1 and C_2, from the compensated grafted groups (lesioned side).

TABLE III

CATECHOLAMINE AND DOPAC CONCENTRATIONS IN FOREBRAIN EXCLUDING STRIATUM (mean ± S.E.M.)

For symbols, see TABLE II

GROUP	n	DOPAMINE ng/mg	DOPAC ng/mg	DOPAC/DA	NORADRENALINE ng/mg
N. NORMAL CONTROLS	6	0.806 ± 0.006	0.122 ± 0.006	0.156 ± 0.014	0.375 ± 0.023
L. LESIONED CONTROLS	6				
intact side		0.935 ± 0.054	0.129 ± 0.010	0.141 ± 0.013	0.300 ± 0.020 N
lesioned side		0.068 ± 0.021*N,C	0.054 ± 0.005*N,C	0.844 ± 0.155*N	0.057 ± 0.008*N
C. COMPENSATED 9 µl GRAFTS	6				
intact side		0.979 ± 0.044	0.124 ± 0.009	0.128 ± 0.013	0.350 ± 0.026
lesioned side		0.104 ± 0.0018*N,L	0.094 ± 0.007*N,L	1.118 ± 0.293*N	0.060 ± 0.011*N

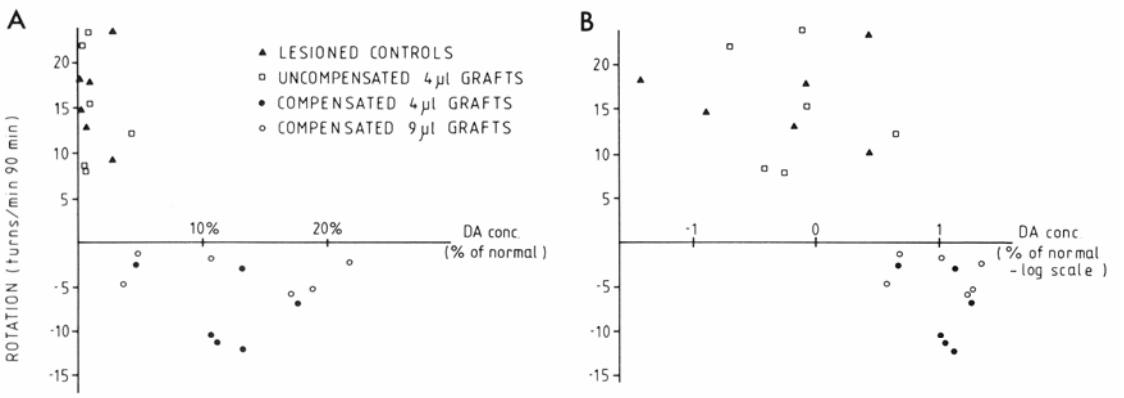

Fig. 1 Relationship between the DA concentration values (in the animals of the DOPAC experiment) and the rotation scores in the final amphetamine-induced rotation test. Turns/min towards the lesion side are given positive values, and away from the lesion side negative values. The DA concentrations are in *A* given on a normal scale, and in *B* on a logarithmic scale.

in Table I have been plotted against their final scores in the amphetamine-induced rotation test. The rotational bias remained in all rats with DA levels below 3% of normal. With values of 4.5% or higher the ipsilateral rotation was eliminated, and in 6 of the 9 rats with values above 10% of normal it was replaced by a strong rotational bias in the contralateral direction. Graphically, the relationship between DA concentration and rotational bias seemed to follow an exponential curve. This impression finds some support in the semi-logarithmic plot in Figure 1B where the different values scatter fairly well around a straight line.

DOPAC levels and DOPAC : DA ratios

DOPAC levels were approximately 1.8 ng/mg in the normal striatum and were in a similar range, 1.11-1.49, on the intact side of the lesioned rats (Table II). The DOPAC:DA ratio was 0.09 in the normal rats and 0.08-0.10 in the intact side of the lesioned rats. In the lesioned control rats the striatal DOPAC level was reduced by 95.6% and the average DOPAC:DA ratio was 1.1, which was more than 10-fold higher than that recorded in the normal striatum. In the compensated grafted rats the DOPAC levels were restored to an average of about 20% of normal, and the DOPAC:DA ratio was markedly reduced as compared to both the lesioned control rats and to the uncompensated grafted animals. The average DOPAC:DA ratio in the caudate-putamen of the compensated rats, 0.15, was however still significantly higher than the ratios recorded in the normal control animals or in the intact contralateral sides of the grafted animals.

As shown in Table III, the 6-OHDA lesion caused a marked, about 4- to 5-fold, increase in the DOPAC:DA ratio also in the forebrain areas outside the striatum. In contrast to the striatum, however, the ratio in the rest of forebrain was unaffected by the graft, which indicates that the graft-induced effect on DA turnover was confined to the primary target area of the grafted neurones.

In Fig. 2 the individual striatal DOPAC:DA ratios have been plotted against the striatal DA concentration values recorded in the same animals. With a couple of exceptions, the animals with less than 10% of the normal DA level (i.e. <1.35 ng/mg) had markedly increased DOPAC:DA ratios (above 0.15), whereas the

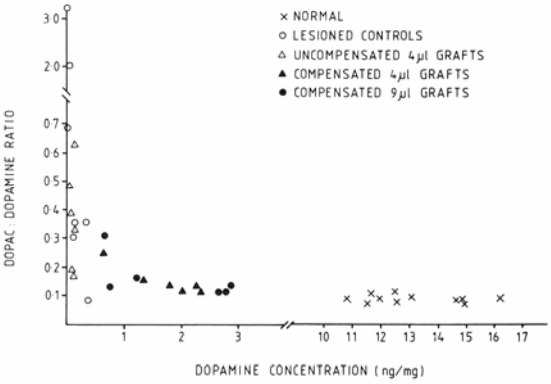

Fig. 2 Relationship between DA concentrations and DOPAC : DA ratios in five different groups of animals, as indicated.

TABLE IV

CATECHOLAMINE AND DOPA CONCENTRATIONS IN CAUDATE-PUTAMEN
30 MIN AFTER NSD-1015 (mean ± S.E.M.)

For symbols, see TABLE II

GROUP	n	DOPAMINE ng/mg	DOPA ng/mg	DOPA/DA	NORADRENALINE ng/mg
N. NORMAL CONTROLS	6	16.58 ± 0.47	1.467 ± 0.093	0.089 ± 0.006	0.197 ± 0.019
L. LESIONED CONTROLS	5				
intact side		15.19 ± 0.50	1.337 ± 0.100	0.088 ± 0.005	0.177 ± 0.010
lesioned side		0.11 ± 0.04*N,C	0.089 ± 0.033*N,C	1.037 ± 0.245*N,C	0.001 ± 0.001*N
C. COMPENSATED 9 µl GRAFTS	8				
intact side		17.68 ± 0.95	1.214 ± 0.118	0.071 ± 0.008	0.192 ± 0.010
lesioned side		3.05 ± 0.85*N,L	0.403 ± 0.051*N,L	0.185 ± 0.031*N,L	0.039 ± 0.009*N,L

TABLE V

CATECHOLAMINE AND DOPA CONCENTRATIONS IN FOREBRAIN EXCLUDING
STRIATUM 30 MIN AFTER NSD-1015 (mean ± S.E.M.)

For symbols, see TABLE II

GROUP	n	DOPAMINE ng/mg	DOPA ng/mc	DOPA/DA	NORADRENALINE ng/mg
N. NORMAL CONTROLS	5	0.778 ± 0.120	0.056 ± 0.003	0.080 ± 0.027	0.333 ± 0.036
L. LESIONED CONTROLS	5				
intact side		0.761 ± 0.053	0.123 ± 0.025	0.169 ± 0.038	0.261 ± 0.031
lesioned side		0.030 ± 0.010*N,C	0.046 ± 0.003*	3.039 ± 1.222*N	0.062 ± 0.011*N
C. COMPENSATED 9 µl GRAFTS	8				
intact side		1.014 ± 0.091	0.108 ± 0.020	0.109 ± 0.029	0.288 ± 0.049
lesioned side		0.100 ± 0.027*N,L	0.058 ± 0.017*	0.870 ± 0.453*N	0.046 ± 0.010*N

compensated grafted animals with higher DA levels exhibited ratios close to the normal range (i.e. between 0.10 and 0.15).

DOPA accumulation

The rate of accumulation of DOPA was measured 30 min after DA synthesis inhibition through administration of the DOPA decarboxylase inhibitor NSD 1015. Tables IV and V summarize the resulting DOPA levels, which reflect the overall DA synthesis in the area analysed, and the DOPA:DA ratios, which can be taken as a measure of the rate of synthesis and turnover of DA in the individual intrinsic or implanted DA neurones.

In the absence of DOPA-decarboxylase inhibition the DOPA level in the striatum averaged 39 pg/mg in the caudate-putamen and 16 pg/mg in the remaining forebrain. These values were increased to 1467 pg/ng and 60 pg/mg, respectively, by 30 min after the NSD 1015 injection.

In the lesioned controls the overall DOPA accumulation was reduced by 94% in the caudate-putamen on the lesioned side. Concomitantly, the average DOPA:DA ratio (i.e. the synthesis rate) was increased more than 10-fold as compared with the ratios obtained from the normal controls or the intact contralateral side. The nigral grafts restored DA synthesis to an average of about 30% of normal. This was accompanied by a reduction of the DOPA:DA ratio, from a mean of 1.04 in the lesioned controls to a mean of 0.19 in the grafted animals. Nevertheless, the ratio in the grafted rats remained significantly increased (about 100%) above that of the normal controls or the intact contralateral sides in the lesioned animals.

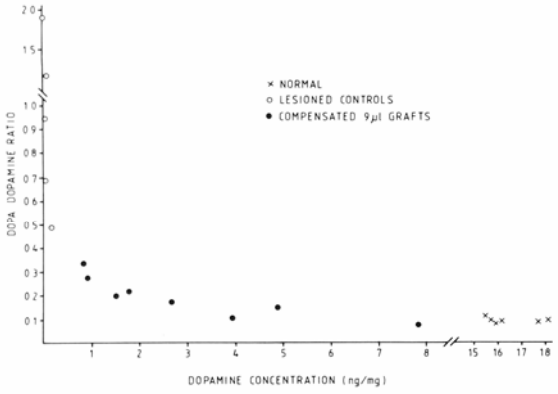

Fig. 3 Relationship between DA concentrations and DOPA : DA ratios in three groups of rats, as indicated.

In Fig. 3 the individual striatal DOPA:DA ratios have been plotted against the DA concentrations recorded in the same animals. The relationship between DA depletion and DOPA:DA ratio was similar to (but perhaps even more clear-cut than) that seen for the DOPAC:DA ratios in Fig. 2. The lesioned rats, the DA depletions of which were greater than 98%, all had ratios above 0.4; the grafted rats with DA levels between 5 and 11% of normal had ratios ranging between 0.19 and 0.33; and the grafted rats with DA levels above 11% had ratios of 0.17 or less. As in the DOPAC experiment this effect of the graft was not seen in the forebrain areas outside the primary target area in the striatum (Table V).

DISCUSSION

The present biochemical data substantiate the histochemical observations in Chapter II that nigral suspension implants can provide a significant reinnervation of the surrounding striatal target, and they provide some quantitative assessment of the magnitude of DA fibre outgrowth obtained by such implants.

The 6-OHDA lesioned rats used in the present study (which were selected on the basis of the amphetamine-induced rotational criterion of at least 7 turns/min) had an average ipsilateral DA depletion of 99% in the caudate-putamen and of about 95% in remaining forebrain. The nigral suspension implants restored the striatal DA level to an average of 13-18%, with the best individual values reaching about 50% of control. These figures are similar to those previously obtained with solid nigral grafts placed in a cavity in the parietal cortex (11), suggesting that the present suspension grafts are as efficient as the solid implants in the reinnervation of the denervated striatum. The DA level in the remaining forebrain was by contrast only marginally affected, which is consistent with the parallel histochemical observation that the graft-derived DA fibre outgrowth is largely confined to the part of the dorsal caudate-putamen surrounding the graft. Grafts placed in a single brain region, such as in the present study, will thus innervate only a limited part of the total innervation territory of the mesotelencephalic DA system. The values in

Table I show that some 3-6% of the total forebrain DA content (equivalent to 15-40 ng) remained in the 6-OHDA lesioned animals on the lesioned side. The 9 µl nigral grafts contributed on the average a further 35-80 ng, which is equivalent to about 10% of the total forebrain content. It seems likely, therefore, that with the present technique, a complete reinnervation of the dopaminergically denervated forebrain will require many, perhaps up to 10, nigral suspension implants placed in different forebrain sites.

The total DA content of the grafts may also give an idea of how many DA neurones survived and grew in the new location. The mean DA content of an intact mesencephalic DA neurone in situ (cell body plus dendrites and axons) has been estimated at about 30 pg (2). In the compensated grafted animals in the present study, the graft-derived striatal DA content ranged between a minimum of about 6 ng and a maximum of about 120 ng, equivalent to between 200 and 5000 normal DA cells. These figures are well compatible with the estimates of surviving DA neurones reached in the parallel histochemical study (Chapter II).

The DA levels reached with the 4 µl and 9 µl implants were not significantly different from each other despite the difference in volume injected (cf. Figs. 2 and 3). This may simply reflect variability in the yield of surviving DA neuroblasts from one suspension to another, but it could also be due to problems of back-leakage of suspension along the needle tracks when larger volumes of fluid are injected. Nevertheless, the highest DA concentration values were obtained with the 9 µl implants, and the highest single value (7.8 ng/mg) was more than 3-fold higher than the highest value obtained in the 4 µl group (2.4 ng/mg).

The nigral suspension grafts provided a substantial restoration of dopaminergic neurotransmission in the initially denervated caudate-putamen. This was evidenced both by the increase in striatal DOPAC, from about 5% of normal in the lesioned controls to about 20% of normal in the grafted animals, and by the increase in overall DOPA accumulation after DA synthesis inhibition, from about 6% to about 30% of normal. The DOPAC:DA and DOPA:DA ratios signify that the implanted DA neurons had a spontaneous turnover of the transmitter at rates that were on the average some 50-100% higher than that of intact intrinsic nigrostriatal DA neurons. These increased ratios are, however, well in the range of those recorded in the intrinsic DA system after partial dopaminergic denervation of the striatum (7).

The DA turnover ratios of the present suspension grafts are close to those previously recorded in animals with solid nigral grafts reinnervating the dorsal caudate-putamen (11). In the solid grafts there was a tendency for the turnover ratios to be higher in the animals with the lowest striatal DA concentration values. This tendency for an inverse relationship between the degree of DA reinnervation and DA turnover rate stands out even more clearly in the suspension grafted animals. Moreover, this same relationship is seen in the intact nigrostriatal system subjected to partial destruction by varying the amount of 6-OHDA injected (7). These similarities suggest that the mechanisms regulating transmitter function in the suspension grafted DA neurones are similar to those operating in the solid grafts and, at least in part, in intrinsic nigrostriatal neurones, despite the dissociation of the tissue prior to grafting. This may suggest either that the intrinsic organization and the connectivity of the grafted DA neurones are relatively unimportant in this regulation, or else that the intrinsic organization and neuronal connexions of the suspension grafts are sufficient to maintain this function.

With both solid and suspension grafts, recovery of the motor asymmetry, as assessed by the rotation test, occurred in animals with as little as 3% of the normal striatal DA levels restored. However, in contrast to the solid grafts where the compensation of the turning response levelled off at zero (i.e. no rotational bias) with increasing levels of DA restored, the suspension grafted animals expressed an increasing degree of contralateral turning, i.e. an overcompensation of the rotational bias, with increasing DA levels (compare Fig. 1 in the present study with Fig. 3 in the study of Schmidt et al., 11). This "overcompensation" has been a general feature of the amphetamine-induced turning response in several of our experimental groups with nigral suspension grafts, but it has not been seen in any other test performed in these animals, including apomorphine-induced turning behaviour (Chapter IV). This difference between solid and suspended nigral grafts thus seems to be due to some peculiarity in the action of amphetamine on the different types of grafts.

In conclusion, the present results show that nigral suspension grafts implanted into the depth of the caudate-putamen are capable of restoring dopaminergic transmission in the previously denervated striatum. The biochemical measures of DA synthesis and release indicate that the implanted dopaminergic neurons are spontaneously active and utilize their transmitter machinery at a rate that is even above that of the intact intrinsic nigrostriatal DA neurones.

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