The Effect of Fetal Dopaminergic Grafts On The Distant Neural Tissues

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Abstract : Fetal substantia nigra grafts (obtained from 15-17-day-old-foetuses) were transplanted into cavities overlying the caudate nucleus of rats following destruction of the nigrostriatal dopaminergic pathways by intraventricular injection of the 6-Hydroxydopamine (6-OHDA). We investigated the effect of fetal substantia nigra grafts on the adrenal gland and sympathetic ganglia.

Three months after grafting, tyrosine hydroxylase activity of the adrenal gland, celiac and lumbar sympathetic ganglia of grafted rat groups showed significant increases of 154 %, 114 %, 128 % respectively. There were no increases in the superior and inferior cervical ganglia.

These results indicate that fetal nigrostriatal dopaminergic grafts had an augmenting effect upon the distant CNS tissues.

Key Words : Dopaminergic neurons, mesencephalic fetal grafts, substantia nigra, sympathetic ganglia.

INTRODUCTION

Much research has been devoted to transplanting fetal brain tissues into adult host animals. One of the most commonly studied systems is the dopamine (DA) containing substantia nigra (6,16,15,22). Most previous studies utilized fetal substantia nigra tissue grafted into the DA innervated caudate nucleus of adult recipient animals (1,2,3,7,17). Fetal nigral grafts have been shown to be capable of re-establishing the DA innervation of a host caudate, forming ultrastructurally normal synapses within a previously denervated corpus striatum (20,27). In addition, graft-derived catecholamine axons appear to release DA within the host central nervous system (7). These grafts have also been shown to restore a variety of motor and sensorymotor deficits associated with destruction of the DA pathway (1,2,3,7,17,19). However, no previous study has investigated the effects of nigral grafts upon the distant CNS tissues, especially on sympathetic ganglia.

MATERIALS AND METHODS

Twenty randomly-bred Norvegicus Albino rats (weight 175-250 grams) were used to evaluate the effects of nigral grafts on the adrenal and sympathetic ganglia. For destruction of dopaminergic neurons intraventricular 6-OHDA (250 g / 25 l) injections were given to 15 rats under light ether anaesthesia at coordinates 1 mm posterior and 1 mm lateral (right side) to the bregma and 3.5 mm below the dura, as described by Freed et al., König and Klippel (7,12). The solution was made up just before use and kept on ice in order to retard auto-oxidation. The 6-OHDA, expressed as free base, was injected in 25 increments. Control animals were injected with an equal volume of the saline vehicle solution.

Four weeks after 6OHDA injection, a 2x3 mm cavity way made by suction unilaterally on the surface of the right caudate nucleus of 15 rats, as described by Stenevi and Björklund (22). Two weeks later the cavity was re-opened and in 10 rats, fetal nigral grafts were inserted. These monoamine containing

ventral mesencephalon grafts were obtained from rat embryos of 17-18 day gestation (crown to rump length 19-22 mm), as described by Olson and Seiger (16). The remaining 5 rats served as sham grafted controls, undergoing all surgical procedures except graft insertion. Five vehicle injected rats served as normal controls.

Three months after grafting, adrenal gland, celiac, lumbar, superior and inferior cervical ganglia were removed, weighed and homogenized in 10 volumes of Tris-HCl buffer (50 mM, pH 6.2). Tyrosine hydroxylase activity was measured in 0.05 ml of homogenates by the radioenzymatic method described by Waymire et al (23).

RESULTS

The months after grafting all the grafts appeared grossly to have survived. Table I shows the level of tyrosine hydoxylase activity measured on the adrenal, celiac, lumbar-2, superior and inferior cervical ganglia of normal control, sham grafted and grafted rats.

Table I : Results and comparison of the measurement of tyrosine hydroxylase activity (nmol^{14} CO_2 / gland or ganglia / hr)

	Normal (n:5)	Sham grafted (n:5)	Grafted (n:5)
Adrenal gland	4.65+0.59	3.97+0.50	6.12+0.41*
Celiac ganglion	1.02+0.08	1.12+0.04	1.28+0.08**
L-2 ganglion	0.70+0.04	0.70+0.04	0.97+0.03x
Superior cervical ganglion	1.390+0.060	1.15+0.15	1.31+0.07
Inferior cervical ganglion	1.49+0.11	1.70+0.16	1.73+0.23

* $p<0.008$ $t=3.37$
** $p<0.02$ $t=2.56$
x $p<0.05$ $t=2.10$
(Compared to normal) Values are means + S.D.

The increase in enzyme activity was 154 % - 114 % and 128 % in adrenal gland, celiac and second lumbar ganglia respectively. No significant increases were found for the superior and inferior ganglia.

DISCUSSION

Research during the last several years has provided support for the idea that fetal nervous tissue can survive in the adult mammalian brain and can modify the function and behaviour of the host.

Brain tissue transplantation has been used in several CNS areas to correct hormone deficiencies or the reverse the effects of lesions produced by various methods (1,2,3,6,7,13,14,15,17,22). Although numerous investigators have performed many studies of the effects of substantia nigra grafts on drug induced rotational behaviour (1,2,3,7,17,17), DA reinnervation and reestablishment of DA content (1,2,3,7,17), no previous study has investigated the effects of nigral grafts on sympathetic ganglia and the adrenal gland. It was previously shown that stimulation of central dopaminergic activity by D-amphetamine (20), L-dopa (18) apomorphine (8) and ET-495 (18) cause increase in the tyrosine hydroxylase activity in the adrenal gland and sympathetic ganglia.

The observed increase in the tyrosine hydoxylase activity in our study, strongly suggests that fetal substantia nigra grafts in rats pretreated with 6-OHDA leads to dopaminergic receptor supersensitivity. It is known that intraventricular injection of 6-OHDA produces long-lasting depletion of dopamine and central nervous system dopaminergic supersensitivity and increases the number of central dopaminergic receptors (4,5,9,10,11,21). Nigral neural grafts restore central dopaminergic receptor binding (11). The present study demonstrates that after destruction of dopaminergic neurons by 6-OHDA, centrally-implanted neural grafts not only correct the local receptor changes, but also produce dopaminergic type effects in peripheral organs such as the adrenal gland and some sympathetic ganglia which receive regulatory input from central dopaminergic neurons.

In conclusion, nigral grafts can mimic normal dopaminergic neuronal function.

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REFERENCES

1. Björklund, A., Stenevi, U.: Reconstruction of brain circuitries by neural transplants. Trends in Neuroscience, 2:301-306, 1979.
2. Björklund, A., Stenevi, U.: Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res., 177:555-560, 1979.
3. Björklund, A., Dunnett, S.B., Stenevi, U.: Reinnervation of the denervated striatum by substantia nigra transplants: Functional consequences as revealed by pharmacological and sensorimotor testing. Brain Res., 199:307-333, 1980.
4. Breese.E.R., Taylor, T.D.: Depletion of brain noradrenalin and dopamine by 6-OHDA. Br. J. Pharmacol. 42:88-89, 1971.
5. Breese, G.R.: Chemical and immunochemical lesions by spesific neurotoxic substances and antisera. In: Handbook of Pharmacology. Vol. 1. Eds.: Iversen, L.L., Iversen, S.D., Snyder, H.S., Plenum Press, NY-London, 1975, pp. 137-189.
6. Das G.D., Hallas, B.H.: Transplantation of brain tissue in the brain of adult rats., Experientia, 34:1304-1306, 1978.
7. Freed, W.J., Perlow, M.J., Karoum, F., Seiger, A., Olson, L., Haffer, B.J., Wyatt, R.J.: Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: Long term behavioral, biochemical and histochemical studies. Ann. Neurol, 8:510-519, 1980.
8. Gagner J-P, Gauthier S., Sourkes, T.L.: Participation of spinal monoaminergic and cholinergic systems in the regulation of adrenal tyrosine hydroxylose: Neuropharmacol. 22:45-53, 1983.
9. Iversen, L.L., Uretsky, N.J.: Regional effects of 6-OHDA an catecholamine containing neurons in rat brain and spinal cord. Brain Res., 24:364-367, 1970.
10. Iversen, L.L., Uretsky, N.J.: Biochemical effects of 6-OHDA on catecholamine containing neurons in the rat CNS. In: 6-OHDA and catecholamine Neurons. Eds: Malmorms, T., Thoenen, H., North Holland Pub. Co. Amsterdam, London, 1971, pp. 171-186.
11. Korfali, E., Knowles, W.D., Uysal S., Ulus, İ.H., Özmen, T.: Fetal substantia nigra grafts, effect on dopamine receptors in the rat corpus striatum. Cleve Clin J. Med. 56:259-262, 1989
12. König, J.F.R., Klippel, R.A.: The Rat Brain. A stereotaxic atlas of the forebrain and stem. Robert E. Krieger Publiching Co. Inc. NY, 1974.
13. Krieger, D.T., Perlow, M.J., Gibson, M.J.: Brain grafts reverse hypogonadism of gonadotropin-releasing hormone deficiency. Nature, 298:468-471, 1982.
14. Kromer, L.F., Björklund, A., Stenevi, U.: Intracephalic implants: A technique for studying neuronal interactions, Science, 204: 1117-1119, 1979
15. Lund, R.D., Hauschka, S.D.: Transplanted neural tissue develops connections with host rat brain. Science, 193:582-584, 1976.
16. Olson, L., Seiger, A.: Early prenatal ontogeny of central monoamine neurons in the rat: Flourorescence histochemical observations. Z.Anat. Entwickl. Gesch., 137:301-316, 1972.
17. Perlow, M.J., Freed, W.J., Haffer, B.J.: Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science, 204:643-647, 1970.
18. Quic M., Sourkes LT: Regulation of adrenal tyrosine hydroxylase activity: Neuronal versus local control studied with apomorphine. Biochemical Pharmacol 25:1157-1166, 1976.
19. Ungerstedt, U., Arbuthnott, G.W.: Quantitative recording of rotational behavior in rats after 6-OHDA lesions of the nigrostriatal dopamine system. Brain Res, 24: 485-493, 1970.
20. Ulus I.H., Wurtman, R.J.: Selective response of rat peripheral sympathetic nervous system to various stimuli. J. Physiology. 293:513-525, 1979.
21. Uretsky, N.J., Iversen, U.: Effects of 6-OHDA on catecholamine containing neurons in the rat brain. J. Neuro Chem., 17:269-278, 1970.
22. Stenevi, U., Björklund, A., Svendgaard, N.A.: Transplantation of central and peripheral monoamine neurone to the adult rat brain. Techniques and conditions for survival. Brain Res., 114:1-20, 1976.
23. Waymire, J.C., Bjur, R., Weiner, N.: Assay of tyrosine hydroxylase by coupled decarboxylation of dopa formed from (^{14}C) L-tyrosine. Analyt. Bioc. 43:588-600, 1971.