Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 443–448, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0103-0

Modulation of Acrylamide-Induced Neurochemical and Behavioral Deficits by Cerebellar Transplants in Rats

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Received 26 May 1992

HUSAIN, R., A. K. AGRAWAL, M. HASAN AND P. K. SETH. *Modulation of acrylamide-induced neurochemical and behavioral deficits by cerebellar transplants in rats.* PHARMACOL BIOCHEM BEHAV 49(3) 443–448, 1994.—Acrylamide (30 mg/kg body wt.) administered intraperitoneally daily to young adult male rats, five times a week for 3 consecutive weeks, affected the cerebellar functions, as exhibited by a significant reduction in rotarod performance, spontaneous locomotor activity, glutathione-*S*-transferase activity, and ^{3}H-flunitrazepam binding in cerebellum. Transplantation of dissociated fetal cerebellar cells (E14) to cerebellum resulted in a significant recovery in behavioral and neurochemical parameters evaluated 9 weeks after transplantation. Light- and electron-microscopic studies confirmed the viability and specificity of cerebellar grafts.

Acrylamide Purkinje cells Neuromodulation Spinocerebellar ataxia Rotarod performance GABA-ergic functions Cerebellar grafts

ENVIRONMENTAL contaminants of diverse chemical structures, such as heavy metals, pesticides, plastic monomers, and certain solvents, have been implicated in the etiology of a variety of neurologic and neurodegenerative disorders, including Parkinson's disease, Alzheimer's, heterodegenerative ataxia, and amyotropic lateral sclerosis (5). The role of the axonal transport of essential proteins has also been indicated in system degeneration (26,28).

Several reports to date have established the efficacy of striatal, adrenomedullary, or nigral transplants in treating Parkinson's disease patients (4,10,21,23). However, the underlying mechanisms by which the neural transplants help to restore the lost functions and the extent to which they do so are not fully understood.

Repeated exposure to acrylamide, a commonly used monomer, in the production of polymeric plastic devices is known to produce central, peripheral distal axonopathy of the dying back type in humans and experimental animals; its behavioral, biochemical, and histopathologic effects are well documented (13,20).

The selective loss of cerebellar Purkinje cells by acrylamide and subsequent hind limb weakness, cerebellar ataxia, and failure to perform on the rotarod has been reported earlier (6). Cerebellar Purkinje cells are enriched in GABA-benzodiazepine receptors (1); therefore, ^{3}H-flunitrazepam, a specific ligand for GABA-benzodiazepine type I receptor complex, was used to evaluate the viability and extent of damage to cerebellar Purkinje neurons. Involvement of mixed-function oxidases in acrylamide neurotoxicity has also been reported (9), and the specific role of glutathione-*S*-transferase in catalyzing the conjugation of acrylamide with glutathione (thereby serving as the major pathway for its detoxification) prompted us to include it in the present study. Furthermore, we examined the restorative potential of transplantation of fetal cerebellar tissue on the neurochemical and behavioral deficits caused by systemic exposure to acrylamide. In addition, morphologic studies were conducted to demonstrate the viability of the graft in the damaged host cerebellar tissue.

METHOD

Animals and Treatment

One hundred Wistar strain male albino rats (100 ± 10 g) from our own animal breeding stock were housed in stainless-

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steel cages under standard conditions with a 1-12-h light-dark cycle and free access to pellet food (Hindustan Lever Ltd.) and water.

Acrylamide (30 mg/kg body wt.) dissolved in physiologic saline was injected intraperitoneally (IP) daily five times a week for 3 consecutive weeks in 70 rats.

Transplantation

The donor tissue for grafting was obtained from gestation day 14 rat embryos, as described by Das et al. (8). Gestation day 1 was counted from the presence of sperm in the vaginal smears of rats allowed to mate overnight.

Briefly, adult control and acrylamide-treated rats were anesthetized with ketamine hydrochloride (0.5 mg/kg body wt.) and fixed in a stereotaxic frame. Craniotomy was performed bilaterally by drilling two symmetrical holes in the occipital bone so as to expose the cerebellum, using the stereotaxic coordinates according to the atlas of Pelligrino et al. (24). Coordinates were: anterioposterior, 1.0 mm; mediolateral, 1.0 mm; and dorsoventral, 1.0 mm, relative to λ. The group designated to be control received only an injection of Ringer's lactate solution under identical conditions.

Grafting of cerebellar primordia and neocortical neurons was performed 24 h after the last exposure to acrylamide. Cerebellar primordia and neocortex from GD-14 rat embryos were dissected out and a slurry of embryonic neurons in Ringer's lactate solution (5 μl) was prepared by repeated aspiration through a Pasteur pipette with a small bore. Three microliters of this suspension was stereotactically injected at the border between vermis and hemisphere through a glass capillary needle fixed to a glass tuberculin syringe. The different groups of test rats were as follows:

Group I (control): Forty rats received normal saline IP. Surgery was performed as in group III and Ringer's lactate solution was injected.

Group II (acrylamide): Sixty rats received acrylamide, 30 mg/kg body wt., IP, for 5 consecutive days per week for 3 weeks.

Group III (transplanted): Twenty rats in which cerebellar suspensions were grafted were selected from group II.

Group IV (sham control): Twenty rats in which neocortical suspensions were grafted were selected from group II.

Behavioral Studies

Rotarod performance. Six rats from each of the four experimental groups were assessed for rotarod performance by the method of Kaplan and Murphy (17). Each rat was given 2 consecutive days of training, with four trials per day separated by an intertrial interval of 5-10 min. The time elapsed from the onset of rotation until the subject fell from the rod (or completed the trial) served as the measure of performance.

Spontaneous locomotor activity. Six rats from each of the four experimental groups were placed individually in the test chamber of a photoactometer (Techno, India), and spontaneous locomotor activity was recorded for a period of 10 min by the method of Kuhn and Van Maanen (19). All testing was done between 1000 and 1400. The box was wiped clean before each rat was tested. Activity scores are expressed as counts per minute.

Ten rats from each treatment group were decapitated and their brains were rapidly excised; cerebellar and neocortical tissue was dissected according to the method of Glowinski and Iversen (11) and stored frozen at −20°C until further processing.

Glutathione-S-Transferase Activity

Briefly, cerebellar and neocortical tissue was homogenized in phosphate buffer (4 vol., 0.1 M, pH 7.4) containing KCl (0.15 M) and centrifuged at 14,000 × *g* for 20 min at 0°C to obtain postmitochondrial supernatant, which was carefully decanted and used for the estimation of Glutathione-S-Tranferase activity according to the method of Habig et al. (12) using 1-Chloro-2,4-Dimitrobenzene as a substrate.

Neurochemical Studies

^{3}H-Flunitrazepam binding. GABA-benzodiazepine (BZ) receptor complex was studied in a synaptic membrane preparation of the cerebellar region using ^{3}H-flunitrazepam as radioligand after the method of Agrawal et al. (2), with a slight modification. Briefly, crude cerebellar synaptic membrane fraction was prepared by homogenizing the tissue in 19 vol. of 0.32 M sucrose followed by centrifugation (50,000 × *g* for 10 min). The pellet from this step was then homogenized in 5 mM Tris-HCl buffer, pH 7.4, and recentrifuged at the same speed. This procedure combined with the previous freezing step caused major lysis of structural cell components such as nerve endings. The final pellet was suspended in Tris-HCl buffer (40 mM, pH 7.4) at a concentration of 50 mg wet-weight tissue/ml.

Binding incubations were performed in triplicate in a final volume of 1.0 ml containing 40 mM Tris-HCl, pH 7.4, together with ^{3}H-flunitrazepam (0.7 nM, sp. act. 85 Ci/mmol) and 100 μl membrane equivalent to 250 μg protein. The incubation was carried out at 4°C in ice for 30 min. Receptor ligand complex was separated by filtration through glass fiber filter discs (GF/C) and washed twice with 5.0 ml cold Tris-HCl, pH 7.4. Filter discs were vacuum-dried and counted in a β-counter with an efficiency of 50% for tritium. Parallel incubations were performed in the presence of excess unlabeled diazepam (1 μM) to determine the extent of nonspecific binding. Results are expressed as picomoles of ^{3}H-ligand bound per milligrams of protein.

Light and Electron Microscopy

The brains of all rats were fixed by perfusion with phosphate-buffered glutaraldehyde-paraformaldehyde solutions prepared along the lines recommended by Karnovsky (18). Rats were anesthetized with sodium pentobarbital (30 mg/kg body wt., IP) and perfused through the heart. The perfusion fluid was kept at a height of about 100 cm above the level of the animal's heart. The needle was introduced into the left ventricle and guided into the ascending aorta. The right auricle was cut with a sharp pair of scissors as soon as the perfusion was started, to drain out the perfusate after circulation. The duration of perfusion was 20-30 min, and the volume for each animal was approximately 300 ml (at the rate of about 10 ml/min). The brains were carefully removed from the cranium, taking care to avoid trauma. Small pieces of the cerebellum were dissected out and quickly immersed in the fixative used for perfusion for 3 h at 4°C. The tissue was then rinsed in 0.1 M phosphate buffer (pH 7.3) and postfixed in 1% osmotically adjusted osmium tetroxide for 1-2 h. After dehydration in graded alcohols, the material was embedded in araldite. The desired regions of the brain were sectioned to be 1 μm thick with an LKB ultratome Model III and stained with toluidine blue for light microscopy. Adjacent ultrathin sections were obtained from the area of interest and were stained with uranyl acetate (29) and lead citrate (25) and examined with a Philips 410 LS electron microscope.

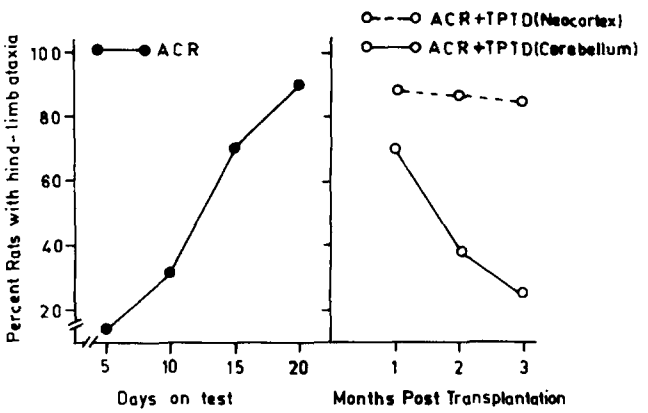

FIG. 1. Restorative effect of cerebellar (GD14) grafts on acrylamide-induced hind limb ataxia in rats.

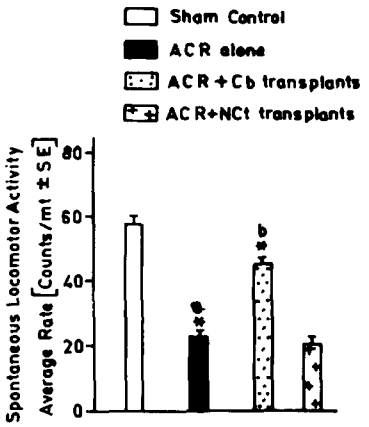

FIG. 2. Ameliorating effect of fetal cerebellar transplants on spontaneous locomotor activity in acrylamide-exposed male albino rats. Data are expressed as mean activity counts per minute ± SE of five rats. Two-way analysis of variance, $F(3, 13) = 177.76, p < 0.001$. a, vs. control; b, vs. acrylamide $*p < 0.001$.

Protein Estimation

Protein content in postmitochondrial fraction and cerebellar membrane was estimated by the method of Lowry et al. (22), using bovine serum albumin as a reference standard.

Statistical Analysis

Fisher's Exact test was applied to analyze the rotorod performance data. Two-way analysis of variance (randomized block design) was used to analyze the mean significant differences of the parameters—that is, spontaneous locomotor activity, ^{3}H-flunitrazepam binding, and glutathione-*S*-transferase activity. Post-hoc analysis was performed to compare the means between the two groups by calculating their least significant differences.

RESULTS

The animals exposed to acrylamide exhibited a significant loss in average body weight compared with controls (data not shown). Signs of hind limb weakness (measured by hind limb splays, i.e., distance between the hind paws while walking) were evident by the end of 1st week of treatment. The severity of the weakness increased with further exposure, and by the end of the treatment schedule rats had difficulty in walking as a result of the increase in hind limb splay. In contrast, acrylamide-treated transplanted rats with cerebellar tissue showed a gradual improvement in gait, and the number of animals with hind limb ataxia was considerably reduced (Fig. 1) 9 and 12 weeks after transplantation. No other sign of toxicity or increased rate of mortality was evident in the treated group compared with controls.

TABLE 1

ROTAROD PERFORMANCE ON 2ND DAY OF TESTING

Group	Proportion of Rats Reaching at Least Two Complete Trials (30 s at 3 rpm)
Sham	6 : 6
Acrylamide (3 wk)	0 : 6*
Acrylamide (9 wk)	1 : 6*
Acrylamide + Cb tpt	4 : 6*†
Acrylamide + Nct tpt	1 : 6

*Significantly different from control group ($p < 0.01$). +Significantly different from acrylamide group (3 wk): $p < 0.05$ (Fisher Exact test).

Neurobehavioral Changes

The acrylamide-treated rats revealed progressive truncal ataxia that advanced with treatment. A significant decrease in their ability to stay on the rotating rod compared with sham controls was evident. However, a significant recovery was exhibited by acrylamide-exposed transplanted rats in neuromuscular coordination, as assessed by rotarod performance (Table 1).

The spontaneous locomotor activity of the animals, expressed as the average of overall activity scores, is shown in Fig. 2. The acrylamide-exposed animals showed a significant reduction in motor function compared with sham controls. However, the acrylamide-exposed transplanted rats with cerebellar tissue were able to score more counts whereas the acryl-

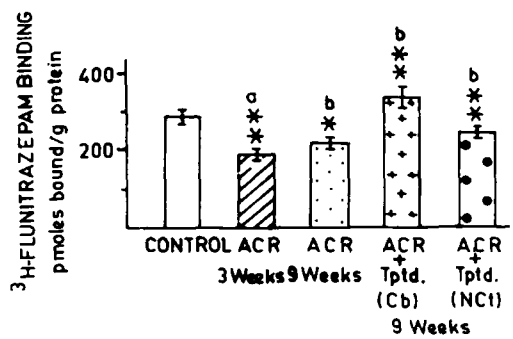

FIG. 3. Ameliorating effect of fetal cerebellar transplants (GD-14) on GABA-ergic binding in the cerebellum of host rats with acrylamide-induced Purkinje cell damage, 9 weeks after transplantation. Data are means ± SE of five rats per group. Two-way analysis of variance, $F(4, 16) = 109.38, p < 0.01$. a, vs. control; b, vs. acrylamide (3 weeks). $*p < 0.01$. $**p < 0.001$.

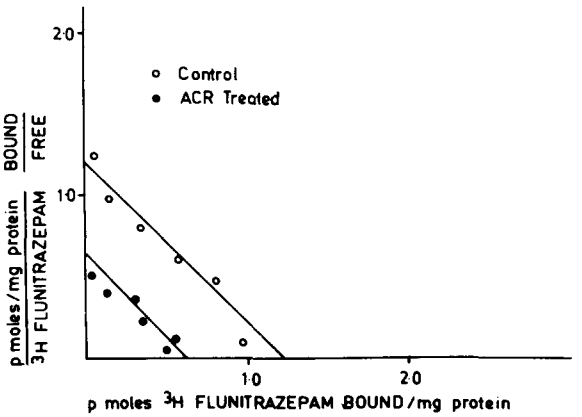

FIG. 4. Scatchard analysis of binding of ^{3}H-flunitrazepam to cerebellar membranes of control and acrylamide-treated rats. Each data point is the mean of three separate experiments performed in triplicate.

amide-exposed and -transplanted rats with neocortical tissue failed to show improved motor function.

Neurochemical Changes

Figure 3 shows the effect of the acrylamide treatment and fetal cerebellar transplants on ^{3}H-flunitrazepam binding. A significant reduction in specific binding was observed in the acrylamide-exposed group at 3 weeks, which persisted up to the last dose at 9 weeks. The acrylamide-exposed animals transplanted with fetal cerebellar suspensions exhibited an increase in binding compared with acrylamide-exposed transplanted rats with neocortical tissue. Scatchard analysis revealed that this increase in binding resulted from an increase in the maximum number of binding sites (B_{max} (transplanted): 1.2 pmol; B_{max} (acrylamide): 0.6 pmoles) without significantly

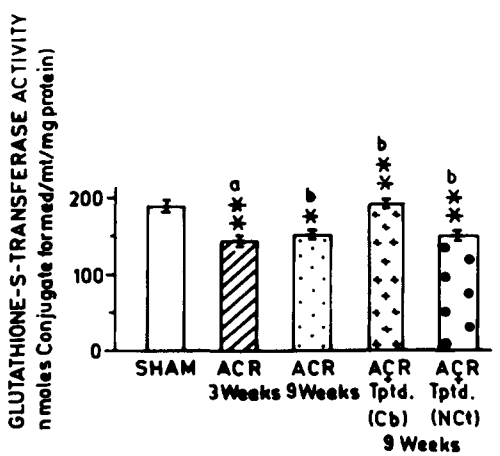

FIG. 5. Recovery of Glutathione-S-Transferase activity (expressed as nanomoles of conjugate formed per milligram protein per min) in acrylamide-treated rats with grafts of cerebellar suspensions (GD-14), 9 weeks after transplantation. Data are mean ± SE of five rats per group. Two-way analysis of variance, $F(4, 16) = 87.794$, $p < 0.01$. a, vs. control; b, vs. acrylamide (3 weeks). $^*p < 0.01$. $^{**}p < 0.001$.

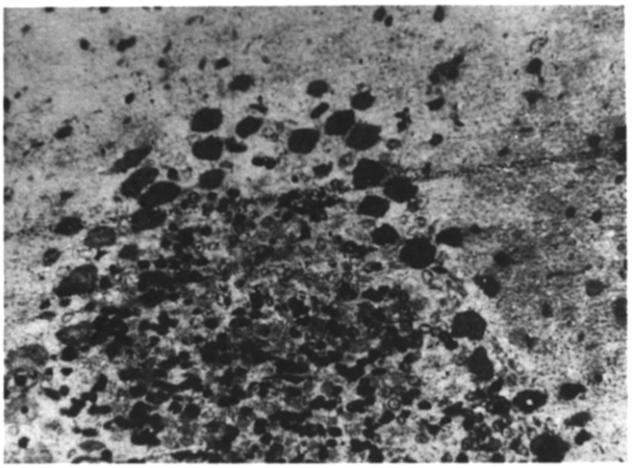

FIG. 6. Photomicrograph of part of the cerebellar cortex of the acrylamide-treated rat. Note the disorganization of the Purkinje cell layer showing dark, irregular, and degenerating neurons. The underlying granular layer depicts pleomorphic round cells; some are aggregated and vacuolated at places (×200).

affecting the affinity of receptor (K_d [transplanted]: 0.96 nM; K_d [controls] :0.83 nM) (Fig. 4).

The activity of glutathione-*S*-transferase was significantly decreased at 3 and 12 weeks after exposure to acrylamide compared with animals not treated. The animals receiving cerebellar transplants exhibited a recovery of enzyme activity that was highly significant at 9 weeks after transplantation (Fig. 5) whereas rats exposed to acrylamide and transplanted with neocortical grafts failed to show improvement in enzyme activity.

Morphologic Changes

Light micrographs clearly demonstrated that the cerebellar cortex exhibited a characteristic trilaminar structure with

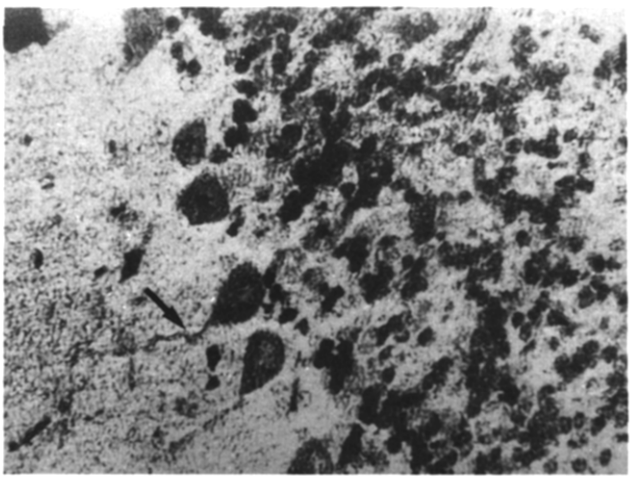

FIG. 7. Photomicrograph of a segment of the cerebellar cortex of the control rat. Note the well-preserved flask-shaped Purkinje neurons in a single row. One shows a clearly defined apical dendrite (arrow). The underlying granular layer is composed of normal-looking round cells (×200).

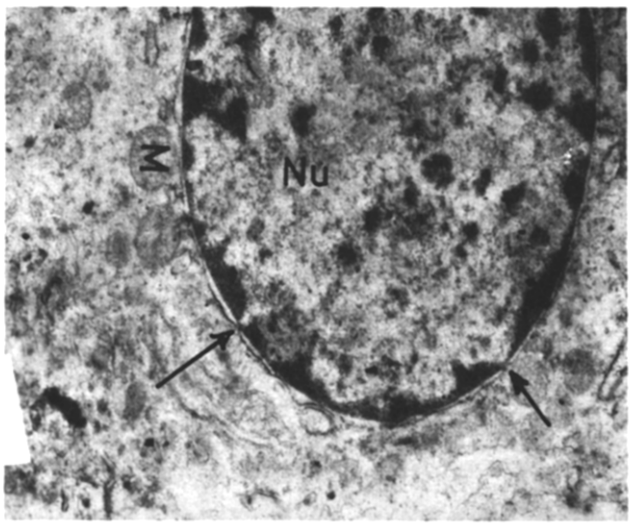

FIG. 8. Electron micrograph of a neuron from the cerebellar cortex-transplanted material. A segment of the nucleus (Nu) is discernible. Note the well-preserved double nuclear membranes and clearly visualized nuclear pores (arrow). Peripheral clumping of the nuclear chromatin is seen. The cytoplasmic organelles are equally well preserved. A number of mitochondrial profiles (M) are seen in the perikaryon (×12,800).

flask-shaped Purkinje neurons. The acrylamide-treated material showed pyknotic and degenerating Purkinje neurons of variegated morphology, some irregular in outline (Fig. 6). On the other hand, the control material exhibited well-preserved, flask-shaped Purkinje neurons organized in a single row, and their apical dendrites were often discernible (arrow, Fig. 7). The acrylamide-treated transplanted material showed the ultrastructural features of a well-preserved neuron, depicting a segment of a nucleus (Nu, Fig. 7) with double nuclear membranes and nuclear pores (arrow, Fig. 8). Cytoplasmic organelles were also well-preserved, and a number of mitochondrial profiles (M) were noticeable in the perikaryon (Fig. 8).

DISCUSSION

The mechanism of neurotoxicity for acrylamide has been studied in our laboratory and elsewhere (15,16,26). In the present study using acrylamide, we have shown that fetal neural transplants were able to restore lost function, as judged by behavioral, biochemical, and morphologic parameters. We earlier reported both an improvement in acylamide-induced hind limb ataxia and spinocerebellar degeneration by fetal medullary transplants, which lends support to our present observations (14).

The decrease in cerebellar synaptic membrane ^{3}H-flunitrazepam binding may also be related to the loss of these cells; the decrease in the glutathione-*S*-transferase activity could be due to the direct action of acrylamide with the enzyme. Perhaps degeneration of these and other neuronal cells may cause the loss of glutathione, which offers a mechanism of removal for acrylamide, and thus may increase the bioavailability of free acrylamide for interaction with the enzyme. However, further studies are needed to confirm this.

Cerebellum acts as a modulator of spinal reflexes. The selective death of cerebellar Purkinje cell due to acrylamide can be attributed to the perturbations in energy-linked functions by the monomer, as observed in our earlier study (14). In the cerebellum, neuronal networks are organized in a point-to-point manner, indicating cell-cell interactions between immature and adult neuronal cells. In an elegant series of experiments, Sotello and Alvardo-Mallart (27) and Agrawal et al. (3) demonstrated that when transplanted in the cerebellum of Purkinje cell-degenerated mutant mice, fetal cerebellar Purkinje neurons reconstruct the deficient cerebellar circuitary in a point-to-point manner. The restoration of acrylamide-induced biochemical and behavioral deficits by fetal cerebellar transplants, especially rotarod performance and spontaneous locomotor activity at 9 weeks after transplantation, suggests partial restoration of the damaged cerebellar circuitary. Histologic studies further confirm that cerebellar grafts are anatomically integrated within the host cerebellum. The inability of neocortical grafts to reverse acrylamide-induced central nervous system deficits lends support to the specificity of cerebellar grafts. In conclusion, a possible mechanism for functional recovery in the current investigation could be the graft-induced substitution of GABA-ergic deficits in Purkinje neurons of acrylamide-damaged cerebellum. The involvement of a trophic influence of the graft, by way of releasing neuron-promoting and nerve growth factors in enhancing the repair process, may also be possible (7).

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Director of the Industrial Toxicology Research Centre for his support in conducting this study. The kind help of Dr. R. C. Murthy in light microscopy is gratefully acknowledged. The authors also thank Prof. P. N. Tandon and G. Gopinath for their encouragement and support.

REFERENCES

1. Agrawal, A. K.; Ansari, A. A.; Seth, P. K. Effect of procarbazine on benzodiazepine receptor in mouse brain. Toxicol. Lett. 9: 171–175; 1981.
2. Agrawal, A. K.; Squibb, R. E.; Bondy, S. C. The effect of acrylamide treatment upon the dopamine receptors. Toxicol. Appl. Pharmacol. 58:88–99; 1981.
3. Armengol, J. A.; Sotelo, C.; Angaut, P.; Alvarado-Mallart, R.-M. Organisation of host afferents to cerebellar grafts implanted into kainate lesioned cerebellum in adult rats. Hololological evidence for the specificity of host-graft interactions. Eur. J. Neurosci. 1:75–93; 1989.
4. Bjorklund, A. Neural transplantation an experimental tool with clinical possibilities. Trends Neurosci. 14:319–322; 1991.
5. Calne, D. B. Neurotoxins and degeneration in the central nervous system. Neurotoxicology 12:335–340; 1991.
6. Cavanagh, J. B.; Nolan, C. C. Selective loss of Purkinje cells from the rat cerebellum caused by acrylamide and the responses of β-glucuronidase and β-galactosidase. Acta. Neuropathol. 58: 210–214; 1982.
7. Cohen-Cory, S.; Dreyfus, C. F.; Black, I. B. NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. J. Neurosci. 11:462–471; 1991.
8. Das, G. D.; Hallas, B. H.; Das, K. G. Transplantation of neural tissues in the brain of laboratory mammals: Technical details and comments. Experientia 35:143–153; 1979.
9. Das, M.; Mukhtar, H.; Seth, P. K. Effect of acrylamide on brain

and hepatic mixed function oxidases and glutathione-*S*-transferases in rats. Toxicol. Appl. Pharmacol. 66:420–426; 1982.
10. Dunnet, S. B.; Bjorklund, A.; Schmidt, R. H.; Stenevi, V.; Iverson, S. D. Intracerebral grafting of neuronal cell suspension. V. Behavioral recovery in rats with bilateral 6-OHDA lesions following implantation of nigral cell suspensions in different brain sites. Acta. Physiol. Scand., Suppl. 522:39–47; 1982.
11. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine, ^{3}H-dopa in various regions of the brain. J. Neurochem. 13:655–669; 1966.
12. Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione-*S*-transferase. The first step in mercapturic acid formation. J. Biol. Chem. 249:7130–7139; 1974.
13. Hashimoto, K.; Aldridge, W. N. Biochemical studies on acrylamide a neurotoxic agent. Biochem. Pharmacol. 19:2591–2604; 1981.
14. Husain, R.; Agrawal, A. K.; Sunderaman, V.; Hasan, M.; Seth, P. K. Functional recovery following fetal medullary transplants in the spinal cord of rats with acrylamide-induced spinocerebellar lesions. Restor. Neurol. Neurosci. 4:18; 1992 (abstr.).
15. Husain, R.; Dixit, R.; Das, M.; Seth, P. K. Neurotoxicity of acrylamide in developing rat brain: Changes in the levels of brain biogenic amines and activities of monoamine oxidase and acetylcholine esterase. Industrial Health 25:19–28; 1987.
16. Husain, R.; Srivastava, S.; Srivastava, S. P.; Seth, P. K. Effect of acrylamide on energy-linked functions in rat brain. Bull. Environ. Contam. Toxicol. 37:427–432; 1981.
17. Kaplan, M. L.; Murphy, S. D. Effect of acrylamide on rotarod and sciatic nerve B-glucuronidase of rats. Toxicol. Appl. Pharmacol. 22:259–268; 1972.
18. Karnovsky, M. J. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137; 1965 (abstr.).
19. Kuhn, W. L.; Van Maanen, E. F. Central nervous system effects of thalidomide. J. Pharmacol. Exp. Ther. 134:60–68; 1961.
20. LeQuesne, P. M. Acrylamide. In: Spencer, P. S.; Schaumberg, H. H., eds. Experimental and clinical neurotoxicology. Baltimore: William & Wilkins; 1981:309–325.
21. Lindvall, O.; Brundin, P.; Widner, H.; et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. Science 247:574–577; 1990.
22. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement by the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
23. Madrazo, I.; Leon, V.; Torres, C.; Aguilera, D. C.; Varela, G.; Alvarez, F.; Fraga, A.; Drucker-Colen, R.; Ortrosky, F.; Skurovich, M.; Frango, R. Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. N. Engl. J. Med. 318:51; 1988.
24. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
25. Reynolds, E. S. The use of lead citrate of high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208–212; 1963.
26. Sickles, D. W. Toxic neurofilamentous axonopathies and fast anterograde axonal transport. I. The effects of single dose of acrylamide on the rate and capacity of transport. Neurotoxicology 10:91–102; 1989.
27. Sotelo, C.; Alvarado-Mallart, R.-M. The reconstruction of cerebellar circuits. Trends Neurosci. 14:350–355; 1991.
28. Spencer, P. S.; Griffin, J. W. Disruption of axoplasmic transport by neurotoxic agents. The 2,5-hexanedione model. In: Weiss, D. G.; Gario, A., eds. Axoplasmic transport in physiology and pathology. Berlin: Springer-Verlag; 1982:92–103.
29. Watson, M. L. Staining of tissue sections for electron microscopy with heavy metal. J. Biophys. Biochem. Cytol. 4:475–478; 1958.