tumorigenic phenotype. All other animals have remained tumor-free. For animals without tumors, histopathologic examination of the site of injection of the (+6) microcell hybrids after 2 months shows only occasional reactive fibroblasts with no evidence of melanoma cells. In contrast, the histology of the parental and revertant tumors grown in nude mice are indistinguishable from the patient's melanoma.

As a control for the introduction of chromosome 6 into melanoma cell lines, we have introduced, by electroporation, psv_2neo into the parental UACC-903 melanoma cell line (Table 1). In contrast to the introduction of chromosome 6, the addition of the selectable marker did not alter in vitro morphology or effect tumorigenicity, suggesting it has no role in the suppression of tumor formation.

Several studies have been reported on the genetics of tumor suppression by somatic cell fusion (*8, 9*). However, only recently has intraspecies hybridization of a single human chromosome been possible (*10*). On the basis of our current observations, it is reasonable to speculate that genetic information present on chromosome 6 can suppress the malignant phenotype of human melanoma cells. If [as suggested by Vogelstein (*5*)] tumor suppressor genes have both a hierarchical and incremental effect on the regulation of cell growth, it is possible that a gene, or genes, on chromosome 6 may be acting early in the pathway of tumor formation.

REFERENCES AND NOTES

1. P. Armitage and R. Doll, *Br. J. Cancer* **8**, 1 (1954); A. Knudson, Jr., *Cancer* **35**, 1022 (1975).
2. A. Knudson, Jr., *Cancer Res.* **45**, 1437 (1985); M. Hansen and W. Cavenee, *ibid.* **47**, 5518 (1987); E. J. Stanbridge, *Adv. Virol. Oncol.* **6**, 83 (1987); R. Weinberg, *Cancer Res.* **49**, 3713 (1989).
3. E. J. Stanbridge, *Bioessays* **3**, 252 (1985); G. Klein, *Science* **238**, 1539 (1987); S. Friend, T. Druja, R. Weinberg, *N. Engl. J. Med.* **318**, 618 (1988).
4. S. Friend *et al.*, *Nature* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987); Y.-K. T. Fung *et al.*, *ibid.* **236**, 1657 (1987); S. Baker *et al.*, *ibid.* **244**, 217 (1989); T. Takahaski *et al.*, *ibid.* **246**, 491 (1989).
5. B. Vogelstein *et al.*, *Science* **244**, 207 (1989).
6. K. Kok *et al.*, *Nature* **330**, 578 (1987); S. Naylor, B. Johnson, J. Minna, A. Sakaguchi, *ibid.* **329**, 451 (1987); H. Brauch *et al.*, *N. Engl. J. Med.* **317**, 1109 (1987); S. Orkin, D. Goldman, S. Sallan, *Nature* **305**, 172 (1983); E. Fearon, B. Vogelstein, A. Feinberg, *ibid.* **309**, 176 (1984); A. Koufos *et al.*, *ibid.* **316**, 330 (1985); W. Cavenee *et al.*, *ibid.* **305**, 779 (1983); C.-T. Fong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3753 (1989).
7. N. Dracopoli, A. Houghton, L. Old, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1470 (1985).
8. H. Harris *et al.*, *Nature* **223**, 363 (1969); H. Harris, *Somatic Cell Genet.* **5**, 923 (1979).
9. E. Stanbridge, *Nature* **260**, 17 (1976); E. Stanbridge and J. Wilkinson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1466 (1978); H. Klinger, *Cytogenet. Cell Genet.* **27**, 254 (1980); E. J. Stanbridge *et al.*, *Science* **215**, 252 (1982); W. Benedict, B. Weissman, C. Mark, E. Stanbridge, *Cancer Res.* **44**, 3471 (1984).
10. C. McNeill and R. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5394 (1980); R. Fournier, *ibid.* **78**, 6349 (1981); B. E. Weissman *et al.*, *Science* **236**, 175 (1987).
11. J. M. Trent, S. B. Rosenfeld, F. L. Meyskens, *Cancer Genet. Cytogenet.* **9**, 177 (1983); R. Becher *et al.*, *Cancer Res.* **43**, 5010 (1983); S. Pathak, H. L. Drwinga, T. C. Hsu, *Cytogenet. Cell Genet.* **36**, 573 (1983); G. Balaban *et al.*, *Cancer Genet. Cytogenet.* **11**, 429 (1984); M. I. Pedersen, J. W. Bennett, N. Wang, *ibid.* **20**, 11 (1986); J. M. Cowan, R. Halaban, A. T. Lane, U. Francke, *ibid.*, p. 255; A. H. Parmiter, G. Balaban, M. Herlyn, W. H. Clark, P. C. Nowell, *Cancer Res.* **46**, 1526 (1986).
12. J. M. Trent *et al.*, *Cancer Res.* **49**, 420 (1989).
13. Microcell hybrids are constructed by treating a proliferating population of donor cells [containing a chromosome with an integrated dominant selectable marker] with colcemid (0.15 μg/ml) for 72 hours. The cells are initially blocked in mitosis, but over time the majority of cells overcome the colcemid block and enter G_1. Because no spindle has formed, the chromosomes recondense into multiple micronuclei with each nucleus containing one to several chromosomes. The population of multinucleate cells is enucleated with a combination of cytochalasin B (2 μg/ml) and centrifugal force. The resulting microcells are filtered to produce a population that consists predominantly of micronuclei containing single chromosomes. These microcells are then fused to recipient cells and grown in medium containing 600 μg of G418 per milliliter. Only recipient cells into which the chromosome containing the integrated *neo* has been introduced are capable of surviving in this selective medium.
14. Cells grown on cover slips were fixed 5 min in cold methanol (4°C) and permeabilized by dipping in acetone. The primary antibody, HMB-45 [A. M. Gown *et al.*, *Am. J. Pathol.* **123**, 195 (1986)] was incubated for 30 min. Slides were then washed three times with phosphate-buffered saline (PBS). Secondary fluoresceinated rabbit antibody to mouse antigen was incubated for 30 min. Again the slides were washed three times with PBS and observed under a cover slip with an epifluorescent microscope. As described by Gown *et al.*, HMB-45 recognizes 97% of malignant melanomas (both pigmented and nonpigmented) while failing to react with normal skin melanocytes. The antibody recognizes an unknown cytoplasmic antigen. In direct screening of tumor tissues, the HMB-45 antibody in general strains "epitheloid" melanoma cells more uniformly positive than more "sarcomatous" melanoma cells (analogous to the situation following the introduction of chromosome 6 into our cell lines).
15. Exponentially growing cells were harvested for karyotypic analysis as described previously [J. Trent and F. Thompson, in *Methods in Enzymology*, M. Gottesman, Ed. (Academic Press, New York, 1987), pp. 267–278]. A minimum of 50 cells per line were analyzed, with results expressed according to International System for Human Cytogenetic Nomenclature (ISCN) recommendations [*Cytogenet. Cell Genet.* **21**, 1 (1985)]. The model chromosome number of the UACC-903 cell line was 47, including clonal structural alterations of chromosome 1 [t(1;11)(q23;q23) and derinv(1) (p36q25)]; chromosome 11 [t(1;11) and del(11) (q23)]; and chromosome 17 [t(17;?)(q25;?)]. No evidence of chromosome 6 alterations were observed in any cell (Fig. 3A). The UACC-091 cell line had a hypertriploid modal chromosome number (78 chromosomes per cell) with clonal structural alterations of chromosome 1 [del(1)(p22)]; chromosome 3 [del(3)(q13)]; chromosome 5 [t(5;?)(p14;?)]; chromosome 6 [del(6)(q16)]; chromosome 7 [iso(7q)]; chromosome 12 [del(12)(p12)]; chromosome 13 [t(13:?)(p11;?)]; chromosome 15 [t(15;?)(p11;?)]; and chromosome 16 [t(16;?)(q25;?)]. Fig. 3A documents chromosome 1 and 6 alterations from UACC-091 and chromosome 1 alterations from the UACC-903 cell line.
16. Y. Nakamura *et al.*, *Nucleic Acids Res.* **16**, 4743 (1988).
17. J. Trent, unpublished data.
18. H. McBride, B. Stea, A. Hill, J. Trent, *Int. J. Radiat. Oncol. Biol. Phys.*, in press.
19. A. Leibovitz and K. Mazur, *Tissue Cult. Assoc. Man.* **3**, 655 (1977).

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2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: A Neuroprotectant for Cerebral Ischemia

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2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) is an analog of the quinoxalinedione antagonists to the non–*N*-methyl-D-aspartate (non-NMDA) glutamate receptor. NBQX is a potent and selective inhibitor of binding to the quisqualate subtype of the glutamate receptor, with no activity at the NMDA and glycine sites. NBQX protects against global ischemia, even when administered 2 hours after an ischemic challenge.

THE DISCOVERY OF THE QUINOXALINEDIONES (*1*), a series of potent and selective antagonists at non-NMDA excitatory amino acid (EAA) receptors, has greatly facilitated the study of the pharmacology of the quisqualate and kainate receptor subtypes. Here we report a new non-NMDA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) (Fig. 1), which is more potent than the previously described compounds DNQX (6,7-dinitroquinoxaline-2,3-dione) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) in displacing [^{3}H]AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) binding and is less potent in inhibiting [^{3}H]kainate, [^{3}H]CPP [3(2-carboxypiperazine-4-yl)propyl-1-phosphonic

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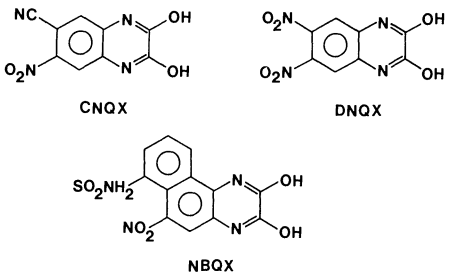

Fig. 1. The structures of three quinoxalinedione non-NMDA EAA antagonists.

acid], and [^{3}H]glycine binding to rat brain cortical membranes. NBQX is a selective antagonist of quisqualate, AMPA, and kainate electrophysiological responses in rat neocortex slices and is a neuroprotective agent against cerebral ischemia.

Binding experiments were performed at 4°C with rat brain cortical membranes that had been extensively washed (*2*). Kainate receptors were studied with [^{3}H]kainate (5 n*M*) binding in 50 m*M* tris-citrate buffer (*3*); quisqualate receptors were studied with [^{3}H]AMPA (5 n*M*) binding in 30 m*M* tris-HCl buffer with 2.5 m*M* $CaCl_2$ and 100 m*M* potassium thiocyanate (*2*); NMDA receptors were studied with [^{3}H]CPP binding (5 n*M*) in 30 m*M* tris-HCl buffer with 2.5 m*M* $CaCl_2$ (*4*); and glycine receptors were studied with [^{3}H]glycine (20 n*M*) binding in 30 m*M* tris-HCl buffer with 2.5 m*M* $CaCl_2$. Binding was selectively inhibited at the quisqualate site by NBQX, the concentration that inhibited 50% (IC_{50}) of [^{3}H]AMPA binding was 150 n*M*, and the Hill coefficient was close to one, indicating no noncompetitive or cooperative interactions.

The affinity of NBQX for the kainate receptor was about 30 times less than its affinity for quisqualate receptors, the IC_{50} being 4.8 μ*M*. NBQX has no or very low affinity for the CPP or glycine binding sites. The values compared to those for CNQX, DNQX, and some standard compounds are shown in Table 1. The compound is also inactive (IC_{50} > 100 μ*M*) at the following binding sites: [^{3}H]spiperone in the presence of the dopamine agonist (±)-2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (5-HT receptors), [^{3}H]WB 4101 (α-noradrenergic receptors), [^{3}H]3-quinuclidinol benzilate (muscarinic receptors), [^{3}H]spiperone, and [^{3}H]SCH 23390 (dopamine D1 and D2 receptors), [^{3}H]naloxone (opiate receptors), [^{3}H]flunitrazepam (benzodiazepine receptors), [^{3}H]GABA (γ-amino-butyric acid receptors), and [^{3}H]strychnine (inhibitory glycine receptor). NBQX was inactive (IC_{50} > 30 μ*M*) at phencyclidine sites on the NMDA-coupled ionophore as measured by [^{3}H]*N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP) binding.

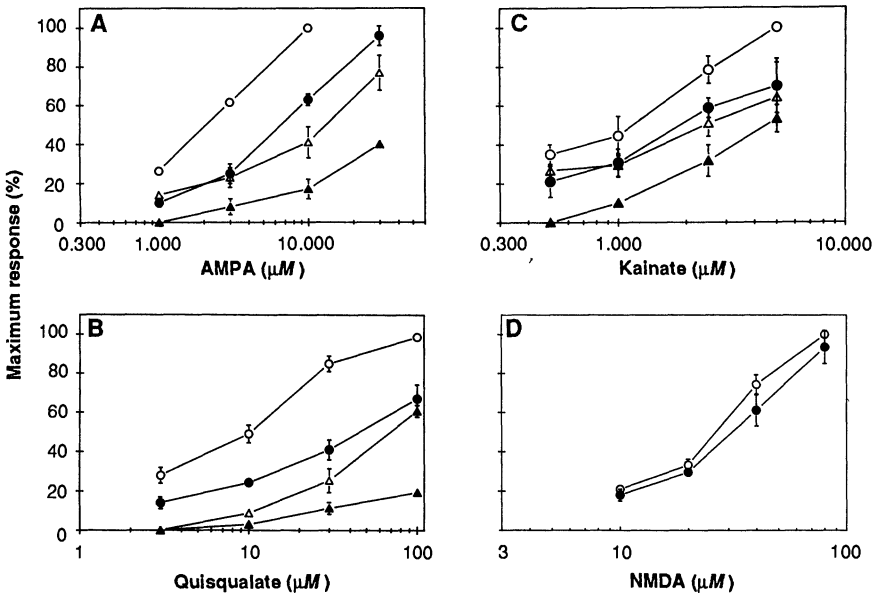

Fig. 2. The effect of NBQX on responses of rat neocortical slices to (**A**) AMPA, (**B**) quisqualate, (**C**) kainate, and (**D**) NMDA. In all the graphs the *x* axis is the log concentration of agonist and the *y* axis is the percentage maximum response (depolarization). (A) Open circles, control AMPA dose-response curve; closed circles, AMPA plus NBQX (0.1 μ*M*); open triangles, AMPA plus NBQX (0.3 μ*M*); and closed triangles, AMPA plus NBQX (1 μ*M*). (B) Open circles, control quisqualate dose-response curve; closed circles, quisqualate plus NBQX (0.3 μ*M*); open triangles, quisqualate plus NBQX (1 μ*M*); and closed triangles, quisqualate plus NBQX (3 μ*M*). (C) Open circles, control kainate dose response curve; closed circles, kainate plus NBQX (0.3 μ*M*); open triangles, kainate plus NBQX (1 μ*M*); and closed triangles, kainate plus NBQX (3 μ*M*). (D) Open circles, control NMDA dose-response curve; and closed circles, NMDA plus NBQX (30 μ*M*). Points represent means ± SEM (where error bar is greater than the symbol); *n* = 7 to 10 responses for each point. The maximum concentrations of the agonists used in the dose-response curves were 30 μ*M* AMPA, 100 μ*M* quisqualate, 5 μ*M* kainate, and 80 μ*M* NMDA. Higher concentrations produced neurotoxic effects, making it difficult to obtain reproducible control responses to the agonists.

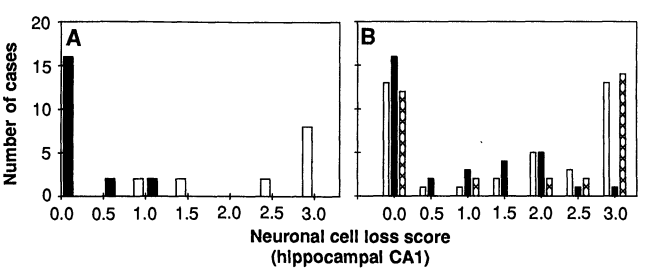

Fig. 3. The protective effect of NBQX against hippocampal CA1 neuronal cell loss evoked by 5-min global ischemia in Mongolian gerbils. The *x* axis neuronal cell loss score [0, no cell loss; 1, 25% to 50% cell loss; 2, 50% to 75% cell loss; and 3, almost total cell loss (>90%)]. The *y* axis represents the number of individual hippocampi with a particular score (number of cases). (**A**) NBQX (30 mg/kg) administered intraperitoneally 15 and 5 min before ischemia and 10 min after (solid bars). (**B**) Doses of 10 (solid bars) and 3 mg/kg (hatched bars) administered as in (A). Empty bars, control.

To show the pharmacological effects of NBQX, we studied the electrophysiological responses of rat neocortex slices to excitatory amino acids by grease-gap techniques (*5*). This simple method is suitable for studying slow steady-state effects of agonists and antagonist selectivity, but does not measure very fast quisqualate currents (*6*). NBQX produced large shifts of the dose-response curves to quisqualate and AMPA at concentrations of 0.1 to 1.0 μ*M* and kainate at 0.3 to 3.0 μ*M*, while having no effect on NMDA responses at a concentration of 30 μ*M* (Fig. 2). This result shows that NBQX is a highly selective antagonist of non-NMDA EAA receptor–evoked responses in the central nervous system.

Transient complete forebrain ischemia was produced in female Mongolian gerbils (60 to 70 g) anesthetized with a mixture of 2% halothane, 70% nitrous oxide, and 30% oxygen. Cerebral ischemia was induced by occlusion of the carotid arteries for 5 min. After 4 days, the animals were killed, the brain was removed, and frozen coronal sections of 20 μ*M* were taken of the hippocampal area and stained with cresyl violet and hematoxylin-eosin. The CA1 subfield of the hippocampus was assessed for neuronal cell death on a scale ranging from 0 for a normal hippocampus to 3 for an almost total loss of

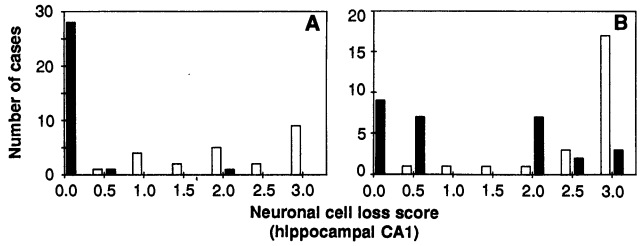

Fig. 4. (**A**) NBQX (30 mg/kg) (solid bars) administered intraperitoneally at 60, 70, and 85 min after ischemia and (**B**) at 120, 130, and 145 min (solid bars) after ischemia produces a neuroprotective effect against cerebral ischemia. Axes are as in Fig. 3. *P* values = 0.001 and 0.0015, respectively, with the randomized Wilcoxon rank-sum test. Empty bars, control.

Table 1. Inhibitory activity of selected glutamate antagonists and agonists. Rat cortical membranes were prepared as in (*2*). The final pellet was homogenized in buffer (50 volumes per gram of original tissue) and used for binding assays. Portions (0.5 ml) in triplicate were incubated for 1 hour at 0°C with [^{3}H]kainic acid (Du Pont, Biotechnology Systems; 60.0 Ci/mmol], 30 min at 0°C with [^{3}H]AMPA (Du Pont, Biotechnology Systems; 27.6 Ci/mmol), and 30 min at 0°C with [^{3}H]CPP (Tocris; 20 Ci/mmol) in the absence or presence of 0.6 m*M* L-glutamate for determination of specific binding. For [^{3}H]glycine binding the final pellet was homogenized in 75 volumes of buffer per gram of original tissue. Portions (1 ml) were incubated for 10 min at 0°C with [^{3}H]glycine (Du Pont, Biotechnology Systems; 49 Ci/mmol) in the absence or presence of D-serine (100 μM) for determination of specific binding. Free and bound radioactivity were separated by filtration through Whatman GF/C glass fiber filters followed by three washes with 5 ml of ice-cold buffer. The IC_{50} values were obtained with at least four different concentrations of the inhibitor and the software EBDA from Elsevier Biosoft. Results shown are means ± SEM of three determinations; otherwise results are means of two determinations.

Compound	IC_{50} (μM)			
	[^{3}H]AMPA (±SEM)	[^{3}H]Kainate (±SEM)	[^{3}H]CPP (±SEM)	[^{3}H]Glycine
NBQX	0.15 (±0.014)	4.8 (±0.47)	>90	>100
CNQX	0.30 (±0.15)	1.5 (±0.30)	25	14
DNQX	0.5 (±0.10)	2.0 (±0.10)	40	9.5
CPP	450		0.050 (±0.25)	
Glycine				0.16
AMPA	0.045 (±0.005)	45	>100	
Kainate	20	0.0045 (±0.001)	>100	
Quisqualate	0.025 (±0.005)	0.2 (±0.01)	40	
MK-801	>1000	>1000	>100	
Pentobarbitone	>1000	>1000	>1000	

neuronal cells (>90%). NBQX was administered intraperitoneally at doses of 3, 10, and 30 mg per kilogram of body weight at 15 and 5 min before and 10 min after the onset of occlusion, or at 30 mg/kg 60, 70, and 85 min or 120, 130, and 145 min after occlusion. In each experiment matched controls received the vehicle only at the same time intervals.

When administered before and after occlusion (Fig. 3), NBQX produced a dose-dependent protection against delayed neuronal cell death of CA1 neurons; 30 mg/kg totally protected, whereas 3 mg/kg showed no protection. When administered at 60, 70, and 85 or 120, 130, and 145 min after the onset of cerebral ischemia, NBQX (30 mg/kg) showed a clear neuroprotective effect (Fig. 4). In order to control for the effects of anesthesia, animals were reanesthetized with pentobarbitone (50 mg/kg) 30 min after the onset of ischemia. Pentobarbitone showed no neuroprotective effect. In order to check for a possible drop in brain temperature induced by the drug, a group of five animals were given 30 mg/kg NBQX 15 and 5 min before the onset of ischemia and temporal muscle temperature, which is linearly related to brain temperature (7), was monitored. The fall in temporal muscle temperature after NBQX was 2.5° ± 1.2°C compared with 2.0° ± 0.7°C in animals treated with saline (P = >1.0, t test). No drop in core temperature was seen in gerbils after three doses of NBQX (30 mg/kg) administered over a 25-min period.

Our results show that NBQX protected hippocampal neurons from ischemic damage after complete forebrain ischemia, not only when given before ischemia but also when given up to 2 hours after ischemia. Since NBQX is a highly selective non-NMDA EAA antagonist, the results suggest that delayed neuronal cell death after a period of global ischemia is mediated not only by NMDA receptors as previously suggested (*8*), but also by a mechanism involving quisqualate or kainate receptors. In support of this conclusion, it has been shown that quisqualate-evoked dark cell degeneration in rat hippocampal slices can be prevented by the non-NMDA glutamate receptor antagonist CNQX when the compound is administered after the quisqualate challenge (*9*). Furthermore, quisqualate receptors can evoke a biphasic increase in intracellular Ca^{2+} (*10*), the second (plateau) phase being sensitive to CNQX and Ca^{2+} antagonists. On the basis of these findings, we hypothesize that glutamate released during ischemia (*11*) may trigger a post-ischemic release of glutamate or another endogenous substance, which acts at quisqualate receptors. This would evoke neuronal cell loss in a manner sensitive to NBQX administered up to 2 hours after ischemia. Another possible mechanism for the protective effect of NBQX is that non-NMDA receptor blockade may reduce NMDA receptor–evoked depolarization during the post-ischemic period. As the results presented show NBQX to be highly selective for non-NMDA excitatory amino acid receptors, a protective mechanism not involving non-NMDA excitatory amino acid receptors seems unlikely, but cannot be ruled out. However, the lack of effect of pentobarbitone when given post-ischemically to gerbils and the lack of effect of NBQX on temporal muscle temperature suggest that the neuroprotective effect of NBQX is not exerted via a nonspecific sedative or hypothermic action.

Our data also support previous suggestions that kainate-evoked depolarizations are mediated by the low-affinity kainate sites, which are identical to quisqualate receptors (*2, 12*). In the binding studies, NBQX showed a 30-fold higher affinity for the [^{3}H]AMPA binding sites as compared to the high-affinity Ca^{2+}-sensitive [^{3}H]kainate binding site. However, NBQX shows little selectivity between quisqualate- and kainate-evoked depolarization in rat neocortex slices.

In conclusion, NBQX is a highly sensitive non-NMDA EAA receptor antagonist that can act as neuroprotectant in global ischemia.

REFERENCES

1. T. Honoré *et al.*, *Science* **241**, 701 (1988).
2. T. Honoré and M. Nielsen, *Neurosci. Lett.* **54**, 27 (1985).
3. T. Honoré, J. Drejer, M. Nielsen, *ibid.* **65**, 47 (1986).

4. T. Honoré, J. C. Watkins, H. J. Olverman, *Eur. J. Pharmacol.* **136**, 137 (1987).
5. M. J. Sheardown, *ibid.* **148**, 471 (1988).
6. C.-M. Tang, M. Dichter, M. Morad, *Science* **243**, 1474 (1989).
7. J. R. Busto *et al.*, *J. Cereb. Blood Flow Metabol.* **7**, 729 (1987).
8. A. C. Foster, R. Gill, G. N. Woodruff, *J. Neurosci.* **8**, 4745.
9. G. Garthwaite and J. Garthwaite, *Neurosci. Lett.* **99**, 113 (1989).
10. S. N. Murphy and R. J. Miller, *Mol. Pharmacol.* **35**, 671 (1989); H. Sugiyama, J. Ito, C. Hirono, *Nature* **325**, 531 (1987).
11. H. Benveniste, J. Drejer, A. Schousboe, N. H. Diemer, *J. Neurochem.* **43**, 1369 (1984).
12. T. Honoré, J. Drejer, M. Nielsen, *Neurosci. Lett.* **65**, 47 (1986).

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Grafts of Fetal Dopamine Neurons Survive and Improve Motor Function in Parkinson's Disease

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Neural transplantation can restore striatal dopaminergic neurotransmission in animal models of Parkinson's disease. It has now been shown that mesencephalic dopamine neurons, obtained from human fetuses of 8 to 9 weeks gestational age, can survive in the human brain and produce marked and sustained symptomatic relief in a patient severely affected with idiopathic Parkinson's disease. The grafts, which were implanted unilaterally into the putamen by stereotactic surgery, restored dopamine synthesis and storage in the grafted area, as assessed by positron emission tomography with 6-L-[^{18}F]fluorodopa. This neurochemical change was accompanied by a therapeutically significant reduction in the patient's severe rigidity and bradykinesia and a marked diminuation of the fluctuations in the patient's condition during optimum medication (the "on-off" phenomenon). The clinical improvement was most marked on the side contralateral to the transplant.

WHEN GRAFTS OF FETAL DOPAmine (DA)-rich mesencephalic tissue are implanted into the DA-depleted caudate-putamen of rodents and nonhuman primates with neurotoxin-induced parkinsonism, they can improve many of the motor impairments (*1*). In rats, such graft-induced amelioration of motor deficits is critically dependent on the ability of the grafted neurons to restore dopaminergic neurotransmission in the deafferented area surrounding the transplant, and sustained graft effects require survival and continuous function of the implanted dopaminergic neurons (*2, 3*).

Clinical trials with transplanted fetal mesencephalic tissue have been initiated in patients with Parkinson's disease in the last 2 years. In the few cases reported (*4, 5*), some symptomatic improvement has been observed, but it remains unclear if any of these changes can be attributed to graft-induced restoration of dopaminergic transmission in the striatum, or if they have been caused by nonspecific aspects of the surgical intervention (*6*). For the further development of this therapeutic approach, it is critical to establish (i) whether fetal nigral allografts can survive in the environment of the diseased parkinsonian brain; (ii) whether such grafts are able to restore DA functions in the affected striatum; and (iii) whether the survival of DA-synthesizing neurons can be correlated to a therapeutically valuable recovery of affected motor function. This study was designed to address these questions.

A severely affected patient with dramatic diurnal fluctuations in disability, despite optimum medical therapy, was selected after having given his consent. The patient is a 49-year-old man with Parkinson's disease, which began with unilateral tremor and rigidity in the right arm in 1977. Initial treatment with L-dopa was successful, but in 1984 he developed progressively worsening "on-off" phenomena, with rapid, often unpredictable, fluctuations in motor performance from a mobile, or on, state to an off, or rigid state, with manifest symptoms of Parkinson's disease. At the beginning of the study (April 1988) he was rated stage III on the scale of Hoehn and Yahr (*7*). During off periods he had severe rigidity, hypokinetic movements, and a moderate tremor in the right arm; less marked symptoms were evi dent in the left arm and legs. During on periods he displayed only very minor symptoms. The patient was taking daily doses of 700 mg of L-dopa (combined with benserazide), 10 mg of bromocriptine, and 6 mg of benzhexol chloride; these doses remained unchanged during the period of the study both before transplantation and in the 5 months thereafter. For 11 months before the operation the patient was assessed clinically and kept a daily log of his disability, scoring motor symptoms every 30 min (Fig. 1A). The duration and frequency of off periods were relatively stable preoperatively. On the average he had four to five daily off periods and spent 40 to 50% of the time in a severe off state. A preoperative 6-L-[^{18}F]fluorodopa positron emission tomographic (PET) scan showed the left putamen to be markedly deficient in DA-synthesizing capacity (Table 1); the right putamen was also affected, but to a lesser extent.

Immunosuppression was begun 2 days before transplantation (*8*). Dissociated ventral mesencephalic tissue from four fetuses (aged 8 to 9 weeks) was implanted stereotactically in the anterior, middle, and posterior part of the left putamen (*9*), the side contralateral to the most affected limbs. There were no complications. The implantation procedure was similar to one we have used previously (*5*) with three potentially important changes: the implantation cannula was considerably thinner (1.0-mm versus 2.5-mm outer diameter); the medium used for storage and dissociation of the tissue was a balanced, *p*H-stable salt solution rather than saline; and the technique of loading the cannula was improved so that virtually all the tissue could be used. In addition, the time of storage before transplantation was shorter for this patient.

During the second month after transplantation there was a marked reduction of both the time spent in off periods and the number of daily off periods (Fig. 1A). The patient noted a progressive reduction of rigidity, particularly in his right arm, and improvement of mobility during the night and in the

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