Brain Research, 459 (1988) 200–203
Elsevier

BRE 23078

Neurotoxicity of *N*-methyl-D-aspartate is markedly enhanced in developing rat central nervous system

John W. McDonald[1], Faye S. Silverstein[2,3] and Michael V. Johnston[1–4]

[1]*Neuroscience Training Program and Departments of* [2]*Pediatrics and* [3]*Neurology, University of Michigan Medical School and* [4]*Center for Human Growth and Development, Ann Arbor, MI (U.S.A.)*

(Accepted 17 May 1988)

Key words: Central nervous system; Development; Excitatory amino acid; Glutamate; Hypoxia–ischemia; Neurotoxicity; *N*-Methyl-D-aspartate; Rat

The neurotoxic lesion produced by direct injection of 25 nmol of *N*-methyl-D-aspartate (NMDA) into the corpus striatum of 7-day-old rats was compared to the effects of injecting 75 nmol into the striatum or hippocampus of adults. The area of histopathology in the immature striatum was 21× larger than the striatal lesion in adults. Damage from NMDA injected into the immature striatum also extended into the dorsal hippocampus and produced an area of destruction which was 16× larger than observed after direct injection into the adult hippocampus. Several studies have implicated excessive *N*-methyl-D-aspartate receptor activation in the pathogenesis of hypoxic–ischemic and hypoglycemic injury and our results suggest that this neurotoxic mechanism is extremely active in the immature brain.

Glutamate is one of the principle excitatory amino acid (EAA) neurotransmitters in the mammalian brain[7,31]. Glutamate mediates different cellular responses by interacting with several receptor subtypes named by their preferential agonists: *N*-methyl-D-aspartate (NMDA), quisqualic acid (QA) and kainic acid (KA)[15,31]. The excitatory responses of glutamate are terminated by a presynaptic high capacity uptake system that clears glutamate from the synaptic cleft[1]. Imbalance between the mechanisms responsible for synaptic release and uptake of glutamate could cause glutamate accumulation in the synaptic cleft and excessive excitation of EAA receptors. Overexcitation of EAA receptors can initiate a cascade of events[20,24] leading to excessive calcium entry, neuronal injury and death[9,17,18]. Synthetic and alkaloid agonists of each of the EAA receptors have been found to be neurotoxic in both the developing[4,25,28] and mature[6,8,29] rat central nervous system (CNS). Furthermore there are compounds endogenous to the brain that are not themselves neurotransmitters but which have neurotoxic actions mediated at EAA receptors. For example, quinolinic acid (QUIN), an endogenous metabolite of tryptophan, is an NMDA receptor neuromodulator and a potent neurotoxin in the adult CNS[23].

Neuronal susceptibility to the toxicity produced by EAA receptor agonists changes during development. For example, QA appears to be more toxic in the developing CNS[25] than in the CNS of adults[11,32] whereas the capacity of KA to induce neuronal injury increases with age and parallels the development of glutamatergic pathways[2]. The non-selective glutamate agonist ibotenate appears equally toxic in the developing and mature CNS. In the present study, we report that the neurotoxicity of NMDA is greatly enhanced in the immature rat brain.

Intracerebral injections of NMDA were performed in 7-day-old (n = 10) and in adult (3-month-old, n = 10) Sprague–Dawley albino rats briefly anesthetized with ether[13]. Stereotaxic injections in adults were made through a burr hole in the calvarium by means of a Hamilton syringe (26 gauge) into the corpus striatum (n = 5; bregma, AP 0, ML 3.0, V 5.5 mm) or the dorsal hippocampus (n = 5; bregma, AP 3.5, ML 3.5, V 3 mm). Seven-day-old rat pups

Correspondence: M.V. Johnston, Neuroscience Laboratory Building, 1103 E. Huron, Ann Arbor, MI 48104, U.S.A.

only received injections in the corpus striatum (n = 10; bregma, AP 1, ML 2.5, V 4 mm). Pups received 25 nmol/0.5 μl of NMDA dissolved in 0.01 M Tris, pH 7.4, and adult rats were injected with 75 nmol/1.5 μl of NMDA (n = 5, intrastriatal; n = 5, intrahippocampal). Control animals received equivalent volumes of Tris-buffer solution. Injections were made over a 2-min period to limit leakage. All animals were sacrificed 5 days after injection of NMDA and coronal frozen brain sections were prepared for Nissl stain.

The perimeter of neuronal destruction and gliosis in coronal brain sections was traced and the cross-sectional area measured with the aid of a video-based computerized image analyzer. Data were obtained from adult rats injected with NMDA (75 nmol/1.5 μl) in the corpus striatum (n = 5) or in the dorsal hippocampus (n = 5). Similar data were obtained from 7-day-old rat pups that were injected with 25 nmol/0.5 μl NMDA intrastriatally (n = 10). Cross-sectional areas were measured from brain sections at both the level of the corpus striatum and dorsal hippocampus (3 sections per level). Data from sections through the striatum or hippocampus were averaged and statistical comparisons were made at each level between adult and 7-day-old groups using independent two tailed t-tests. In control 7-day-old rats, intrastriatal injection of Tris-buffer produced no significant difference between the cross-sectional areas of the cerebral hemispheres (injected hemisphere, 30.09 ± 0.48 vs contralateral hemisphere, 30.602 ± 0.54, n = 5).

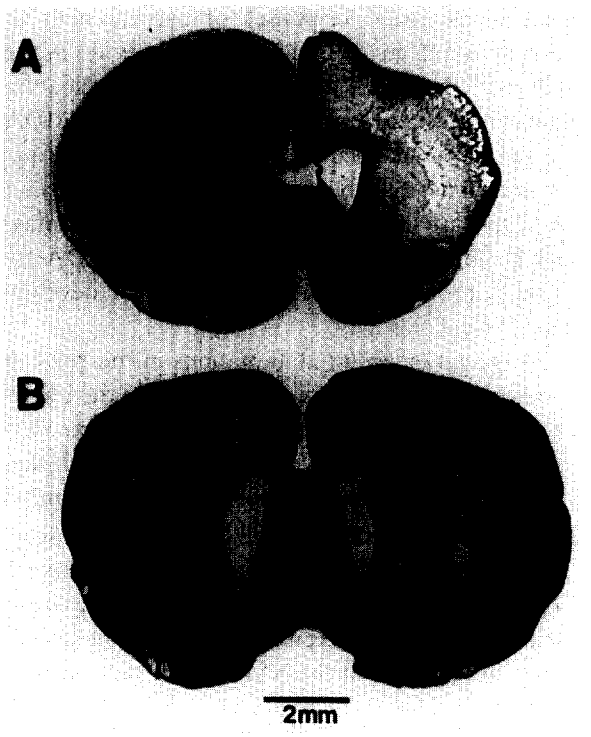

Fig. 1. Nissl-stained coronal brain sections at the level of the corpus striatum (C) illustrating the differential neurotoxicity produced by intrastriatal injection of the specific glutamate agonist *N*-methyl-D-aspartate (NMDA) in the developing (A) and mature (B) CNS. Seven-day-old (n = 10) and adult (n = 5) rats were injected intrastriatally with NMDA (25 nmol/0.5 μl, 7-day-old; 75 nmol/1.5 μl, adult) and were sacrificed 5 days later. NMDA injections in immature rats resulted in tissue damage that was much more extensive than the damage induced by a similar injection of 3 times as much NMDA in adult rats.

NMDA (25 nmol) injected intrastriatally in 7-day-old pups resulted in extensive areas of confluent necrosis, deformity and tissue loss involving the corpus striatum, dorsal hippocampus, and overlying neocortex of the injected hemisphere (Fig. 1A). These regions correspond to areas enriched in NMDA-preferring glutamate receptors. Histological signs of neuronal injury were confined to the injected hemisphere. In contrast, injection of 75 nmol of NMDA in adults rats produced neuronal necrosis and reactive gliosis that was limited to a small area surrounding the injection site (Fig. 1B).

Quantitative analysis of lesion size also indicated that the developing CNS is more susceptible to the toxicity of NMDA. Intrastriatal injection of 25 nmol NMDA into 7-day-old rats produced a significantly larger lesion than that resulting from 75 nmol NMDA injected intrastriatally in adults ($P < 0.001$, adult (n = 5) vs 7-day-old (n = 10), independent t-test). Cross-sectional area of the striatal lesion site in adults was 0.74 ± 0.14 mm^2 and in immature rats was 15.81 ± 1.02 mm^2 (mean ± S.E.M.), or 21.3 times the adult. Lesion size in adult rats injected in the dorsal hippocampus with 75 nmol NMDA resulted in an average lesion size of 1.10 ± 0.25 mm^2 whereas intrastriatal injection of 25 nmol NMDA in rat pups produced a cross-sectional lesion area of 17.56 ± 0.91 mm^2 in brain sections at the level of the dorsal hippocampus (16 times adult; $P < 0.001$, adult (n = 5) vs 7-day-old (n = 10), independent t-test).

Behavioral seizures were manifested in both the 7-day-old and adult rat. The behavioral signs of seizures were much more pronounced in immature rats and were of longer duration. Pups exhibited severe generalized tonic and tonic–clonic seizure activity

for 8 h whereas behavioral signs of seizures in adults only lasted for 3 h and consisted of ipsiversive turning and counterclockwise corkscrew rolling.

These results demonstrate that NMDA is extremely toxic in the immature CNS as opposed to the effects observed in the adult CNS. Foster et al.[8] cited unpublished observations that NMDA was equally toxic in 7-day-old and mature rat CNS. However, details of the dosages and experimental protocols were not provided. Differences in experimental protocol may account for the discrepancies between these reports.

Differences in the distribution, uptake, metabolism, and/or elimination of NMDA between immature and adult rats could account for part of the observed age-related effect. However, the uptake system for NMDA is relatively inefficient in the adult[26] and there is no report regarding metabolism of NMDA. There is no information to suggest that its distribution within the brain following injection is different in immature versus adult rats. However, the more heavily myelinated adult brain may limit diffusion. A possible prolonged duration of action of NMDA in pups would be consistent with the longer duration of behavioral seizure activity manifested in seven-day-old rats. However it seems more likely that the prolonged seizure activity may result from the greater extent of neuronal damage present in the pups. The developing CNS is very susceptible to seizure afterdischarges[27] especially following hypoxic- or trauma-induced brain damage and this may result in longer periods of seizure activity and delayed neuronal injury.

The results are consistent with the growing body of evidence suggesting that the biochemistry and pharmacology of glutamate receptors in the developing CNS differs from the adult CNS. For example, glutamate-stimulated phosphoinositol hydrolysis is markedly increased in immature brain[16]. Additionally the rank order of potency of EAA-induced neurotoxicity is markedly different between adult and developing CNS. The ability of specific EAA agonists to produce neuronal damage in adult rat CNS is KA > NMDA $\geq$ QA in contrast to the order of NMDA > QA > KA in 7-day-old rat CNS[2,5,11,19,25,32]. Interestingly, quinolinate, an endogenous NMDA receptor neuromodulator, is neurotoxic in the adult brain like NMDA, however it is not neurotoxic in the immature brain[8] which is highly susceptible to NMDA-induced damage.

Furthermore, the receptor binding characteristics of EAA recognition sites are altered during development. There is a transient developmental expression of QA-preferring glutamate receptors in the immature brain[10]. In addition, the immature brain expresses an uncharacterized fourth glutamate binding site (unpublished data). The enhanced susceptibility of the immature brain to NMDA neurotoxicity may also reflect alterations in NMDA receptor–ligand interaction, receptor–channel coupling, allosteric modulation (e.g. by glycine), or in the biochemical cascade leading to the neuronal damage that may follow excessive NMDA receptor activation.

Electrophysiological studies suggest that there is considerable shift in the effectiveness of NMDA receptors during development. NMDA receptor antagonists block visual responses of cortical neurons much more effectively in kittens that they do in the adult cat[30]. Neuronal development in the immature brain is exquisitely sensitive to the effects of NMDA antagonists. Visual ocular dominance column formation in the kitten can be disrupted and reversed by a 1 hour exposure to the non-competitive NMDA antagonist ketamine[21]. Moreover, competitive NMDA antagonists produce a similar disruption of developmental visual plasticity[3,12].

Congenital or acquired alterations in the NMDA receptor/channel complex may predispose the immature CNS to damage. Several studies have linked hypoxic–ischemic injury to activation of NMDA receptors[14,22] and these results suggest that this mechanism is extremely active during development. The susceptibility of the immature brain to NMDA toxicity offers a sensitive and rapid in vivo model for exploring these issues and for screening prospective neuroprotective drugs acting at the NMDA receptor–channel complex.

Supported by USPHS Grants 1P01NS19613 (M.V.J.), MSTP Grant 5 T32 6M07863-07 (J.W.M.), 1K08NS01171 (F.S.S.), Grant R-326 from the United Cerebral Palsy Research and Education Foundation (M.V.J.) and a grant from the Epilepsy Foundation of America (F.S.S.). MK-801 was a gift of Dr. P. Anderson, Merck, Sharp and Dohme, West Point, PA.

1 Balcar, V.J. and Johnston, G.A.R., The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices, *J. Neurochem.*, 19 (1972) 2657–2666.

2 Campochiaro, P. and Coyle, J.T., Ontogenic development of kainate neurotoxicity: correlates with glutamatergic innervation, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 2025–2029.

3 Cline, H.T., Deloski, E.A. and Constantine-Paton, M., N-methyl-D-aspartate receptor antagonist desegregates eye-specific stripes, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 4342–4345.

4 Cook, T.M. and Crutcher, K.A., Intrahippocampal injection of kainic acid produces significant pyramidal cell loss in neonatal rats, *Neuroscience*, 18 (1986) 79–92.

5 Coyle, J.T., Excitatory amino acid neurotoxins. In L.L. Iversen, S.D. Iversen and S.H. Snyder, Eds., *Handbook of Psychopharmacology, Vol. 15*, Plenum, New York, (1982) 237–269.

6 Coyle, J.T. and Schwarcz, R., Lesion of striatal neurones with kainic acid provides a model for Huntingtons chorea, *Nature (Lond.)*, 263 (1976) 244–246.

7 Fagg, G.E. and Foster, A.C., Amino acid neurotransmitters and their pathways in the mammalian central nervous system, *Neuroscience*, 9 (1983) 701–719.

8 Foster, A.C., Collins, J.F. and Schwarcz, R., On the excitotoxic properties of quinolinic acid, 2-3-piperidine dicarboxylic acids and structurally related compounds, *Neuropharmacology*, 22: 12A (1983) 1331–1342.

9 Fuxe, K., Roberts, P. and Schwarcz, R., Eds., *Excitotoxins*, Plenum, New York (1983).

10 Greenamyre, T., Penney, J.B., Young, A.B., Hudson, C., Silverstein, F.S. and Johnston, M.V., Evidence for transient perinatal glutamatergic innervation of globus pallidus, *J. Neurosci.*, 7:4 (1987) 1022–1030.

11 Hasting, M.H., Winn, P. and Dunnett, S.B., Neurotoxic amino acid lesions of the lateral hypothalamus: a parametric comparison of the effects of ibotenate, N-methyl-D,L-aspartate and quisqualate in the rat, *Brain Research*, 360 (1985) 248–256.

12 Kleinschmidt, A., Bear, M.F. and Singer, W., Blockade of 'NMDA' receptors disrupts experience-dependent plasticity of kitten striate cortex, *Science*, 238 (1987) 355–358.

13 McDonald, J.W., Silverstein, F.S., Cardona, D., Uckele, J., Chen, R. and Johnston, M.V., Neuroprotective effects of MK-801 and other compounds on perinatal hypoxic-ischemic injury. In E. Domino (Ed.), *Sigma Opioid-PCP-Like Compounds as Molecular Probes in Biology*, NPP Press, Ann Arbor, in press.

14 McDonald, J.W., Silverstein, F.S. and Johnston, M.V., MK-801 protects the neonatal brain from hypoxic-ischemic damage, *Eur. J. Pharmacol.*, 140 (1987) 359–361.

15 McLennan, H., On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system, *Adv. Biochem. Psychopharmacol.*, 27 (1981) 253–262.

16 Nicoletti, F., Iadorula, M.J., Wroblewski, J.T. and Costa, E., Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism. Developmental changes and interaction with alpha$_1$-adrenoreceptors, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 1931–1935.

17 Olney, J.W., Excitotoxins: an overview. In K. Fuxe, P. Roberts and R. Schwarcz (Eds.), *Excitotoxins*, Plenum, New York, 1983, pp. 82–96.

18 Olney, J.W., Ho, O.L. and Rhee, V., Cytotoxic effects of acidic and sulfure containing amino acids on the infant mouse central nervous system, *Exp. Brain Res.*, 14 (1971) 61–76.

19 Peterson, G.M. and Moore, R.Y., Selective effects of kainic acid on diencephalic neurons, *Brain Research*, 202 (1980) 165–182.

20 Raichle, M.E., The pathophysiology of brain ischemia, *Ann. Neurol.*, 13 (1983) 2–10.

21 Rauschecker, J.P. and Hahn, S., Ketamine–xylazine anaesthesia blocks consolidation of ocular dominance changes in kitten visual cortex, *Nature (Lond.)*, 326 (1987) 183–185.

22 Rothman, S. and Olney, J., Glutamate and the pathophysiology of hypoxic–ischemic brain damage, *Ann. Neurol.*, 19 (1986) 105–111.

23 Schwarcz, R., Whetsell Jr., W.O. and Mangano, R.M., Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain, *Science*, 219 (1983) 316–318.

24 Siesjo, B.K., Cell damage in the brain: a speculative synthesis, *J. Cereb. Blood Flow Metab.*, 1 (1981) 155–185.

25 Silverstein, F.S., Chen, R.C. and Johnston, M.V., The glutamate agonist quisqualic acid is neurotoxic in striatum and hippocampus of immature rat brain, *Neurosci. Lett.*, 71 (1986) 13–18.

26 Skerritt, J.H. and Johnston, G.A.R., Uptake and release of *N*-methyl-D-aspartate by rat brain slices, *J. Neurochem.*, 36 (1981) 881–885.

27 Smith, K.L. and Swann, J.W., Carbamazepine suppresses synchronized afterdischarges in disinhibited immature rat hippocampus in vitro, *Brain Research*, 400 (1987) 371–376.

28 Steiner, H.X., McBean, G.J., Kohler, C., Roberts, P.J. and Schwarcz, R., Ibotenate-induced neuronal degeneration in immature rat brain, *Brain Research*, 307 (1984) 117–124.

29 Stewart, G.R., Price, M., Olney, J., Hartman, B.K. and Cozzari, C., *N*-Methylaspartate: an effective tool for lesioning basal forebrain cholinergic neurons of the rat, *Brain Research*, 369 (1986) 377–382.

30 Tsumoto, T., Hagihara, K., Sato, H. and Hata, Y., NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats, *Nature (Lond.)*, 327 (1987) 513–514.

31 Watkins, J.C. and Evans, R.H., Excitatory amino acid neurotransmitters, *Annu. Rev. Pharmacol. Toxicol.*, 21 (1981) 165–204.

32 Zaczek, R. and Coyle, J.T., Excitatory amino acid analogues: neurotoxicity and seizures, *Neuropharmacology*, 21 (1982) 15–26.