Journal of Neurochemistry
Raven Press, New York

Perinatal Hypoxia-Ischemia Disrupts Striatal High-Affinity [^{3}H]Glutamate Uptake into Synaptosomes

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Abstract: We examined the impact of hypoxia-ischemia on high-affinity [^{3}H]glutamate uptake into a synaptosomal fraction prepared from immature rat corpus striatum. In 7-day-old pups the right carotid artery was ligated, and pups were exposed to 8% oxygen for 0, 0.5, 1, or 2.5 h, and allowed to recover for up to 24 h before they were killed. High-affinity glutamate uptakes in striatal synaptosomes derived from tissue ipsilateral and contralateral to ligation were compared. After 1 h of hypoxia plus ischemia, high-affinity glutamate uptake in the striatum was reduced by 54 ± 13% compared with values from the opposite (nonischemic) side of the brain ($p < 0.01$, *t* test versus ligates not exposed to hypoxia). There were similar declines after 2.5 h of hypoxia-ischemia. Activity remained low after a 1 h recovery period in room air, but after 24 h of recovery, high-affinity glutamate uptake was equal bilaterally. Kinetic analysis revealed that loss of activity could be attributed primarily to a 40% reduction in the number of uptake sites. Hypoxia alone had no effect on high-affinity glutamate uptake although it reduced synaptosomal uptake of [^{3}H]3,4-dihydroxyphenylethylamine. Addition of 1 mg/ml of bovine serum albumin to the incubation medium preferentially stimulated high-affinity glutamate uptake in hypoxic-ischemic brain compared with its effects in normal tissue. These studies demonstrate that hypoxia-ischemia reversibly inhibits high-affinity glutamate uptake and this occurs earlier than the time required to produce neuronal damage in the model. **Key Words:** Perinatal — Ischemic — Brain — Injury — Glutamate—Uptake. **Silverstein F. S. et al.** Perinatal hypoxia-ischemia disrupts striatal high-affinity [^{3}H]glutamate uptake into synaptosomes. *J. Neurochem.* **47,** 1614–1619 (1986).

Recent studies in experimental models of ischemic brain injury indicate that neurotransmitters may play an important role in the pathogenesis of irreversible neuronal damage (Meldrum, 1985; Rothman and Olney, 1986). Increased neuronal excitability induced by ischemia and membrane depolarization could both stimulate neurotransmitter release. Several lines of investigation have suggested that enhanced release of excitatory amino acid neurotransmitters such as glutamate may contribute to neuronal damage.

For glutamate and related compounds that are not degraded in the synaptic cleft, reuptake by presynaptic nerve terminals and glia is important for terminating the excitatory effects of released transmitter (Fonnum, 1984). Failure of reuptake could significantly augment the impact of released neurotransmitters. Specific high-affinity sodium-dependent glutamate uptake activity is normally linked to neuronal firing rates so that when glutamate release increases, uptake increases concurrently (Nieoullon et al., 1983). However, ischemia may inhibit specific uptake mechanisms even though transmitter release rises.

In experimental animals, and in neuronal and glial tissue cultures, ischemia and/or hypoxia inactivates sodium-dependent uptake of several neurotransmitters (Pastuszko et al., 1982). In mature animal stroke models, declines in uptake activity have been interpreted to be sequelae of ischemic injury and to reflect irreversible damage to nerve terminals (Weinberger and Cohen, 1982; Strong et al., 1983). However, depletion of high-energy phosphates, accumulation of toxic metabolites, or the effects of endogenous regulatory factors could produce functional but reversible uptake failure. For example, in vitro, accumulation of free fatty acids inhibits synaptosomal amino acid uptake, and addition of albumin, which binds fatty acids, can reverse this effect (Rhoads et al., 1982).

In this study we examined the impact of hypoxia-

Received March 21, 1986; revised May 27, 1986; accepted June 2, 1986.

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Abbreviations used: BSA, bovine serum albumin; dopamine, 3,4-dihydroxyphenylethylamine.

ischemia on high-affinity glutamate uptake in the immature rat brain. In immature animals, mechanisms of brain injury may differ in some respects from responses to similar insults in mature animals. Differences in local metabolic rates, maturity of specific enzyme systems, and complexity of synaptic connections could all affect the evolution of neuronal damage. We used a well-characterized small-animal model for perinatal hypoxic-ischemic encephalopathy (Rice et al., 1981) to learn more about the role of alterations in high-affinity glutamate uptake in the pathogenesis of neuronal injury in this setting.

Unilateral carotid artery ligation and subsequent timed exposure to 8% O_2 in 7-day-old rat pups leads to progressive ischemia in striatum and fronto-parietal cortex ipsilateral to ligation (70% reduction in perfusion after 2 h of hypoxia) and produces a relatively predictable pattern of ischemic neuronal injury limited to forebrain, unilaterally (Silverstein and Johnston, 1984; Johnston, 1983). There is a time threshold (1.5 h) for duration of hypoxic exposure that elicits permanent morphologic damage (Silverstein and Johnston, 1984).

We studied high-affinity glutamate uptake in the striatum, which in adult animals has a dense glutamatergic innervation, because it is a major target area for injury in this animal model. We examined the relationship between duration of hypoxic-ischemic exposure and severity of disruption of high-affinity glutamate uptake and we assessed factors that contributed to the recovery of uptake activity.

EXPERIMENTAL PROCEDURES

Animal preparation

In 7-day-old Sprague-Dawley rat pups, anesthetized with ether, the right common carotid artery was ligated as previously described by Johnston (1983). Pups were returned to the dam to feed for 1 h and were then placed in plastic chambers warmed to 37°C for 2.5 h. Pups were exposed to 8% O_2, 92% N_2 environment for 0, 0.5, 1, or 2.5 h. Pups exposed to either 0 or 0.5 h of hypoxia were placed in the chambers, separate from the dam, for at least 1 h. After hypoxic exposure animals were allowed to recover for up to 24 h. Either immediately after hypoxic exposure or 1–24 h later, animals were killed by decapitation and striata (caudate, putamen, and globus pallidus) were quickly dissected out on ice. Each experiment included 10–14 ligates and two control pups. In a single experiment all ligates were exposed to the same duration of hypoxia, and allowed to recover for the same time interval before they were killed. Corresponding striata from two or three pups were pooled for each sample.

High-affinity glutamate uptake assays

Well-established methods were used to measure sodium-dependent high-affinity glutamate uptake (Bennett et al., 1973). Tissue and reagents were kept on ice. Striatal tissue (two or three striata per sample) was homogenized 1:20 in 0.3 *M* sucrose. The P2 fraction was isolated by repeated centrifugation and resuspended in cold sucrose. Three 25-μl aliquots of the synaptosome pellet (duplicate samples and blank) were incubated at 37°C in 950 μl of Krebs-Ringer phosphate buffer, pH 7.4. The buffer for the experimental samples contained NaCl. Choline chloride was substituted in the buffer for blanks. After a 10-min preincubation, 20 μl (1 mCi/ml) of [^{3}H]glutamate (39 Ci/mmol, Amersham) was added; the final glutamate concentration was 0.5 μM. Four minutes later the incubation was terminated by addition of excess cold sodium-deficient buffer. The suspension was recentrifuged at 20,000 *g* at 5°C for 15 min and the pellet was washed, dissolved in tissue solubilizer, and accumulated radioactivity was counted. To calculate high-affinity glutamate uptake, counts (dpm) in each blank (no sodium) were subtracted from total counts in the experimental sample. The protein concentration in an aliquot of the synaptosomal suspension was assayed (Bradford, 1976). Specific uptake per milligram of protein or tissue was calculated.

In pilot studies, we found no side-to-side differences in striatal high-affinity glutamate uptake in untreated 7-day-old pups or in pups exposed to 2 h of 8% O_2 and that glutamate uptake after hypoxic exposure was no different from control values. However, there was significant interassay variation in absolute values for glutamate uptake. Therefore, in the first set of experiments, to incorporate results from multiple assays, high-affinity glutamate uptake in striatum ipsilateral to ligation was expressed as a fraction of uptake activity in the contralateral striatum.

In the second set of experiments, for kinetic analysis of high-affinity glutamate uptake ipsilateral and contralateral to ligation after 2.5 h of hypoxia, uptake was compared bilaterally using seven concentrations of glutamate (0.06–6 μM) with the same specific activity.

In the third set of experiments, the effects of addition of 1 mg/ml of bovine serum albumin (BSA) to the incubation medium were assessed in ischemic tissue and in controls. In three experiments, synaptosomes prepared from untreated 7-day-old pups and ligates exposed to 2.5 h of 8% O_2 were compared. For each sample, high-affinity glutamate uptake was assayed in four incubation media: +BSA, +Na; +BSA, −Na; −BSA, +Na; −BSA, −Na. Specific BSA-stimulated high-affinity glutamate uptake was calculated by subtracting counts in sodium-deficient blanks (i.e., [+Na, +BSA] − [−Na, +BSA]). For each experimental sample, the effect of albumin was calculated by comparison of values for specific BSA-stimulated high-affinity glutamate uptake and values for sodium-dependent high-affinity glutamate uptake in the absence of BSA.

In the fourth set of experiments, to assess the specificity of alterations in high-affinity glutamate uptake activity observed with hypoxia-ischemia, striatal [^{3}H]3,4-dihydroxyphenylethylamine ([^{3}H]dopamine) uptake was assayed in tissue from 7-day-old rat pups after hypoxia alone, and unilateral carotid ligation followed by exposure to 8% O_2. Similar methods were used to measure [^{3}H]dopamine uptake. Each aliquot of synaptosomes was suspended in Krebs buffer, pH 7.4, and equilibrated at 37°C; for blanks, 10 μM benztropine was included. Then [^{3}H]dopamine (1 mCi/ml) was added (1 μCi/assay, final concentration of dopamine 5×10^{-8} *M*) and after 5 min the reaction was terminated. The pellet was isolated by centrifugation and radioactivity per milligram of tissue was calculated.

Statistical analysis

In the first and fourth set of experiments, for each time point, striatal high-affinity glutamate uptake ipsilateral to ligation was expressed as a percent ± SD of uptake activity

in the contralateral striatum. Percent change at each time point was compared with values at "time 0" with one-tailed t tests. In the second set of experiments Lineweaver-Burk plots were constructed using linear regression analysis. The maximum number of binding sites, B_{max}, equaled $1/y$-intercept, and the affinity construct, K_D, equaled $-1/x$-intercept. In the third group of experiments, albumin's effects on uptake were compared in control and ischemic striatum with a one-tailed t test.

RESULTS

In a single uptake experiment, there is little intraanimal and interanimal variation (results not shown) in striatal high-affinity glutamate uptake. However, there is often significant interassay variation in absolute values for high-affinity glutamate uptake. To incorporate results from multiple experiments, striatal high-affinity glutamate uptake ipsilateral to ligation was expressed as a fraction of the value in the contralateral striatum (mean ± SEM).

Figure 1 demonstrates the reversible suppression of striatal high-affinity glutamate uptake that evolves after unilateral carotid artery ligation and timed exposure to 8% O_2. Each bar represents high-affinity glutamate uptake ipsilateral to ligation expressed as a percent of uptake in the contralateral striatum (n = 5–8, at each point). In unoperated animals, exposure to 2 h of 8% O_2 does not alter high-affinity glutamate uptake (data not shown). Carotid ligation alone had no effect on uptake activity nor did exposure to 0.5 h of 8% O_2. In contrast, after 1 h of hypoxic exposure, there was a significant reduction in high-affinity glutamate uptake ipsilateral to ligation (−54 ± 13%, $p < 0.01$, t test for comparison with values from ligates not exposed to hypoxia). High-affinity glutamate uptake activity in ischemic striatum remained depressed to a similar degree after 2.5 h. After a 1-h recovery period in room air, high-affinity glutamate uptake was still suppressed (−42 ± 18%, $p < 0.05$, t test). However, after a 24-h recovery period, high-affinity glutamate uptake activity was restored and was almost equal bilaterally (95 ± 14%). At 24 h, values were in the same range as for untreated controls (data not shown). As well, calculation of uptake activity per milligram of tissue (rather than per milligram of protein) yielded similar results (data not shown).

Figure 2 compares high-affinity glutamate uptake at multiple substrate concentrations (Lineweaver-Burk plot) in striatal synaptosomes derived from each hemisphere after right carotid ligation and 2.5 h of 8% O_2. The points are the observed values and the lines were calculated by regression analysis. The results of a single experiment are plotted and the number of binding sites and the rates of the uptake reactions are derived from the x- and y-intercepts. The calculated number of uptake sites (V_{max}) in synaptosomes derived from ischemic striatum is decreased by 40% compared with contralateral striatum. However, the calculated affinity constant (K_m) for the reaction in ischemic tissue is slightly lower than on the opposite side (i.e., higher affinity). Thus, loss of uptake sites appears to be the major factor contributing to loss of high-affinity glutamate uptake in ischemic synaptosomes. Similar results were obtained in two other experiments.

Table 1 compares the extent of stimulation of high-affinity glutamate uptake produced by addition of albumin to the incubation medium in control and hypoxic-ischemic striatum. In tissue from control, untreated 7-day-old animals, addition of 1 mg/ml of BSA to the incubation buffer increased high-affinity glutamate uptake by 51 ± 12.7% (mean ± SEM, n = 7). In hypoxic-ischemic synaptosomes used for this group of experiments, high-affinity glutamate uptake was reduced by 36 ± 7.4% (n = 5) compared with values from the corresponding contralateral striatum. Using aliquots from these synaptosome preparations, assays were repeated in the presence of 1 mg/ml of BSA. Addition of BSA markedly stimulated high-affinity glutamate uptake (+193 ± 62.5%, n = 5, p

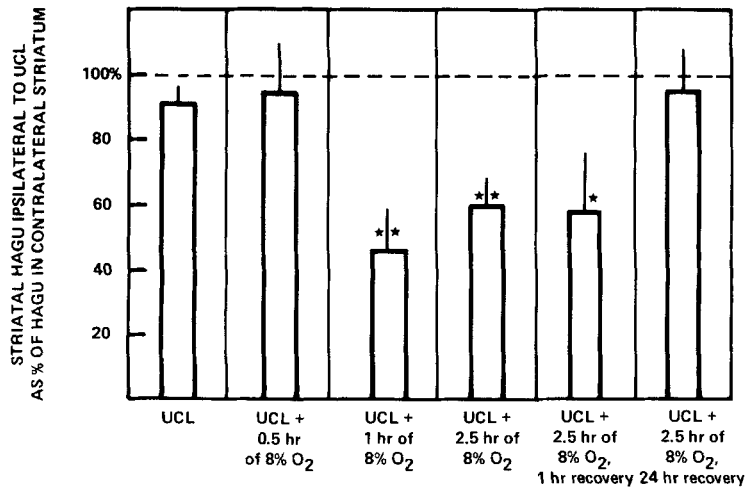

FIG. 1. Bilateral striatal high-affinity glutamate uptake was measured immediately after unilateral carotid ligation (UCL) and subsequent timed exposure to 0, 0.5, 1, or 2.5 h of 8% O_2. Striata from ligates exposed to 2.5 h of 8% O_2 and allowed to recover in room air, with dam for 1 h or 24 h before they were killed, were also assayed. In these studies striatal glutamate uptake ipsilateral to ligation was expressed as a fraction of uptake in the contralateral striatum (mean ± SEM, n = 5–8). After 1 h, glutamate uptake ipsilateral to UCL decreased markedly ($p < 0.01$, t test for comparison with values at time 0), and remained depressed to the same degree after 2.5 h. There was considerable functional recovery 24 h later. **$p < 0.025$; *$p < 0.05$.

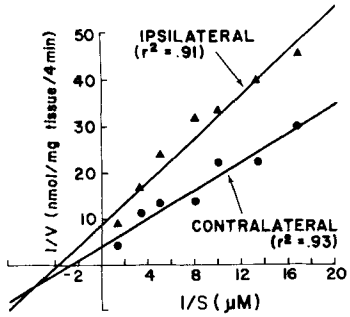

FIG. 2. Lineweaver-Burk plot for kinetic analysis of high-affinity glutamate uptake. The number of binding sites and the rates of the uptake reactions were compared in striatal synaptosomes derived from each hemisphere after unilateral carotid ligation and 2.5 h of 8% O_2; "ipsilateral" indicates tissue derived from the side of ligation and "contralateral" indicates tissue from the opposite side. The calculated number of uptake sites, V_{max} (=1/*y*-intercept) in synaptosomes derived from ischemic striatum is decreased by 40%. The calculated affinity constant for the reaction, K_m (= −1/*x*-intercept) is slightly lower (i.e., higher affinity) in the ischemic tissue so that the loss of uptake sites appears to be the major factor in loss of high-affinity glutamate uptake.

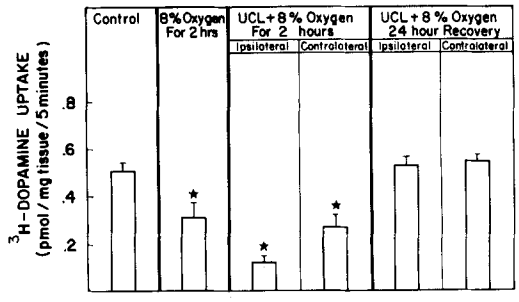

FIG. 3. Synaptosomal [^{3}H]dopamine uptake in 7-day-old rat pups. Controls received no treatment; "hypoxia" animals were exposed to 2 hours of 8% O_2; "ligates" refer to animals subjected to unilateral carotid ligation (UCL), allowed to recover, and then exposed to 8% O_2 for 2 h; "contralateral" indicates that tissue is from the side of the brain opposite the UCL, whereas "ipsilateral" tissues are those on the same side as UCL. Each bar (mean ± SEM; n = 6–9) represents results from at least two experiments. In each sample tissue from three striata were pooled. *p < 0.01.

= 0.025, *t* test for comparison of stimulation in ischemic striatum and controls). In tissue derived from the striatum contralateral to ligation, BSA stimulated high-affinity glutamate uptake to the same degree as in controls (+86 ± 23%, n = 5).

Figure 3 compares bilateral striatal [^{3}H]dopamine uptake in controls, 7-day-old pups exposed to 2 h of hypoxia, and pups with unilateral carotid ligation and 2 h of hypoxic exposure killed immediately or after a 24-h recovery period. Since there was little interassay variation in absolute values for [^{3}H]dopamine uptake per milligram of tissue, values from several experiments were combined. Exposure to hypoxia alone reduced uptake by 40% in comparison with untreated controls. Maximal depression of uptake (−76 ± 6%) occurred ipsilateral to carotid ligation after 2 h of 8% O_2. After a 24-h recovery period, uptake activity was normal bilaterally.

TABLE 1. *Stimulation of striatal high-affinity glutamate uptake by addition of BSA*[a]

Source of tissue	n (no. of samples)	Percent stimulation (mean ± SEM)[b]	Comparison percent stimulation versus controls[c]
Control striatum	7	151 ± 12.7	—
Striatum, ipsilateral to ligation	5	293 ± 62.5	p = 0.025
Striatum, contralateral to ligation	5	186 ± 32.2	NS

All tissue was obtained from 7-day-old rat pups. "Controls" received no treatment. In experimental animals, unilateral carotid ligation was followed by exposure to 2.5 h of 8% O_2 before the animals were killed. Striata were immediately dissected out, and synaptosomes were prepared as described in the text.

[a] BSA, 1 mg/ml, was added to the Krebs solution in which synaptosomes were suspended (see Experimental Procedures for details).

[b] Calculated as: (Na-dependent high-affinity glutamate uptake + BSA)/(Na-dependent high-affinity glutamate uptake − BSA).

[c] Student's *t* test used.

DISCUSSION

Several lines of evidence implicate the excitatory amino acid neurotransmitter glutamate in the pathogenesis of ischemic neuronal injury. Brain regions with a dense glutamatergic innervation and high concentration of specific glutamate receptors appear to be particularly vulnerable to ischemic neuronal injury (Jorgensen and Diemer, 1982; Meldrum, 1985; Rothman and Olney, 1986). Direct local injection of the glutamate antagonist 2-amino-7-phosphonoheptanoic acid prevented acute ischemic neuronal damage in hippocampus of experimental animals (Simon et al., 1984). Using in vivo microdialysis in hippocampus, several studies have demonstrated increased levels of glutamate and aspartate in the extracellular fluid during ischemia (Benveniste et al., 1984; Hagberg et al., 1985). As well, in tissue culture, release of excitatory neurotransmitters appears to mediate anoxic cell death (Rothman, 1984).

Neuronal and glial reuptake remove released glutamate from the synaptic cleft (Fonnum, 1984). Inhibition of uptake mechanisms could contribute to accumulation of glutamate at the synapse and amplify the effects of released glutamate. The lack of toxicity of intracerebroventricular injections of glutamate is attributed to the efficiency of uptake processes. After lesions of corticostriatal tracts, intrastriatal injections of glutamate produce neuronal damage (Kohler and Schwarcz, 1981). Similarly, direct local injection of the specific glutamate uptake inhibitor *threo*-3-hydroxyaspartate into striatum leads to neuronal necrosis (McBean and Roberts, 1985). Normally, neuronal

reuptake activity increases with increased neuronal firing rates (Nieoullon et al., 1983). In vitro, under conditions simulating some of the biochemical features of hypoxia-ischemia, glutamate uptake is inhibited (Pastuszko et al., 1982; Hauptman et al., 1984) or fails to increase with stimulation; these results suggested that with ischemia specific neuronal defense mechanisms may be impaired (Drejer et al., 1985). Hagberg et al. (1985) found a marked accumulation of both excitatory and inhibitory amino acid neurotransmitters in hippocampus during ischemia. Although it is possible that these neurotransmitters are all released in excess, it is also conceivable that failure of uptake systems accounts for this pattern.

Our data demonstrate reversible inhibition of striatal high-affinity glutamate uptake in this experimental model of perinatal hypoxic-ischemic brain injury. In a previous study, we showed that there is a time threshold at approximately 1.5 h of hypoxic exposure after carotid ligation when morphologic damage occurs ipsilateral to ligation. This coincided with the time necessary to produce an acute increase in striatal dopamine metabolism (depletion in endogenous dopamine and concurrent accumulation of homovanillic acid) (Silverstein and Johnston, 1984). In contrast, inhibition of glutamate uptake occurs earlier; uptake is reduced by >50% after 1 h of hypoxia. This feature of the time course has several implications.

First, it suggests that failure of uptake does not reflect irreversible neuronal damage. Since each animal can be examined only at a single time point, it is impossible to prove that in the animals studied after the 24-h recovery period uptake activity was inhibited earlier during hypoxia-ischemia. However, the preparation is quite consistent, each set of results incorporated assays from two separate experiments, and the results were found for both transmitter uptake systems assayed. In adult stroke models, loss of specific glutamate, dopamine, and γ-aminobutyric acid (GABA) uptake activity, of varying severity, have been noted but the patterns differ from those in our study (Weinberger and Cohen, 1982; Strong et al., 1983). For example, in gerbils after unilateral carotid ligation, there was an interval of 16 h after the insult before onset of uptake inhibition; this time lag was interpreted as an indication that loss of uptake reflected destruction of nerve terminals. And no studies in mature animals have reported recovery of neurotransmitter uptake activity after a period of inhibition. The second implication of the time course data is that uptake failure in this setting occurs relatively early and could contribute to the evolution of neuronal injury by augmenting the effects of released glutamate.

However, the neurotoxic potential of glutamate in immature animals has been questioned. Kainate, a rigid analogue of glutamate, does not cause neuronal damage when injected into striatum of 7-day-old rat pups (Campochiaro and Coyle, 1977); nor does injection of the glutamate uptake inhibitor *threo*-3-hydroxy-aspartate cause damage at this age (McBean and Roberts, 1985). These results have been interpreted as reflecting the immaturity of corticostriatal glutamatergic innervation at this developmental stage. However, high-affinity glutamate uptake can consistently be measured in striatum of 7-day-old rat pups, and there is a high density of postsynaptic glutamate receptors in striatum at this age (Greenamyre et al., 1984). It is possible that developmental differences in receptor pharmacology account for the resistance to compounds such as kainate, but pre- and postsynaptic markers of glutamatergic synapses are well-established in these immature animals.

The data demonstrated reversibility of glutamate uptake inhibition both in vivo and in vitro and the kinetic analysis indicated that hypoxia-ischemia reduced the number of functional recognition or binding sites for glutamate (and/or sodium). Many changes in the synaptic microenvironment develop during the evolution of ischemic brain injury. Factors that could contribute to loss of functional activity include depletion of metabolic substrates required to maintain membrane integrity, membrane depolarization secondary to K^+ and Ca^{2+} fluxes, accumulation of toxic metabolites, structural damage to recognition or carrier sites, or destruction of the nerve terminal. However, incubation of ischemic tissue in a solution with physiologic concentrations of substrates and ions, as was done in these experiments, might be expected to correct reversible defects related to substrate depletion or ionic shifts.

In contrast, addition of albumin overcame the inhibition of uptake in ischemic tissue. Rhoads et al. (1982) attributed the stimulatory effect of BSA on amino acid uptake to binding of free fatty acids. Free fatty acids accumulate as the integrity of the cell membrane is disrupted by ischemia. It is possible that they are not washed from the synaptosome membranes during routine tissue preparation. Free fatty acids may affect glutamate or sodium binding directly, or diminish the Na^+ gradient that drives the uptake reaction (Rhoads et al., 1982). The preferential stimulation by BSA of ischemic tissue suggests that removal of such compounds could contribute to the restoration of normal high-affinity glutamate uptake activity in ischemic synaptosomes. As well, if accumulation of a toxic metabolite during ischemia was responsible for loss of uptake, this would be consistent with the subsequent late recovery of uptake activity.

There is little information about neurochemical regulation of neurotransmitter uptake activity in immature animals (Coyle, 1977; Johnston, 1984). Studies in mature animals have demonstrated that several neurotransmitters affect glutamate uptake rates. There are dopaminergic receptors on corticostriatal glutamatergic nerve terminals, and in mature animals, dopamine inhibits high-affinity glutamate uptake. Previously, we have shown that striatal dopamine release increases acutely with hypoxia-ischemia. Stimulation of dopamine receptors on presynaptic

glutamate nerve terminals could also contribute to the decrease in glutamate uptake as has been described in vitro (Nieoullon et al., 1983).

The pattern of inhibition of striatal dopamine uptake was similar to that observed for glutamate uptake. There was a marked decline in dopamine uptake ipsilateral to ligation and activity was restored 24 h later. In contrast to high-affinity glutamate uptake, hypoxia alone also inhibited dopamine reuptake into synaptosomes, but to a lesser degree. This differential sensitivity of neurotransmitter uptake systems to hypoxia has also been noted in vitro (Pastuszko et al., 1982). The basis for such variations in susceptibility are unknown but this observation provides additional evidence to suggest that specific neurotransmitter uptake inhibition does not necessarily reflect global neuronal membrane damage. The physiologic consequences of inhibition of dopamine uptake are unknown since released dopamine is degraded to homovanillic acid (even during hypoxia).

Clinical experience and experimental data suggest that neuronal injury is progressive after an acute ischemic event and that there may be a time period during which intervention could prove beneficial. If uptake inhibition can be prevented or reversed more quickly, the extent of ischemia-induced neuronal damage may be decreased. A better understanding of the neurobiology of these synaptic events could provide a basis for important new specific drug therapies.

Acknowledgment: This work was supported by PHS Grants NS 00603 (M.V.J.) NS 17642, NS PO1 19613 (M.V.J.), Grant 326 from the United Cerebral Palsy Research and Education Foundation (M.V.J.), a grant from the Michigan Memorial Phoenix Project (F.S.S.) and a postdoctoral fellowship from the Medical Research Council of Canada (F.S.S.).

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