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Cerebral Synthesis and Release of Kynurenic Acid: An Endogenous Antagonist of Excitatory Amino Acid Receptors

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Excitatory amino acid (EAA)–mediated synaptic transmission is the most prevalent excitatory system within the mammalian brain. Activation of EAA receptors has been postulated to contribute to neuronal cell death in stroke, epilepsy, hypoglycemia, and Huntington's disease. Kynurenic acid is an endogenous substance that inhibits EAA receptors and may therefore influence important physiologic and pathologic processes. The release of intracerebrally synthesized kynurenic acid into the extracellular fluid (ECF), where it may act at EAA receptors, has not been established *in vivo*. We studied the synthesis and release of kynurenic acid in the rat striatum using intracerebral microdialysis coupled with high performance liquid chromatography and fluorescence detection. The basal ECF concentration of kynurenic acid in the rat corpus striatum was 17.1 ± 1.1 nM. Peripheral administration of the immediate biosynthetic precursor of kynurenic acid, L-kynurenine, resulted in marked dose-dependent increases in striatal ECF concentrations of kynurenic acid, peaking at 2–2.5 hr. The highest dose of L-kynurenine (100 mg/kg), administered peripherally, resulted in a 108-fold increase in plasma kynurenic acid levels and a 37-fold increase in cerebral ECF levels. Peripheral administration of kynurenic acid, at a dose that caused plasma levels to increase 430-fold, resulted in only 4-fold increases in striatal ECF concentrations. The precursor responsiveness of striatal ECF kynurenic acid to peripherally infused L-kynurenine was blocked by the central application (via the dialysis probe) of aminooxyacetic acid, an inhibitor of the immediate synthetic enzyme for kynurenic acid, kynurenine aminotransferase. Administration of L-tryptophan was less effective than L-kynurenine in increasing ECF kynurenic acid concentrations and did so at a considerably later time interval (6 hr). Infusion of L-kynurenine, but not L-tryptophan, through the dialysis probe dramatically increased striatal ECF concentrations of kynurenic acid. Administration of glutamate, NMDA, quinolinic acid, kainic acid, or quisqualic acid via the dialysis probe for 10 min had no effect on basal ECF levels of kynurenic acid. The conclusions drawn from the present study are that (1) kynurenic acid is present in ECF within the CNS, (2) the CNS can synthesize kynurenic acid and release it into the extracellular space, (3) the majority of CNS kynurenic acid synthesis results from the transport of L-kynurenine across the blood–brain barrier, and (4) extracellular levels of kynurenic acid can be dramatically and selectively increased by pharmacologic manipulation of precursor levels.

As a broad-spectrum antagonist of excitatory amino acid (EAA) receptors, kynurenic acid can antagonize electrophysiological responses to NMDA and kainic acid (KA) receptor agonists, and to a lesser degree, quisqualate (QUIS) receptor agonists (Perkins and Stone, 1982, 1985; Ganong et al., 1983; Stone and Connick, 1985; Ganong and Cotman, 1986; Stone and Burton, 1988). Recent evidence suggests that kynurenic acid acts at both the glycine allosteric site and the agonist recognition site on the NMDA receptor complex, as well as at non-NMDA receptors (Birch et al., 1988a, b; Danysz et al., 1989). Interest in kynurenic acid centers around its possible involvement in several neurologic diseases with proposed excitotoxic etiologies (Schwarcz et al., 1984; Stone and Connick, 1985; Schwarcz and Ben-Ari, 1986; Choi, 1988; Dingledine et al., 1988; Stone, 1989), as well as its potential relevance for physiologic regulation of excitatory synaptic transmission mediated by endogenous EAAs (Forsythe et al., 1988; Foster and Kemp, 1989a; Mayer et al., 1989; Thomson et al., 1989).

Kynurenic acid was one of the first metabolites of tryptophan to be isolated and characterized in mammals (Ellinger, 1904; Homer, 1914); the intermediary role of L-kynurenine was subsequently demonstrated (Heidelberger et al., 1949; Fig. 1). Synthesis of kynurenic acid from L-kynurenine is catalyzed by kynurenine aminotransferase (EC 2.6.1.7), which has been extensively studied in mammalian liver, kidney, and intestine (Mason, 1954, 1957, 1959; Mason and Gullekson, 1960; Noguchi et al., 1973; Okuno et al., 1980) and has also been found in the rat brain (Minatogawa et al., 1974). Recently, the presence of kynurenic acid in the mammalian brain has been unequivocally demonstrated (Carla et al., 1988; Moroni et al., 1988a, b; Turski et al., 1988; Swartz et al., 1990). Using brain slice preparations *in vitro*, Turski and colleagues (1989) demonstrated the synthesis and release of kynurenic acid when L-kynurenine was added to the incubation media; however, no kynurenic acid could be found in the absence of added L-kynurenine (in all cases, ≥25 μM).

While it has been shown that brain tissue can catalyze the formation of kynurenic acid from L-kynurenine and that kynurenic acid is present in brain tissue, it remains unclear whether the CNS synthesizes and releases kynurenic acid *in vivo*. Kyn-

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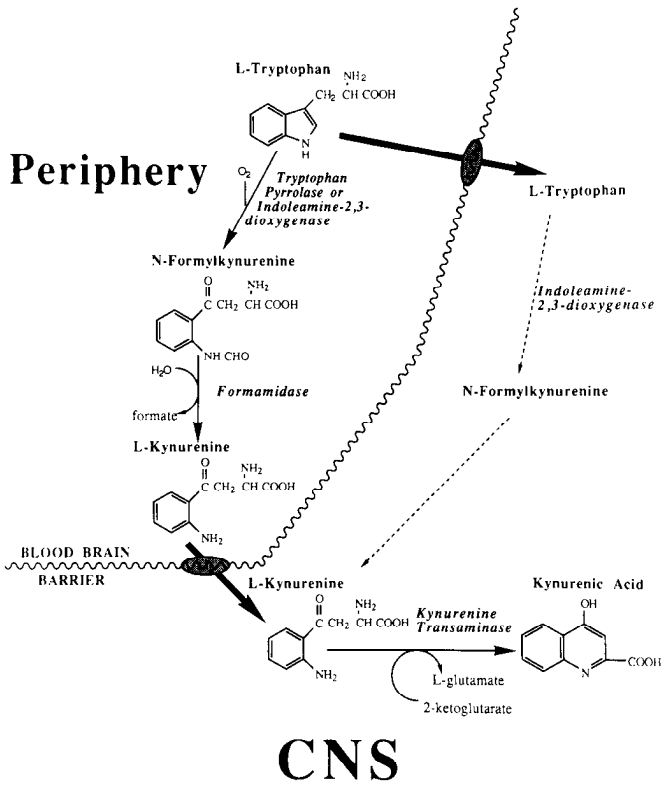

Figure 1. Proposed biochemical pathway for the metabolism of L-tryptophan to kynurenic acid. The diagram depicts the peripheral conversion of tryptophan to kynurenine, which enters the CNS on the large neutral amino acid transporter and is converted to kynurenic acid within the CNS (see Figs. 2–6). More than likely, only minimal tryptophan is converted to kynurenic acid within the CNS (see Fig. 4).

urenic acid found in the brain could originate from peripheral pools and then transfer into the brain from the blood. Furthermore, kynurenic acid synthesis from physiologically supplied precursors has not been demonstrated *in vivo* or *in vitro*. In this report, we used *in vivo* microdialysis coupled with a highly sensitive and specific assay to measure kynurenic acid in striatal extracellular fluid (ECF). Our objective was to establish the presence and origin of kynurenic acid in cerebral ECF and to determine the responsiveness of kynurenic acid to peripheral loading of precursors in a dynamic *in vivo* system composed of extracerebral metabolism, a blood–brain barrier, and CNS metabolism. The results presented in this report demonstrate that the brain synthesizes and releases kynurenic acid and that cerebral ECF levels of this neuroprotective agent can be manipulated pharmocologically.

Materials and Methods

Experimental animals. Male Sprague-Dawley rats (200–300 gm) were purchased from Charles River Laboratories (Wilmington, MA) and maintained under standard conditions with ad lib access to water and Rodent Lab Chow 5001 (Purina Mills Inc.) under a 12-hr dark/light cycle. All experiments were started between 7:00 and 8:00 A.M. Rats were anesthetized with freshly prepared chloral hydrate (350 mg/kg, i.p.), with small additional doses throughout experimentation to maintain stable anesthesia. Anesthetized rats were placed in a Kopf stereotaxic frame and maintained on a homeostatic heating pad at 37°C. Dialysis probes were implanted into anterior striata (either unilaterally or bilaterally) through small burr holes drilled in the skull at the following coordinates: 0.5 mm anterior to the bregma, 2.6 mm lateral to the midline, and 7.0 ventral to the skull surface (Paxinos and Watson, 1986). Accurate placement of probes was verified postmortem by gross and light microscopic visualization of coronal sections cut through and on either side of the probe path.

Microdialysis. The dialysis probes were of concentric design and had a 4-mm length of exposed membrane so the diffusion surface spanned the entire dorsoventral coordinates of the rat striatum. Probes were constructed using the design of Sandberg et al. (1986), with minor modifications. Hollow dialysis fibers (5-kDa cutoff; Cuprophan, Hospal, Edison, NJ) were sealed at 1 end with epoxy resin (Devcon, Danvers, MA). A length of hollow vitreous silica fiber (0.17-mm outer diameter; Anspec, MI) was inserted into the dialysis tube flush to the sealed end. The dialysis tubing with the vitreous silica fiber *in situ* was then inserted through a length of 23-gauge stainless-steel tubing into which another length of vitreous silica fiber (outlet) had been placed. Both ends of the 23-gauge tubing were sealed with epoxy resin with the dialysis membrane protruding from 1 end of the probe. The length of the exposed membrane surface was controlled by coating the dialysis membrane with epoxy resin. The probe was perfused using a Carnegie Medicin microperfusion pump (model CMA/100) via PE-50 tubing. The perfusate consisted of an artificial extracellular fluid (ECF) containing the following: 135 mM Na^+, 2.8 mM K^+, 1.0 mM Mg^{2+}, 1.2 mM Ca^{2+}, 200 μM ascorbic acid, and 2.0 mM phosphate at pH 7.4. The flow rate in all experiments was 2.0 μl/min, with fractions collected at 30-min (L-kynurenine studies) or 60-min (L-tryptophan studies) intervals. Probes were calibrated *in vitro* by placing them in standard solutions containing 20.0. and 200.0 nM authentic kynurenic acid and measuring the recovery of kynurenic acid in the dialysate. The *in vitro* recovery of all probes used (n = 10) was 11.3 ± 0.4% (mean ± SEM).

Analytical measurements of kynurenic acid. Kynurenic acid was measured using HPLC with fluorescence detection (Swartz et al., 1990). The HPLC system used for the analysis of kynurenic acid consisted of the following: pump A, Waters Model 510 Solvent Delivery System; pump B, Waters M45 Solvent Delivery System; a BIORAD Automatic Sampler (refrigerated) Model AS-48; a Waters 470 Scanning Fluorescence Detector; a Shimadzu C-R5A Chromatopac Integrator; and a BBC Goerz Metrawatt SE-120 chart recorder. The HPLC-fluorescence method utilized a mobile phase of 4.5% acetonitrile, 50 mM sodium acetate (pH, 6.20, adjusted with glacial acetic acid) pumped through an 8-cm HR-80, C-18, 3 u reverse-phase column (ESA Inc.) at a flow rate of 1.0 ml/min. Zinc acetate (0.5 M, not pH adjusted) was delivered post-column at a flow rate of 1.0 ml/min. The injection volume was 50 μl. The fluorescence detector was set at an excitation wavelength of 344 nm (18-nm band width) and an emission wavelength of 398 nm (18-nm band width). The retention time of kynurenic acid under these conditions was 4.3 min, with a sensitivity of 15 × 10^{-15} mol/injection (signal : noise ratio, 5). For dialysate measurements, the recovered dialysate (60 μl) was diluted 1:1 with fresh artificial ECF, and 50 μl of the mixture was directly injected onto the HPLC system. Aminooxyacetic acid (AOAA), L-kynurenine, and L-tryptophan did not interfere with the measurements of kynurenic acid when added to standard solutions or to ECF containing exogenous or endogenously synthesized kynurenic acid. Plasma kynurenic acid measurements were made using the same HPLC system, with minor modifications in sample preparation procedures. Freshly collected blood (200 μl) was centrifuged at 12,000 rpm for 10 min. The resulting plasma (50 μl) was separated off, diluted to 1.0 ml with 0.5 M $HClO_4$, and centrifuged at 12,000 rpm for 10 min to remove precipitated protein. Fifty μl of the resulting supernatant was directly injected onto the HPLC system.

Systemic precursor loading studies: effects on striatal ECF kynurenic acid. In several experiments, precursors (L-kynurenine or L-tryptophan) were administered peripherally while striatal ECF was sampled. Dialysis probes were implanted for a minimum of 1.5 hr before baseline fractions were collected. Three 30-min fractions were collected before any manipulation was attempted. L-Kynurenine sulfate and L-tryptophan (Sigma, St. Louis, MO) were dissolved in sterile 0.9% saline (pH, 7.4) and administered intraperitoneally in a vol of 3–4 ml/kg. One group of rats received either saline alone (n = 5) or L-kynurenine at doses of 100 mg/kg (n = 4), 75 mg/kg (n = 4), 50 mg/kg (n = 4), or 25 mg/kg (n = 2). The quantity of L-kynurenine sulfate given was calculated as the free base. Another group of rats received either saline alone (n = 4) or 100 mg/kg L-tryptophan (n = 5). Microdialysis was continued for up to 4 hr at 30 min intervals when L-kynurenine was given and for 10 hr at 1-hr intervals when L-tryptophan was given.

Cerebral precursor administration studies: effects on striatal ECF kynurenic acid. In order to determine which reactions of the kynurenine

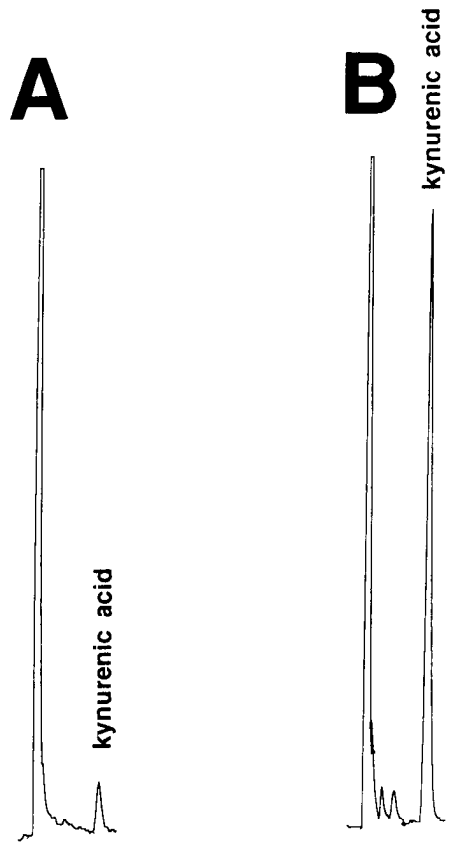

Figure 2. Chromatograms illustrating the measurement of kynurenic acid in rat striatal ECF. *A*, Sample representing basal kynurenic acid levels that was collected 2 hr after dialysis probe implantation. *B*, Sample collected 1 hr after peripheral administration of L-kynurenine (50 mg/kg).

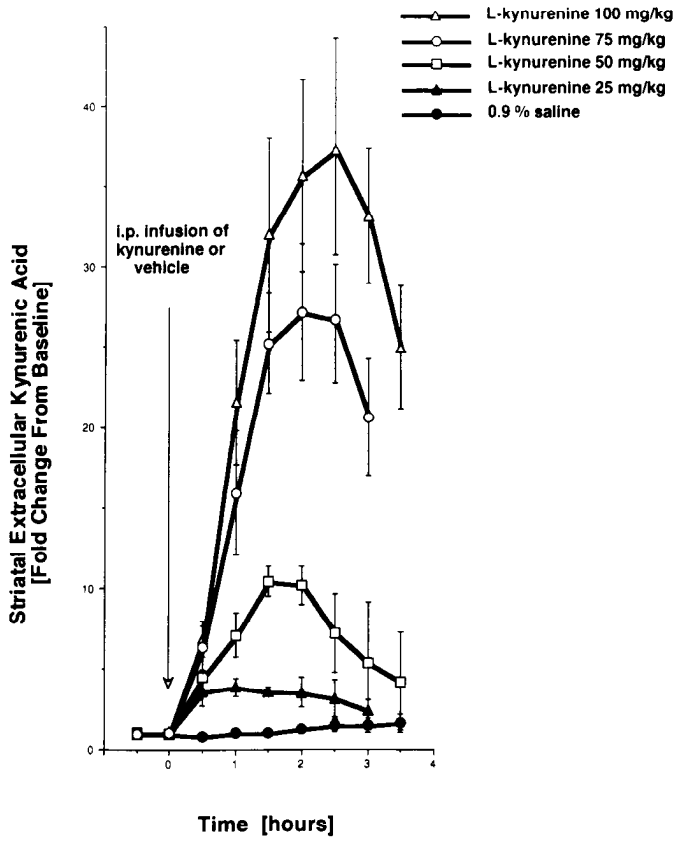

Figure 3. Striatal extracellular levels of kynurenic acid following peripheral (i.p.) infusion of saline or L-kynurenine at doses of either 25, 50, 75 or 100 mg/kg. The vertical axis represents the fold change in striatal concentrations of kynurenic acid, and the horizontal axis represents time (t) in hr. The peripheral infusion of L-kynurenine or vehicle occurred at t_0. Error bars represent SEM. All points after infusion of L-kynurenine were significantly different ($p < 0.01$) from baseline values by repeated measures ANOVA or from saline controls by Mann-Whitney *U* test (see Materials and Methods and Results).

pathway (leading to kynurenic acid) exist in the brain, we compared the ability of centrally applied precursors to increase striatal ECF concentrations of kynurenic acid. Dialysis probes were implanted into rat striata for a minimum of 1.5 hr before baseline fractions were collected. After 3 30-min fractions were collected to establish basal concentrations of kynurenic acid, artificial ECF containing either 100 μM L-kynurenine ($n = 7$) or 100 μM L-tryptophan ($n = 10$) was perfused through the dialysis probe for the next 2.5 hr (when L-kynurenine was administered) or 5 hr (when L-tryptophan was administered). Fractions were collected every 30 min, and kynurenic acid concentrations were determined.

Systemic precursor loading studies: effects on plasma kynurenic acid. We compared the induction of peripheral kynurenic acid formation after administration of L-kynurenine to the effects of L-kynurenine on intracerebral kynurenic acid synthesis. Rats were cannulated with intrafemoral catheters prior to implantation of dialysis probes. Three baseline plasma samples were taken from each rat and 6 dialysis samples were collected prior to any manipulation. One group of rats ($n = 3$) received an intraperitoneal infusion of the highest dose of L-kynurenine administered in the precursor ECF studies (100 mg/kg). Subsequent measurements of plasma kynurenic acid determined the magnitude of the increase in plasma kynurenic acid. In a separate group of rats ($n = 3$), kynurenic acid (10 mg/kg) was given intraperitoneally to cause an increase in plasma kynurenic acid of a greater magnitude than that observed with L-kynurenine administration. In both groups, simultaneous striatal ECF was monitored using microdialysis.

Enzyme inhibition studies. In order to establish that the kynurenic acid measured in striatal ECF was synthesized in the CNS rather than transported into the CNS from the periphery, we examined the ability of AOAA, an established inhibitor of kynurenine transaminase, to inhibit the kynurenic acid surge produced by peripheral infusion of L-kynurenine (Metcalf, 1979; Turski et al., 1989). We designed a model with internal controls to negate the effects of variable L-kynurenine absorption from the periphery. Dialysis probes were implanted bilaterally into the striata of 5 rats. Initially, both probes were infused with artificial ECF. After 1.5 hr of prebaseline perfusion, 3 consecutive 30 min baseline fractions were collected from each probe. After the last fraction was collected, artificial ECF containing either 1.0 mM ($n = 3$ rats) or 2.0 mM ($n = 2$ rats) AOAA was perfused through one probe, while the contralateral probe was perfused with artificial ECF alone as a control. Both probes were perfused for 1.5 hr (3 consecutive 30-min fractions). After the last fraction was collected, L-kynurenine (75 mg/kg) was administered peripherally via an intraperitoneal route. Dialysis fractions were then collected at 30 min intervals for the next 3.0 hr. Ipsilateral (AOAA-perfused) and contralateral (control) fractions were analyzed for kynurenic acid.

Striatal release studies: effects of excitatory amino acids. The effects of various EAAs on kynurenic acid release into striatal ECF was examined. Glutamate, KA, quisqualic acid, NMDA, and quinolinic acid were dissolved in artificial ECF at concentrations of 0.5 and 1.0 mM. Two rats were used to test each concentration of EAA. Rats were implanted bilaterally with dialysis probes into the anterior striatum and perfused with artificial ECF for 2 hr. Three 30-min baseline fractions were collected, EAAs were infused via the dialysis probe for 15 min, and 30-min fractions collected for the next 2 hr. The dialysate concentration of kynurenic acid was measured in all fractions.

Data analysis and statistics. All kynurenic acid values were calculated and expressed in units of either nM (nmol/liter) or fold-change from baseline levels (mean ± SEM). Statistical comparisons were made using repeated measures analysis of variance (ANOVA) and the Mann-Whitney *U* test.

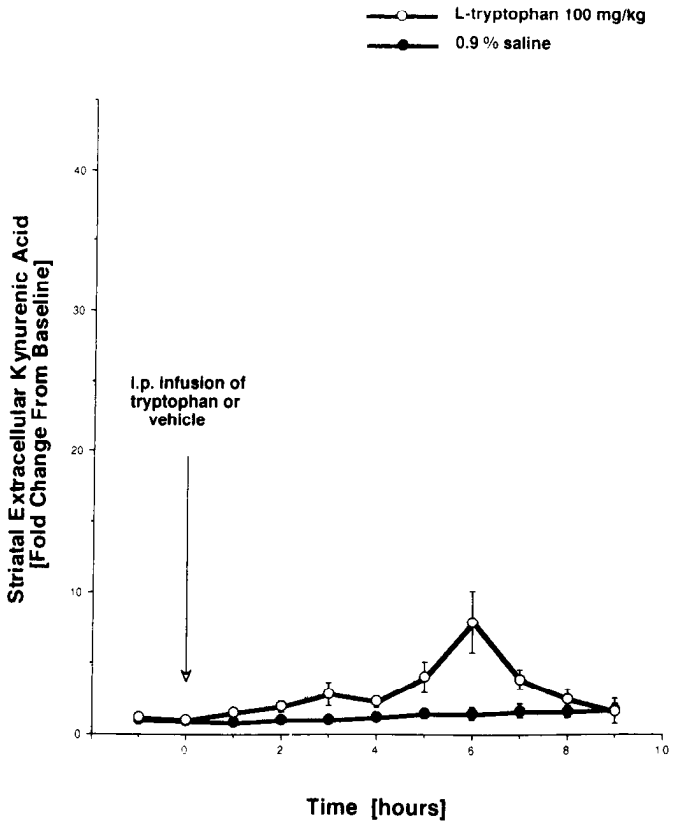

Figure 4. Striatal extracellular levels of kynurenic acid following peripheral (i.p.) infusion of saline or L-tryptophan, 100 mg/kg. The vertical axis represents the fold change in striatal concentrations of kynurenic acid, and the horizontal axis represents t in hours. The peripheral infusion of L-tryptophan or vehicle occurred at t_0. Error bars represent SEM. The point corresponding to the peak in kynurenic acid concentration (t = 6 hr) following L-tryptophan infusion was significantly different ($p < 0.01$) from baseline values by repeated measures ANOVA or from saline controls by Mann-Whitney U test (see Materials and Methods and Results).

Results

Basal levels of kynurenic acid in striatal ECF were determined in 124 samples collected at 30-min intervals in 45 anesthetized rats. A representative chromatogram illustrating the measurement of kynurenic acid in striatal ECF is shown in Figure 2. These fractions were collected either prior to any experimental manipulation or during saline (0.9%) infusion into the peritoneal cavity. Baseline samples and samples collected during saline administration did not significantly differ in kynurenic acid concentration. The basal level of kynurenic acid measured in these dialysates was 1.94 ± 0.12 nM (mean ± SEM). After correction for *in vitro* recovery (11.31% in 10 dialysis probes; see Materials and Methods), the ECF level of kynurenic acid, under basal conditions, was 17.1 ± 1.1 nM.

In initial experiments, we examined the effect of peripheral infusion of L-kynurenine or saline (0.9%) on striatal ECF kynurenic acid concentrations. As illustrated in Figures 2 and 3, doses of L-kynurenine from 25–100 mg/kg caused dose-responsive increases in striatal ECF concentrations of kynurenic acid. At 25 mg/kg, dialysate levels rose 3.85 ± 0.58-fold; at 50 mg/kg, 9.47 ± 1.26-fold; at 75 mg/kg, 27.10 ± 4.26-fold; and at 100 mg/kg, 37.11 ± 7.04-fold. With the exception of the lowest dose, the response of striatal ECF kynurenic acid peaked between 2.0–2.5 hr after infusion of L-kynurenine. The slope of the rise in striatal ECF kynurenic acid increased with increasing doses of L-kynurenine; however, the difference in slope between the 75 and the 100 mg/kg groups was minimal, suggesting that a maximal rate of precursor uptake, synthesis, and release was reached. As illustrated in Figure 4, administration of L-tryptophan (100 mg/kg, i.p.) also caused striatal ECF concentrations of kynurenic acid to increase significantly. This increase in striatal ECF kynurenic acid was substantially smaller and occurred at a considerably later time interval as compared to L-kynurenine administration. Saline infusions (i.p.) had no significant effect on striatal ECF concentrations of kynurenic acid for up to 9 hr (Figs. 3, 4).

The involvement of cerebral and peripheral metabolism in the synthesis of kynurenic acid from L-tryptophan was examined by comparing the ability of precursors to increase striatal ECF concentrations of kynurenic acid when infused through the dialysis probe directly. As shown in Figure 5*A*, L-kynurenine (100 μM) infusion via the dialysis probe resulted in marked increases (68.4 ± 13.1-fold) in striatal ECF concentrations of kynurenic acid. L-Tryptophan (100 μM) infusion via the dialysis probe resulted in minimal, nonsignificant increases in striatal ECF concentrations of kynurenic acid (Fig. 5*B*).

In order to determine whether L-kynurenine was converted to kynurenic acid within the CNS, we examined the ability of an inhibitor of kynurenine aminotransferase (applied locally within the CNS) to block the striatal ECF kynurenic acid response to peripherally infused L-kynurenine. AOAA inhibits the immediate synthetic enzyme for kynurenic acid, kynurenine aminotransferase (Turski et al., 1989). AOAA containing artificial ECF was infused into one striatum, and control artificial ECF was infused into the contralateral striatum. As illustrated in Figure 6, when L-kynurenine (75 mg/kg) was given peripherally, the striatum receiving the AOAA showed only a small increase in kynurenic acid, while the contralateral (control) striata showed a dramatic increase in kynurenic acid, equivalent to that seen in previous experiments (see Fig. 3). The inhibition of striatal ECF kynurenic acid response by AOAA was dose dependent. Infusion of control artificial ECF into the contralateral striata resulted in a 29.56 ± 10.01-fold increase in kynurenic acid, while the striatum receiving 1.0 and 2.0 mM AOAA resulted in only a 6.29 ± 1.79-fold and 4.75 ± 0.40-fold increase, respectively.

The peripheral formation of kynurenic acid from L-kynurenine was also examined. In 6 rats, basal levels of kynurenic acid in plasma were 137.9 ± 25.5 nM. As shown in Figure 7, at the highest does of L-kynurenine (100 mg/kg), plasma levels of kynurenic acid increased 108.7 ± 11.7-fold (peaking at 1 hr), while striatal ECF levels of kynurenic acid increased 37.1 ± 7.0-fold (peaking at 2.5 hr). From data obtained in other preliminary experiments, we estimated that a dose of 10 mg/kg kynurenic acid administered peripherally would cause increases in plasma kynurenic acid of equal or greater magnitude than seen with L-kynurenine (100 mg/kg). As shown in Figure 7*B*, intraperitoneal administration of kynurenic acid (10 mg/kg) caused plasma levels of kynurenic acid to increase 430.7 ± 52.8-fold (peaking at 30 min). Simultaneous striatal dialysis measurements showed that under these conditions, the striatal ECF concentrations of kynurenic acid increased only 4.6 ± 0.4-fold (Fig. 7*A*). The peripheral infusion of kynurenic acid (10 mg/kg) therefore caused 4-fold larger increases in plasma kynurenic acid than

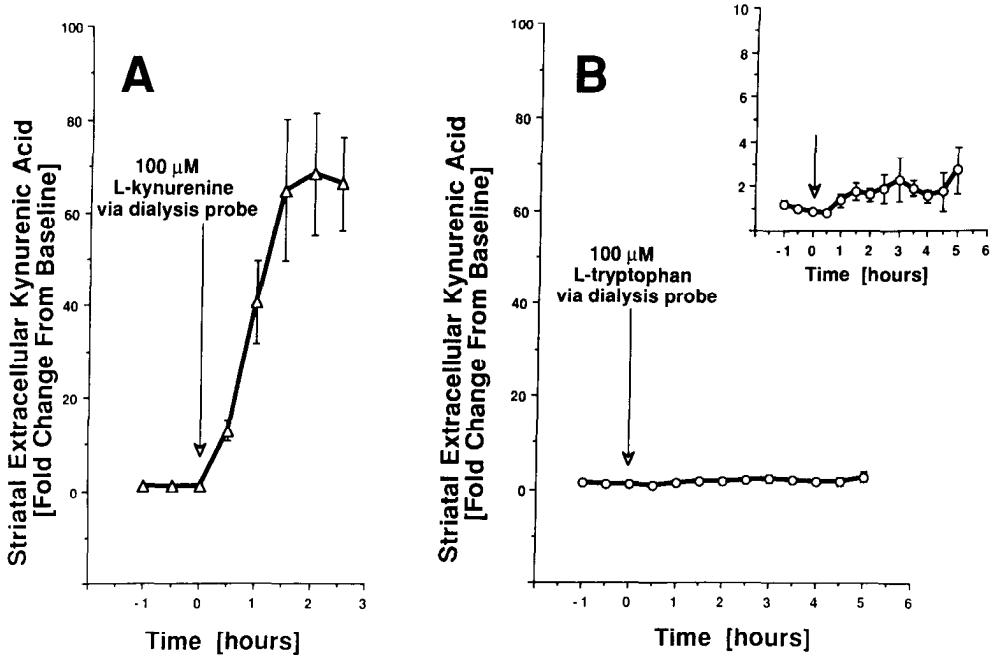

Figure 5. Striatal extracellular levels of kynurenic acid following continuous cerebral (via dialysis probe) infusion of 100 μM L-kynurenine (*A*) or 100 μM L-tryptophan (*B*). The vertical axis represents the fold change in striatal concentrations of kynurenic acid, and the horizontal axis represents *t* in hours. The L-kynurenine or L-tryptophan infusion via the dialysis probe was started at t_0. Error bars represent SEM. The inset in *B* is scaled to 10-fold full scale to show the minimal increase in kynurenic acid that occurred after infusion of L-tryptophan. All points after infusion of L-kynurenine were significantly different ($p < 0.01$) from baseline values by repeated measures ANOVA. Kynurenic acid concentrations in striatal ECF following L-tryptophan infusion were not significantly different from baseline values by repeated measures ANOVA.

did L-kynurenine (100 mg/kg), but striatal ECF levels of kynurenic acid were increased 10-fold more by peripheral infusions of L-kynurenine than by peripheral infusions of kynurenic acid.

The ability of EAAs to stimulate kynurenic acid release into the ECF was also examined. Among the EAAs tested (glutamate, KA, quisqualic acid, NMDA, and quinolinic acid), none could be seen to change the ECF concentration of kynurenic acid for up to 2 hr after the infusion (15 min in duration) of the EAA (data not shown).

Discussion

Kynurenic acid is the only known endogenous inhibitor of EAA receptors in the mammalian CNS. Although its presence in the CNS has been established, it remains unclear whether it plays a modulatory role in EAA neurotransmission under physiologic conditions and whether it may be involved in the pathogenesis of neurologic disease. For kynurenic acid to be of physiological or pathological relevance, it must be established that it can be synthesized by CNS tissue and released into the ECF in concentrations that can act at EAA receptors. This report presents initial findings that begin to address these fundamental questions.

We recently developed a highly sensitive and specific assay for kynurenic acid using HPLC with fluorescence detection. Using this assay, the basal level of kynurenic acid in striatal dialysate was above the sensitivity of the method (15×10^{-15} mol/injection; Swartz et al., 1990). The basal concentration of kynurenic acid in 124 samples (45 rats) of striatal dialysates was 1.94 ± 0.12 nM (mean ± SEM). *In vitro* recovery of kynurenic acid from the microdialysis probe (see Materials and Methods) was $11.3 \pm 0.4\%$. Correction using this estimate of recovery suggests that basal striatal ECF levels of kynurenic acid are approximately 17 nM. However, correction for recovery using saline solutions is thought to underestimate the actual ECF concentrations of many neurotransmitters and their metabolites by 5- to 10-fold (Benveniste, 1989; Benveniste et al., 1989).

Based on previous findings and those presented here, we propose the metabolic pathway leading to the cerebral formation of kynurenic acid depicted in Figure 1. Kynurenic acid is a metabolite of L-tryptophan that is formed by transamination of L-kynurenine. Kynurenine aminotransferase (EC 2.6.1.7), the synthetic enzyme for kynurenic acid, is thought to be localized in glial cells within the CNS (Minatogawa et al., 1974; Turski et al., 1989). Brain tissue can synthesize L-kynurenine from tryptophan (Gal, 1974), but L-kynurenine can also be transported across the blood–brain barrier (Gal and Sherman, 1978). L-Kynurenine uptake systems also exist in rat brain astrocytes (Speciale et al., 1989a). It has previously been estimated that approximately 40% of brain L-kynurenine is endogenously synthesized from tryptophan by the enzyme indolamine-2,3-dioxygenase (EC 1.13.1.12), while the remaining 60% is transported across the blood–brain barrier (Gal and Sherman, 1980; but see below). We examined the responsiveness of striatal ECF kynurenic acid concentrations to peripheral administration of precursors. Striatal ECF kynurenic acid showed dramatic increases following intraperitoneal administration of L-kynurenine (see Fig. 3 and Results). Administration of L-tryptophan (100 mg/kg, i.p.) also caused significant increases in striatal ECF concentrations of kynurenic acid (see Fig. 4). This increase in striatal ECF kynurenic acid was substantially smaller and occurred at a considerably later time interval than that seen with L-kynurenine administration. This lag would be consistent with peripheral formation of L-kynurenine from L-tryptophan and transport of L-kynurenine across the blood–brain barrier into glia. The finding that cerebral infusion of L-kynurenine, but not L-tryptophan, resulted in increases in striatal ECF kynurenic acid concentrations further suggests that L-kynurenine is the predominant precursor that crosses the blood–brain barrier and is subsequently transaminated to kynurenic acid (see Figs. 1, 5).

Although the increases in striatal ECF kynurenic acid were observed in a dialysis system thought to be localized within the blood–brain barrier (Benveniste, 1989; Benveniste et al., 1989), the possibility that kynurenic acid diffuses through, or is transported across, the blood–brain barrier could not be ruled out. However, we found that a centrally applied inhibitor of kynurenine aminotransferase blocked the increases in striatal ECF kyn-

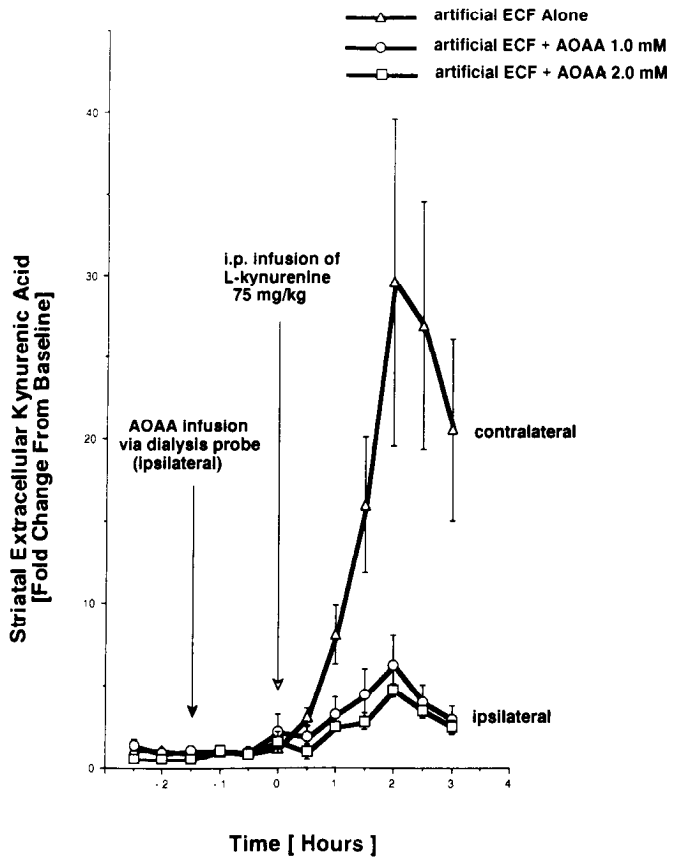

Figure 6. Striatal extracellular levels of kynurenic acid following peripheral (i.p.) infusion of L-kynurenine, 75 mg/kg. One probe (designated ipsilateral) was perfused with artificial ECF containing 1.0 or 2.0 mM AOAA, and the other contained standard artificial ECF. The vertical axis represents the fold change in striatal concentrations of kynurenic acid, and the horizontal axis represents t in hours. The ipsilateral infusion of AOAA was initiated at $t = -1.5$, and L-kynurenine was intraperitoneally injected at t_0. Error bars represent SEM. The contralateral ECF concentrations of kynurenic acid, after infusion of L-kynurenine, were all significantly different ($p < 0.01$) from baseline concentrations by repeated measures ANOVA. Both striatal responses to peripheral L-kynurenine with AOAA containing ECF differed significantly ($p < 0.01$) from the response to L-kynurenine with standard ECF alone by ANOVA.

urenic acid after peripheral L-kynurenine administration (see Fig. 6). These results indicate that kynurenic acid is synthesized from L-kynurenine within the CNS.

The permeability of the blood–brain barrier to kynurenic acid was also examined by simultaneous plasma and striatal ECF measurements. At the highest dose of L-kynurenine (100 mg/kg), plasma kynurenic acid levels increased by 100-fold, and striatal ECF levels increased by 37-fold. However, when kynurenic acid (10 mg/kg) was administered peripherally, plasma levels increased by 400-fold, and striatal ECF levels increased by only 4-fold (see Fig. 7 and Results). These results, taken together with the results of the enzyme inhibition studies and cerebral precursor administration studies, provide strong evidence that the CNS synthesizes and releases kynurenic acid *in vivo*. Whether this release represents an active process or simply diffusion from cellular compartments remains unclear.

It will be of interest to examine what, if any, mechanisms exist that control the release of kynurenic acid from glial populations. One possibility is that endogenous EAAs mediate the release of kynurenic acid from glial cells, because several types of glia have electrophysiologic responses to EAAs (Barres et al., 1988, 1990a, b; Barres, 1989; Cull-Candy et al., 1989; Gallo et al., 1989; Usowicz et al., 1989; Cornell-Bell et al., 1990). In several experiments, the ability of glutamate, NMDA, quinolinic acid, KA, or quisqualic acid to stimulate kynurenic acid release was examined. The above EAAs were infused through the dialysis probe, at concentrations ranging from 0.5 to 1.0 mM, for 15 min. No effects on kynurenic acid release were observed for up to 2 hr after EAA infusion (data not shown). These preliminary results suggest EAAs do *not* play a pivotal role in the release of kynurenic acid. Thus, given the findings that (1) peripheral L-kynurenine administration freely increases cerebral ECF levels of kynurenic acid, and (2) tissue levels of kynurenic acid are in the same range of ECF levels (low nM; Swartz et al., 1990), it would seem plausible that control may be exerted at the level of kynurenine release from peripheral stores (liver or kidney).

Whether kynurenic acid concentrations in ECF are within the physiologically relevant range is as yet unclear. In the mammalian CNS, several types of EAA receptors are believed to be activated by glutamate (Watkins and Olverman, 1987). As classified by prototypical EAA agonists, they are NMDA, KA, and QUIS. Because concentrations required to block non-NMDA receptors (activated by KA and QUIS) are in the millimolar range (Stone and Connick, 1985; Kessler et al., 1989), it is unlikely that kynurenic acid concentrations in ECF would be sufficient to modulate these non-NMDA receptors.

Johnson and Ascher (1987) first demonstrated that glycine potentiates NMDA electrophysiologic responses and proposed the existence of an allosteric modulator site on the NMDA-receptor complex that is sensitive to glycine (Llano et al., 1988). Glycine binding appears to be a prerequisite for activity of the NMDA receptor (Kleckner and Dingledine, 1988). Several NMDA-receptor antagonists, including kynurenic acid, have been shown to act at the glycine site on the NMDA-receptor complex (Foster, 1988; Honore et al., 1988; Kemp et al., 1988; Birch et al., 1989a, b, c; Foster and Kemp, 1989b; Lester et al., 1989; Shalaby et al., 1989). While kynurenic acid has affinity for both the agonist recognition site and the glycine allosteric site on the NMDA-receptor complex, it is several times more potent at the glycine allosteric site (Danysz et al., 1989). The EC_{50} of kynurenic acid for the glycine site is approximately 15 μM, whereas its affinity for the NMDA recognition site is approximately 200–500 μM (Kessler et al., 1989). Binding studies therefore suggest that low micromolar concentrations of kynurenic acid are required to have activity at the glycine site on the NMDA-receptor complex. From data presented in this report, basal striatal ECF levels of kynurenic acid in the rat were ~17 nM (but see Discussion above). These concentrations of kynurenic acid are significantly lower than those that clearly block the glycine allosteric site on the NMDA-receptor complex *in vitro*. Microdialysis techniques, however, only allow the measurement of kynurenic acid after diffusion and dilution from the site of release, and therefore may underestimate the concentration at the site of action (i.e., the synapse). These data suggest that for kynurenic acid release to be functionally important in rats, release may need to occur close to its site of action. We have recently found levels of kynurenic acid in human ECF ($n = 2$) to be an order of magnitude higher than those found in rats (18.3 nM in 12 human dialysates, 0.183 μM after correction

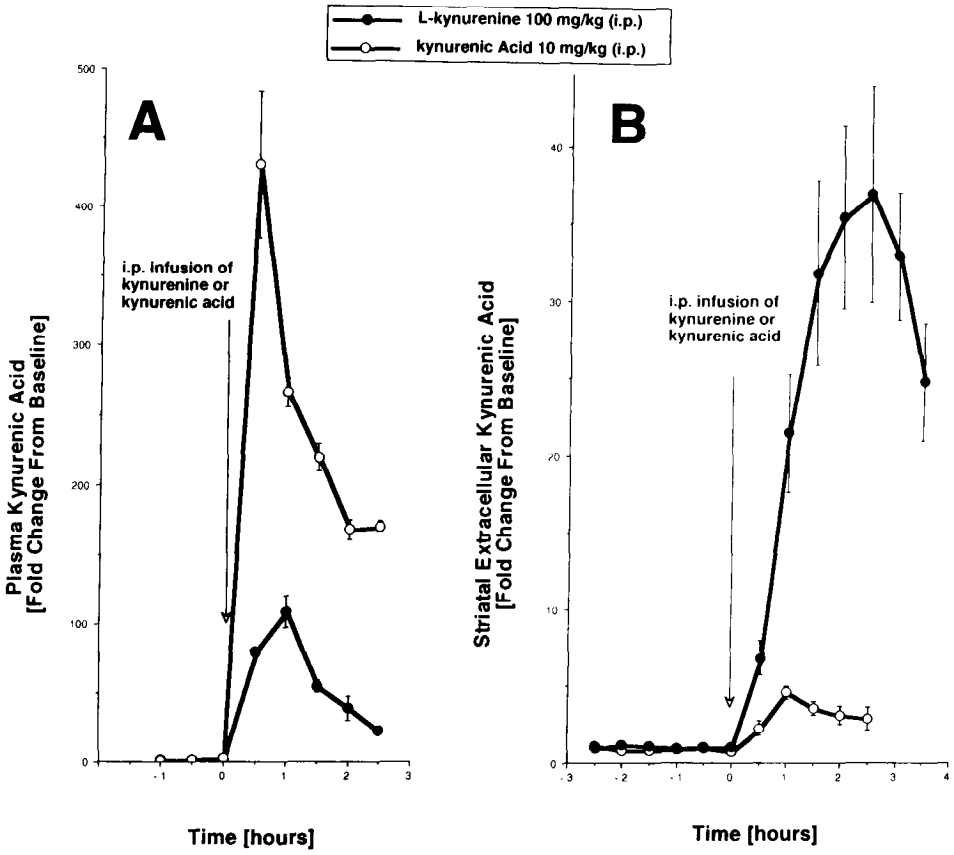

Figure 7. Plasma and striatal extracellular concentrations of kynurenic acid after peripheral injections (i.p.) of L-kynurenine or kynurenic acid. *A*, Plasma concentrations of kynurenic acid after L-kynurenine (100 mg/kg) or kynurenic acid (10 mg/kg) peripheral infusions. Plasma kynurenic acid concentrations after kynurenic acid infusions were significantly larger ($p < 0.01$) than changes after injection of L-kynurenine by ANOVA. *B*, Striatal ECF concentrations of kynurenic acid after L-kynurenine (100 mg/kg) or kynurenic acid (10 mg/kg) peripheral injections. Changes in striatal ECF concentrations of kynurenic acid after L-kynurenine infusions were significantly larger ($p < 0.01$) by ANOVA than changes after intraperitoneal injections of kynurenic acid.

for *in vitro* recovery; M. J. During and K. J. Swartz, unpublished observations). This is consistent with previous data suggesting that regional brain-tissue content of kynurenic acid is an order of magnitude higher in humans and monkeys as compared to rats (Swartz et al., 1990). Furthermore, in these 2 patients, kynurenic acid concentrations increased 15-fold upon oral administration of L-tryptophan. Recent evidence suggests that the glycine allosteric site is *not* saturated *in vivo* and may therefore be modulated by kynurenic acid (Thomson et al., 1989). Mayer et al. (1989) have suggested that glycine acts as a positive modulator of the NMDA receptor by blocking receptor desensitization. Kynurenic acid could therefore facilitate NMDA-receptor desensitization via its action as an antagonist of the glycine allosteric site on the NMDA-receptor complex.

Although it is as yet unclear that basal levels of kynurenic acid can physiologically modulate EAA receptors, the present studies indicate that peripheral administration of L-kynurenine or L-tryptophan can increase brain kynurenic acid concentrations by 37- or 8-fold, respectively, into a range that may have activity at the glycine allosteric site on the NMDA receptor. While acute L-tryptophan loading leads to dramatic increases in the ECF levels of the NMDA-receptor agonist quinolinic acid (During et al., 1989), extremely high doses of L-kynurenine (600 mg/kg), only result in 6-fold increases in brain tissue levels of quinolinic acid (Speciale et al., 1987). Furthermore, Speciale et al. (1989b) infused 1 mM L-kynurenine via dialysis probes and found no change in ECF quinolinic acid; however, basal ECF levels could not be detected using their assay. We are presently examining the effects of peripheral L-kynurenine administration on cerebral ECF levels of quinolinic acid using mass spectroscopy, where basal ECF levels of quinolinic acid are within the limit of detection. Because peripheral L-tryptophan loading dramatically increases ECF concentrations of quinolinic acid and L-kynurenine loading apparently does so only minimally, we suggest that there may be an alternative pathway from L-tryptophan to quinolinic acid that does not require L-kynurenine entry into the brain. In the periphery, L-tryptophan can be metabolized to anthranilic acid, which can readily cross the blood–brain barrier, probably via the large neutral amino acid transporter (K. J. Swartz et al., unpublished observations). In brain slice preparations, anthranilic acid can be metabolized to 3-hydroxyanthranilic acid, which can subsequently be metabolized to quinolinic acid (Baran and Schwarcz, 1989). While it does not appear that L-kynurenine is a major precursor to quinolinic acid in the CNS, it can be metabolized to quinolinic acid in the periphery, possibly resulting in systemic side effects, because NMDA receptors exist in peripheral tissues (Moroni et al., 1986). Furthermore, the peripheral metabolism of L-kynurenine would be expected to decrease L-kynurenine available for transport into the brain. The administration of kynureninase inhibitors, which would block the peripheral metabolism of L-kynurenine, could be used in combination with L-kynurenine administration to increase cerebral synthesis of kynurenic acid. Thus, it appears possible to increase kynurenic acid formation within the CNS without concomitantly increasing quinolinic acid production. Such an approach would represent a novel therapeutic strategy for diseases such as ischemia, hypoglycemia, epilepsy, and Huntington's disease, in which excitotoxic

mechanisms have been implicated (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Foster et al., 1984; Schwarcz et al., 1984; Simon et al., 1984; Wieloch, 1985; Beal et al., 1986, 1989; Schwarcz and Ben-Ari, 1986; Choi, 1988).

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