3-Hydroxyanthranilic Acid Oxygenase–containing Astrocytic Processes Surround Glutamate-containing Axon Terminals in the Rat Striatum

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Glutamate, the major transmitter of the corticostriatal pathway, is present in abundance in the striatum. 3-Hydroxyanthranilic acid oxygenase (3HAO) is the biosynthetic enzyme for quinolinic acid, an endogenous agonist of the NMDA glutamate receptor subtype and a potent neurotoxin. In order to explore the anatomical basis of possible functional interactions between glutamate and quinolinic acid in the rat striatum, pre- and postembedding immunocytochemical methods were used to localize 3HAO immunoreactivity (-i) and glutamate-i at the electron microscopic level. In accordance with previous light microscopic and biochemical studies, 3HAO-i was detected exclusively in astrocytes throughout the striatum. Notably, 3HAO-i was present in fine-caliber glial processes that often surrounded or abutted synaptic profiles, both asymmetric and symmetric. Glutamate-i was heavily deposited (3–13-fold higher gold particle density than tissue average) in axon terminals forming asymmetric synapses with spines and, occasionally, dendrites. In contrast, terminals forming symmetric synapses, dendrites, neuronal somata, and glial cells contained significantly less labeling than terminals forming asymmetric synapses. In double-labeled material, 3HAO-i was observed in glial processes that partially surrounded or were adjacent to glutamate-labeled terminals forming asymmetric synapses. 3HAO-labeled glial processes were also adjacent to unlabeled terminals forming symmetric synapses. Since quinolinic acid is known to enter the extracellular compartment readily, these results suggest that astrocytic quinolinic acid may participate in the regulation of glutamatergic neurotransmission in the rat striatum.

[Key words: quinolinic acid, excitotoxicity, basal ganglia, caudate, astrocytes, NMDA]

The ubiquitous excitatory neurotransmitter glutamate plays a critical role in the normal function of the mammalian neostriatum. A number of studies have established that striatal neuro-

transmitter glutamate is derived largely from corticofugal striatal afferents. Thus, both glutamate content (Kim et al., 1977) and high-affinity glutamate uptake (Divac et al., 1977; McGeer et al., 1977) are reduced in the striatum following cortical ablation. Conversely, glutamate is released in the rat striatum during electrical stimulation of the cortex (Perschak and Cuénod, 1990). Moreover, corticostriatal excitatory postsynaptic potentials are clearly mediated by excitatory amino acid (EAA) receptors (Herrling, 1985), which have been identified and extensively characterized in the striatum (Monaghan and Cotman, 1985; Monaghan et al., 1989; Martin et al., 1992, 1993). Classical anatomical studies have demonstrated that striatal afferents originate largely from the cortex (Kemp and Powell, 1971) and form asymmetric synapses onto striatal projection neurons (Pasik et al., 1980). Finally, in ultrastructural studies using anti-glutamate antibody, glutamate has been localized in axon terminals which form asymmetric synapses in the striatum (Meshul et al., 1992; Snyder et al., 1993).

In addition to its physiological role in the striatum, glutamate has the potential to be excitotoxic, and thus may play a role in neurodegenerative brain disorders involving the striatum, such as Huntington's disease (HD) (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). However, when injected directly into the rat striatum, glutamate causes only very limited damage, even when administered at high concentrations (Mangano and Schwarcz, 1983), probably due to its effective removal from the synaptic cleft (Drejer et al., 1982; Fonnum, 1984; Erecińska and Silver, 1990). The most potent endogenous excitotoxin discovered so far is quinolinic acid (QUIN), a selective *N*-methyl-D-aspartate (NMDA) receptor agonist (Stone and Perkins, 1981; Wolfensberger et al., 1983) which readily produces axon-sparing neuronal destruction in the rat striatum (Schwarcz et al., 1983). Since QUIN-induced striatal lesions mimic many of the neuropathological features of HD (Schwarcz et al., 1983; Beal et al., 1986; Roberts et al., 1993), QUIN has been implicated in the neurodegeneration occurring in HD. The enzyme directly responsible for the synthesis of QUIN, 3-hydroxyanthranilic acid oxygenase (3HAO), is present in brain tissue (Foster et al., 1986), and its activity is substantially elevated in the brain of HD victims (Schwarcz et al., 1988a). Rat 3HAO has been purified from liver and brain, and anti-3HAO antibodies have been produced and used for the immunocytochemical localization of 3HAO in the rat brain (Okuno et al., 1987). Studies at the light microscopic level, which included double labeling with antibod-

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ies against glial fibrillary acidic protein (Köhler et al., 1988a), showed that the enzyme is contained in astrocytes throughout the adult rat brain including the striatum.

The available data therefore suggest an involvement of NMDA receptors in normal and perhaps in abnormal striatal function, and a role of QUIN in the modulation of striatal NMDA receptors in healthy and diseased states. The aim of the present study was to examine the relationship between glutamate immunoreactivity (-i) and 3HAO-i in the adult rat striatum at the electron microscopic level. Particular attention was paid to the relationship between 3HAO-i processes and synaptic profiles involving glutamate-labeled axon terminals.

A preliminary report of this work has been published in abstract form (McCarthy et al., 1993).

Materials and Methods

Animals

Twenty-eight adult Sprague-Dawley rats (200–220 gm) were deeply anesthetized with a mixture of xylazine/ketamine (1 mg xylazine + 5 mg ketamine/100 gm body weight) and perfused through the heart with 50 ml saline followed by 350 ml of fixative containing (1) 4% paraformaldehyde, 0.03 M periodate, and 0.1 M lysine in 0.1 M phosphate buffer (PB), pH 7.4 (McLean and Nakane, 1974) ($n = 17$); (2) 1–1.5% glutaraldehyde and 1–4% paraformaldehyde in 0.1 M PB, pH 7.4 ($n = 10$); or (3) 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M PB, pH 7.4 ($n = 1$). The brains were postfixed for 3 hr in the same fixative, stored in PB for 2–24 hr (4°C), and sectioned with a cryostat (20 μ m thick) or a vibratome (40 μ m thick). Coronal sections throughout the rostrocaudal extent of the striatum were collected and one series of sections out of six was processed for immunocytochemistry.

Localization of 3HAO-i using pre-embedding techniques

As described previously, 3HAO was purified to homogeneity from liver, and liver and brain 3HAO were confirmed to be identical using a spectrum of biochemical, physicochemical, and immunological techniques (Okuno et al., 1987).

Sections were processed for the immunocytochemical localization of rabbit anti-3HAO antibody, according to a modification of the pre-embedding protocol of Köhler et al. (1987). Briefly, free-floating sections from rats perfused with fixative 1 ($n = 17$) or 2 ($n = 8$) and cut with the cryostat ($n = 7$) or vibratome ($n = 18$) were incubated in 2% normal goat serum in PB for 30 min prior to incubation in anti-3HAO (1:3000) for 72 hr (4°C). Sections were then processed using reagents from the avidin-biotin kit using recommended dilutions (Hsu et al., 1981), followed by reaction with diaminobenzidine (6 mg/10 ml PB) containing 0.03% hydrogen peroxide for 5–15 min. Tissue cut on the cryostat was mounted on gelatin-coated slides and coverslipped, whereas tissue cut on the vibratome was prepared for electron microscopic analysis (see below).

Preparation of tissue for electron microscopy

Tissue sections from all brains cut on the vibratome were processed for electron microscopy. (1) Tissue processed for 3HAO-i (fixative 1) was embedded using standard techniques. Briefly, sections were immersed in 1% osmium tetroxide for 1 hr, stained with 1% uranyl acetate for 1 hr, dehydrated in alcohols, embedded flat in Epon, and heated at 60°C for 72 hr. (2) To prepare tissue for postembedding immunolabeling with anti-glutamate antibody, tissue processed for 3HAO-i (fixative 2) or unprocessed tissue (fixative 3) was embedded using the following protocol. Sections were immersed in 1% osmium tetroxide for 1 hr, dehydrated with a series of alcohol dilutions, flat embedded in Durcupan ACM resin, and heated at 56°C for 48 hr. Areas from the striatum containing optimal 3HAO staining or random areas in unstained tissue were excised, mounted on Epon blocks, and thin (90 nm) sectioned. Thin sections were collected onto Formvar-coated slot grids (for single label analysis of 3HAO-i) or nickel mesh grids (for the postembedding immunogold procedure).

Localization of glutamate using postembedding techniques

Striatal tissue sections were processed using a modification of the procedure of Somogyi and Hodgson (1985). The sections, collected on

nickel mesh grids and dried overnight at 50°C, were immersed sequentially in the following solutions: (1) 1% periodic acid (HIO_4) in double-distilled water (ddW) for 7 min; (2) 8% sodium m-periodate (NaIO_4) in ddW for 15 min; (3) 1% human serum albumin (HSA) in ddW for 10 min; (4) 1% HSA in Tris phosphate-buffered saline (TPBS, 0.4 gm KCl, 0.2 gm NaN_3 , 7.0 gm NaCl per liter of 0.01 M PB + 0.01 M Tris/HCl) for 10 min; (5) glutamate antiserum (#607; diluted 1:100–1:900 in TPBS containing 2% HSA and preadsorbed with 100 μ M aspartate-glutaraldehyde complex, 200 μ M glutamine-glutaraldehyde complex, and 200 μ M β -alanine-glutaraldehyde complex) for 2 hr (Ottersen et al., 1986; Broman et al., 1993); (6) 1% HSA in TPBS for 10 min; (7) 0.05 M Tris and 0.5 mg/liter polyethyleneglycol in ddW ("Tris buffer") for 3 min; and (8) colloidal gold (20 nm) coupled to anti-rabbit IgG (diluted 1:20 in Tris buffer) for 2 hr. All steps were followed with thorough rinses in either ddW or TPBS. After drying, the sections were stained with 1% uranyl acetate (23 min) and 0.3% lead citrate (3 min). The tissue sections were incubated together with test sections carrying a series of different amino acid conjugates (Ottersen et al., 1992) for continuous monitoring of specificity.

Quantitative ultrastructural analysis

Analysis of 3HAO-i. Electron micrographs were taken at a magnification of 15,000 $\times$ and were analyzed at a final magnification of 30,000 $\times$. Ten fields from the striatum were photographed per rat (six animals perfused with fixative 1) near the edge of the tissue, where immunoreactivity was present but background staining was minimal. All synapses were counted, and the proportion of synapses in each field that had immunoreactive glial processes in direct contact with synaptic profiles, either adjacent to or surrounding the synaptic profiles, was determined. 3HAO-i profiles were deemed adjacent if they were directly adjacent to one or both synaptic profiles. 3HAO-i profiles were judged to surround synaptic profiles if they encircled at least 50% of the circumference of the synaptic profiles including the region of the synapse.

Analysis of glutamate-i. Electron micrographs were taken from single- and double-labeled material at a magnification of 17,000 $\times$, and analyzed at 34,000 $\times$. Ten fields from the striatum were photographed per rat (six animals perfused with fixative 2 and one animal perfused with fixative 3). The distribution of glutamate-i was analyzed using SIGMA-SCAN software to quantify the number of colloidal gold particles per square micron in the following profiles: axon terminals forming asymmetric synapses; axon terminals forming symmetric synapses; astrocytic processes; dendrites and the lumen of capillaries. Axon terminals forming asymmetric synapses were not subdivided according to subtypes of Hassler et al. (1978). Gold particle density values do not include mitochondrial labeling. Group means were compared using an independent Student's *t* test. A Bonferroni correction was used to correct for multiple comparisons.

Controls

Controls consisted of (1) omitting the 3HAO antibody, (2) preadsorbing the 3HAO antibody with 31 μ g pure 3HAO per 1 ml diluted antibody for 24 hr (Okuno et al., 1987), (3) preadsorbing the glutamate antibody with 300 μ M of glutamate-glutaraldehyde complex (Ottersen et al., 1986), and (4) exposing tissue labeled with 3HAO antibodies to paraformaldehyde fumes prior to incubation with glutamate antibody to denature free rabbit anti-IgG sites (Wang and Larsson, 1985; Ottersen et al., 1992). This final control was necessary to avoid interference between the first and second incubations because both antibodies were produced in rabbit. Therefore, the possibility existed in double-labeled material that the secondary antibody in the glutamate protocol (colloidal gold conjugated anti-rabbit IgG) would attach to free 3HAO IgG sites, producing a certain amount of nonspecific gold labeling in astrocytic processes (the only cellular compartment we measured where both 3HAO and glutamate are present).

Results

3HAO immunoreactivity

At the light microscopic level, 3HAO-i was detected in glial cells which appeared to be homogeneously distributed throughout the striatum (Fig. 1A). Immunoreactivity was present in the cell bodies of astrocytes, in their widely ramifying proximal processes, and in an intricate network of fine-caliber processes (Fig. 1C). Utilizing the present methods, 3HAO-i was not detected in

Figure 1. Light micrographs showing the distribution of 3HAO-i in the normal rat striatum (fixative 1) sectioned in the coronal plane with a cryostat (30 μ m). **A**, 3HAO-labeled cells are numerous throughout the striatum. Region indicated by *asterisk* is enlarged in **C**. **B**, Specific staining is eliminated in the preadsorption control. **C**, High-magnification view showing immunoreactive astroglial cells (*arrows*). *fb*, fiber bundle (**B**, **C**). Scale bars: **A**, 1000 μ m; **B** and **C**, 100 μ m.

astrocytes in the white matter, though preliminary results using a more sensitive technique (Adams, 1992) indicate the presence of some labeled astrocytes in white matter. This apparent difference in staining intensity may be related to heterogeneous features of astrocytes in gray and white matter (Miller and Raff, 1984; Shehab et al., 1990). The pattern of immunoreactivity was virtually identical between cryostat and vibratome sections. Immunolabeling of the tissue from rats prepared with fixative 1 was superior to that from tissue prepared with fixative 2 in that more extensive staining of processes was achieved. Specific staining was eliminated by the preadsorption control in sections cut on the cryostat (Fig. 1*B*) and vibratome (not shown).

Confirming our light microscopic observations, 3HAO-i was detected exclusively in the cell bodies and processes of astrocytes (Fig. 2*A–C*). All immunoreactive astrocytes exhibited cytoplasmic labeling (Fig. 2*A–C*), which was deposited diffusely on various organelles including rough endoplasmic reticulum, fibrils, and some outer mitochondrial membranes (Fig. 2*C*). Moreover, nuclear labeling was also detected in a subset of immunoreactive astrocytes (Fig. 2*B,C*). Immunoreactive processes emanated from the astroglial somata (Fig. 2*A*) and were also abundant throughout the neuropil in small-caliber processes (Fig. 3*A–C*), which partially engulfed synaptic profiles (Fig. 3*B,C*). Several types of synaptic profile, including asymmetric axospinous and axodendritic (Fig. 3), symmetric axodendritic (see Fig. 5) and symmetric axospinous synapses (not shown), were contacted or encircled by labeled glial profiles.

Quantification of 3HAO-labeled astrocytic processes and synaptic profiles is summarized in Table 1. Approximately 36% of asymmetric and 29% of symmetric synapses formed between terminals and either spines or dendrites were closely associated with (i.e., adjacent to or surrounded by) a 3HAO-labeled glial process. Therefore, 3HAO-i processes do not appear to be preferentially related to either type of synapse. Taken together, 35% of all synapses (excluding axosomatic, which were not tabulated) were contacted by 3HAO-containing processes.

Glutamate immunoreactivity

Glutamate-i was deposited throughout the neuropil in varying densities in several types of subcellular profiles including axon terminals, dendrites, spines, neuronal somata, astrocytes, and astrocytic processes (Fig. 4). Based on qualitative inspection, the density of gold particles was much higher in terminals forming asymmetric synapses than in other cellular profiles. However, even in areas exhibiting maximal labeling, some terminals forming asymmetric synapses did not contain a high density of gold particles. Nevertheless, within axon terminals forming asymmetric synapses, gold particles were overlying or closely associated with synaptic vesicles. Within most cellular profiles (except spines which lack mitochondria), gold particles were often associated with mitochondria. Specific staining was eliminated with the glutamate preadsorption control (not shown). Specificity of staining was verified using test sections that accompanied the tissue sections, as described in Ottersen et al. (1992).

Figure 2. Electron micrographs illustrating 3HAO-i in astroglial cell bodies and processes (fixative 1). Labeled processes (*solid arrows*) emanate from the cell bodies and are present in abundance throughout the neuropil. **A**, Labeled astrocyte with reaction product deposited in the cytoplasm, but not in the nucleus (*n*). **B**, Labeled astrocyte with reaction product in both the cytoplasm (*open arrows*) and the nucleus (*n*). **C**, Enlargement of the boxed area in **B** showing the subcellular distribution of 3HAO-i. Reaction product is deposited in the nucleus (*thick solid arrow*), around mitochondria, and throughout the cytoplasm on rough endoplasmic reticulum (*open arrows*) and fibrils (*thin arrow*). Scale bars: **A** and **C**, 1 μ m; **B**, 5 μ m.

Figure 3. Electron micrographs illustrating 3HAO-i in striatal neuropil (fixative 1). *A*, 3HAO-i is abundant throughout the neuropil in a fine network of astroglial processes adjacent to or partially surrounding synaptic profiles (arrows). *B*, Enlarged view of the boxed area in *A* showing 3HAO-i processes (solid arrows) partially surrounding synaptic profiles. *C*, 3HAO-labeled processes (solid arrows) of small caliber extend up to the synapse. *d*, dendrite; *s*, spine. Open arrows (*B*, *C*) indicate asymmetric synapses in close association with 3HAO-labeled glial processes. Scale bars, 1 μ m.

Quantification of glutamate labeling in cellular profiles is summarized in Table 2. The density of gold particles in terminals forming asymmetric synapses was significantly higher than in other subcellular domains, confirming our qualitative observations. This relationship was verified in each of the seven individual animals studied. Moreover, when the data were pooled, the mean gold particle density in axon terminals forming asymmetric synapses was significantly higher than in other subcellular compartments. Ratios of the number of gold particles in terminals forming asymmetric synapses compared to other cellular profiles are presented in Table 3. These ratios ranged from 3.2:1

to 13.5:1, indicating higher binding of glutamate in axon terminals forming asymmetric synapses as compared with other profiles.

Double labeling with 3HAO and glutamate antibodies

In double-labeled material, 3HAO-i was found predominantly in large-caliber processes and, less frequently than in the single-labeled tissue, in fine astrocytic processes (Fig. 5*A–D*). The compromised immunoreactivity was due to the high glutaraldehyde concentration in the fixative which was necessary to obtain specific glutamate staining. Nevertheless, 3HAO-labeled

Figure 4. Electron micrographs of glutamate-i in striatal neuropil. Glutamate-i was deposited in various cellular profiles with varying intensity. It was most heavily deposited (2–14-fold higher gold particle density than tissue average; see Table 3) in axon terminals (*at*) forming asymmetric synapses (arrows) with spines (*s*). *A*, Field of neuropil showing several synapses. Fixative 2. *B* and *C*, Higher-magnification views of individual labeled glutamatergic axon terminals forming synapses (fixative 3). *g*, glial process. Scale bars, 0.5 μ m.

Table 1. Quantification of 3HAO-labeled astrocytic processes and synaptic profiles

Animal	Asymmetric synapses			Symmetric Synapses			Total synapses		
	Adjacent to 3HAO-i	Total	% of Total adjacent	Adjacent to 3HAO-i	Total	% of Total adjacent	Adjacent to 3HAO-i	Total	% of Total adjacent
1	9.2	44.2	20.8	0.0	6.7	0.0	9.2	50.9	18.1
2	22.5	59.2	38.0	1.7	9.2	18.5	24.2	68.4	35.4
3	23.1	52.3	44.2	2.3	4.6	50.0	25.4	56.9	44.6
4	17.7	50.0	35.4	2.4	5.3	45.3	20.1	55.3	36.4
5	14.6	37.7	38.7	1.5	2.3	65.2	16.1	40.0	40.2
Mean	17.4	48.7	35.7	1.6	5.6	28.6	19.0	54.3	35.0
SEM	2.6	3.6	3.9	0.4	1.1	11.7	2.9	4.6	4.5

3HAO-i was deemed "adjacent" if labeled glial processes abutted or surrounded the synaptic profiles. Values are the means per picture $\times 10$, as described in the text.

processes, albeit large, were found adjacent to or abutting synaptic profiles, many of which were glutamate containing (Fig. 5). Thus, 3HAO-i astrocytic processes were most frequently encountered adjacent to asymmetric synapses formed between spines and axon terminals heavily labeled by the glutamate antibody. 3HAO-i processes were also found adjacent to symmetric synapses formed between dendrites and glutamate-negative axon terminals (Fig. 5A), though the number of such appositions was low due to the scarcity of symmetric synapses.

Discussion

The main results of the present study are the following: (1) 3HAO-i is abundant throughout the rat striatum in astrocytic cell bodies and in an extensive network of fine-caliber processes which encircles synaptic profiles; (2) glutamate-i is distributed in various cellular profiles throughout the striatum, but is most heavily deposited in axon terminals forming asymmetric synapses; and (3) 3HAO-containing astrocytic processes are frequently found in close apposition to glutamate-containing terminals forming asymmetric synapses.

The demonstration of 3HAO in striatal astrocytes is consistent

with immunohistochemical studies performed in other brain regions, where 3HAO-i was localized in astrocytes or colocalized with astrocytic markers such as glial fibrillary acidic protein and S100 (Okuno et al., 1987; Köhler et al., 1988a,b; Schwarcz and Du, 1991). Although 3HAO-i astrocytes appear to constitute a homogeneous population at the light microscopic level, two subtypes can be distinguished by electron microscopy, based on the presence or absence of nuclear staining. While poorly understood, the nuclear labeling is clearly not unspecific since it was found only in cells that also showed cytoplasmic labeling. Notably, striatal 3HAO-i is remarkably pervasive, particularly in fine processes encircling synapses. The close relationship between 3HAO-labeled processes and synaptic profiles may be a common feature of most brain structures. For example, the striatum appears to have a similar proportion of synaptic profiles in contact with 3HAO-i processes as the substantia nigra pars compacta, the only other brain area where an ultrastructural analysis of the anatomical features of 3HAO-i elements and their micro-environment has been performed to date (Roberts et al., in press). However, it is our impression that the proportion of total synaptic profiles *surrounded*, rather than abutted, by 3HAO-i

Table 2. Quantification of glutamate labeling in cellular profiles

Profile	Animal							Mean
	1	2	3	4	5	6	7	
Asym. syn. terminals	39.3 $\pm$ 2.7 (31)	26.1 $\pm$ 1.8 (35)	31.1 $\pm$ 2.8 (26)	32.4 $\pm$ 2.8 (24)	30.5 $\pm$ 3.2 (27)	75.4 $\pm$ 2.4 (20)	19.5 $\pm$ 2.6 (29)	36.3 $\pm$ 6.9
Sym. syn. terminals	7.1 $\pm$ 1.1*	6.5 $\pm$ 1.5*	8.5 $\pm$ 1.6*	9.6 $\pm$ 1.5*	8.3 $\pm$ 1.3*	14.0 $\pm$ 2.0*	5.4 $\pm$ 1.6*	8.5 $\pm$ 1.1**
Dendrites	8.2 $\pm$ 0.8*	7.5 $\pm$ 0.8*	9.6 $\pm$ 0.7*	5.0 $\pm$ 0.8*	6.0 $\pm$ 0.8*	25.3 $\pm$ 4.1*	3.2 $\pm$ 0.4*	9.3 $\pm$ 2.8**
Dendritic spines	7.4 $\pm$ 0.9*	6.1 $\pm$ 0.8*	9.3 $\pm$ 1.1*	9.9 $\pm$ 1.8*	10.1 $\pm$ 1.4*	20.8 $\pm$ 2.7*	4.8 $\pm$ 1.0*	9.8 $\pm$ 2.0**
Astroglial processes	5.6 $\pm$ 0.8*	5.7 $\pm$ 0.9*	7.6 $\pm$ 0.9*	5.8 $\pm$ 1.0*	4.9 $\pm$ 0.9*	18.1 $\pm$ 2.1*	2.0 $\pm$ 0.4*	8.0 $\pm$ 2.1***
Capillary luminae	1.2 $\pm$ 0.2*	1.2 $\pm$ 0.1*	1.0 $\pm$ 0.2*	1.9 $\pm$ 0.2*	0.6 $\pm$ 0.2*	6.1 $\pm$ 0.5*	0.6 $\pm$ 0.2*	1.8 $\pm$ 0.7**

The mean number of colloidal gold particles per $1 \mu\text{m}^2 \pm \text{SEM}$ is listed for each type of subcellular profile. The data do not include gold particles present on mitochondrial profiles. The total number of profiles counted in each case is given in parentheses. Asterisks indicate significant differences (independent Student's *t* test, * $p < 0.001$, ** $p < 0.005$) in mean gold particle density between terminals forming asymmetric synapses and other subcellular profiles. Values for capillary luminae were considered background labeling. Animals 1, 2, 3, 4, and 7 were perfused with fixative 2 and double labeled with anti-3HAO antibody. Animal 6 was perfused with fixative 3. Animal 7 was additionally treated with paraformaldehyde vapor before incubation with glutamate antibody to prevent nonspecific staining with the secondary antibody. Such nonspecific staining would occur only in astroglial processes, where both 3HAO and glutamate are localized.

*Mean value for astroglial processes does not include animal 7.

Table 3. Glutamate label in axon terminals forming asymmetric synapses compared to other profiles

Profile	Animal							Mean
	1	2	3	4	5	6	7	
Sym. syn. terminals	6.5:1	4.7:1	4.0:1	4.0:1	3.9:1	8.8:1	3.9:1	5.1:1
Dendrites	5.4:1	3.9:1	3.5:1	9.8:1	5.5:1	3.6:1	7.3:1	4.6:1
Dendritic spines	6.1:1	5.1:1	3.6:1	3.8:1	3.1:1	4.7:1	4.5:1	4.3:1
Astroglial processes	8.9:1	5.5:1	4.6:1	7.8:1	7.0:1	5.8:1	13.5:1*	5.6:1 ^a

Ratios of the number of gold particles/ μm^2 in terminals forming asymmetric synapses are compared to other subcellular profiles. In all cases, values were adjusted for background labeling by subtracting values for capillary luminae prior to determining ratios.

* High ratio between terminals forming asymmetric synapses and astroglial processes due to low labeling of glial processes in the paraformaldehyde vapor-treated animal 7.

^a Mean ratio for astroglial processes does not include animal 7.

processes is lower in the striatum than in the substantia nigra pars compacta.

The results of the experiments with the anti-glutamate antibody provide a quantitative description of the subcellular distribution of glutamate in the striatum, and are consistent with current concepts regarding the nature and function of the striatal glutamatergic system (see introductory remarks). Thus, striatal profiles containing the most intense glutamate labeling were axon terminals forming asymmetric synapses, typical of cortical and thalamic input (Kemp and Powell, 1971). In agreement with previous studies in the hippocampus and the cerebellum, many gold particles in boutons were associated with synaptic vesicles (Somogyi et al., 1986; Broman and Ottersen, 1992; Ottersen et al., 1992). Consistent with the fact that glutamate also exists in nontransmitter pools, for example, as a participant in intermediary metabolism (Fonnum, 1984), glutamate labeling was also observed in several other subcellular compartments. For example, glutamate is transported into mitochondria (Dennis et al., 1976; Minn and Gayet, 1977), the primary site of glutamate synthesis by phosphate-activated glutaminase (Salganicoff and DeRobertis, 1965; Erecińska and Silver, 1990); accordingly, mitochondrial profiles were moderately to heavily labeled. Astrocytes, which actively accumulate glutamate (Drejer et al., 1982; Fonnum, 1984), possessed lower intensities of glutamate labeling than axon terminals forming asymmetric synapses (Ottersen et al., 1992 and references therein).

As a selective yet rather weak NMDA receptor agonist (Stone and Perkins, 1981), QUIN can be expected to affect NMDA receptors provided that its synaptic concentrations are sufficiently high. The concentration of 10–20 nM, which is usually given based on QUIN measurements in cerebrospinal fluid (Moroni et al., 1986; Schwarcz et al., 1988b; Heyes et al., 1991) or brain microdialysates (During et al., 1989; Speciale et al., 1989), has been repeatedly judged to be too low for receptor activation, which reportedly requires at least 1000 times more QUIN (Martin and Lodge, 1987; Peters and Choi, 1987). 3HAO-labeled astrocytic processes were found to be in close apposition to asymmetric synapses which typically mediate glutamatergic neurotransmission, as well as with symmetric synapses which are preferentially linked to inhibitory neurotransmission (Ribak et al., 1979). These anatomical arrangements imply that discrete synaptic QUIN concentrations may in fact exceed previous estimates, and suggest a role of QUIN as an endogenous participant in NMDA receptor function and dysfunction in the rat striatum. Notably, neostriatal neurons, including medium-sized and large cells, contain the NMDAR1 receptor subunit (Petralia et al., 1994) and the mRNA encoding the NMDA receptor (Ge et

al., 1992). It is therefore certainly possible that striatal synapses engulfed by QUIN-producing processes carry functional NMDA receptors. Colocalization of the NMDA receptor and 3HAO at the ultrastructural level is currently underway in our laboratories.

In spite of the anatomical features of QUIN-producing astrocytes, which are suggestive of a role of QUIN in glutamatergic transmission in the striatum, definitive proof of such a role will have to await a better understanding of QUIN neurobiology in the normal and abnormal brain. For example, while it has been established that astrocytes have a limited capacity to store newly synthesized QUIN and are capable of releasing QUIN into the extracellular compartment (Speciale and Schwarcz, 1993), the metabolic control of brain 3-hydroxyanthranilic acid, the substrate of 3HAO, has so far not been elucidated. Moreover, the function of the QUIN-degrading enzyme quinolinic acid phosphoribosyltransferase, which is also largely contained in astrocytes (Köhler et al., 1987; Du et al., 1991), has not been clarified to date. Eventually, relevant information is expected to come from studies using selective pharmacological agents such as 3HAO inhibitors (Walsh et al., 1991, 1994).

The astrocytic localization of 3HAO is in agreement with biochemical studies which have shown large increases in 3HAO activity following destruction of neurons in rats (Speciale et al., 1987; Schwarcz et al., 1989; Saito et al., 1993) or in HD (Schwarcz et al., 1988a). Thus, the increased production and release of QUIN in lesioned tissue, which has been verified directly in neuron-depleted striatal slices (Speciale and Schwarcz, 1993), is likely due to the astroglial reaction to neurodegeneration (Björklund et al., 1986). Although alternative interpretations regarding the lesion-induced increase in QUIN production, and of the cellular source of brain QUIN in general, have been purported (Moffett et al., 1993; Saito et al., 1993), recent ultrastructural studies of 3HAO-i in the excitotoxin-lesioned rat striatum have confirmed the prominent location of the enzyme in normal and reactive astrocytes (McCarthy et al., 1993).

While the QUIN model of HD, effected by acute or subchronic (DiFiglia, 1990; Bazzett et al., 1993) intrastriatal injection of the toxin into experimental animals, has been widely used, arguments have been raised against the proposition that a pathological overabundance of endogenous QUIN is causally involved in the neurodegenerative process. Thus, in spite of increased 3HAO activity in the HD brain, which is particularly prominent in the basal ganglia (Schwarcz et al., 1988), QUIN levels measured in brain tissue homogenates (Reynolds et al., 1988) or cerebrospinal fluid (Schwarcz et al., 1988b) are not elevated in HD and in fact show a tendency to decrease (Heyes et al., 1991). In light of the physical proximity of QUIN-pro-

Figure 5. Several examples of striatal neuropil double labeled with antibodies against 3HAO and glutamate (fixative 2). 3HAO-labeled glial processes (*g*) are adjacent to glutamate-labeled axon terminals (*at*) forming asymmetric synapses (*s*) with spines (*s*). *A*, Glutamate-labeled axon terminals forming asymmetric synapses are abutted by 3HAO-labeled glial processes. Note the presence of an unlabeled terminal (*) forming a symmetric synapse (*arrowhead*) with a dendrite. The *open arrow* indicates a thin 3HAO-labeled glial process. *B–D*, Glutamate-labeled axon terminals forming asymmetric synapses abutted or partially surrounded by 3HAO-labeled glial processes (fixative 2). (Note that fixative containing glutaraldehyde is necessary for glutamate immunocytochemistry, but results in the confinement of 3HAO labeling mainly to large-caliber glial processes, though fine 3HAO-labeled processes can be occasionally detected). Scale bars, 0.5 μ m.

Figure 6. Diagrammatic representation of postulated actions of astrocyte-derived quinolinic acid at a glutamatergic synapse in the rat striatum. 3HANA, 3-hydroxyanthranilic acid.

ducing astrocytic processes and glutamatergic synapses, it now seems prudent to view the putative neurotoxic roles of QUIN and glutamate *in tandem*; that is, to study the possible functional interdependence of the classical neurotransmitter glutamate and astrocyte-derived QUIN (Fig. 6). Synergism between these two excitatory amino acid receptor agonists has been demonstrated in hypoxic hippocampal slices (Schurr and Rigor, 1993), and similar scenarios, involving interactions at and beyond the NMDA recognition site, can be envisioned to play a role in HD neurodegeneration. Moreover, it may be of significance that kynurenine aminotransferase, the biosynthetic enzyme for the endogenous neuroprotectant kynurenic acid, too, is localized in astrocytic processes adjacent to asymmetric striatal synapses (Roberts et al., 1992). Therefore, potential interactions at the synaptic level of glutamate, QUIN, and kynurenic acid, a broad-spectrum glutamate receptor antagonist, must be considered with regard to the occurrence or prevention of neuronal damage. More generally, such mechanisms may constitute a conceptual modification of classic excitotoxic theory and could be applicable to a spectrum of neurodegenerative brain diseases.

References

- Adams JC (1992) Biotin amplification of biotin and horseradish peroxidase signals in histochemical stains. *J Histochem Cytochem* 40:1457–1463.
- Bazzett TJ, Becker JB, Kaatz KW, Albin RL (1993) Chronic intrastriatal dialytic administration of quinolinic acid produces selective neural degeneration. *Exp Neurol* 120:177–185.
- Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KF, Martin JB (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* 321:168–171.
- Björklund H, Olson L, Dahl L, Schwarcz R (1986) Short- and long-term consequences of intracranial injections of the excitotoxin quinolinic acid as evidenced by GFA immunohistochemistry of astrocytes. *Brain Res* 371:267–277.
- Broman J, Ottersen OP (1992) Cervicothalamic tract terminals are enriched in glutamate-like immunoreactivity: an electron microscopic double-labeling study in the cat. *J Neurosci* 12:204–221.
- Broman J, Anderson S, Ottersen OP (1993) Enrichment of glutamate-like immunoreactivity in primary afferent terminals throughout the spinal cord dorsal horn. *Eur J Neurosci* 5:1050–1061.
- Coyle JT, Schwarcz R (1976) Lesion of striatal neurons with kainic acid provides a model for Huntington's chorea. *Nature* 263:244–246.
- Dennis SC, Land JM, Clark JB (1976) Glutamate metabolism and transport in rat brain mitochondria. *Biochem J* 156:323–331.
- DiFiglia M (1990) Excitotoxic injury of the neostriatum: a model for Huntington's disease. *Trends Neurosci* 13:286–289.
- DiFiglia M, Christakos S, Aronin N (1989) Ultrastructural localization of immunoreactive calbindin-D_{28k} in the rat and monkey basal ganglia, including subcellular distribution with colloidal gold labeling. *J Comp Neurol* 279:653–665.
- Divac I, Fonnum F, Storm-Mathisen J (1977) High affinity uptake of glutamate in terminals of corticostriatal axons. *Nature* 266:377–378.
- Drejer J, Larsson OM, Schousboe A (1982) Characterization and L-glutamate uptake into and release from astrocytes and neurons cultured from different brain regions. *Exp Brain Res* 47:259–269.
- Du F, Okuno E, Whetsell WO Jr, Köhler C, Schwarcz R (1991) Immunohistochemical localization of quinolinic acid phosphoribosyltransferase in the human neostriatum. *Neuroscience* 42:397–406.
- During MJ, Heyes MP, Freese A, Markey SP, Martin JP, Roth RH (1989) Quinolinic acid concentrations in striatal extracellular fluid reach potentially neurotoxic levels following systemic L-tryptophan loading. *Brain Res* 476:384–387.
- Erecińska M, Silver IA (1990) Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* 35:245–296.
- Fonnum F (1984) Glutamate: a transmitter in mammalian brain. *J Neurochem* 42:1–11.
- Foster AC, White RJ, Schwarcz R (1986) Synthesis of quinolinic acid by 3-hydroxyanthranilic acid oxygenase in rat brain tissue *in vitro*. *J Neurochem* 47:23–30.
- Ge P, Aizawa H, Lester J, Sapp E, Aronin N, VonSattel J-P, DiFiglia M (1992) Distribution of NMDA receptor mRNA in rat neostriatal neurons. *Soc Neurosci Abstr* 18:308.
- Hassler R, Chung JW, Rinne V, Wagner A (1978) Selective degener-

- ation of two out of nine types of synapses in cat caudate nucleus after cortical lesions. *Exp Brain Res* 31:67–80.
- Herrling PL (1985) Pharmacology of the cortico-caudate excitatory postsynaptic potential in the cat: evidence for its mediation by quisqualate- or kainate-receptors. *Neuroscience* 14:417–426.
- Heyes MP, Swartz KJ, Markey SP, Beal MF (1991) Regional brain and cerebrospinal fluid quinolinic acid concentrations in Huntington's disease. *Neurosci Lett* 122:265–269.
- Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29:577–580.
- Kemp JM, Powell TPS (1971) The site of termination of afferent fibres in the caudate nucleus. *Philos Trans R Soc Lond [Biol]* 262:413–427.
- Kim J-S, Hassler R, Haug P (1977) Effect of frontal cortex ablation on striatal glutamic acid level in rat. *Brain Res* 132:370–374.
- Köhler C, Okuno E, Flood PR, Schwarcz R (1987) Quinolinic acid phosphoribosyltransferase: preferential glial localization in the rat brain visualized by immunocytochemistry. *Proc Natl Acad Sci USA* 84:3491–3495.
- Köhler C, Eriksson LG, Flood PR, Hardie JA, Okuno E, Schwarcz R (1988a) Quinolinic acid metabolism in the rat brain. Immunohistochemical identification of 3-hydroxyanthranilic acid oxygenase and quinolinic acid phosphoribosyltransferase in the hippocampal region. *J Neurosci* 8:975–987.
- Köhler C, Eriksson LG, Okuno E, Schwarcz R (1988b) Localization of quinolinic acid metabolizing enzymes in the rat brain. Immunohistochemical studies using antibody to 3-hydroxyanthranilic acid oxygenase and quinolinic acid phosphoribosyltransferase. *Neuroscience* 27:49–76.
- Mangano RM, Schwarcz R (1983) Chronic infusion of endogenous excitatory amino acids into rat striatum and hippocampus. *Brain Res Bull* 10:47–51.
- Martin D, Lodge D (1987) Biphasic effect of quinolinate on frog spinal, but not rat cortical, neurones: *N*-methyl-D-aspartate-like depolarization and a novel type of hyperpolarization. *Neurosci Lett* 75:175–180.
- Martin LJ, Blackstone CD, Haganir RL, Price DL (1992) Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron* 9:259–270.
- Martin LJ, Blackstone CD, Levey AI, Haganir RL, Price DL (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* 53:327–358.
- McCarthy KE, Ottersen OP, Okuno E, Schwarcz R, Roberts RC (1993) The ultrastructural relationship of glutamate immunoreactive terminals and 3-hydroxyanthranilic acid oxygenase labeled astroglial processes in the rat substantia. *Soc Neurosci Abstr* 19:782.
- McGeer PL, McGeer EG (1976) Duplication of biochemical changes of Huntington's chorea by intrastratial injection of glutamic or kainic acids. *Nature* 263:517–519.
- McGeer PL, McGeer EG, Scherer U (1977) A glutamatergic corticostriatal path? *Brain Res* 128:369–373.
- McLean IW, Nakane PK (1974) Periodate-lysine-paraformaldehyde fixative: a new fixative for immunoelectron microscopy. *J Histochem Cytochem* 22:1077–1083.
- Meshul CK, Stallbaumer RK, Taylor B, Janowsky A (1992) Haloperidol-induced morphological changes in striatum are mediated via glutamate synapses. *Soc Neurosci Abstr* 18:1042.
- Miller RH, Raff MC (1984) Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. *J Neurosci* 4:585–592.
- Minn A, Gayet T (1977) Kinetic study of glutamate transport in rat brain mitochondria. *J Neurochem* 29:873–881.
- Moffett JR, Espey MG, Gaudet SJ, Aryan Namboodiri MA (1993) Antibody to quinolinic acid reveal localization in select immune cells rather than neurons or astroglia. *Brain Res* 623:337–340.
- Monaghan DT, Cotman CW (1985) Distribution of *N*-methyl-D-aspartate-sensitive L-[³H]glutamate-binding sites in rat brain. *J Neurosci* 5:2909–2919.
- Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu Rev Pharmacol Toxicol* 29:365–402.
- Moroni F, Lombardi G, Carlà V, Lal S, Etienne P, Nair NPV (1986) Increase in the content of quinolinic acid in cerebrospinal fluid and frontal cortex of patients with hepatic failure. *J Neurochem* 47:1667–1671.
- Okuno E, Köhler C, Schwarcz R (1987) Rat 3-hydroxyanthranilic acid oxygenase: purification from the liver and immunocytochemical localization in the brain. *J Neurochem* 49:771–780.
- Ottersen OP, Storm-Mathisen J, Madsen S, Skumlien S, Stromhang J (1986) Evaluation of the immunocytochemical method for amino acids. *Med Biol* 64:147–158.
- Ottersen OP, Zhang N, Walberg F (1992) Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. *Neuroscience* 46:519–534.
- Pasik P, Pasik T, DiFiglia M (1980) The internal organization of the neostriatum in mammals. In: *The neostriatum* (Divac I, Oberg RGE, eds), pp 5–36. Oxford: Pergamon.
- Perschak H, Cuénod M (1990) *In vivo* release of endogenous glutamate and aspartate in the rat striatum during stimulation of the cortex. *Neuroscience* 35:283–287.
- Peters S, Choi DW (1987) Quinolinate is a weak excitant of cortical neurons in cell culture. *Brain Res* 420:1–10.
- Petralia RS, Yokotani N, Wenthold RJ (1994) Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci* 14:667–696.
- Reynolds GP, Pearson SJ, Halket J, Sandler M (1988) Brain quinolinic acid in Huntington's disease. *J Neurochem* 50:1959–1960.
- Ribak CE, Vaughn JE, Roberts E (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. *J Comp Neurol* 274:406–421.
- Roberts RC, Du F, McCarthy KE, Okuno E, Schwarcz R (1992) Immunocytochemical localization of kynurenine aminotransferase in the rat striatum: a light and electron microscopic study. *J Comp Neurol* 326:82–90.
- Roberts RC, Ahn A, Swartz KJ, Beal MF, DiFiglia M (1993) Intrastriatal injections of quinolinic acid or kainic acid: differential patterns of cell survival and the effects of data analysis on outcome. *Exp Neurol* 124:274–282.
- Roberts RC, McCarthy KE, Du F, Okuno E, Schwarcz R (1994) Immunocytochemical localization of 3-hydroxyanthranilic acid oxygenase (3HAO) in the rat substantia nigra. *Brain Res*, 650:229–238.
- Saito K, Nowak TS Jr, Suyama K, Quacary BJ, Saito M, Crowley JS, Markey SP, Heyes MP (1993) Kynurenine pathway enzymes in brain: responses to ischemic brain injury versus systemic immune activation. *J Neurochem* 61:2061–2070.
- Salganicoff L, DeRobertis E (1965) Subcellular distribution of the enzymes of the glutamic acid, glutamine and γ -aminobutyric acid cycles in brain. *J Neurochem* 12:287–309.
- Schurr A, Rigor BM (1993) Quinolinate potentiates the neurotoxicity of excitatory amino acids in hypoxic neuronal tissue *in vitro*. *Brain Res* 617:76–80.
- Schwarcz R, Du F (1991) Quinolinic acid and kynurenine acid in the mammalian brain. In: *Kynurenine and serotonin pathways: progress in tryptophan research* (Schwarcz R, Young SN, Brown RR, eds), pp 185–199. New York: Plenum.
- Schwarcz R, Whetsell WO Jr, Mangano RM (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* 219:316–318.
- Schwarcz R, Okuno E, White RJ, Bird ED, Whetsell WO Jr (1988a) 3-Hydroxyanthranilic acid oxygenase activity is increased in the brains of Huntington disease victims. *Proc Natl Acad Sci USA* 85:4079–4081.
- Schwarcz R, Tamminga CA, Kurlan R, Shoulson I (1988b) Cerebrospinal fluid levels of quinolinic acid in Huntington's disease and schizophrenia. *Ann Neurol* 24:580–582.
- Schwarcz R, Okuno E, White RJ (1989) Basal ganglia lesions in the rat: effects on quinolinic acid metabolism. *Brain Res* 490:103–109.
- Shehab SAS, Cronly-Dillon JR, Nona SN, Stafford CA (1990) Preferential histochemical staining of protoplasmic and fibrous astrocytes in rat CNS with GFAP antibodies using different fixatives. *Brain Res* 518:347–352.
- Snyder GL, Fisone G, Morino P, Gundersen V, Ottersen OP, Hökfelt T, Greengard P (1993) Regulation by the neuropeptide cholecystokinin (CCK-8S) of protein phosphorylation in the neostriatum. *Proc Natl Acad Sci USA* 90:11277–11281.
- Somogyi P, Hodgson AJ (1985) Antisera to γ -aminobutyric acid. III.

- Demonstration of GABA in Golgi-impregnated neurons and in conventional electron microscopic sections of cat striate cortex. *J Histochem Cytochem* 33:249–257.
- Somogyi P, Halasy K, Somogyi J, Storm-Mathisen J, Ottersen OP (1986) Quantification of immunogold labelling reveals enrichment of glutamate in mossy and parallel fiber terminals in cat cerebellum. *Neuroscience* 19:1045–1050.
- Speciale C, Schwarcz R (1993) On the production and disposition of quinolinic acid in rat brain and liver slices. *J Neurochem* 60:212–218.
- Speciale C, Okuno E, Schwarcz R (1987) Increased quinolinic acid metabolism following neuronal degeneration in the rat hippocampus. *Brain Res* 436:18–24.
- Speciale C, Ungerstedt U, Schwarcz R (1989) Production of extracellular quinolinic acid in the striatum studied by microdialysis in unanesthetized rats. *Neurosci Lett* 104:345–350.
- Stone TW, Perkins MN (1981) Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. *Eur J Pharmacol* 72:411–412.
- Walsh JL, Todd WP, Carpenter BK, Schwarcz R (1991) 4-Halo-3-hydroxyanthranilic acids: potent competitive inhibitors of 3-hydroxyanthranilic acid oxygenase *in vitro*. *Biochem Pharmacol* 42:985–990.
- Walsh JL, Wu H-Q, Ungerstedt U, Schwarcz R (1994) 4-Chloro-3-hydroxyanthranilate inhibits quinolinate production in the rat hippocampus *in vivo*. *Brain Res Bull* 33:513–516.
- Wang BL, Larsson LI (1985) Simultaneous demonstration of multiple antigens by indirect immunofluorescence or immunogold staining. Novel light and electron microscopical double label and triple staining method employing primary antibody from the same species. *Histochemistry* 83:47–56.
- Wolfensberger M, Amsler U, Cuénod M, Foster AC, Whetsell WO Jr, Schwarcz R (1983) Identification of quinolinic acid in rat and human brain tissue. *Neurosci Lett* 41:247–252.