EXPERIMENTAL NEUROLOGY 120, 177–185 (1993)

Chronic Intrastriatal Dialytic Administration of Quinolinic Acid Produces Selective Neural Degeneration

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The excitotoxic hypothesis of Huntington's disease pathogenesis suggests that selective striatal neuronal loss results from excessive activation of striatal excitatory amino acid receptors. Using a microdialysis probe mated to an Alzet 2002 mini-osmotic pump three different concentrations of quinolinic acid or vehicle were administered to the striata of rats over a 3-week period. Animals that received a total of 3.3 μmol of quinolinic acid had significant striatal atrophy that could be attributed to two distinct areas of neuronal loss. First, an area of necrosis surrounding the probe was marked by inflammatory infiltrate and a lack of neurons. In the second region, surrounding the necrotic area, there was a significant reduction in nissl-stained cells, with relative sparing of NADPH–diaphorase-staining neurons. In addition, there was a reduction in cytochrome oxidase staining throughout both of the areas of cell loss. Beyond the area of cell loss, the striatum appeared normal in all respects. The striata of animals that received 880 nmol quinolinic acid appeared identical to those that received vehicle. The striata of animals that received 8.8 μmol quinolinic acid showed severe nonselective atrophy of the striatum and some surrounding structures. We conclude that dialytic delivery of 3.3 μmol quinolinic acid produces an area of neuronal destruction that resembles the selective neuronal loss seen in Huntington's disease. This selective neurodegeneration produced by chronic exposure to quinolinic acid simulates more closely the course of Huntington's disease than previously described methods.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disease marked by involuntary movements, psychiatric disorders, and dementia. The neuropathological hallmark of HD is striatal atrophy resulting from loss of striatal neurons. Current evidence suggests that neuronal degeneration in HD particularly affects the GABAergic medium spiny projection neurons that form the great majority of striatal neurons (6, 21). Some populations of striatal interneurons are spared in HD (22). One group of selectively spared neurons has been identified as the striatal aspiny cells containing somatostatin, neuropeptide Y, and the enzyme NADPH–diaphorase/nitric oxide synthase (19, 20, 22).

Although the HD gene has been localized to chromosome 4, the processes underlying neurodegeneration in HD are still unknown. One popular theory, the excitotoxic hypothesis, suggests that neurodegeneration may be the result of excessive activation or abnormal function of excitatory amino acid (EAA) neurotransmitter receptors (1, 5, 14, 17, 21, 28, 39).

Quinolinic acid (QUIN), is a tryptophan metabolite of the kynurenine pathway and is an agonist at one subtype of EAA receptor, the *N*-methyl-D-aspartate (NMDA) receptor (14, 36, 37). Beal and colleagues have reported that acute administration of a high concentration of QUIN into rat striatum produces selective neurodegeneration similar to that seen in HD (6, 7, 9). Other researchers using similar administration procedures for QUIN have been unable to duplicate the selective sparing of neuronal subpopulations (12, 13, 18, 33). In addition, there are conceptual difficulties in using an acute high-dose injection procedure to produce a model of a disease that is chronic and progressive in nature. Chronic administration of a low dose of QUIN may produce a more accurate model of the progressive degenerative processes hypothesized to occur due to excitotoxic neuronal injury in HD.

In the present report, we describe the effects of chronically administered QUIN on striatal morphology and amphetamine-induced rotational behavior. We have recently developed a technique with which excitotoxins can be delivered to the rat striatum over approximately 3 weeks (4). This method utilizes *in vivo* microdialysis techniques and allows chronic elevation of QUIN in the striatum via diffusion down a concentration gradient without introducing additional fluid into the brain. The parameters of this method more closely approximate those suggested by the excitotoxic hypothesis of HD than do other currently described methods.

0014-4886/93 $5.00

METHODS

Animals and Protocol Overview

Adult male and female Sprague–Dawley rats were housed under a 14:10 (light:dark) cycle with food and water available *ad libitum*. Both aged (12–24 months) and young (6–9 months) rats were used. Quinolinic acid solutions (4, 15, and 40 m*M*) or vehicle flowed continuously from an Alzet mini-osmotic pump through a dialysis probe apparatus held in place within the striatum via a chronically implanted guide cannula. Forty millimolar QUIN was administered to 4 young and 6 aged male rats that were used for final analysis. Four millimolar QUIN was administered to 4 young and 6 aged female rats and to 3 young male rats that were used for final analysis. Data from young and aged animals were combined since no differences were found between these two groups. In addition no differences were apparent between male and female rats receiving 4 m*M* QUIN. Fifteen millimolar QUIN was administered to a total of 13 young male rats that were used for final analysis. A total of 23 rats received vehicle (aged: 4 female, 5 male; young: 4 female, 10 males). The time course for dialytic delivery was determined using the mean fill volume and mean delivery rate for minipumps. The average time course of delivery was 18 days. All probes were left in the striatum for at least 21 days.

Surgery

Cannula implantation. For surgery, animals were anesthetized with pentobarbital (30 mg/kg) supplemented with methoxyfluorane. For each rat, a stainless steel cannula (8 mm, 21 gauge) was implanted through the skull aimed at the right striatum (stereotaxic coordinates: 0.5 mm anterior, 2.6 mm lateral from bregma, 1 mm ventral from skull surface). The cannula was secured in place with dental acrylic and kept patent with a stylet.

Dialytic apparatus and implantation. The construction and detailed design of the chronic dialysis probe apparatus are described elsewhere (4). Briefly, the dialysis probe is constructed of 26-gauge thin-wall stainless steel tubing with a 4-mm dialysis fiber tip. When inserted into the guide cannula, the dialysis fiber tip of the probe extends from approximately −3.5 mm to −7.5 mm ventral from the skull surface. The mini-osmotic pump provides continuous flow through the dialysis probe via an 8-cm length of PE20 tubing. The apparatus is also designed with a 6-cm length of outlet tubing and collection vesicle made of silastic tubing sealed with medical-grade silastic adhesive.

One day prior to probe implantation osmotic pumps (Alzet Model 2002, mean fill volume approx 220 μl) were filled with 0, 4, 15, or 40 m*M* quinolinic acid (Sigma) dissolved in 0.1 *M* phosphate-buffered 0.9% saline (PBS, pH 7.4). Pumps were then allowed to activate for 24 h at room temperature in PBS. The total amounts of QUIN delivered were: 0 nmol, 880 nmol (2.0 nmol/h), 3.3 μmol (7.6 nmol/h), and 8.8 μmol (20.4 nmol/h) respectively.

One week after baseline testing for rotational behavior, animals were anesthetized with pentobarbital (30 mg/kg). For each animal a chronic dialysis probe was inserted through the guide cannula and secured in place with dental acrylic. The mini-osmotic pump and collection vesicle were secured subcutaneously through a small incision in the back of the neck.

Rotational Behavior

One week following cannula implant surgery, animals were tested for rotational behavior following D-amphetamine sulfate administration (3.0 mg/kg, i.p.; AMPH). Test periods were 2 h, and 360° turns were monitored using automated rotometers (27). One week following removal of dialysis probes, animals were retested for AMPH-induced rotational behavior once a week, for 5 weeks.

Tissue Preparation

All animals were given an overdose of pentobarbitol and perfused through the heart with PBS followed by 4% paraformaldehyde in 0.1 *M* phosphate buffer. Brains were postfixed overnight at 4°C and cryoprotected in 20% sucrose/phosphate buffer.

Histology and Histochemistry

For histology and histochemistry, sucrose-protected brains were frozen in crushed dry ice and consecutive 40-μm coronal sections were cut on a sliding microtome and collected in 10 vials. Sections were stored at 4°C in 0.1 *M* phosphate buffer plus 0.02% sodium azide.

NADPH–diaphorase. Free-floating sections were rinsed 2× 1 min in 0.1 *M* Tris-buffered saline (TBS, pH 8.0) and then incubated for 20 min at 37°C in a NADPH–diaphorase staining solution containing 12 mg malic acid (Sigma), 10 mg nitro blue tetrazolium (Sigma), and 4 mg NADPH (Sigma) in 10 ml TBS plus 0.8% Triton X-100. Following incubation, sections were again rinsed 2× 1 min in TBS, mounted on gelatin coated microscope slides, and coverslipped with DPX mountant.

Cytochrome oxidase. Free-floating sections were incubated for 6 h at 37°C in a cytochrome oxidase solution containing 2.5 mg cytochrome c (Sigma), 2 mg catalase (Sigma), 5 mg diaminobenzidine (Sigma), and 400 mg sucrose in 10 ml 0.1 *M* phosphate buffer. After the ini-

tial incubation period, the tissue was incubated in the same solution overnight at room temperature. Sections were mounted on gelatin-coated microscope slides and coverslipped with DPX mountant.

Cresyl violet. Sections were stained in 0.5% cresyl violet, dehydrated in graded alcohols and xylene, and coverslipped with Permount.

Histochemical Quantification

For animals receiving 4 and 15 m*M* QUIN, quantification of nissl-stained cells was accomplished using the optical dissector technique as described by Coggeshall (16). Cells were counted using a 10 × 10-mm ocular lens grid. Briefly, nissl-positive cells were identified by positive cresyl violet staining that distinguished cytoplasmic features from the nucleus or membrane in neurons with a diameter ranging from approximately 10 to 16 μm. Cresyl violet-stained cells were not excluded from the analysis for morphological abnormalities (i.e., swelling or elongation) or reduced intensity of nissl staining. NADPH–diaphorase neurons were identified by a positive reaction to the diaphorase staining solution. NADPH-diaphorase stained cells were not excluded for morphological abnormalities (i.e., varicose fibers or reduced dendritic branching) or for mildly reduced intensity of NADPH–diaphorase staining.

For each animal, neurons were counted in the section with the most clearly defined dialysis probe tract. Areas evaluated were designated with respect to the area of necrosis surrounding the probe tract (as indicated by inflammatory infiltrate and a lack of larger nissl staining cells). Beal and colleagues (9) previously termed this necrotic area the lesion core. Six regions were quantified: 0–200 μm and 200–400 μm, medial and lateral from the lesion core, and 400–600 μm and 600–800 μm lateral from the lesion core. Cell counts from four to eight 200-μm^2 areas were used to quantify neuronal populations for each region in the lesioned striatum. The number of cells for each area in the lesioned striatum was then compared to the number of cells in the same area of the intact striatum. Cells were expressed as mean number per volume (1.6×10^{-3} mm^3) of tissue for each region. There was no significant difference between medial and lateral regions, so this data was combined to yield a single value for the regions 0–200 and 200–400 μm radial to the lesion core.

Statistics

Paired Student's *t* tests were used to compare pre- and posttreatment behavioral test scores and to compare the cell number in the probe vs that in intact striatum.

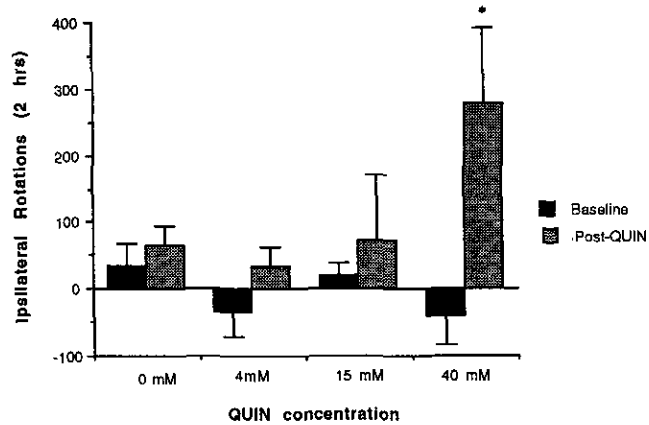

FIG. 1. Effects of quinolinic acid on amphetamine-induced rotational behavior (3 mg/kg, i.p.). Only animals receiving a concentration of 40 m*M* (8.8 μmol total) QUIN showed a significant increase in ipsilateral rotations using a paired Student *t* test (*$P < 0.05$). Solid bars represent preprobe implant (+SEM). Hatched bars represent post-QUIN administration (+SEM).

RESULTS

Rotational Behavior

The group of animals that received 40 m*M* ($n = 10$) QUIN showed a significant increase in AMPH-induced ipsilateral rotations compared to their baseline scores before probe implantation. Rotations in animals receiving 0 ($n = 17$), 4 ($n = 10$), and 15 m*M* ($n = 7$) QUIN were not significantly different from their baseline scores (Fig. 1).

Histology and Histochemistry

Cresyl violet staining shows the effects of dialytic delivery of 0, 4, 15, and 40 m*M* QUIN over a 3-week period (Fig. 2). Four millimolar QUIN ($n = 10$) produced no apparent changes when compared to vehicle ($n = 17$) administration. Fifteen millimolar QUIN ($n = 10$) produced a necrotic region surrounding the probe and some atrophy of the striatum. Forty millimolar QUIN ($n = 10$) caused extensive destruction with atrophy of the entire striatum and some surrounding structures.

Cytochrome oxidase staining was also used to examine the extent of injury in animals receiving 15 m*M* QUIN compared to those receiving vehicle. The striata of animals that received 15 m*M* QUIN ($n = 10$) had an area of decreased cytochrome oxidase staining extending beyond the lesion core, compared to striata of animals receiving vehicle ($n = 17$) administration (Fig. 3).

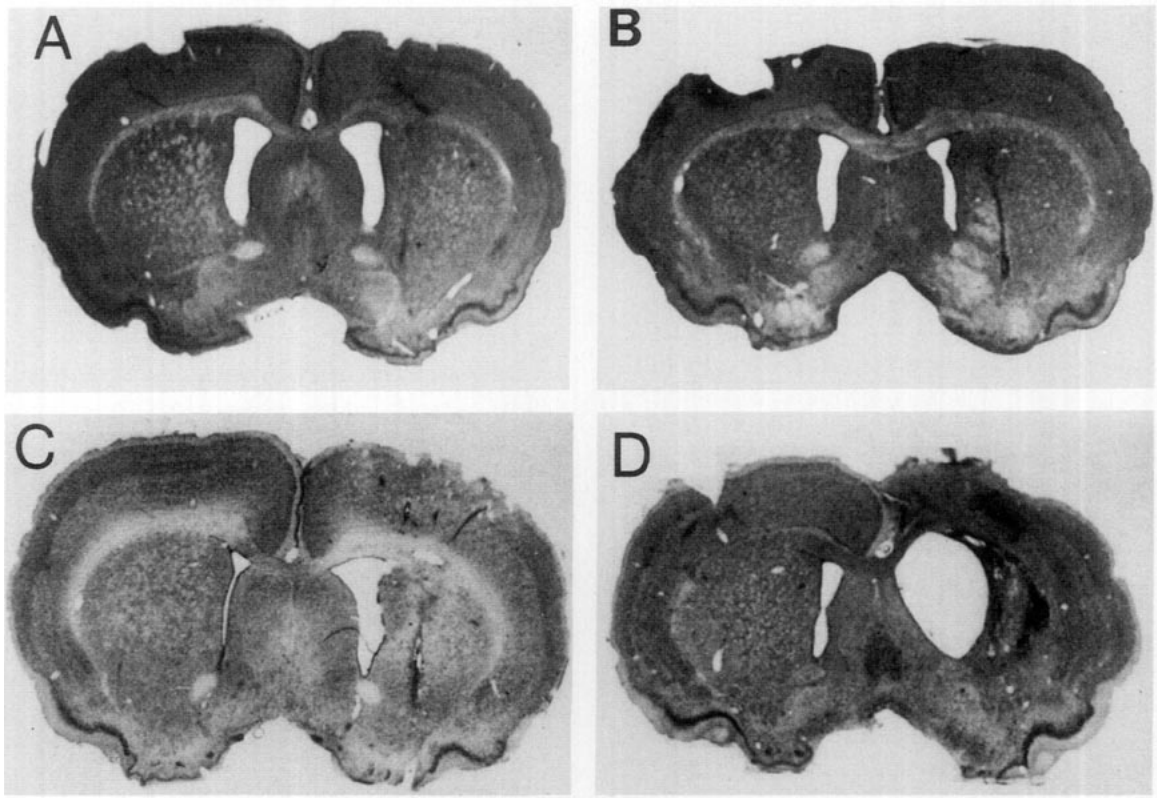

FIG. 2. Cresyl violet-stained sections from animals receiving concentrations of 0, 4, 15, and 40 m*M* QUIN. (A) Vehicle. (B) 4 m*M* (880 nmol total) QUIN. No apparent damage was evident surrounding the probe tract. (C) 15 m*M* (3.3 μmol total) QUIN. A necrotic zone surrounding the probe tract is apparent. In addition, some atrophy of the striatum is apparent compared to the intact side. (D) 40 m*M* (8.8 μmol total) QUIN. Severe atrophy of the entire striatum and some surrounding structures is apparent compared to the intact side.

Quantification of Nissl and NADPH–Diaphorase Cells

Nissl and NADPH–diaphorase striatal cells were quantified in seven animals that received 15 m*M* QUIN. There was a significant decrease in nissl-stained cells in the regions 0–200 and 200–400 μm from the lesion core, when compared to the same regions in the intact striatum. Furthermore, this decrease appeared to represent a gradient of cell loss, since there were significantly fewer cells from 0–200 μm than from 200–400 μm. No significant decrease in nissl-stained cells was found in the regions 400–600 or 600–800 μm from the lesion core (Fig. 4a).

The only region showing a decrease in the number of NADPH–diaphorase cells was the region 0–200 μm from the lesion core (Fig. 4b). Although this decrease indicates that there was not absolute sparing of NADPH–diaphorase neurons in this region, the percentage of these cells remaining (approx 65%) compared to that of nissl-stained cells remaining (approx 23%) suggests a relative sparing in this region. There were no significant changes in the density of NADPH–diaphorase neurons beyond 200 μm.

Additional features of these lesions should also be noted. First, NADPH–diaphorase neurons were equally distributed within the region of 0–200 μm surrounding the lesion core, including some directly adjacent to the area of dense necrosis (Fig. 5). In addition, some morphological changes in NADPH–diaphorase-stained neurons were also apparent. Marshall and Landis (26) have reported that one feature of surviving NADPH–diaphorase in human HD is a beading of axonal and/or dendritic projections. This feature was also apparent in a number of our QUIN animals (Fig. 6). Although these "beaded" neurons were not apparent in all brain sections analyzed, they were restricted to striata receiving QUIN treatment and were not found in control striata.

Cells were not counted in striata of animals receiving 40 m*M* QUIN since these striata showed a near complete loss of both nissl and NADPH–diaphorase cells. Analysis of nissl-stained cells in animals receiving 4 m*M* QUIN ($n = 5$) revealed no significant difference in average cell number per 1.6×10^{-3} mm^3 in the region 0–200 μm (QUIN 37.46, SEM 4.16; Control 37.08, SEM 3.41) or 200–400 μm (QUIN 38.33, SEM 3.86; Control 37.98, SEM 3.80) from the area of nonspecific dialysis

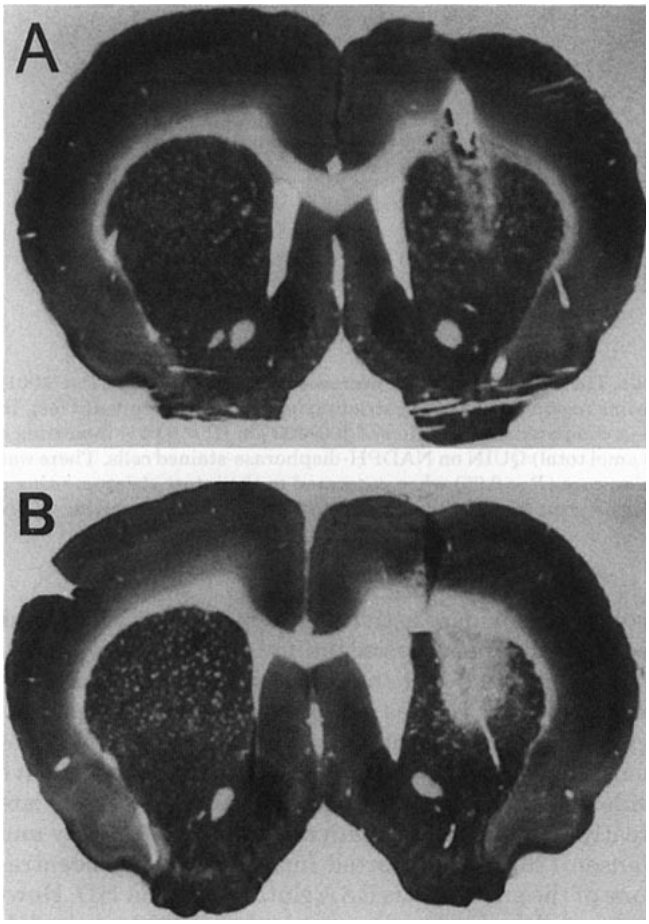

FIG. 3. Cytochrome oxidase-stained sections from animals receiving vehicle and 15 m*M* QUIN. (A) Vehicle. Reduced staining is apparent only in an area of nonselective damage caused by the dialysis probe. (B) 15 m*M* (3.3 μmol total) QUIN. Reduced staining is apparent throughout a relatively extensive region surrounding the probe site.

fiber damage, when compared to the same regions in the control striatum.

DISCUSSION

Three different concentrations of QUIN or vehicle were introduced into rat striatum over 3 weeks using dialytic delivery. Animals that were chronically exposed to a solution of 15 m*M* QUIN exhibited a gradient of striatal destruction beginning with total tissue necrosis in the lesion core immediately surrounding the probe. Adjacent to the lesion core, an area extending approximately 200 μm could be identified by a 75 to 80% decrease in nissl-stained cells. In the area extending 200 to 400 μm from the lesion core, there was a 25 to 30% decrease in nissl-stained cells. A reduction in cytochrome oxidase staining was also apparent in the region of neuronal loss surrounding the lesion core. There was a significant decrease in the number of NADPH–diaphorase staining cells in the region 0–200 μm from the lesion core. However, in this region the decrease in NADPH–diaphorase neurons represented a loss of approximately 35%, indicating a relative sparing of this subpopulation of neurons compared with nissl-stained cells. There was no significant change in the number of NADPH–diaphorase-stained neurons in the region 200–400 μm from the lesion core. Beyond 400 μm, the striatum appeared normal in all respects.

A concentration of 40 m*M* QUIN produced nonselective damage to the entire striatum and some surrounding structures, while administration of a concentration of 4 m*M* QUIN did not produce any striatal injury.

Previous reports have indicated that acute administration of QUIN into the rat striatum can produce selective neuronal degeneration similar to that seen in the striatum of HD patients (6, 7, 9, 30). Although the method of chronic dialytic delivery differs significantly from acute injections, similarities are apparent in the results following QUIN administration with both these techniques. In both cases a central lesion core was characterized by necrosis surrounding the site of administration. Adjacent to the lesion core a transition zone was identified by an extensive loss of nissl-stained cells with a less extensive decrease in NADPH–diaphorase neurons, indicating a relative sparing of NADPH–diaphorase neurons.

In addition, the present report notes a number of spared NADPH–diaphorase neurons had obvious varicose dendritic processes. Beal and colleagues (10) also produced noted varicose fibers after acute injections of the EAAs kainic acid and ibotenic acid. Similar varicose NADPH–diaphorase fibers have been observed in autopsied striatal tissue from HD patients (26). Thus, it appears that intrastriatal dialytic QUIN administration can reliably produce a region of neurodegeneration that resembles several aspects of HD pathology.

A number of research groups have reported no selective sparing of NADPH–diaphorase neurons following acute (12, 13, 33) and chronic (24, 38) intrastriatal QUIN administration. This discrepancy may reflect differences in histological analysis between laboratories. For example, two research groups (6, 7, 9, 30), as well as the present study, report that NADPH–diaphorase sparing is not absolute, but rather, relative to a reduction in the number of nissl-stained cells. In the lesion core, there is a near complete loss of all neurons. Within the transition zone, on the other hand, there is extensive loss of nissl-stained cells with a relatively small decrease in the density of NADPH–diaphorase neurons. A more detailed analysis of regional effects on striatal neurons in these earlier reports refuting selective sparing may have revealed similar findings.

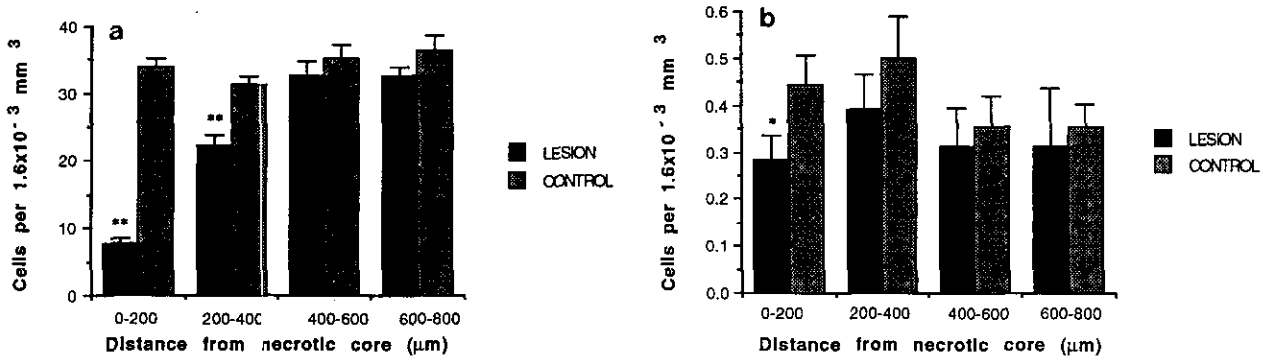

FIG. 4. (a) Effects of 15 m*M* (3.3 μmol total) QUIN on nissl-stained cells. There was a significant decrease in cells 0–200 μm (**$P < 0.001$) and 200–400 μm (**$P < 0.001$) from the lesion core when compared to the same regions in the intact striatum using a paired Student *t* test. In addition, there were significantly fewer cells in the region of 0–200 μm when compared to the region of 200–400 μm ($P < 0.001$) indicating a gradient of destruction radial to the necrotic core. (b) Effects of 15 m*M* (3.3 μmol total) QUIN on NADPH-diaphorase-stained cells. There was a significant decrease in NADPH–diaphorase cells 0–200 μm from the lesion core ($P < 0.05$) when compared to the intact striatum using a paired Student *t* test. No other significant differences in NADPH–diaphorase neurons were found. Solid bars represent striata receiving QUIN (+SEM). Hatched bars represent contralateral (intact) striata (+SEM).

Qin and colleagues (30) have suggested that inconsistent findings of neuronal sparing following acute administration of QUIN may be a result of variability in experimental conditions. Two factors possibly contributing to the inability to replicate selective sparing are the rate and volume of QUIN injections. Variation in either of these factors could subsequently affect the area of diffusion and, in turn, the relative concentration of QUIN. Supporting this suggestion, Figueredo-Cardenas *et al.* (23) have recently shown that selective sparing of NADPH–diaphorase neurons is evident following a rapid (3 min) injection of QUIN. In contrast, no relative sparing of NADPH–diaphorase neurons was observed after slow (15 min) injection of the same concentration of QUIN. Such methodological differences could explain results showing no selective sparing of NADPH–diaphorase neurons after acute QUIN injections, despite a detailed regional analysis of the striatum (2, 18).

In the present report, the effects of chronic dialytic delivery of QUIN on rotational behavior and neuronal metabolism were also examined. The results of behavioral studies indicated that amphetamine induced rotational behavior following unilateral QUIN administration is an insensitive measure of striatal injury. Behavioral abnormalities were apparent only in animals that received the highest dose (40 n*M*) of QUIN, a dose which produced massive striatal destruction.

The results of cytochrome oxidase staining indicate that although a substantial number of neurons remain intact within 400 μm of the lesion, there may be reduced metabolic activity in these neurons. Cytochrome oxidase levels are governed by neuronal functional activity (41). In the present study, a reduction in cytochrome oxidase staining was apparent throughout the region 400 μm beyond the necrotic core. Similarly, depressed cytochrome oxidase activity in some nuclear groups has been observed in autopsied brains from HD patients (41).

To date, researchers have focused primarily on overproduction of EAAs as a possible cause of HD pathology. Although data indicate that kynurenine metabolism is abnormal in HD (11, 34), there is no conclusive evidence to show that endogenous QUIN levels are greatly elevated in HD patients (25, 31, 35). Perry and Hansen (29) have reported increased CSF concentrations of the endogenous EAA glutamic acid in HD. However, some doubts have been raised about the methodology used to determine glutamate levels in this study (42).

It has more recently been proposed that HD neurodegeneration may be a result of increased sensitivity of postsynaptic responses to EAAs, and in particular, increased sensitivity to NMDA agonists (1, 5). Regardless of whether presynaptic or postsynaptic alterations underlie the pathogenesis of HD, the apparent similarity of QUIN-induced lesions to HD histopathology suggests that excessive activation of NMDA receptors is involved in the process of cell death in HD. Future research is needed to investigate the possible role of postsynaptic mechanisms, alone or in combination with presynaptic aberrations, in the neuropathology of HD.

The present experiment describes a method which consistently and accurately simulates the pattern of histopathology associated with HD and has advantages over the prior acute injection model. Acute administration of QUIN fails to accurately model the chronic progressive neurodegeneration characteristic of HD. Bakker and Foster (3) have shown that acute intrastriatal injection of a dose of QUIN that produces selective neuronal loss (200 n*M*) in the rat, also produces peak extracellular QUIN concentrations of greater than 13 m*M* within 30 min. In addition, QUIN concentrations

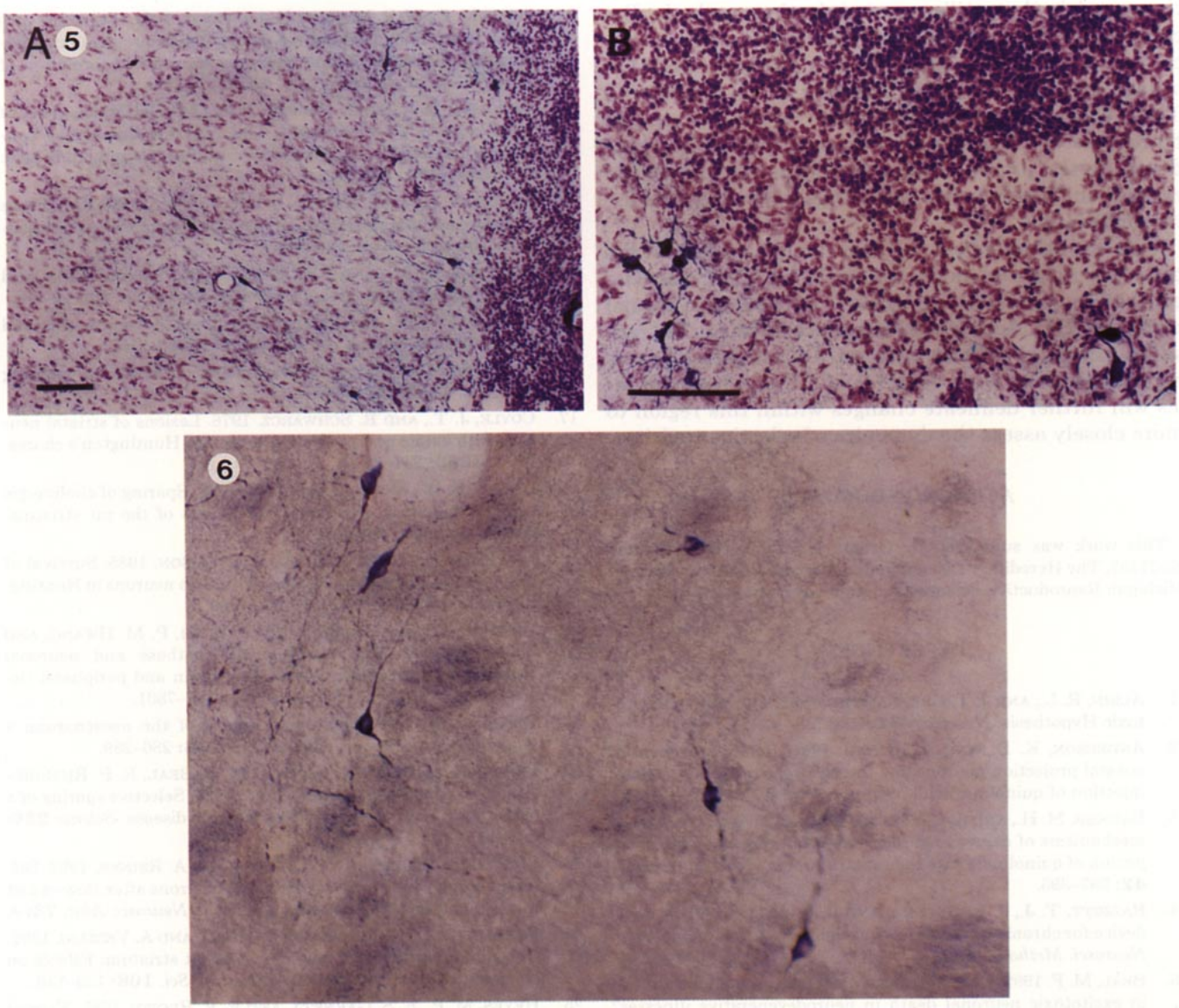

FIG. 5. NADPH–diaphorase-stained sections counterstained with cresyl violet from an animal receiving 15 m*M* (3.3 μmol total) QUIN. (A) The lesion core (right side) was easily identified by inflammatory infiltrate and lack of neurons (scale bar = 100 μm). (B) High-power magnification shows diaphorase neurons directly adjacent to the lesion core (scale bar = 100 μm).

FIG. 6. NADPH–diaphorase-stained neurons in the transition zone of a striata that was exposed to a concentration of 15 m*M* QUIN. The beaded projections of these spared neurons resemble coarse varicose projections found in some spared NADPH–diaphorase neurons of HD patients.

remained in the millimolar range for at least 2 h. Though it is likely that acute elevation of extracellular levels of EAAs is responsible for neuronal death in acute neurological disorders (1, 5, 15), it is unlikely that an acute high level of EAAs causes neuronal death in HD. Considering the gradual progression and the duration of HD, a more plausible hypothesis would be that neurodegeneration is caused by a chronic accumulation of low levels of EAAs. Supporting this contention, recent studies employing long-term administration of EAAs in low concentrations have shown excitotoxic effects that selectively spare NADPH–diaphorase neurons *in vitro* (40) and *in vivo* (32).

The dialysis apparatus allows chronic delivery of a low concentration of QUIN over a relatively large portion of the striatum without increasing striatal fluid volume. The result is a selective loss of neurons that can be attributed to a long-term increase in the striatal concentration of QUIN. This model may prove more valuable for preclinical testing of potential drug therapies

than models that utilize acute injection methods. Because acute injection produces such high transient concentrations of QUIN (3) only very high doses of NMDA antagonists are effective in reducing QUIN toxicity (8). These doses are unrealistically high for use in a chronic human disease. The low-dose chronic intoxication produced by the present model should permit preclinical evaluation of neuroprotective drugs under more realistic conditions.

Finally, it should be noted that further analysis of the presently proposed model for producing EAA neurodegeneration is needed to realize an accurate animal model for HD. Additional characterization of the transition zone is of primary interest. Degeneration in this region resembles some features of HD and future studies will further delineate changes within this region to more closely assess the dynamics of selective toxicity.

ACKNOWLEDGMENTS

This work was supported by Grants NS01300, NS19613, and NS22157, The Hereditary Disease Foundation, and The University of Michigan Reproductive Sciences Program Grant HD07048.

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