Local and Distant Neuronal Degeneration Following Intrastriatal Injection of Kainic Acid

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Abstract. After intrastriatal injection, the neurotoxin, kainic acid, was cleared from the rat forebrain in a biphasic manner with 70% eliminated within 2 hours; by 24 hours after infusion, less than 1% of the kainic acid remained in the forebrain. The kainic acid diffused into adjacent brain structures, achieving μmolar concentrations in several regions ipsilateral to the injected striatum. At various times after intrastriatal injection of 9.3 nmoles of kainic acid, the brain was serially sectioned; the sections were stained for Nissl substance with cresyl violet or for degenerating neurons with the ammoniacal silver method. Neuronal degeneration spread unevenly into contiguous structures from the central sphere in the injected striatum and affected the ipsilateral pyriform cortex and amygdala, the deep layers of the overlying cerebral cortex, and the medial aspects of the bed nucleus of the stria terminalis and of the nucleus accumbens. In half of the rats, the pyriform cortex contralateral to the side of injection also underwent degeneration. A subpopulation of pyramidal cells in layer IV of the lateral neocortex and the CA3-CA1 pyramidal cells in the ipsilateral hippocampus were selectively affected, whereas adjacent neurons remained intact. The distribution of agyrophile fibers and terminals in subcortical structures was consistent with the degeneration of neurons of origin in the affected striatal and extrastriatal regions. Brain sections stained by the gold sublimate technique from rats perfused 20 days after injection revealed an intense astrocytic response in all areas affected by acute neuronal degeneration. Extrastriatal damage could be markedly reduced by injection of lower doses of kainic acid (2.3 nmoles) with brief anesthesia; under these conditions, however, the subpopulation of large striatal neurons were relatively resistant, as compared to the Golgi II neurons.

These studies demonstrate significant and variable neuronal degeneration beyond the primary site of the lesion after intracerebral injection of kainic acid; several factors affect the pattern of degeneration, including the amount of kainic acid injected, its biological activity, its diffusion, duration of anesthesia, and variable sensitivity of neurons. Consequently, care must be exercised in the use of this neurotoxin to determine the extent and selectivity of neuronal damage, particularly with reference to neuronal vulnerability beyond the central sphere of intrinsic neuronal degeneration.

INTRODUCTION

Kainic acid, a conformationally restricted analogue of L-glutamate, is a potent neuroexcitant in the central nervous system of mammals (Biscoe et al., 1976). Intrastriatal injection of kainic acid produces a rapid and selective degeneration of neurons with their cell bodies located in the striatum, but spares axons passing through or terminating in the region that come from neurons

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outside the area directly affected by the toxin (Coyle and Schwarcz, 1976; Coyle et al., 1978). The selectivity of action is supported by extensive neurochemical studies that have shown marked reductions in presynaptic markers for cholinergic and GABAergic neurons intrinsic to the striatum but maintenance of the presynaptic markers for the dopaminergic terminals innervating the region (Schwarcz and Coyle, 1977). Electron microscopic analysis (Coyle et al., 1978; Hattori and McGeer, 1978) and studies of the retrograde transport of horseradish peroxidase (Divac et al., 1978) have also shown that internal capsule fibers of cortical origin, which traverse the lesioned striatum, remain intact. The pattern of neuronal degeneration and the neurochemical alterations following intrastriatal injection in the rat closely resemble those occurring in the hereditary neurodegenerative disorder, Huntington’s disease (Coyle and Schwarcz, 1976).

Neurochemical and histologic studies in the past have focused primarily on the specificity of the neurotoxic effects of kainate in the injected striatum and have not systematically assessed neuronal damage in regions distant from the primary site of injection. That extrastriatal neuronal degeneration may be a significant factor has been suggested by the observation that the CA3-CA4 pyramidal cells in the ipsilateral hippocampus degenerate following striatal or intraventricular injection of kainate (Coyle et al., 1978; Nadler et al., 1978). In addition, a recent report has noted significant damage to the cerebral cortex overlying the kainate-injected striatum (Wuerthule et al., 1978). Kainate lesions of the cerebellum have further dramatized the marked variation in neuronal vulnerability, with the cerebellar granule cells being remarkably insensitive to its neurotoxic action (Herndon and Coyle, 1977). Since there is growing interest in the use of the kainate lesion to map neuronal projections and to determine the behavioral consequences of selective brain lesions, it has become apparent that a more precise description of variation in neuronal vulnerability is warranted.

In this report, we have characterized neuronal cell loss following striatal injection of kainic acid in the rat by several techniques. To visualize directly the degenerating neuronal perikarya as well as their axons and terminals, brain sections were stained by the ammoniacal silver impregnation technique (Eager, 1971). As an alternative and indirect means for monitoring neuronal degeneration, the reactive astrocytes that proliferate in affected areas were stained in the subacute stage after lesion by Cajal’s gold sublimate method. The results from these two methods were correlated with neuronal cell loss in Nissl-stained sections. The clearance of the kainic acid from the injection site and its spread into adjacent brain structures were monitored.

**MATERIALS AND METHODS**

*Preparation of tissues.* Male Sprague-Dawley rats (Sprague-Dawley, Madison, Wis.) weighing 155 to 165 grams were used for all experiments. Most animals were anesthetized by intraperitoneal injection of 0.6 ml of Equithesin (Jensen-Salsbury Labs, Kansas City, Mo.); this agent, which contains a mixture of pentobarbital, chloral hydrate, ethanol, and magnesium sulfate, results in anesthesia lasting from 90 to 120 minutes.
Some animals were anesthetized briefly with ether, which was administered by nose cone during the period of intracerebral injection; within 5 minutes of removal of the nose cone, the lesioned animals were responsive to pain and were spontaneously active. The anesthetized animals were placed in a David Kopf small animal stereotaxic apparatus and the calvarium was exposed. A 0.3 mm Hamilton cannula was inserted into the striatum through a burr hole in the calvarium at the following coordinates: 7.9 mm anterior to the intra-aural line, 2.6 mm lateral to the midline, and 4.8 mm ventral to the pial surface. Kainic acid (Lot 116C-0404, Sigma Chemical Company, St. Louis, Mo.) dissolved in artificial cerebrospinal fluid solution titrated to pH 7.4 was infused over a period of 1 minute; the total volume injected was 0.5 μl, containing either 9.3 or 2.3 nmoles of kainic acid. After injection, the scalp wound was closed with metal sutures.

Disposition of [3H]kainic acid. For studies of the clearance of kainic acid, 500,000 cpm (0.12 nmoles) of [3H]kainic acid (4.1 Ci/mmol, Amersham-Searle, Arlington Heights, Ill.) was added to 9.3 nmoles of unlabeled kainic acid prior to injection. At various times after injection, the animals were sacrificed by decapitation and their brains were rapidly removed and dissected on an aluminum block resting on ice at 2°C. The brain were hemisected at the midline, and various regions ipsilateral and contralateral to the injection were dissected according to the method of Glowinski and Iversen (1966). The brain parts were weighed, sonicated in 95% ethanol, and the homogenate was then centrifuged at 20,000 × g × 20 minutes in a Sorval refrigerated centrifuge at 5°C. The supernatant fluid was decanted and counted for radioactivity by scintillation spectrometry. Portions of the extract were concentrated in a vacuum centrifuge and subjected to thin layer chromatography on Eastman silica gel plates (Eastman Kodak, Rochester, N.Y.). The chromatograms were developed in a system of N-butanol, glacial acetic acid, and water (95:4:1); 5 μg of authentic kainic acid was spotted at the origin with the extract, and the kainic acid was identified after chromatography by staining with ninhydrin. The entire strip from the origin to the solvent front was cut at 1 cm intervals, and the silica from the individual segments was scraped into counting vials, eluted with 1 ml of water, and counted for radioactivity in Aquasol (New England Nuclear Corp., Boston, Mass.) in a scintillation spectrometer with efficiency monitored by internal and external standards.

Histology. At various times after injection of kainic acid, the rats were anesthetized with chloral hydrate and the heart was exposed through a midline incision. The rats were perfused via an intracardiac cannula with ice-cold phosphate buffered formalin fixative solution. The brain was rapidly removed and fixed for an additional ten days to two weeks at 5°C. Blocks were cut from the brain and cryoprotected by incubation in formalin fixative containing 30% sucrose (wt/vol) at 4°C until the blocks sunk. The blocks were then frozen onto a cryostat chuck and sectioned at 20 to 40 μm. Sections were stained for Nissl substance using cresyl violet. To reveal degenerating neurons and their axonal projections, sections were stained by the ammoniacal silver impregnation technique, as described by Eager (1971). Tissue was examined at several intervals after injection to determine the interval between lesion and sacrifice optimal for impregnation; this varied for different brain regions and types of processes. Degenerating neuronal perikarya near the injection site reacted with silver at 6 hours after injection, while the terminals of striatal projections stained best at 96 hours after injection. Sections obtained from rats sacrificed 21 days after striatal kainate lesion were stained for astrocytes by the gold sublimate technique (Railes et al., 1973). Observations reported for the silver impregnation, gold sublimate, and Nissl staining techniques are based upon at least three preparations at each time point. Craigie’s Neuroanatomy of the Rat (Zeman and Innis, 1963) was used as the basis for neuroanatomical nomenclature.
RESULTS

Diffusion and clearance of [\( ^3\text{H} \)]kainic acid injected into the striatum. The [\( ^3\text{H} \)]kainic acid (9.3 nmoles) exhibited a biphasic clearance after injection into the striatum (Fig. 1). Within two hours after injection, more than 70% of the radiolabel was cleared from the forebrain, with an estimated half-life of 48 ± 12 minutes. Subsequently, there was a slower rate of clearance of the tritium, with a half-life of 4 ± 0.5 hours. By 24 hours after injection, only 0.5% of the injected radiolabeled kainic acid remained in the forebrain. Chromatographic characterization of the [\( ^3\text{H} \)] product extracted from the forebrain revealed that 90, 84, and 78% of the radiolabel migrated isographically with authentic kainic acid at 20 minutes and 2 and 6 hours after injection, respectively. Up to 6 hours after injection, less than 3% of the radiolabel was associated with protein precipitated by the ethanol extraction. Kainic acid therefore remained largely unmetabolized after injection, and negligible amounts were bound covalently or were incorporated into striatal protein.

Various amounts of the radiolabeled kainic acid gained access to regions outside the injected striatum (Table 1). Whereas the level of [\( ^3\text{H} \)]kainic acid

![Graph of clearance of [\( ^3\text{H} \)]kainic acid from forebrain.](image)

Fig. 1. Clearance of [\( ^3\text{H} \)]kainic acid from forebrain. Rats received a stereotaxic injection of [\( ^3\text{H} \)]kainic acid (500,000 cpm in 9.4 nmoles) in the striatum as described in Methods. At various times after injection, the rats were sacrificed and the amount of tritium in the forebrain (striatum and frontal cortex) was measured. Each point is the mean of three or more samples.
TABLE 1
Distribution of $^{3}$H]Kainic Acid in Brain After Intrastriatal Injection

<table>
<thead>
<tr>
<th>Region</th>
<th>10 min</th>
<th>20 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>4 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral striatum</td>
<td>124 ± 13</td>
<td>101 ± 9</td>
<td>94 ± 12</td>
<td>40 ± 8</td>
<td>34 ± 8</td>
<td>0.06</td>
</tr>
<tr>
<td>Ipsilateral frontal cortex</td>
<td>12 ± 3</td>
<td>10 ± 2</td>
<td>12 ± 4</td>
<td>4.5 ± 0.8</td>
<td>5.6 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Ipsilateral olfactory cortex</td>
<td>0.31 ± 0.06</td>
<td>0.83 ± 0.23</td>
<td>0.90 ± 0.21</td>
<td>0.72 ± 0.300</td>
<td>0.87 ± 0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Ipsilateral lateral cortex</td>
<td>0.10 ± 0.02</td>
<td>0.13 ± 0.04</td>
<td>0.09 ± 0.02</td>
<td>0.31 ± 0.06</td>
<td>0.34 ± 0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Ipsilateral hippocampus</td>
<td>0.17 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.32 ± 0.06</td>
<td>0.36 ± 0.06</td>
<td>0.26 ± 0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>0.18 ± 0.01</td>
<td>0.35 ± 0.10</td>
<td>0.51 ± 0.08</td>
<td>0.88 ± 0.45</td>
<td>0.52 ± 0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>0.25 ± 0.06</td>
<td>0.59 ± 0.26</td>
<td>0.38 ± 0.10</td>
<td>1.51 ± 0.75</td>
<td>0.39 ± 0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Medulla-Pons</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.12 ± 0.03</td>
<td>0.11 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Contralateral olfactory cortex</td>
<td>0.02 ± 0.01</td>
<td>—</td>
<td>—</td>
<td>0.07 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>—</td>
</tr>
<tr>
<td>Contralateral striatum</td>
<td>0.01 ± 0.01</td>
<td>—</td>
<td>—</td>
<td>0.09 ± 0.03</td>
<td>0.12 ± 0.04</td>
<td>—</td>
</tr>
</tbody>
</table>

Rats received a stereotoxic injection in the striatum of 9.4 nmoles of kainic acid, containing 500,000 cpm of $^{3}$H]kainic acid. At various times after injection, the regions of the brain were measured for apparent kainic acid by scintillation spectrometry of tissue extracts as described in Methods. Each value is the mean ± s.e.m. of three to five samples, except for the 24-hour value, which is the mean of two samples.

decreased progressively in the injected forebrain, it tended to exhibit a delayed increase in the extrastriatal regions, achieving a peak at one hour after injection in the septum and six hours after injection in the diencephalon; by 24 hours after injection, all areas contained only traces of radioactivity. On the assumption that the molecule remains largely intact, as suggested by the chromatographic studies, the apparent peak concentrations achieved in extrastriatal regions ranged from 0.4 pmole per mg tissue in the hippocampus to 6.8 pmoles per mg tissue in the septum nucleus accumbens. Concentrations of kainic acid in the micromolar range were therefore achieved in extrastriatal regions following the striatal injection.

Neuronal cell degeneration following intrastriatal injection of 9.3 nmoles of kainic acid. As previously described (Coyle et al., 1978), all neuronal perikarya degenerated within a radius of approximately 1.5 mm around the cannula tip (Fig. 2); a cylinder of nonspecific damage and hemorrhage with a radius of 0.1 mm was evident along the tract of the cannula. Striatal intrinsic neurons at the primary site of the lesion were not as well impregnated with silver at any time from 3 to 72 hours after the lesion was made, by which time their degeneration was complete; instead, the affected perikarya stained a pale brown with irregular silver precipitates in their Nissl substance. The inadequate impregnation of these neurons is in contrast to the fairly complete staining of many extrastriatal neurons that undergo degeneration (see below).

In a much larger area surrounding the central sphere of complete neuronal cell loss, neurons were variably affected by the kainate. Anteriorly, the lateral half of the nucleus accumbens was moderately to severely affected by intrinsic
neuronal degeneration; an abrupt border separated the neurons lying in the medial half of the nucleus, which were completely spared by the neurotoxin. A
finger-like extension of neuronal degeneration invaded the lateral half of the bed nucleus of the stria terminalis; this represented the most medial extent of marked neuronal loss. Severe neuronal degeneration was seen in the amygdaloid nuclei as far anterior as the tuberculum olfactorium and posterior to the subiculum. At the anterior aspects of the pyriform cortex and the amygdaloid nuclei, this degeneration was contiguous to that in the injected striatum; in its caudal reaches, however, the degeneration was restricted to these structures, with the adjacent ventral hippocampus and entopeduncular nucleus remaining unaffected. The large polymorphic and pyramidal cells in the pyriform cortex and amygdaloid nuclei were the most profoundly affected, whereas granule cells appeared more resistant. The degeneration occurred rapidly and was associated with profound local edema (Fig. 3); occasionally, hemorrhage into the affected areas of pyriform cortex was observed. In over half of the brains examined (10 of 19), the pyriform cortex and amygdaloid nuclei contralateral to the side of injection underwent degeneration, although in a somewhat more restricted degree than in the ipsilateral side.
Fig. 3. Degeneration in pyriform cortex ipsilateral to the striatal kainic acid injection. A 30-micron section through the pyriform cortex adjacent to the striatal kainic acid injection (9.3 nmoles) was stained by the ammoniacal silver technique; the rat was perfused 24 hours after injection. Note the pallisade of agyrophilic fibers perpendicular to the pial (p) surface and the marked edematous (E) changes in the underlying gray matter. ×160.

An isthmus of profound neuronal degeneration projected up from the corpus callosum and spread medially and laterally from the cannula tract in the dorsal parietal cortex. Coronal and parasagittal sections through the parietal and frontal cortex revealed a moderate to severe degeneration of neurons in the deep layers of the cerebral cortex overlying the injected striatum. In the lateral parietal cortex extending inferiorly to the insular cortex, a population of pyramidal cells in layer 4 were exquisitely and selectively sensitive to kainic acid (Fig. 4). Within 12 hours of injection, these neurons became intensely agyrophilic, revealing their perikarya and apical and basilar dendrites; notably, other pyramidal cells in the same layer and neurons in adjacent layers were unaffected.

An additional area distant from the primary site of injection affected by a neuronal degeneration was the CA3-CA4 pyramidal cell layer in the hippocampus ipsilateral to the site of injection (Fig. 5). The affected pyramidal cells, in their most caudal location, lay approximately 3.0 mm posterior to the site of the striatal injection. These cells became intensely agyrophilic within 36 hours after injection. The selective vulnerability of these neurons was emphasized by the integrity of surrounding granule cells in the dentate gyrus and by the abrupt demarcation between the degenerating CA3 and the spared CA2 pyramidal cells.

Fiber and terminal degeneration following intrastratal injection of 9 nmoles of kainic acid. Agyrophilic axonal processes were observed coursing from
degenerating pyramidal cells in the affected deep layers of the frontal and parietal cortex anterior to and overlying the injected striatum (Fig. 6). Isolated degenerating fibers were present in the internal capsule bundles within the injected striatum; there appeared to be little correlation between the density of the agyrophilic fibers in these bundles and the severity of cell loss in the surrounding gray matter. The intensity of fiber staining in the internal capsule bundles was much less severe than that following ipsilateral cortical ablation;
the degeneration of corticofugal axons probably reflected the damage to the pyramidal cells in the affected and the deep layers of frontal and parietal cortex rather than damage incurred locally at the site of injection. The corpus callosum was stippled with moderate fiber degeneration, which could be followed caudally into the cerebral peduncles and into the corticospinal tract in the pons. The density of degenerating axons increased in the globus pallidus, with some coursing medially and superiorly via the ventral thalamic and the anterior thalamic nuclei, which were also marked by degenerating terminals. Fiber and terminal degeneration were noteworthy in the zona incerta; more caudally, the substantia nigra and zona reticularis contained many agyrophilic fibers and terminals, whereas the zona compacta was virtually devoid of degenerating processes (Fig. 7). Consistent with the destruction of the CA3-CA4 pyramidal cells, degenerating axons were visible in the fimbria, fornix, and medial septal nuclei. As a result of direct toxic affects on polymorphic and pyramidal cells in the amygdaloid nuclei and pyriform cortex, rather intense fiber degeneration was apparent in the stria terminalis, and fragmented terminals were visible in the posterior and lateral nuclei of the hypothalamus.

Astrocytic response following intrastratial injection of kainic acid. Reactive astrocytes were selectively stained by the gold sublimate method in rats that had received an intrastratial injection of 9 nmoles of kainic acid three weeks
prior to sacrifice. An intense astrocytosis involved the entire striatum, the lateral half of the nucleus accumbens, the deep layers of the frontal and parietal cortex overlying the injected striatum, and the pyriform cortex and amygdaloid nuclei (Fig. 8). In the injected striatum, a dense mat of darkly staining fibers occupied the area between the internal capsule fiber bundles; only the processes and not the cell bodies of these astrocytes reacted with the gold (Fig. 9). Although Nissl-stained sections did not reveal a significant proliferation of astrocytes within the bundles, the gold sublimate stain indicated a considerable investment of the bundles by astrocytic processes. The astrocytic response in the ipsilateral pyriform cortex, the amygdaloid nuclei, the lateral nucleus accumbens, and the lateral half of the bed nucleus of the stria terminalis was particularly intense with their somata, and processes stained in their entirety. More caudally, reactive astrocytes completely occupied the globus pallidus and spread medially into the ventral thalamic nucleus; astrocytic infiltrates were present bilaterally in the medial dorsal nuclei of the thalamus. A marked astrocytosis was seen in the area formerly occupied by the CA3-CA4 pyramidal cells in the dentate gyrus of the hippocampus (Fig. 10). In nearly half of the affected animals, reactive astrocytes were present in the contralateral pyriform
cortex and amygdaloid nuclei. Except for the dorsomedial portion of the thalamus, the reactive astrocytosis involved areas affected by neuronal cell body degeneration.

Effect of intrastriatal injection of 2.3 nm of kainic acid in ether anesthetized rats. In an attempt to intensify the specific neurotoxic action of kainic acid in the striatum (Zacze et al., 1978), rats were anesthetized with ether rather than Equithesin during the period of injection, and a fourfold lower dose of kainic
Fig. 8. Reactive astrocytes following striatal kainic acid injection. Schematic representation shows the areas affected by astrocytic reaction 20 days following the intrastratial injection of 9.3 nmoles of kainic acid. The results are abstracted from four rats.

acid was injected (2.3 nm). Under these conditions, a nearly spherical volume of striatal tissue with a radius of 1.2 mm was affected by neuronal degeneration (Fig. 11). The large striatal neurons appeared to be relatively resistant to the neurotoxic action of the agent, whereas the loss of the smaller Golgi II neurons was nearly complete (Fig. 12). Only a small area of degeneration, limited to the immediate vicinity around the cannula tract, was present in the dorsal parietal cortex. This restricted cortical damage was consistent with the occasional agyrophilic fibers present in the internal capsule and, to a lesser extent, in the corpus callosum. The lesion did not extend into the globus pallidus, thalamus, or the nucleus accumbens; the ipsilateral or contralateral pyriform cortex, amygdaloid nuclei, and the CA3-CA4 region of the ipsilateral hippocampus were unaffected. Striatal efferent degeneration was clearly visible in the globus pallidus and in the zona reticulata of the substantia nigra.

DISCUSSION

Previous studies on the neurochemistry and the neuropathological features of kainic acid lesions of the striatum have focused primarily on the specificity of neuronal degeneration at the site of injection (Coyle and Schwarcz, 1976; Hattori and McGeer, 1978; Coyle et al., 1978). In most cases, histologic analysis has been limited to the study of Nissl-stained preparations. With the possible
Fig. 9. Reactive astrocytes in the striatum with lesions due to kainic acid. Low-power photomicrographs were taken of the contralateral uninjected (A) and kainic acid-injected striatum (B). The rat was perfused 20 days after injection of 9.3 nmoles of kainic acid, and the forebrain section containing both striata was processed by Cajal's gold sublimate technique. Note the virtual absence of stained astrocytic cell bodies in the uninjected striatum and the dense mat of astrocytic processes in the side injected with kainic acid. ×160.
Fig. 10. Reactive astrocytes in the dentate gyrus of the hippocampus following striatal kainic acid lesion. Low-power photomicrographs were taken of the dentate gyrus contralateral (A) and ipsilateral (B) to the kainic acid-injected striatum. The rat was perfused 20 days after injection of 9.3 nmoles of kainic acid and the forebrain sagittal section containing both hippocampi was processed by Cajal's gold sublimate technique. Note the marked astrocytosis in the area between the granule cell layers (G) formerly occupied by the CA1 pyramidal cells (P). ×160.
Fig. 11. Neuronal degeneration following intrastratal injection of 2.3 nmoles of kainic acid in the ether-anesthetized rat. Schematic representation shows the area affected by neuronal degeneration as abstracted from five rats processed with the ammoniacal silver method and two rats stained with Cajal's gold sublimate technique.

exception of a recent report by Schwok et al. (1978), there has been little use of histologic techniques that positively stain for neuronal degeneration or its sequelae. Positive techniques may be particularly helpful in light of evidence that some neurons distant from the primary injection site exhibit unusual sensitivity to the neurotoxin (Coyle et al., 1978). In the present study, we have
Fig. 12. Survival of large striatal neurons in the striatum with a kainate lesion. Photomicrographs of cresyl violet-stained sections through the contralateral (A) and kainic acid-injected (B) striatum demonstrate the large striatal neurons (arrows). The rat received an intrastratal injection of 2.3 nmole of kainic acid under ether anesthesia and was perfused 48 hours later. Note the absence of intact Golgi II neurons in the neuropil of the injected striatum (B). ×160.
used a combination of Nissl stain, ammoniacal silver stain for degenerating neurons, and the gold sublimate stain for reactive astrocytes to identify areas affected by neuronal, axonal, and terminal degeneration following striatal kainate injection. The silver stain offers a particular advantage because, under proper conditions, it can reveal degeneration of isolated sets of neurons that might be overlooked in Nissl-stained preparations.

As previously reported, injection of 9 nm kainic acid into the striatum of a rat anesthetized with a combination of pentobarbital and chlorohydrate (Equithesin) produced an area of profound neuronal degeneration with a radius of approximately 1.5 mm (Coyle et al., 1978). The present study revealed the presence of significant neuronal degeneration in areas contiguous to, as well as distant from, the injected striatum. In reviewing sections from previous histologic studies done in this laboratory, however, it has become apparent that significant variation in the extent of the lesions occurs with different batches of kainic acid. This may reflect the fact that kainic acid is a biological substance whose neurophysiologic and neurotoxic actions are dependent upon the maintenance of its structure and stereoisomerism (Shinozaki and Shibuya, 1976). It is conceivable that this variation results from differences in purity and molecular integrity of different batches of the compound, as well as from the role of volume and rate of injection and duration of anesthesia (McGeer and McGeer, 1978; Zaczek et al., 1978; Schwarcz et al., 1978).

In the present study, several extrastriatal areas were notable for their sensitivity to kainic acid. Neuronal cell loss occurred in the lateral half of the nucleus accumbens and the lateral half of the bed nucleus of the stria terminalis and extensively involved the pyriform cortex and amygdaloid nuclei ipsilateral to the site of injection. In over half of the injected animals, the neurons in the pyriform cortex and amygdaloid nuclei contralateral to the site of injection also underwent degeneration. The reason for this inconsistent pattern of degeneration in the contralateral pyriform cortex remains unclear. A notable feature of the neuronal degeneration in the pyriform cortex is that it is associated with considerable cellular edema that occasionally results in hemorrhagic necrosis of the area. As previously reported by Coyle et al. (1978) and further characterized by Nadler et al. (1978), the hippocampal CA3-CA4 pyramidal cells are quite sensitive to kainic acid and represent the group of neurons most distant from the injection site that undergo degeneration.

The studies on diffusion and clearance of radiolabeled kainic acid injected into the striatum are consistent with the pattern of neuronal degeneration in the striatum and the affected extrastriatal areas. The injected kainic acid diffuses to all areas in the forebrain ipsilateral to the injection as well as, to a lesser extent, the contralateral forebrain. Although the percentage of the injected dose that reaches the ipsilateral hippocampus and pyriform cortex was quite small (less than 2%), the absolute concentration of kainate attained in these regions approached the micromolar level. The receptor binding sites that appear to mediate the neurotoxic action of kainic acid have affinity constants (Kd’s) of 5 and 50 nM (London and Coyle, 1979); thus, the concentration of kainate attained in the extrastriatal regions affected by neuronal degeneration surpasses
that required to saturate the receptors. Since diffusion occurs at a finite rate, it is noteworthy that the peak accumulation of kainic acid in the extrastriatal regions occurs 2 to 4 hours after the injection. In previous studies, we have shown that anesthetics attenuate the neurotoxic action of kainic acid in proportion to the duration of action (Zaczek et al., 1978). The anesthesia commonly employed for kainate lesions consists of a combination of chlorohydrate and pentobarbital; the duration of the anesthesia with these agents is 1½ to 2 hours. Thus, when the protective effects of the anesthesia are remitting, the concentration of kainate in the extrastriatal regions is reaching its peak.

Because of the finding that anesthetics can attenuate the neurotoxic effects of kainic acid in proportion to the duration of their action, we have explored the effects of lower doses of kainic acid injected into the striatum of rats anesthetized briefly with ether. In rats anesthetized with the chlorohydrate-pentobarbital combination, 2.3 nmole of kainic acid produced a small lesion with a radius of approximately 0.3 mm. In the ether-anesthetized rat, the same dose of kainate produced a lesion of primary neuronal degeneration of approximately 1.1 mm in radius (e.g., a radius 75% of that occurring with 9.2 nmole injected in the pentobarbital-chlorohydrate-anesthetized animals). With the lower dose and a brief anesthesia, however, there was negligible damage to extrastriatal regions; in particular, the pyriform cortex, the amygdaloid nuclei, the globus pallidus, and the hippocampal pyramidal cells were spared. These results suggest that with lower doses of kainic acid the amount which diffuses into adjacent structures is below the threshold required for neuronal degeneration. The use of brief anesthesia with lower doses of kainic acid offers an important strategy to limit the extent of the lesion, while at the same time producing specific neuronal degeneration in a significant area around the primary site of injection.

Recent studies indicate that the mechanism of neurotoxic action of kainic acid is complex. It was originally hypothesized that kainic acid destroyed neurons by directly activating excitatory glutamate receptors that are present on nearly all neurons in the central nervous system of mammals (Olney et al., 1974; Coyle and Schwarcz, 1976). It has become evident that the afferent input to neurons specifies their vulnerability. Destruction of the cortical-striatal projections blocks the neurotoxicity of kainic acid injected into the striatum (Biziore and Coyle, 1978; McGeer et al., 1978); these afferents appear to use glutamate as their excitatory neurotransmitter (Divac et al., 1977). Neurophysiologic and biochemical studies of the receptors activated by kainic acid suggest that these receptors are highly specific for the kainic acid molecule and are not primary receptor sites for glutamic acid (Hall et al., 1978; London and Coyle, 1979). A combination of factors, including receptor density and characteristics of afferent input, may contribute to the unusual sensitivity of the hippocampal CA3-CA1 pyramidal cells and the amygdaloid nuclei or the relative resistance of the large striatal neurons.

With the demonstration that kainic acid is a selective neurotoxin which kills neurons on the basis of the localization of their perikarya, there has been a growing interest in the use of this technique in neurobiology (McGeer et al.,
1978). Although the present study serves to further confirm the selective nature of the neurotoxic action of kainic acid, it nevertheless underlines the wide variation in neuronal vulnerability to the agent. This technique of producing lesions may be particularly hazardous for behavioral studies because of the possibility of damage to highly sensitive neurons distant from the primary site of injection. Because of the extensive damage to extrastriatal regions, in particular the pyriform cortex, bed nucleus, nucleus accumbens, and hippocampus, following striatal kainate lesions, interpretations about the specificity of behavioral alterations must be accepted with caution. Although, in the present study, lesions highly localized to the striatum were obtained with a low dose of kainic acid injected into ether-anesthetized animals, some neuronal damage still occurred in the overlying cerebral cortex.

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